International Union for the Study of Social Insects
International Congress

13 - 18 July 2014 | Cairns Convention Centre | Queensland, Australia

ABSTRACTS

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Oral Presentations

OR001

Natural selection on the regulation of foraging in harvester ants

Deborah M. Gordon

A long-term study of a population of colonies of the desert seed-eating ant *Pogonomyrmex barbatus* has made it possible to investigate how natural selection is shaping collective behavior. It appears that colonies that regulate foraging so as to conserve water in current drought conditions are more likely to have offspring colonies. Behavioral studies show that colonies regulate foraging activity according to food availability and current humidity. The collective behavior that regulates foraging activity is based on positive feedback from interactions of outgoing and returning foragers. Demographic data and observations of foraging activity show no evidence of any behavioral or ecological differences between the two lineages of this dependent-lineage population. There is variation among all colonies in the regulation of foraging activity, and we are investigating how this variation arises from differences among colonies in how individual ants respond to interaction. Using microsatellite variation at 5 loci, we matched parent and offspring colonies, as colonies founded by daughter queens of a known parent queen, for 270 colonies of known age. We were able to estimate a life table for this population of colonies and to estimate the female component of colony lifetime reproductive success. Only about 25% of colonies have offspring colonies, and surprisingly, we found no evidence of reproductive senescence in the 20-30 lifespan of a colony. There is evidence of heritability from parent to offspring colonies in the regulation of foraging, and we are investigating this further. Since variation among colonies in the regulation of foraging behavior is associated with variation in colony lifetime reproductive success, it appears that natural selection is shaping collective behavior so as to favor less foraging activity in poor conditions.

OR002

Individual personalities within a honeybee colony

Alexander Walton, Amy Toth

Individual differences, often referred to as personalities or behavioral syndromes, are an important new area of investigation in the biological and social sciences and are being investigated in many animals and humans. Differences in personalities are something we hold dear as humans because they not only make us who we are, but lead to role specialization, and thus contribute to the maintenance of a functioning society. It is likely that personality differences can serve a similar role in an insect society. I have hypothesized that some individuals in the hive have an ‘affiliative’ personality: that throughout their lifetime they perform tasks that require interacting with their nestmates. I have also hypothesized that there are ‘non-affiliative’ bees that perform non-interactive tasks throughout their life. Detailed observations on individual bees can be achieved with an observation hive, a glass-walled bee hive that allows for monitoring of activity within the colony without disturbing the insects. We built observation hives and recorded the behaviors of individually marked, same-age bees throughout their 5 week lifetime for four different hives. Tasks were placed into the categories of affiliative or non-affiliative based on the criteria of whether the task does or does not require interaction with another individual. Analyses are ongoing but results thus far suggest there are inter-individual differences in several social behaviors. Investigation of individual differences in personalities within the social insect colony could provide insight into the role of specialization during social evolution. Further research can illuminate how personalities may contribute to the fitness and success of the colony

OR003

Adult-larva epistasis affects development and fitness in a clonal ant

Serafino Teseo, Nicolas Châline, Pierre Jaisson, Daniel J.C. Kronauer

In social species, the phenotype and fitness an individual depend not only on its genotype, but also on the genotype of its social partners. However, how these indirect genetic effects affect genotype fitness in competitive situations is poorly understood, for example because of the lack of control over the genetic composition of social groups. In our study, we investigate how indirect genetic effects affect phenotypic plasticity and fitness of two clones (A and B) of the parthenogenetic ant *Cerapachys biroi*, both in monoclonal and chimeric colonies. We show that, while clone B has lower fitness in isolation, it consistently outcompetes clone A in chimeras. The reason is that, in chimeras, clone B produces more individuals specializing in reproduction rather than cooperative tasks, behaving like a facultative social parasite. A cross-fostering experiment shows that the proportion of larvae that develop in high-reproductive individuals depends on intergenomic epistasis between larvae and nursing adults, explaining the flexible allocation strategy of clone B. In *C. biroi*, larval fate depends thus not only on the genotype of the larvae, but also on the genotype of the nursing adults, which exert a control over larval development. This mechanism has possibly evolved to maintain an optimal colony-level allocation in reproductive vs. ergonomic tasks within monoclonal natural colonies, but its clone-specific calibration leads to differential fitness levels for different clones mixed within the same colony. Our study shows that intergenomic epistasis could be a possible proximate mechanism for social parasitism in ants, and reveals striking analogies between social insects and social microbes.

OR004

Social synchronization of activity rhythms and the temporal organization of honeybees

Taro Fuchikawa, Moshe Nagari, Ada Eban-Rothschild, Guy Bloch

Temporal organization of activity may improve the coordination and efficiency of insect societies. Young honeybee workers typically perform in-hive activities such as brood care and therefore spend most of the time inside the dark and theromoregulated hive. Nevertheless, when removed from the hive they show circadian rhythms in locomotor activity and brain clock gene expression that are synchronized to the ambient day-night cycles. We examined the relative importance of social and light synchronization in free-foraging colonies by exposing young nest bees to conflicting social and photic environmental cycles. We established hives with conflicting information for photic (illumination regime) and social (forager activity) environmental cycles. To determine the phase relationships between the bee rhythms and these two conflicting environmental cycles we transferred bees from the hive to individual cages in constant lab conditions and monitored their locomotor activity. We found that: (1) the rhythms of foragers were synchronized to the hive entrance opening and closing cycle, (2) the rhythms of nurse-age bees that were housed in a cage adjacent to the observation hive were synchronized to the illumination regime, (3) the rhythms of nurses were similar to that of foragers in that the offset of activity was comparable to the time of hive entrance closing. In a complementary set of experiments we caged young bees in single- and double-mesh enclosures inside the hive. These experiments showed potent social synchronization even in bees deprived of direct contact with the brood or other bees. Together our results suggest that nest honeybees adjust their activity rhythms to environmental cycles through volatile, vibration, or microenvironmental cues related to forager activity. These findings are consistent with the hypothesis that the evolution of sociality in honeybees was associated with modifications in the circadian clock and its environmental regulation by social cues.

OR005

What is the relationship between altitude and ant colony size?

Yi-Huei Chen, Elva Robinson

Bergmann's rule describes an increase of body size from tropical to polar latitude; the rule also applies from low to high altitude. In terms of physiology and genetics, the colony is the unit of selection for ants; colony size (worker number in a colony) can be considered the body size of a colony. Combining these two ideas, this project uses two wood ant species, *Formica lugubris* and F. *paralugubris*, to investigate changes in colony size with altitude. We firstly developed an accurate and non-destructive method to estimate wood ant nest size (worker number) and also found that mound volume was feasible in representing nest size. We tested the hypothesis that nest size increases with increasing altitude in Swiss Jura Mountains during the summer 2012. Mound volume was used to estimate nest size and colony size. We found that canopy cover was probably a more important factor than altitude related to nest size variation. For a more comprehensive study with a wider altitudinal range, we investigated the relationship between altitude, canopy cover and polydomy of these two wood ant species along an altitudinal gradient (1200-2000 m) in the Swiss Alps in 2013. Overall, our results showed that colony size was larger both with rising altitude and with rising canopy cover. Temperature is the most likely underlying cause of these effects, due to the generally colder thermal environments both in shady areas and in higher altitude. Taken together, our findings demonstrate a positive trend between colony size and altitudinal gradients and follow Bergmann's rule. This study has implications for understanding the effects of climate change on social insects.

OR006

Genetic diversity in a honeybee colony influences the behavioral performance

Tanja Gempe, Silke Stach, Kaspar Bienefeld, Martin Beye

Honeybees can detect and remove diseased brood from their nest. This so-called hygienic behavior plays a major role in the colonie's overall resistance against pathogens. Here we describe the molecular differences associated with the hygienic performance of honeybee workers which stem from backcrosses with high (xH) or low (xL) hygienic drones. We showed that xH worker bees were more often recruited to engage in hygienic tasks than xL worker bees ($P < 0.01$). We analyzed the genome-wide transcription level differences of 496 hygienic and non-hygienic worker bee brains in a combined loop/replicate design on a total of 82 whole-genome oligonucleotide microarrays. We detected a list of 1408 candidate genes which were associated with the performance of hygienic behavior in xH and xL worker bees ($P < 0.01$). 501 out of 1408 candidate genes differ in transcription level due to the behavior effect attributed to xH backcrosses, 342 out of 1408 candidate genes relate to xL backcrosses; the overlap between hygienic behavior associated genes relating to xH and xL backcrosses is minor (21 genes). GO analysis of the assigned orthologs in *Drosophila melanogaster* revealed that an annotation cluster including the GO terms 'olfactory receptor activity', 'sensory perception of smell', and the Interpro domain 'olfactory receptor', is significantly enriched in the xH candidate gene set when compared to the total set of *Drosophila* orthologs represented on the microarray (enrichment score: 2.8, $P < 0.01$). In the xL candidate gene set, an annotation cluster including the GO terms 'negative regulation of signal transduction', 'negative regulation of cell communication', and 'negative regulation of protein kinase cascade' is enriched (enrichment score 2.0, $P > 0.05$). Combined, these findings suggest that the distribution of hygienic alleles in a colony strongly influences the behavioral performance of the worker bees and the gene transcription associated with it.

OR007

The long reach of a parasite in Temnothorax *ants.*

Sara Beros, Evelien Jongepier, Felizitas Hagemeier, Susanne Foitzik

The consequences of parasite infections are well-studied on the individual level, but the potentially complex outcomes on social groups are less clear. Insect societies could buffer individual-level effects, but parasites could also try to extend their influence to non-infected members by chemical or behavioral manipulation. Parasite-induced alterations of host phenotypes can be either explained by the parasite's interest to survive, develop and to increase its transmission or by the host trying to limit infection costs, or they can be non-adaptive by-products of infection. The tapeworm *Anomotaenia brevis* causes distinct changes in its intermediate host, the ant *Temnothorax nylanderi*. Infected individuals display a lighter coloration, changed chemical profile and behavior, and as we can show in this study an increased longevity. As infected ants are well-cared for but inactive, it is reasonable to assume that the presence of infected animals might negatively influence colony traits and reveal fitness consequences of parasitism. However, in field colonies this was not the case for a direct fitness measure like per-capita productivity. Only when we controlled for external conditions in the lab, we could detect a slight reduction in colony productivity due to parasitation. We wondered why infected individuals were accepted in their colony albeit they show a divergent chemical profile. We experimentally manipulated the parasitation status of field colonies by adding and removing infected workers, and determined colony aggression towards infected and healthy non-nestmates. Our results show that current parasitation status clearly influences the colonies´ responses to intruders. Parasitized colonies behaved less aggressive towards non-nestmates and this change in colony-level behavior is based on lower aggression in uninfected workers. Our findings are consistent with colony-level changes in aggression with parasite presence and extended to non-infected nestmates, possibly to facilitate acceptance of infected workers in the nest.

OR008

Expression of the red imported fire ant foraging gene and colony-level variation in behavior

Alison Bockoven, Craig Coates, Micky Eubanks

Intraspecific behavioral variation has important consequences for ecology and evolution. In social insects, genetic variation in a behavior can allow a colony to exhibit more flexible, rapid responses to environmental changes and promote homeostasis. In addition, genetic variation in traits enables adaptive evolution. In a number of social insects, variation in foraging behaviors and division of labor has been associated with variation in expression of the foraging gene (for), a gene encoding cGMP-dependent protein kinase (PKG). We assayed field colonies of the red imported fire ant (*Solenopsis invicta*) in order to quantify colony-level variation in foraging-related behaviors such as activity, exploration, macronutrient preferences, and recruitment to transient and stable resources. In order to correlate behavior with gene expression, we flash froze foragers and workers from the interior of the nest and used qPCR to quantify *Solenopsis invicta* for expression (sifor) Behavioral assays revealed a significant effect of colony of origin on all foraging-related behaviors ($p<0.001$, all traits). Fire ants varied in sifor expression levels at both the colony and regional level, and foragers exhibited increased expression compared to interior workers. In addition, colony-level variation in sifor expression was significantly correlated with colony-level variation in foraging-related behaviors. These results demonstrate an important role of sifor expression and the associated cGMP/PKG signaling pathway in fire ant foraging behavior. This intraspecific variation in behavior may provide insight into variation in the ecological effects of fire ants, their success as invaders, and methods necessary for their control.

OR009

Preservation and degradation in stored pollen of honeybees

Kirk Anderson, Vanessa Corby-Harris

The honeybee colony is a complex homeostatic unit, filled with nutritionally rich resources and a broad spectrum of microbial microenvironments. Increasing evidence points to a core gut microbiota, but the structure and function of microbial communities throughout the hive and food stores are relatively unknown. Pollen stored in wax cells, or 'beebread', is a critical aspect of colony health, supplying the bulk of proteins, vitamins and lipids for colony growth. Because beebread is warm, moist, and sugar rich, it provides a suitable niche for potentially pathogenic or spoilage microorganisms. To explore the structure and function of beebread, we used 454 amplicon sequencing of 16S rDNA to reveal beebread bacterial communities from two neighboring colonies sampled at six equally spaced time points over the course of a year. Community signatures were indicative of preservation and degradation, the former defined as an abundance of lactic and acetic acid bacteria, the later characterized as a complex nitrogen processing community. Consistently found in all beebread samples were facultative anaerobes typically abundant in nitrogen rich environments. These included many different bacteria capable of digesting complex plant polymers providing access to the pollen protoplasm. In these beebread samples, the release of nitrogen rich cell contents of pollen is evidenced by the strong presence of a comprehensive nitrogen processing bacterial community. During pollen degradation, carbon slowly made available from cellulose and other complex plant polymers would provide sustained fuel for nitrogen processing and these metabolic systems may be mutually reinforcing.

OR010

Genome sequencing reveals host specialization in bee gut symbionts

Waldan Kwong, Philipp Engel, Hauke Koch, Nancy Moran

Host specialization occurs in many organisms participating in symbiotic lifestyles. For bacteria, different strains of the same species may evolve to favour the colonization of one host type over others. This has been of considerable interest in the study of pathogens and endosymbionts but little attention has been given to this process for the normal gut microbiota. In this study, we present the first complete genome sequences of two gut symbionts (*Gilliamella apicola* and *Snodgrassella alvi*) found in both honeybees and bumblebees. We found that the strains in the two bees are deeply divergent and possess a large host-specific accessory gene set. Furthermore, there were signs of lateral gene transfer between the two symbiont species, thus suggesting the gut microbial community, as a whole, plays a key role in shaping the evolution of its individual members. We also conducted in-vivo experiments, inoculating lab-cultured strains into germ-free honeybees and bumblebees to show that the bacteria can better colonize and compete in their native hosts, supporting the idea that these symbionts are specialized to living with their particular hosts. As ecologically and economically important insects with relatively simple microbiotas, bees hold great promise as a model for understanding symbiotic gut microbial communities.

OR011

Life-history trades-offs and within-host competition in honeybee brood parasites

Sophie Evison, Annette Jensen, William Hughes

Many parasite infections consist of multiple parasite strains with conflicting interests. The resulting within-host competition between a genetic diversity of parasites is predicted to result in, and select for, increased virulence. Understanding the dynamics of within-host competition is therefore important when considering disease epidemiology. Currently, empirical studies of the evolution of parasites in social insects is very limited, but considering that social insect colonies are often exposed to multiple parasites which may compete within the host, understanding these dynamics is important for understanding how social insects manage disease. Using honeybees and multiple strains of their fungal brood parasite, *Ascosphaera apis*, we tested the prediction that increased within-host genetic diversity of parasites will heighten competition, resulting in increased virulence and decreased parasite fitness. We were also interested in the precise within-host dynamics between competing stains of the parasite, and tracked the course of an infection using quantitative PCR. The results suggest that different strains of this parasite have evolved different life history strategies, which is reflected by their virulence when infecting alone. However, when in competition with other strains, their differential strategies result in variable outcomes in terms of virulence evolution. The consequences of this for disease management social insects will also be discussed.

OR012

Investigating interactions between land use and honeybee - microbiota associations

Julia Jones, Thierry Gosselin, Camilla Ip, William Hughes

There is growing concern about the serious losses of honeybee colonies in many areas due to a multifactorial combination of new and existing threats, principally parasites and disease, pesticide exposure and food availability. Honeybees and other bees host a community of mutualistic and commensal microbes in their guts which play an important role not only in the nutritional biology of a honeybee , but also in its general health, resistance to disease and potentially susceptibility to pesticides or pollutants. While the microbe community appears to be broadly similar across honeybee populations, it is very likely that its precise composition will change depending on pesticide exposure and the particular plant species that a honeybee colony has foraged on, and thus upon land use in the area of the colony. However, the potentially profound impact of land use on honeybee microbiota, and thereby on honeybee health, has not previously been investigated. Here we analyse the variation of the 16S rRNA sequence using Illumina next-generation sequencing technology to characterise the gut microbial community of honeybee foraging in agricultural and suburban habitats in the southern United Kingdom. We compare the communities to determine the impact of land use on the microbiota, and the implications of this for honeybee health.

OR013

Viruses and the functional toolkit in social insect biology

Patrick Abbot

Many insects harbor persistent infections by pathogenic and sometimes mutualistic viruses. Social insects are no different. However, the role of viruses as mutualistic agents in social insect biology is unclear. I review some known and potential mutualistic interactions between viruses and social insects. I then discuss dicistroviruses and social aphids. Dicistroviruses are picorna-like viruses that infect arthropods. Most are pathogenic, but some are not. Recent discoveries in the our lab have revealed dicistroviruses infecting two species of aphids in the genus *Pemphigus*. Aphids are insects that attack plants, and are economically important pests of most of the plant crops worldwide. They also transmit over half of all known plant viruses. A small fraction of of aphid species are eusocial. We have identified viruses in the genus *Cripavirus*, and I discuss experiments using various surveys (PCR, ELISA and Illumina sequencing) in which we examine the qualitative and quantitative patterns of virus prevalence, distribution and functional roles within and across eusocial aphids species.

OR014

Advanced farming ants rear polyploid crop fungi

Pepijn Kooij, Duur Aanen, Morten Schiott, Jacobus Boomsma

Polyploidy is common in plants, presumably because advantages of increased functional heterozygosity often surpass costs of destabilized mitosis or epigenetic instability, but rare in fungi, where 'diploid' cells retain the two parental haploid nuclei rather than merging them into a zygote. Such dikaryotic mycelia are actively maintained in Basidiomycota where clamp connections ensure honest propagation of nuclei during cell division, but the same mechanism may constrain the evolution of multinucleate cells. Previous research has indicated that the domesticated basidiomycete crop-fungus of leafcutter ants, which lacks clamp connections, is a functional polyploid, but without pursuing this further. We microscopically estimated the mean number of nuclei per somatic cell for 42 fungal symbionts reared by 14 species (eight genera) of fungus-growing ants in Panama, and mapped these numbers on a fungal symbiont phylogeny. This showed that all higher attine ants that rear specialized fungi without free-living close relatives had 7-17 nuclei per cell, whereas non-specialized fungal crops of the basal attines were dikaryons. Analysis with ten microsatellite markers revealed that almost 40% of the additional nuclei represented novel haplotypes, yielding an estimate of average ploidy in higher attine crop fungi of 5-6. We hypothesize that functional polyploidy in crop symbionts evolved because benefits of increased heterozygosity outweighed costs that could be met by the farming ants. Polyploid crops may thus represent a form of symbiont chimerism for the sake of enhanced genetic diversity, similar to multiple queen-mating that evolved in the leafcutter ants to generate higher genetic diversity among colony workers. The transition to crop-polyploidy coincided with at least an order of magnitude increase in colony size of farming ants.

OR015

Ant-microbe metabolic integration supporting the nutrition of agricultural pest leaf-cutters

Mauricio Bacci, Ana Carolina Marchiori, Milene Ferro, Aline Silva

Leaf-cutter ants (Hymenoptera: Attini) are important neotropical agricultural pests. This pest status is related to the high population size and geographic density of ants nests. In this work, we propose that high biomass production by leaf-cutters depends on the association with two classes of key microbes. The fist one is the mutualistic fungus *Leucoagaricus gongylophorus* that we show able to degrade plant polysaccharides and to accumulate in the extracellular space simple sugars which are taken up by the ants. Hemicellulose and starch are the major plant polysaccharides supporting mutualistic nutrition. A metabolic pathway is described involving starch degradation by fungal enzymes to generate maltose and maltose degradation, likely by an ant maltase, to generate glucose, which is a major food source for the mutualists. This mutualistic pathway may have evolved in the ant gut by selection of enzymes which are not sensitive to proteolytic inactivation or catabolic repression. We also show that ant nutrition on plant matter does not occur in the absence of fungal carbohydrases, which are thus essential for ants to access carbons from plants. A second class of mutualistic microbes seem involved on leaf-cutter ant nutrition on nitrogen. Nitrogen from plant leaves is present in very low amounts which are not enough to support the generation of billions of individuals living in a leaf-cutter ant colony. Using metagenomic analysis, we show that the guts of leaf-cutters harbor a narrow variety of microbial species closely related to nitrogen-fixing bacteria. These microbes are proposed as mutualists mediating ant feeding on N2. Therefore, metabolic integration is assumed as a central force modulating the evolution of the association between leaf-cutters and microbes. This association has likely enabled the ants to assimilate great amounts of nutrients necessary to reach high population size and to become major agricultural pests.

OR016

Phylogenetic distribution, stability and function of attine ant gut microbiota

Panagiotis Sapountzis, Lars H. Hansen, Søren J. Sørensen, David R. Nash, Jacobus J. Boomsma, Morten Schiott

The attine fungus-growing ants are a monophyletic group that switched to an almost exclusive fungal diet ca. 50 MYA. They have become a model of complex symbiosis after several additional fungal and bacterial symbionts were discovered, but their gut bacteria have never been studied. Here we present the first comparative and functional gut-microbiome data for the attine fungus-growing ants based on 454 sequencing and FISH confocal microscopy. We show that gut-microbiomes of nine Panamanian species, representing eight genera, are dominated by few bacterial species belonging mostly to the Alpha-Proteobacteria and Mollicutes and that closely related bacterial species belonging to these clades are often mutually exclusive. The dominant bacterial species are located in specific gut tissues and some of them are clearly intracellular. They appear to mediate the acquisition and/or preservation of nitrogen as prokaryote nifH genes were found in almost all ant samples, particularly in Rectal Papillae and Malpighian Tubules where nitrogenous waste-excretion normally takes place. Guts and surrounding organs of higher attine, and particularly leaf-cutting, ants harbored most of these bacteria, consistent with the symbiosis remaining protein-limited in spite of evolutionary transitions in specialization of the crop fungus and later adaptations to using fresh leaves as fungal substrate. The composition of the gut microbiomes was correlated by the presence or absence of a cuticular microbiome of actinomycete bacteria: attine species that have cuticular actinomycetes appear to have more stable gut microbiomes, possibly because cuticular antibiotics protect gut microbiomes against inadvertent invasions by non-symbiotic bacteria. Our results suggest that gut microbiomes and their interactions with external microbiomes may have played important roles in shaping the mutualism between attine ants and their fungal crops over evolutionary time.

OR017

Nutritional homeostasis from individuals to insect societies

David Raubenheimer, Stephen Simpson

Nutritional homeostasis results from the fine-tuned interactions within a tightly integrated network of behavioural, physiological, morphological and developmental traits. Achieving this is a complicated challenge, given that animals need to ingest a large number of nutrients simultaneously, each at its own particular level. Considering the importance of nutrition for fitness, as well ecological interactions, understanding how animals solve these challenges is a high priority. In recent years an integrative framework, termed nutritional geometry, has been applied to investigate how individuals of a wide range of species solve the challenges of nutritional homeostasis. More complex yet are social animals, such as social insects, where individuals (e.g. foragers) cooperate to provide nutrition for others (e.g. queens and larvae). Only recently, however, have studies begun to investigate nutritional homeostasis in this more complex, group-level, context. In this talk we demonstrate how nutritional geometry has been applied to investigate individual-level homeostasis, and discuss its extension to social species with nutritional division of labour.

OR018

The nutritional dimensions of animal collective behaviour

Jerome Buhl, Mathieu Lihoreau, Michael Charleston, Gregory Sword, David Raubenheimer, Stephen Simpson

There are many well described examples of collective behaviour phenomena where local interactions between individuals scale up to produce rich group level dynamics and patterns often beneficial to group members. Such self-organised phenomena allow groups to synchronise, achieve cohesive mass migration, and perform collective decisions and complex task partitioning. Here we revisit some of the classic collective behaviour models applied to group living insects and embed them in a nutritional framework where individuals attempt to regulate their nutrient intake to reach their desired target. Using these simple models, we illustrate how integrating nutritional constraints in the framework of animal collective behaviour opens new opportunities to study and understand the evolution of group-living and sociality. In particular, we study how individual nutritional requirements can affect collective decisions and synchronisation in simple gregarious groups. We show how the influence of individual nutritional states can affect their movement and shape locust marching bands. Finally, we revisit a model initially used to explore the emergence of dominance hierarchy in wasps to study how competition for nutrients can shape the distribution of nutritional states in a group and provides a basis for the emergence of division of reproductive labour.

OR019

Understanding foraging patterns that achieve colony-level macronutrient regulation

Theodore Pavlic, Stephen Pratt

Several studies of ants and honeybees have shown that colonies forage so as to regulate macronutrient intake to specific targets. Moreover, when a regulating mixture of diet choices is not available (e.g., if one macronutrient is over-represented in all choices), colony survival declines -- ostensibly because of the accumulation of unavoidable surpluses of macronutrients. These observations are consistent with predictions of the geometric framework (GF) of Simpson and Raubenheimer, but little is known about the behavioral mechanisms that eusocial insects use to achieve macronutrient regulation. To this end, we have developed a novel economic framework (EF) of nutrition that encapsulates the GF while also modeling the effect of ecologically rational behaviors that achieve regulation under typical dietary conditions and lead to deleterious allocations otherwise. The EF suggests designs for new experiments that use two mutually deficient diets to probe foraging behavior further than is currently possible with the GF. Additionally, it provides testable predictions for how foragers will allocate to three or more food sources that vary in only two macronutrients. In this latter case, a continuum of foraging allocations is available that satisfies the regulating condition of the GF. However, the EF is able to predict allocations that will be preferred within that continuum. Moreover, the EF predicts how macronutrient supplements commonly given to agricultural honeybee colonies can destabilize an existing healthy foraging allocation. So the EF provides new insights into apiculture and the nutritional components that have been implicated in colony collapse disorder. More generally, this foraging framework also encapsulates two classical results from solitary and social foraging theory, the ideal free distribution (IFD) and the marginal value theorem (MVT), and explains why these results may not apply when there is macronutrient co-limitation. Thus, the EF potentially is a unifying framework for solitary, social, and eusocial foraging theory.

OR020

Food dissemination in ant colonies - from interactions to global distributions

Efrat Greenwald, Ofer Feinerman

Collective nutrition is a fundamental process in the communal life of ants. During this process, food gathered by a small number of foragers is relayed between ants to satisfy the various nutritional demands of the entire colony. We are interested in understanding how the regulation of trophallaxis at the level of single pairs leads to the dissemination and resulting distributions of food as observed on the collective level. To approach this question, we have developed a novel experimental system that combines fluorescent labeling and a 2D barcode identification system. These enable us to continuously track the amount of food held in the crop of each and every identified ant, the amounts of food transferred during trophallaxis events, and of the distribution of food within the nest. We used this system to study the dissemination of liquid food within a *Camponotus sanctus* colony. The identification system allows us to construct the complete trophallaxis network from the time at which food was discovered and until steady state is reached. We show that workers tend to adopt one of three behaviors: (1) Foragers that alternate between collection and rapid dissemination, (2) delayed food distribution and (3) food storage. Such local decision rules dictate the timescales, spreading rates and food distribution at the level of the colony. We further study the dependency of the collective process on the nutritional makeup of the food source. These observations contribute to our understanding of the distributed dissemination activity by uncovering the underlying network structure and its regulation mechanisms at the level of single interactions.

OR021

Modelling food storage management in ants: mechanisms and social implications.
Olivier Bles, Jean-Louis Deuneubourg

As a consequence of work division in ants only a few workers, the foragers, leave the nest for food gathering. Foragers return to the nest and provide food to other workers through a trophallaxis network. Here we first develop a computational model of collective food management on the basis of previous empirical works on food flow dynamics and storage. The core of the model includes two types of workers: foragers (food collection) and domestics (food receivers, inside the nest) and considers several states for each individual where the evolution from one state to another is determined by a set of individual behaviour/empirically based parameters. We explore a model based on these simple rules, deriving the number of feeding domestics during foraging after different periods of starvation. Results of the model are in rough agreement with empirical data. Confronting and analysis experimental results and theoretical predictions of the model reveals key mechanisms involved in food flow adaptation to the colony's needs, particularly a negative feedback modulating foragers' activity and numbers during food collection. Theoretical exploration of set parameters reveals an optimal ratio of foragers and domestics that minimizes the time to fill up the colony's needs. Surprisingly, a maximum number of foragers is under optimal. Based on these insights on foraging modulation we then developed an individual based model in order to investigate the question of social network organization and functionality in the nest. It is well known that interaction networks operate through ant nests, however our simulations allowed us to better understand how determinant factors of food flow regulation (e.g., foragers/ domestics ratio) and individual behaviour affect the structure of interaction networks and how this ensures an effective and optimal collective management of food resources.

OR022

Nutrition and behavioural plasticity in the solitary spider Agelena labyrinthica
Pierre Lesne, Marie Trabalon, Alfonso Perez-Escudero, Raphael Jeanson

The role of nutrition on the physiology, behaviour and fitness of individuals is increasingly recognized as a major factor regulating interactions within social groups. Understanding the impact of the nutritional status is of particular importance for species showing transient gregariousness. Variations in diet experienced by group members can impact their decision to initiate dispersal and, thereby, the maintenance of cohesion. Spiders are relevant models to address this issue as all species show a transient gregarious phase. The duration of this phase is a highly plastic trait under the dependence of food availability. Several studies have investigated the feeding habits of spiders (e.g. foraging behaviour) but only recently attention has been paid to their nutritional ecology (nutrients required for optimal growth, survival and reproduction). For the most part however, the influence of the nutritional state on the regulation of social behaviours and their physiological determinants has been largely overlooked. The important role of food availability on the duration of gregariousness in solitary spiderlings nevertheless suggests a major influence of nutritional status on the initiation of dispersal. In this context, our study aimed to examine the interplay between food supply, energetic stores and short range communication on the maintenance of social cohesion during the gregarious phase of the spider *Agelena labyrinthica* (Araneae, Agelenidae). In our experiments, spiderlings were reared under different diets differing in prey quantities. At different developmental stages following hatching, spiderlings were introduced in circular arenas to quantify their degrees of mutual tolerance and interattraction. After the completion of behavioral assays, we characterized the profiles of cuticular lipids and we quantified energetic stores (triglycerides and cholesterol). Our results suggest a decoupling between the nutritional status and the maintenance of tolerant social interactions in spiderlings. This study provides valuable insight for understanding the proximal causes of social transitions in spiders.

OR023

Larval egg cannibalism and kin conflict in ants

Eva Schultner, Heikki Helanterä

In ant colonies individuals cooperate in the aim of maximizing offspring production. But cooperation is only flawless from afar. In fact, because adults can differ in their relatedness to brood they often have contrasting inclusive fitness interests, which may lead to outbreaks of social conflict, i.e. individuals attempting to allocate resources according to their conflicting optima. So far, conflict studies have been restricted to adults. We provide an example of selfish conflict behavior in larvae by demonstrating that *Formica* ant larvae readily engage in egg cannibalism. Although larvae are typically assumed to be powerless and rely on food provided by workers, egg consumption allows larvae to increase survival and positively affects the expression of key growth-related genes. Levels of cannibalism across species decrease when relatedness between larvae and eggs is high, which suggests that cannibalism is a selfish trait that can underlie social control, and makes it the first example of a behavioral means of power in ant larvae. Cannibalism appears to be plastic in F. aquilonia, where levels increase when larvae are presented with foreign eggs compared to sibling eggs. In addition, cannibalism intensity is highly dependent on larvae sex and size across eight species, indicating that benefits from cannibalism vary with individual traits. We conclude that ant larvae are far from powerless. Instead, cannibalism may allow larvae to influence important determinants of individual fitness such as caste fate or size. By consuming eggs, larvae may furthermore affect queen - worker ratios, total reproductive output and sex ratios, and ultimately overall colony fitness. For the first time, our study identifies larvae as actors with selfish interests that have the power to act in social conflict, thus adding a new dimension to our understanding of colony dynamics in social insects.

OR024

Fruit flies in the nutrient space

Mathieu Lihoreau

Animals select foods and eat them in appropriate amounts in order to maintain physiological states maximising growth and reproduction. These nutritional strategies have profound impacts on the physiology, behaviour and fitness of individuals, and thus affect the ways individuals interact within groups and societies. Social insects, for instance, have evolved extreme levels of nutritional interdependence in which food collection, processing, storage and disposal are performed by different individuals with different requirements. This raises the fundamental question of how nutrient regulation is achieved at multiple organizational levels, by individuals and groups. Here I will discuss how a gregarious insect, the fruit fly (*Drosophila melanogaster*), solves these nutritional trade-offs through collective foraging decisions. I will describe a series of laboratory experiments in which adult flies were observed foraging on chemically defined foods with controlled ratios of protein to carbohydrate. When flies were given a choice between a nutritionally balanced and several nutritionally unbalanced foods, groups made faster and more accurate decisions than individuals. In the presence of several complementary foods, grouped flies balanced their diet collectively by simultaneously switching from food to food, ultimately leading to complex spatio-temporal group dynamics. Agent-based models integrating the concepts of the Geometric Framework for nutrition help unravelling the mechanisms of these collective strategies. These preliminary results in ‘simple' insect groups provide a framework to further explore the role of nutrition as a potential organizer of social life in a wider range of species and social systems.

OR025

Honeybees balance essential fatty acids and suffer cognitively from deficiency

Sharoni Shafir, Yael Katz, Shlomi Zarchin, Arnon Dag

Honeybees depend on pollen for many nutrients, including essential linolenic and linoleic fatty acids (eFA). We tested whether honeybee (*Apis mellifera*) foragers complement a deficiency in an eFA when foraging for pollen and the effect of such deficiency on cognitive performance. Colonies were maintained in netted enclosures to control their diets. In the first experiment, colonies were deprived of pollen for a week, and then allowed to forage for several days for only one pollen. Marked foragers were allowed to forage from a single pollen dish and we monitored their round dance at an observation hive. Then the dish was replaced by another pollen. We presented three pollens repeatedly in pseudorandom order: the same pollen fed during the previous days, another pollen similar in eFA composition, or a complementary pollen rich in the eFA lacking from the pollen fed during the previous days. Dance rates were highest for the complementary pollen showing that foragers evaluated them as more attractive, regardless of floral pollen identity. In the second experiment, we used soy-flour based artificial diets enriched by vegetable oils, either poor (corn and sesame) or rich (flax and sage) in linolenic acid. Colonies were fed for several weeks one of four treatments: corn, sesame, a mixture of the four oils, or a pollen control. We then tested by proboscis-extension conditioning olfactory and tactile learning of nurse bees, which were raised on the examined diets. Learning performance in the two linolenic acid deprived groups was greatly impaired relative to the other two groups. We conclude that maintaining proper linolenic acid levels is critical for proper cognitive function, and that foragers can assess pollen eFA composition and selectively recruit foragers to sources that complement colony needs. This study highlights the importance of balanced eFA diet in honeybee natural and artificial diets.

OR026

How much protein is good for honeybees?

Sue Nicolson, Christian Pirk, Ruth Archer, Henrika Bosua, VinetteOosthuizen, Geraldine Wright

Pollen is the natural source of protein for bees, essential for growth, brood rearing, and gland and ovarian activation. It is assumed that pollens of high protein content are best for bees. Recent colony declines around the world have drawn attention to malnutrition in honeybees and its interactions with pesticides and disease. We are investigating the importance of protein to carbohydrate (P:C) ratio in honeybee nutrition and the implications for stress resistance. Bees on an imbalanced diet must eat more to obtain deficient nutrients, thereby ingesting excess amounts of other nutrients and incurring fitness costs such as reduced lifespan. We tested the effect of different protein sources and P:C ratios on food consumption and survival of caged worker bees (*Apis mellifera scutellata*): under these brood-free conditions consumption is directly related to worker needs. When offered complementary agar-based diets containing casein and sucrose, bees selected a carbohydrate-biased intake target (P:C 1:6.5). Although protein improved their survival when challenged with the dual stressors of nicotine and cold, caged workers given a choice did not shift their intake towards higher protein. However, bees offered casein hydrolysate, which is easier to digest than casein, adjusted their intake to consume less protein. Taking protein breakdown even further, we fed bees liquid diets composed of specific proportions of essential amino acids (eAAs) and sucrose: their intake target was more carbohydrate-biased because amino acids are assimilated more efficiently. However, there were survival costs of overconsuming eAAs, and antioxidant supplementation did not improve survival on these diets. Pollens vary greatly in protein content and amino acid composition, and dietary diversity is essential to avoid nutritional deficiencies and to dilute toxins. Surprisingly, individual honeybee foragers do not appear to select pollens of high protein content; however, pollen quality affects the probability of attracting other foragers to the pollen source.

OR027

Amino acid role in high protein diet toxicity in ants

Sara Arganda, Sofia Bouchebti, Sepideh Bazazi, Gerard Latil, Steve Simpson, Jacques Gautrais, Audrey Dussutour

In solitary and social insects, the balance between the amount of ingested proteins (P) and carbohydrates (C) affects the performance of tasks such as reproduction, growth and survival. For example, *Drosophila melanogaster* females die faster under a high protein diet (high P:C ratio), while their reproductive output increases. Similarly to flies, ant workers also die faster when restricted to a high P:C ratio. This could be caused by digestion problems (low level of midguts proteases or blockage of their narrow petiole) or by toxic effects of the amino acids resulting from protein digestion. Supporting this latter idea, it has been found that lifespan of flies depends on the composition of a supplementary mix of free amino acids. Our aim was to investigate the causes of high protein diets toxicity on ants, both in social and isolated condition. To do so, we restrained worker colonies and isolated ants of *Linepithema humile* to a single diet containing either proteins or their translation in free amino acids. We explored the nutritional landscape of those two nitrogen sources using a range of different P:C ratios and concentrations. We have found that for all P:C ratios, ants die sooner when proteins are replaced by free amino acids, and that the higher the P:C ratio, the higher the mortality. We have also observed that isolated ant workers live shorter that in groups, but the effect of P:C ratio and amino acid sensibility on lifespan remains similar in both social conditions. To identify whether some amino acids were more toxic than others, we constrained isolated ants to diets in which just one amino acid was overrepresented. We identified Methionine, Serine, Threonine and Phenylalanine as especially toxic.

OR028

Nutrition and colony investment in Solenopsis invicta *workers*

Bill Wills, Cody Chong, SM Wilder, DA Holway, AV Suarez

In social insects, investment into worker size versus worker number is thought to play an important role in determining colony success. Additionally, colony investment into the worker force may shift in response to the availability of resources. Access to carbohydrate-rich resources can influence colony growth, and the monopolization of such resources has been implicated in the ecological success of certain groups of ants. Here we conduct a diet-manipulation experiment to test how access to carbohydrates and amino acids affects colony investment in worker number, mean worker body size, worker size distributions, and individual worker fat content (condition) of a polymorphic ant species (*Solenopsis invicta*). Field collected colonies (n = 15) were divided into four experimental sub-colonies, each consisting of two queens, ~ 1200 workers, and ~ 50 brood. Each experimental subcolony was reared on a diet of insects and one of four macronutrient treatment solutions: water, amino acids, carbohydrates, and amino acid + carbohydrates). Having access to carbohydrates increased the colony biomass after 60 days. This increase in biomass resulted from shifts in worker number and worker size, but not an increase in worker fat content. Increased access to carbohydrates altered the worker body size distributions of colonies such that colonies increased production of larger ‘minor’ workers but not larger ‘major’ workers. These changes in colony investment shed insight into how macronutrient limitation shapes colony demography and in turn may contribute to ecological success.

OR029

Polistes *castes' nutrient levels parallel corresponding generations of bivoltine wasps*

Timothy Judd, Matthew Fasnacht

The diapause ground plan hypothesis is that the worker and gyne paper wasp castes correspond to the non-diapausing (G1) and diapausing (G2) generations of solitary bivoltine wasps. Recent work on social wasps suggests that caste differentiation occurs at the larval stage, in reflection of different levels of nourishment. In a previous study, we have found that levels of several micro and macronutrients differ between worker and reproductive destined larvae of the paper wasp *Polistes metricus*. Thus far, however, no information is known about potential nutritional differences in G1 and G2 generations of solitary wasps. We measured the levels of macro and micronutrients in several species of solitary trap-nest wasps and compared levels in G1 and G2 larvae and adults to those previously found in paper wasp worker and gyne larvae and adults. We found that several of the nutritional differences that exist between worker and reproductive destined larvae of P. metricus also occur in the G1 and G2 generations of trap nest wasps. These similarities provide support for the diapause ground plan hypothesis.

OR030

How do polydomous ant colonies correct nutritional imbalances between nests?

Samuel Ellis, Elva Robinson

Many important and well-studied behaviours in social insects are directed towards collecting food from the environment, returning it to the nest and, ultimately, transferring the nutriment to brood and reproductive individuals. Less well studied, is how this complex system of nutritional transfer is affected by a single colony nesting in several different spatially separated, but still socially connected, locations. This distributed nesting system is called polydomy and is found in many ant species. A distributed nesting system means that one part of the colony may have more food than other parts of the colony at any particular time. Understanding the behaviour which is used to correct these within-colony nutritional imbalances is important to understanding how the colony as a whole functions. We observed and marked workers travelling on the trails between nests in the wood ant *Formica lugubris*, to investigate the flow of resources between nests and how it is mediated by the behaviour of individual workers. We found that there is a class of workers consistently travelling without food to neighbouring nests, taking food from that nest and then returning to their home nest carrying food. In effect, the workers are treating other nests of the colony as food sources. In the stable nutritional environment of wood ant colonies this is likely to be an efficient and reliable way to move resources through the colony. This mechanism demonstrates how a simple self-organising behaviour can solve the complex task of correcting within-colony nutritional imbalances in a complex nesting system.

OR031

Diversity of infestation and foraging strategies in bark beetles.

Etienne Toffin, Marceau Louis, Jean-Louis Deneubourg, Jean-Claude Grégoire

Bark beetles are considered major disturbance agent in forest ecosystems worlwide, able to efficiently exploit frequently nutrient poor, often ephemeral resources, to reproduce, develop and disseminate. This success is due to the large diversity of infestation strategies used by the different species. Hence, variations can be observed at the different life stages - from tree infestation by adults, ranging from mass attack to solitary colonization, to larval feeding, where highly aggregative behaviours deeply constrast with strong individual avoidance . These changes are observed at both the outer surface of the trees through the pattern and density of the attacks, and under surface of the bark where larvae feed collectively in chambers, or individually in tunnels. Between these extreme behaviours and patterns, there is a gradient in aggregative tendencies, or even qualitative shifts in behaviours during its life history, leading to even more intermediate collective patterns. All these patterns are species-specific, and it is hypothesized that the variations in individual behaviours (aggregative tendencies) and the subsequent change in collective patterns are linked to the feeding resources (quality, availability). For instance, larval aggregative behaviours and collective larval chambers are observed in species attacking healthy trees, the cohesion of the group being hypothesized to facilitate both feeding and counteracting the defensive response of the tree (resin, chemistry). Until recently, this topic in bark beetle ecology has received scarce attention, despite being a promising model to investigate the link between transition from solitary to collective patterns and resource quality and availability. Here we will illustrate this topic with our experimental insight on larval foraging patterns and aggregative behaviours in species that represent the extremes of both aggregation tendencies and cavity patterns.

OR032

The general stress response syndrome in the honeybee

Naila Even, Jean-Marc Devaud, Andrew B. Barron

The biological concept of stress originated in mammals, where it describes a set of common integrated physiological responses to diverse noxious agents. Physiological mechanisms of stress in mammals have been extensively investigated through diverse behavioral and physiological studies. One of the main elements of the stress response pathway is the endocrine hypothalamo-pituitary-adrenal (HPA) axis, which underlies the ‘fight-or-flight’ response via a hormonal cascade of catecholamines and corticoid hormones. Physiological responses to stress have been studied more recently in insects. Here I present a hypothetical integrated stress response system in bees that has functional and organisational analogies to the mammalian HPA. The bee stress response system involves signaling elements such as biogenic amines (octopamine, dopamine, serotonin), neuropeptides (allatostatin, corazonin) and metabolic hormones (adipokinetic hormone, diuretic hormone) that coordinate responses between the brain (particularly the neurohaemal organ corpora cardiaca) and its peripheral targets, including energy storage organs (fat body and crop). Understanding these mechanisms is highly important as the accumulation of new stressors is suspected to be the cause of honeybee population decreases in various parts of the globe.

OR033

Nutritional stress, behavioral development and honeybee health

Miguel Corona, Jay Evans, Judy Chen, Jeff Pettis

In the United States populations of honeybees have experienced serious losses during recent years. Multiple possible causes for colony losses have been proposed, however no independent factor has been shown to be consistently associated to this phenomenon. Colony losses can be predicted by measuring the extent of open land relative to developed land area, suggesting that nutritional stress due to habitat loss is an important underlying factor associated to colony losses. Pollen is the main source of proteins and lipids for honeybees and its continuous supply is essential for colony growth and survival. We tested the effects of pollen deprivation at colony level to gain insight into the mechanisms connecting nutrition, behavioral development and honeybee health. For this purpose, we determine the effect of pollen deprivation on behavioral development, expression of molecular markers of behavior, expression of immune genes and virus load. We used triple cohort colonies to control colony size and demography and uncouple the effects of age and behavior on nurses and forager collected after two and three weeks of pollen deprivation. Our results showed that pollen deprivation induced accelerated behavioral development and that the behavioral state has a major effect on the expression of immune genes and virus load, with foragers having higher expression of immune genes and virus load compared with nurses. Our results also demonstrated that nutrition has a significant effect on the expression of immune genes and virus load: foragers of restricted colonies showed reduced levels of humoral immune genes and increased virus load compared to forager of non-restricted colonies. Overall, our results reveal that nutritional stress induced abnormal behavioral development, decreased immune function and higher susceptibility to diseases and support the proposal that nutritional stress is a important contributing factors associated with colony losses.

OR034

BEEHAVE: modelling multifactorial causes of honeybee colony losses

Matthias A Becher, Jack CO Rumkee, Juliet L Osborne

Honeybee colonies can be subject to multiple stressors like parasites (e.g. varroa mites and varroa transmitted viruses), insufficient forage availability and pesticide exposure. We developed a honeybee model that integrates colony dynamics, agent-based foraging in realistic landscapes and population dynamics of varroa mites, acting as vectors for viruses (Deformed Wing Virus and Acute Paralysis Virus). We explore the potential impact of pesticide applications by increased mortality rates of foragers and in-hive stages and how this is affected by food availability in the landscape. Our results suggest that the timing of the pesticide exposure is crucial and the impact is more severe, if colonies are already under food stress. The BEEHAVE model is available for free download at http://beehave-model.net/ and comes together with a detailed model description and a user manual.

OR035

Evaluation of pesticide toxicity to the honeybee: a new approach

Yannick Poquet, Marie Dupre, Jean-Baptiste Philibert, Marianne Cousin, Sylvie Tchamitchian, Luc Belzunces

Chemicals are used in agriculture (fertilizers, pesticides) and can be potentially found in the environment. In order to know their impacts on the non-target organisms and to limit their undesirable effects, these products are evaluated according to several tests. One of the first steps is the establishment of the hazard quotient to evaluate the level of risk presented by the use of a molecule to an organism. This ratio is obtained by comparing the toxicity of the molecule to this organism and the quantity or the concentration to which it can be exposed in the environment. In honeybee tests, the environmental exposure is based on the amounts of active substance spread on one hectare of cultivated surface. This factor does not take into account the amounts that can be in contact with a forager during pulverization and thus does not enable a real evaluation of the risk. Our study proposes a new approach to evaluate the exposure and the potential toxicity of the pesticides to honeybees. We tested 20 of the most common commercial pesticide preparations on bees in order to know the rates of residues deposited on the bees during the pulverization in the field. This enabled us to determine an exposure surface of around 1 cm^2 per honeybee and then to convert an amount per hectare into an amount per bee. This new approach of the risk is more accurate and could become a tool for decision-making aid in the evaluation of the toxicity of pesticides.

OR036

Levels of neonicotinoids found in United Kingdom oilseed rape

Kristopher Wisniewski, William D.J Krik, Falko Drijfhout

Honeybees, bumblebees and stingless bees only represent a small proportion of insect pollinators; however, they provide important and economically valuable pollination services to agriculture and many terrestrial ecosystems. The recent documented global decline of social pollinators has been a central concern to the scientific and wider communities, with pesticides identified as a key contributing factor to the falling numbers. Chronic exposure to low levels of pesticides, whereby direct mortality does not occur, has been shown to induce sublethal effects, which can have a profound outcome on both the individual and ultimately the overall functioning and survival of a colony. Various methods of pesticide exposure have been identified; including contaminated pollen and nectar collected during foraging; but at what levels? Here we present the levels of three neonicotinoids found in the pollen and nectar, collected from oilseed rape, within multiple locations of the United Kingdom.

OR037

Review of recent Syngenta bee field study data on Thiamethoxam

Peter Campbell, Helen Thompson, **Mike Coulson**

Neonicotinoid residues in nectar and pollen from crop plants have been implicated as one of the potential factors causing the declines of honeybee populations. Indeed the European Commission has introduced a restriction for the use of Imidacloprid, Thiamethoxam and Clothianadin on selected bee attractive crops. However, much of the data that has implicated neonicotinoids in the decline of honeybee health has been generated either under laboratory conditions and/or have used unrealistic exposure conditions. In this review of Syngenta higher tier field data i.e. conducted under realistic field use conditions, the risk to bee colonies is investigated. Included within this review is a field study program investigating the risk to honeybee colonies following four years consecutive single treatment crop exposures to flowering maize and oilseed rape grown from thiamethoxam treated seeds at rates recommended for insect control. During the study honeybee mortality, foraging behavior, colony strength, colony weight, brood development, food storage levels and over wintering success are monitored and reported. The results from all these field studies confirm a low risk to bees from systemic residues in nectar and pollen following the use of thiamethoxam as a seed treatment on oilseed rape and maize. These results contribute towards reducing the gap in our understanding of exposure and risk to bees from the use of neonicotinoids as seed treatments under field conditions.

OR038

Abnormal behavior of honeybee workers due to contamination of Imidacloprid

En-Cheng Yang, Hui-Chun Chang, Yu-Cheng Chuang

The threat of insecticide is always the major concern to the decline of honeybee population. Mortality rate is normally calculated as the evaluation of harmfulness and lethal dose was used as the indicator of toxicity. However even a small amount of insecticide will affect the behavior of honeybees, and the chronic effects to honeybee colony may be caused by continuous poisoning with tiny dose of insecticide brought from environmental residues by forager's daily activity. In the past decade, an increasing number of studies imply that imidacloprid, a systematic neonicotinoid neurotoxic insecticide, could be associated with colony disorder. Our study shows that the honeybee workers delayed their return visit when they were treated orally with sugar water containing imidacloprid. This time delay in their return visit is concentration-dependent. When treated with higher concentrations, they showed abnormalities in revisiting the feeding site and. some of them went missing. Besides, the capped-brood, pupation and eclosion rates of the honeybee larvae were recorded after treating them directly in the hive with different dosages of imidacloprid. The brood-capped rates of the larvae decreased significantly when the dosages increased from 24 to 8000 ng/larva. However, there were no significant effects of DMSO or 0.4 ng of imidacloprid per larva on the brood-capped, pupation and eclosion rates. Although the sublethal dosage of imidacloprid had no effect on the eclosion rate, we found that the olfactory associative behavior of the adult bees was impaired if they had been treated with 0.04 ng/larva imidacloprid in the larval stage. These results demonstrate that a sublethal dosage of imidacloprid given to the larvae affects the subsequent associative ability of the adult honeybee workers. Thus, a low dose of imidacloprid may affect the survival condition of the entire colony, even though the larvae survive to adulthood.

OR039

Migratory stopover sites of giant honeybees: A plea for conservation

Willard Robinson

Populations of the giant honeybee, *Apis dorsata*, face many threats, but paramount among them is habitat loss. The forests of South Asia, the habitat of the giant bee, face the most rapid deforestation occurring anywhere in the world. I recently discovered and reported on a site--an orchard along the Pai River in Thailand--where 16 colonies of migrating giant honeybees were bivouacking in close proximity to one another. The same time the following year >2x as many bivouacs gathered there. Informants assert the site is used annually; apparently the colonies are resting as they move to higher altitudes and flowering plants as the dry season begins. The site affords remarkable opportunities to study bees as they migrate. Although some colonies perch high, observable only with binoculars, others rest within ~1-5 m, easily accessible. E. g., I was able to describe their migratory dances in preparation for departure flights. Also, in 6 weeks the first year I observed >40 swarms of the Indian hive bee, *Apis cerana*, hopscotching through the orchard, eluding predatory hornets. This behavior had not previously been recorded. To make matters even more interesting, a black dwarf honeybee swarm, *Apis andreniformis*, occurred in the same orchard, and I studied interesting facets of its absconding behavior. The orchard is an astounding site for research, but probably not unique in South Asia. Most likely such sites exist wherever bees undertake long seasonal migrations. I describe attributes of the site, e.g. its location along a major river, and other possible navigational cues. I recommend researchers search for congregation sites wherever Asian honeybees migrate. Stopover sites--or hornet-shedding sites--may be essential to these species' life histories. Humans have drastically altered much riparian habitat in South Asia, but doubtless there still exist essential bivouacking sites that warrant conservation.

OR040

Museum specimens reveal resilience to disease in feral bees

Alexander Mikheyev, Mandy Tin, Jatin Arora, Thomas Seeley

For several decades colonies of honeybees (*Apis mellifera*) managed by beekeepers have suffered high mortality due to parasites and pathogens, most notably the ectoparasitic mite *Varroa destructor* and its associated viruses. Consequently, beekeepers depend on regular applications of pesticides and antibiotics to maintain their colonies, but this way of beekeeping is not sustainable. There is, therefore, a pressing need for information about natural ways to strengthen the robustness of honeybee colonies. In contrast to the losses of managed colonies, feral colonies are thriving in many places, including some parts of the United States. Little is known about the historical abundance of feral colonies in the United States, but one well-studied population near Ithaca, NY had the same density in 2011 as it did in 1978, despite the arrival of *Varroa* and other diseases in the intervening years (Seeley, 2007). To investigate what selective events occurred in this population during this time, we conducted whole-genome re-sequencings of worker bees collected from feral colonies living near Ithaca, NY in 1977 and 2011, using a novel low-error PCR-free library preparation technique. An analysis of 516,702 SNPs reveals evidence for numerous 'soft' selective sweeps in the genomes of these feral honeybees. These sweeps, however, were localized around targets of selection, and do not correlate with a reduction of the effective population size. Indeed, the effective population size increased in the intervening 34 years, probably as a result of migration from other honeybee populations, such as African honeybees, whose characteristic alleles increased in frequency. These results suggest that feral honeybee colonies may be resilient to disease and capable of rapid selective responses when faced with novel parasites and pathogens. These studies of feral honeybee colonies suggest natural ways to maintain the health of managed honeybee colonies.

OR041

Disease associations between honeybees and bumblebees: a threat to pollinators

Matthias A. Fuerst, Dino P. McMahon, Juliet L. Osborne, Robert J. Paxton, Mark J.F.Brown

Pollination is one of the key ecosystem services and it is provided mainly by insects, bees in particular. The recent dramatic decline in honeybee populations as well as wild bee populations is therefore of significant concern, especially since the driving forces are largely unexplained to date. A variety of potential causes have been proposed and, among others, diseases have come into focus. Emerging infectious diseases (EIDs) are recognized as particularly problematic, as the absence of co-evolutionary history between pathogen and host favours highly virulent pathogens in comparably poorly-defended hosts. Prominent examples of EIDs are viruses, fungi and mites in honeybees. Some of these honeybee diseases have recently been detected in a variety of non-*Apis* pollinators including bumblebees (*Bombus sp*.). Even though potentially detrimental to these wild bee populations, next to nothing is known about the basic epidemiology of the newly detected diseases in these vital and charismatic pollinators. To advance our understanding we collected data on prevalence of the microsporidian *Nosema ceranae* and the Iflavirus DWV (deformed wing virus) in the most common bumblebees across the UK along with data on honeybee densities and disease prevalence in honeybees at our collecting sites to assess the degree of disease advance and the potential role the original host plays. Both pathogens were present and infective in bumblebees as well as honeybees across the UK, with strong evidence for disease transmission from honeybees to bumblebees. These results have important implications for potential wild pollinator conservation actions.

OR042

Impacts and dynamics of two emergent parasites on bumblebee colonies

Matthias Fuerst, **Mark Brown**

Emergent diseases are one of the major threats to global biodiversity, and this is also true for pollinators. Whilst the impact of emergent diseases on honeybees has been recognised for decades, the potential for emergent disease to impact wild bee populations has only recently been explored. Recently we have shown that Deformed Wing Virus and the fungal parasite *Nosema ceranae*, both major parasites and causes of decline in honeybees, are widespread and emergent in UK bumblebee populations. However, determining the impact of such parasites requires large-scale experiments. We used a fully-crossed design to examine the dynamics and impacts of these parasites on bumblebee colony growth and fitness under laboratory conditions. We report the results of these experiments, and discuss the implications for the health of our wild bumblebee populations.

OR043

Impacts of multiple stressors on bumblebee queens

Gemma Baron, Mark J. F. Brown, Nigel E. Raine

Bumblebees have an annual colony cycle, several stages of which may be particularly vulnerable to environmental stress. Founding queens represent such a stage; they must forage, nest search and initiate a colony alone, often under challenging environmental conditions. Loss of the queen at this stage, or even sub-lethal impacts on her behaviour or ability to lay eggs could lead to failure of the colony. A range of stressors, such as parasites, pesticides, adverse weather and difficult foraging conditions could all have negative impacts on queens during this critical phase in their lifecycle. While much is known about the impacts of natural parasites and hibernation duration on queen success, almost nothing is known about the impacts of pesticides at this stage of the colony cycle. The large size of queens may buffer them from the impacts of low level of pesticide exposure, but when faced with additional potentially stressful events, such as diapause, parasitism, or reduced food availability, these stressors may interact to negatively impact the health and fitness of queens. Here I present data on the survival, egg-laying and colony founding ability of *Bombus terrestris* queens when challenged with multiple stressors, and discuss the implications for wild bee populations.

OR044

Chronic impairment of bumblebee foraging induced by sublethal pesticide exposure

Richard Gill, Nigel Raine

Continued human population growth means food security is an increasingly pressing issue. Pesticides are considered essential to maintain high crop yields, and systemic pesticide use has increased significantly in the last two decades. Systemic pesticides, e.g. neonicotinoids, provide pest protection throughout the plant for an extended period of time. However, residues are also found in the nectar and pollen of flowering crops: an unintended route of pesticide exposure for beneficial pollinating insects. Bees contribute a substantial proportion of insect pollination so they are an insect group that could be at particular risk. As the most widely used class of systemic pesticide, neonicotinoids have been implicated as a causal factor in reported bee declines. Yet there is much debate over whether neonicotinoid residues in the environment are detrimental to bee health with empirical evidence providing mixed support. Most studies of sublethal impacts of neonicotinoids have focused on the effects of acute exposure on bee behaviour. However, bees in the field are likely to be exposed to pesticide residues over extended periods of time, and there is a paucity of data showing how bees respond to persistent exposure. We investigated the effect(s) of field-level neonicotinoid exposure on the bumblebee *Bombus terrestris* (40 colonies), including examining natural foraging behaviour using radio frequency identification (RFID) tagging (259 foragers performing 8751 foraging bouts), over four weeks. We provide detailed data on the day-to-day natural foraging patterns to assess the temporal dynamics of how foraging behaviour was affected. We show that prolonged pesticide exposure has both acute and chronic impacts on fundamental aspects of forager behaviour and performance. These findings will improve our understanding of how persistent pesticide exposure might affect pollination efficiency, identifies whether subtle pesticide induced behavioural impairments accumulate over time, and has implications for how we should conduct risk assessment protocols.

OR045

Investigating sublethal pesticide effects on bumblebee navigation and foraging

Dara Stanley, Nigel Raine

Bumblebees are essential pollinators of many important agricultural crops and wild plants. While foraging in agricultural farmland bees are likely to be exposed to pesticides applied for crop protection. Although bees typically encounter these pesticides at sublethal levels, exposure may still have impacts on factors such as behaviour or reproduction with potential consequences for colony fitness. Neonicotinoid pesticides, which are often applied systemically, are found in the nectar and pollen of mass flowering crops where bees and other pollinators become orally exposed while provisioning their brood/colonies with food. We examined the impact of field realistic doses of a neonicotinoid pesticide, thiamethoxam, on foraging and navigation in a common bumblebee *Bombus terrestris*. We used Radio Frequency IDentification (RFID) tag technology to monitor colonies in a semi-field experiment. Colonies were located in the lab but had free access to forage for nectar and pollen outside. Our results indicate varying effects of pesticide exposure on both bumblebee foraging and homing ability. Our work shows that exposure to field-realistic, sub-lethal concentrations of pesticides may not affect reproduction, but can still impact a critical aspect of bumblebee foraging, pollen collection. This reduced foraging efficiency in colonies exposed to neonicotinoid pesticides could have knock-on impacts for colony fitness not measured in this study.

OR046

Emerging RNA virus threats to honeybees and bumblebees

Dino McMahon, Myrsini Natsopoulou, Matthias Fuerst, Vincent Doublet, Jessica Casper, Eva Frey Peter Rosenkranz, Mark Brown, Robert Paxton

Emerging infectious diseases (EIDs) have contributed significantly to the current biodiversity crisis, leading to widespread epidemics and population loss. Bees are under severe pressure globally, and although EIDs have been implicated in bee losses, the extent and causal role of EIDs - particularly RNA viruses - in declining managed and wild bee populations remains poorly understood. We combine field and laboratory experiments with a systematic field survey to: 1) address the role of RNA viruses in honeybees (*Apis mellifera*) overwinter losses, 2) investigate the prevalence, infection level and extent of spillover of RNA viruses in and between managed (*A. mellifera*) and wild (*Bombus spp.*) bees. We show that an emerging virus strain, *Varroa destructor* virus (VDV-1), is more virulent (in *A. mellifera*) and more widespread in the landscape than its well-known close relative, deformed wing virus (DWV). Significantly, it is causally responsible for overwinter honeybee loss. Furthermore, we find widespread circulation and high levels of infection of multiple RNA viruses in a range of wild bumblebee species - and evidence of their spillover between managed honeybees and wild bumblebees. Our findings indicate that RNA viruses are major stressors of bee pollinators, and that their possible contribution to bee declines should not be overlooked.

OR047

Chemical communication during column foraging in Nearctic Messor *species*

Nicola Plowes, Bert Hoelldobler

Our research focuses on chemical communication during foraging in seed harvesting ants found in Nearctic *Messor*. This group is particularly interesting from an evolutionary perspective, because, like *Pogonomyrmex*, it has species which display a variety of different foraging strategies from individual to group foraging. Three Nearctic *Messor* species employ a spectacular mode of foraging where thousands of individuals follow columns for distances of up to 40m before individual ants disperse and forage independently. The foraging columns function both to lead workers to rich ephemeral food sources, as well as to avoid conflict with neighboring colonies. We present data on a combination of field, laboratory, and chemical analyses which have elucidated a multi-modal orientation and navigation strategy during foraging in *M. pergandei*. In addition, we will describe differences in the chemical ecology between several species of Nearctic *Messor*. Our results lend further support linking Nearctic *Messor* with *Novomessor* rather than Palearctic species of *Messor*.

OR048

Extinction of ants' feeding and social foraging on myrmecochorous seeds

Audrey Bologna, Claire Detrain

Myrmecochory - seed dispersal by ants - is a mutualistic interaction in which seeds are dispersed by ants, who consume a nutrient-rich appendage - the elaiosome - as a reward. Yet, real benefits received by ants are still unclear since elaiosome consumption can lead to a positive or a negative impact on colony fitness. Consequently, harvesting of myrmecochorous seeds is expected to be a dynamic process in which ant-plant interaction evolves depending on the outcomes obtained by each partners. We studied the stability of the association between *Myrmica rubra* ants/*Viola odorata* seeds at short and long time scale. While seed harvesting was complete on the 1st week, seed exploitation by ants decreased on the 2nd week until a complete extinction during the following 3 weeks. Extinction is a persistent phenomenon which lasted at least 7 weeks and is specific since it was not generalized to other myrmecochorous seeds (i.e. *Chelidonium majus*) or to other individually transportable items (i.e. fruit flies). The extinction of seeds exploitation was related to a decrease in the flow of foragers arriving at the food source as well as to a reduced probability for an ant contacting a seed to retrieve it. A drastic decrease in elaiosome consumption during the 5 weeks of exposition was concurrently observed with this seed harvesting extinction Even if chemical cues (e.g. oleic acid) stimulate ants to gather diaspores, ants faced with toxic compounds (alkaloids) or energetically non beneficial elaiosomes could, by gustatory learning, modulate their behavior towards the cessation of seeds exploitation. Myrmecochory appears as a complex process in which the ability of ants to evaluate the nutritive value of elaiosomes, and then by gustatory learning to accordingly adapt their foraging strategy, is of first importance for the stability of this association between the social and the plant partner.

OR049

Foraging leaf-cutting ants reject plants based on the gardeners' experience

Flavio Roces, Nicole Saverschek

Leaf-cutting ants are polyphagous herbivores that, despite their catholicity, show distinct preferences in the choice of plants as substrate for their fungus. Their foraging patterns are the result of an intricate interplay between the different colony members: foragers, gardeners and their symbiotic fungus. We investigated to what extent avoidance learning and memory for plant unsuitability for their fungus underlie plant selection by foragers, and whether foragers are influenced by gardening workers, which do not forage at all but are responsible for the processing of the harvested fragments. The rationale of the experiments was to test the acceptance of a novel plant by naïve foragers in two different situations. In the first, a suitable plant was previously incorporated into the colony and gardeners were therefore familiar with it. In the second, a previously-acceptable plant was experimentally made unsuitable by infiltrating leaves with a fungicide not detectable to the ants, but harmful to the symbiotic fungus, so that gardeners experienced these leaves as unsuitable after their incorporation into the garden. Naïve foragers readily accepted a novel plant species after having interacted with gardeners that knew this plant as suitable. However, when naïve foragers interacted with gardeners in the presence of untreated, but previously-unsuitable plant fragments, they showed a significant decrease in acceptance. Since the experienced gardeners were the only source of information about the former unsuitability of the leaves, it can be concluded that interactions with experienced gardeners suffice for naïve foragers to learn about substrate quality if the substrate is present. The gardeners' negative past experience with a plant lowered the acceptance of naive foragers to a now suitable substrate. It is argued that foragers are able to learn about substrate suitability not only while foraging, but also inside the nest from their gardening nestmates.

OR050

Towards isolating and identifying feeding stimulants in honeybee pollens

Richard Bridgett, Falko Drijfhout

One contributing factor to the decline of honeybees could be that reduced colony strength during the winter period, and in times of sparse natural forage, leaves bees more susceptible to parasites, disease, and starvation. Beekeepers may attempt to enhance colony health during such times by feeding high protein supplemental diets to colonies. Unfortunately, these tend not to be as readily consumed as pollen. The addition of natural pollen to such diets can increase uptake by bees. It is therefore believed that pollens contain naturally occurring feeding stimulants to honeybees. Modern analytical techniques provide the best hope of being able to conclusively isolate and identify such stimulants. Current work is looking at the novel application of Counter-current Chromatography, within a process of bio-guided fractionation of mixed-species pollen extracts, to attempt to isolate and identify compounds within pollen that elicit an increased feeding response in pollen consuming bees. Results obtained through feeding trials indicate that the majority of the common sugars and amino acids present in pollens may have a limited effect on increasing consumption. Work to isolate compounds responsible for increasing the consumption of diets in feeding trials continues, with initial screening suggesting sterols may be present in the more active extracts. If stimulant identification can be achieved it is hoped a future range of more palatable supplemental diets, which more effectively maintain colony strength, may be produced for use by beekeepers. Such diets could also be of significant benefit to commercial beekeeping and industrial pollination services.

OR051

The larval pheromone beta-ocimene regulates foraging in honeybees

Rong Ma, Juliana Rangel-Posada

Social insects communicate in part using pheromones that are capable of eliciting behavioral and physiological responses across a broad spectrum of time scales. Honeybee larval pheromones have emerged as a robust system for deciphering the behavioral consequences of nuanced chemical communication. Brood ester pheromone, which is comprised of 10 esters, suppresses worker ovary development, modulates the behavioral transition from nursing to foraging, and regulates the level of pollen foraging by the colony. However, it is still not clear how workers, especially foragers, detect these brood pheromones. The recently discovered larval pheromone E-beta-ocimene induces long-term physiological effects similar to those produced by brood ester pheromones. Because beta-ocimene is a volatile compound, it is hypothesized that worker bees throughout the hive can directly assess beta-ocimene and modulate their behavior accordingly. In this study, we tested the hypothesis that bees could directly assess volatile larval pheromones by investigating the role that beta-ocimene plays in the regulation of foraging activity. Four naturally-foraging honeybee colonies were exposed to a one-hour pulse of either synthetic beta-ocimene or a paraffin oil control, and the number of returning pollen or nectar foragers was recorded once an hour thereafter. Exposure to synthetic beta-ocimene significantly increased overall foraging activity - but not pollen foraging - thus verifying that honeybee larvae can communicate directly with foragers via volatile chemical signals. Further, this study demonstrates the potential for synthetic chemicals in the study of pheromones and raises questions about the relationship between two classes of brood pheromones: beta-ocimene, a lightweight volatile and brood ester pheromone, a complex blend of 10 non-volatile compounds.

OR052

Exploiting honeybee learning and foraging behavior for biosecurity

Flore Mas, Colin Henderson, Jerry Bromenshenk, Lloyd Stringer, Max Suckling

Honeybee social organization and communication enables rapid recruitment to exploit floral resources. This behavior presents unique opportunities to employ honeybees for detection and localization of emergent biosecurity risks. Prior research has shown conclusively that bees have the capability to detect several components in military explosives. A series of carefully designed experiments performed under varying conditions at geographically widespread locations has shown in every instance that whole colonies of bees can be trained to search for and find isolated targets emitting selected volatiles in small quantities and vapor concentrations at low parts per trillion. In the course of proving honeybees' potential, standard procedures for behavioral conditioning and tracking, and mapping forager density patterns were developed that can be implemented for a variety of volatile compounds. Tested compounds include a variety of illicit drugs, decomposition products, and more recently plant volatiles. With the continued pressure of biological invaders in natural and primary ecosystems, the ability to rapidly train honeybees to odors associated with infestation by introduced animals or plants and monitor their position on the landscape, offers potential for efficient targeted surveillance of rare pests on the landscape. We will discuss the training of bees to signature and whole organism odors and how current technology can make use of bees' ability to rapidly seek out and find these odor sources

OR053

Neurophysiological and behavioral study of olfaction in an invasive hornet

Antoine Couto, Karine Monceau, Olivier Bonnard, Denis Thiery, Jean-christopheSandoz

The yellow-legged hornet *Vespa velutina*, was accidentally introduced from China to Europe (France) before 2004. As this hornet is well adapted to the West European climate, it extends its territory each year and now shows high population densities throughout invaded areas (France since 2004, Spain since 2010, Belgium and Portugal since 2011). To feed their larvae with proteins, hornets prey on numerous insect species, but feed predominantly from honeybee colonies. Western Europe previously harbored only one hornet species, *Vespa crabro*, which never represented a strong threat to honeybee colonies due to its solitary hunting strategy. European bees *Apis mellifera* are thus not able to protect their colonies from the cooperative predation strategy of the yellow-legged hornet. For this reason, hornet predation is becoming a major problem in Europe and methods for limiting *Vespa velutina* populations are needed. Olfaction plays a key role in the biology and predatory behavior of hornets. One promising strategy for controlling hornets would be to interfere with their olfactory behavior using specific olfactory baits. We therefore first investigated with neuroanatomical methods, the organization of the olfactory system in the different castes of *Vespa velutina* aiming to understand its intraspecific communication system. By coupling fluorescent dye injections and confocal microscopy, we described hornet's brain and olfactory system, with a special emphasis on the first olfactory relay, the antennal lobe. Our results identify promising structures for studying sexual pheromone processing in hornet males and revealed remarkable anatomical similarities between ant and hornet antennal lobes. Next, we tested hornet workers' attraction toward honeybee hive components and honeybee-emitted odorants in a multiple-choice test. The results reveal strong and specific attraction of hornets towards hive products, paving the way for the development of efficient trapping strategies.

OR054

Recent advances in trail pheromones and sex pheromones in termites

David Sillam-Dusses, Jan Sobotnik, Robert Hanus, Jana Krasulova, Ping Wen, Etienne Simon, Paulo Fellipe Cristaldo, Og DeSouza, Michael J. Lacey

Because termites are blind, their communication largely relies upon chemical cues. Trail-following behaviour is mediated by trail pheromones, which mark the path from the nest to foraging sites. As for the mate localization, it is performed by sex pheromones. These last years, much progress has been made in the study of trail pheromones and sex pheromones in termites. The chemical structure of the female sex pheromone of *Zootermopsis nevadensis* and *Z. angusticollis* has been identified as (5E)-2,6,10-trimethylundeca-5,9-dienal and the male sex pheromone as syn-4,6-dimethyldodecanal. The trail pheromone of both species was shown to be composed of the same compound as the male sex pheromone. In female alates of *Hodotermopsis sjoestedti*, the major sex-specific compound was identified to be identical with the female sex pheromone of *Zootermopsis*. In male alates, the major sex-specific compound was identified as syn-4,6-dimethylundecanal, a homolog of the male sex pheromone of *Zootermopsis*. As for the trail-following pheromone of *H. sjoestedti*, it is syn-4,6-dimethylundecan-1-ol. These results highlight the conservative nature of the chemical communication in termites and an obvious chemical proximity in communication strategies between the Indomalayan termite *Hodotermopsis* and the nearctic termite *Zootermopsis*, which confirms the recent classification of both species in a new family, the Archotermopsidae. (10Z,13Z)-Nonadeca-10,13-dien-2-one has been recently identified as the trail pheromone of *Glossotermes oculatus* and *Serritermes serrifer* which confirms the close relationship between both species and the recent transfer of *Glossotermes* genus in this family. These results show that trail pheromones generally consist of a single compound in basal termites, while multi-component trail pheromones have been identified very recently in the advanced termites (Termitidae). A mixture of (3Z,6Z)-dodeca-3,6-dien-1-ol and (3Z)-dodec-3-en-1-ol is the female sex pheromone and the trail pheromone of *Odontotermes formosanus* (Macrotermitinae) whereas the trail pheromone of *Inquilinitermes microcerus* (Termitinae) and of all Nasutitermitinae studied consists of neocembrene and (3Z,6Z,8E)-dodeca-3,6,8-trien-1-ol.

OR055

Formation of efficient transportation networks in the Argentine ant

Simon Garnier, David Neiman, Subashkusum Ray, Andrea Perna, Guy Theraulaz, Iain Couzin

Transportation networks play a crucial role in the success of human societies and ant colonies. Their topology and morphology can dramatically affect the distribution of individuals, materials and information, and the overall productivity of a population. In a recent study, we discovered that the asymmetrical organization of bifurcations in ant pheromone trail networks was responsible for a 3-fold increase in the amount of food transported to the nest compared to a symmetrically organized network. Such asymmetrically organized networks have been found in several ant species but the mechanisms responsible for their formation remain unknown. Here we present for the first time experimental and theoretical evidence that the formation of asymmetrical bifurcations in ant trail networks is caused by a simple interaction between the trail-following behavior of ants and properties of their walking behavior. We measured the trajectories of ants following a pheromone trail that bifurcated with different angles into two trails. For narrow angles, ants tended to alter their course after crossing the point at which the trail bifurcated. For wide angles, they tended to alter their course before the bifurcation point. For intermediate angles similar to the angles found in natural networks, ants altered their course around the trail bifurcation point. We used computer simulations to show that the repetition of this behavior at a bifurcation point leads to a stable, asymmetrical organization of the bifurcation and we explored the impact of the linear and angular speeds of ants on the final shape of the bifurcations. Our results demonstrate that efficient transportation networks can emerge solely from the activity of network users, without prior planning. Uncovering simple mechanisms that lead to the formation of robust and efficient transportation networks is critical to understanding the ecological success of ants and to proposing new solutions for man-made systems.

OR056

ASSISIbf: A new pathway to examine collective behaviours in honeybees

Martina Szopek, Michael Bodi, Sibylle Hahshold, Ronald Thenius, Thomas Schmickl

Collective behaviours in honeybees is a challenging field of research due to the various communication channels and cues the animals use for decision making and the performance of collaborative tasks. In previous experiments in a temperature arena with a complex thermal gradient we found that only a minority of tested single young bees is able to locate themselves at their temperature optimum of 36°C. In contrast to that we found that a sufficiently large group of bees aggregates at the optimum and is even able to discriminate it from a suboptimal temperature spot. Thus we conclude that the bees collectively choose the optimum and perform collective decision making. Furthermore, we were able to interfere with the group decision by introducing confined bees in a suboptimal temperature spot. The bees were then collectively lured to the suboptimum. In the presented EU funded project ASSISIbf we now aim for developing a novel method to further examine the underlying mechanisms of collective behaviours. We are investigating various stimuli (e.g., vibration, light) with regard to the behavioural response of the bees (e.g., attraction, repulsion). The findings will lead to the development of static robotic devices able to interact with the honeybees and interfere with the group decision by using multiple communication channels and signals. We will establish an experimental setup with a robotic array that will be able to interact autonomously with the bees and adapt by evolutionary algorithms. We aim for generating a mixed group of robots and honeybees, enabling us to investigate the collective processes from the inside of this bio-hybrid society. Adapted to various other species this technology may lead to new methods in e.g., live stock or pest management in the future.

OR057

Does increased contact rate increase foraging efficiency in leaf-cutting ant?

Sofia Bouchebti, Luiz Carlos Forti, Audrey Dussutour, Vincent Fourcassie

Trail traffic in ants always involves a high rate of head-on encounters between workers. These encounters decrease the speed of nestbound laden ants and thus lead to an overall decrease of the rate of resources arrival to the nest. Previous experiments have shown however that in leaf cutting ants placed in crowding conditions this decrease can be compensated by an increase in foraging efficiency (proportion of laden ants in the nestbound flow). The aim of our study was to test the hypothesis that this could be due to the fact that, following multiple contacts with nestbound laden ants on their way to the foraging area, outbound ants could be stimulated to carry leaf fragments when returning to the nest. We worked on the leaf cutting ant *Atta laevigata* and manipulated the rate of contact between workers travelling on a bridge placed between their nest and a foraging area. To increase the rate of contacts between outbound ants and leaf fragments, we hanged leaf fragments to an endless thread suspended a few millimeters over the bridge. During the experiments the leaf fragments were then moved nestward at the average speed of a laden ant. Conversely, to decrease the rate of contacts between outbound and inbound ants, we removed at the foraging area end of the bridge one out of two laden (or unladen) ants in the flow of returning workers. The foraging efficiency measured in these experimental conditions was then compared to that observed in control condition, i.e. without any contact manipulation. Overall, our results fail to show any statistically significant effects of contact rate manipulation on foraging efficiency, suggesting that the increase in foraging efficiency observed in crowding conditions may be linked to other phenomena, e.g. a higher density of trail pheromone deposits or a particular traffic organization.

OR058

Emergence of colony-specific architectures in termite shelter-tube construction

Nobuaki Mizumoto, Kazuya Kobayashi, Kenji Matsuura

Social insects build sophisticated and complex architectures such as huge nests and underground galleries based on self-organizing rules. The structures of these architectures vary widely in size and shape within a species. Some studies have revealed that the current difference of environmental and/or social conditions can cause differences in the architectures that emerge from collective building. However, little is known about the colony variation of the structure under the same condition. Here, we show that termite colonies build colony-specific architecture using shelter-tube construction as a model system. When we divided a colony into multiple groups of individuals, groups drawn from the same colony performed similar patterns of construction, whereas groups from different colonies exhibited different patterns. Some groups laid many shelter tubes from the nest, while others constructed fewer but longer shelter tubes. We also found groups that formed no shelter tubes but simply covered the bottom of the container with mats. Our two-dimensional lattice model demonstrated that these colony variations in shelter-tube patterns can be attributed to the following two factors: 1) the sensitivity of workers to the cement pheromone which is involved in indirect local communication among workers; 2) the number of workers that engaged in shelter-tube construction. These results indicate that dramatic variations of architectures emerge from the difference of the workers' property among colonies even under the identical condition. The colony variations of architectures can be interpreted as the result of the adjustment of each colony to the original situation in the field. We will discuss the fitness consequence of the different structural patterns of constructions.

OR059

Effects of nest architecture on collective behavior of ant colonies

Noa Pinter-Wollman

Evolution acts on variation in structures that determine function, thus potentially affecting animals' fitness. In social insects, the relationship between a colony and its nest is an example of the relationship between morphology and behavior, or structure and function. Colony behavior is organized without central control through local interactions among workers. When and where ants interact with one another depends on their walking trajectories which are constrained by nest architecture. For example, the width of a chamber entrance, and obstructions within it, affect how fast ants move in and out of the chamber. Therefore, the collective behavior of a colony, such as recruitment to food, which emerges from the interaction network among workers, is likely affected by nest architecture. Indeed, the speed at which colonies of the black harvester ant, *Messor andrei*, respond to food and to disturbance changes when they relocate to a new nest site. However, it is unknown whether nest structure dictates these behavioral changes or what architectural features influence the collective behavior of the colony. I examine the relationship between the collective response of a colony to food and disturbance and features of their nest architecture. Because *M. andrei* colonies often relocate among nest sites I can examine nest architecture by creating plaster casts of nests that have recently been vacated. I then relate features of the nest cast to the collective behavior of the colony, as measured before it left the nest. I found a relationship between various architectural features of the nest and the speed at which a colony collectively recruits to food. Thus, my work provides new insights about the rules that underlie the emergence of collective behavior. Furthermore, understanding which architectural features influence collective behavior may inspire novel architectural designs that optimize efficient communication and movement.

OR060

Emergence of a Lévy-like searching through agents' local interaction

Tomoko Sakiyama, Yukio-Pegio Gunji

It is well discussed whether animals show Lévy-like searching or not. Lévy-like algorithms are considered to be efficient in case of extinction because of their supper-diffusion property. The problem is however, how one can achieve searching efficiency without assuming power-law distributed step lengths. Therefore, we developed new agent-based algorithm in which the agent changed the directional rule using others' directional information in its limited visual field. Foragers, which don't know any available food location information, must get some ambiguous information from other agents. If the agents accept that information absolutely, then the agents don't refer the global property which the swarm has and will get to their goal as only a result by just obeying that information such like chemical pheromone recruitments. However, changing the directional rule by expecting the others moving directional information in local area, the agent might hold ambiguous global information within local information. We investigated whether rule change based on local agents' interaction would achieve effective random searching or not. We developed two random walk algorithms and checked whether the searching efficiency and power-law distributed step lengths were achieved in those algorithms. Comparing only interaction model in which the agent obeyed others' moving direction in it visual field only on that time step, rule change model, in which the agent changed its own directional rule based on others' moving direction in it visual field showed more searching efficiency. We also could obtain that power-law distributed step lengths were achieved in our rule change model.

OR061

Fire ants as an active self-healing material

David Hu

Fire ants are excellent model organisms for studying active self-healing materials. By linking their legs together, they build highly interconnected networks that can quickly rearrange themselves in response to applied stress. In this talk, we present our combined experimental and theoretical work on ant rafts and ant towers. We use time-lapse photography and micro-CT scanning to visualize the outside and inside of these structures. Our experiments reveal spherical rafts of ants can morph into pancakes within minutes. Once a raft docks onto land, ants build Eiffel-tower-like structures whose shape ensures each ant supports an equal load. In our associated modeling, particular attention is paid to rationalizing the limits of these behaviors using the sensory and locomotory constraints of a single ant. Lastly, we also use plate-on-plate rheology to show how similar ants assemblages are to complex fluids like corn starch.

OR062

Social evolution and behavior of the queenless clonal raider ant

Daniel Kronauer

The clonal raider ant *Cerapachys biroi* has an unusual reproductive system: colonies are queenless and consist entirely of totipotent workers that reproduce via thelytokous parthenogenesis. As a consequence, all workers in a natural colony are essentially clonally identical. This allows us to set up large numbers of arbitrarily sized experimental colonies from stock colonies, without the need to mate the species in the lab. It also allows us to experimentally control and replicate the genetic composition of social groups, something that is impossible in most social insects due to the difficulties associated with creating inbred lines. Colonies of *Cerapachys biroi* undergo stereotypical reproductive cycles of about one month that alternate between reproductive and brood care phases. The colony cycles emerge from the interaction between workers and larvae: larvae develop in discrete cohorts, suppress ovarian activity and induce brood care and foraging behavior in adult workers. Therefore, a discrete cohort of age-matched young workers emerges once per cycle, providing easy experimental control over individual age and group demography. Finally, despite the fact that queens are absent, *Cerapachys biroi* workers show some level of phenotypic plasticity. For example, the number of ovarioles in workers ranges from two to six, and correlates with external morphological characters. As in other social insects, ovariole number also correlates with reproductive output, behavior, and longevity. Because different types of individuals can be arbitrarily mixed in experimental colonies, the unusual biology of *Cerapachys biroi* provides unparalleled control over the composition of social groups with respect to important factors that are known to affect social insect behavior and development, and thereby division of labor: group size, genotype, age, and reproductive physiology. Based on this experimental accessibility, I will discuss how the species can be leveraged as an interesting model system to provide novel insights into social evolution and behavior.

OR063

Evolutionary consequences of and selection on recombination in automictic populations

Jan Engelstaedter

Many animal species, including many social insects, reproduce asexually through automictic parthenogenesis. Here, a modified version of meiosis takes place where diploidy is restored in the eggs through fusion of two meiotic products. In contrast to clonal reproduction, this entails a reduction in heterozygoity among the offspring. This reduction in heterozygosity depends on the type of automixis (e.g., central fusion or terminal fusion) but may also depend on the rate at which crossover events between the locus under consideration and its linked centromere take place. I present results from a mathematical model of a population reproducing through automixis that makes predictions on the consequences of recombination on standing genetic variation, in particular levels of heterozygosity. The model considers two loci, mutation and selection with arbitrary levels of dominance. It is demonstrated that with overdominance (heterozygote fitness advantage), heterozygotes may reach a higher or lower frequency than with sexual reproduction but may also go extinct when the rate at which they are eliminated through automixis is too high. For example, with central fusion automixis there exists a maximum recombination rate between locus and centromere above which heterozygosity is lost in the population. These results are of particular relevance for species with complementary sex determination systems. I also explore the mutation-selection balance of deleterious mutations and the resulting genetic load as well as the expected genetic variation at neutral loci linked to loci under natural selection. Finally, I also investigate how natural selection acts on recombination rates in automictic populations. Overall, the results from this model should provide useful predictions on evolutionary patterns in automictic species against which empirical results can be tested.

OR064

*Asexual queen succession in soil-feeding termites (*Cavitermes tuberosus*)*

Yves Roisin, Robert Hanus, Denis Fournier

Asexual queen succession (AQS) is a process by which a termite queen gets herself replaced by multiple parthenogenetic daughters, which mate with the founder king. AQS is known to occur in subterranean termites (*Reticulitermes* spp.), where it might boost the egg production rate without inbreeding (1). In a totally different context, some soil-feeding Termitidae from tropical rainforests commonly display multiple neotenics as well. We investigated the genetic and social pathways of neotenic production in one such species, *Cavitermes tuberosus* (Emerson), in French Guiana. Nests were dissected and searched for reproductives. The reproductive female function was assumed by the primary queen in 39 colonies, and by neotenic females (up to 450 individuals) in 28 colonies. A primary king was accompanying the primary or neotenic queen(s) in most nests, but only one neotenic male was found. Nests headed by a primary queen were never found to contain neotenic females, but they often contained female nymphs of an atypical form, which might be future neotenics. Eighteen microsatellite markers were specifically developed. Our first genetic analyses strongly support AQS as near-exclusive source of neotenic females in this species. Most neotenic females were completely homozygous, as expected under parthenogenesis with terminal fusion. All other individuals appeared to be produced sexually. The finding of AQS in an arboreal-nesting, soil-feeding tropical rainforest species, thus ecologically opposite from wood-feeding, subterranean *Reticulitermes*, raises the question of its adaptive significance. In addition, *Reticulitermes* (Rhinotermitidae) and *Cavitermes* (Termitidae) are phylogenetically distant, which indicates either an unlikely convergence or an ancient origin of facultative terminal fusion parthenogenesis in termites. It suggests that AQS may be much more widespread in termites that previously thought.
(1) Matsuura et al. (2009) Science 323, 1687. Vargo et al. (2012) Proc. R. Soc. B. 279, 813-819. Luchetti et al. (2013) Insect. Soc. 60, 203-211.

OR065

Asexual queen succession in the Neotropical higher termite Embiratermes neotenicus

Robert Hanus, Romain Fougeyrollas, Klara Dolejsova, David Sillam-Dusses, Chantal Poteaux, Yves Roisin, Virginie Roy

Sexual and asexual reproductions both have undisputable advantages as well as drawbacks. The best option for the breeding queens of social insects thus would be to combine these modes of reproduction and benefit from an increased genetic contribution to next generations produced by asexual reproduction while still maintaining sufficient genetic diversity of their offspring through sexual reproduction. There is now rising evidence that termite queens are able to do so. Asexual queen succession (AQS) in which workers and alates are produced sexually while multiple neotenic queens arise through thelytokous parthenogenesis has recently been described in three species of the subterranean genus *Reticulitermes*. However, there are more termite species and genera in which neotenic reproductives replace the founding primary queen at a certain stage of the colony development, including a few species of higher termites. The Neotropical humivorous species *Embiratermes neotenicus* (Termitidae: Syntermitinae) is notorious for the occurrence of numerous neotenics in its large and populous colonies. Using nine newly developed polymorphic microsatellite markers, we studied the genetic structure in *E. neotenicus* colonies sampled in French Guiana. The colonies contained either a single primary queen with a primary king or up to 150 neotenic queens with a primary king and rarely a few neotenic males. Neotenic females never carried exclusive paternal alleles at the studied loci unlike the sterile castes (workers and soldiers). This strongly supports the hypothesis that in *E. neotenicus*, the primary queen is replaced by asexually produced neotenic queens. Thus, it appears that AQS is not restricted to a single genus of lower termites and can be expected to be found in more termite species, including higher termites. What are the circumstances promoting the occurrence of this unusual reproductive strategy in phylogenetically and ecologically distant termites, remains an open question.

OR066

Queens close sperm-gates of eggs for asexual reproduction in termites

Toshihisa Yashiro, Kenji Matsuura

The near-ubiquity of sexual reproduction in animal species despite the two-fold cost of sex has long been an enigma. In the evolution of parthenogenesis (female reproduction without male fertilization), males and females may be in conflict over genetic transmission to the next generation because parthenogenetic reproduction enhances female's reproductive outputs but completely deprives of male's genetic contribution. For these males, any trait coercing these females into sexual reproduction should increase their fitness and should be under positive selection. In the termite *Reticulitermes speratus*, however, queens produce their replacements (neotenic queens) parthenogenetically while using normal sexual reproduction by mating with kings to produce other colony members. Here, we report that queens of this termite species produce parthenogenetic offspring in the presence of kings by closing micropyles (sperm-gates, tiny openings for sperm entry) of unfertilized eggs. Our field survey demonstrated that there is a large variation in the number of micropyles in the eggs, and that some of the eggs have no micropyle. Microsatellite DNA analysis indicated that micropyleless eggs develope parthenogenetically without fertilization, whereas eggs with micropyles get fertilized and develope sexually. Comparison of the number of micropyles between the eggs laid by old- and young-queens showed that only old-queens produce micropyleless eggs. Moreover, sampling of the field colonies revealed that most of the micropyleless eggs are produced in early egg-laying season. Our results demonstrate that the queens control egg fertilization without interference from the kings and produce their replacements parthenogenetically as needed. This suggests that parthenogenesis can evolve in favour of the females' own interests independently of males' interests. Furthermore, the maintenance of sexual reproduction even under the strong control of egg-fertilization by the queens may be explained by the importance of genetic diversity among colony members in social insects.

OR067

Male territoriality in African and Caribbean populations of Cardiocondyla venustula

Susanne Jacobs, Juergen Heinze

The genus *Cardiocondyla* is characterized by a pronounced male polyphenism with wingless fighter males with lifelong spermatogenesis and winged disperser males with the limited sperm supply typical for ant males. Winged males have been lost secondarily in several species, and while lethal fighting among wingless males appears to be the ancestral stage, several species have evolved mutually tolerant males. In the phylogeny of *Cardiocondyla*, *C. venustula* occupies an intermediate position between species with fighting males and species with tolerant males. The behavior of its males is therefore of particular interest. Wingless males of *C. venustula* spread out in their natal nests and defend ‘territories’ against rivals. While injuries are common among males, fighting with lethal outcome is rare. Behavioral observations of 25 *C. venustula* colonies from South Africa (native) and Puerto Rico (introduced) revealed a high intraspecific variability in male behavior. While some colonies had several highly territorial males, males of other colonies did not show any territorial behavior. Similarly, the degree of aggressiveness varied substantially among the observed colonies. Ongoing behavioral and molecular genetic analyses will allow further insights into male competition and might thus help understanding the evolution of male mating strategies in *Cardiocondyla*.

OR068
Incipient social parasitism in the microgyne form of Myrmica rubra
Sämi Schär, David Nash

Ant microgynes are miniaturized queen forms found together with normal queens (macrogynes) across the ant phylogeny. Their status is not yet fully understood: in some cases they seem to be alternative reproductive morphs, in others incipient social parasites and thus potential models for studying the evolution of social parasitism. Whether they are regarded as parasitic or not has traditionally been based on genetic differentiation from syntopic macrogynes rather than measuring fitness traits. We measured virulence and infectivity of microgynes of *Myrmica rubra* from the Danish island of Læsø¸ in a controlled laboratory experiment. Nests headed only by macrogynes (controls), only by microgynes, and naturally and artificially mixed nests were kept under identical conditions. We found reduction in host fitness in both naturally and artificially infected nests compared with controls, suggesting that they act primarily as social parasites. On the other hand, there was surprising variation in fitness of nests headed only by microgynes, suggesting that in some cases they can also act as independent alternative reproductive morphs (i.e. intraspecific temporary social parasites). Microgynes did not themselves reproduce in artificially mixed nests, but reproduced most in orphaned host nests. This, together with higher mortality of field-collected macrogyne queens from naturally infested colonies, suggests that they preferentially exploit older host colonies.

OR069
Hybridogenesis shapes complex phylogeographic patterns in Cataglyphis *desert ants*
Pierre-Andre Eyer, Laurianne Leniaud, Serge Aron

Ant species of the *Cataglyphis altisquamis* group are characterized by a hybridogenetic mode of reproduction. Two distinct genetic lineages co-occur in each population and queens mate with males originating from the alternative lineage than their own. Queens use sexual reproduction to produce an inter-lineage hybrid worker force, but they use asexual reproduction (parthenogenesis) for the production reproductive offspring. Thus, sterile workers systematically arise from the hybridization of two genetic lineages, whereas the sexual forms belong all to the queen lineage. Recently, it has been shown that new reproductive queens are occasionally produced by gynogenesis. In gynogenetic species, sperm cells are required to trigger embryogenesis but usually do not transmit their genome to the zygote. However, this mode of reproduction provides an opportunity for incidental leakage of sperm DNA into clonal queen-destined eggs (Schlupp 2005). Using both mitochondrial and nuclear genes, we examined phylogeographic patterns of 3 species belonging to the *Cataglyphis altisquamis* group where hybridogenesis and gynogenesis co-occur. Our results reveal three important insights. (1) For each species, a single pair of nuclear lineages exists across all populations sampled. Reproductives from each genetic lineage interbreed to produce sterile workers; by contrast, new queens and males are asexually produced. (2) Remarkably, genetic analyses reveal strong incongruences between mitochondrial and nuclear variations: variation at mtDNA shows a geographic structure independent of nuclear lineages segregation. This indicates a substantial mitochondrial gene flow between the two divergent nuclear lineages. (3) Consistent with these results, we found a higher mitochondrial diversity within species than between species. Such uncommon pattern of mitochondrial distribution most likely stems from recombination events between mitochondrial genomes from distinct lineages. Overall, this study shows how alternative reproductive strategies can shape complex phylogeographic patterns.
Schlupp, I. 2005. The evolutionary ecology of gynogenesis. Annual Review of Ecology Evolution and Systematics.

OR070

Evolution of social hybridogenesis in Cataglyphis *desert ants*

Hugo Darras, Serge Aron

Social hybridogenesis has evolved repeatedly in ants (e.g., *Pogonomyrmex*, *Solenopsis*). Hybridogenetic populations consist of self-sustainable pairs of hybridizing lineages. Queens of each pair mate with males of the opposite lineage to produce workers. By contrast, reproductive individuals arise from intra-lineage mating and maintain pure-lineage genomes. Recently, a new form of social hybridogenesis was discovered in the desert ant *Cataglyphis hispanica*. All workers are inter-lineage hybrids, whereas male and female sexuals are produced by asexual reproduction through parthenogenesis. As a consequence, only maternal genes should be perpetuated across generations. This system, as it stands, is expected to be evolutionarily short-lived due to long-term costs of asexual reproduction and selection against males, which do not contribute any genes to reproductive offspring. Contrary to this expectation, we show (i) that hybridogenetic lineages in *Cataglyphis* ants have been maintained over a long evolutionary time and across a large geographic scale. Hybridogenetic lineages occur in a whole complex of species that have diverged several millions years ago and are distributed from Northwest Africa to West Asia. (ii) Furthermore, a detailed survey of colonies structure in *C. hispanica* reveals that the workers are hybrids of the same two genetic lineages across the whole distribution range of the species. (iii) Remarkably, hybridogenetic *Cataglyphis* ants escape the costs of asexual reproduction by the sporadic production of new queens by intra-lineage mating, instead of parthenogenesis. Rare intra-lineage mated queens were found in polygynous colonies. Genetic analyses indicate that they lay fertilized eggs; these eggs fail to develop into workers but can achieve queen development. Thus, intra-lineage mated queens produce new queens and males only. Though they do not contribute to the production of the worker-force, these ‘social parasites’ promote genetic diversity and ensure non-null male fitness.

OR071

Causes and consequences of thelytoky in the ant Cerapachys biroi

Ingrid Fetter-Pruneda, Peter Oxley, Daniel J.C. Kronauer

Workers of the queenless ant *Cerapachys biroi* reproduce by thelytokus parthenogenesis, but the cytological mechanism is currently unknown. We therefore determined the mode of asexual reproduction in *C. biroi* by observing chromosomes stained with DAPI during oogenesis and early embryogenesis. Our results show that eggs undergo regular meiotic division within the first half hour after oviposition. Following the second meiotic division, the two central haploid nuclei fuse to restore diploidy, showing that *C. biroi* reproduces via automixis with central fusion. Automixis with central fusion incurs loss of heterozygosity (LOH) due to meiotic recombination as has been observed in other thelytokous social insects such as the Cape honeybee and the ants *Wasmannia auropunctata* and *Cataglyphis cursor*. LOH in these species also leads to high proportions of sterile diploid males. Using RAD-Seq, we show that the rate of LOH in *C. biroi* is as low as 0.0013% per generation: substantially lower than in the other thelytokous ants studied, and ca. 100 times lower than in the Cape honeybee . As a consequence, the genomes of *C. biroi* nestmates are almost clonally identical. Furthermore, diploid males are exceedingly rare in this species, with only ca. one out of 10,000 diploid individuals being male. It is currently not known whether the low rate of LOH is due to reduced levels of recombination, selection against recombinants, or both. We are currently aiming to measure the strength of selection against recombinants by genotyping individuals at the egg and adult stage. We are also studying potential molecular players, such as recombinase proteins, that could be involved in lowering recombination rates in *C. biroi*.

OR072

Thelytoky in the honeybee

Frances Goudie, Benjamin Oldroyd

Thelytoky, the asexual production of females, is rare in honeybees. However, it is ubiquitous in workers of the Cape honeybee *Apis mellifera capensis*. Thelytoky allows some workers to be reincarnated into the queen phenotype, and thereby selects for reproductive competition among workers. Thelytoky also acts as an exaptation for the emergence of reproductive parasites, the most extreme example of which is an entirely clonal 'cancerous' lineage of workers (the Clone) that lethally parasitises colonies of another subspecies *Apis mellifera scutellata*. The Clone is an enigma because thelytoky results in the accumulation of homozygosity at any loci that are free to recombine, yet the Clone retains considerable heterozygosity. The Clone pays a cost for its thelytoky: the selective removal of homozygous offspring at each generation. We propose that workers, queens and Clones have differing abilities to endure the costs and benefits of sex and asexuality, accounting for the heterogeneous distribution of reproductive strategies across the *A. mellifera capensis* population. We further suggest that multiple factors must fall into place for thelytoky to emerge as an effective reproductive strategy in a honeybee population, and that geographic isolation resulting in genetic drift and founder effects may have enabled thelytoky to emerge in *A. mellifera capensis*. Finally, we consider the honeybee in the broader context of haplodiploid Hymenoptera, and argue that constraints on the evolution of sex in non-haplodiploid taxa may make sexual reproduction an evolutionary 'one-way street'.

OR073

Collective decision-making in Asian honeybee swarms on the move

James Makinson, Timothy Schaerf, Atsalek Rattanawannee, Wandee Wattanachaiyingcharoen Benjamin Oldroyd, Madeleine Beekman

When a honeybee swarm leaves the colony it is faced with a tough dilemma; it must collectively locate, choose between and coordinate movement to the best quality nesting cavity it can find. The process of nest-site selection in the Western honeybee (*Apis mellifera*) is the best studied example of collective decision-making in the social insects. But *A. mellifera* is only one of eleven species within the genus *Apis*. Furthermore, the genus can be split into three categories based on nesting biology; cavity nesters (e.g. *A. mellifera*), dwarf open nesters (e.g *A. florea*) and giant open nesters (e.g. *A. dorsata*). Both open nesting groups are migratory, following seasonal nectar flows. Dwarf open nesters build small colonies on shrub and tree branches, while due to their size giant open nesters are limited to nesting on large smooth surfaces such as the branches of large trees. We test whether differences in nesting biology influence the decision-making processes used by these species. By creating swarms of *A. florea* and *A. dorsata* we found that unlike *A. mellifera*, neither of these species go through a process of waggle dance decay. In contrast to *A. mellifera*, *A. florea* scout bees did not frequently leave the swarm surface to re-evaluate sites being danced for, while *A. dorsata* took off from the swarm surface regularly. Our results demonstrate that the decision-making process of *A. florea* is the simplest within the genus, with the decision-making process of *A. dorsata* appearing to be intermediate between the quality independent process of *A. florea* and the quality dependent process of *A. mellifera*.

OR074

Visualization of neural activity of forager honeybee brain by IEGs

Taketoshi Kiya

One of the most interesting social behaviors of the honeybees is 'dance communication'. Foragers that successfully find a rich food source return to the hive and transmit the information of the location of the food source to their nestmates using a symbolic waggle dance. Although there are considerable amount of ecological researches for the dance communication, the underlying neural mechanisms remains mostly unknown. To elucidate the neural mechanisms of this remarkable ability, we aimed to identify active brain regions in the foragers that might be involved in dance communication and/or information integration during the foraging flight. To this end, we constructed neural activity mapping methods with the immediate early genes (IEGs). IEGs are genes expressed in a neural activity-dependent manner and thus can be used to visualize neural activity. We identified a novel IEG, kakusei, from honeybee brain and revealed that a specific mushroom body (MB) neuron subtype is preferentially active in in the forager brain. In addition, we revealed that the MB neuron activity depends on the foraging frequency, whereas the number of active MB neurons is related to the pattern of visual input received during foraging flight. Recently, we identified an evolutionally conserved IEG, Hr38, whose expression is higher than kakusei. Taking advantage of the high expression and long intron of Hr38, we are establishing novel neural activity mapping methods with high sensitivity and time-resolution. These methods will enable us to differentiate the neural activities related to the dance and foraging behaviour, which will lead to the identification of dance-specific brain regions and neurons.

OR075

Vibration processing and olfactory locomotion related to honeybee communication

Hiroyuki Ai, Kazuki Kai,Hidetoshi Ikeno

Honeybees (*Apis mellifera* L.) display sophisticated behaviors including learning and communication. Honeybee foragers learn different types of flower-related information and transfer such information to their hive mates by species-specific stereotyped in-hive behaviors. For information receivers, vectors pointing to profitable flower patches and associated floral odors are key informations to locate the indicated flowers. We have studied mechanisms to decode these informations using an interdisciplinary approach. The distance and direction of vectors are encoded in the number of pulses of air-borne vibration and in the body axis during the waggle dance, respectively. We have identified the primary center of these informations and the candidates for interneurons related to distance coding. We are now collaborating with a group of computational neuroscientists at LMU Munich to evaluate the morphological and physiological changes of the critical identified interneurons, depending on the age and labor-state of the bee, to clarify the development of the candidates for interneurons related to distance coding (http://projects.g-node.org/ginjang/). Honeybees also communicate plant odors by unique behavior, trophallactic contact in which the recipients learn plant odors associated with nectar rewards and then orientate toward corresponding odor sources. We investigated the locomotion pattern toward the reward-associated odor (CS+), compared with those toward a reward un-associated odor (CS-). Stimulation with CS- induced exploratory walks that had no clear relation to the stimulus position. In contrast, stimulation with CS+ induced localized search walks around the initial position at which the stimulus was received. This locomotor pattern consists of typical alternation of left/right turns less than 180 degrees. We will propose the possible mechanism for integrating the reward-associated odor and vector informations in the brain. This research was supported by the Ministry of Education, Science, Technology, Sports and Culture of Japan; Grant Number: 22570079 and Strategic International Cooperative Program, Japan Science and Technology Agency (JST).

OR076

Footprint of increased anthropogenic disturbance elevates termite pest status

Kok-Boon Neoh, Vuong Nguyen Tan, My Nguyen Thi, Huy Nguyen Quoc, Masayuki Itoh, Osamu Kozan

Among tropical regions, Southeast Asia is known to be ecological dominance of termites, yet also one of the highest rates of natural forest loss for urbanization (e.g., human settings, agricultural intensification). Local and landscape-scale modification has posed a major threat to global biodiversity as hectares of natural forest were razed to make way for more human dominating landscapes. Soil-dwelling invertebrates such as termites in particular are susceptible to rapid habitat modification owing to changes in microclimate, food availability, and light regimes. This, in turn, disrupts the integrity of their habitat and alters the biological functioning in an ecosystem. Using the example of land-used transition from natural forest to intensified cropland in Vietnam, this presentation will explain how the anthropogenic footprint driven by urbanization and socio-economic demand, elevate termite pest status following the termite dominance shift in line with increased land-use gradients.

OR077

The effect of urbanization on ant abundance and diversity

Grzegorz Buczkowski

Numerous studies have examined the effect of urbanization on species richness and most studies implicate urbanization as the major cause of biodiversity loss. However, no study has identified an explicit connection between urbanization and biodiversity loss as the impact of urbanization is typically inferred indirectly by comparing species diversity along urban-rural gradients at a single time point. A different approach is to focus on the temporal rather than the spatial aspect and perform ‘before and after’ studies where species diversity is cataloged over time in the same sites. The current study examined changes in ant abundance and diversity associated with the conversion of natural habitats into urban habitats. Ant abundance and diversity were tracked in forested sites that became urbanized through construction and were examined at 3 time points - before, during, and after construction. On average, 4.3 ± 1.2 unique species were detected in undisturbed plots prior to construction. Ant diversity decreased to 0.7 ± 0.8 species in plots undergoing construction and 1.5 ± 1.1 species in plots 1 year after construction was completed. With regard to species richness, urbanization resulted in the permanent loss of 17 of the 20 species initially present in the study plots. Recovery was slow and only 3 species were present right after construction was completed and 4 species were present 1 year after construction was completed. The second objective examined ant fauna recovery in developed residential lots based on time since construction, neighboring habitat quality, pesticide inputs, and the presence of invasive ants. Ant diversity was positively correlated with factors that promoted ecological recovery and negatively correlated with factors that promoted ecological degradation. Taken together, these results address a critical gap in our knowledge by characterizing the short- and long-term the effects of urbanization on the loss of ant biodiversity.

OR078

Invasive impacts of the Asian paper wasp across different landscapes

Darren Ward, Ana Ramon-Laca, Fraser Morgan

We have recently completed two projects on the ecological impacts of the Asian paper wasp, *Polistes chinensis antennalis*, an invasive species in New Zealand. Firstly, we developed a hierarchical modelling approach to estimate the extent of its impacts across New Zealand. We used a baseline distribution layer modelled via MaxEnt; paper wasp nest density (from >460 field plots); and rates of foraging intensity. Using geographic information systems this data is combined and modelled across different landscapes. The highest densities of Asian paper wasps were in herbaceous saline vegetation (nests densities of 34 per ha, and occupancy rates of 0.27), followed closely by built-up areas, and then scrub and shrubland. However, the extent of impacts of the Asian paper wasp in New Zealand remains relatively restricted because of narrow climate tolerances and spatial restriction of preferred habitats. Secondly, we used molecular techniques for determining the prey of paper wasps, based on removing paper wasp larva from the nest and examining their gut contents using COI. A total of 42 taxa were identified from 211 samples. Lepidoptera were the majority of prey, with 91% of samples. Conclusive species-level identification of prey was possible for 66% of samples, and genus-level identification for another 12% of samples. Small differences in diet were evident between urban and native habitats. The results greatly extend the prey range of this invasive species, and we believe is a more effective and efficient technique than relying on the collection of pellets, or morphological identification of prey, for the study of paper wasp diets. Ward DF, Ramon-Laca A. 2013. Molecular identification of the prey range of the invasive Asian paper wasp. Ecology and Evolution. DOI:10.1002/ece3.826 Ward DF, Morgan F. 2014. Modelling the impacts of an invasive species across landscapes: a hierarchical approach. Submitted PLOS.

OR079

Ants and urbanization: the case of Parma (Italy)

Fiorenza Spotti, Cristina Castracani, Donato Grasso, Alessandra Mori

Urban development is transforming natural ecosystems, inducing deep changes at landscape level. As the process of urbanization is not homogeneous, different urban land uses may affect all the ecosystem components to various extents. Studying plant and animal assemblages in anthropogenic habitats may help to better plan urban growth in the perspective of a sustainable development that preserves biodiversity. Ants have been frequently used as bioindicators in ecological studies and monitoring programs because they respond rapidly to environmental change, represent a variety of trophic levels and they are easy to collect. Moreover, several studies show that ants are widespread in urban environments. In this work, we used the town of Parma as a model to study the effects of urbanization on ant community structure. We selected 6 types of green spaces classified according to management and human exploitation: residential, industrial, business, agriculture, park and greenway. In each sampling area, pitfall traps associated with food baits were used to gather information on species richness, abundance and interspecific hierarchies of food dominance. Samplings were conducted during summers 2012/2013, recording 30 species. Our results suggested that different urban environments support different ant assemblages. The high biodiversity degree we observed in this work highlights the importance to lead studies on urban areas in order to allow the conservation of key habitats and their species.

OR080

Termite infestation in the urban landscape of Japan

Tsuyoshi Yoshimura, Wakako Ohmura

Japan is an urbanized country and has millions of wooden houses in the urban landscape. Four termite species, two subterranean and two drywood, are recognized as major urban pests. Witnessed from the recent earthquake disasters, termite infestation can be a cause of serious damages of houses. Thus, termite management in Japan has an important role not only to achieve a long service life of wooden houses, but also to save human lives. Results of four country-wide surveys on termite infestation in 2001, 2007, 2009/2010, and 2012 are available at present. The period during 2001 to 2012 represents a drastic change of housing structures in Japan, as most of newly-built houses have a concrete foundation with an increasing use of synthetic insulation materials to save energy. The results of the surveys show a decrease of infestation rate from 34.34% (5,644/16,435) in 2001 to 7.34% (192/2,615) in 2012. However, in over 20-years old soil-foundation houses, nearly 15% infestation was still reported in 2012 survey. The most important cause might be the change of the foundation system from soil foundation to concrete foundation. The concrete-foundation may be effective for deterring the termite invasion. The entry points of subterranean termites to houses are the contacts of untreated synthetic insulation materials with soil. Other entries from small utility holes and defects of the concrete-foundation are often reported lately. Now the most common infestation parts of house are located near the entrance and service-door. This clearly shows that termites are smart enough to find the insecticide-free and easy-accessible areas in housing structures when approaching from the soil.

OR081

An integrated framework for risk assessment of invasive urban ants

Chin-Cheng (Scotty) Yang, Shu-Ping Tseng, Han-Chih Ho, Rong-Nan Huang, Wen-Jer Wu, DeWayne Shoemaker

Accurate forecasting of biological invasions is extremely difficult especially given the trends of increasing global commerce, transportation and travel. Invasive ants are among the worst invaders worldwide due to their negative impacts, and the magnitude of impacts is expected to be more profound in human-altered areas (ex. urban or agricultural settings) presumably as a result of pre-adaptation to such environments. Risk assessment analysis (RAA) represents one of few reliable approaches that effectively assist in preventing introductions of invasive species including ants. We developed an integrated framework that focuses on ''likelihood of entry'', one of the critical elements of RAA, using population genetic data along with trade records. We hypothesized that the volume of international trade can serve as a predictor of propagule pressure of invasive ants, which generally transforms into the invasion success. Our genetic data coincide with intensity of trade where the most likely source population of invasive ants determined genetically appears to be at or near areas that rank highest in exportation of goods to a recipient country. Such patterns provide support for tight linkage of introduction routes and invasion success to global trade and transportation networks. Integration of population genetic data and trade estimates into future RAA may assist in establishing early warning systems as well as identifying routes of risk that potentially introduce invasive ants.

OR082

Termite baits for management of structural infestations of Reticulitermes flavipes

Susan Jones

The eastern subterranean termite, *Reticulitermes flavipes*, is the most economically important, widely dispersed native species in the United States. It is a common pest in homes, since wood is a dominant structural element. As discussed in this presentation, studies of bait effectiveness in infested homes in Ohio have demonstrated that a number of bait systems can provided structural protection, often within a matter of months once termites begin feeding on the active ingredient. In addition to effectiveness of the active ingredient in the bait itself, refining bait placement is an important consideration, particularly since one or more colonies may be associated with a structure. Furthermore, a moisture meter is a useful tool to position aboveground bait stations since areas of 18 to 20% or more moisture often are indicators of potential termite activity in a structure. This capitalizes on our knowledge of termites' ability to relocate free water to dry resources. Hence, it is important to investigate underlying biological and ecological mechanisms that enable termites to exploit their environment, with the ultimate goal of developing new and improved termite detection and control procedures.

OR083

X-Ray CT analysis of nest-gallery development of Incisitermes minor

S. Khoirul Himmi, Tsuyoshi Yoshimura, Yoshiyuki Yanase, Toshiyuki Torigoe, Masao Oya

The biology of the drywood termite, *Incisitermes minor* (Hagen) is poorly understood. To date no published data are available regarding in situ nest-gallery development of *I. minor*. Three naturally infested spruce (*Picea sitchensis* Bong. Carriere) timbers were biannually analyzed by X-ray computer tomography. Two timbers were infested by five pairing reproductives from nuptial flight. Other timber was infested by a group of individuals from outside foraging. CT images revealed that *I. minor* reproductives showed anatomical selectivity in nest-founding activity as well as nest-development, for instance, excavating the chambers in the early wood of sapwood. Structure of initial chambers varied adjusting to anatomical texture of the wood, which resembles a pear-shape and a cashew nut-shape. The first six months CT analyses indicated two kinds of strategies to initiate a new colony; two pairing reproductives distributed the energy to brood and slowed down in foraging simultaneously and other three concentrated in foraging. The pairing reproductives showed no hibernation period and continuously excavated the galleries in the first six months. The nest-gallery was excavated cavernously in a particular annual growth ring where the breeding chamber was established. All the chambers showed significant accretion in volume and structure. After one year, one pairing reproductive showed no brooding activity, while the other four had 2 - 5 new colony members. The infestation by a group of individuals isolated from its natal nest resulted in showing the different nest development pattern with that of the nuptial flight infestation. The group established the first chamber, excavated transversely in several growth rings. After one year, nest-gallery systems were established which consisted of seven chambers with extensive galleries across both sapwood and heartwood. Young colony members were encountered, which indicated the emergence of supplementary reproductives.

OR084

Change in an urban ant community after 87 years.

Andrew Suarez, Andrea Walker, May Berenbaum

Quantifying the long-term impacts of urbanization on taxa requires historic records and long-term surveys. In the mid-1920s, Dr. Marion R. Smith conducted a 3-year survey of urban ants in Urbana, Illinois. He reported species diversity, relative abundance, and the economic impact of control measures. We replicated this study in 2012-2013 to examine how the ant fauna and measures used for their control have changed over this 87-year time frame. We complemented visual surveys with pitfall traps and leaf litter samples to characterize the urban ant fauna from which house-infesting ants were being drawn. In addition to urban sampling, we used similar methods to sample three remnant forest fragments in Champaign County. The forest fragments and urban site were all once part of a contiguous forest (known as The Big Grove). Our surveys revealed long-term trends in the incidence and abundance of ants that persist in urban environments and the relative economic impact of these ants on households.

OR085

3D Distribution of drywood termites in structures for southern California

Vernard Lewis

Approximately one thousand wooden frame apartments were visually inspected for drywood termite (*Incisitermes minor* (Hagen): Blattodea, Kalotermitdae) and the extent of their infestations were determined using termite feeding detection devices. The 40 year old site on 9-hectares includes two three-story structures each containing about 150 apartments enclosing a large central courtyard, and surrounded by approximately 28 two-story structures containing about 6 to 20 each (total apartments, 981). All top-floor apartments (approximately 336) have open-beam ceilings with beams extending as rafters to the outside of the buildings. Each apartment also has an outdoor wooden deck. A four person, in-house pest control service used acoustic emission devices to verify the active or non-active status of all infestations found inside each apartment and for all exterior attached wooden decks. It took 2 years to inspect, monitor, map out and locally treat all infestations discovered. The work was completed in 2003. The analyses of these data allows for a snap-shot-in-time view of the distribution of infestations at the site. Overall the distribution of infestations was clumped, occurring mostly on the upper floors and exterior decks. Further analyses of the distribution of infestations suggest an orientation pattern concentrated on the eastern side of the complex. This pattern suggests the possibility of wind aided movement of swamers and incipient colony formation starting at the top floor exterior decks and through the years, moving progressive down the buildings. Since *I. minor* is a day time swarmer during late summer and fall for Southern California, the distribution pattern suggest 'Santa Ana' wind conditions (strong off shore direction) may explain this phenomenon. The orientation of drywood termites to external stimuli from previously lab and field investigations will also be discussed and compared to this new finding.

OR086

Invasion genetics of two termite species: sources and breeding structure

Edward Vargo, Claudia Husseneder, Elfie Perdereau, Franck Dedeine, Simon Dupont, Anne-Genevieve Bagneres

Compared to ants, there are surprisingly few truly invasive termite species. Two of the most successful and destructive termite invaders are the subterranean termites *Reticulitermes flavipes* and *Coptotermes formosanus*. We conducted phylogeographic studies using nuclear microsatellite markers and mitochondrial DNA sequence data to determine potential source populations for these species as well as patterns of spread in introduced areas. We also characterized colony breeding structure in native and introduced populations to determine whether their invasion success is associated with shifts in breeding structure in introduced populations toward more spatially expansive colonies with greater numbers of reproductives, as seen in many invasive ants. In the case of *R. flavipes*, a native of North America, our results indicate that introduced populations in Europe and Chile most likely originated from the vicinity of New Orleans. Colony breeding structure in introduced populations is similar to those in the putative source region, with a high proportion of colonies headed by inbreeding neotenics, but the numbers of neotenics appears greater in the introduced range and this appears to be associated with larger colonies. In the case of *C. formosanus*, native to China, introduced populations in Japan and the USA appear to be unrelated and likely arose through separate introduction events. Our data suggest there were multiple introductions from China to Hawaii, followed by at least two secondary introductions from Hawaii to the mainland USA. So far we have no strong evidence pointing toward specific source populations in China, although evidence favors a southern China origin. Colony breeding structure in this species is highly variable and introduced populations show no pattern of having greater numbers of reproductives. Thus, a shift to larger colonies with more reproductives may have played a role in the invasion success of *R. flavipes* but not *C. formosanus*.

OR087

Comparison of colony dynamics in native and invasive ant species

Gloria Luque, Franck Courchamp

The ability to achieve big colony sizes has been attributed as an important characteristic of invasive social insects. Increasing colony size improves colony defense, foraging success, and productivity of new workers and reproductives. However, invasive social insects are often highly polygynous and both the number of workers and queens determine colony size. Differential effects of queens and workers on colony survival and productivity have been demonstrated in the Argentine ant. These may lead to positive feedbacks between worker and queen abundance that contribute to the large colony sizes of invasive ants. Native ants may show biological resistance to the spread of invasive ants. A native ant, *Tapinoma nigerrimum*, shows the potential to outcompete the Argentine ant under certain colony sizes whereby this potential depends on its ability to achieve colony sizes large enough relative to those of the Argentine ant. We explored the colony dynamics of these two species experimentally. While varying the initial number of queens and workers, we measured individual survival and productivity at different time periods over two months. Overall queen and worker survival was higher in colonies of Argentine ant. Patterns did not vary over the different time period measured. The positive effect of the number of queens found in the Argentine ant was not evident in *T. nigerrimum*, suggesting less potential positive feedbacks that encourage large colonies.

OR088
Argentine ants prefer semi-natural sites over urban sites
Merav Vonshak

Urban ecosystems are becoming more common, converting countryside and natural ecosystems into urban environment, hostile to many organisms, but appealing to others. We focused on the fragile boundaries between urban and natural ecosystems and how native and invasive ants are distributed between them. In the suburbs of the San Francisco Bay Area, we set a nonlinear gradient of 12 sites, from a well-protected nature reserve to the business district of Palo Alto. We monitored ant communities for a year, studied ants' foraging behavior, and compared food webs by analyzing ants' nitrogen and carbon stable isotopes. We found a bimodal distribution of ant species richness, with peaks at both natural and urban sites. In the natural sites we found only native species, while in the urban sites we found both native and invasive species. We found only two species in the semi-natural sites, the native winter ant (*Prenolepis imparis*) and the invasive Argentine ant (*Linepithema humile*). Interestingly, ant abundance showed the opposite trend, with highest abundance at semi-natural sites. We think that competition with other invasive species, in addition to some native species, was keeping *L. humile* abundance low in the urban sites. Distance from buildings was found to be the most important human related environmental factor affecting ants' distribution. When comparing foraging characteristics in field conditions, we found that *L. humile* scored highest on every parameter. The species' remarkable foraging abilities are probably one of the factors making it such a successful invader. Stable isotope analyses revealed that dietary overlap in food preferences was much higher in urban and semi-natural sites, compared with natural sites, were the species diet was more diverse. Moreover, *P. imparis*'s diet was found to highly overlap with *L. humile*'s diet. This is interesting, as they coexist in many habitats, in spite of the strong competition.

OR089
Thelytoky in honeybee invaders
Ros Gloag, Madeleine Beekman, Ben Oldroyd

Some social insects are highly invasive, quickly establishing as pests in new locations where they threaten agricultural services and the environment. What makes these social insects such successful invaders? We investigate the role of thelytoky in a recent social insect invasion: the Asian Hive Bee (*Apis cerana*) to Australia. Thelytoky is a form of asexual reproduction in which unmated queens or workers produce daughters. It occurs in some ants and honeybees, including *A. cerana*. Thelytoky might increase the invasive potential of a species because it permits the species to overcome the problem of locating mates in initially low-density populations. *A. cerana*'s introduction into Australia presents an excellent opportunity to assess the role of thelytoky in a social insect invasion. Samples of queens and workers were collected throughout the invasion as part of the Biosecurity response, beginning with the first observed swarms in Cairns in 2007 to the present day. We genotype these samples to determine if there is evidence of past thelytoky either by queens or by workers. This allows us to generate a timeline of the reproductive strategies used by *A. cerana* over its past 5 years of rapid population growth. In addition, we assess the number of source colonies from which the invasion spread, the degree of inbreeding in the current population, and the heterozygosity of the sex locus. Understanding the role of thelytoky in this honeybee invader may help in the prevention and management of future social insect invasions.

OR090

Unique reproduction system of invasive ants avoids genetic bottlenecks

Misato Miyakawa, Alexander Mikheyev

In the Hymenoptera, sex is usually determined by a single locus. Heterozygotes are female and hemizygotes are male. Inbreeding leads to homozygosity at the sex determining and the production of diploid males, which are typically sterile, and act as a major burden for the colony. Hymenoptera employ a range of strategies such as outbreeding, or gender-specific reproduction to avoid diploid male production. Recently, an extraordinary reproduction system has been reported in the ants *Wasmannia auropunctata*, *Vollenhovia emeryi*, and *Paratrechina longicornis*. In this system queens and males are produced clonally, receiving only maternal and paternal genomes, respectively. Workers are produced sexually, but are sterile. Thus, gene pools are separated between sexes. Because all three of these species employing this strategy are invasive, it may have some strategic advantage, namely to avoid inbreeding during low population sizes at the start of an invasion (Pearcy et al. 2011, Proc. R. Soc. B). However, the existence of clonal males has never been documented in these species, so the extent to which inbreeding will affect their production remains unknown. Using the facultatively clonal species *V. emeryi*, we crossed sexually produced queens (with both parental genomes) with their brothers, with which the queens shared half their genomes. 25 to 30% of the resulting offspring were diploid males. Ordinarily, haploid males are produced only in autumn in nature and lab. By contrast, we observed continuous diploid male production from March to December, suggesting a high cost of inbreeding in this species. The significant deviation from a 50:50 Mendelian ratio it is clear that at least two loci underlie sexual development. Using FASSST ddRAD tagging, we compared genomes of diploid males and their full sisters in order to identify regions containing gender-determining elements. These data should help to narrow the field of candidate genes related to sex determination.

OR091

Cricket community acoustics: a new tool to detect invasive ants.

Jeremy Anso, Amandine Gasc, Laure Desutter-Grancolas, Eric Vidal, Herve Jourdan

New Caledonia archipelago, as a biodiversity hotspot of high endemism, is highly threatened by biological invasions. Among invasives, ants are recognised as major threats for New Caledonian fauna and flora, local agriculture and population. Three main invasive ants are established in the archipelago: *Wasmannia auropunctata*, *Pheidole megacephala* and *Anoplolepis gracilipes*. In this context, early detection is important to promote regulation, control and eradication in order to promote conservation of ecosystems with high biodiversity values. Here, we investigate the impact of 2 invasive ants (*W. auropunctata* and *A. gracilipes*), on the structure and composition of cricket fauna, which are recognized as a major component of forest floors, according to their richness and high abundance all year round. Their diversity and high level of endemism suggest they may be good candidate to be used in community assessment. Crickets also produce consistent sounds through the environment, as males call to attract females at long range. They contribute greatly to acoustic environments. Then acoustic environments can be related, through analysis of sound signal complexity, to the richness of the community and then to perturbation such as ones linked to ant invasions. We investigate how meta-acoustic can be used as a non invasive, innovative, and efficient method to identify biological invasions from ants, to manage natural and invasive free reserves, or to assist traditional methods in conservation and management of endangered ecosystems. We tested its efficiency in the southern part of New Caledonia, contrasting different biotopes invaded or not by one of the three invasive ants found in the area. Our results already demonstrate that crickets communities modifications are efficient biomarkers to detect invasive ants, especially for *W. auropunctata*, as the ants modify the composition and profile of cricket communities. Crickets may be used as an innovative tool to early detect biological invasion, using meta-acoustics.

OR092

Isotopic analysis of Solenopsis geminata *diets in invaded Indonesian savanna.*

Rebecca Sandidge

The Tropical Fire Ant (TFA), *Solenopsis geminata*, is considered an invasive pest throughout much of the global tropics, however very few studies have been conducted on TFA biology. Like most invasive ants, the TFA is omnivorous and attains large colony sizes. Among the fire ants, TFAs display a unique major worker caste with physical adaptations for seed consumption. In order to better understand the process of TFA invasion we examined colonies utilize food resources across habitat boundaries. Sites were located on small, isolated islands dominated by native tropical savanna in southeastern Indonesia. Behavioral assays and stable isotope analysis were used to describe TFA dietary differences between villages and disturbed savannas, two commonly invaded habitats. Environmental data and foraging observations were used along with analysis of C13, N15, and S34 isotopic ratios to clarify major food resources and trophic position of the TFA. The trophic position of the TFA is described relative to co-occurring species ranging from herbivores (Acrididae) to predators (Lycosidae). Results show that adaptations for seed consumption may play an important role in the distribution and invasion biology of the TFA. We show that the TFA does not rely on grass seed availability; colonies are able to shift from animal-based village food waste resources to C4 plant-based resources during range expansion. An isotopic signal from nutrients present in village waste declines with distance from development boundaries without a corresponding decline in TFA abundance in disturbed savanna. However, land use practices influencing the abundance of favored grass species and availability of these grasses may limit the spread of the TFA beyond disturbed grasslands into native savanna.

OR093

Carbohydrate availability correlates with yellow crazy ant abundance and trophic position

Lori Lach, Benjamin D. Hoffmann, Melinda L. Moir

Recent studies have elucidated the importance of carbohydrates for colony growth of invasive ants. The yellow crazy ant (*Anoplolepis gracilipes*) has invaded many tropical islands as well as parts of mainland Australia and poses a threat to native invertebrates when it reaches high abundance. We hypothesized that if carbohydrates are driving high abundances of yellow crazy ant, yellow crazy ant abundance should be 1) positively correlated with carbohydrate availability in the environment, and 2) negatively correlated with yellow crazy ant trophic position. We tested our hypotheses with stable isotope analyses and field measurements of carbohydrate resources (tended honeydew producing insects, extrafloral and floral nectaries) in maquis vegetation in New Caledonia and savanna in northern Australia. Our data supported both hypotheses. In both locations, we found a positive correlation ($p<0.05$) between the abundance of tended honeydew-producing insects and the abundance of yellow crazy ant at baits. In northern Australia where acacias with extrafloral nectaries are common, the number of extrafloral nectaries correlated positively ($p<0.05$) with yellow crazy ant nest density, abundance of yellow crazy ants at baits, and card counts. Our second hypothesis was supported by a negative correlation ($p<0.05$) between mean trophic position and the abundance of yellow crazy ants at baits in both locations. This is the first study to link carbohydrate availability, trophic position, and invasive ant abundance in field conditions, and it provides further evidence of the importance of carbohydrate availability in ant invasions. However, the potential for effects of the more predacious, less abundant populations of yellow crazy ants on invertebrates should not be ignored.

OR094

Bacteria diversity and virus detection in the invasive Argentine ant

Alexandra Sebastien, Phil Lester, Monica Gruber, Richard Hall, Jing Wang, Nicole Moore

Argentine ant (*Linepithema humile*), originally from South America, can now be found worldwide in Mediterranean like environments, threatening native fauna and flora. One key of the Argentine ant's invasive success is the high density colonies reach in their new environments like it is the case in New Zealand. However unexplained colony collapses have been reported in well-known invaded sites in New Zealand. As some pathogens and viruses have previously been shown to have dramatic effects on populations, the role of infections in the population decline of Argentine ant in New Zealand was investigated. For the first step of this study, samples were collected in two cities with a high density of ants and two cities with a low density in 2011 and 2012. Using universal 16S primers and 454 sequencing, bacteria diversity was shown to be dominated by beneficial and non-pathogenic bacteria from the phylum Proteobacteria - including the endosymbion *Rickettsia-*, and the phylum Firmicutes - mostly gut bacteria such as the acid lactic bacteria *Lactobasillus* and *Pedioccocus*. Bacteria diversity did not to differ between cities or years. Preliminary results from Illumina sequencing indicated the presence of *Solenopsis invicta* virus -2 (SINV-2), bee Kashmir virus and Israeli acute paralysis bee virus, three viruses known for their involvement in ant and bee population drastic decline. Further sampling and specific targeting of those viruses will confirm their presence throughout New Zealand which might explain at length the observed population collapse in Argentine ant. The second step of this study will later involve the infection with chosen bacteria / virus and treatment with antibiotics of colonies in the laboratory to characterize the evolution of the colony fitness throughout time. Characterizing the bacteria diversity and access the presence of viruses in Argentine ant might lead to a better understanding of invasive ants' population fluctuations.

OR095

Evolutionary processes in invasive and non-invasive ant supercolonies

Heikki Helanterä

Supercolonies have evolved independently in several ant lineages. While supercolonial lifestyle is usually associated with invasive ants, large supercolonies exist also in non-invasive groups such as *Formica* wood ants. Both native and invasive supercolonies are predicted to be exposed to evolutionary consequences of extremely low kinship among nestmates, namely evolution of selfish traits, and mutation accumulation due to lack of indirect selection on worker traits. The actual threat of such possible evolutionary meltdowns, and timescales involved remain unknown. In this talk, I will draw on behavioural, population genetic and genomic analyses in order to explore what studies of non-invasive supercolonies can tell us about the evolutionary future of invasive supercolonies.

OR096

The paradox of supercolonies in ant invasions

Jes Søe Pedersen, Luigi Pontieri, Dora B Huszár

The evolution of advanced eusocial insect colonies is based on kin-selected benefits from helping close relatives. Consequently, most ant, bee, wasp, and termite colonies are well-defined families competing against other such families. However, some ants seem to have secondarily abandoned the inclusive fitness laws of evolution to form large networks of interconnected nests whose individuals are unrelated but fail to show territorial aggression. This can lead to the emergence of massive supercolonies that may extend over hundreds or even thousands of kilometres as seen in the most successful and problematic species of invasive ants. The iconic pest species is the Argentine ant *Linepithema humile* that I will use to demonstrate the life-history characteristics that appear to define supercolony-forming ants, and to argue that supercoloniality is a distinct category of social organisation. However, recent studies in our group on other important pest species indicate that supercolonies may not always be as standardised as previously thought. The Pharaoh's ant *Monomorium pharaonis* has genetically extremely divergent colonies, even over a small spatial scale, suggesting that this species has efficient mechanisms to keep lineages of colonies separate. Nevertheless, after a brief initial aggression period, most colonies fuse readily in the laboratory. In contrast, the common red ant *Myrmica rubra* often forms populations with a mix of different colony types where local supercolonies may depend on recruiting new queens from isolated family-based colonies, and these may in turn expand to new supercolonies. These complexities suggest that there may be more than a single evolutionary route to the paradoxical state of supercoloniality. Importantly, the existence of alternative routes implies that the constraints from limited genetic diversity and dispersal faced by the 'standard' supercolony can be overcome, likely leading to increased invasiveness of supercolonial species.

OR097

Pathogens of common wasps in their native and invaded range.

Phil Lester

The common wasp (*Vespula vulgaris*) is native to Eurasia but has invaded and attained high densities in Argentina, Australia and New Zealand. The first aim in this study was to define the native range of invasive populations. The second aim was to examine pathogens and parasites of these wasps within the introduced and specific native ranges. Using variation in two mitochondrial genes, the likely home range of invasive populations of these wasps was found to be the United Kingdom, Ireland or nearby countries in Western Europe. Populations within the invaded range appear to have arisen from a small number of introductions, limiting the likelihood of the co-introduction of wasp parasite and pathogens. Using proteomic methods, the presence and diversity of pathogens and parasites was examined in wasps from the native (Belgium and England) and invaded range (New Zealand and Argentina). Contrary to expectations, no evidence of 'enemy release' was observed, as the number of pathogen taxa observed in the native range was similar in both the introduced and native range. The pathogens observed in wasps were similar to a variety of taxa found in honeybees. Given the importance of *Nosema* spp. as pathogens in honeybees, wasps were examined for microsporidian infection prevalence. Rates of microsporidian infection were similar in the native and introduced ranges. Wasps appear to carry *Nosema* spp. observed in honeybees and bumblebees. Perhaps many of the pathogens and parasites affecting wasps are 'generalist' species affecting bees and wasps, which has major implications for wasp and bee disease management.

OR098

Foraging in invasive species: evading the limitations of polydomy

Elva Robinson

Unicoloniality is characteristic of invasive ant species: in these species, colonies spread across many socially connected nests. Forming new, connected nests provides a low risk way to enter new territory, but splitting between multiple nests (polydomy) also impacts the way colonies forage. Polydomous colonies must trade-off the number of nests with the size of each nest. We developed a model of how colony success relates to the food environment under different forms of social organisation (from monodomy to extreme polydomy). The model predicts a potential cost of polydomy: constraints on exploitation of large resources. This is because in highly polydomous colonies, there is a limited pool of workers in each nest from which foragers can recruit. Empirically testing the predictions of this model, we show two ways in which successful invasive species can use their polydomous nest networks to evade this constraint. Firstly, we use data from radio-tagged foraging Pharaoh's ants (*Monomorium pharaonis*) to show very rapid cross-communication between nests during active foraging. Traditionally, in studies of ant foraging, we think of ants passing back and forth between their nest and the food. Our results challenge this 'linear' idea of ant foraging, suggesting that foraging and the redistribution of information or resources between nests can be concurrent processes. Secondly, the formation of a polydomous trail network is flexible and responsive to the environment. We show that yellow crazy ants (*Anoplolepis gracilipes*) spread into new areas by building networks centred around sources of food. These mechanisms allow invasive species to evade the limitations of the nest-number nest-size trade-off by forming large numbers of small nests, but maintaining rapid and effective communication between neighbouring nests, and siting them responsively in relation to the environment. Understanding the behaviours involved in the success of invasive species is essential for informing their control.

OR099

Synthetic pheromone reduces the competitive ability of an invasive ant

Fabian L. Westermann, David M. Suckling, Philip J.

The globally distributed Argentine ant *Linepithema humile* has been reported to break the trade-off between interference and exploitative competition, achieve high population densities, and overpower nests of many endemic ant species. One of the factors contributing to the success of Argentine ants is their effective recruitment system, which allows them to quickly divert workers from depleted to newly discovered resources. We tested if the dominance and competitive ability of this invasive species could be reduced, by the use ofa synthetic trail pheromone. Argentine ant foraging success was reduced with increasing pheromone concentration, which benefited competing resident species and allowed them to increase foraging. The mechanism for the observed increase in resource acquisition of resident species appears to be a decrease in aggressive behaviour displayed by the Argentine ant, which created an opportunity for other resident species to forage more successfully. Our demonstration of modified species interactions with increasing trail pheromone concentrations is the first known case of reduced competitive dominance under a pheromone treatment in ants. Reducing the competitive ability of an invader could have important applications for invasive species management and enable native species to reclaim native habitats.

OR100

Pollinator harassment by invasive ants alters floral utilization by bees

Erin Wilson Rankin, C. Sheena Sidhu

Harassment of pollinators at floral resources influences both plants and their pollinators. In dry or drought conditions, pollinators may be particularly sensitive to disruption of the plant-pollinator mutualism because of limited resource availability. Here, we examined pollinator harassment by invasive ants on blooming succulents (*Aloe spp*) under drought conditions in southern California. In such dry areas, aloe and other blooming succulents serve as valuable resources, especially early in the year when few other plants are blooming. The Argentine ant (*Linepithema humile*) is one of the most detrimental invasive ants and is known to disrupt pollination services. While invasion impacts on plant-pollinator mutualisms have received increasing attention in recent years, our understanding of their impact on the pollinators themselves is incomplete. The presence of these invasive ants on aloe flowers led to an alteration of honeybee (*Apis mellifera*) foraging behavior and a decrease in resource collection. To quantify the effects of Argentine ants on bee foraging behavior, we manipulated ant density in aloe flowers and observed subsequent bee foraging behavior and resource collection. Ant occupation within the flower had strong negative effects on the number of bees landing on and handling the flower. Interestingly, ant activity outside the flower had no effect on bee visitation or resource collection. The duration of floral visits increased three-fold when ants were excluded. Furthermore, by isolating the chemical and visual cues of foraging ants and explicitly manipulating nectar levels, we quantified the contributions of exploitative and interference competition to harassment. Further research is needed to assess how resource limitation due to pollinator harassment may interact with drought and drought-induced stresses as these factors have important implications for bee health.

OR101

Some like it sweet: floral nectar fuels Argentine ant success

Theresa Wossler, Natasha Mothapo

Invasive ants opportunistically form mutualistic associations with plants and arthropods that produce carbohydrate exudates such as nectar and honeydew. Argentine ants, *Linepithema humile*, shows ecological plasticity in resource use and behaviour which is thought to enhance its invasion success. The ability of *L. humile*, to utilise temporarily available floral nectar was compared to that of the dominant native ant, *Anoplolepis custodiens*, within the Cape Floristic Region (CFR), South Africa. The abundance and species composition of ground foraging ants as well as floral arthropod visitors in inflorescences of two Proteacea species were assessed, as well as comparing the foraging activity of the two ant species on the Proteacea nectar, during different flowering periods. Elemental Stable-isotope analysis of Carbon and Nitrogen, as well as the ratio of Carbon to Nitrogen (C:N), was used to investigate patterns of resource assimilation in *L. humile* and *A. custodiens*, and how they respond to increased carbohydrate resource availability. Compared to *A. custodiens*, *L. humile* showed strong dietary flexibility, showing a trophic position similar to that of herbivores during the flowering period, and a higher trophic position when floral nectar was depleted. *Linepithema humile* presence altered the composition of floral visitors by 29% compared to the dominant native ant *A. custodiens*, and negatively affected species abundance and composition of epigaeic native ant species. The CFR is rich in floral resources, providing ample opportunity for *L. humile* to spread further in this biodiversity hotspot. Through the effective utilisation of this fluctuating and temporarily available carbohydrate resource abundant in the CFR, coupled with the lack of competition from native ant species, *L. humile* has the potential to expand into more pristine areas, which may exacerbate its negative impacts in this biodiversity hotspot.

OR102

Population-level differentiation between Yellow Crazy Ant supercolonies in South-East Asia

Jochen Drescher, Heike Feldhaar, Nico Bluthgen, Thomas Schmitt, Damayanti Buchori, Stefan Scheu

The Yellow Crazy Ant (YCA) *Anoplolepis gracilipes* ranks among the most destructive social insect invaders in South-East Asia and the Indopacific. It is believed that their ability to form vast polydomous, polygynous supercolonies is the key to their ecological success. This is particularly true in Arnhem Land, NE-Australia, where a single supercolony spans up to 80km across, covering more than 15.000km² in total (Gruber et al. 2011). In Sabah, North-East Borneo, however, YCA population structure is very different. Here, YCA supercolonies are no larger than 300m across and many supercolonies of varying sizes compete for resources within the same population. Using data from two independent populations of supercolonies, we will demonstrate that YCA supercolonies in NE-Borneo are genetically and chemically (Cuticular Hydrocarbons) differentiated to such an extent that it suggests lack of gene flow between them. We argue that positive feedback between behavioral, genetic and chemical differentiation will further intensify intercolonial segregation, possibly leading to reproductive isolation between different YCA supercolonies and thus, speciation. Preliminary experiments using a limited set of laboratory colonies suggest a reproductive barrier between distantly related YCA supercolonies. It is currently unclear whether this is due to worker-policing or the inability of queens mated with males from foreign supercolonies to produce vital offspring. We aim at further studying the potential reproductive barrier between YCA supercolonies along a gradient of genetic similarity by cross-breeding males and queens from variably related supercolonies. To identify suitable supercolonies, we performed a population genetic analysis of YCA populations in Jambi, Central Sumatra, sampling from over 30 supercolonies that are as far as 100km apart. We thus present first genetic data on that population and discuss the experimental design with which we intend to identify the degree of genetic distance at which males and queens from different YCA supercolonies are reproductively isolated.

OR103

Sugar preference and the importance of viscosity in Apis cerana*, the Asian honeybee*

David Guez

The Asian honeybee, *Apis cerana java* (AHB) is a tropical bee species that recently breached quarantine in northern Australia. It is classed as an invasive pest with the potential to detrimentally impact upon Australian beekeeping and other agricultural industries. New research is investigating the behaviour and ecotoxicology of AHB with the aim of developing and optimising trapping stations to impede colony spread. Whilst sugar feeding preferences in the temperate species *Apis mellifera* have been extensively investigated, preferences in AHB are much less known. This information is crucial to the design of an efficient AHB trapping station. Here, we investigate the sugar solution preferences of AHB using the sugars most common found in nectar. We tested glucose and fructose (hexose sugars), sucrose (a disaccharide sugar) and various mixtures. We also used manipulated solution viscosity through the addition of tylose. We found that, as for *Apis mellifera*, AHB displayed a strong preference for equicaloric solutions of sucrose over hexose solutions, with both hexoses equally attractive. However, whilst *Apis mellifera* displayed a strong preference for glucose-fructose-sucrose over an equicaloric sucrose solution, AHB displayed no preference between equicaloric solutions of sucrose, glucose-fructose and glucose-fructose-sucrose. In addition, when sucrose concentrations were fixed and viscosity was manipulated using tylose, AHB showed a preference for more viscous solutions than was predicted by a recently published model of nectar-feeding. We discuss these results in light of this model and the ecology of AHB.

OR104

Signaling pathway integration in honeybee, Apis mellifera*, caste development*

Klaus Hartfelder, Gustavo Tiberio, Mario Cervoni, Douglas Santos

Caste polyphenism is the morphological manifestation of the highly efficient and evolutionarily successful division of labor in social insects, but understanding how a single genotype can give rise to two very distinct phenotypes in response to environmental signals is a challenging task for developmental biologists. In the honeybee , queen larvae receive copious amounts of royal jelly throughout larval development, while workers are fed less frequently and initially receive a diet less rich in sugar. This triggers differential responses in several signaling pathways, including juvenile hormone (JH), EGF receptor, insulin/insulin-like signaling and the TOR pathway. All of these are major developmental regulators in insects and conserved response systems to nutritional status. The JH titer is much elevated in queen larvae and plays a key role in inhibiting programmed cell death in the larval ovary. Similarly, TOR signaling is also an important factor for queen development. Unexpectedly, insulin signaling has now been shown to be of only minor importance for queen development, even though the sugar concentrations in royal jelly is higher in worker jelly. This has led us to investigate the role of the role of the respiratory metabolism in caste development, revealing overexpression of hypoxia core genes in worker larvae, thus indicating an endogenous hypoxia condition in these. With all these signaling pathway data in mind, and seeing that their gene expression patterns in bee larvae diverge from current views on their function in Drosophila and other model organisms, we are now mapping the integration of these pathways to reveal molecular mechanisms underlying caste morphology. Financial support: FAPESP (Brazil)

OR105

The evolutionary genetic basis of social regulation of caste development

Timothy Linksvayer

Increasingly, researchers have used transcriptomic approaches to identify genes and gene networks affecting the caste developmental trajectory of social insect larvae. These results have largely been interpreted as supporting evolutionary genetic scenarios (e.g., the reproductive groundplan and gene toolkit hypotheses) whereby highly conserved gene networks found in solitary ancestors are rearranged and decoupled to produce the alternative reproductive castes that form the basis of insect societies. Intuitively, these conserved gene networks must play important roles in the expression of phenotypic plasticity for reproductive traits. However, these scenarios do not help to explain how development is increasingly socially controlled in derived insect societies. Because of strict social regulation of development and caste expression, the social insect developmental program includes genes expressed in social partners, as well as genes expressed in developing larvae. I will discuss recent results from honeybees and ants highlighting how socially acting genes affect the genetic basis and evolution of social insect caste.

OR106

Evolution of bigger helpers in ants: stronger head and prothorax

Roberto A Keller, Abdou Fofana, Christian Peeters

Ants are a remarkable example of phenotypic plasticity at the service of behavior, exhibiting morphological castes that are well-adapted to specific tasks within the colony. Much of caste variation in ants involves allometric differences in structures that have high functional significance. For example, the large head capsule of major workers and soldiers accommodates massive mandibular muscles that, together with mandibles having specialized shapes, convert the head into a powerful tool. It is well known that head size scales with positive allometry relative to body size: bigger helpers have proportionally much larger heads, thus giving more power to the mandibles. A recent morphological analysis (http://dx.doi.org/10.7554/eLife.01539) has shown that an enlarged prothorax is also important in the use of the head-as-tool, since it houses specialized musculature that supports the head and controls its movements. A corollary of this functional coupling is that the prothorax should scale in size following head size rather than overall body size. To test this prediction we analyzed the static allometry between the head, pronotum and body size in various ant species, belonging to several genera and subfamilies, having workers highly variable in size or discrete soldiers. We also performed comparative anatomy of the internal skeletomuscular system of the prothorax. Our results show that the prothorax scales as predicted, and that its specialized function is further enhanced by unique muscle-bearing invaginations of the cuticle present only in the bigger helpers. We discuss the impact that these types of allometries have in the integration of form and function in the context of sociality, and in the evolution of novel phenotypic diversity.

OR107

Wing polyphenism in ants: new insights from the Mystrium *genus*

Julien Behague, Romain Paronnet, Ehab Abouheif, Mathieu Molet

Winged queens and wingless workers in ants represent an extreme example of phenotypic plasticity, the phenomenon by which a single genotype produces various phenotypes in response to environmental cues. Studying the developmental mechanisms responsible for winglessness is important to understand the evolution of the worker caste. For instance, Abouheif et al. (2002) compared the genetic network required for wing development in larvae of several species and they demonstrated that it is conserved across species in winged queens, while it is surprisingly interrupted at different points in workers, suggesting its evolutionary lability. Workers are not the only wingless caste in ants. Other castes such as soldiers and ergatoid queens evolved repeatedly. The aim of our study is to understand how development could produce ergatoid queens during ant evolution. Our hypothesis is that ergatoid queens evolved by recombining traits from the already existing winged queen and worker castes (Molet et al., 2012). To address this issue, we focus on wing polyphenism in two closely related Poneroid species: *Mystrium rogeri* and *Mystrium oberthueri*. While queens are winged in *M. rogeri*, they are ergatoid in *M. oberthueri*. We extensively study the wing genetic network in imaginal discs of developing larvae by combining whole mount in situ hybrizations and immunostainings. This allows us to compare the molecular signatures of ergatoid queens to workers and winged queens. We expect similar molecular signatures between wing discs of ergatoid queens and workers, indicating that parts of the worker developmental program is being recycled and incorporated for the evolution of a new ergatoid queen caste. Abouheif E and Gregory A. Wray, Science 297, 249 (2002) Molet M., Wheeler D. E., Peeters C. American Naturalist (2012)

OR108

Mosaic nature of intercastes and evolutionary implications in ants

Sylvain Londe, Thibaud Monnin, Mathieu Molet

Ant colonies occasionally produce individuals called intercastes that are morphologically highly variable but always intermediate between queens and workers. Qualitative characters such as ocelli number and presence of wing buds suggest that these developmental anomalies are mosaics of queen and worker traits. Because of their rarity, intercastes have been little studied. However, they may be involved in the evolution of novel morphological castes such as ergatoid queens. Indeed, ergatoid queens can also be interpreted as mosaics recombining a queen reproductive apparatus with a worker thorax. In order to test whether intercastes are effectively mosaic phenotypes for quantitative characters, we used morphometric techniques to compare the co-variation between head, pronotum, mesonotum and propodeum in 28 queens, 123 workers and 37 intercastes of *Mystrium rogeri*. In accordance with our hypothesis, we found that these four modules are more independent from one another in intercastes than in queens and workers. Interestingly, our sample of intercastes partially bridges the gap between queen and worker morphologies, resulting in a continuous reaction norm from small workers to large queens, contradicting the classic view of a discrete queen/worker caste polyphenism. The shape of the reaction norm differs among modules, and this may determine the panel of intercaste phenotypes that can be produced. The evolution of ergatoid queens from intercastes is plausible only if some intercastes have behaviors consistent with reproduction. Behavioral tests showed that intercastes are often dominant and that some of them can attract males and mate, thereby demonstrating their reproductive potential. Our results therefore support the hypothesis that intercastes may be at the origin of the evolution of novel castes.

OR109

Evolution of termite defence

Jan Sobotnik, Katerina Kutalova, Thomas Bourguignon

Termite colonies consist of several castes, which, unlike in other social insects, result from distinct ontogenetic pathways. Primitive species reveal very plastic ontogeny, while advanced ones show rigid ontogeny with early and irreversible split between sterile and fertile colony members and task specialization among up to three worker and soldier subcastes. Soldier caste is a synapomorphy of all termites. Particular lineages reveal different defensive strategies with soldiers being classified as biting (crushing, slashing, reaping), phragmotic, snapping (symmetrical or asymmetrical) or ejecting defensive fluids (nasutes, nasutoids). Apart from mandibles, soldiers may possess two major defensive glands (labial and frontal), whose development is usually mutually exclusive. Soldiers also differ in other aspects, like defensive behaviour, size difference compared to workers, or proportion in the colony. Although soldiers are defenders of prime importance, other castes are often involved in colony defence. Workers always fight during conflicts, and their role is especially important in (i) building defensive structures (nests and galleries); (ii) soil-feeding species, which in general have lower soldier proportion and complete soldier caste disappearance occurred in several linages, with workers showing several features typical of soldiers (high aggressiveness, presence of defensive glands); (iii) conspecific conflicts of chemically well-defended species which developed specific auto-detoxification mechanisms. Imagoes defend against predators before all by synchronization of dispersal flights, but many species reveal presence of the frontal gland, which may in some cases exceed in size the frontal gland of soldiers. All above-mentioned characters evidence interesting evolutionary history with many examples of convergent evolution. In this talk I will disentangle termite defensive strategies, place them in their ecological context, and emphasise the evolution morphological novelties.

OR110

Presoldier cuticle contributes to the soldier morphogenesis in termites

Yasuhiro Sugime, Kota Ogawa, Dai Watanabe, Hiroyuki Shimoji, Shigeyuki Koshikawa, Toru Miura

In all termite species, the soldier differentiation occurs through a presoldier stage and the drastic morphogenesis with body-part modifications occurs through the molts into presoldier and into soldier. However, it was considered that the morphological alternation might occur even during the presoldier stage because, during the presoldier stage, termites possess soft bodies covered with whitish or transparent cuticles. Thus, we hypothesized that presoldiers possess elastic cuticles that enable the drastic morphogenesis even after the molt into presoldiers, leading to the formation of soldier-specific morphologies. In this study, using the damp-wood termite *Hodotermopsis sjostedti*, we artificially induced the presoldier molt by applying a juvenile hormone analog and then measured the sizes of 5 body parts after the molt. Results indicated that the head length significantly increased during 7 days after the presoldier molt in contrast to the pseudergate molt (stationary molt). Furthermore, on the 14th day after the presoldier molt, the head length showed a further increase. To elucidate the cuticular characteristics underlying the head elongation after the presoldier molt, we observed head cuticles by scanning electron microscopy, showing the folding structure on the head of newly-molted presoldiers which was then unfolded during 7 days after the molt. Histological observations also indicated that the folding structure was unfolded due to the additional cuticle synthesis during the 7 days. Then, the thickened cuticle was stretched by 14th day after the molt. Our results firstly showed that the soft and elastic cuticle in presoldiers contribute to the head elongation, like body cuticle of holometabolous larvae. This study suggests that, as well as the molts itself, this developmental process during the intermolt stage after the presoldier molt contributes to the formation of soldier-specific exaggerated morphologies.

OR111

Epigenetic maternal effects on caste development in Polistes *wasps*

Jennifer Jandt, Robert Jeanne, John Hermanson, Amy Toth

Caste differences in most social insects result from differences in environmental input, such as nutrition and exposure to pheromones. Recent evidence for *Polistes* wasps suggests that maternal vibrational signaling influences caste development. Early in the season, queens frequently perform antennal drumming on the nest. Larvae subjected to this drumming develop into adults with low, worker-like fat stores. Later in the season, when drumming is rarely performed, larvae develop into gynes (reproductive females), with high levels of fat. What is less understood is the extent to which this maternal drumming behavior influences caste-biased gene expression in the developing larvae, or division of labor among newly emerged adults. To test these effects, we subjected late-season nests, in which natural drumming had ceased, to three experimental treatments: (1) simulated drumming at a constant, low vibrational frequency; (2) vibration events at random frequencies; (3) no vibration (control); all received food ad libitum. We predicted that larvae experiencing vibrations that simulated drumming, but not random-frequency vibrations or no vibration, would exhibit worker-like gene expression related to fat metabolism and heat shock, and develop into adults that performed higher rates of colony-maintenance tasks. Our results confirmed these predictions, suggesting vibrational signals may be an epigenetic factor that can affect caste-related gene expression. This is the first study to show the potent influence of the maternal environment on the development of plasticity in adult behavior, physiology, and gene expression.

OR112

Can social pheromones regulate reproduction in non-social insects?

Alison Camiletti, Graham Thompson

Insect sociality has evolved multiple times, and in each case has presumably involved a modification of regulatory mechanisms already present in solitary ancestors. It is not clear, however, how similar social and non-social taxa remain with respect to the cues and pathways used to regulate personal reproduction. In this talk we present a series of experiments that test how closely social and non-social insects regulate reproduction in response to a common social pheromone. First, we show that honeybee queen mandibular pheromone inhibits virgin *Drosophila* ovaries in much the same way as it typically suppresses worker bee ovaries. That is, flies exposed to pheromone showed a reduction in ovary size, produced fewer eggs, and generated fewer viable offspring, relative to unexposed controls. Fruit flies therefore respond to an interspecific social cue to which they would not normally be exposed. Why a non-social fly responds to a highly social bee's pheromone is not clear, but one possibility is that the social and non-social insects represented here share pathways associated with female reproduction, as predicted by the ‘groundplan' hypothesis of social evolution. Second, we show that the cross-species effect of bee pheromone on fly ‘sterility' depends in part on the fly's genotype. Rover flies are less responsive to ovary-inhibiting pheromone than are sitters, and this differential response parallels the response from forager and nurse subcastes of the bee. Sitter flies, like nurse bees, are apparently more sensitive to queen pheromone, and our results thus extend further the analogy between this well-known bee-fly polymorphism. Beyond implicating the foraging gene in the regulation of reproduction, our comparative experiments suggests a striking degree of functional homology between bee and fly, and highlight the potential for *Drosophila*-based models in ‘socio-evo-devo' research.

OR113

Repeated evolution of a derived feature: insights from complementary sex determination

Martin Beye, Marianne Otte, Vasco Koch, Inga Nissen, Bjorn Schmitt

Complementary sex determination is shared among many social hymenopteran insects and determines femaleness (workers and queens) by heterozygosity and maleness by homo-/ hemizygosity. This mechanism couples the control of sexual development in fertilized and unfertilized eggs (enabling a female biased sex ratio) and the promotion of outbreeding. I will present our findings on the evolutionary trajectory of complementary sex determination in honeybees and some other hymenopteran insects showing a repeated evolution of the complementary sex determination process. In detail I will present our findings on the mechanisms underlying the evolutionary origin of the complementary sex determiner gene and single alleles in honeybees. I will also show studies which link the evolutionary signatures with the molecular functioning of the gene which is regulating the switch between male or worker/queen development. Our comprehensive understanding of this derived feature in hymenopteran insects highlights the limits of comparative genomics and emphasizes the requirement to study the function of homologous genes in different species and major hymenopteran lineages.

OR114

Commitment in social life, sex and symbiosis

Jacobus J. (Koos) Boomsma

Hamilton s rule shows that positive relatedness can induce cooperation, but kin selected behaviours remain conditional as long as relatedness varies. The rule can therefore not explain the evolutionary origins of morphologically differentiated castes that are either unmated for life or destined to permanently rely on distinct helpers for breeding. Such fundamentally novel and irreversible social arrangements appear to have come about by absolute life-time commitments. This logic applies to eusociality (two strictly monogamous parents) and the origins of eukaryote multicellularity (two gametes committed to a single zygote allowing somatic differentiation), and likely also to the origin of the eukaryote cell (obligate commitment between a single mitochondrial clone and a single nucleated host cell). The facultative versions of these novel stages of complexity belong to previous, not the new domains of social evolution that emerged after major evolutionary transitions. We can further extend this logic to evolutionarily derived forms of eusociality. For example, *Megalomyrmex* guest ants function as a mercenary soldier caste for colonies of *Sericomyrmex* host ants because the guest ant colony is life-time committed to a single host colony. Eusocial elaborations such as fungus-farming in ants and termites are also characterized by life-time commitments between a single insect family and a single clone of fungus, which makes these mutualisms unusually robust no matter whether the fungal symbiont is vertically or horizontally acquired.

OR115

Caste determination in eusocial bees: A key role of terpenoids?

Stefan Jarau

Reproductive division of labour is an identifying feature of eusocial insects. It is based on the ability of female larvae to develop into either of two distinct female castes, queens and workers. A key to castes in eusocial insects lies in the regulation of growth and development in the larval stages by colony internal factors, particularly by a modification of the larval food. The food modifications subsequently translate into endocrine signals that control the caste specific developmental patterns. The importance of royal jelly for queen development and, thus, caste determination in honeybees is well known. Information about proximate caste determining mechanisms in the remaining eusocial bees is rare. The only species for which a direct influence of a compound from larval food on caste development has been demonstrated is the stingless bee *Melipona beecheii*. We found that in this species the terpene geraniol, secreted from labial glands of nurse bees, can induce queen development in larvae when mixed to the larval food in brood cells. We recently identified terpenoid compounds from labial gland secretions of another stingless bee species, *Plebeia remota*, as well as in the bumblebee *Bombus terrestris*. These terpenoids are abundant in the gland secretions of nurse bees but almost lacking in foragers, indicating their importance for larval nutrition and, potentially, for caste determination. I hypothesize that the mode of action of terpenoids in queen development is linked to the biosynthesis of juvenile hormone, which is a terpenoid compound, too, and which is produced in higher amounts by queen larvae as compared to worker larvae. Terpenoids are also common in pollen and nectar, the primary food of bee larvae. Thus, caste determining mechanisms in eusocial bees may have their evolutionary origin in phenotypic plasticity during larval development due to variations in terpenoid contents in larval provision.

OR116

Thrips soldiers winged but grounded

Andrew Chaulk, Peterson Coates, Holly Caravan, **Tom Chapman**

Within the gall-inducing thrips of Australia, genus *Kladothrips*, is a single origin of a soldier caste. A subsequent radiation has led to at least seven social species, and two species that are likely to have independently lost the soldier caste. Both losses of soldiers are connected to a shift in the insects' host-plant. A third inferred host shift is correlated with life history changes in a species with soldiers, *K. intermedius*, which might suggest this defensive caste may be in transition. The soldiers of this species have variable wing lengths that overlap with that of the flight-capable dispersers. Our study was designed to assess the possibility that some soldiers in this species retain the ability to disperse by wing. A morphological assessment shows that a proportion of soldiers have body dimensions and wing lengths that fall within the range of dispersers, with males more so than females. However, longer wing length in soldiers (sample of primarily females) did not correspond with a disperser like walking behaviour. Furthermore, histological sections suggest that wing muscles of soldiers are deteriorated or absent, which is similar to what was observed in their foundress mothers. The presence of long wings did not correspond with being flight-capable in this caste. Soldiers are more likely to be deeply, and in their lifetime, irreversibly committed to their helping role. If *K. intermedius* does represent a reemergence of the soldier caste, fighting ability, the development of robust forelimbs, and flight muscle degeneration all evolved rapidly. Lagging slightly behind is the evolution of microptery.

OR117

Matedness does not matter in queenship formation in Polistes snelleni*.*

Kazuhisa Yamasaki, Toshiharu Akino, Takahumi Mizuno, Koji Tsuchida

In colonies of primitively eusocial wasps, some dominant workers become successive queens and inherit queenship after the death of the foundress queens. Although workers in many species do not mate, workers of *Polistes snelleni* are capable of mating and female production. We experimentally removed foundress queens from colonies of *P. snelleni* to evaluate the effects of queen loss on the dominant-subordinate relationships among the remaining workers and the productivity of colonies in this species. We also analysed wasps' cuticular hydrocarbons (CHCs), and compared the profiles among the females. The foundress queens were the sole egg layers in almost all of the queen-right colonies. The frequency of dominance behaviors among the wasps in the queen-right colonies was significantly less than that in the orphan colonies. The frequency of dominance behaviors in the successive queens after queen removal was significantly more than in the foundress queens before her removing. Multiple daughter workers had developed ovaries, including the successive queens in 66.7% (10/15) of the orphan colonies after the foundress queen removal. The orphan colonies produced significantly more cells and eggs than the queen-right colonies. The foundress queens possessed characteristic CHC profiles, and which were different from those of the daughter workers including the successive queens. Our results suggest that the reproductive capacity of the successive queens in the orphan colonies is not lower than that of the foundress queens, and that the productivity of the orphan colonies is maintained, irrespective of potential conflict over direct reproduction among daughter workers. The differences in the frequency of dominance behaviour and the CHC profiles between the foundress and the successive queens suggest that the successive daughter queens are not as totipotent as the foundress queens in physiological aspects. Pre-imaginal caster determination could play a role in a primitively eusocial wasp, *P. snelleni*.

OR118

Impacts of nymph/worker genotypes on termite incipient colony fitness

Osamu Kitade, Kaori Takatsuto

In *Reticulitermes* termites, caste fate of a young larva (whether to become functionally sterile, wingless 'worker' or to become 'nymph' with reproductive opportunities) is strongly affected by the genotypes of a X-linked locus (Hayashi et al., 2007). This Mendelian system predicts that offspring in a normal incipient colony of *Reticulitermes speratus* (Kolbe) founded by primary reproductive pairs has worker-oriented genotype, while that of parthenogenetically produced offspring in a colony founded by a female-female primary pair has nymph-oriented genotype. To understand the function of the gene in the incipient colony and evolutionary basis of the commitment mediated by it, we compared colony traits between the colonies expected to have different offspring genotype ratios: (1) colonies founded by normal alate pairs and female-female pairs, and (2) colonies introduced 100 worker-orineted eggs and 100 nymph-oriented eggs. While almost no nymphs were produced in the normal colonies, 5.8-17.9% of the offspring in female-female colonies became nymphs. Even in the presence of primary pairs, 15-20% of the nymphs were differentiated into neotenic reproductives. Replacements of the primary reproductives by the neotenic reproductives took place significantly higher rates in female-female colonies than in normal colonies. The fatal aggression between primary and neotenic reproductives significantly decreased the colony size and survival rate of female-female colonies. Sexual colonies introduced nymph-oriented eggs exhibited the same trends as female-female colonies. These results indicate that parthenogenesis and production of nymph-genotype individuals considerably decrease the fitness of the incipient colonies through the promotion of intra-colonial conflicts among reproductives. In the normal incipient colonies, the genetic system functions to coerce offspring into becoming workers and increases competitive advantage of the colonies. It possibly enables the large colony size of *Reticulitermes* and other relatively advanced termite taxa with worker caste.

OR119

Ant colony development and transfer of juvenile hormone by trophallaxis

Adria C LeBoeuf, Colin S Brent, Richard Benton, Laurent Keller

Communication is essential in high-functioning animal groups, from social insects to humans. How do ants maintain homeostasis or bring about colony-wide changes without language or top-down control? While ants are traditionally thought to communicate mostly through volatile or secreted pheromones, we hypothesized that trophallaxis ‘mouth-to-mouth liquid transfer’ is also an important means of chemical communication. Given the power of trophallaxis to rapidly distribute liquids throughout the colony, it would provide an excellent means of private information transfer, especially for compounds unstable outside the body, potentially enabling a form of biochemical crowdsourcing. To date, the contents of trophallaxis fluids have not been thoroughly analyzed and thus many function(s) of this striking behavior beyond food sharing may be unappreciated. Using nano-liquid- and gas-chromatography mass spectrometry to analyze the protein and small molecule contents of fluid passed during trophallaxis in *Camponotus floridanus* workers, we have identified a number of endogenous ant proteins implicated in regulation of juvenile hormone levels, as well as juvenile hormone (JH) itself. We found that workers have significant levels of JH in their trophallaxis fluid, comparable to JH levels found in their hemolymph. Using radioactive JH, we have detected the passage of JH between individual workers and from workers to larvae. We are currently testing the effect of JH on larval fate by feeding queenless groups of workers food supplemented with JH or methoprene, a JH analog, while workers raise larvae. Preliminary results indicate that the ants that develop from these larvae are larger than those developing in groups fed food without JH supplement. These observations suggest that the JH content of the workers' trophallaxis fluids may alter the caste fate and development of the colony's brood.

OR120

Social dominance modifies ontogeny of behavioural rhythm in queenless ant

Yasukazu Okada, Taro Fuchikawa, Takahisa Miyatake, Kazuki Tsuji

Social insects' caste system is quite diverse. In this study, castes are analyzed from the viewpoint of chronobiological polytheism. Typically, reproducing queen exhibits behavioral arrhythmicity for around-the-clock egg laying, whereas foraging workers exhibit clear circadian rhythmicity for temporal foraging. In the queenless ant genus *Diacamma*, colony members lack morphological castes; hence, reproductive differentiation occurs because of dominance hierarchy formation. Specialized dominance interaction, termed 'gemmae-mutilation', provides an precious opportunity for investigating the effect of social dominance on rhythmic ontogeny. In this study, the measurement of individual rhythms showed that *Diacamma* ants exhibited clear circadian rhythm at eclosion, but that it is diminished by gemmae mutilation. Moreover, unlike highly eusocial species, *Diacamma* exhibit sexual-individual-specific rhythms even after mating. The measurement of colony-level rhythms showed that gemmae mutilations occur at specific times of the day, whereas foraging occurs at day and night. These findings confirm a novel form of temporal organization in social insects, providing a new insight into morphologically casteless species. We discuss the causes and consequences of rhythmic variability in social organization.

OR121

Caste plasticity maximises personal fitness at the origin of sociality

Emily Bell, Robin Sounthon, Solenn Patalano, Andy Radford, Seirian Sumner

The evolution of sociality is a major evolutionary transition, the peak of which is found in the eusocial insects. One of their defining features is the emergence of phenotypic commitment and division of labour whereby individuals specialise as reproductives (queens) or provisioners/carers (workers). Inclusive fitness theory explains why reproductive division of labour can evolve, and provides some mechanistic predictions for how phenotypic commitment arises at the origin of sociality. Specifically, it predicts that both direct and indirect fitness are important for all group members (irrespective of caste) in the early stages of social evolution, enabling each individual to adopt different strategies to maximise their own personal genetic fitness, depending on the opportunities available. An alternative hypothesis is that workers and queens should become committed to their roles in stable, established societies, possibly maximising group-level productivity. Here we show, through manipulation experiments, that females in colonies of the primitively eusocial paper wasp, *Polistes canadensis*, retain the behavioural plasticity necessary to maximise personal genetic fitness, through direct and indirect reproduction, irrespective of their original phenotype or stage in colony development. By isolating queens and workers on nests before and after worker emergence, we show that all females retain equivalent abilities to reproduce and provision young, regardless of phenotype and stage of colony cycle. All females therefore, retain the ancestral ability to switch reproductive strategies to exploit both direct and indirect fitness, suggesting that personal fitness is the driver at the early stages of social evolution.

OR122

Standardising morphological trait schemes: Introducing the global ant database

Catherine Parr, Heloise Gibb, Nathan Sanders, Rob Dunn

While traditionally much of ecology has focused on species richness and composition in communities, it is recognised that not all species are equal. Consequently functional trait approaches to ecology are now increasingly commonplace. Functional traits are features of organisms that have demonstrable links to the organism's ecosystem role or performance. Using functional traits can be of particular value because a trait-based understanding of community responses to global change drivers can enable generalisations across ecosystems. Here we introduce a new trait scheme for ants which builds on an established database, the global ant database (this contains community data on species and their abundances). We describe a set of standardised morphological traits for use in functional ecology work. The traits proposed, if used broadly, will enable direct comparison across different study regions facilitating understanding of the generality of patterns, how communities are structured and how their responses can vary. Given access to online specimens (e.g., AntWeb) and the increasing interest in functional traits, there is much scope to build a significant resource for current and future myrmecologists.

OR123

The morphological structure of neotropical and temperate forest ant communities

Carlos Roberto F. Brandão, Rogerio R. Silva

Morphological approaches can provide insights into the mechanisms that drive community organization and species coexistence and it is increasingly recognized that tests of community assembly need to incorporate information about functional characteristics of species. In the present study we compared the morphological space of ant communities in tropical and temperate forests. Further, we used a trait-based approach to determine the relative importance of niche and environmental filters in determining ant community structure, comparing trait dispersion between tropical and temperate regions. We surveyed ant communities at 26 sites along a latitudinal gradient of twenty degrees in the Atlantic forest (Eastern Brazil) and at 67 sites spanning 10 degrees of latitude in temperate forests (Northeastern United States), at 1000 meters of elevation in both datasets. We used mini-winklers apparatuses to sample leaf-litter ants in tropical forests, pitfall traps and systematic hand collecting to sample ants in temperate forests. We measured fifteen morphological traits on the basis of their putative function in 516 tropical ant species and 92 temperate ant species. Our morphological data set comprises 31,000 measures from 608 ant species, representing 2,000 individuals from Neotropical and 600 ant individuals from temperate-zone forests. Our results shall identify the major functional traits and environmental factors that shape assemblies of tropical and temperate ant communities. The relationship between species richness and the morphological space occupied may provide insights into the processes that outline patterns of ant biodiversity. Fapesp (Grant 98/05083-0 to CRF Brandão; grants 06/02190-8, 10/51194-1 and 10/20570-8 to RR Silva)

OR124

Exploring functional diversity of arboreal ants in New Guinea rainforests

Nichola Plowman Kate Parr, Vojtech Novotny, Petr Klimes

Functional diversity indices may be a more appropriate indication of ecosystem functioning than traditional taxonomic diversity indices. For ants, there is an increasing number of studies on the effects of disturbance in the functioning of terrestrial, ground dwelling ant communities. However, in tropical forests, trees harbour a large proportion of insect diversity and ants play a major role in canopy dynamics. To explore for the first time how functional diversity of arboreal ant communities can be affected by forest disturbance, we measured a selection of morphological traits of 124 species of arboreal ants collected from 0.5ha whole forest plots of primary and secondary lowland rainforest in Papua New Guinea. We compare the multidimensional trait space of the two habitats and investigate how vegetation characteristics such as tree size, species and leaf biomass influence ant functional diversity and which ecological traits, related to feeding or foraging, are responsible for these differences. Additionally, we explored if certain ant traits are correlated with particular nesting habitats. We hypothesise that the more open environment in secondary forest communities may select for ant communities with more heat tolerant and dessication resistant traits such as large body size and increased sculpturing

OR125

Taxonomic and functional beta diversity of montane ant communities

Tom Rhys Bishop, Mark Robertson, Berndt van Rensburg, Catherine Parr

Ants (Hymenoptera: Formicidae) are a dominant and ubiquitous group in nearly all terrestrial systems. Consequently, understanding ant beta diversity, variation in the composition of their communities, is a key goal for ecologists and conservationists. Whilst there is a body of literature describing differences in ant communities across spatial or environmental gradients, three key areas require further study. Firstly, in which ways do communities differ? Compositional differences can be due to complete species turnover or to nested patterns where a species poor community is simply a subset of a richer one. This distinction has important consequences for our understanding of which processes are driving community assembly, yet is rarely made for ants. Secondly, do changes in functional traits mirror those seen for species identity? If there is high functional redundancy among species within a region, then high taxonomic beta diversity may simply be the product of stochastic processes, rather than deterministic assembly rules. Thirdly, how do ant communities vary through time? Single instances of sampling give us only a snapshot of the patterns and processes shaping ant communities. We attempt to address these gaps in the ant ecology literature by utilising a spatially and temporally extensive community dataset, morphological and physiological functional traits and a new method for partitioning taxonomic and functional beta diversity into turnover and nestedness components. This will not only allow us to better understand the altitudinal ecology of southern African ants, but will give us greater insight into the mechanisms shaping ant communities in general.

OR126

The global termite functional diversity anomaly: are there ecological consequences?

Paul Eggleton

Termites have a long-established role as ecosystem engineers, modifying environments at a number of different scales. This effect is perhaps most clearly seen in species of *Macrotermes*, whose mounds are important providers of environmental and ecological heterogeneity, particularly in savannah environments. More perhaps than any other ecologically-important major lineage, however, the distribution of key termite clades has been determined by biogeographical factors. This has led to a large functional anomaly, with the global distribution of functional traits (and synthetic functional groups) being far from uniform. In this talk I will concentrate on two subfamilies that display some of the most striking of these anomalies: the Macrotermitinae and the Cubitermitinae (both in the Termitidae). The Macrotermitinae are fungus-growing termites, known, in concert with their mutualistic fungi, to have the most efficient lignocellulose degradation systems on the planet. The Cubitermitinae, in contrast, are true soil-feeders that break down soil organic matter, predominantly by the alkaline hydrolysis of recalcitrant protein residues. Both lineages have their highest biomass densities in Africa, in both rain forests and savannahs, and are absent from South America, Australia and Madagascar. I hypothesise that these absences cause between-continent differences in ecosystem process, particularly in plant decomposition rates and nitrogen cycling. I will examine the evidence for the existence of these predicted differences in a functional trait context. Finally, I will discuss the key experiments that will be required to test these predictions fully.

OR127

Which processes govern community assembly of West African savanna termites

Janine Schyra, Judith Korb

What are the determinants of species community assembly in tropical ecosystems? This is a specifically challenging question because here many similar species coexist at the local scale. There are two major theories that can explain community assembly, composition and diversity. First, the classical niche theory, which states that species have to differ in their niches to coexist. Here deterministic processes structure communities (e.g. environmental filtering and species interactions). On the other side, there is the unified neutral theory of biodiversity, which says that species are demographically equivalent and niche differences are not essential for coexistence. Here species distribution is due to random effects like dispersal, disturbance and stochastic processes. Our study organisms, termites, seem to have nearly identical niche requirements but still more than 20 species coexist in African savanna regions. We tested the importance of deterministic vs. neutral processes for termites in a West African savanna (Togo), where they are important ecosystem engineers. We combined specific pattern analyses of termite communities in natural, undisturbed ecosystems with cross-sectional studies of communities representing different assembly stages after anthropogenic disturbance (agriculture). Here the dynamics of community assembly could be followed. We will analyse community structure across assembly stages to show the impact of phylogeny, niche traits, and environmental factors on community assembly. With this we can test whether and how community structure changes during the assembly process.

OR128

Competition or environmental filtering - A trait-based perspective of ant communities
Silvija Budaviciute, Mar Cabeza, F. Guillaume Blanchet, Tomas Roslin

That ant communities are structured by competition is currently a widely held view among myrmecologists. However, recent evidence questions the importance of competition in ant communities. Thus, the aim of this study was to explicitly compare the role of competition and environmental filtering as forces structuring ant communities. For this purpose, we examined Malagasy ant assemblages across seven major habitats, measuring eleven morphological traits of ant workers. As signs of environmental filtering, we expected to see a convergence of trait values among habitats. Conversely, a divergence in trait values would reflect competitive pressure. The distribution of trait values observed was thus compared with those expected under a null model, where trait values were randomly assigned a specific habitat. Yet, the dispersion of trait values observed at this national scale did not conform to either those expected due to environmental filtering or to those expected due to competition. Our results thereby indicate that neither competition nor environmental filtering were major forces structuring ant communities across Malagasy macro-habitats. As a likely explanation for the observed patterns, the traits selected for measurement may be only weakly related to competition and/or environmental filtering at this scale. Instead, we propose that the traits of the larger colony (for example queen ability to disperse, colony size and structure, etc.) rather than the traits of individual workers may be the factors structuring ant community assembly.

OR129

Trophic functions, a structuring trait for tropical ant assemblages.
Mickal Houadria, Alex Salas-Lopez, Jerome Orivel, Nico Bleuthgen, Florian Menzel

Interspecific competition is high in ants, with few species dominating food resources and displacing other species. Therefore, niche differentiation is an important mechanism to maintain the high local species richness, which is typical for tropical communities. Species composition and the ecosystem functions performed by ant communities can drastically change with habitat disturbance. However, detailed information on the functional niches is only available for a few ant species, hampering our understanding of the importance of niche differentiation in ant communities in general. To assess community structure in relation to trophic functions performed by ants we conducted studies in primary and secondary rainforests of the neo- and paleotropics. We used eight bait types that reflected different natural resources. On a grid system, each type of bait was offered night and day, in order to estimate niche position and breadth for each ant species. Our results demonstrate that ant communities consist of a range of dietary and temporal specialists and generalists. Functional diversity and functional redundancy increased with ant diversity, indicating that the alteration of community structure does not invariably lead to a degradation of trophic functions. Some ant species play key roles in structuring the food webs of tropical rainforests and we can show that this is due to their numerical abundance, type of food specialization and temporal breadth. Our findings furthermore indicate that taxonomically related species can exhibit highly distinct trophic niches. Overall, we discovered clear parallels between the ant communities in the neo- and paleotropics in the trophic functions and their role in shaping community structure.

OR130

Global life trait spectra of resource exploitation in European ants
Xim Cerda, Javier Retana, Xavier Arnan, Elena Angulo, Raphaél Boulay

The leading dimensions of ecological variation have been widely discussed in plants in the last decade using the effort of generating large datasets about species traits. However, relationships among traits, function and the environment are poorly understood in animals, where large databases are hardly available. Here we seek to identify the main functional spectra of variation among many of the most important functional life traits related to resource exploitation in ants. We have created a global ant trait database of eleven traits recognized as important in resource exploitation by ants from 150 European species including a wide range of species from different biomes. The overall results of the study show wide-ranging evidence that resource exploitation strategies are to a great extent arrayed along two fundamental spectra, with similar patterns of trait relationships seen globally and with species grouped by taxonomy, habitat and climate. The first syndrome is the behavioral dominance spectrum and reflects that behaviorally dominant ants are frequently characterized by large colony size, different nests per colony, worker polymorphism and collective foraging strategies. The second spectrum of resource exploitation is the foraging strategy/diet spectrum, which mainly separates subordinate species and runs from species with diet based on liquid food and group foraging to those with diet based on insects and seeds, individual foraging, larger worker size and strictly diurnal activity. The combination of life traits that define these two spectra of resource exploitation allows classifying ant species into clearly separated guilds. These general patterns have been obtained bringing together a high number of life traits across many taxa and most biogeographical regions in Europe, something very difficult to find in animals.

OR131

Top predators, habitat complexity and the biodiversity of litter-dwelling ants
Terrence McGlynn, Peter Tellez, Walter Carson, Robert Dunn, Nathan Sanders

The loss of top predators in ecosystems can have cascading effects, including the loss of social insect biodiversity. We demonstrate how ground-dwelling ants responded to 17-year exclosure of large vertebrates in a Costa Rican rainforest. The biomass of leaf litter was unchanged, and there was no alteration in the density of ants regardless of vertebrate exclusion. However, the physical structure of the leaf litter environment was significantly altered, and was more compacted when unmanipulated densities of terrestrial vertebrates were permitted access to the research plots. The species richness of ground-dwelling ants reduced by one half as a result of terrestrial vertebrate access. Because there was no effect on density, this is likely a non-trophic cascade caused by a structural change in the leaf litter related to habitat complexity. Investigations into the thermal microclimates of leaf litter suggest that the loss of biodiversity may be connected to a loss of environmental heterogeneity, in which species with functional roles unsuited to lost microclimates may disappear.

OR132
Effects of habitat disturbance on the morphology of ant assemblages
Heloise Gibb, Kate Parr, Nathan Sanders Rob Dunn

A functional traits-based understanding of organismal communities is critical for understanding the principles that underlie community assembly, and predicting likely responses of assemblages to environmental change. This is particularly true for terrestrial arthropods, of which only 20% are described and whose biology is poorly understood. Using a collaboration of over fifty ant researchers, we constructed a database containing the abundance of pitfall-trapped ants in over one thousand local assemblages around the globe. Morphological and life history traits were recorded for each species in a subset of these assemblages. We expected that habitat disturbance would alter habitat structure, resulting in changes in metrics of assemblage morphology due to species turnover. We tested the effect of habitat disturbance and climate-related covariates on the mean, variance and range of morphological traits of ant assemblages. Here, we focus on morphological traits representative of ant size and limb length. We also consider the role of phylogeny in determining morphological responses to disturbance. Our findings contribute to our growing understanding of the functional responses of species assemblages to habitat disturbance and enhance our ability to predict changes in assemblages in response to anthropogenic disturbance.

OR133
Ant functional responses along environmental gradients
Xavier Arnan, Xim Cerda, Javier Retana

Understanding species distributions and diversity gradients is a central challenge in ecology and requires prior knowledge of the functional traits mediating species survival under particular environmental conditions. While the functional ecology of plants has been reasonably well explored, much less is known about that of animals. Ants are among the most diverse, abundant, and ecologically significant organisms on earth, and they perform a great variety of ecological functions. In this study, we analyze how the functional species traits present in ant communities vary along broad gradients in climate, productivity, and vegetation type in the Southwestern Mediterranean. To this end, we complied one of the largest animal databases to date: it contains information on 211 local ant communities (including eight climate variables, productivity, and vegetation type) and 124 ant species, for which 10 functional traits are described. We calculated two complementary functional trait community indices (trait average and trait dissimilarity) for each trait, and we analyzed how they varied along the three different gradients using generalized least squares (GLS) models that accounted for spatial autocorrelation. Our results show that productivity, vegetation type, and, to a lesser extent, each climate variable per se might play an important role in shaping the occurrence of functional species traits in ant communities. Among the climate variables, temperature and precipitation seasonality had a much higher influence on functional responses than their mean values, whose effects were almost lacking. Our results suggest that strong relationships might exist between the abiotic environment and the distribution of functional traits among Southwestern Mediterranean ant communities. This finding indicates that functional traits may modulate the responses of ant species to the environment. Since these traits act as the link between species distributions and the environment, they could potentially be used to predict community changes under future global change scenarios.

OR134

How does anthropization affect the functional characteristics of ant communities?

Alex Salas-Lopez, Jean-Romain Roussel, Isabelle Kozon, Florian Menzel, Jerome Orivel

Linking species diversity and traits to habitat properties and their effect on the rules governing community assembly is essential to understanding the impact of human activity on ecosystem functioning. Species coexistence is determined by a number of factors among which local adaptation and competition act in opposite ways. Indeed, whereas environmental filtering promotes trait convergence, competition favours niche divergence. We aimed to quantify the nutritional and foraging strategies of tropical ant communities to understand (i) how the ecological optima may contribute to species coexistence and (ii) how human-driven modifications to habitats affect species traits and thus community assembly and functioning. To that end, various food items representing different ecosystem functions were used to attract ants in forests and slash-and-burn croplands. Then, the most representative bait was offered (but displayed in different ways) to assess how species are able to discover and/or appropriate resources, and how the amount of food or the effect of a microhabitat act on their foraging strategies. We show that species diversity, as well as the composition and functional characteristics of the communities, differ greatly between habitats. Niche-overlap was greater in slash-and-burn croplands than in the forest where species differed more in their feeding and foraging optima. This may be linked to the heterogeneous conditions present in the forest due to habitat complexity and stratification. On the contrary, the simplified structure and homogeneous environmental conditions found in the croplands are likely to favour the high dominance of generalist species, regardless of bait type. We conclude that the conversion of forest areas to open and simplified habitats favours a limited guild of ant species adapted to such conditions. This has consequences for the functional structure of the ecosystem, although we did not observe a decline in most of the tested functions.

OR135

Myrmecochory in relation to soil disturbances in south-eastern Australia

Zsofia Palfi, Peter Spooner, Wayne Robinson, Levente Palfi

Myrmecochory, or seed dispersal by ants, is a significant ant-plant mutualistic relationship worldwide. Dispersal services provided by ants are largely influenced by habitat disturbances, primarily due to changes in the composition of ant species. In much of south-eastern Australia, an extensive network of road corridors exist, which contain linear tracts of native vegetation. As much of the landscape has been cleared, roadsides often provide critical refuge for native species and are of high conservation status. In Australia and elsewhere, minor rural roads are maintained by anthropogenic disturbances (i.e. road grading operations) which create novel environments for native species to persist. Despite the severity of disturbances from roadworks, some authors have suggested that certain ant species may prefer to nest such areas owing to foraging or habitat benefits (e.g. cleared substrate) provided by roadwork disturbances. However little is known about the effect of soil disturbances on seed dispersing ant communities. Field studies were conducted in a typical fragmented agricultural landscape in southern NSW, Australia. The selected area contains a large network of minor rural roads of gravel construction that require periodic management. Seed removal experiments were performed in 30 road segments that can be divided into two distinct zones: (1) a disturbed zone maintained by grading operations, and (2) a non-disturbed zone or roadside, which possessed *Acacia* shrubs. Seeds of *Acacia pycnantha*, a common myrmecochorous shrub found throughout the region, were offered to ants at multiple stations at each site. The composition of ant species interacting with seeds, rates of seed removal and seed dispersal distances by ants was recorded. We hypothesized that removal rates, dispersal distances, and seed dispersing ant assemblages would differ between the two zones as a consequence of differences in habitat conditions which influence the foraging ability of particular ant species.

OR136

How forest fragmentation affects functional diversity of soil fauna?

Mika Yasuda, Kyle Tomlinson, Ferry Slik

Fragmentation is the process of land clearance and habitat modification that leaves small-isolated remnants of native vegetation in disturbed lands. Xishuangbanna, Yunnan, China is included in the Indo-Burma Biodiversity Hotspot, but its native forests are now fragmented into many small, isolated patches due to rubber plantation expansion. The conservation value of the remaining small fragments still needs to be investigated. To understand mechanisms behind observed biodiversity patterns in these fragments, monitoring should include functional components of communities. Our objective was to investigate the effects of forest patch size and adjacent rubber plantations on functional diversity of the soil fauna. Leaf litter arthropods including ants, spiders, wasps, thrips, beetles and collembolans in forest fragments and adjacent rubber plantations were sampled in Xishuangbanna using the shuffling technique and winkler extractors. Adult specimens were identified and their individual numbers were counted. Morphological traits (e.g. for ants: length of mesosoma, eye, total hind leg and mandible) were measured individually. Trait values were used to calculate the mean trait value per community (mT) and functional dispersion (FDis). These variables were used as response variables, and elevation, area of forest within different radius from the sampling points and depth of leaf materials set as explanation variables to investigate the effects of fragmentation on the functional composition of the soil fauna. Preliminary analysis of the ant fauna indicates that mTs and FDis of forest fragments and rubber plantations were not significantly different. mTs increased with elevation and with respect to the area of forest in a 1000 m radius. FDis increased with litter depth and as the area of forest within a 1000 m radius decreased. Our results suggest that large forest fragments are required to preserve large-bodied ants, but that leaf litter thickness is more important for the diversity of ant functional types within a community.

OR137

Predicting morphology of Iridomyrmex *in response to changes in climate*

Katayo Sagata, Heloise Gibb, Steve Shattuck

The interaction between species traits and habitat characteristics is central to the existence of a species because habitat selects and favours certain traits that enable a species to occur in a habitat. However, climate change is altering habitat characteristics, which may alter both the abundance and traits of species. Here, we use annual mean temperature and precipitation to predict the potential morphologies of *Iridomyrmex* ants in Australia for 2080. We measured seven morphological traits from 19-71 replicate individuals in ten species using museum specimens. Through correlation tests, head length (HL) was identified as representative descriptor of overall ant body size. Eye size was least correlated with other traits so residuals from head length and eye length (EL) were used as a second response variable. A simple linear regression was used to test for the response of HL and eye length residuals to current temperature and rainfall. Potential morphological change of each species that responded was then predicted. Traits in 50% of the species responded to climate, with rainfall having negative effect on head length of *I. discors*, I*. dromus*, *I. sanguineus*, while temperature had a negative effect on *I. reburrus* HL, but a positive effect on *I. viridiaeneus* HL. Potential morphological change of these species corresponded with predicted temperature and rainfall, but varied within species with *I. discors*, *I. dromus* and *I. viridiaeneus* showing opposite responses. Such trait plasticity indicates that species need to be adaptive. Species showing traits plasticity will adapt better to changing climates than those showing limited trait plasticity.

OR138

Heating the superorganism: colony-level responses to environmental change

Clint Penick, Sarah Diamond, Rob Dunn

The response of social insects to their environment is dynamic, and results from interplay between individual traits and social interactions. Research on thermal tolerance in ants has focused primarily on forager traits, such as upper thermal limits of individual workers (e.g. CTmax), but temperature also drives colony-level patterns in brood production, metabolism, and growth. Here we combine studies on thermal tolerance of adult workers with measurements of brood development rates. For 15 dominant ant species from the eastern United States, we measured pupal development rate as a function of rearing temperature. Surprisingly, all species converged on nearly the same maximum development rate independent of worker size (~10 days for pupal development). Cold-tolerant species, however, reached this maximum at significantly cooler temperatures than heat-tolerant species. This suggests a tradeoff between brood development rate and worker thermal performance, such that species specialized to forage under warm conditions also require warmer temperatures to fuel colony growth. These findings highlight how epistatic interactions between developing brood and adult workers could shape colony-level responses to warming. Taking into account traits associated with colony growth, in addition to foraging traits, should improve models that seek to understand how social organisms will respond to environmental change.

OR139

Trait-based characterisation of invasiveness in ants

Cleo Bertelsmeier, Sebastien Ollier, **Franck Courchamp**

In order to outdo the descriptive framework of species-specific responses to ecological processes and to endeavour better generalisations and projections, ecologists are increasingly moving from taxonomic-based approaches to those based on sets of ecological traits. One good illustration of a discipline where trait-based approaches are especially welcome is invasion biology. Although such information has been long sought after, one of the most frustrating issues remains the persisting lack of robust patterns of ecological traits of invasive species that might be used to identify them before they become invasive. Typically, a database of ecological traits of invasive and non-invasive species of similar ecology and/or taxonomy would be a very potent tool to answer this, and many other ecological questions, such as identifying the relationships between any given ecological characteristics. Ants are a very large group of species which ecology is becoming increasingly well known, albeit often by specialists. Recent approaches to gather this wealth of dispersed information, such as the Global Ant Database, or our Antprofiler database (www.antprofiler.org), provide opportunities to extract generalised features related to specific ecological traits or community responses. Here, we show how our dataset of ants provides very promising information with regards to, among other things, biological invasions. Preliminary analyses of 2200 ant species, including the 20 most invasive species, for 25 life history traits, already suggested that some life history traits could be good predictors of invasiveness. Notably, this approach enables us to classify the 200+ ant species that are exotic but not yet invasive into species likely to become invasive, and those that should remain mere exotics. We hope that this demonstration will stimulate specialists to join those who already have contributed in filling up Antprofiler, for the greater benefit of the scientific community.

OR140

Why does kin recognition exist in subsocial Stegodyphus *spiders?*

Andre Walter, Trine Bilde

More than 99% of the arachnid species are solitary predators. In a few species social behaviours are found without reaching the level of eusociality. Kin recognition as a key feature for the evolution of cooperation and sociality has also been revealed for some arachnids. Like in other social animals the ultimate benefits of cooperation are well studied, but proximate mechanisms explaining the maintenance and early evolution of kin recognition as a mediator are still poorly understood. Social recognition mechanisms are well investigated in spiders while they are understudied in other taxa. We briefly review various recognition abilities and sociality levels arachnids. In contrast to eusocial insects, the adaptive value of kin recognition seems context-dependent in this arthropod group and thus a generalisation of its adaptive value is not possible. There is some evidence for the concept of kin recognition facilitating the evolutionary transition from subsocial to permanently social living. Interestingly, kin recognition has not yet been demonstrated in social arachnids. It might have been lost during evolution due to a lack of encounters with unrelated individuals in permanently social societies. Finally, we present our recent research on subsocial *Stegodyphus lineatus* spiders as a case study, demonstrating the difficulties in revealing the adaptive value of kin recognition. These animals only hunt and feed communally over a certain period of time. Groups of close relatives perform better resulting in a higher individual fitness. However, behavioural differences do not explain the better performance. A lack of discrimination against non-kin suggests that the benefits of group living are higher than an exclusive association with kin, raising the question why kin recognition exists in the species. Since these spiders feed communally using extra-oral digestion we suggest that the better performance of kin groups may manifest on molecular level, potentially through differential enzymatic compatibilities.

OR141

*Genomic imprinting mediates social interactions within honeybee (*Apis mellifera*) colonies.*

David Galbraith, Sarah Kocher, Tomas Glenn, Greg Hunt, Istvan Albert, Joan Strassmann, David Queller, Christina Grozinger

Genomic imprinting is an epigenetic mechanism that facilitates the differential expression of inherited alleles. The kinship theory of genomic imprinting predicts that differential relatedness of inherited alleles to other individuals in the colony will result in different expression patterns between maternally and paternally inherited alleles favoring different phenotypic outcomes. Honeybees provide an excellent system in which to test the kinship theory of genomic imprinting because their haplodiploid genetics creates relatedness asymmetries for maternally and paternally inherited alleles within colonies, and because there are a myriad of complex social interactions among the colony members predicted to be influenced by imprinting. Since the honeybee queens typically mate with multiple males, this leads to relatedness asymmetries of paternally-inherited alleles among workers within a single colony. Workers are facultatively sterile, but under certain circumstances they may activate their ovaries to produce haploid males. In this case, kinship theory predicts that paternally-inherited alleles will favor worker reproduction, while maternally-inherited alleles will disfavor it. To test this prediction, multiple reciprocal crosses of two honeybee races (Africanized and European) were created. Workers from these colonies were reared under conditions favoring ovary activation. Our results indicate that paternity significantly affects a worker's reproductive potential in terms of ovary size and ovarian activation. Transcriptomic analysis of expression levels of maternal and paternal alleles in reproductive vs. sterile workers is ongoing. These studies suggest that imprinting may play a significant role in regulating social dynamics within insect colonies.

OR142

Altruistic worker policing in honeybees, a multi-level approach

Claire Narraway, Peter Nonacs

Kin selection is the dominant paradigm to explain the evolution of cooperation and, worker policing is one of its most convincing examples. Here, workers aggress upon laying workers or consume their eggs to increase overall colony relatedness and therefore, a workers inclusive fitness benefits. However, examples of worker policing when the queen is monogamous or when workers reproduce thelytokously do not fit the predictions of kin selection. As such, other levels of selection may be important. Here, we present results from a simulation model that examine how three different levels of selection interact under varying starting conditions. The levels of selection are the individual level, colony level, and intragenomic level. Preliminary results suggest that conflict between the two portions of a workers genome may play a much more significant role than previously thought.

OR143

Grouping synergy generates the kin-selected benefit of bee workers

Tatsuhiro Yamamoto, Norihiro Yagi, Eisuke Hasegawa

Roles of grouping and kinship are recent focuses in the evolution of cooperation, but there is no empirical data. Sympatric occurrences of both solitary and multiple-female nests are confirmed in *Lasioglossum baleicum* bees. We showed that brood survival rates in Sapporo populations are much higher (ca. nine times) in the multiple-female nests than in the solitary nests, and that direct fitness of a queen and inclusive fitness of a worker is significantly higher than fitness of a solitary female. These results indicate that a worker gains the kin-selected benefit via the mother queen. However, the results also show that a synergistic improvement of brood survival results in the fitness benefit of a worker. The synergistic improvement of brood survival rate in multiple-female nests is arisen with no relation to the kinship among nest members. In fact, there were several multiple-female nests that consist of only unrelated females. Such a non-kin female laid her own eggs and gained higher direct-fitness than a solitary female. To explain this improvement, we provided a hypothesis that grouping is an anti-predation strategy. If grouping generates a synergistic benefit against the predation threat, individual fitness can increase irrespective of kinships between egg-laying nest-members. We conducted a simulation in which the effect of grouping on the brood survival against predation was examined. The simulation demonstrated that when the predation threat becomes high, the grouping results in a synergistic increase in brood survival comparing with solitary nests. This synergistic improvement is caused to decrease the period in which all females leave from the nest. Therefore, this synergistic benefit of grouping generates the higher fitness in a cooperative female, but in many nests, such a benefit returns to a worker via kinship. Our studies show the importance of both grouping and kin-selection in the evolution of cooperation.

OR144

Kin selection and reproductive strategies in a facultatively social bee

Jess Vickruck, Miriam Richards

Facultatively social insects offer a unique opportunity to study the role that kin selection may play in social group formation. The eastern carpenter bee, *Xylocopa virginica*, can nest solitarily or socially and displays multiple reproductive strategies in social nests. Large primary females dominate foraging and oviposition, but many die before the end of the brood production phase of the colony cycle. Secondary females, which are also large, forage and lay eggs at a reduced rate, and often attempt to relocate to other nests where reproductive opportunities are higher. Small tertiary females neither forage nor lay eggs but remain in the nest and may live for two years. We test two hypotheses generated under the framework of kin selection to examine the role of each reproductive strategy. First, we predicted that social females should often be sisters who overwintered together in their natal nest. Using nine microsatellite loci, we found high relatedness in small colonies containing only two females, but that relatedness was lower in larger groups. Second, we predicted that the extent of helping behaviour by secondaries and tertiaries should correlate with their relatedness to the primary female. Microsatellite analyses indicate primaries and tertiaries are more closely related than primaries and secondaries. Intensive behavioural observations show that tertiaries are the main helpers, guarding the nest from potential usurpers. In contrast, secondary females contribute little to the colony. Moreover, when primary females are experimentally removed from the nest, a secondary immediately begins to forage, while a tertiary does not. Thus, in *X. virginica*, the degree of helping behaviour exhibited by subordinate females correlates with their relatedness to dominant egg-layers, as predicted under kin selection. This creates a novel social dynamic in which related subordinates help the dominant, whereas unrelated subordinates either do not help, or actively compete for reproductive opportunities.

OR145

Sex ratio biases in termites provide evidence for kin selection

Kazuya Kobayashi, Eisuke Hasegawa, Kazutaka Kawatsu, Edward L. Vargo, Jin Yoshimura, Kenji Matsuura

Inclusive fitness theory, also known as kin selection theory, is the most general expansion of Darwin's natural selection theory. The idea that relatedness among individuals can drive the evolution of altruism has been the subject of much debate in evolutionary biology. One of the strongest lines of support for this theory comes from female biased investment by female workers in eusocial Hymenopterans where relatedness from the worker to sisters is higher than to brothers. These evidences of kin selection obtained from the studies of eusocial Hymenoptera are attributed to their special genetic system 'haplodiploidy'. Therefore, such a strong test of the theory has proven difficult in diploid social insects because they lack such relatedness asymmetry. Here we show that kin selection can result in sex ratio bias in eusocial diploids. Our model predicts that allocation will be biased toward the sex that contributes more of its genes to the next generation because of sex-asymmetric inbreeding over generations such as mother-son mating. Prediction of the model matches well with the empirical sex allocation of *Reticulitermes* termites. In *R. speratus*, *R. virginicus* and *R. lucifugus*, queens produce replacement queens asexually but use normal sexual reproduction to produce the other castes including replacement king, which results in mother-son inbreeding. Thus, the genes of primary queens were more transmitted to the next generation than that of primary king. In these three species, the sex allocations were significantly female biased while other non-parthenogenetic species (*R. flavipes*, *R. okinawanus* and *R. yaeyamanus*) showed equal allocations. These results suggest that kin selection is an important force in both diplodiploid and haplodiploid organisms. Previously, the validity of inclusive fitness theory has relied merely on the empirical studies of haplodiploid organisms. Our findings open broad new avenues to test inclusive fitness theory beyond the well-studied eusocial Hymenoptera.

OR146

Testing inclusive fitness theory in a lower termite

Judith Korb, Katharina Hoffman

Kin selection theory has been repeatedly debated and while it has been most successfully applied to understand conflict and sex allocation in social Hymenoptera, rigorous tests for other social taxa are scarce. Termites evolved sociality independently from social Hymenoptera and due to a lack of haplodiploidy conflicts such as those observed in Hymenoptera are predicted to be absent. Yet, termites offer a promising test case to study the interaction between relatedness and altruism because different termite castes vary in degree of altruism: Soldiers are always sterile and can only gain indirect fitness. In contrast, workers of wood-dwelling lower termites are immature instars that are less altruistic and have the full capability to become reproductives. They can either become winged sexuals that found new colonies or they can inherit the natal colony as replacement reproductives. Inclusive fitness theory predicts that relatedness should be more crucial in influencing soldier's behavioural interactions (kin discrimination) than that of workers. This we tested in the drywood termite *Cryptotermes secundus* where within-colony relatedness varies dramatically due to the common occurrence inbreeding ($r > 0.5$) and fusions of colonies ($r < 0.5$). As predicted, soldiers were more nepotistic than workers. Even more interesting, in both castes the adjustment was a phenotypic plastic response and kin discrimination only occurred during critical ecological conditions (e.g. food restriction). Our study demonstrates that as predicted by inclusive fitness theory it is the interaction between benefits/costs and relatedness that is key to social evolution. Hence, studies that investigate relatedness only, can be misleading and a holistic approach including all factors of Hamilton's rule should be applied.

OR147

Nowak-style models refute Nowak's conclusions about eusociality

David Queller, Sean Liao, Stephen Rong

In 2010, Nowak, Tarnita, and Wilson challenged the utility of the inclusive fitness approach, using the evolution of eusociality as a test case. The paper has drawn many responses defending inclusive fitness, but little response on the alternative method for modeling the evolution of eusociality, which the authors claimed to be superior. They used their model to support three claims that differ from standard inclusive fitness results. First, they argued that relatedness was unimportant in the evolution of eusociality. Second, they argued that eusociality is much more difficult to evolve than inclusive fitness theory seems to predict. Third, though inclusive fitness theory predicts conflict between queen and workers, they argued that the queen and her colony were selected as a whole and the distinction between queen and worker genes was not important. Here we investigate these three claims, using exactly the kinds of models advocated by Nowak et al. but guided by inclusive fitness thinking. All three of their claims turn out to be flawed. First, we show that relatedness is essential by showing that eusociality does not evolve in a model with zero relatedness. Second, we show that eusociality is much easier to evolve if we employ more reasonable fitness functions and decision rules. Finally, models with eusociality genes expressed in queens give different results than models with eusociality genes expressed in workers, generating worker-queen conflict, as previously shown by inclusive fitness models. In each case, Nowak et al. overgeneralized from specific assumptions or parameter values. More thorough use of their own preferred modeling strategy does not support these generalizations but instead supports the conventional inclusive fitness results. While their methods can be good for modeling complexity, they can also make it easy to miss the important generalities that inclusive fitness makes so clear.

OR148

The role of kin value and queen traits in reproductive partitioning

Liselotte Sundström, Martina Ozan

Reproductive cooperation confers benefits, but simultaneously creates conflicts among cooperators. When one breeding pair forms a society, relatedness among offspring, and thus the genetic incentive to cooperate, is high. In such societies most conflicts arise between the breeders and helpers. By contrast, when multiple breeding pairs form societies, relatedness among offspring is usually low, reducing the genetic incentive to cooperate. While conflicts between generations remain, new ones arise within generations and castes. Thus, when queens in multi-queen colonies of ants share a nest and its resources, reproductive competition arises among queens and conflicts over which queen to favour arises among workers. This often results in unequal reproduction of queens. Two mutually non-exclusive factors may produce such inequality in reproduction: worker intervention or queen traits. Workers may intervene by favouring some queens over others, owing to either kinship or queen signals, or queens may differ in their ability to extract resources, convert these to egg production, and/or produce offspring of different quality. Here, we test the role of queen kin value (relatedness) to workers and queen traits in determining the maternity of offspring, and show that both factors play a role in shaping reproductive partitioning in ant colonies.

OR149

Kin conflicts and the polyandry/polygyny paradox

William Hughes

Kin selection theory predicts the evolution of cooperation when rb > c, but it also predicts the emergence of potential social conflicts when the relatedness of individuals in a group is reduced. Polyandry (queens being inseminated by multiple males) and functional polygyny (colonies containing multiple, reproductively active queens) both reduce intracolonial relatedness, and have evolved as derived traits in many eusocial insects, with effective policing mechanisms appearing to largely prevent the resulting increase in potential conflicts over caste fate and worker reproduction translating into actual conflicts. There is now strong evidence of the benefits that social insect colonies can gain from polyandry or polygyny, making it a paradox that more obligately eusocial taxa have not evolved the traits. Here I will present experimental and comparative evidence showing that increased intracolonial genetic diversity can, in keeping with kin selection predictions, result in increased actual conflict under some conditions, and that the heightened conflict may then make greater genetic diversity costly rather than beneficial for the colony. The results suggest that the evolution of polyandry/polygyny is constrained not only by the direct costs of the traits, but also by indirect costs associated with heightened social conflicts, both providing an explanation for why more obligately eusocial taxa have not evolved polyandry/polygyny and providing support for kin selection theory.

OR150

Hidden diversity in the gut microbiota of Apis mellifera

Philipp Engel, Ramunas Stepanauskas, Nancy A. Moran

Honeybees (*Apis sp.*) and bumblebees (*Bombus sp.*) harbor characteristic gut communities composed of only a small number of bacterial species. This contrasts the immense diversity found in the guts of other animals and renders these social insects simple model systems for gut microbiology. Yet, only little is known about the functions and evolution of these communities. We sequenced a metagenome of the gut microbiota of the Western honeybee to gain insights into the functional gene repertoire of these bacteria. Our comparative analyses showed that different species of the bee gut microbiota harbor distinct functional capabilities linked to adhesion, biofilm formation, and carbohydrate breakdown. These functions are likely to be involved in symbiosis with the host, affecting immunity, pathogen colonization, and nutrient utilization. We further detected marked degrees of genetic diversity within species of the bee gut microbiota. We examined the extent of this intraspecific diversity by sequencing genomes from single bacterial cells of two species: *Snodgrassella alvi* and *Gilliamella apicola*. In both species, we found striking variation in genome divergence, despite uniform levels of 16S rRNA similarity. Some genotypes revealed genome cohesion indicated by high levels of recombination and low levels of sequence divergence. Other genotypes were highly divergent, constituting distinct and irreversibly separated lineages. Gene repertoires differed markedly among strains in both species, suggesting functional differences, which could be important for symbiosis with the host. Our findings from the honeybee parallel observations from mammals, suggesting that in situ diversification of a few bacterial lineages is a common pattern in the evolution of gut communities. In the future, we want to understand which factors drive this diversification and how it affects the interaction with the host.

OR151

Bumblebee gut microbiota diversity and their interaction with parasites

Hauke Koch, Nancy Moran, Paul Schmid-Hempel

I will present an overview of the diversity of the gut bacteria in European and North American bumblebee species and compare it to the honeybee microbiota. Bumblebees harbor species specific communities, but community composition is less stable than in honeybees and may contain high representations of environmental bacteria in some species. I will look in more detail at the strain diversity of two of the main core gut symbionts, *Snodgrassella alvi* and *Gilliamella apicola*. Symbiont phylogenies match with host phylogeny, but not with geography, but both host switching and horizontal gene transfer can also be found. To look at the potential functional significance of the microbiota, I looked at the interactions of gut bacteria with the trypanosomatid parasite *Crithidia bombi*. Germ-free individuals of both the European bumblebee species *Bombus terrestris*, and the North American species *Bombus impatiens* are highly susceptible to parasitic infections compared to individuals containing a complex microbiota. Furthermore, microbiota form different colonies appear to interact with parasite genotypes in a specific manner. Inoculations with pure cultures of different isolated members of the gut microbiota suggested *Snodgrassella alvi* as the main taxon reducing parasite loads.

OR152

Bumblebee immune response upon faecal transplant and microbiota community structure

Kathrin Napflin, Paul Schmid-Hempel

Microbial symbionts are suspected to closely interact with the host immune system. Recently, their role in mediating levels of resistance in host-parasite interactions has thus come into focus. So far, however, experimental investigations of the impact of the microbiota on the host immune system, and vice versa, are virtually absent. In the bumblebee, *Bombus terrestris*, the gut community is socially acquired by callows, likely via the uptake of faeces from nest mates. Experiments demonstrated that the microbiota provides a protective function and mediates the specificity of the host interaction with the trypanosome gut parasite, *Crithida bombi*. In this study, we investigated how faecal transplants of 'resistant' and 'susceptible' microbiota into microbe-free worker bees affect the activity if the host immune system. Following the transplant, we measured the primary expression response of a number of candidate genes in order to disentangle the importance of host versus microbiota-mediated effects on the host immune response. Although both affected the outcome, we found a major effect of the host resistance type (i.e. the genotypic background of the receivers) compared to the resistance type of the donors, i.e. the transplanted gut microbiota community. This result underlines the importance of host genotype; most likely, the host genotype so exerts selection upon the establishing gut community. In a second step, we discuss implications of these gene expression patterns (focusing on antimicrobial peptide and melanisation) in the context of the microbiota community of the two resistance types.

OR153

Identifying the core microbial community in fungus-growing termite guts

Saria Otani, Aram Mikaelyan, Tania Nobre, Lars Hansen, Søren Sørensen, N'Golo Kone, Duur Aanen, Jacobus Boomsma, Andreas Brune, Michael Poulsen

Gut microbes play a crucial role in decomposing lignocellulose to fuel termite societies, with predominantly protists in the lower termites and predominantly prokaryotes in the higher termites providing these services. However, a single basal subfamily of the higher termites, the Macrotermitinae, also domesticated a plant biomass-degrading fungus (*Termitomyces*), but it has remained enigmatic how this additional symbiont has affected the fungus-growing termite gut microbiotas. Here, we compared the intestinal bacterial communities of five genera (nine species) of fungus-growing termites to characterize extant lineages and to identify elements of the microbiota that are common to, and characteristic for, members of the Macrotermitinae. Using 454-pyrosequencing of the 16S rRNA gene, we identified representatives of 26 bacterial phyla, which were dominated by Firmicutes, Bacteroidetes, Spirochaetes, Proteobacteria and Synergistetes. A set of 42 genus-level taxa was present in all termite species and accounted for 56â€“68% of the species-specific reads. Gut communities of termites from the same genus were more similar than from distantly related species, suggesting that host phylogeny influences community structure, possibly in connection with genus-specific differences in their ecological niches. This lineage-specific microbial diversity likely represents more recently evolved digestive syndromes. Finally, by comparing the abundance of Macrotermitinae-specific bacterial lineages with their abundance in other representative dictyopteran insects, we found that the gut microbiota of fungus-growing termites is more similar to that of cockroaches than to lower termites or non-fungus-growing species of higher termites. These results suggest that the obligate association with *Termitomyces* has forced the bacterial gut communities of the fungus-growing termites towards higher similarity with their omnivorous relatives than to their more closely related termites.

OR154

The compartmentalized microbiota of a herbivore ant gut

Pedro A P Rodrigues, Michele Lanan, Piotr Lukasik, Jacob A Russel, Diana E Wheeler

Herbivores such as cows and rabbits evolved specialized portions of their gut where symbiotic microorganisms are found. These symbionts contribute to their host nutrition, by either digesting plant tissues (e.g. cellulose) or increasing nitrogen availability (e.g. nitrogen fixation). Such a relationship is not as well investigated in insects, with the exception of termites. *Cephalotes* is a diverse genus of herbivorous ants that harbor a mass of bacteria in a pouch-like sac (ileum) in their hindgut. The specific identity and function of these bacteria is still unknown, as only analyses of whole guts of ants have been done to date. In this study we investigated how symbiotic bacteria are distributed along the digestive tube of the Sonoran Desert turtle ant, *Cephalotes rohweri*. Using next-generation technology, we sampled field collected colonies and sequenced morphologically distinct regions of the gut. We found a clear compartmentalization of the microbiota. Opitutales were found to be the dominant bacteria in the midgut, a compartment markedly less diverse than other parts of the gut such as the crop and hindgut. Phylotypes found in each compartment were also consistent with a functional partitioning of the gut: upper gut bacteria are related to bacteria associated with nitrogen fixation and complex carbohydrate degradation, while hindgut dwellers are related to groups able to use simple carbohydrate and amino acids as sources of energy. *C. rowheri*, as other species in this genus, have an unusual feeding behavior and our future work involving enzymatic assays on the different parts of the gut will shed light on the nutritional role of these microorganisms.

OR155

Morphological adaptations for gut microbiota partitioning in the ant Cephalotes rohweri

Michele Lanan, Pedro Rodrigues, Diana Wheeler

Separating the crop and the midgut of ants there is a complex and beautiful small valve called the proventriculus. This valve regulates the flow of food between the crop or ‘social stomach’ and the midgut or ‘individual stomach’, an important division for the regulation of nutrient flow at the colony level. Ants in the genus *Cephalotes* have a proventriculus that exhibits a particularly complex form, the purpose of which is not clearly understood. We experimentally determined that the proventricular valve in the ant *Cephalotes rohweri* functions as a filter, playing a key role in partitioning of the bacterial microbiome between regions of the gut. We discuss the role of microscopic morphological adaptations for separating and maintaining distinct microbial communities within the ant gut, and the importance of the proventriculus as a pump, filter, and potentially also a substrate for symbiotic biofilms.

OR156

Interplay between the ant Cardiocondyla obscurior *and its two bacterial endosymbionts*

Antonia Klein, Lukas Schrader, Martin Kaltenpoth, Dave Wheeler, Jürgen Heinze, Jan Oettler

Intracellular endosymbiotic bacteria are vertically transmitted via maternal, cytoplasmatic inheritance. In social insects, only queens are reproductive, whereas workers are a dead end for the bacteria. Obligate endosymbionts in insects are often characterized by specialized organelles, which indicate an important role of the prokaryote in the host´s metabolic system. Whole genome sequencing revealed two bacterial symbionts in the invasive ant *Cardiocondyla obscurior*, which has been distributed throughout the subtropical range with human commerce. A *Sodalis*-like endosymbiont is present exclusively in a Brazilian population, whereas *Wolbachia* was detected in the Brazilian as well as in a Japanese population. Using fluorescence in situ hybridization (FISH), we found that *Wolbachia* is distributed throughout the queen's abdomen, with focus on ovary tissue. By contrast, *Sodalis* is localized in abdominal bacteriomes, indicating an obligate relationship with the host. A genome size reduction (543 Mb) of the *Sodalis* genome furthermore points to a symbiosis formed over longer evolutionary time. Surprisingly, the Japanese *C. obscurior* population does not exhibit *Sodalis*. To unravel the interactions between the bacteria and the ant host, we analyzed *Sodalis* and *Wolbachia* density in the ant tissue in relation to sex, morph and age using real-time quantitative PCR. The results show a close association of both bacterial lineages with female fertility. *Wolbachia* as well as *Sodalis* titers increase with queen age. Whereas *Wolbachia* infection state remains constant with worker aging, surprisingly, *Sodalis* titers even decline with worker age. This points to an adaptation of *Sodalis* to their eusocial host, as the steril *C. obscurior* workers are a dead end for the endosymbionts. Taken together, the obligate, well-adapted relationship to the ant host makes the absense of *Sodalis* in the Japanese population even more staggering.

OR157

Microbiomes of Megalomyrmex *social parasites and their fungus-growing ant hosts*

Joanito Liberti, Panagiotis Sapountzis, Lars H. Hansen, Søren J. Sørensen, Rachelle M. M. Adams, Jacobus J. Boomsma

Bacterial symbionts are important fitness determinants of insects. Some hosts have independently acquired microbes of the same lineage to meet similar challenges, but whether distantly related hosts that live in symbiosis can maintain similar microbial communities has not been investigated. The varying degrees of nest-sharing between *Megalomyrmex* social parasites (Solenopsidini) and their fungus-growing ant hosts (Attini) allowed us to address this question, as both ant lineages rely on the same fungal diet, interact through a variety of parasitic relationships, and are distantly related. We used tag-encoded FLX 454 pyrosequencing and diagnostic PCR to map bacterial symbiont diversity across the *Megalomyrmex* phylogenetic tree, which also contains clades of free-living generalist predators. We show that social parasites sharing the nest with their hosts or merely consuming host brood and fungus garden, harbour microbial communities that are partially overlapping with those of the attine species that they exploit. Particularly abundant were Entomoplasmatales, Bartonellaceae, *Acinetobacter, Wolbachia and Pseudonocardia*, in many cases co-infecting associated hosts and parasites with identical 16S rRNA genotypes. We further reconstructed population level infection dynamics for Entomoplasmatales and Bartonellaceae species in the particularly well sampled species-pair of *M. symmetochus* guest ants and *Sericomyrmex amabilis* hosts. Our results suggest that hosts and socially parasitic *Megalomyrmex* ants share a number of prevalent bacterial symbionts, as these bacteria may be transmitted via consumption of shared fungus gardens, predation on host brood by the social parasites, or parasite grooming by host workers.

OR158

A mutualistic network of ants, aphids and gut microbes

Aniek Ivens, Daniel Kronauer

Gut bacteria are increasingly recognized as crucial players in digestion and nutrient uptake in social insects. In fact, colonization of termite and ant guts by specialized intestinal bacteria may have facilitated the use of new food sources by their insect hosts. Likewise, aphid endosymbionts play important roles in amino acid synthesis from nutrient poor plant sap, thereby allowing aphids to survive on a diet of plant phloem sap only. In ant-aphid mutualisms, ants protect and groom aphids in exchange for sugar-rich honeydew excreted by the aphids. Microbes are active on both sides of this nutrient transfer: while endosymbiotic microbes in the aphids determine the biochemical composition of the honeydew, those in the ant gut facilitate nutrient uptake from the honeydew. Ant-aphid mutualisms therefore provide a unique opportunity to study the coevolutionary dynamics between microbiomes in distinct but interacting insect hosts. Here we focus on a subterranean ant-aphid-microbe interaction network found in forests in the North-Eastern United States. In this hitherto poorly characterized system, three Lasius ant species tend aphids inside their nests. The tended aphids belong to multiple species in the genera *Prociphilus* and *Stomaphis*. Using a combination of field sampling, DNA barcoding and next-generation microbiome sequencing, we characterized this mutualistic interaction network in terms of species interactions, host and microbiome genotypic covariance, and levels of specialization. Our findings show that the network is of moderate complexity, with varying levels of specialization between the players involved. This, in combination with the ease with which the species can be sampled in the field and kept under laboratory conditions, indicates that this is a potentially powerful new model system to study the role of microbiomes in both nutrient uptake and excretion across species boundaries.

OR159

Host-microbiome co-propagation: a novel agricultural tool copied from fungus-growing ants

Ulrich Mueller

Fungus-growing ants engineer complex microbiota during the gardening of their cultivated fungi. Specifically, the ants co-propagate their fungal crop together with diverse communities of beneficial bacteria and yeasts, thereby engineering a beneficial crop-microbe consortium. I adapted these fungicultural principles to develop an efficient method of plant-bacterial-community co-propagation that enhances plant growth by selecting indirectly on root-associated (rhizosphere) bacterial communities while selecting directly on plant biomass. Next-gen surveys confirm that bacterial communities rapidly evolve differences between replicated selection-lines that are selected for either growth-enhancing versus growth-attenuating effects on plants. Beneficial rhizosphere bacteria can greatly enhance fitness of host-plants, for example by solubilizing nutrients or secreting protective antibiotics, but the exact mechanistic effects of the microbiota evolving in my experiments remain to be studied. Because my co-propagation methods were bio-inspired by fungus-growing ants, natural-history observations (e.g., study of outlandish ant-fungus symbioses) therefore can generate innovative ideas with economic applications.

OR160

Evolution of task allocation: selection for efficiency or robustness?

Anna Dornhaus

Division of labor is the central innovation after each major transition in evolution, and is thus at the root of biological complexity. Task allocation is the process that achieves this. An understanding of why division of labor evolves, and why particular task allocation strategies evolve, is thus fundamental to our understanding of life. Social insect research has uncovered many such strategies, i.e. behavioral rules or physiological mechanisms that allow workers to choose which task to engage in; but we still lack evolutionary explanations for the diversity in task allocation strategies across species. In an experiment with evolving artificial (in silico) organisms, we showed that even in a very simple system several different task allocation strategies can evolve, including task allocation by spatial position, by developmentally induced stochastic individual differences, and by signaling. All of these strategies have also been proposed to exist in social insects. What environmental or social factors, or what properties of tasks determine which task allocation strategy evolves in a particular species or for a particular task type? Task allocation strategies differ in their accuracy, cost, flexibility, and ability to allow individual specialization. We have shown that specialization is not the only, and not always a beneficial result of task allocation. Here we investigate first how different task allocation strategies are expected to perform in different environmental and social conditions, and second quantify in the ant *Temnothorax rugatulus* how the factors contributing to task allocation differ between task types. We use a semi-automated video tracking system that can dramatically increase the quantity and quality of information on individual behavior in the colony. We show that task allocation mechanisms are likely the outcome of a complex interplay of colony-level and individual-level selection, but also that different measures of performance are selected for in different task types.

OR161

Group size, division of labor and fitness in clonal ant societies

Yuko Ulrich, Jonathan Saragosti, Daniel Kronauer

Testing hypotheses on division of labor requires the ability to manipulate colony composition. In particular, the distribution of individual age and genotype across members of a social group, as well as the size of the group, are expected to affect division of labor and colony performance. However, how each of these factors affects individual and group level behavior is still poorly understood. We present the first results from a series of experiments manipulating these factors independently from one another in *Cerapachys biroi*, a queenless, parthenogenetic ant. The unconventional biology of *C. biroi* affords experimental control over 1) individual age and group demography, because of cyclic reproduction leading to discrete age cohorts; 2) individual genotype and the genetic composition of groups, because of clonal reproduction and 3) group size, because colonies of any size can be set up from totipotent workers. In a first experiment, we set up replicate colonies of various sizes, but composed of individuals of exactly the same age and genotype. Automated tracking of individual ants in 100+ experimental colonies in parallel over a month allows us to quantify the effect of group size on division of labor and group fitness. We show a positive effect of group size on different components of fitness (adult survival, reproduction, development time), and link this to differences in individual and group-level behavior.

OR162

Fitness costs of division of labour in a social insect

Evelien Jongepier, Susanne Foitzik

Although division of labor is thought to be the key to the success of societies, the fitness consequences of division of labour have not been experimentally explored. Under stable conditions, specialization can increase individual efficiency and consequently group performance. However, when conditions are dynamic and unpredictable, a too rigid division of labor could constrain flexible task allocation in response to rapid shifts in group demand. We manipulated the division of labor in colonies of the ant *Temnothorax longispinosus* and show that worker specialisation is disadvantageous under dynamic conditions posed by the slavemaking ant *Protomognathus americanus*. During slave raids, host colonies composed of generalist workers saved more worker and sexual brood than specialist colonies, because they carried their brood faster to safety. Generalist colonies also caused more casualties among slavemaker colony members, without paying a price in terms of increased worker or queen mortality. These fitness costs of specialisation imply that slavemakers select for less division of labor in their hosts. Indeed, when comparing three parasitized and three unparasitized T. longispinosus populations, we found that hosts that co-occur with *P. americanus* exhibit a lower degree of division of labor. Thus, colonies appear to organize themselves in accordance with an important source of environmental unpredictability. This corroborates with our experimental results showing that worker specialisation conflicts with flexibility in task performance, which has detrimental fitness consequences for colonies under dynamic conditions.

OR163

Scaling of energetics and division of labor in harvester ants

Jon Harrison, James Waters, Carter Tate Holbrook, Jennifer Fewell

Complex social groups, such as social insect colonies, show striking similarities to organisms in their scaling relationships between body size, metabolism and the organization of work. Organisms and colonies both show a combination of increased specialization (in cell function and worker task performance) and decreased per unit metabolic costs at larger size. One of the central challenges in biology is understanding the causes of the hypometric scaling of metabolism, which appears robustly in individuals, social groups and even cities. We describe recent experiments focused on elucidation of the behavioral mechanisms responsible the hypometric scaling of metabolic rate using harvester ant colonies (*Pogonomyrmex californicus*). We show that larger, intact, functioning colonies of this species (of the same age) exhibit decreased mass-specific metabolic rate, whereas ants in random groups outside of the nest show isometric scaling of metabolic rate. Larger colonies (of the same age, or during ontogeny) also show increased division of labor, specialization of individuals, and a greater focus on maintenance-related tasks. Larger, same-aged colonies also had increased variance in walking speeds, with an increasing fraction of slow-moving ants, consistent with increased division of labor in locomotory activity. Experimental manipulations of colony size reproduced some of these features (such as hypometric metabolic rate scaling) but not all (e.g. no effect on division of labor), so understanding the causal linkages between colony size, metabolic rate and behavior remains elusive. However, studies of scaling in social insect colonies have the potential to provide fundamental information necessary to understand the evolution and ecology of colony size, solutions to the universal paradox of metabolic hypometry, and insights into general organizational principles of social groups. This research was partially supported by NSF grants to JW, JFH, CTH and JF.

OR164

Evolution of self-organized division of labor

Jianlei Zhang, Chunyan Zhang, Ana Duarte, Franz J. Weissing

Division of labor (DOL) is characterized by the coordinated interaction of individuals that collectively achieve a common goal by specializing on certain tasks. It is undisputed that DOL is a key feature for the ecological success of social insects. Yet, the evolution of DOL is not well-understood. Natural selection on DOL is indirect, since DOL is not a heritable property in itself but the result of self-organization. The challenge is to understand how selection acting on individual behavior results in the evolution of well-organized collective behavior. To meet this challenge, we develop and analyze models for the evolution of heritable neural networks that determine the response of individuals to external stimuli. Within a colony of insects, all workers are endowed with a neural network that reflect the genetic information inherited from their parents. These networks steer the behavior of individual workers and collectively the functioning and productivity of the colony. Colony performance determines the rate at which reproductives are produced and, hence, colony fitness. Well-performing colonies (where the neural networks of the workers lead to the emergence of DOL) transmit the genes underlying the neural networks at a higher rate than less well performing colonies. Previous studies revealed that this process can lead to the evolutionary emergence of efficient DOL from scratch. The models developed thus far are not fully satisfactory, since they focus on DOL under constant conditions, where a fixed distribution of tasks is selectively favored. In contrast, the flexible rescheduling of tasks in response to a changing state of the colony or to changing environmental conditions is a hallmark of DOL in social insects. Here we present some first results on the evolution of flexible DOL, which illustrate both the scope and the limitations of neural network models for understanding the evolution of self-organized DOL.

OR165

Modular approaches to the genetic basis of division of labor

Brian Johnson, Cameron Jasper, Joel Atallah

Honeybee workers pass through four distinct developmental stages as they age. These temporal castes are specialized for particular task sets such as nursing, food processing and nest building, and foraging. Some of these castes work together requiring tight coordination of activity. The genetic (and general mechanistic) basis of this social system has received much attention, with the regulatory basis of the transition to foraging receiving the most attention. Thousands of genes have been identified that differ between nurses and foragers, for example, many of them transcription factors or other regulatory genes. This work has focused primarily on brains and has used microarrays. This talk will present work using RNA-Seq that expands on this work to consider the genetic basis of the nurse bee to forager transition across ten separate honeybee tissues. In particular, we focus on how expression of genes associated with juvenile hormone and insulin/insulin-like signaling differ across the tissues, implying modular regulation of tissue specific social functions with caste transitions. This work expands greatly our already impressive understanding of the regulatory genetic basis of complex social behavior in bees.

OR166

Molecular evolution of the honeybee brain transcription regulatory network

Daria Molodtsova, Amro Zayed

Honeybees are highly social animals, and worker bees exhibit division of labour by specializing on specific tasks over the course of their life. Shifts in worker behaviuor are coordinated by a complex transcription regulatory network (TRN) that has been recently elucidated in the honeybee *Apis mellifera* (Chandrasekaran et. al. 20122, PNAS 108: 18020-18025). I studied patterns of molecular evolution of the honeybee's brain TRN to understand the degree to which pleiotropy constrains adaptive protein and regulatory sequence evolution. I estimated the coefficient of selection acting on coding and regulatory sequences of transcription factors and their target genes. My results indicate that pleiotropy constraints molecular evolution of transcription factors and their targets via different mechanisms.

OR167

The neuroecology of social organization in the Australian Weaver Ant Oecophylla smaragdina

J. Frances Kamhi, SKA Robson, W Gronenberg, JFA Traniello

Collective arboreal nest construction and territorial defense have earned the Australian Weaver Ant, *Oecophylla smaragdina*, the reputation of being the ‘pinnacle of ant social evolution’. Division of labor between worker subcastes underscores collective sophistication and serves a significant role in weaver ant ecology and social organization. Small minor workers specialize in brood care and larger major workers forage and defend territory when mature. Cognitive capabilities are thus distributed among workers according to their size and age. The impact of division of labor on weaver ant worker social brain evolution is not known, nor is the phylogenetic origin of neurobiological traits associated with their advanced social complexity. We tested the hypothesis that relative investment in neural processing decreases with increased behavioral specialization, and macroscopic neuroanatomy, synaptic organization in visual and olfactory processing regions, and neurochemical differentiation mirror division of labor as neurobiological responses to ecological and social challenges of task performance. Our results suggest that majors invest more in primary visual input regions than minors and increase investment in mushroom bodies, a region of sensory integration, with maturation. Microglomeruli, synaptic complexes in the mushroom bodies, are affected by experience during development: light deprivation leads to synaptic pruning in the visual input region although brood deprivation increases microglomeruli densities in the olfactory input region. Brain octopamine titer is positively correlated with territorial aggression, a prominent behavior of majors. Neuroanatomical comparisons between *O. smaragdina* and a sister clade species *Formica subsericea*, which represents a social phenotype characterized by less social complexity, show differences in investment in sensory systems and integrative regions correlated with their disparity in collective organization. Results support the hypothesis that brain architecture reflects behavioral phenotype and the corresponding sensory and processing requirements of workers, and neurochemicals modulate subcaste-related task performance.

OR168

The development of task performance across the worker lifespan

Mario Muscedere, Ysabel Giraldo, Darcy Gordon, Hannah Waxman, James Traniello

The developmental approach to studying division of labor has yielded considerable insight into endocrine, neural, and gene regulatory mechanisms that underlie worker task performance. Spurred by Wilson's description of temporal polyethism in *Pheidole dentata*, much of this work in ants and other social hymenopterans has focused on age-related developmental events that occur relatively soon after adult eclosion. Our recent efforts integrate physiological, neurobiological, behavioral, and comparative evolutionary analyses of division of labor in ants. I will discuss the relative contributions of canalized and experience-dependent physiological development to worker labor patterns; the use of antennal ablations, social manipulations, and efficiency measurements to test the importance of early-life sensory experience for worker behavioral and neural development; how aging workers continue to develop (and whether they senesce) throughout their adult lifespan; and how similar physiological and neurobiological mechanisms may regulate the behavioral differentiation of workers from different subcastes and species. Variation in worker larval and pupal developmental rates may be correlated with species ecology and impact adult task performance patterns, providing novel insight into the factors that affect worker task capabilities early in adult life. We emphasize the challenges of identifying the proximate mechanisms of division of labor in the context of the social and ecological factors that select for behavioral complexity.

OR169

Plasticity and the organization of division of labour.

Sofia Ibarraran, James Traniello, Marc Seid, Ehab Abouheif

According to Holldöbler and Wilson the role of plasticity in division of labour labour is one of the outstanding challenges of insect sociobiology. We address this fundamental question by focusing on temporal polyethism which is the overall pattern of behavioural variation through time. Temporal polyethism has been hypothesized to depend on age, physiology and experience. Our works shows that plasticity contributes significantly to the overall pattern of division of labour through by generating inter-individual variability. Inter-individual variability in both trajectories and endpoints of behavioural ontogeny appears to be the rule rather than the exception. To address the how inter-individual variability is generated, we assessed the behavioral progression of same-age individuals that share a social context. On day five of behavioural progression, we clearly identified two distinct behavioural groups: (1) ‘NO GO’ ants that remain inside the nest performing brood care and other tasks inside the nest; and (2) ‘GO’ ants that leave the nest, look for food, and eat food before returning to the nest. Surprisingly, NO GO and GO ants have similar behavioural capacities, biogenic amine titres as well as receptor expression levels, suggesting that this striking inter-individual variability must be a consequence of their social micro-environment. In order to address this hypothesis we assessed social interactions during the period between post eclosion day 2 and day 5. Our results suggest social interactions have a role in the generation of inter-individual variability. Inter-individual variability is an important mechanism that enhances robustness and resilience of the colony, fundamental features of division of labour. These results may lead us to reassess the way we have traditionally thought about division of labour in insect societies.

OR170

Why are there 'lazy' ants? How worker inactivity can arise

Daniel Charbonneau, Neil Hillis, Karen Kierstead, Maxwell Akorli, Anna Dornhaus

Most animals are remarkably lazy, spending upwards of 50% of their waking hours resting. This behavior is pervasive across a wide range of ecologies and life histories. However, inactivity also varies widely within and across species, even among the genetically similar nestmates of social insect colonies. By measuring circadian activity profiles, and comparing field and lab activity levels, we have eliminated the possibilities that inactivity results from temporal sampling biases and lab artifacts, thus validating the ecological relevance of this behavior. Using automated spatial tracking, we linked high levels of inactivity to a suite of characteristics, including slower walking speeds, and spatial fidelity zones within the nest. Here, I test leading hypotheses explaining inactivity in social insects. We pay special attention to the notions that (1) inactivity is a form of social cheating in which egg-laying workers selfishly invest in their own reproduction rather than contribute to colony fitness, and (2) inactivity results from immaturity or senescence of workers. We show that highly inactive workers in the ant *Temnothorax rugatulus* have more oocytes in their ovaries, indicating a significantly greater reproductive potential compared to other workers. This suggests that inactive workers may be benefitting from the work of their nestmates, and selfishly diverting colony resources towards their own reproduction. We also found that very young workers (<10 days) are significantly more inactive than their older nestmates, but quickly reach similar activity levels (after ~20 days). Colony inactivity was strongly affected by brood/worker ratios which increased as more workers emerged and less brood were left to care for. These results provide insight into the complex nature of inactivity, and the many ways variation among workers can arise, including selfish worker reproduction, and stochastic and predictable fluctuations in work demand, and worker surplus.

OR171

Detailed analysis of division of labor using long-term tracking

Jonathan Saragosti, Daniel Kronauer

Division of labor is a fundamental feature of insect societies and thought to play a major role in the ecological success of social insects. Despite the absence of centralized control, insect colonies must constantly adapt to environmental changes through dynamical transitions between the tasks performed by the workers. To decipher the mechanisms regulating division of labor we study the queenless clonal ant *Cerapachys biroi*, whose unusual biology allows us to set up large numbers of experimental replicate colonies of arbitrary size, genetic composition and demographic structure under controlled laboratory conditions. We built a custom video tracking setup, designed to be affordable and versatile. This setup allows us to track individual ants tagged with simple color marks for up to six weeks, and to measure individual behavior as well as interactions between workers. Dimensionality reduction schemes are then applied to the behavioral trajectories to extract the relevant tasks and to measure transitions between these tasks. This approach enables us to quantitatively describe how division of labor changes over time and to understand how interactions between individuals affect task allocation. The unique experimental control provided by the study system allows us to directly compare the social organization of replicate colonies and to obtain a statistical description of division of labor and its plasticity. The next step will be to perform manipulative experiments where division of labor is perturbed and its adaptation measured. For instance, how does a colony respond to the sudden replacement of foragers by nurses and vice-versa? And how does this dynamical adaptation depend on the demography and genetic composition of a colony?

OR172

Dynamics of division of labor in the social space using individually tracked ants

Raphael Braunschweig, Danielle Mersch, Paolo De Los Rios, Laurent Keller

Division of labor among workers is considered one of the important causes of the ecological success of ants. Workers change tasks throughout their life, but a precise quantification of the dynamics of this task change at an individual level remains understudied. Having previously shown that task, spatial structure and social groups are correlated in *Camponotus fellah* (Mersch et al. 2013), we use six colonies of this species where all ants were individually tracked over 11 days to precisely quantify, model and predict the dynamics of division of labor using social group transition dynamics. Our refined network analysis reveals that the network consists of two overlapping groups: nurses and foragers, and that about a third of the workers belong to both groups. To dissect the dynamics, we estimated the foraging membership, i.e. how much an individual belonged to the forager group, for each ant and each day. Using the first seven days of this simplified time series of all workers of each colony, we show that the group transition dynamics of an individual worker can be mapped onto a single colony-specific sigmoidal (slow-fast-slow) curve. To test our model, we compared the observed foraging membership values of the last four days to those predicted by the sigmoidal curve. Our results show that the sigmoidal curve predicts future individual behavioral trajectories better than a model assuming a linear change or no change at all. Together, these results highlight that ants that are intermediaries between nurses and foragers have fast social dynamics suggesting that they might be the first ones to switch tasks if the colonies social structure is disrupted. Ref: Mersch, Crespi, Keller, Science 340: 1090

OR173

Slow behavioral reorganization following experimental manipulation of caste ratios

Danielle Mersch, Raphael Braunschweig, Alessandro Crespi, Laurent Keller

Ants live in organized societies with a dynamic division of labor among workers. We previously showed that workers of *Camponotus fellah* colonies organize themselves in three social groups, each representing a functional behavioral unit with workers moving from one group to the next as they age (Mersch et al. 2013). To investigate the dynamics of these behavioral transitions, we tracked all workers in 15 colonies over a week to determine their social group. We then performed targeted removal experiments, removing either 25% of nurses, 25% of foragers, or 25% randomly selected workers. Subsequent tracking of these colonies for another two weeks revealed that the remaining workers need more than a week to reorganize and compensate the lost workforce, suggesting that slow physiological maturation processes might underlie the behavioral and social organization of division of labor. Ref: Mersch, Crespi, Keller, Science 340: 1090

OR174

*Neighbor removal increases forager longevity, slows progression through temporal castes (*Pogonomyrmex badius*)*

Christina Kwapich, Walter Tschinkel

In the Florida harvester ant (*Pogonomyrmex badius*), aging workers move through a sequence of labor roles, culminating in foraging. By wire marking age-cohorts in field colonies, we demonstrated that a 5 fold difference in the age at first foraging exists between workers born in summer and autumn (43 vs. 200+days). The interaction of both development rates generated a seasonal pattern of forager allocation, conserved across years, for more than 55 field colonies. Here, we ask how these patterns are influenced from the top-down by forager longevity. In this study, removal of conspecific, neighboring colonies resulted in an immediate and significant increase in monthly forager survival (+40%), which in turn suppressed the movement of younger workers into the forager population, resulting in a maintenance of forger number. Likewise, when foraging-range was limited, longevity increased under both starved and fed conditions, delaying the movement of younger workers into the forager population and increasing their tenure in prerequisite labor roles. In contrast, removal of 50 -75% foragers did not increase per diem forager addition, workers were not drawn from other behavioral castes to fill induced labor gaps, and larvae died in proportion to foragers lost. Overall, this study suggests that development rate is influenced by increased worker longevity but not death rate (as might be suggested if labor roles were filled due to low occupancy). Furthermore, our results challenge the idea that foragers in social insects are disposable and that selection has favored a lifespan that predicts external mortality. We predict colony growth to occur when death rate falls below 3% per day, at 1 colony per 670 M2; resulting in increased forager tenure and suppression of younger workers. Development rate was also correlated with early adult and larval nutrition (15N), and percent fat in newly enclosed adults, suggesting multi-directional effects.

OR175

Individual differences in honeybee trophallaxis network connectivity

Tim Gernat, Vikyath Rao, Weibing Deng, Nigel Goldenfeld, Martin Middendorf, Harry Dankowicz, Gene Robinson

Liquid food exchange (trophallaxis) is a common social interaction that fulfills important functions in many social insect societies. In honeybees, trophallaxis has been extensively described in the context of feeding and the transport of materials. In addition, it has been implicated as a communication channel through which honeybees may transmit chemical signals to coordinate their flexible age-related division of labor. As a first step to experimentally test this hypothesis, we studied the pattern of trophallaxis interactions in single-cohort colonies made of 1000 individually marked day-old bees, in which there is initially no division of labor. Within one week these colonies partition into hive bees and foragers, allowing us to study if there is a relationship between the timing of an individual’s age at onset of foraging and the pattern of trophallaxis interactions she experienced. To enable an experiment of this nature, we developed a novel method to automatically identify and track all bees living inside a glass-walled observation hive with high temporal resolution. We used these observations in conjunction with new computer vision algorithms to automatically detect when two honeybees engage in trophallaxis. Compiling this information into a time-aggregated social interaction network revealed striking differences in the connectivity between individual bees. We will discuss whether this diversity has possible functional consequences with respect to the remarkable ability of honeybees to establish and maintain division of labor at the colony level while being faced with environmental changes.

OR176

Reduction of fecundity precedes onset of foraging in ants

Bartosz Walter

An age-independent division of labour is common in workers of many eusocial insects (ants, bees, wasps, termites), where individuals divide into nest-dwelling nurses and outside-nest working foragers. In addition, foragers exhibit ovarian atrophy or even lack of ability to lay male destined eggs when compared to highly fecund nurses. As the differences in ovary development are not strictly age dependent, it is not clear whether decreased fecundity precedes onset of foraging or is a consequence of foraging wear and tear. In the study, I examined fecundity (ovarian development and ability to lay male designed eggs) of nurses and same-age 'new' foragers, just after the onset of foraging in the ants *Temnothorax unifasciatus* and *Myrmica rubra*. I found that 'new' foragers had significantly reduced fecundity compared to nurses already at the onset of foraging, indicating that ovarian atrophy precedes the onset of the behaviour. The findings are consistent with the subfertility hypothesis, proposing that differences in fecundity potential between cooperatively breeding insects resulted in division of labour, and thus, were important for the origin and evolution of eusocial insects.

OR177

What makes a reproductive worker in a clonal ant?

Abel Bernadou, Giacomo Alciatore, Danon Clemes Cardoso, Maykon Passos Cristiano, Jürgen Heinze

Division of labour in insect societies is one the tenets of eusociality. However, the proximate factors underlying task allocation and specialization in social insects are not yet fully understood. For example, there is firm evidence for genetic variation, morphology, individual experience or developmental factors in the propensity of individuals to take over particular tasks. Nevertheless, a division of labor still exists among individuals that lack these differences. A case in point is in clonal ants. Little is known about the factors causing task allocation in these cases. Workers of the tropical ant *Platythyrea punctata* are capable of producing female offspring from unfertilized eggs. Clone-colonies are characterized by a clear division of labor between one (occasionally several) reproductive worker and a number of non-reproductive workers. This reproductive division of labor is based on rank orders established by workers by fighting, but what determines rank differences among nestmates remains unanswered. In this study, we first investigated whether division of labor in *P. punctata* is based on age-polyethism between nestmates and if it correlates with the egg-laying capacities of workers. Then, we tested whether clone origin of workers influence their propensity to take over particular tasks in the society, i.e. the reproductive division of labor. First results reveal that there appears to be an age-based division of non-reproductive labor between nurses and foragers. By chimeric assemblies of clones, we followed the formation of hierarchies in young worker groups. Our results show that a hierarchy readily appears between workers in few days but their clonal origin does not affect division of labor.

OR178

Ergatoid queens actively contribute to colony emigration in Mystrium oberthueri

Diane Bouchet, Christian Peeters, Brian Fisher, **Mathieu Molet**

Nest emigrations are perilous for social insect colonies. Outside their nests, adults and brood are exposed to dangers. The behavioural mechanisms of emigration are thus likely to be under strong selective pressures. Most studies on emigration focused on monogynous species where survival of the queen is paramount, but emigration processes are largely unknown for species having several queens per colony. In colonies of *Mystrium oberthueri*, members of the morphological queen caste are as numerous as workers although only a few of them mate and reproduce (polygyny). All queens perform intranidal tasks such as brood care, whereas workers focus on hunting and nest defence. Accordingly, we expected queens to actively participate in emigration and to be less protected. Using four colonies, we studied the dynamics of 16 emigrations with special focus on individual behavioural profile. Non-reproductive queens were actively involved in recruitment and brood transport, although not as much as workers. Reproductive queens and young ants (including virgin queens and workers) preferentially walked directly to the new nest without carrying brood. Accordingly, the physiological status of individuals had more impact on their behavioural profile than their morphological caste. A chemical trail was probably used. This highly organized emigration process may underpin dependent colony foundation as they both involve the coordinated movement of nestmates.

OR179

Rescue of callows in Cataglyphis cursor *ants*

Elise Nowbahari, Celine Amirault, Karen L. Hollis

Previously we have shown that *Cataglyphis cursor* ants are capable of highly sophisticated rescue behavior in which individuals are able to identify what has trapped a nestmate victim and to direct their behavior toward that specific obstacle. In addition, we have shown that rescue behavior is constrained by a division of labor: Whereas foragers are able both to give and receive the most help, inactives neither give nor receive any help whatsoever, and nurses give and receive intermediate levels of aid. This profile of rescue behavior across the three castes suggests that workers' age may be a critical variable. In this species, as in others in which a sensitive period for nestmate recognition exists, young ants (callows) that are less than four days postemergence - and thus younger than inactives in our previous study - are able to be adopted by heterospecifics, suggesting that they are not yet recognized as belonging to a specific colony. However, it is not known if callows carry the colonial odors and if they are able to solicit or receive help when trapped. In the present study, we artificially ensnared individual *C. cursor* callows and tested them with a group of five potential rescuers, either foragers or nurses. In addition, we performed biochemical analyses of released substances by trapped callows and adults using Solid Phase Micro Extraction (SPME). Our results show that callows are able to trigger rescue behavior in both foragers and nurses. Nonetheless, homospecific callows are rescued for a longer duration than either heterocolonial or heterospecific callows. Finally, biochemical analyses revealed the presence of volatile components in adults, but not in callows, suggesting that, although the call for help in callows may be different than the call for help in adults, it releases the same precisely-directed rescue behavior.

OR180

Priority effects in ants: implications for behavioral dominance and tradeoffs

Roxana P Arauco-Aliaga, Frederick R Adler, Donald H Feener

Studies of priority effects in ant communities have focused on the slow time scale of competition for space, in which the first species to colonize a patch can resist invasion. Priority effects at this colonization stage are most relevant for species that establish territories. Ground dwelling ants, which rarely establish territories, compete for food resources on a much faster time scale in a race for exploitation with other colonies. In this context, priority effects influence competitive outcomes by favoring the species that mobilized foragers to the resource first regardless of its relative behavioral dominance. If priority effects were common, they should weaken the strength of important ecological tradeoffs, such as the behavioral dominance-resource discovery ability tradeoff. Behaviorally dominant species would not always be able to successfully usurp resources from behaviorally subordinate species. We explored the role of priority effects and its impact on measures of behavioral dominance in one of the most diverse Amazonian ground-dwelling ant communities (Madre de Dios, Peru). We monitored competitive interactions at baits (980) almost continuously for two hours, keeping track of the timing of their arrival, interactions and recruitment for all species in 12 quadrats of 25m by 25m distributed over an area of ~670 ha. To account for priority effects, we distinguished ‘wins’ at local resources in which a species successfully defends the resource from an intruder (‘repels’) from those in which the species usurps the resource from a previous owner (‘takeovers’). We recorded 1085 pairwise interactions that resulted in either repels or takeovers. This study was restricted to the 635 interactions involving the most common species. Using generalized linear models, we found that priority effects increase the probability that a species will repel an intruder but did not favor subordinate species consistently enough to mask the effect of the behavioral dominance-discovery ability tradeoff.

OR181

Fossil versus molecular datings of major ant subfamilies

Vincent Perrichot

Despite the interest the ants (Formicidae) have long generated, the group's evolutionary history has become more clearly established only recently, and yet our understanding of the diversity and phylogeny of these organisms is far from complete. Substantial advances have been made in the last decade by the discoveries of primitive fossil and extant lineages, and by molecular phylogenetic analyses. Recent molecular divergence dating estimates suggest that crown group ants arose some time between 115 and 168 million years ago, depending on the methodology applied (BEAST, penalized likelihood, etc.) and the assignment of fossils as stem group or crown group calibration points. This time interval is devoid of fossil ants, however, and the earliest known fossils are currently reported from mid-Cretaceous (ca. 100 Mya) ambers from France and Myanmar. These included only members of extinct or uncertain subfamilies thus far, but new discoveries presented here have revealed the presence of three of the 'big four' modern subfamilies in this time frame, i.e. Dolichoderinae, Formicinae, and Ponerinae. These findings and an overview of other known Cretaceous ants show that molecular divergence datings proposed respectively for each three subfamilies, which did not exceed 90 Mya in previous studies, were all underestimated. Meanwhile, this argues for an age of crown group ants within the 139-158 Mya as proposed by the most recent study, thus closer to the Jurassic/Cretaceous boundary than previously thought.

OR182

The evolution, diversification, and biogeography of the ants

Corrie Moreau

The ants (Hymenoptera: Formicidae) are undoubtedly one of the major evolutionary success stories. With over 13,000 described species distributed on all continents except Antarctica and exhibiting ecological dominance in almost all terrestrial habitats understanding the factors that have facilitated their diversification may shed light on broader evolutionary patterns. With a long evolutionary history dating back ~140 million years ago for the modern ants, investigating the role biogeography and shifts in rates of diversification play may provide insights into the factors that have facilitated their evolutionary success. Also, understanding the role co-evolved microbes play in diet shifts and habitat use have provided new insights into how these coevolved systems have facilitated the diversification of the ants. Taken together our work demonstrates that several factors have promoted the evolutionary success of the ants including the rise of the flowering plants and expansions of the associated tropical ecosystems, as well as associations with novel gut bacteria.

OR183

Ant global diversity: opening new possibilities in ant-biology.

Benoit Guenard, Michael Weiser, Evan Economo

The study of global diversity patterns has been restricted to a few groups of organisms belonging to vertebrates or plants. Yet, insects represent the vast majority of living species. Our comprehension of biodiversity patterns, the processes that underpin them, and the future prospects for biodiversity, is thus largely incomplete. Here, we present the global diversity of the most diverse group of social insects, ants, with over 14000 described species. We compiled distribution data from over 7700 publications, which combined with museum records represents the core of the Global Ant Biodiversity Informatics (GABI) database. This work represents the first global assessment of the distribution of a diverse group of insects. The compilation of over a million records of ant species distribution allows us to solve important questions in biodiversity and conservation such as the identification of main diversity and endemism hotspots, or the importance of biological invasions across the world. Finally, I will briefly introduce some of the possibilities offered by this database in the study of social insects ecological and evolutionary questions. GABI will represent an important new tool for ant research and provide guidance for the development of global database for other insect groups as well.

OR184

Biogeography - Contributions from systematics and collections

Steve Shattuck

Specimen-based information is crucial for exploring ecological and biogeographic patterns along both the taxonomic and geographic axes. However, significant challenges exist in determining the quality of specimen data and selecting records which are ‘fit for purpose’ for any given analysis. We are developing methods for detecting errors in existing specimen-based data by examining multiple data sources for inconsistencies. These inconsistencies can then be used to detect both errors and potentially interesting ‘outlier’ phenomena. Additionally, recent advances in modelling methods allow us to interpolate existing data to fill gaps in our biogeographic understanding of individual taxa. Combined, these methods provide robust datasets which can be reliably used to answer a wide range of biological questions.

OR185

The impact of climatic changes on ant distributions

Gracen Brilmyer, Corrie Moreau

Understanding how and if human induced climate change is impacting biodiversity will require large and extensive biological collection data spanning at least the last 100 years. Leveraging the ant collections of the Field Museum of Natural History in Chicago, USA we are addressing this question for species found across the United States. Ants (Hymenoptera: Formicidae) are widely considered biological indicators of ecosystem health and our recent efforts to database, digitize, and georeference the >25,000 ant collections provide us with a unique opportunity to produce an extensive snapshot of ant biodiversity spanning 1915 to the present. The depth and breadth of this project will allow us to address questions surrounding the effects of climatic changes on ant biodiversity by determining if ant species are shifting their geographical distributions within the United States through time. Our preliminary ecological niche modeling suggests a northward shift in ant distributions for many low elevation species and an upward shift in elevation for species found in mountain regions, which is consistent with models of global warming and climate change. Although these findings have potential impacts for all ants, species with limited geographic ranges are the most likely to face serious extinction threats.

OR186

Biogeography, ecology and evolution of ant genus Myrmica *from Himalaya*

Himender Bharti

Ants of genus *Myrmica* are restricted to high altitude regions of Himalaya. The genus is represented by 31 species placed in 6 species groups with few species still uncertain about their placement. These species have pocketed themselves at different elevations in Himalaya. Most of the Himalayan *Myrmica* species are endemic to this region. Some of the species have restricted distribution being topographic specialists, while few are weedy. Morphologically, most of the Himalayan species represent relic forms/old lineages with plesiomorphic features and differ considerably from rest of the Palearctic species. It seems quite probable that after the formation of Himalaya, these lineages got isolated from rest of the *Myrmica* fauna and diversified here. This view has been authenticated by the recent discoveries. Being subject to high altitude stress in the form of short summers, sub-zero temperatures in winter, paucity of resources and almost negligible nesting sites, these ants have developed different survival strategies. These include various forms and phases of parasitic interactions ranging from temporary parasitism to inquilism (the highest form of which has been discovered recently from this region, the parasite being younger than host and the discovery also points towards sympatric mode of speciation). The other strategies are topographic specialization; polygyny; polydomy. endogenously heterodynamous life cycles (in which the compulsory diapause of queen, workers and immatures, help the evolutionary novel ants to tide over hostile weather conditions). To work out the evolutionary relationships of these species, molecular markers are being used, which will unfold species relationships, work out level of interspecific variations, and unravel cryptic species.

OR187

Wingless workers are the innovation underlying the evolutionary success of ants

Christian Peeters

Ant colonies are organized similarly to those of wasps and bees: reproductive altruism, age polyethism, complex communication and perennial nests. Yet ants have achieved much greater ecological dominance, and their lifestyles and diets are more diverse. Thus factors other than sociality must be involved, and I argue that the basic design of ant workers was decisive. An ancestral commitment to ground-living is reflected by both a shift to prognathy and the evolution of wingless helpers. First, forward-pointing mandibles allow for effective manipulation of objects or prey. Mandibles thus become multi-purpose tools that are assisted by specialized musculature in the head and prothorax (Keller et al. 2014). Second, winglessness permitted a complete dissociation between reproductives and helpers, hence phenotypes well-suited for novel foraging behaviours and ecological niches. The design of ant workers also allowed lowering per capita costs: loss of wing muscles, smaller size, thinner cuticle, elimination of superfluous organs, and age-related reduction of metabolic costs (e.g. brain, muscles, poison gland) in the context of polyethism. Cheaper workers removed limitations on increased colony size unseen in wasps and bees, leading to new strategies of resource utilization. Conversely, ant queens became more expensive because of the acquisition of substantial metabolic reserves prior to dispersal, further increasing caste dimorphism. This means that queens of most formicoid species do not need to forage while they found alone - an adaptation unknown in social wasps and bees. I compare poneroid and formicoid species to highlight some of the modifications in morphology from aculeate wasps. The evolution of cheaper wingless workers and costly queens is unique to the ants. Flying constraints in wasp and bee workers - infertile just as in ants - make such divergence impossible. Keller R.A., C. Peeters & P. Beldade (2014) eLife 3:e01539

OR188

Utilizing genotyping-by-sequencing to elucidate Neotropical army ant evolution

Max Winston, Daniel Kronauer, Corrie Moreau

As keystone predators in Neotropical forests, hundreds of vertebrate and invertebrate organisms associate with the 12 known species of *Eciton*, which exert strong top-down effects on community structure in the leaf litter. Despite their clear ecological importance and strong history of research, a comprehensive phylogenetic and biogeographic study is lacking for the important clade. This absence of phylogenetic resolution has been a substantial impediment to determining ecological and evolutionary dynamics, and presents an opportunity to investigate broader mechanisms of diversification in the Neotropics. Using the high-throughput method genotyping-by-sequencing (GBS), we harvested SNPs from across the Eciton genome to infer the clade's phylogenetic history and their colonization of Neotropical habitats. This work contributes both to a better understanding of the ecological impact of Neotropical army ants as well as the significance of biogeographic processes in macroevolutionary dynamics.

OR189

Physiology and the transition from hunting to farming in ants

Jonathan Z. Shik, Ernesto Gomez, Juan C. Santos, Mike Kaspari, Jacobus J. Boomsma, William T. Wcislo

Most ant colonies are comprised of workers that cooperate to harvest resources and feed developing larvae. Around 50 million years ago, attine ants adopted an alternative strategy, harvesting resources used as compost to produce fungal gardens. While fungus cultivation is considered a major breakthrough in ant evolution, the associated physiological consequences remain poorly understood. Combining lab and field experiments, we use phylogenetically-informed comparative analyses to test for metabolic and nutritional transitions across millions of years of evolutionary diversification corresponding to: 1) the transition to attine colony-farms from ancestral hunter-gatherer colonies ca. 50 MYA, and 2) the adoption of a specifically ‘domesticated' (gongylidia-producing) cultivar lineage by the ‘higher' attines ca. 20 MYA. We find two major metabolic transitions. First, colonies of the earliest ‘lower'-attine farmers shifted significant fractions of biomass from ant tissue to fungus-gardens and transitioned to lower mass-specific metabolic rates. Second, the transition to higher-attine cultivation was associated with increased colony metabolism, and metabolic scaling approximately identical to that observed in hunter-gatherer ants. We next explored the nutritional consequences of the farming transition. We first tested whether the shift from protein-rich to carbohydrate-rich diets, seen when humans adopted agriculture, also applied to the attine ants. We did this by analyzing the nutritional composition of resources harvested among hunting and farming colonies in a diverse tropical-forest ant community. We next used lab experiments to test whether true domestication has yielded cultivars that extract composted nutrients (supplied by ants) more efficiently as to allow better colony performance. We propose that replacing live storage tissue in the form of ant biomass with fungal mutualist biomass provided energetic and nutritional advantages that may have contributed to the further adaptive radiation of the attine lineage, and we outline critical assumptions that, when tested, will help link physiology, farming efficiency, and colony size.

OR190

Global population structure and invasion history of Solenopsis geminata

Dietrich Gotzek, Heather Axen, Sara Helms Cahan, DeWayne Shoemaker, Andrew Suarez

Biological invasions are largely thought to be contemporary, having recently increased sharply in the wake of globalization. However, human commerce already became global by the mid-16th century, when the Spanish connected the New World with Europe and Asia via their Manila galleon and West Indies trade routes. We use a large genetic dataset to infer the population structure and trace the global invasion of one of the world's most widespread and invasive pest ants, the Tropical Fire Ant, *Solenopsis geminata*. We show that Old World populations are introduced and their movement is highly consistent with historic trading patterns, suggesting that Spanish trade introduced the Tropical Fire Ant to Asia in the 16th century. We identify southwestern Mexico as the most likely source for the invasive populations, which is consistent with the early use of Acapulco as the major Spanish port on the Pacific Ocean. From there, the Spanish galleons shipped silver to Manila, which served as a hub for trade with China. The genetic data document a corresponding spread of *S. geminata* from Mexico via Manila to Taiwan and from there throughout the Indo-Pacific. Our descriptions of the worldwide spread of *S. geminata* represent the first documented case of a biological invasion of highly invasive and globally distributed pest species due to the earliest stages of global commerce. We hypothesize that many invasive ants reached their global distributions via early trade networks, when the use of soil as ballast was still common.

OR191

Historical biogeography predicts social complexity in the small carpenter bees

Sandra Rehan

The small carpenter bees, genus *Ceratina*, offer important insights into the early stages of sociality. *Ceratina* provide a unique opportunity to study the evolution and maintenance of social complexity in a group benefitting from detailed behavioural studies and a well-established phylogeny. Small carpenter bees are globally-distributed (excepting Antarctica) and comprise a single genus *Ceratina* with 21 subgenera, most of which are endemic to continents. Species range from solitary to eusocial; solitary species are typically found in temperate environments and social groups are recurrent in tropical regions. Unequal rates of forward and backward dispersal are one mechanism that will produce regional differences in biotic composition. Any such differences will affect how ecosystems are assembled and it is important to understand the implications of niche composition on social evolution. Using sequence data from three genes and 99 species, and ancestral distributions the historical biogeography of this genus is inferred using Bayesian and maximum likelihood approaches. These data show that *Ceratina* had an African origin in the early Eocene followed by rapid radiations around the globe. Once a continent was occupied there have been no back dispersals. These results are similar to studies on two other major bee groups indicating that back dispersals are very rare. It is unlikely that physical barriers can explain these patterns. However, they might be explained by assembly rules such as niche pre-emption or reduced opportunities for escaping into enemy-free space. At a continental level it is likely that the composition of bee faunas is more strongly influenced by very early dispersal events rather than later physical barriers to dispersal. These data highlight the importance of molecular phylogeny and historical biogeography for understanding the relative roles of phylogenetic inertia and regional ecology on the evolution of social phenotypes.

OR192

Evolution and body size: drivers of global termite patterns

Cecilia Dahlsjo

Termites are one of the most important invertebrate decomposers in lowland tropical forests and play a major role in ecosystem processes, such as nutrient turnover and availability, which affect forest ecosystems as a whole. The Termitidae family comprises four of the five termite functional groups which feed on dead organic matter from wood to mineralised soil. Termitidae evolved in Africa and colonised tropical regions in other parts of the world through dispersal events either by crossing land bridges or by rafting. Due to the physiological and functional differences of termite functional groups their dispersion, and subsequent evolution, have affected the assemblage of lineages on different continents. It is known that termite species diversity is different across the tropical regions; and although this is important for understanding assemblage patterns, in order to quantify the importance of termites in ecosystem processes biomass and abundance data are needed. Until now, comparable biomass and abundance data have been sparse and so comparisons across regions have not been possible. Through extensive sampling, using a standardised sampling protocol in Peru, the available comparable biomass and abundance data from Malaysia and Cameroon were complimented and enabled the first intercontinental comparison of its kind. The intercontinental differences were mainly due to the dominance of two large-bodied soil-feeding termite lineages, which are endemic to Africa, and the absence of fungus-growing termites from South America. These findings strongly suggest that the intercontinental patterns may be caused by evolutionary history and not present day climate. Allometric relationships (population density-body size and metabolic rate-body size) in termite functional groups among the three regions were also examined. We show that the evolutionary drivers that influence termite biogeography patterns also affected the Allometric relationships with potential impacts on ecosystem processes.

OR193

Evolution of Syntermitinae from morphological and molecular analysis

Mauricio Martins Rocha, Adriana Coletto Morales, Eliana Marques Cancello

The Syntermitinae, an endemic subfamily of the Neotropical region, is well characterized by the soldiers with large developed frontal glands, opening in a large aperture in the tip of a frontal tube, variable in size among the species. The relationships inside the subfamily taxa have never been deeply studied and this is our main objective. We conducted a series of phylogenetic analysis including 35 of the 99 Syntermitinae species, including all the genera, using morphological and molecular data. For the morphological data we conduct a parsimony analysis, using implicit weighting and 80 characters. For the molecular data we realize a maximum likelihood analysis, including two set of genes (COII and Cytb) and GTR+I+G strategy. The most parsimonious trees of both analyses corroborate the monophyletism of Syntermitinae and most of the subfamily genera; the only polyphyletic groups are Embiratermes and Ibitermes. The most basal genera form a paraphyletic group that mainly consume humus and organic-rich soil (for example, *Curvitermes*, *Cyrilliotermes*), and another group correspond to the litter feeders and grass feeders genera (for example *Syntermes*, *Cornitermes*, *Rhynchotermes*) as a more derivate lineage. Similar defense mechanisms, evidenced by the soldier external morphology, appear independently among Syntermitinae. This data suggest that the divergence among feed niches may play a central role for the diversification of the group.

OR194

Phylogenetics and evolution of Australian Nasutitermitinae

Daej Arab, Stephen Cameron, Theo Evans, Anna Namyatova, Nathan Lo

In Australia, Termites are keystone species and ecosystem engineers in many environments, particularly in arid areas. Australia contains a total of 260 termite species, representing 5 of the 7 generally recognized families worldwide. One important group in Australia is Nasutitermitinae. There are 6 representative genera with over 44 representative species in Australia (of over 200 species worldwide), which are found all across the Australian continent in various ecoregions including temperate forests, semi-deserts, savannah woodland and others. Australian Nasutitermitinae are particularly known for their variability in nest architecture and construction. Despite the ecological and economic importance of the Australian termite fauna, their systematics and evolution is poorly understood. We are constructing a comprehensive phylogeny of the Australian Nasutitermitinae based on mitochondrial and nuclear gene markers and will report our most recent results.

OR195

The evolution of mound building in the Australian Coptotermes termites

Timothy Lee, Stephen Cameron, Theodore Evans, Simon Ho, Dieter Hochuli, Nathan Lo

The mounds of termites are the result of some of the most complex co-operative behaviour in the animal kingdom, but the evolutionary origins of this behaviour remain poorly understood. Mound-building has evolved multiple times independently in the termites, including one occurrence in the Australian *Coptotermes*. Some Australian *Coptotermes* species also nest in living trees, while the most closely related non-Australian species predominantly nest in dead wood. Worldwide, the genus includes some of the most destructive termite pests. We conducted a phylogenetic study of the Australian *Coptotermes* in order to shed light on the evolution of nesting behaviour in this group, sequencing mitochondrial COI and COII and nuclear ITS1 in >100 specimens representing all currently described Australian species. We have found that mound-building behaviour arose at least three times independently within the Australian *Coptotermes*, in all cases resulting in distinctive thick-walled mound architecture. We have used ancestral-state reconstruction and molecular-clock analysis to estimate the timing of the appearance of new nesting types in the Australian *Coptotermes*. Along with the results of logistic a regression analysis using climate data, this suggests that the evolution of new nesting types in the Australian *Coptotermes* coincided with a period of climate fluctuation on the Australian continent. We tentatively conclude that the ancestral Australian *Coptotermes* was a living-tree nester, and that mound-building in the Australian *Coptotermes* arose in response to the increasingly erratic availability of moisture in the Australian environment. Finally, we find that genetic diversity within the Australian *Coptotermes* is greater than previously thought, with a possible three new species discovered.

OR196

Molecular evidences that Heterotermes tenuis *should be a species complex*

Tiago Carrijo, Adriana Morales, Eliana Cancello

Proposed originally by A.R. Wallace, the riverine barrier hypothesis states that major Amazonian rivers significantly reduce or prevent gene flow between populations inhabiting opposite river banks. Thus, the alignment of Amazonas-Madeira-Mamoré Rivers divides the Neotropical region into two areas of endemism for a diversity of taxa. We used the mitochondrial gene COII intending to test whether the Madeira River was a geographic barrier to the genetic flow of the populations of *Heterotermes tenuis*. We sampled in 12 areas along 200 km near the banks of the Madeira River, in the state of Rondônia, Brazil, being six on the right bank and eight on the left. We used 84 sequences of *H. tenuis* from Rondônia, six from the Cerrado biome, and three from GenBank (from Manaus-Brazil, French Guiana and Ecuador); and one of *H. longiceps* as outgroup. We found a strong genetic structure within our samples, however, without any relation to the side of the river or any other spatial feature. In a tree built with a Bayesian approach in the software BEAST, using the model HKY+I, we were able to distinct two groups: one with the individuals from French Guiana, Manaus and 28 individuals from RO, and the second with the individuals from Ecuador, those from the Cerrado and the other 56 individuals from RO. Besides, the haplotype network, F statistics and K2P genetic distance, also pointed to the same direction, corroborating the isolation of these two groups. The divergence between them was estimated with an uncorrelated lognormal relaxed clock, and dated from approximately 20 million years, in the Miocene. All these evidences led us to suggest that the name *H. tenuis* should comprises at least two morphological-cryptic species possibly isolated during the Miocene marine incursions, much earlier than the riverine barriers, as first hypothesized by us.

OR197

Macroevolution of Trap-jaw Ants in the genera Anochetus *and* Odontomachus

Fredrick Larabee, Andrew Suarez

Mandibles are an important trait in the evolutionary success of ants, and are the primary structures they use to physically interact with their environment during activities like foraging, predation, food processing, defense, nest excavation, and brood care. Although these essential functions constrain their morphology, ant mandibles display a remarkable amount of diversity, with some of the most extreme specializations found among trap-jaw ants in the genera *Anochetus* and *Odontomachus*, whose spring-loaded mandibles snap shut at some of the fastest speeds ever recorded for an animal movement. These rapid mandible movements are used to capture fast or dangerous prey (e.g. springtails or chemically defended termites), and to escape from predators. Trap-jaws may be an example of a key morphological innovation, a trait that allows an organism to interact with its environment in a novel way and may lead in increased ecological and species diversification. We used phylogenetic comparative methods to answer the following questions about diversification and mandible evolution in this lineage of trap-jaw ants: (1) What is the closest living non-trap-jaw ant relative to *Anochetus* and *Odontomachus*? (2) Are *Anochetus* and *Odontomachus* monophyletic sister groups? (3) Is trap-jaw performance (speed and force) correlated with species diversification? We constructed a time-calibrated molecular phylogeny for 90 species of *Anochetus* and *Odontomachus* based on two mitochondrial and four nuclear genes. This phylogeny was then used to infer patterns of trap-jaw evolution, examine the relationship between trap-jaw morphology and performance, and measure correlations between trap-jaw performance and diversification rate. Together our results provide insight on how a morphological innovation has influenced the patterns of species distribution in a remarkable social insect.

OR198

Congruence of morphological characters in habitats occupied by Strumigenys *species?*

Douglas Booher

Strumigenys is a large and morphologically diverse genus of litter-dwelling ants that inhabit a broad array of different habitats. Jaw structure, dentition, spongiform tissue, and hair patterns are morphological traits that are highly variable between species, yet exhibit little or no variation within species. Whether variation in these traits is associated with habitat differences is unknown. Using data I collected on 33 species across the southeastern U.S. in 280 sampled habitats, I evaluated whether species co-occurring in similar habitats have more similar morphological traits than expected by chance. I quantified habitat differences using 13 abiotic and biotic variables. I used clustering methods to quantify habitat similarities, and then tested the degree of congruence between habitat similarities and ant morphological similarities. The results of the analysis help provide insights regarding niche conservatism, phylogenetic conservatism, and habitat filtering in *Strumigenys* ant communities.

OR199

Ant mosaics in primary rainforests across four continents

Maurice Leponce, Jacques H.C. Delabie, Petr Klimes, Thibaut Delsinne, Justine Jacquemin, Alain Dejean

The existence of ant mosaics, or the mutually exclusive distribution of numerically dominant ants (NDA) in tropical tree canopies, has been demonstrated for plantations but remains contentious concerning primary forests mostly due to the difficulty in collecting and observing ants in trees that grow up to 30m in height. Our goal was to overcome this problem and study the three-dimensional distribution of NDA in primary rainforests across four continents. We developed the baitline method allowing us to collect arboreal ants every 5 meters along tree trunks and to conduct aggressiveness tests between ants collected from neighboring trees. These direct observations allowed us to define ant numerical dominance, species coexistence and to delineate NDA spatio-temporal extension. Ant colonies were mapped in quarter hectare forest plots in French Guiana, Brazil, the Democratic Republic of the Congo (RDC), Mozambique, Laos and Papua New Guinea (PNG). Our results indicate that territorial NDA species are found in every lowland forest site investigated. However, there seems to be a gradation in the ant mosaic structure according to the high (e.g. in PNG, RDC) to low (e.g. in Laos) prevalence of NDA on the trees. In some instances (e.g. in Mozambique or in PNG), *Crematogaster* supercolonies completely dominated the plots. Trees where NDA only forage intermittently can be observed at the border of a large NDA territory (e.g. that of Azteca in French Guiana) and create temporary gaps. Two mutually aggressive NDA are sometimes briefly observed on the same tree (e.g. the vertical segregation of *Crematogaster* and *Oecophylla* foragers along a tree trunk in PNG). These results emphasize the three-dimensional and dynamic structure of ant mosaics. They also stress that, in the absence of behavioral observations, statistical analyses of species co-occurrence on individual trees, often used to detect ant mosaics, must be interpreted with care.

OR200

Biodiversity and life history adaptations of army ant myrmecophiles

Christoph von Beeren, Daniel Kronauer

Over the last decades, biologists have begun to explore the last biological frontiers, from the deep sea to tropical rainforest canopies. Despite these efforts, such frontiers of largely unexplored biodiversity still exist today, albeit on a smaller scale. One example is the microcosm of myrmecophiles associated with tropical army ants. For instance, more than 300 myrmecophile species, primarily arthropods, are associated with a single species of Neotropical army ant, Eciton burchellii. It is clear, however, that this is only the tip of the iceberg and that many more species await scientific discovery and formal taxonomic description, especially because myrmecophiles of other army ant species are notoriously understudied. Furthermore, very little is known about the myrmecophiles' life cycles and basic life history adaptations. We study the myrmecophile communities associated with six *Eciton* army ant hosts at La Selva Biological Station, Costa Rica, by combining DNA barcoding for rapid species identification with detailed taxonomic descriptions. For each specimen, we provide species name, DNA barcode, digital voucher images, and detailed collection records. Such a reference database will form an essential baseline for further studies of these complex myrmecophile communities. A group of staphylinid beetles (genus *Vatesus*) exemplifies the benefits of our approach well. Our DNA-based species identification protocol uncovers cryptic species in this genus, and thereby provides evidence for unexpectedly high host specificity. Using DNA barcodes to match larval and adult stages, we demonstrate that both adults and larvae of all *Vatesus* species participate in colony emigrations, and that reproduction and larval development are remarkably well synchronized with the hosts' reproductive cycles. Besides revealing the biodiversity and life history adaptations of army ant myrmecophiles, a large-scale comparative analysis of different myrmecophile taxa will also unveil general evolutionary trends in such diverse multi-species networks.

OR201

Through the Looking-Glass: reflection of ant-diversity in ant-mimics

Yoshiaki Hashimoto, Tomoji Endo, Takao Itioka, Fujio Hyodo, Takashi Yamasaki

Ants show enormous diversity is the tropics, and high diversity of ant-mimics is also found in the regions. Focusing on ant-mimicking salticid genus *Myrmarachne*, we examined whether high diversity of ant-mimic is reflected on the ant-diversity in tropics, or not, as follows: 1) Ants and spiders are collected from canopy and ground layer respectively in tropical forest in different locations of Borneo, from 2004 to 2014. 2) We developed computer-vision tools, using the image-recognition technique, for assessment the mimetic resemblance by objective and quantitative indexes, and detected species-to-species association for *Myrmarachne* spiders to their ant models, by similarity index of shape, color and size. 3) Based on the judgments of mimic-model association, we tested whether the mimetic and species-diversity patterns of *Myrmarachne* were matched to morphological and species-diversity pattern of sympatric ants. 4) Using of null-model analysis and logistic regression analysis, we examined occurrence pattern of *Myrmarachne* spiders and ants in same habitat, and ascertained whether co-occurrence pattern was found between *Myrmarachne* spiders and their ant-models. As a result of the study, we found mimetic patterns, species diversity and occurrence patterns in ant-mimicking *Myrmarachne* spiders were closely matched with morphological and species diversity and occurrence patterns of ants in same habitat. In this presentation, we show supporting evidence that the enormous diversity of tropical ants play as a mechanism to creates high biodiversity in tropical forest, though ant-mimicking.

OR202

How do terrestrial ant-plant symbiotic networks change with altitude?

Tom Fayle, Nichola Plowman, Amelia Hood, Petr Klimes, Conor Redmond, Vojtech Novotny

Mutualistic interactions between ants and plants are widespread, particularly in the tropics. While these interactions, and the costs and benefits for the partners involved, have been reasonably well quantified, the way that the balance of these factors are impacted by changing altitude are very poorly understood. Understanding these shifts is important, as this is expected to shed light on responses of interaction networks to climate change. Here we investigate how the mutualistic interaction network between ants and their host plants changes along an altitudinal gradient (up to 1600m asl) on Mt Wilhelm in Papua New Guinea. We then focus on the most common interaction, that between *Myristica subalulata* and *Anonychomyrma sp.* and quantify how costs and benefits for the host plant change with altitude by conducting patrolling censuses, herbivory assays and experimental exposure of model herbivores. We find that in the lowlands multiple ant and plant species are involved in the mutualistic network, with little apparent specialisation. At higher altitudes, the interaction network becomes simpler, with the highest elevations being dominated by *Anonychomyrma*-inhabited *Myristica subalulata*, before a reduction in the abundance of even this species at 1600m. At higher elevations, the *Anonychomyrma* inhabiting *Myristica* plants patrol less and take longer to find a model herbivore. This seems to have resulted in increased levels of herbivory on plants at higher elevations, and may be the cause of the limitation of this plant species to lower elevations. We speculate that the driver of this pattern is reduced ant activity due to lower temperatures at higher altitudes, thus reducing the benefits for plants of ant inhabitation. We have demonstrated that interaction networks shift with altitude, and also, for one particular interaction, that the corresponding change in temperature may drive a shift in the balance of costs and benefits for one partner.

OR203

The role of Rhytidoponera metallica *in facilitating post-fire seed germination*

Kieren Beaumont, Duncan Mackay, Molly Whalen

Within fire-prone vegetation, ants may benefit myrmecochorous plants by protecting seeds within their nests during fire and by burying seeds at depths where seed dormancy can be broken by increased soil temperatures. In Australia, species of *Rhytidoponera* are predominant seed dispersers of myrmecochorous plants, yet little is known of the post-fire fates of seeds that they disperse. We investigated the role of *R. metallica* (Formicidae: Ectatomminae) in seed burial and post-fire seed germination of the three legume species; namely *Pultenaea daphnoides*, *Acacia pycnantha* and *A. myrtifolia*. Burial depth requirements for heat stimulated germination of the three plants species were determined by burying seeds within aluminium cans at a site prior to being burnt by a prescribed fire and at an adjacent site that remained unburnt. The fire increased germination levels for seeds buried at 1 and 2 cm below the soil surface. Prior to the fire, seeds were also fed to colonies of *Rhytidoponera metallica* and the proportions of buried *P. daphnoides* and *A. pycnantha* seeds that germinated from ant nests and non-nest locations were higher in the burnt than in the unburnt site. For *A. myrtifolia*, 8.1% of the seeds fed to *R. metallica* colonies located in the burnt site germinated, whereas 1.1% germinated from nests located in the unburnt site. These results show that *R. metallica* ants can facilitate post-fire seed germination, although this disperser also buries some seeds at depths at which temperatures required to break seed dormancy are unlikely to occur, and also discards seeds from their nests, so that discarded seeds would require reburial in order to be protected from fires.

OR204

Effects of prescribed burning on ant communities on Kangaroo Island

Sara Norwood, **Duncan Mackay,** Molly Whalen

We investigated the effect of variation in fire intensity on the diversity and abundance of ant communities in mallee remnants that were subjected to prescribed burning as part of a conservation management program (Eastern Plains Fire Trial). Three un-grazed roadside sites, burnt in spring 2009, were used in this study. Ants were sampled from pitfall traps over four sample years, 2009-2012, six months before, and six months,18 months and 30 months after burning. Within each site there were three burn treatment plots; a control unburnt plot, a low intensity burn plot, and a high intensity burn plot. Ants were collected in pitfall traps and identified to morphospecies level. Analyses were conducted on the richness and diversity of genera and morphospecies and on functional group composition. There were no significant effects of variation in fire intensity on species diversity, richness, composition of functional groups, or abundance of genera, although there were significant effects on the presence/absence of particular morphospecies. In all analyses, effects varied between sites and there were significant effects of sampling year. There was a general trend for a return to pre-burn abundances and presences and absences by 30 months after burning. These results suggest that the ant communities sampled are resilient to the disturbances caused by prescribed burning programs in this habitat.

OR205

Climate-mediated cooperation promotes niche expansion

Syuan-Jyun Sun, Dustin R. Rubenstein, Bo-Fei Chen, Shih-Fan Chan, Jian-Nan Liu, Mark Liu, Wenbe Hwang, Ping-Shih Yang, Sheng-Feng Shen

The ability to form complex, cooperative societies may explain why humans and social insects have come to dominate the earth. The shift from being a habitat specialist to a generalist as a result of being cooperative is thought to underlie the ecological dominance by these group-living species, yet this social conquest hypothesis has proven difficult to test empirically. Here we examine the ecological consequences of cooperation by quantifying the fitness values of cooperative (large groups) and non-cooperative (small groups) phenotypes in burying beetles (*Nicrophorus nepalensis*) along an elevational and temperature gradient. The primary benefit of cooperative breeding behavior in beetles is to jointly prepare carcasses more rapidly than their primary competitor, flies. We experimentally created large and small groups along the temperature gradient and manipulated levels of interspecific competition by heating carcasses to increase fly activity. Cooperative groups had similarly high breeding success at all temperatures and elevations, whereas non-cooperative groups only bred successfully at intermediate elevations. Moreover, cooperative groups had relatively higher breeding success than non-cooperative groups at lower elevations where climates are warmer and higher interspecific competition with flies reduces the fitness of small groups. Together, these results suggest that that cooperative groups performed as thermal generalists and non-cooperative groups as thermal specialists. Ultimately, studying the ecological consequences of cooperation may not only help us to understand why so many species of social insects have conquered the earth, but also to determine how climate change will affect the success of these and other social species, including our own.

OR206

Are local adaptations possible in Polistes *wasps?*

Perttu Seppä, Mariaelena Bonelli, Sanja Hakala, M Cristina Lorenzi

Colony founding stage in primitively eusocial wasps is characterized by complex and intertwined interactions among the wasps, their predators and intra and interspecific parasites. The result is a geographic mosaic of coevolution, where reproductive and social strategies are adapted to the presence of the parasite (Lorenzi & Thompson 2011). Most importantly, heavily parasitized populations can virtually lack the worker caste (Fucini et al. 2009), but the prevalence of both intra and interspecific parasites and the selection pressure they instigate varies widely among populations (Lorenzi & Thompson 2011). Adaptations for hosts to escape parasitism arise when local populations are spatially separated and lack important gene flow (Kaltz & Shykoff 1998). In this work, we studied whether local adaptations are possible in *Polistes biglumis* populations to escape parasitism from its social parasite *P. atrimandibularis*. We sampled both species from five well-separated mountain localities in the Alps (France, Italy, Switzerland). The prevalence of parasitism in sampled populations varied from zero to one fourth of the nests being parasitized and was roughly constant over years. We extended the study temporally by sampling same populations repeatedly between 2005 and 2013 and geographically by adding samples from southern Spain and central Italy. We evaluate the prerequisites of populations for local adaptations by assessing genetic variation and spatial structure in both host and parasite by using DNA microsatellites AFLP markers. From the genetic data, we explored how the parasite prevalence affects the level of genetic variation and demographic stability of host populations. Then we assessed how populations are connected by gene flow by using standard analysis and model-based Bayesian clustering.

Lorenzi MC, Thompson JN 2011, Evolution 65:3527-3542, Fucini S et al. 2009, Insect. Soc. 56:347-358, Kaltz O, Shykoff JA 1998, Heredity 81:361-370

OR207

Candidate markers for adaptive conservation genetics of bumblebees

Jonathan Ellis, Lucy Turner, Mairi Knight

Assessing genetic variation underlying ecologically important traits is increasingly of interest and importance in population and conservation genetics. For some groups generally useful markers exist for examining the relative role of selection and drift in shaping genetic diversity e.g. the major histocompatibility complex in vertebrates and self-incompatibility loci in plants. For invertebrates there is no such generally useful locus. However, phosphoglucose isomerase (Pgi) has been proposed as a useful functional marker in the conservation genetics of invertebrates. Where thermal microclimate varies, balanced polymorphisms may be maintained due to trade-offs between thermally stable and kinetically advantageous allelic forms. Loci underlying immunity also make good candidates in this context: they are expected to be important for population persistence and may exhibit diversifying or divergent selection. Predictions regarding the pattern of selection expected at immune system loci have been based on their interactions with pathogens, however, published studies report mixed results as to whether these are borne out or not. Social insects, including bumblebees, make an excellent model system to investigate genetic variation in this adaptive conservation genetic context. Species are variously threatened and isolated; some being ubiquitous and others occurring in highly fragmented populations. Threatened species have also been examined in a conservation genetic context using neutral markers in the past. Here, Pgi and innate immune loci were screened as candidate markers for assessing adaptive variation in bumblebees. Interestingly, in contrast to other taxa, very low levels of Pgi variation were found rendering this locus to be of little use as an adaptive marker in a conservation genetics context in bumblebees. Variation at IIR loci was also found to be very limited. Potential explanations and caveats regarding this lack of variation will be considered.

OR208

The gut microbiota of termites: evolutionary origin and functional adaptations

Andreas Brune

Termites degrade lignocellulose with the help of their intestinal gut microbiota. The general role of the microbial symbionts in the digestive process is slowly emerging, but the specific functions of individual populations and their evolutionary origin are still in the dark. Characterization of the hindgut community in a wide range of termite and cockroach species by pyrosequencing of bacterial 16S rRNA genes revealed strong differences among the major host groups, with dramatic changes in the relative abundance of particular bacterial taxa. When mapped onto the host tree, these changes coincided with major events in termite evolution. The acquisition of cellulolytic protists, which are abundantly present in all evolutionary lower termites, gave rise to large populations of bacterial symbionts that specifically colonize the flagellates. These flagellate symbionts were recruited among the gut bacteria and apparently serve to complement deficits in the nitrogen metabolism of their flagellate hosts. When the flagellates were lost in higher termites (family Termitidae), the availability of wood particles for bacterial colonization opened up new niches for fiber-digesting populations that had until then prevailed only in low numbers. Many of the bacterial lineages characteristic of the termite gut microbiota are present also in many cockroaches. While some members of this core microbiota seem to represent diet-specific lineages that have been independently acquired from the environment (host selection), others probably became associated with an ancestral cockroach already in the early Cretaceous (cospeciation).

OR209

Community profiling and metagenomics of Australian termite gut microbiota

Philip Hugenholtz, Nurdyana Abdul Rahman

Culture-independent molecular techniques are increasingly being applied to termite gut communities revealing novel organisms and functionality in this fascinating habitat. Relatively little molecular data exist for the gut microbiota of Australian termites, so we conducted a 16S rRNA-based survey of 42 samples representing five higher and six lower termite genera. We discovered that co-evolution appears to be the primary force shaping the membership of these communities and that the relative abundance of Archaea is higher (and more variable) than previously reported. We also detected sequences closely related to the ciliate *Nyctotherus* in higher termites consistent with early microscopic observations. We are currently recovering population genomes from metagenomic data via differential coverage binning from a subset of these samples in order to determine the metabolic potential of gut populations of interest.

OR210

Western Australian termites: a hotspot for novel cellulose-degrading bacteria?

Ghislaine Small, Katharine Howell, Tamara Hartke, Boris Baer

Termites are renowned as pests as they can cause major damage to properties. However, not all termite species consume lignocellulose in the form of wood - many Australian termites feed on grass, plant debris or soil, thereby providing a range of crucial ecosystem services. This diversity in feeding habits suggests that the intestinal tracts of these insects are an untapped resource for enzymes with the potential to breakdown regional sources of lignocellulose for the production of biofuels. Utilising this resource requires knowledge of the diet and endosymbionts of the termite species local to the biofuel process and the adaptation of one or the other to optimise the conversion of cellulose into ethanol. Therefore this study aims to: (1) profile the natural gut microbiota of several Western Australian termites and (2) perform feeding experiments to investigate whether the gut population can be selected for efficient breakdown of wheat crop residue and eucalyptus wood, two potential sources of feedstock for future biofuel production in WA. We optimised a metagenomics pipeline, including sample preparation, paired-end sequencing of the V4 hypervariable region of the 16S rRNA gene on the Illumina MiSeq platform and a bioinformatics analysis using the USEARCH and QIIME software packages. We profiled the gut bacteria from the lower termite *Coptotermes acinaciformis*, and two higher termites, *Tumulitermes westraliensis* and *Amitermes obeuntis*. We found that WA termites indeed harbour a large number of novel bacterial species while retaining the major phyla present in other termite species. Furthermore, there are differences between the gut microbiota of individuals from different *T. westraliensis* colonies, suggesting local adaptations of gut bacterial populations. Our findings indicate that there is a rich diversity and natural variation of gut communities present in Western Australian termites, warranting further experiments to understand the functioning and adaptability of these gut communities.

OR211

The functional implications of Termitomyces *domestication on fungus-growing termite gut microbiomes*

Michael Poulsen, Haofu Hu, Cai Li, Saria Otani, Duur Aanen, Jacobus Boomsma, Guojie Zhang

Approximately 30 MYA, the ancestors of the higher termite sub-family Macrotermitinae and the basidiomycete fungus *Termitomyces* joined forces in what was to become one of the most sophisticate plant biomass decomposition symbioses on Earth. At that time, the termites had already lost their Protist gut symbionts and replaced them with bacteria, but the degree to which the innovation of fungiculture induced a functional shift in the gut microbiota has remained unclear. We used 16S pyrosequencing to describe the community composition of the core gut microbiota associated with the Macrotermitinae and offer the first metagenomic insight into the functional roles of these gut communities. Focusing on the carbohydrate-active enzymes (CAZymes) encoded by *Termitomyces* and gut microbes in *Macrotermes natalensis*, we show that the gut bacteria primarily contribute enzymes for the final digestion of simple polysaccharides after *Termitomyces* has degraded the more complex carbohydrates. We also provide the first comparative analysis of the enzymatic potential of gut microbiota for decomposing fungal cell walls in *Macrotermes natalensis* and *Odontotermes yunnanensis*. Despite belonging to different termite genera and occurring on different continents, the gut communities had remarkably similar enzyme profiles for this function. The bacterial genera making these enzymes are consistently over-represented in fungus-growing termites relative to other higher termites that have a higher preponderance of cellulolytic bacteria. The shift in gut metagenome function after *Termitomyces* domestication thus appears to involve both complementary division of labor and targeted digestion of the novel food offered by *Termitomyces*.

OR212

Evolution of termite gut microbiota in Coptotermes *and* Heterotermes

Thomas Bourguignon, Manping Zhang, Theodore A. Evans

Termites are dominant decomposers of organic matter in tropical and subtropical terrestrial ecosystems and this is partly due to their association with gut microbes. Termites have acquired the capacity to digest wood and sustain themselves on this nutrient poor diet as gut fauna provides help in digestion of cellulose and nutrients acquisition. Here, we intend to document the evolution of gut microbial communities in related termite species inhabiting distinct ecosystems and geographical regions. To do so, we selected 13 termite colonies belonging to nine species of *Coptotermes* and two species of *Heterotermes*, that we sampled in lowland tropical forests, peat swamp forests and in man-made habitats. We used a meta-genomic approach and sequenced two fragments of the 16S rRNA gene of the termite gut microbiota for each colony using Ion Torrent. Altogether, we obtained 325,000 and 310,000 sequences for both fragments. Both 16S fragments provided comparatively similar results, although they suggested different species diversity. Microbial communities were dominated by *Candidatus Azobacteroides* (Bacteroidetes) and *Treponema* (Spirochaetes). These two genera are involved in nitrogen fixation and made up between 70% and 90% of the microbial abundance. Microbiota species diversity was higher for colonies collected in peat swamp and lower for colonies collected in man-made habitats. No correlations were found between species composition and phylogeny or habitat. Overall, our results show that the taxonomic composition of termite gut fauna is relatively similar among *Coptotermes* and *Heterotermes* species and is dominated by nitrogen fixating bacteria.

OR213

Molecular studies of termite-gut protists on cellulose utilization

Moriya Ohkuma

Symbiotic digestion of gut protists in lower termites is well known but only limited information is available because of the difficulty of cultivation of them, their uniqueness in nature, and their presence in a complex microbial community. Recent advance in meta-transcriptomic studies of the gut community of protists discloses genes for enzymes of cellulose degradation and the following metabolisms. Identification of organismal origins of these genes is of particular interest in order to understand the difference in the abilities and roles among protists species as well as their evolution. Indeed, the organismal identifications of certain genes and biochemical studies of encoding enzymes reveal similarity and difference of degradation systems and metabolisms of cellulose depending on protisit species. Lateral gene transfers between protist species and from bacteria have had great impacts on their evolution and adaptation by acquiring efficient cellulose utilization systems and likely have caused differentiations of their roles in symbiotic digestion in the gut.

OR214

Cellulose catabolism in the gut of the termite, Hodotermopsis sjostedti

Gaku Tokuda, Jun Kikuchi

Increasing evidence for the presence of endogenous cellulase genes in many invertebrates has raised intriguing questions on the mechanisms by which they digest cellulose and the role played by gut symbionts in their metabolism. Although DNA- and RNA-based approaches have provided important insights in recent decades, we are still far from a comprehensive understanding of in situ digestive processes of these invertebrates. In this talk, we present the first comprehensive metabonomic investigation of 13C-labeled cellulose digestion in a termite. Two-dimensional nuclear magnetic resonance analyses of the gut extract resolve 256 signals, which are assigned to 46 known metabolites. Temporal and spatial variations of these signals along the gut segments provide the first direct evidence for immediate cellulose breakdown by endogenous cellulases in insects, and the previously neglected contribution of hindgut bacteria, which we show phosphorolyze cellodextrins or cellobiose. Temporal ordination analyses of the metabolites along the gut segments show that essential amino acid acquisition by termites is most likely occurring via digestion of microbes. Soil-burrowing behavior contributed to accumulation of amino acids and essential minor minerals, providing firm evidence for the nutritional importance of soil to these insects. These findings provide the first comprehensive metabolic network analysis of an insect gut and reveal the temporal transitions of cellulose-derived catabolites along the gut and with respect to the intestinal cellulosic symbiosis. Our techniques with 13C-labeled cellulose should be applicable to diverse xylophagous insects to understand their digestive processes and will greatly contribute to shedding light on the complex metabolic networks in symbioses.

OR215

Evolution of nitrogen-fixing endosymbionts of termite-gut protists

Yuichi Hongoh

Wood-feeding termites generally need taking up nitrogen from the atmosphere with the aid of N2-fixing gut bacteria to balance the low nitrogen content in their food materials (1). One of such N2-fixing bacteria, *Candidatus Azobacteroides pseudotrichonymphae* (order Bacteroidales), is an intracellular symbiont of the cellulolytic protist *Pseudotrichonympha grassii* in the gut of the Formosan subterranean termite *Coptotermes formosanus*. *A. pseudotrichonymphae* predominates in the gut microbiota, accounting for more than 50% of the total bacterial cells (2), and the *Azobacteroides* bacteria, the *Pseudotrichonympha* protists, and the host rhinotermitid termites have strictly cospeciated (3). Although it is difficult to examine the functions of *Azobacteroides* because of their unculturability, our genome analysis of *A. pseudotrichonymphae* has previously suggested that the bacterium fixes nitrogen and synthesizes various amino acids and cofactors. In addition, we suggested that the bacterium potentially utilizes the waste products of the host protists: it recycles urea and ammonia as nitrogen sources and consumes hydrogen as an energy source (4, 5). Thus, it is likely that the bacterium and the hosts have a close mutualistic relationship. In my talk, I will present our recent results of comparative genomics of *Azobacteroides* endosymbionts of the *Pseudotrichonympha* protists in the gut of several rhinotermitid termite species. We performed whole genome amplification and successfully reconstructed complete or nearly complete genomes of these unculturable bacteria. Our study demonstrates how the ecology and evolution of the host termites are in harmony with the functions of gut bacteria. (1) Hongoh Y (2011) Cell Mol. Life Sci 68: 1311-1325 (2) Noda S et al (2005) Appl Environ Microbiol 71: 8811-8817 (3) Noda S et al (2007) Mol Ecol 16: 1257-1266 (4) Hongoh Y (2008) Science 322: 1108-1109 (5) Inoue J et al (2007) Eukaryot Cell 6: 1925-1932

OR216

Blattabacterium function, genome degradation and loss in primitive termites

Nathan Lo

The low levels of nitrogen in wood means that wood-feeding termites must find ways of obtaining and conserving this element. *Blattabacterium cuenoti* is an intracellular bacterium found in specialised bacteriocytes of the fat body of cockroaches and the primitive termite *Mastotermes darwiniensis*. The bacteriocytes are found adjacent to urocytes (containing uric acid), and it was long thought that the function of the bacterium was to convert uric acid (an end product of nitrogen metabolism) back into usable nitrogen products for the host. The absence of genes encoding uricases in all *B. cuenoti* genomes thus far sequenced was, therefore, unexpected. One hypothesis to explain this absence is that gut bacteria are responsible for recycling uric acid, and that the breakdown product urea is transported back to the fat body where *B. cuenoti* converts it into essential amino acids for its host. I will examine the evidence for this hypothesis as well as alternative hypotheses on uric-acid degradation in cockroaches and termites. I will then compare the *B. cuenoti* genomes thus far sequenced and discuss the connection between social behaviour and the degradation and eventual disappearance of the B. cuenoti genome during the evolution of termites.

OR217

Dissecting the fire ant social supergene

John Wang

The fire ant *Solenopsis invicta* is characterized by a remarkable form of social polymorphism, with the presence of one or several queens per colony being completely associated with allelic variation at a single Mendelian factor marked by the gene Gp-9. Because additional phenotypic differences in physiology, fecundity, and behavior are also completely associated with Gp-9, it was hypothesized that the locus actually consists of multiple genes that form a supergene. Recently, genetic and cytogenetic analyses revealed that this is the case with Gp-9 being associated with ~600 genes locked together by a large inversion. Despite the discovery of a social supergene, a detailed understanding of how this social system works is still lacking. Currently, we are examining one of the drivers of the fire ant social form polymorphism, which is the reciprocal interaction between the queen odor and worker perception or response, leading to the monogyne or polygne colony phenotype. I will report our ongoing progress using transcriptomic profiling of antennae and brains to identify the genes important for the worker perception of or response to queens. Additionally, I will present our preliminary progress at examining the transposon landscape within the supergene.

OR218

Convergent genetic architecture underlies social organization in ants

Jessica Purcell, Alan Brelsford, Yannick Wurm, Nicolas Perrin, Michel Chapuisat

Complex adaptive polymorphisms are common in nature, yet their underlying genetic basis is rarely known. Intraspecific variation in social organization has evolved multiple times in ants. The convergent evolution of similar social polymorphism in two independent ant species provides a great opportunity to investigate how genomes evolved under parallel selection. Here, we demonstrate that a large, non-recombining 'social chromosome' is associated with social organization in the alpine ant *Formica selysi*. This social chromosome shares architectural characteristics with that of the fire ant *Solenopsis invicta*, but the two show no detectable similarity in gene content. In line with recent theoretical studies, these findings demonstrate that suppression of recombination and chromosomal rearrangement play a key role in shaping phenotypes involving multiple co-adapted traits, such as social organization.

OR219

Population genomics approach identifies recent adaptation in invasive fire ants

Eyal Privman, DeWayne Shoemaker, Laurent Keller

Solenopsis invicta and other closely related fire ant species evolved high invasiveness. A sub-clade of *Solenopsis* species have recently evolved a polymorphism in their social structure that may have contributed to their invasiveness: a colony can either have one reproductive queen (the ancestral monogyne form) or many reproductive queens breeding cooperatively (the derived polygyne form). It has been suggested that the polygyne social structure allows more efficient cooperation and faster population growth, which facilitate invasiveness. Therefore, we hypothesize that this transition was accompanied by positive selection on related adaptive traits. The polymorphic social form is determined by the so-called "social chromosome": a super-gene of at least 600 genes with suppressed recombination that evolved less than one million years ago. We set out to identify additional genes outside the social chromosome that contributed to this evolutionary innovation. We used a population genomic approach to infer recent selection pressures in populations of S. invicta. We identified SNPs and genotyped individuals from different populations. We employed a combination of RAD (Restriction site Associated DNA) sequencing and whole genome sequencing to achieve either high accuracy in allele frequency estimation or high resolution of SNPs, respectively. These extensive data sets allow for high inference power in different types of tests for positive selection. We analyzed these data using population structure inference methods and genome-wide scans for positive selection. Finally, we identified specific gene families and molecular functions under selection that could be implicated in the evolution of alternative social structure. These analyses provide for the first time a genomic view of selection pressures and recent adaptation in a polymorphic social insect.

OR220

Genomic basis of a social polymorphism in a halictid bee

Sarah Kocher, Cai Li, Hopi Hoekstra, Guojie Zhang, Naomi Pierce, Douglas Yu

Species exhibiting natural phenotypic variation are ideal for ecological genomic studies aimed at identifying some of the key genetic and environmental factors underlying the evolution of these traits. *Lasioglossum albipes* is a polymorphic halictid bee that varies in social behavior across populations. Common-garden experiments have suggested that this variation is likely to have a strong genetic component. A draft genome for this species is now complete, facilitating a population genomic approach to identify the key genetic differences underlying the behavioral polymorphism in this species. Whole-genome resequencing of solitary and social individuals identified a small number of genes that appear to be rapidly diverging between social forms. These genomic resources also facilitate comparative studies among additional solitary, social, and polymorphic halictid species to search for common genetic mechanisms associated with social transitions in this group.

OR221

The cost of inbreeding in a socially polymorphic ant population

Dora B. Huszar, Louise S. Pedersen, Rasmus S. Larsen, Sarah Carlsen, Jacobus J.Boomsma, Jes Søe Pedersen

Monogynous and polygynous populations of the same ant species tend to have different dispersal rates and inbreeding coefficients, but the cost-benefit ratios of philopatry and inbreeding have rarely been compared in populations where monogynous and polygynous colonies interact in sympatry. We studied the relative costs of inbreeding (in terms of body size, fluctuating asymmetry and diploid male production) in a *Myrmica rubra* population where monogynous, polygynous and supercolonial nests coexist, and where genetic analysis showed that these colony types had distinct patterns of relatedness and inbreeding. Despite variation in inbreeding we could not detect any difference in wing asymmetry of males, nor did we find any diploid males. However, outbred monogynous nests with high relatedness had males with lower leg-asymmetry and these nests stood out by also having larger workers and males but smaller queens than the other two types. Polygynous nests had both higher relatedness and higher inbreeding than supercolony nests, but there was no difference in morphological traits between the two groups, suggesting that their dispersal strategies are similar. Significant genetic isolation by distance in both polygynous and supercolony nests suggested that queens are inseminated inside or close to the nest where they were raised, which implies that higher numbers of male and female sexuals breeding in such supercolonies could potentially explain lower inbreeding. However, winged virgin queens were exclusively produced in outbred monogynous nests, corroborating earlier findings that supercolonial patches tend to produce only males. Our results show that dispersal, inbreeding, morphology, relatedness and colony sex ratio co-vary when different types of colonies occur in sympatry, and offer relevant understanding of the complex selection forces that gave this ant species the potential to become invasive in Eastern North America. Ongoing genomic studies will help to further understand how social polymorphism is regulated in this species.

OR222

Population genomics of the honeybee , Apis mellifera

Brock Harpur, Clement Kent, Amro Zayed

The honeybee, *Apis mellifera*, has a global distribution resulting from ancient expansions out of Africa. The incredible phenotypic diversity observed among the different geographic subspecies of the honeybee is in-part caused by genetic divergence across the genome. We re-sequenced 39 *A. mellifera* honeybee workers from progenitor populations using Illumina Hi-Seq sequencing Using this large dataset, we identified regions of the honeybee genome with signatures of positive selection associated with local adaptation. We have identified clear signals of selection acting across the honeybee genome, and we used this knowledge to better understand the evolutionary and genetic basis of phenotypic diversity in honeybees.

OR223

Genomic and physiological regulation of diapause in bumblebee queens

Etya Amsalem, Jonathan Cnaani, Christina Grozinger

Bumblebees provide critical commercial and wild pollination services throughout the world. However, populations of many wild bumblebee species are declining, while commercial mass rearing operations are also reporting reduced survival, fertility and health. In wild populations, the majority of the losses occur during the winter diapause, a sensitive period during life cycle associated with physiological changes in hormones, fats, metabolism and immunity. Similarly, treatment of managed bumblebees with CO2 to bypass diapause results in variable losses and there is evidence for both short- and long-term negative effects on queen performance and survival. The underlying molecular mechanisms associated with diapause or the impacts of CO2 on queens have not been characterized. Here we studied the behavior, physiology and genome-wide gene expression patterns of *Bombus terrestris* and *Bombus impatiens* queens as they transition through emergence, mating, diapause and colony founding, either under artificial diapause or CO2 treatment. Genes involved in core nutrient storage and metabolic pathways, including insulin signaling, seem to play a major role in the process, similar to other non-social insects, despite differences in social organization and patterns of diapause (complete vs. reproductive diapause). However, pathways that have been modified during the evolution of eusociality, such as vitellogenin and juvenile hormone, do not show a typical pattern of expression. We further explore the critical role of the insulin pathway in regulating diapause by RNA interference of FoxO, a downstream molecule in the insulin signaling pathway. CO2 treatment affected the expression levels of several genes and was associated with lower lipid mass in fat body, higher flying activity and mortality. Understanding the physiological and molecular processes underlying diapause and CO2 treatment can lead to recommendation to improve commercial and wild bumblebee management.

OR224

Caste and life stage biased gene expression in Formica exsecta

Claire Morandin, Kishor Dhaygude, Jenni Paviala, Christopher Wheat, Kalevi Trontti, Heikki Helanterä

Social insects provide an ideal opportunity to study polyphenism and the impact of gene expression on the existence and maintenance of distinct phenotypes. Based on differential expression of the same set of genes, workers and queens develop morphological, physiological and behavioral differences. During social insect life cycles, differential expressions of still unknown sets of genes maintain the female caste differences. In order to understand the extent of the impact of developmental stage on caste biased expression in *Formica exsecta*, we used RNA sequencing (RNA-seq) to explore gene expression differences between queens and workers at several life stages. We performed a de novo transcriptome assembly of the ant *Formica exsecta*, with RNA-seq libraries constructed from queen and worker samples originating from three developmental classes, pupal, emerging adult and adult stages. The analyses revealed that the number of caste biased genes increases with age, with adults displaying the largest expression differences among castes. Furthermore, no genes were found to be consistently biased towards one caste across all three life stages. This suggests that the gene expression of caste differences is highly context specific. Despite obvious polymorphic differences between the queens and the workers, the differences in gene expression patterns among life stages were higher than between castes. These results indicate that high polymorphic divergences between castes may persist even with relatively small differences in gene expression. In addition, when comparing caste-biased expression with between-developmental stages expression, we determined a subclass of caste specific genes. These genes represent a small proportion of genes exclusively linked to caste bias for each studied developmental stage. Our results suggest that further studies on polyphenism, focusing on gene expression patterns and caste development, cannot overlook the importance of life stages.

OR225

Conserved microsatellites as broadly applicable genetic markers: a case study in ants

Ian Butler, Kimberly Siletti, Peter Oxley, Daniel Kronauer

Because of their abundance in eukaryotic genomes and high degree of polymorphism, microsatellites have proved to be especially useful population genetic markers. However, the breadth of their application has been limited by their specificity to one or a few closely related species. Additionally, developing new microsatellite markers for new species can be both expensive and time consuming. To overcome this hurdle and to broaden the applicability of microsatellites, we asked whether it would be possible to identify loci that are conserved across a wide range of species, but still sufficiently variable within species to be useful as population genetic markers. We chose the ants as a particularly suitable case study because of the age and diversity of the group, as well as the number of sequenced ant genomes. Additionally, the popularity of ants as study organisms would provide numerous and immediate applications for conserved population genetic markers. Using all eight currently available ant genomes, we identified a set of 45 conserved microsatellite loci and designed primer pairs for PCR amplification. We tested these primers in six different species covering six different ant subfamilies. Most primers amplified well for all species tested. We then studied a subset of 24 of these primer pairs in detail, and found that the number of polymorphic loci in Hardy-Weinberg Equilibrium in any given species ranged from 4 to 21, with a median of 9. The average number of alleles per informative locus was 4.83 (±2.49 SD). From this set of primers, a subset can now be selected for use in any particular ant species without having to spend time and money designing microsatellite primers de novo. We therefore hope that these primers will significantly increase the utility of microsatellites in ants and will facilitate research on less studied or novel ant species.

OR226

Nesting and genetic diversity of Melipona subnitida *in Brazil*

Marcela Barbosa, Rafael Pinto, Márcia Rêgo, Vanessa Bonatti, Tiago Francoy

The species *Melipona subnitida*, Ducke, 1910, is considered endemic from the Brazilian northeast backwoods and was uncovered lately in 2006 in the sandbank of National Park of Lençóis Maranhenses and has been adjusted to other places. Even though the important role played by these bees to ecosystems maintenance, they are threatened by extinction due the high rates of deforestation into the region. Thus, the goal of this study is to understand patterns of distribution and nesting of *M. subnitida* into the sandbank region and the genetic diversity between other populations from Maranhão seaside. The search for nests in Lençóis Maranhenses was established through a systematic methodology from a meliponary well known and from it transects were surveyed in different distances totaling 18 hectares. Therefore, three different groups of *M. subnitida* were genetically compared: individuals collected in the wild nests in Park of Lençóis Maranhenses; individuals identified through informations from communities in the Parnaíba Delta and a group previously studied from Barreirinhas to determine genetic diversity among them. The majority of nest sites were represented by *Humiria balsamifera* Mart harboring 88% of the nests of *M. subnitida*. However, uncommon results were observed, as *M. subnitida* nesting in mangrove forest, in *Avicennia germinans* L. Density on nests per hectare was 1.02 and distribution index Variance/Average was equal 2 into the National Park of Lençóis Maranhenses and the species showed an aggregated spreading pattern which, possibly, is related to vegetation composition besides dependence behavior between daughter and mother colonies. Molecular results demonstrated absence of genetic variability among the groups of *M. subnitida* involving common ancestral origin; nonetheless it was observed haplotypes formation in each one of the communities. The absence of genetic variability might be explained for being a young species with low rate of fecundity and restricted spreading.

OR227

Correlates of miniaturization in Neotropical stingless bees

Meghan Duell, David Roubik, William Wcislo, Brian Smith, Jon Harrison

When a species evolves extremely small body size with respect to close relatives, is said to be miniaturized. This condition is found throughout the animal kingdom but we do not have a sufficient understanding of the physiological mechanisms responsible or their functional consequences. Evolution towards miniaturized body size has occurred in at least 11 separate genera of stingless bees with species ranging in size over three orders of magnitude. These miniaturized species were originally identified using unique characters, such as wing structure and size, to pinpoint costs inherent to small body size, though actual costs are difficult to quantify. We observed foraging behaviors of over 15 species of Neotropical stingless bees in the Panama and Colon provinces of the Republic of Panama before collecting them and removing their brains to determine if major differences in foraging regimes correlate with body size, head size, and brain structure. We also examined wing size and structure to determine whether wing characteristics are behaviorally limiting to miniaturized bees. Tiny bees had relatively larger heads but smaller thoraxes and abdomens relative to larger bees. Relative wing size varied greatly among miniaturized species. One interpretation of these data is that miniaturized bees may over-invest in the brain to maintain behavioral capacity, at the expense of flight and transport capabilities. However, wing size does not impose the same costs on all small bees. We found that bees of all sizes collected displayed similar foraging strategies, suggesting that miniaturized stingless bees might not be behaviorally limited by the physiology of small size in terms of foraging abilities.

OR228

Crop fidelity of two species of stingless bees

Tim Heard, Giorgio Venturieri, Chris Fuller

Tetragonula carbonaria and *T. hockingsi*, (Apidae: Meliponini) are the two most common stingless bee species in Australia both in the nature and in managed hives. They are increasingly being used for pollination of horticultural crops but their propensity to forage on crops is unknown. We determined the foraging preferences of these two species on five crops, macadamia, avocado, lychee, blueberry and strawberry. We surveyed 46 hives situated in 12 orchards each visited twice during the flowering season. Of the 7 128 bee checked, 20% foraged for pollen, with the majority of the remaining collecting nectar. The 603 bees checked carried pollen of 39 species. 98% of these bees carried pure pollen loads. The overall number of individual bees foraging on the target crop was 52%. This varied between species, being highest on macadamia (85%) and lowest on blueberry (12%). *Tetragonula carbonaria* was more strongly oriented to foraging for pollen on macadamia and lychee but *Tetragonula hockingsi* was more oriented to strawberry and perhaps also avocado. The presence of attractive flowering plant species affected fidelity to the target crop. It was not possible to reliably determine the foraging sites of the nectar foragers. This may not be a major limitation of this trial as these foragers are probably not important in pollination. These results demonstrate that the majority of pollen foragers from introduced hives of these two species of stingless bees will visit the target crops.

OR229

Conservation of stingless bees through beescape (landscaping for bees)

Abu Hassan Abdul Jalil, Ibrahim Shuib

Much has been studied on the pesticides effects on bees, but little has been studied on the impact of the birds nest industry on the survival of the Indo-Malayan stingless bees in South East Asia. How the rapid growth in major agricultural land developments are also rapidly decreasing the wild colonies of stingless bees. For every 100 acres, there are an average of 30 bee colonies (personal encounters) that need to be contend with. major developments involving vast virgin areas for clearing need to look into saving wild bee colonies prior to clearing. These log hives can be removed and placed in forest reserves or new Apiaries and Meliponaries established . Considering the fact that birds usually capture bees above the canopy of trees, we can tell our stingless bees not go where the birds frequent. We do this by planting downward blooming flowers like *Syzygiums*, low flowering shrubs and beneficial ground covers like *Portulacas* and *Zephyranthes* and numerous types of flowering weeds. Palm tree flowers are favourites of stingless bees and are always under the protection of the Palm canopy. The height of these Palm blooms are a great deterrent to frogs and toads and cleverly designed barriers on the Palm trunks can deter lizards as well. Concentrating the bees efforts in hive temperature regulation and nest building and brood health. Avoiding predating birds and freeing themselves from spider webs are a stressful day in the life of a stingless bee. Reducing this stress for them, allows them to pool their efforts into building stronger colonies. Assuming that the bee colonies as a single super organism will require all the help it can get to muster survival in the three dimensional zone of varied and endless predators and dangers.

OR230

Antimicrobial production and the evolution of eusociality in the gall-inducing thrips of Australia

Peterson Coates, Adam Stow, Tom Chapman, Christine Turnbull, Andrew Beattie

Social insects show the most extreme levels of altruistic behaviour. One group of social insects that have recently received more attention are the gall-inducing thrips of Australia. *Kladothrips intermedius*, a gall-inducer with an altruistic caste (soldiers), was found to produce antimicrobials; this observation increases the evolutionary complexity of taking on the helping role in this species complex. Our work is focused on the evolution of the thrips castes dual role of fighting and antimicrobial ability. We focused on the solitary species *Kladothrips arotrum* and *Kladothrips tepperi* that are closely related to the social thrips. Antimicrobial compounds were extracted from full broods and their ability to retard the germination and growth of a common entomopathogenic fungus was tested via spectrophotometry. Our work suggests that antimicrobial production predates the evolution of the soldier caste in gall-inducing thrips, and that individual foundresses antimicrobial abilities are much higher than that of her brood. Additionally, we found that the most probable source of antimicrobial production is the defensive droplet produced when the individual is under physical attack. Fighting and antimicrobial production were present at the origin of eusociality in this group and, subsequently, these behaviours became specialized in the soldier caste.

OR231

Social insect antimicrobial secretions: prevention rather than cure

Andrew Beattie

Fossil entomopathogenic fungi provide evidence that microbial pathogens were present in the environments in which insect sociality first evolved and consequently may have been major selective agents affecting the very earliest colonies. If fungi such as these were sociality's primordial enemies, then some evidence of this may remain in the form of covariance between levels of sociality and antifungal defences in extant lineages: antifungals becoming stronger with group size and increasing social organisation. Our work shows this to be generally true with respect to cuticular anti-entomopathogenic fungal activity in bees, wasps, thrips - and social spiders. In bees the greatest increment in antifungal activity appears in the transition from solitary to primitively social organisation, suggesting the necessity for antifungals from sociality's earliest stages. In some thrips and termites, soldier anti-fungal activity may be as critical to colony survival as their physical ability to repel larger enemies. These findings suggest the importance of cuticular secretions as the front-line mechanism in the prevention of disease and prompt at least two lines of thought. First, we suggest that elevated levels of activity of antimicrobial compounds were so crucial to social evolution that the traits that produce them should be considered to be part of the shortlist of major traits conventionally thought to be essential for social evolution. Second, the evolutionary arms race between nascent colonies and microbial pathogens might have been a driver of increasing colony size as limits to the potency of antimicrobial compounds could have been offset by increasing the numbers of individuals producing those secretions. Our data provide insights into these possibilities but leave us with the question: Was there a role for the arms race between microbial pathogens and insect antimicrobials in social evolution?

OR232

Social immunity and sanitary risks management in ant Myrmica rubra

Jean-Baptiste Leclerc, Claire Detrain

Eusociality in insects has led to the emergence of various evolutionary benefits but brought new constraints. One of the strongest is the increased risk of transmission of pathogens due to the close physical and genetic proximity of individuals living at high density in the confined environment of a nest or a beehive. In addition to individual innate immunity, eusocial insects have therefore developed a range of individual and social behaviours that limit the entry and spread of pathogens within the colony. Among these, we are interested in the behavior of spatial and social isolation of infected individuals with a pathogen. Although this phenomenon is considered to be one of the simplest forms of social immunity, mechanisms underlying it remain poorly understood. First of all, we have found in *Myrmica rubra* no significant differences in aggression levels toward healthy and infected nestmates by *Metarhizium anisopliae*. We also found no differences in social interaction rates in the nest entrance area. It seems that there is no chemical mechanism for early detection of infection by this fungus, and thus no perceptible signal by nestmates. This spatial isolation behaviour is therefore an active phenomenon and not a rejection by congeners, which do not change their behaviours in their presence. We however found changes in behavioral and spatial patterns of infected individuals. Once infected, individuals spend less and less time in the nest, but spend more time on its periphery when they are in. In addition, contaminated individuals groom themselves longer before entering the nest. Infected individuals seem to perceive their morbid state, probably due to physiological changes induced by the fungus. This is supported by the fact that these changes took place 3 days after infection, when the spores through the cuticle of individuals.

OR233

Social insect soldiers double up as medics

Holly Caravan, Christine Turnbull, Tom Chapman, Andy Beattie

The gall-inducing thrips of Australia are a group of insects that are an important model-clade for studies of the evolution of altruism. The self-sacrificing soldier caste is colony-bound and possesses enlarged forelimbs used in the defense of the nest. Observations of laboratory fighting arenas (microcentrifuge tubes containing a soldier and a colony-invader), in the species *Kladothrips intermedius*, show that the dimorphic sexes of these soldiers are no different in their fighting ability, nor was male or female forelimb variation linked to fighting success. These outcomes have potentially eroded the importance of the forelimb morphology in physical defense, but the soldiers were also found to have strong antifungal properties. Washes of soldier bodies were significantly more effective at inhibiting fungal growth than washes of their winged colony mates (had no ability to retard fungal growth). This dual role for the soldier caste will impact our understanding of the evolutionary trajectory of altruism. A new trajectory for this altruistic caste may include selective forces surrounding antifungal defense, or a combination of selective forces on macro- and micro-fighting ability.

OR234

Molecular mechanism of self-sacrificing gall repair by soldier aphids

Mayako Kutsukake, Shuji Shigenobu, Xian-Ying Meng, Takema Fukatsu

Together with bees, wasps, ants and termites, aphids contain many social species with individuals altruistic to their colony mates, called soldiers. Their primary social role is colony defense against predators, but some gall-forming social species also perform such altruistic tasks as gall cleaning and gall repair. Gall repair is a unique social behavior reported from the social aphid, *Nipponaphis monzeni* (Aphididae: Hormaphidinae) that forms a completely closed gall on the host tree. Since the wall of the growing gall is soft in early spring, gall-feeders such as lepidopteran larvae often invade the gall by tunneling through the wall. When a hole was bored in the gall wall, the soldiers immediately gathered around the hole, discharged a large amount of body fluid from their cornicles on the damaged area, and mixed the fluid with their legs. The discharged fluid soon became viscous and solidified, whereby the hole was filled up completely. In an attempt to understand the molecular basis of the gall repair, especially the mechanism of the body fluid solidification, we analyzed protein components of the secretion. We found that the secretion contained six major components, one of which was phenoloxidase, a key enzyme involved in melanization and scab formation in insects. The phenoloxidase was expressed in peculiar, soldier-specific large globular cells that occupied the body cavity of soldiers. RNAseq analysis of the large globular cells revealed that many genes involved in the process of melanization were predominantly expressed in these cells. We suggest that aphid's innate immune and wound-healing mechanisms might have been enhanced and recruited to the social task, gall repair, in the lineage leading to *N. monzeni*.

OR235

Context-dependent detection of fungal parasites in four ant species

Christopher Tranter

The ability of an organism to detect threats is fundamental to mounting a successful defence and this is particularly important when resisting parasites. Early detection of parasites allows for initiation of defence mechanisms which are vital in mitigating the cost of infection and are likely to be especially important in social species, particularly those whose life-history makes parasite pressure more significant. However, understanding of the relative strength of behavioural responses in different species is still limited. Here I test the response of ants from four species with differing life-histories to fungal parasites in three different contexts. The results show that ants can detect fungi on their food, environment and nestmates, and suggest that ants from different species respond differently depending on the source of contamination. The response to contaminated food correlated with the vulnerability of each ant's food store; from highly discriminatory leaf-cutting ants with their delicate fungal crops, to less selective weaver ants which tend not to store food. Ants showed a tendency to avoid fungal-contaminated surfaces and increase grooming levels in response to fungal-contaminated nestmates, showing that ants are capable of recognising fungal threats in various contexts and suggesting that species may differ in their behavioural responses in ways that correlate broadly with their life histories and their specific vulnerabilities to pathogens.

OR236

Defensive strategies of Azteca *ants against phorid fly parasitism*

Kaitlyn Mathis, Neil Tsutsui

Among the natural enemies of social insects, phorid fly parasitoids are some of the most devastating. Phorid parasitism not only causes individual ant mortality, it may also significantly reduce ant foraging behavior to the detriment of the colony. As such, many ants have both individual and colony level lines of defense against phorid fly parasitism. Three species of *Pseudacteon* phorid fly parasitize the aggressive arboreal ant, *Azteca sericeasur* in southern Mexico. We used observations in the field to examine ant defense behavior from different colonies in our field sites during phorid fly attacks. Our results show a suite of contextual defensive postures the ants will use to attempt to either gain a better vantage point to attack the parasitoids or to hide from them. During these observations we also found that when ants were successfully parasitized, they were often subsequently attacked and consumed by two species of ant-associated beetles in the genus *Myrmedonota*. We then hypothesized that the parasitized ants may be self-sacrificing to prevent phorid egg development. To determine whether parasitized ants and unparasitized ants have different defensive strategies against the *Myrmedonota spp.* beetles, we combined the use of both behavioral assays and extraction and collection of headspace volatiles of *A. sericeasur*. Our results show that *Myrmedonota spp*. beetles are attracted to a component of the alarm pheromone of *A. sericeasur*, which allows them to selectively locate parasitized ants. We then show that, ants parasitized by some species of phorid fly show a reduced level of aggression towards both species of beetle, thus allowing the beetles to consume the parasitized ants alive. The reduced defensive strategy in the parasitized ants against the beetles leads to ant mortality as well as preventing phorid egg development, reducing the overall phorid population and may potentially benefit the colony as a whole.

OR237

Collective defense portfolios shift consistently with social parasite pressure

Evelien Jongepier, Isabelle Kleeberg, Sylwester Job, **Susanne Foitzik**

The evolution of host defence traits depends not only on parasite pressure but also on the efficacy of other defences. The interdependence of concurrently expressed traits can lead to divergent defence portfolios. Their apparent depth in hosts of social parasites makes them ideal models to study defence-in-depth strategies. We investigated the interplay between multi-trait defence portfolios and social parasite pressure in 17 populations of two *Temnothorax* ants, which are hosts of the common slavemaking ant *Protomognathus americanus*. Overall, we found that albeit the expression level of defence traits varied between hosts, expression changes in relation to parasite pressure were highly consistent across the two species, indicating that parasite pressure lead to similar evolutionary trajectories in different hosts. The first experiments analysed the aggression level of host colonies and their response to chemical cues of a dead conspecific or slavemaker. Whereas host colony aggression against the slavemaker was invariably high, aggression against conspecifics clearly increased with parasite pressure in both hosts. In a third trial series, in which we analysed collective fight and flight strategies, host colonies from highly parasitized host populations responded to an intruding slavemaker by nest evacuation, whereas colonies from populations where the slavemaker is rare or absent relied on collective aggression. Colonies succeeding in aggressive nest defence evade the costs of searching and competing for new nestsites. However, counter-fighting is selected against when hosts lack the physical strength or numerical advantage to defend their nest successfully. Flight may thus remain the only effective defence mode to hosts that frequently face large slavemaker colonies during raiding attacks. Degeneration of the first defence line and the evolution of subsequent anti-parasite strategies has been invoked in hosts of brood and social parasites, but we are the first to demonstrate consistent shifts in host defence portfolios along social parasite pressure gradients.

OR238

The specialist predator protects termite colonies from generalist predators

Wataru Suehiro, Kenji Matsuura

Predator-prey relationship is generally recognized as an interaction in which a predator alone benefits unilaterally at the expense of a prey. In solitary species, the prey cannot gain any benefit from being killed by the predator. In social insects, however, the death of a colony member can increase its inclusive fitness if it contributes to the survival of the colony. Here we show that a specialist predator promotes the survival of termite colonies by keeping generalist predators out of the colonies. In this study, we investigated the relationship among the specialist predator ant 'the termite hunter' *Pachycondyla chinensis*, the generalist predator ant *Lasius productus*, and the prey termite *Reticulitermes speratus*. We found that *P. chinensis* has a sustainable foraging strategy avoiding the annihilation of the termite colony, while *L. productus* quickly terminated the prey colony. In addition, *P. chinensis* eliminated *L. productus* when both of the ant species were placed in the same container. These results suggest that termite colonies nesting in the same wood with the specialist predator *P. chinensis* would have higher survivorship under the presence of another predator. Additionally, our field survey revealed that termite colonies coexist with *P. chinensis* more frequently than with other ant species, which strongly supports our experimental results. In conclusion, even though a portion of members were preyed on, living with *P. chinensis* is advantageous to the termite colony defense. Consequently, the victims of predation can gain inclusive fitness benefits. This is the first demonstration of 'the benefit of being preyed on' in a true predator-prey relationship.

OR239

Novel defense by honeybees against mass attack by giant wasps

Heather Mattila, Gard Otis, Hanh Duc Pham, Lien Nguyen, Olivia Knight

Despite being some of the most studied insects in the world, honeybees continue to surprise us with the complexity of their social behaviors. In parts of its range, *Apis cerana* is known to employ spectacular tactics to defend colonies against mass attack by giant hornets (*Vespa mandarinia japonica*). *A. cerana* avert attack by these large predators by balling scout wasps and then simultaneously heating them to lethal temperatures and asphyxiating them until they are dead. In Vietnam, we observed a new response of *A. cerana* to attack by the closely related and similarly sized wasp *Vespa soror* - worker bees search for, collect, and then plaster animal dung (and other 'filth' substances, such as urine, soap scum, and mud) around their colony entrances to thwart attack. Collection of dung and other filth was induced only after visitation by *V. soror* and rates of 'spotting' varied across colonies. Wasps were less inclined to land and chew on hive entrances that were covered with filth spots, preventing predator access to the inside of the nest. Spotting responses were significantly stronger after presentation at entrances of van der Vecht gland extracts from *V. soror* compared to major gland components from *V. mandarinia*. Although we observed *A. cerana* balling smaller species of wasps, we did not observe attempts to ball *V. soror*. A survey of beekeepers in Vietnam indicated that spotting is widespread. Not only is this the first report of an exciting new defense of honeybees against giant wasp predators, it is also the first observation of the collection and manipulation of filth by any *Apis* species. Our findings raise many questions about the behavioral mechanisms that generate *A. cerana*'s coordinated spotting response, as well as the evolutionary path that produced two distinct defenses by *A. cerana* to attack by giant wasps.

OR240

Role of exocrine glands in social insect defence

Johan Billen

Social insects form colonies with an impressive cooperation among their numerous individual members, but at the same time also represent valuable resources for predators. They therefore are equipped with various defence mechanisms, that mainly include mechanical weapons such as powerful mandibles and stings, as well as defensive secretions produced in a variety of exocrine glands. Social Hymenoptera especially rely on the often poisonous secretions of their venom glands, that have different anatomical characteristics in the various groups. Also mandibular, metapleural and pygidial glands may be involved in hymenopteran defence. Termites mainly use frontal, labial and labral gland secretions, while also the recently discovered crystal gland has a defensive role. Spectacular defence showing ultimate altruism is found in some termite and ant species, that have ‘exploding’ individuals that can burst upon disturbance, and thus release frontal gland resp. mandibular gland products that disentangle the enemy. At a much smaller level, social insects also need to defend themselves and their nests against microorganisms. This involves glandular activity through the elaboration of antibiotics, as is known for the frontal gland in termites, the venom gland in bees, the larval labial glands in wasps, and the metapleural gland in ants. This talk aims to give a survey of the various glands that are involved in defence systems of social insects.

OR241

Unraveling the unexplored biochemical complexity of ant venoms

Axel Touchard, Alain Dejean, Jennifer Koh, Graham Nicholson, Pierre Escoubas, Jerome Orivel

Venoms are sophisticated chemical weapons employed by ants to subdue prey, defend against predators and pathogens and to communicate. Peptides are major components in most animal venoms; among the ca. 9,000 ant species that sting, most possess peptide-rich venoms. However, ant venoms, particularly peptidic toxins, remain largely unexplored. In light of the enormous chemical, taxonomical and ecological diversity of ants, their venoms represent a promising source of still unknown molecules. We studied the biochemical diversity and complexity of ant venoms from a threefold perspective: chemical vs. specific, chemotaxonomical and ecological. The MALDI-TOF mass spectrometry of whole venoms demonstrated that ant venoms are mostly constituted of small peptides, although larger peptides have been detected in a few subfamilies. Although previous studies showed the occurrence of mainly linear, non-reticulated peptides, an in-depth exploration permitted us to reveal peptides structured by disulfide bonds in most of the species studied, thus highlighting the extensive complexity of the toxins in ant venoms. Mass spectrometry-based venom fingerprinting also successfully demonstrated the potential of venom peptides as chemotaxonomical markers. Venom profiling is an easy-to-use tool for both species identification and the elucidation of cryptic diversity within species complexes. In a case study using species from the genus *Pseudomyrmex* as models, we show that contrasting ecological preferences, such as nesting mode and foraging strategy, act on the evolution of venom composition. Moreover, we were able to isolate a new class of dimeric peptides structured by disulfide bonds with potentially novel properties. Overall, our results demonstrate that ant venoms display a much higher range of structural complexity than previously thought and that such complexity could be useful in answering evolutionary and ecological questions. Ant venoms also emerge as unique sources of original and potentially valuable molecules with a probable broad array of pharmacological targets.

OR242

Honeybees' detection of foragers with cuticular profile altered by Varroa

Federico Cappa, Claudia Bruschini, Stefania Meconcelli, Ilaria Protti, Giuseppe Pieraccini, Stefano Turillazzi, Rita Cervo

The ecto-parasitic mite *Varroa destructor* represents a crucial driver for the extensive worldwide beehive losses and the parasite is currently considered one of the major threat for apiculture. Therefore, effective methods to stop mite transmission among beehives are of primary importance to save honeybee colonies from further decline. Nevertheless, the strategies adopted by mites to invade a new colony and the potential counter-adaptations of honeybees to defend their hives from outer threats have not been deeply investigated so far. In a previous study we showed that, at low infestation rates, mites remain within the hive and promote their reproduction by riding nurses, distinguished from foragers through different chemical cuticular signatures. When infestation level increases, the cuticular chemical profile of nurses and foragers tends to overlap, promoting mite departure from exploited hives by riding pollen foragers. Once on a forager, the parasites may alter their hosts' chemical profile to make them undetectable in order to promote the mite transmission among colonies. Here, we investigated the alterations induced by *Varroa* mites on the cuticular hydrocarbons profile of honeybees. Our gas chromatography-mass spectrometry analyses showed an increase in cuticular methylated compounds of parasitized bees. Then, we used lure presentation experiments to evaluate the bees ability to distinguish between individuals with a parasite-altered cuticular profile and unparasitized bees. Behavioural assays were conducted by presenting freeze-killed foreign foragers, either experimentally exposed to *Varroa* or unexposed, to honeybees at the entrance of their colonies. We found an increase in the explorative and aggressive responses of the bees towards bee-lures with a parasite-altered cuticular profile, highlighting the fact that *Apis mellifera* honeybees are able to recognize the alterations induced by the *Varroa* mites in the cuticular profile of other bees and they might use such chemical cues to prevent parasitized individuals from entering into a colony.

OR243

Olfactory modulation of honeybee aggressiveness

Morgane Nouvian, Martin Giurfa, Judith Reinhard

Aggression is an innate behaviour and a crucial element in the competition for food, mates and territory as well as a defence mechanism against predators. The defensive behaviour of the honeybee *Apis mellifera* aims at the protection of its nest, which contains the food, brood and the only reproductive individual of the colony, the queen. Despite the common use of this insect as a model in neuroscience, few studies have investigated to date the neural and molecular bases of its aggressive behaviour. One possible reason for this was the lack of a reliable assay to assess the individual aggressiveness of bees under controlled laboratory conditions. Here we introduce a novel, arena-based assay that successfully induces bees to sting in a context different from that of the hive defence. Importantly, this assay triggers stinging responses using jerky movements and dark colour of the target rather than electric shocks, thus providing conditions very similar to the ones naturally experienced by the bees. We then used this assay to investigate whether plant odours or pheromonal compounds modulate aggression in the honeybee, when presented alone or along with the major alarm pheromone component isopentyl-acetate (IPA). We show that three plant odours (linalool, 2-phenylethanol and to a lesser extent lavender) significantly reduce the bees' responsiveness to IPA, even at very low concentrations (10% IPA vs 0.075% plant odours). We also show that citral, an attractive pheromonal component released at the hive entrance, slightly increases the chances of an attack. Using an odour-conditioning assay, we further checked that plant odours do not simply 'mask' IPA when presented together. This suggests that the effect of plant odours may be due to their potential alteration of IPA processing in the olfactory system. Our results highlight the crucial role olfaction plays in eliciting and controlling aggressive behaviour in bees.

OR244

Giant hornet ejecting venom to mark its territory

Ken Tan

The Asian giant hornet *Vespa mandarina* is one of the largest and most dangerous hornets in Asia. I will report on a novel defense strategy of *V. mandarina,* which nest-guarding workers use against ants and other wasp species. When recording the behaviour of the wasps in autumn, we observed that 10.6% of workers ejected or sprayed a substance from their sting chamber at the entrance as they left the nest. Chemical analysis showed that the main components of the sprayed material were the same as the compounds found in venom ejected when hornets were grasped with forceps, and in venom collected from the venom sac by dissection. Video recordings showed that the number of ants seen near the nest entrance was significantly reduced when hornets had sprayed their venom. If hornet venom is applied near a food source being utilised by ants, ant foraging is significantly curtailed.

OR245

Appeasement versus fighting: a new slavemaker employs alternative raiding strategies

Isabelle Kleeberg, Barbara Feldmeyer, Evelien Jongepier, Susanne Foitzik

Social parasites have to break into the host colonies` fortress. Workerless inquilines creep in and avoid detection by chemical insignificance or mimicry. In contrast, slavemaking ants are often openly aggressive as their hosts recognize them by their chemical profile and defend their nests in open fights. At least this holds true for the two North American slavemakers, *Protomognathus americanus* and *Temnothorax duloticus*. However, a related newly described slavemaker species, *T. pilagens*, is using a very different and variable strategy. We can demonstrate that during most raids this slavemaker elicits no aggressive responses from its *Temnothorax* hosts. Not only is the slavemaker allowed to steal the brood unmolested, it was able to carry adult host workers back to its nest and integrated them into the slave workforce. In a few cases, however, in which the host apparently spotted the slavemaker, the latter completely changed its behaviour and killed most adult hosts by stinging. Standardized experiments with colonies of the two hosts *T. ambiguus* and *T. longispinosus* revealed that *T. pilagens* workers elicit only low aggression, much less than workers of its two slavemaker relatives. Indeed, host responses were as aggressive as those towards conspecifics and in one host species even below this level. A preliminary analysis of cuticular chemistry reveals that *T. pilagens* neither employs chemical mimicry nor insignificance. Possibly appeasement substances allow circumventing host aggression so successfully. Our behavioural observations indicate that the benefit of the dual strategy (ingratiation vs. fighting) is that if the slavemaker manages to circumvent detection it can not only increase its workforce by stealing host pupae, but also by re-programming adult host workers to serve as slaves. Future studies will compare adaptations and evolutionary trajectories in these three closely related slavemaker species, which, as phylogenetic analyses demonstrate, evolved slavemaking behaviour independently.

OR246

Unicellular decision making: How slime mould cracks the Two-Armed-Bandit problem

Chris Reid, Hannelore MacDonald, Tanya Latty, Richard Mann, Simon Garnier

A fundamental conundrum in decision making is the exploration-exploitation tradeoff; do I exploit well-known but potentially sub-optimal options, or do I risk further exploration for potentially more rewarding ones? The problem faces casino gamblers and foraging organisms alike, but there remains no known generally optimal solution. Several studies in humans and other animals have examined the tradeoff using the 2-Armed Bandit problem, where a player aims to maximize their gain when faced with two slot machines, each with a distinct but unknown reward rate. Studies thus far have only been undertaken in organisms with brains, yet the exploration-exploitation tradeoff also applies to unicellular foragers, which must tackle the problem without the aid of neurons. Also, solutions offered by collective systems have never been investigated. We tested the slime mould *Physarum polycephalum*, which behaves as a self-organized collective system, with the 2-Armed Bandit problem by assessing the effect of sampling on foraging patch choice in a T-maze. We generate insight into the basic processes of decision making in a unicellular organism, including the use of relative vs absolute reward criteria (in both the frequency of reward, and the combination of frequency and magnitude), and the effect of static vs dynamic exploration environments - factors demonstrated to affect human decision making processes. We then propose several biologically plausible decision criteria the slime mould may be using, and use Bayesian inference to determine which of these models best explains the empirical data. These results can directly inform new models of slime mould decision making and behaviour, improving on the existing, largely biomechanical framework, by incorporating new insight into slime mould 'psychology'. Our study challenges the common view that neurological hardware is required to solve complex problems, and provides insight into basic processes of decision making, beyond phylogenetic boundaries and orders of biological organization.

OR247

Sequential-sampling models of quorum detection in house-hunting ants

Stephen Pratt, Theodore Pavlic

The ability to estimate nestmate numbers plays a key role in insect colony organization. Task allocation, defensive strategies, and collective nest site selection all depend on individual workers responding appropriately to the number of insects around them. How they do so remains unknown, but research has implicated encounter rates as a key source of information. Here we show how sequential-sampling models from psychology provide a cognitively plausible mechanism for an ant to decide whether a critical nestmate density has been achieved. In these models, an individual chooses between two alternatives on the basis of evidence for each one that accumulates over time until a threshold condition is reached. We developed a similar model for quorum sensing by nest site scouts of *Temnothorax* ants. These ants use encounters with nestmates in a candidate site to judge whether a threshold population has been reached, triggering full commitment to the site as the colony's new home. In psychological experiments, the accumulation of evidence cannot be quantitatively observed directly, but it can be inferred by fitting stochastic parameters to statistics from empirical data. In the ant experiments, encounters with other ants are the source of evidence and can thus be observed directly. Hence our model of decision-making takes an encounter rate as an input and predicts the distribution of choices and decision times. This model accounts for rate-dependent choices and decision times observed in *Temnothorax* recruitment experiments.

OR248

Group size and consensus decisions in the ant Myrmecina nipponica

Adam Cronin

Decision accuracy is known to increase with group size, a phenomenon known colloquially as the 'wisdom of the crowds'. Ant colonies vary markedly in colony size, raising the question of what effect this has on decision making capacity and the decision making process. Consensus decisions arise in social insect colonies in a decentralised manner, emerging as the product of numerous semi-independent individual decisions. Decisions are often quorum-based, such that the propensity for an individual to perform an action is a sharply non-linear function of the number of individuals already performing that action. Colonies regularly make consensus decisions over collective actions in which maintaining group integrity is critical, including the selection of a new nest. The Japanese ant *Myrmecina nipponica*, employs a quorum process facilitated by recruitment via pheromone trails during decisions over new nest sites. This species is ideal for studies of consensus decision making as colony size is small (20-50 individuals) and all individuals can be marked and tracked. An accumulation of relocation data for this species data indicate that the mean quorum threshold in increases linearly with colony size. The variance in individual quorum thresholds also increased with increasing mean quorum size in a ratio-dependent manner. Larger colonies tend to find new sites quicker, but are no faster in assessing possible sites or transporting brood to a new site. I summarise recent experimental data for the house-hunting process in this species in different environments and explore how colony size influences the consensus decision making process.

OR249

The Achilles heel of decision making system in termites

Chiho Iwata, Kazuya Kobayashi, Kenji Matsuura

Collective decisions in social insects are made in self-organizing manner where all group members only react to local information. This system exerts a great deal of power in a huge colony where a large number of individuals are performing enormous work. However, it is unknown whether decision making in self-organizing manner can be performed invariably under any social conditions, especially under the conditions with little work and/or few workers. Egg piling behavior in termites is an ideal model system to approach this question. When we randomly arrange eggs in a Petri dish, termite workers gather the eggs together in a single egg pile to take care of them. By using this system, we can investigate the process by which the workers collectively decide the location of the single egg pile. Here we show that the self-organization system in termites is less efficient in the case with less work to do. When workers were given a lot of eggs, they gathered all the eggs into a single egg pile quickly. The speed of egg piling increased as the number of workers. Interestingly, when workers were given only a few eggs, it took them much longer time to finish egg piling regardless of the number of workers. These results matched well with the prediction of our agent-based model with the algorithm that incorporated local communication mediated by a pheromone informing the location of egg pile. This study elucidated the major effects of the amount of work and the number of workers on the efficiency of self-organization system. We propose a novel approach to identify the algorithm of decision making system in social insects.

OR250

Idiosyncrasy effects on collective behaviour in cockroach Periplaneta americana *(L.)*

Isaac Planas Sitja, Gregory Sempo, Jean-Louis Deneubourg

Recently, a large number of publications have been focusing on behavioural syndromes and personality, or in other words: idiosyncrasy. Unfortunately, only few studies are devoted to the existence of personality in arthropods, or more precisely, in insects. Moreover, the implications of idiosyncrasy for ecology and collective decision have been poorly explored. We have focused our study on the most widespread social behaviour: the aggregation. Collective decision that leads to aggregation emerges from the network of feedbacks within the group. The global understanding of these collective decisions requires integration of two levels of analysis: the individual and the collective one. In this respect, most of the studies on collective behaviour underestimate the role of individual idiosyncrasy. Through the use of RFID tagging method, we have highlighted that P. americana shows high rates of inter-variability and a high intra-individual stability of sheltering time along experiments. In addition, we have studied the implications of this variability in collective behaviour during aggregation process. Significant differences observed amongst groups in terms of collective decision-making and sheltering behaviour are due to inter-individual differences. Also, aggregation's dynamics depend on the distribution of personalities inside the group. Furthermore, individuals tested in groups tend to homogenize their differences but they still remain. Finally, we have demonstrated that key-individuals have an asymmetrical influence during collective decision-making and affect the exploitation and exploration of environmental resources by the entire group. For all these reasons, we propose that idiosyncrasy might be studied in a social context and that a global understanding of collective dynamics requires taking into account the idiosyncrasy of individuals.

OR251

Effective leadership in group food retrieval by Longhorn crazy ants

Aviram Gelblum, Ofer Feinerman

Group food retrieval - the cooperative carrying of a food item back to the nest - is a remarkable collective phenomenon observed in several ant species. Foragers can coordinate their efforts and jointly carry food items far heavier than the weight of a single ant. Such coordination is not straightforward, it requires that the ants reach some consensus regarding the desired direction and divide their roles accordingly. Furthermore, carrying a large morsel of food impairs both vision and scent, leading to an inevitable loss of bearings. Indeed, some species display an embarrassingly incompetent form of uncoordinated cooperative carrying. We are interested in understanding how ants carrying the load are able to overcome these obstacles, coordinate their behaviors and successfully retrieve large items. To this end, we developed a joint experimental-image analysis system that enables us to track the behavior of individual *Paratrechina longicornis* ants during collective load carrying over very long distances. Our detailed dataset allows for an in-depth quantitative analysis of collective transport. First, we analyze macroscopic trajectory characteristics such as speed, spin and number of carrying ants. We show that retrieval of food becomes more efficient as the number of carrying ants grows. Second, we use single ant trajectories to show how rules used by individuals lead to the observed behaviors. Specifically, we investigate the role of detachments and attachments of ants from the load and demonstrate the importance of newly joined and detached ants as a driver of the dynamics. Group cognition is thus facilitated by ad-hoc, effective leaders that transfer information from the environment into the system, giving rise to efficiency in both collective decisions and retrieval rates.

OR252

Thinking without chatting: collective decision-making in weaver ants Oecophylla smaragdina *does not require individual communication or recruitment*

Simon Robson, Rhondda Jones, Jean-Louis Deneubourg

Weaver ants are unique among ants in forming chains of living workers to bridge gaps in the canopy. Together, they are capable of making collective decisions when faced with a binary choice of gaps, with chains more likely to bridge the gap between a closer than distant, or larger rather than smaller, target. Although the recruitment dynamics looks identical to that of mass recruiting ants when faced with two food choices, the entire process appears to occur in the complete absence of individual communication or recruitment. Rather, the process is driven by the time at which work commences: the probability of chain initiation at the relevant work site. Flow rates in and out of chains are constant and irrespective of the target type and remain constant once work commences: switching targets in the middle of a choice has no effect on chain dynamics. The probability that a chain forms to a particular target appears to be determined by the optical image size of the target to individual ants. Ants are more likely to bridge a gap to larger rather than smaller targets that are at the same distance, and to closer rather than more distant targets of similar size, but they show no preferences when offered a pairwise choice between two targets with the same visual angle but differing in size and distance. Efforts to replicate collective decision-making in other systems may not require the parallel development of communication and recruitment systems.

OR253

Dynamics of collective worksite selection in weaver ants, Oecophylla smaragdina

Thomas Bochynek, Simon Robson

The nest construction in weaver ants involves the collective rolling of leaves and their subsequent fixation with larval silk. It represents a classical task in social insect societies, in which groups make decisions and perform tasks well beyond the scope of the sensory and physical capacity of the individuals. We investigated what group dynamics lead to the collective selection of worksites for nest construction by providing colonies with an artificial leaf in a laboratory setting. Pulling chain initiation commences when an individual who grasps the edge of the substrate with its mandibles is joined by nest mates, who either grasp the body of the first ant or the leaf perimeter next to them. Only through the combined effort can the leaf be bent into the desired shape. We find that initial site selection by individual workers is not random. When confronted with an artificial leaf individuals more likely to grasp a substrate at its tip rather than along a more broad edge. The activity of additional individuals is also not random, with their activity being grouped in both space and time. Additional individuals are more likely to join an existing biting individual or pulling group. The positive feedback associated with the leaf bending behaviour appears typical for many of the collective actions observed in social insects.

OR254

A mechanism for value-sensitive decision-making

James Marshall

Almost all organisms must make decisions of some kind, and making decisions effectively is crucial for them. I will present a model of collective decision-making based on empirical observations of a novel cross-inhibitory behaviour in house-hunting honeybee swarms. The pattern of interactions observed in collectively-deciding honeybees gives rise to a number of important value-sensitive decision-making characteristics. The model is able to achieve stable deadlock for poor but equal alternatives, but spontaneously choose between good alternatives. This enables sophisticated 'wait and see' decision-making. The model's sensitivity to value is similar to Weber's law of just-noticable-difference from psychology. When differences are large enough to be noticeable, the model exhibits speed-accuracy trade-offs in decision-making. Given the simplicity of the model, the importance of value-sensitivity, and the similar patterns of interaction seen in other decision-making systems, I will ask whether genetic switches and neural circuits may exist that implement the same basic decision mechanism.

OR255

A context-dependent alarm signal in the ant Temnothorax rugatulus

Takao Sasaki, Bert Holldobler, Jocelyn Millar, Stephen Pratt

Because collective cognition emerges from local communication among group members, deciphering communication systems is critical to understanding underlying mechanisms. Alarm signals are widespread in the social insects and can elicit a variety of behavioral responses to danger, but the functional plasticity of these signals has not been well studied. Here, we report an alarm pheromone in the ant *Temnothorax rugatulus* that elicits two different behaviors depending on context. When an ant was tethered inside an unfamiliar nest site and unable to move freely, she released a pheromone from her mandibular gland that signaled other ants to reject this nest as a potential new home, presumably to avoid potential danger. Interestingly, when the same pheromone was presented near the ant's home nest, ants were attracted to it, presumably to respond to a threat to the colony. We used coupled gas chromatography-mass spectrometry to identify candidate compounds from the mandibular gland and tested each one in a nest choice bioassay. We found that 2,5-dimethylpyrazine was sufficient to induce rejection of a marked new nest and also to attract ants when released at the home nest. This is the first detailed investigation of chemical communication in the leptothoracine ants. We discuss the possibility that this pheromone's repellent function can improve an emigrating colony's nest site selection performance.

OR256

Multi-criteria decision making in slime mould amoebas and ant colonies

Tanya Latty, Madeleine Beekman

When making decisions, organisms may need to consider multiple criteria, some of which may be in conflict. For example, the best restaurant may also be the most expensive, the cheapest house might have the longest commute, and the tastiest food might be the most fattening. How do group-level cognitive systems such as slime moulds and social insects deal with multi-criteria decision-making? In this talk I will discuss two types of multi-criteria decision-making: choosing a meal and building a transportation network. In the first half of the talk, I will discuss multi-criteria decision making in the giant amoeba, *Physarum polycephalum*. Despite being brainless, slime moulds are capable of making trade-offs between risk and food quality, are subject to speed-accuracy trade-offs, and even exhibit human-like irrationality when confronted with irrelevant decoy options. In the second part of the talk I will discuss another kind of multi-criteria problem: designing transportation networks. Building a good network is challenging because desirable network features such as low cost, travel efficiency and robustness to damage cannot be optimised simultaneously. We studied network topology in two species of ant: argentine ants (*Linepithema humile*) and meat ants (*Iridomyrmex purpureus*). The two species had different ways of dealing with the trade-offs of transportation network design. In lab experiments, we found that argentine ants built low cost networks using the minimal amount of trail, at the expense of robustness. In contrast field colonies of meat, built trail networks that balanced efficiency and cost.

OR257

House hunting in polydomous ants

Yuval Erez, Ofer Feinerman

Ant colonies exhibit collective decision making in a vast variety of situations, one example of which is house hunting during colony emigration. House hunting in monodomous ants is the subject of numerous studies, which, among other things, provided insight on different strategies for obtaining consensus. Here, house hunting was studied in ant colonies of the polydomous species *Cataglyphis livida*, members of which extensively practice adult carrying. The study varies from other studies on the subject, in that in many cases, nesting choice did not result in a consensus decision. The ants were allowed to choose housing among a large number of symmetrically spread, peripheral possibilities, surrounding an arena. We present a process in which colony members initially distribute among a large number of sites, followed by successive aggregation of the ants in fewer sites. Population distribution between compartments was compared when each had a separate entrance and when the compartments were interlinked through sheltered corridors permitting transition between them without exposure. The comparison was used to assess polydomous vs. intra-nest room distribution interpretations of the results. Experiments were repeated with various initial conditions, quantitative analyses of their results are presented. We also dwell on the division of labor between a minority of decision-making carrier ants, and the passive carried ants, and on its effect on the dynamics of population spatial distribution.

OR258

Economical decision making by Temnothorax albipennis *ant colonies.*

Carolina Doran, Nigel R. Franks

Social insect colonies provide some of the richest examples of complex systems in nature. They are an excellent model for experimental investigation into questions of how group decisions are made as they allow direct manipulation of their components and observation of the collective behaviour. *Temnothorax albipennis* colonies are able to allocate the appropriate effort into gathering information regarding new homes in accordance with the quality of the nest where they currently reside. Furthermore we show that when faced with a risky choice they seem to be risk prone and gamble if the expected payoff is positive, i.e. it represents a gain. However, the time it takes colonies to reach a consensus gradually increases when the gain is smaller. Humanity defies certain classical axioms of economic theory and therefore it is intriguing to determine if animals meet these theoretical criteria. Our results suggest that the violation of standard rationality axioms might make animals better tuned to their natural environment. Specifically by taking into account more than just the final expected utility of each outcome, colonies might avoid unnecessary and costly emigrations and only emigrate when the benefits are really substantial. What mechanisms can explain the variability of individual behaviour? By manipulating nest quality and tracking individual ants with specialized software we plan to analyse the effects such manipulations are causing both at the individual and collective level.

OR259

Follow the streakers - in flight decision-making by honeybees

Timothy Schaerf, Darcy Gray, Mary Myerscough, Ashley Ward, Madeleine Beekman

During the spring, honeybee colonies issue reproductive swarms that go on to form new colonies. A small proportion of the swarm, the scouts, explores the local surroundings to find and assess potential sites for a new home. The location and quality of suitable sites are communicated to other decision making scouts with waggle dances. Once a new home is chosen, knowledgeable scouts have the additional task of guiding naive members of the swarm to the new nest site. It is generally thought that the knowledgeable scouts guide the swarm by flying rapidly through the upper portion of the swarm in the direction of their target (streaking) before dropping down to the lower sections of the swarm, flying slowly to the trailing edge of the swarm and then returning to the top of the swarm to streak again. The level of agreement amongst scouts on the swarm's ultimate destination, reflected by the locations advertised by waggle dances in the final period before take-off, seems to vary between honeybee species. Swarms of the cavity-nesting *Apis mellifera* often show complete agreement on their final destination. In contrast the open-nesting *A. florea* seemingly advertise multiple sites in the final minutes before take-off. If it is assumed that swarm guidance is the same for all species and that scouts will try to guide the swarm in the direction of their own preferred nest-site then a ‘decision’ on the ultimate direction of the motion of the group could be made by the swarm in-flight, governed by the individual level interactions of members of the swarm. I will use a well-established individual based model for collective animal motion to examine how dissent amongst scouts on a preferred home might affect a swarm's final destination.

OR260

Stay cool: social cues influence honeybee thermoregulatory behavior

Chelsea Cook, Michael Breed

A key characteristic to the success of societies is their ability to perform tasks as a group. For example, thermoregulation in honeybee colonies during the summer must be performed by a group of fanner bees. Keeping the colony cool is critical as the developing larvae inside could die if temperatures get too hot. My preliminary results show that honeybees are more likely to fan when in groups than when by themselves, which indicates that there is some critical aspect of being in a group and performing this behavior. This talk presents my current work in which I explore the coordination of individuals within a group. I also offer hypotheses on which cues these fanner bees are using from other workers and larvae to know when to perform fanning behavior. My work provides another layer onto the rules that govern division of labor in societies, and furthers our insight into the complexity that societies exhibit.

OR261

Collective nutritional wisdom: from slime moulds to ant colonies

Audrey Dussutour

A fundamental question in nutritional biology is how distributed systems maintain an optimal supply of multiple nutrients essential for life and reproduction. We address this question using two systems: highly organised societies of ants and a syncitial slime mould (*Physarum polycephalum*). In the case of animals, the nutritional requirements of the cells within the body are coordinated by the brain in neural and chemical dialogue with sensory systems and peripheral organs. Being a social insect adds a first level of complexity to nutritional regulatory strategies. Contrary to other animals, the food entering a social insect colony is assessed and collected by only a small number of workers. These foragers need to adjust their harvesting strategy to the internal demands for nutrients within the nest, where larvae and workers have different needs. So how do foragers reactions to food encountered outside the nest relate to the nutritional demands of the nest as a whole and themselves as individuals? Here, we show that foraging ants can solve nutritional challenges for the colony by making intricate adjustments to their feeding behaviour and nutrient processing, acting both as a collective mouth and gut. Being a slime mould adds a second level of complexity to nutritional strategies. In social insect and other animals, nutritional regulatory strategies involve components specialised to deal with nutrient supply and demand (brains and peripheral organs, foragers and brood). However, slime moulds are acellular organism thus without specialised centres: how do these organisms coordinate the search for multiple nutrients? Here we show how this extraordinary organism demonstrates the capacity to make complex nutritional decisions, despite lacking a coordination centre and comprising only a single vast multinucleate cell.

OR262

The evolution of cuticular hydrocarbon profiles in ants

Florian Menzel, Bonnie Blaimer, Thomas Schmitt

Insect cuticles are covered with hydrocarbons (CHC), which serve two main functions: desiccation barrier and information transfer. The CHC profile of an ant carries information on its species identity, colony, caste, sex, etc. During the last years, our knowledge on causes and functions of intraspecific CHC variation has advanced drastically. Intraspecific variation is usually restricted to quantitative changes in hydrocarbon composition, whereas interspecific variation is characterized by qualitative alterations i.e. variation in substance identities and substance classes. To date, little is known about the evolutionary causes of interspecific variation. Moreover, we do not understand the role of phylogenetic or physiological constraints on CHC evolution: How fast can insects adapt their CHC profiles to e.g. new communication needs? How important are constraints for CHC evolution? Here, we analysed the CHC profiles of 37 *Camponotus* and 39 *Crematogaster* species from around the world. We searched for phylogenetic and/or physiological constraints and for selection pressures on CHC composition. CHC profiles did not appear to experience strong phylogenetic constraints. Even sister species could exhibit completely different profiles. However, we identified several physiological constraints. First, dimethyl alkanes and alkenes rarely co-occurred in the same species. Second, we found correlations between hydrocarbon chain length and the proportions of unsaturated compounds and n-alkanes. They might be caused by the need to maintain a specific viscosity of the epicuticular layer. Finally, climate strongly influenced substance class composition. Tropical rainforest species had higher amounts of unsaturated compounds than species from more arid habitats. We explain this by variance in draught stress, as less waterproofing is required in the humid tropical rainforests. The length of the hydrocarbon chains was unaffected by climate, but was higher among ants in interspecific associations. We conclude that CHC profiles can evolve quickly, but experience constraints posed by climatic conditions, interspecific interactions, and CHC viscosity.

OR263

Asymmetry in olfactory generalization and the inclusion criterion in ants

Nick Bos

Communication is vital for all types of social interactions, and is achieved by the interplay of three separate components, previously described for recognition systems: the expression, perception and action components. Chemical communication is perhaps the most widespread means of communication in the animal world, and the ability to discriminate as well as generalize between chemical stimuli is extremely important. Previously, by using associative learning protocols, we showed asymmetry in generalization in ants. For example, ants trained to substance A treated substance B as similar, but individuals trained to B did not generalize towards A, indicating that generalization is not necessarily only dependent on odorant similarity, but also on the animal's previous experience. This asymmetry follows an inclusion criterion, where the ants consistently generalize from a molecule with a long carbon chain to molecules with a shorter chain, but not the other way around. Here, I will review the evidence for the inclusion criterion, and discuss from which component of communication it might arise, as well as its possible adaptive significance.

OR264

A statistical approach to identify nestmate recognition cues

Jelle van Zweden, Luigi Pontieri, Jes Søe Pedersen

The ability of social insects to discriminate nestmates from non-nestmates is mainly achieved through chemical communication. To ultimately understand this recognition and its decision rules, identification of the recognition cues is very useful, if not essential. Although cuticular hydrocarbons are the prime suspect, identifying the exact recognition cues for specific species has remained a daunting task, partly due to the sheer number of odour variables. Perhaps unsurprisingly, one of the few species where the recognition cues have been identified, *Formica exsecta*, has only around ten hydrocarbons on its cuticle. In this study we use previous results on this species to search for likely nestmate recognition cues in two other species of ants, *Camponotus aethiops* and *Monomorium pharaonis*. Employing chemical distances and observed aggression between colonies, we first ask which type of data normalization, centroid, and distance calculation is most diagnostic to discriminate between nestmate recognition cues and other compounds. We find that using a 'global centroid' instead of a 'colony centroid' significantly improves the analysis. One reason may be that this approach, unlike previous ones, provides a biologically meaningful way to quantify the chemical distances between nestmates, allowing for within-colony variation in recognition cues. Next, we ask which cluster of hydrocarbons most likely represents the cues that the ants use for nestmate recognition, which shows less clear results for *C. aethiops* and *M. pharaonis* than for *F. exsecta*, possibly due to the number of compounds in their respective profiles. Nonetheless, some compound groups behave better than others, showing that this approach can be used to identify candidate compounds to be tested in bio-assays, and eventually crack the sophisticated code that governs nestmate recognition.

OR265

Deconstruct the soup - cuticular hydrocarbon signals of Australian meat ants

Qike Wang, Jason Goodger, Mark Elgar

The capacity to distinguish nestmates from others is critically important for the maintenance and survival of social insect colonies. It is widely accepted that workers use cuticular hydrocarbons (CHC) to distinguish between classes of individuals. Typically, investigations of nestmate recognition report on the individuals' response by measuring the level of aggression, and on the CHC profile of the entire individual. We examined, in Australian meat ant (*Iridomyrmex purpureus*) workers, whether there was an association between where an opposing individual worker directed its antennation behaviour and any CHC differences of different body parts. We discovered that more than 90% of all antennation are directed to antennae and legs, with workers directing more attention to the legs when they encounter nestmates and to the antennae of non-nestmates. Chemical analyses (GC-FID and GC-MS) of CHC on the antennae, head, legs and abdomen of ants from six colonies revealed striking differences between each body part. These differences are due to the concentration rather than type of chemicals, and between-colony CHC profiles vary according to the body part. Principle component analysis (PCA) showed that antennal signals provide better colony discrimination than other body parts. These results suggest that I. purpureus decorate different body parts with different chemical components from various glands, and that these chemicals most likely convey different signals.

OR266

Kin structure and cuticular hydrocarbon complexity in social wasps

Kevin Loope, Patrizia d'Ettorre

Cuticular hydrocarbons (CHCs) are the primary recognition cues in social insects, yet there exists little evidence that social behavior has lead to an adaptive modification of cuticular signatures. In one of the first attempts to link social evolution to the evolution of cuticular chemical profiles, Boomsma et al. (2003 PNAS 100(15):8799) proposed that costly nepotism and other intracolony conflicts in polyandrous species could lead to selection against kin-informative cues, resulting in CHC profiles with low compound diversity dominated by linear alkanes when compared to monandrous, monogynous species. We provide the first comparative test of this hypothesis by determining the CHC profiles of 22 social wasp species and performing a phylogenetically controlled analysis of CHC diversity as a function of queen mating frequency in the Vespinae and queen number in the Polistinae. We find that multiple mating is not associated with a decrease in compound diversity, suggesting that there has not been selection against the presence of these potential cues in polyandrous wasps. In contrast, several species of polygynous, swarm-founding *Polistines* possess simpler profiles with more linear alkanes, relative to the independent-founding species in this clade. The hypothesis that simple profiles have arisen independently due to the evolution of costly nepotism in these species is consistent with their convergently evolved system of cyclical oligogyny, wherein queen number is gradually reduced, sometimes by worker aggression, providing a plausible arena for nepotism. Our results represent an empirical advance in understanding the links between social evolution, CHC diversity and informational constraints by providing support for the costly nepotism hypothesis. We suggest that the polygynous, swarm-founding wasps are an promising group for future work on the role of nepotism in insect societies.

OR267

Navigating in the dark: chemical road signs in the nest

Yael Heyman, Ofer Feinerman

The spatial distribution of ants within the nest is a key factor in a colony's organization, interaction patterns, and function. Although ants are highly mobile they form localized aggregates that remain spatially stable over long periods of time. Moreover, individual ants are known to repeatedly return to specific aggregates, which are therefore termed fidelity zones. The means by which ants find their preferred location and, generally, navigate within the dark interior of the nest are, to a large extent, unknown. Hydrocarbons are known to play a central role in ant communication. It was recently shown that the surfaces of *Lasius niger* nests are covered with cuticular hydrocarbons. It is therefore appealing to study nest floor chemical distribution as a mechanism underlying the ants' navigation and spatial arrangement. To test this hypothesis, we performed a combination of behavioral experiments and chemical analyses. Behaviorally, we used a 2D barcode identification system to automatically track all individuals of a *Camponotus fellah* colony within the nest. Our results suggest that the ants use chemical road signs laid on the nest floor in order to navigate. We show the existence of at least two such road signs which elicit distinct responses from different individuals. Next, we developed a novel technique that allows us to quantitatively measure the distribution of pheromones adsorbed to artificial nest floors. We find that these pheromones are non-homogeneously spread inside the nest, supporting the notion of chemical road signs. The high signal-to-noise ratio of this technique, together with our tracking system, allows us to compare the chemical profiles of nest chambers to those of the ants inhabiting them. Combining both approaches will allow us to decipher the mechanism by which this chemical map is formed and maintained.

OR268

Decoding the biosynthesis of hydrocarbons in ants

Sue Shemilt, Falko Drijfhout

Within social insect species colony signatures are required so that altruistic behaviour can be appropriately directed. It is widely accepted that within ant species nest-mate discrimination is down to a chemical signature determined by the cuticular hydrocarbons present. Previous work involving *Formica exsecta* looked at the incorporation of labelled hydrocarbons within a very simple hydrocarbon profile of just alkenes and alkanes. Using such species as *F. lugubris* we hope to be able to replicate this work and investigate the incorporation of labelled hydrocarbons into the many methyl branched hydrocarbons that this species exhibits. Preliminary studies involve feeding small groups of ants ad libitum a diet containing amino acids, acetates or acids, labelled with either carbon-13 or deuterium atoms. At different time intervals the cuticular hydrocarbons were extracted with hexane and these extracts were analysed using gas chromatography coupled to mass spectrometry. By studying which of the substrates are incorporated into the cuticular hydrocarbon profile and the amount of each present, it is hoped that more information on the biosynthetic pathways that are employed can be determined. Additional experiments using these substrates are planned in order to obtain more insight into the formation of the gestalt odour. It is hoped that results from this study will be presented in view of previous studies looking at the source and maintenance of the CHC profiles in ants.

OR269

Density of antennal sensilla influences signal perception in weaver ant communication

Mark Elgar, Katherine Gill, Ellen van Wilgenburg, David Macmillan

Effective communication requires reliable signals and competent receptors. Theoretical and empirical accounts of animal signaling focus overwhelmingly on the capacity of the signaler to convey the message. However, the impact of receptor organ condition on signal reception and its consequences for functional behavior are poorly understood. Social insects use antennae to detect chemical odors that distinguish between nest-mates and enemies, reacting aggressively to the latter. We investigated the impact of antennal condition, determined by the density of sensilla, on the behavior of weaver ants *Oecophylla smaragdina*. Worker aggression covaried with the condition of their antennae: workers with fewer sensilla on their antennae reacted less aggressively to non-nestmate enemies. These novel data highlight the largely unappreciated significance of receptor organ condition for animal communication, and may have implications for co-evolutionary processes in animal communication.

OR270

Sex/cast specific chemoreceptor analyses from RNAseq data in Camponotus japonicus

Mamiko Ozaki, Masaru Hojo, Midori Sakura, Shuji Shigenobu, Kenichi Ishii, Koichi Ozaki

Ants construct and maintain sophisticated society consisting of workers and reproductive cast. They communicate with each other, using complex chemical signs, which can affect not only physiological states but also stereotypic behaviors. In accordance with previous reports in *Camponotus floridanus* and *Harpegnathos saltator*, we performed comparative RNAseq analyses in the Japanese carpenter ant, *Camponotus japonicus* among workers, virgin queens and males, and constructed sex/cast specific catalogs of chemoreceptor genes. In the antennae, we identified 330, 340 and 297 Or genes and 35, 35 and 30 Gr genes in workers, virgin queens and males, respectively. Because of smaller number of glomeruli in the antennal lobes in males than in females, this quantitative difference in Or genes between males and females seems reasonable. There was dominant expression of an Or gene in male antennae and a Gr gene somewhere in the body without antennae of virgin queens. We also discovered 12 kinds of CSP (chemosensory protein) genes expressed in the worker antennae, and investigated their tissue distribution. They were found not only in the external chemosensory organs but also in their gut. Considering genomic sequence data as well, we will discuss diversity of chemosensory receptor molecules and CSPs with their functional characteristics adequate for sex/cast specific behavior of this social insect.

OR271

Molecular and neural basis of chemosensation in the ant Cerapachys biroi

Sean McKenzie, Peter Oxley, Daniel Kronauer

Cerapachys biroi is an upcoming model for the study of sociogenomics and the neurobiology of social behavior. As a blind myrmecophagous species, *C. biroi* relies heavily on its sense of smell, possessing the largest odorant receptor repertoire of any insect yet sequenced. This makes it an excellent model to study the molecular basis of chemosensation and chemical communication. We have used comparative genomics, transcriptomics, and microscopy to examine the molecular and neuroanatomical basis for chemosensation in *C. biroi*. Two families of small soluble proteins, the odorant binding and chemosensory proteins (OBPs and CSPs), are believed to be important for olfaction. Both families have rapidly evolving clades with many species-specific paralogs which have been hypothesized to be important in chemical communication. However, our comparative expression profiling analysis of OBPs and CSPs reveals that most rapidly evolving proteins are expressed in non-olfactory tissues, suggesting that they are not involved in chemosensation. At the same time, some highly conserved OBPs and CSPs are expressed specifically in antennae and may fulfil important roles in ant olfaction. Light and scanning electron microscopy of the antennae of *C. biroi* reveal adaptations for high reliance on chemosensation, including a five-fold increase in the density of basiconic sensilla relative to other ants. Preliminary genomic and transcriptomic characterization of the odorant receptors (ORs) likewise shows signatures of *C. biroi*'s unusual biology, including expansion of female-expressed OR clades likely important in chemical communication. Our findings shed light on the neurogenetic basis of ant chemosensation in general as well as on specific adaptations of *Cerapachys biroi*.

OR272

Parallel processing of olfactory information in social Hymenoptera

Wolfgang Roessler, Jan Kropf, Martin F. Brill

Social insects face an enormous complexity of chemical signals and cues used for communication, recognition and orientation. The diversity and complexity of odorants involved in many social behaviors requires advanced sensory processing and perception. We investigated neuronal adaptations promoting these amazing capabilities in the honeybee (*Apis mellifera*) and carpenter ant (*Camponotus floridanus*) by combining structural and functional analyses of underlying neuronal circuits. Olfactory information is relayed from primary (antennal lobes) to secondary olfactory centers (mushroom bodies, lateral horn) via a dual olfactory pathway formed by a medial and lateral tract of projection neurons. Comparative studies across insects suggest that this configuration represents a special feature in Hymenoptera (Galizia and Rüssler 2010, Annu Rev Entomol; Rüssler and Brill 2013, J Comp Physiol). Tracing studies show that this dual pathway is sex-specific; the m-tract is reduced in males compared to females. This is associated with the lack of Sensilla basiconica in males - one type of antennal olfactory sensilla. We developed a technique for dual-tract multi-unit electrophysiological recordings to simultaneously monitor odorant induced activity from large numbers of projection neurons in both olfactory tracts (Brill et al. 2013, J Neurosci). The results demonstrate that all tested odorants were processed in parallel via both tracts, but with significant differences regarding quality and temporal coding indicating that the two information streams transfer different features from similar odors. Whereas projection neurons of the medial tract are more narrowly tuned to specific odorants, neurons of the lateral tract respond more generalistic and faster. Based on these features we propose a model for parallel processing and coincidence coding of olfactory information at the level of the mushroom bodies. We suggest that coincidence coding via a dual olfactory pathway supports fast coding and efficient recognition of complex odorant mixtures. Supported by DFG-SPP 1392 (Ro1177).

OR273

Neuroethological study of pheromonal sex communication in honeybee drones

Florian Bastin, Andreas S. Brandstaetter, Gudrun Koeniger, Nikolaus Koeniger, Jean-Christophe Sandoz

During the mating season, male honeybees, the drones, gather in drone congregation areas (DCA). When a virgin queen enters the DCA, drones are strongly attracted by olfactory cues (sex pheromones). The mechanisms allowing drones and virgin queens to find the DCAs are still unclear, and the possible existence of a drone-produced pheromone has been proposed, but not demonstrated. We thus investigated innate odor preferences of drones under controlled laboratory conditions using a locomotion compensator. First, we tested the behavioral responses of drones to 9-oxo-2-decenoic acid (9-ODA; major component of the virgin queen odor bouquet) and to queen mandibular pheromone (QMP; pheromonal mixture specific of mated queens, including proportionally less 9-ODA). Drones were attracted to 9-ODA, but not to QMP. Next, we investigated the potential attractivity of drone-derived odors. We found that honeybee drones are indeed attracted to the odor bouquet from live drones but not to the bouquet of workers. Such attraction between consexuals may play a role in the formation and maintenance of DCAs. The olfactory system of honeybee drones is highly-tuned to queen pheromones. The drones from almost all known honeybee species show a strong attraction to 9-ODA. However, the composition of the mandibular gland pheromone differs clearly between species. How did the drone olfactory system evolve to process these compounds? We performed a comparative neuroanatomical study of the drone antennal lobe (AL; primary olfactory center) to identify pheromone-specific processing units (macroglomeruli). Our results suggest a complexification of AL structure from so-called ancestral species (dwarf and giant honeybees, like *Apis dorsata*) to the most derived ones (cavity-nesting honeybees, like *A. mellifera* and *A. cerana*), with more macroglomeruli. In addition to numerical differences, we also find clear differences in macroglomerulus localization between species. Implication of these results for our understanding of bees sexual behavior will be discussed.

OR274

The evolution of chemosensory proteins in ants

Jonna Kulmuni

Chemical communication has been extensively studied in social insects, yet studies have only begun to look at the genes underlying these sophisticated behaviors. Large gene families contribute to the first steps of receiving chemical messages. Chemosensory proteins (CSPs) and odorant binding proteins (OBPs) are the first ones to interact with the chemical messages. These proteins bind and transport the insoluble odorants to odorant receptors. CSPs are particularly interesting in ants, as they have a role in binding the nest-mate recognition cues. I have studied the evolution of CSPs in ants, and found that the number of functional CSP genes has increased in all the seven ant species studied. Those CSPs that are specific to ants evolve under positive natural selection, suggesting different CSP copies have adapted to slightly different tasks. Furthermore, the ant-specific CSP expansion has originated from the nest-mate cue binder or a protein similar to that, suggesting the expanded genes have adapted to tasks in ant chemical communication. To put these results into a functional context, protein models were built. I was especially interested in which parts and properties of chemosensory proteins have been affected by positive selection. CSPs differ in size, binding pocket and harbor extensive variation in their surface charge. Interestingly, positive selection has targeted the surface rather than the binding pocket, and natural selection seems to drive variation in CSP surface charge. Variable surface charge likely has functional importance for example by affecting ligand binding, interaction between CSPs and odorant receptors, or is connected to the cellular environment of CSP expression. Protein modeling is rarely used in evolutionary context. Yet, these studies show that protein modeling together with comparative genomics can offer unique insights into the evolution and function of genes and proteins.

OR275

Soluble olfactory proteins. A focus on social Hymenoptera

Francesca Romana Dani, Immacolata Iovinella, Antonio Felicioli, Stefano Turillazzi, Paolo Pelosi

Social Insects rely extensively on chemical communication in colonial defense, organization and reproduction. A large diversity of volatile and semivolatile compounds have been reported as semiochemicals, while cuticular lipids and especially hydrocarbons (CHs) are cues involved in nestmate recognition and fertility signaling. Two classes of small soluble proteins, Odorant Binding Proteins (OBPs) and Chemosensory Proteins (CSPs), mediate olfaction perireceptor events. Some OBPs and CSPs are only found in olfactory sensillar limph, where they are believed to transport odorants/pheromones to receptors, while others are present in pheromonal glands or perform unrealted functions in different body tissues and fluids. Based on the importance of chemical communication and the variety of semiochemicals, Social Insects are expected to present high numbers of OBPs and CSPs with complex patterns of expression. Moreover, first reports of CSPs in ant antennae suggested that CSPs could mediate CHs perception. However, so far, analysis of OBP and CSP genes in the genomes or EST libraries of social species have not shown higher complexity with respect to solitary species, while hydrocarbons were never found as endogenous ligands of CSPs. Using different proteomic approaches, we have analyzed the expression of OBPs and CSPs in pheromonal glands, olfactory organs and other tissues/organs of Apis mellifera. We found that the highest number of OBPs (13 out of 21) is expressed in the distal antennal segments and that two are highly expressed in larvae (OBP14) or in juvenile females (OBP13). OBP21 is highly expressed in the mandibular glands. Only two of the six CSPs were detected at the protein level. CSP3 in most tissues, CSP1, instead, more specifically in antennae. A comparison of the expression pattern of OBPs and CSPs in the honeybee will be compared with information available so far in other Social and non social insects.

OR276

Chemical communication and family life: sophisticated signaling system during brood care in the biparental burying beetle

Sandra Steiger

The evolution of parental care can be seen as an important step in the evolution of sociality as it leads to the formation of family groups. As in highly social organizations, in family groups communication and recognition play an essential role in structuring daily life, even such a family group may only consists of a father, a mother and their offspring. I will provide an overview of our recent work on communication processes in the burying beetle *Nicrophorus vespilloides*, a subsocial insect performing elaborate biparental care. I will demonstrate that these beetles have evolved a sophisticated recognition and signaling system based on chemical cues and signals. They do not only recognize their breeding partners and are able to distinguish them from intruding conspecifics of the same sex, but we also found evidence that females communicate their temporary 'sterility' during the time of offspring feeding to avoid costly copulations with their male partner. By revealing the identity of the chemical substance and its linkage to the beetle's hormone system, I will provide an explanation of how honesty of the signal is guaranteed. In general, elucidating the communication system of a subsocial insect may provide additional insights into the evolution of signaling systems in eusocial insects.

OR277

Brood discrimination in the ant Formica exsecta

Unni Pulliainen, Nick Bos, Patrizia d'Ettorre, Liselotte Sundström

In social insects, the ability to recognize and discriminate nestmates from non nestmates plays an important part in maintaining colony integrity and protecting the colony and its resources from unrelated intruders and social parasites. Typical colony intruders are adult individuals, and thus recognition among adults is highly developed and precise. However, whether ants can recognize and discriminate brood of different origins is less clear. From a fitness point of view it would naturally be beneficial for a colony to accept nest mate brood. Accepting alien worker destined brood, especially pupae, might not be harmful either, and it might even be beneficial, as in some cases alien workers, adopted as brood, successfully integrate into adoptive colonies. However, accepting alien sexual brood should be avoided as these individuals may use colony resources without contributing to the colony, and instead act as social parasites. We investigated whether worker ants are efficient in discriminating brood, and whether the discrimination rules differ among brood of different castes. We answer these questions using behavioural assays coupled with chemical analysis in the ant *Formica exsecta*.

OR278

On the use of adaptive resemblance terms in chemical ecology

Sebastian Pohl, Christoph von Beeren, Volker Witte

Many organisms (mimics) show adaptive resemblance to an element of their environment (model) in order to dupe another organism (operator) for their own benefit. We noted that the terms for adaptive resemblance are used inconsistently within chemical ecology and with respect to the usage in general biology. Here we first present how resemblance terms are used in general biology, and then comparatively examine the use in chemical ecology. As a result we suggest the following consistent terminology: ‘chemical crypsis’ occurs when the operator does not detect the mimic as a discrete entity (background matching). ‘Chemical masquerade’ occurs when the operator detects the mimic but misidentifies it as an uninteresting entity, as opposed to ‘chemical mimicry’ in which an organism is detected as an interesting entity by the operator. The additional terms ‘acquired’ and ‘innate’ may be used to specify the origins of mimetic cues.

OR279

Costs and benefits of cooperation: primary polygyny in harvester ants

Jennifer Fewell, Juergen Gadau

Primary polygyny by ant queens creates an interesting layering of social systems, with cooperative associations of nonrelatives layered over eusociality. The harvester ant species, *Pogonomyrmex californicus* has contiguous populations in which queens either found nests singly (haplometrotic) or as groups of nonrelatives (pleometrosis); pleometrotic associations continue on to become polygynous colonies. Queens of these two types can be combined into artificial social groups to test assumptions for the evolution of cooperation. We will present data suggesting that cooperative sociality at the colony founding stage provides individual fitness benefits to pleometrotic queens that are not predicted by current models of cooperation, and that these effects are mediated by social dynamics. We have found that, in the contexts of aggression and division of labor, interactions among queens generate emergent or nonlinear effects on individual queen phenotype. In particular, both conflict escalation and emergent task specialization seem to create unexpected fitness costs for haplometrotic queens. In contrast, pleometrotic queens paired together seem to benefit from potential efficiencies of task coordination. Further, pleometrotic queens may take advantage of normally solitary founding queens in mixed associations, becoming the ‘cheater’ by avoiding more costly tasks. These benefits of cooperation during early colony founding, however, may be balanced by constraints on individual reproductive output later at colony maturity. The collective data from colony founding to reproduction provide a unique opportunity to consider the costs and benefits of cooperative sociality across life history stages.

OR280

The importance of property and privatization in social insect evolution

Joan Strassmann, David Queller

Property is a neglected topic in biology, though examples include territories, domiciles and nest structures, food caching, mate guarding, and the resources and partners in mutualisms. Resources most worth privatizing are often high in value. To be useful to their owner in the future, they are typically durable and defensible. Resources are often privatized by force or threat of force, but privatization can also be achieved by hiding, by constructing barriers, and by carrying or incorporating the property. Property is a topic of particular interest to social insects for two reasons. First, the returns on savings and investments can accrue to relatives, including descendants. This makes it evolutionarily advantageous to build elaborate nests and stores, from the honey of bees to the fungus gardens of some ants and termites. Second, social groups are particularly good at privatization because they can divide tasks among members, so they can simultaneously guard property and forage, for example. These two advantages of privatization enhance the evolution of cooperation through kin selection and explain some of the most spectacular features of social insects.

OR281

Evolving eusociality: The effects of manipulation, relatedness and genetic diversity.

Peter Nonacs

Cooperative sociality has two potential advantages over a solitary life history: (1) Opportunities to ally with close genetic kin and reap inclusive fitness benefits from aiding them; and (2) The ability to create social heterosis through synergistic cooperation across genetically diverse individuals with complimentary skill sets. To maximize one of these benefits, however, requires limiting or losing the other benefit. Both cannot be simultaneously increased. The benefits of high relatedness have often been invoked as being almost a prerequisite for the evolution of cooperative breeding and sterile morphological castes (i.e., the monogamy hypothesis). However, theoretical models on the evolution of these traits have produced mixed results, with some models predicting that monogamy is neutral or can even retard the spread of cooperation in populations. Furthermore, recent data from a facultatively social bee strongly suggest that mothers manipulate daughters into helper roles, rather than daughters maximizing their inclusive fitness from working. If maternal manipulation is responsible for creating the initial stages of a eusocial society, then matedness levels become irrelevant from the standpoint of maternal inclusive fitness (i.e., the threshold for cooperation from Hamilton's Rule, $r > c/b$, is fixed at 0.5). In this presentation I will do two things. (1) Resolve why the alternative models differ in their predictions on the advantages (or lack thereof) from monogamy and haplodiploidy vis-a -vis multiple mating and diplodiploidy. (2) Incorporate the advantages of genetic diversity in a model for the evolution of cooperation that directly tackles the trade-off of kin-directed nepotism versus social heterosis. The latter follows from the realization that r, b and c need not be independent variables. With social heterosis, as relatedness decreases, benefits can increase or costs decrease.

OR282

Population genomic approaches for studying the evolution of sociality

Amro Zayed

Why and how did sociality evolve? There are several adaptive explanations for the evolution of sociality, yet we know little about the prevalence of natural selection acting on the genomes of solitary, primitive and advanced eusocial insects. The emerging field of population genomics holds great promise for answering important questions in the field. Sequencing the genomes of many individuals per species allows researchers to quantify the strength of selection on protein-coding and regulatory loci in an unbiased manner. Population genomic data can thus be used to identify loci with signatures of kin-selection. Additionally, this data can be used to study the fitness effects of mutations in queen-biased and worker-biased loci, and to understand how pleiotropy influences the molecular evolution of loci that affect both queen and worker traits. Ultimately, population genomics will provide the needed link between theory and functional studies, which will allow us to develop and test mechanistically explicit theories for the evolution of social behaviour. I will illustrate the utility of population genomics using two bee datasets.

OR283

Eusocial evolution in termites

Kenneth Howard, Barbara Thorne

Understanding how and why eusociality evolved requires inferences about ecological pressures faced by each clade's non-eusocial ancestors. In the case of prototermites, ancestors co-inhabited resources and would have inevitably met neighboring conspecific families. The extant species *Zootermopsis nevadensis* (Archotermopsidae) forms fused colonies following interactions with neighbors. When laboratory-maintained colonies interact, reproductives are killed, surviving members of both families merge, and developmentally flexible helpers from both original colonies may differentiate into new reproductives within the fused colony. Samples taken one and two years following the interaction also confirm that some individuals, including reproductives, can be hybrids of the two original lineages. The larger fused colony has an advantage in future meetings with smaller colonies occupying the same piece of wood. Colonies collected directly from the field demonstrate that interactions and fusions are relatively common in nature. As a consequence, termite helpers have opportunities to become reproductives in young colonies, providing incentives to stay in their nest, rather than attempt high risk dispersal, despite lowered relatedness within a fused colony. Therefore, direct opportunities for offspring to reproduce may have favored cooperative, fused colonies, and functioned as a key selective pressure impacting prototermites. Fusions of unrelated colonies also occur in some other ‘one piece' (feed within their nest wood; never forage outside that resource; cannot relocate) termites as well as in some more derived species. Comparisons of differing ecological situations that favor colony fusion could yield clues regarding selection for eusocial traits and cooperation with non-relatives. Useful comparisons can be made with colony fusions in ants and honeybees, as well as drifting and usurping behavior of primitively eusocial bees and wasps. The unique ecological contexts affecting each taxon may have favored different suites and convergences of key traits, both early and later in the evolutionary progression from family groups to eusocial colonies.

OR284

Eusocial evolution without fortress defence in aphids

Keigo Uematsu, William Foster

Nesting has been described as a necessary factor in the evolution of eusociality. Closed nests can exclude predators, enabling efficient colony defence. In ‘fortress defenders’, nests also provide valuable food sites such that colony members need not forage outside, leading to high intra-group relatedness. Almost all social species form nests: this includes the aphids, where eusociality is correlated with gall-forming. Nevertheless, in some aphid species, sterile soldier castes have evolved in open colonies, where individuals congregate but do not develop a nest structure. These aphids pose an interesting problem about the preconditions for eusocial evolution. However, the ecological and genetic factors that have favoured soldier evolution in these open colonies have been poorly studied. Here, we compare the kin structures of eusocial and non-eusocial aphid species of the tribe Cerataphidini and investigate whether relatedness has promoted eusocial evolution in this group. In addition, we investigated the presence of altruistic behaviour in non-social species. We show that head-butting between colony members, which appears to be fighting behaviour, may in fact be an altruistic behaviour for young nymphs if they are yielding their feeding sites to older nymphs with higher reproductive value. We will discuss why eusociality in open colonies has evolved in only 1% of the 4400 species of aphids.

OR285

A comparative approach to understanding the evolution of social behavior

Sarah P. Lawson, Patrick Abbot

Since Darwin first wondered about sterile castes in ants, the study of social evolution remains, in many respects, synonymous with the study of ants, bees, and termites. There have been efforts in recent years to broaden the scope, by including such ‘non-traditional insects' as aphids and thrips. The argument for studying these groups is that they directly address the issue of generality in our conceptual understanding of a major thematic problem in evolutionary biology (How do groups suppress conflict such that cooperative integration emerges?) while offering opportunities for novel insights. Experimental work can thus focus precisely on comparing the traits that vary across a ‘major transition’ in evolution. Our research capitalizes on experimental opportunities to broadly test general themes in social evolution, using galling aphids as a model system. Aphids offer key experimental advantages for studying social behavior, such as varying degrees of sociality in closely-related species, clonality, which permits genotype-specific manipulations, and herbivory, which allows us to examine how sociality emerged at the plant-animal interface. By integrating ecological, behavioral, and molecular approaches to compare three aphid species in the genus *Pemphigus*, which vary in sociality, we have begun to 1) Understand the functional basis of defense, which is the defining feature of sociality in aphids, 2) Explore other features of sociality, like communal housekeeping behaviors, and 3) Examine the cost of cheaters. We found that for many ecological and behavioral traits there seems to be a continuum, with the social species on one end, the weakly-social species in the middle and the non-social species on the other end. We will discuss these results in the common light of sociality with the goal of understanding what factors are key in the evolution of advanced sociality in aphids and how this disparate taxa has converged on the phenotype of sociality.

OR286

Social parasites as tools to examine the evolution of eusociality

David Nash

The evolution of eusociality is thought to be tightly bound with the evolution of traits that align the interests of multiple individuals, promoting cooperative over antagonistic behaviour. A fruitful method of examining such traits is through studying cases in which eusocial colonies are exploited by individuals with non-aligned interests. Examining how conspecifics, closely related species and more distantly related social parasites have evolved to exploit insect societies can give valuable insights into how these societies became cooperative in the first place. Here I will give a brief overview of how and to what extent these different types of social parasites can shed light on some of the open questions in understanding eusocial evolution, using microgynes of *Myrmica rubra*, the ant social parasite *Acromyrmex insinuator* and butterflies in the family Lycaenidae as examples. In particular, I will concentrate on how genomics and next-generation sequencing techniques currently, or soon will, allow us to examine evolutionary changes associated with transitions from cooperation to exploitation.

OR289

The superorganism: problems and perspectives

Richard Gawne

The superorganism concept has become a fixture in the literature on social insects, but a number of questions regarding its viability, and capacity to drive research remain unanswered. I explore these existing questions, and raise several new challenges to the legitimacy of the concept. First, I show that a review of the literature reveals that there is not a single superorganism concept, but a host of distinct superorganism concepts, many of which are, at best, vaguely delineated. In short, there seems to be no mutually agreed upon notion of what it means to assert that colonies are superorganisms, and the related question of whether some or all eusocial species form superorganismic colonies has rarely even been discussed. The fact that problems such as these have not been addressed casts doubt on the validity of the concept. I conclude by emphasizing that the question of whether colonies are superorganisms is anything but a superficial semantic quibble. Among other things, this is because the concept has often been used to support the claim that the advent of eusociality represents a major transition in evolution. I analyze the superorganism concept in light of what is known about the other major transitions, and suggest that the perspective provided by such an approach provides additional reason to believe that it is, at best, a convenient heuristic device, and at worst, a deceptive metaphor that has led to a distorted view of evolutionary history.

OR290

Unraveling the mysteries of honeybees in the Mascarene Islands

Maeva A. Techer, Johanna Clemencet, Christophe Simiand, Patrick Turpin, Bernard Reynaud, Helene Delatte

The islands of the South West of the Indian Ocean are a hotspot of biodiversity with high rates of endemism. Honeybees play a major role in theses insular tropical ecosystems, interacting with indigenous and endemic species but also with exotic and invasive species. In Madagascar (heart of the hotspot of biodiversity) the honeybee has already been described as *Apis mellifera unicolor*, an endemic subspecies belonging to the African lineage. However, knowledge about the established honeybee populations from other islands is extremely limited and their origins and diversity remain unknown. We focused on *A. mellifera* populations from the three islands (La Réunion, Mauritius and Rodrigues) of the Mascarene archipelago (> 700km East from Madagascar). This study aimed to 1) characterize the evolutionary lineages and subspecies, 2) determine the genetic diversity and 3) reconstruct the roads of colonization by testing multiples scenarios. Maternal origin of *A. mellifera* was assessed with the study of the mitochondrial tRNAleu-COII intergenic region. Then, nuclear genetic diversity was investigated with 18 microsatellites markers on all samples. A total of 2101 colonies were sampled in La Réunion, 355 in Mauritius and 524 in Rodrigues between 2011 and 2013. Mitochondrial analyses revealed striking differences among islands. On La Réunion, 91% of the sampled colonies were attributed to *A. mellifera unicolor* and 4% to European lineages; in Mauritius the proportion of African and European lineages were equivalent (45% and 43%, respectively) whereas Rodrigues was exclusively represented by exotic European subspecies. Hypotheses for such contrasting patterns between close islands are discussed regarding to original forest loss (75% in La Réunion to 100% in Rodrigues), distance from Madagascar (La Réunion at 700 km to 1400 km for Rodrigues) and past human activities. Nuclear genetic diversity results showed that hybridization occurred between introduced or indigenous subspecies in all islands but at different levels.

OR291

Genetic structure of social bees in Neotropic continental islands

Flavio Francisco, Leandro Santiago, Yuri Mizusawa, Benjamin Oldroyd, **Maria Arias**

Habitat destruction generates forest fragments whose ecological dynamics can be similar to those of islands. Thus evolutionary processes observed on islands can be used to predict outcomes at mainland sites. The continental islands off the south-eastern coast of Brazil are 16000 years old. We studied the genetic structure of the eusocial stingless bee *Tetragonisca angustula* and the primitively eusocial bumblebee *Bombus morio* on these islands as a predictor of the likely impact of habitat fragmentation on these species at mainland sites. We visited 11 islands that have very different sizes and distances from the mainland sampling 1003 stingless bees and 704 bumblebees. *B. morio* was found in all islands whereas *T. angustula* was found only on three islands that are both large and close to the mainland. *T. angustula* populations have low mitochondrial genetic diversity and high population structuring, which we attribute to queen philopatry. Nonetheless, microsatellites showed lower structure and moderate to high genetic diversity, indicating significant dispersal by males. The genetic diversity observed for *B. morio* for both mitochondrial and microsatellite markers was high and no population structuring was detected. For both species, island and mainland populations showed similar genetic diversity, although it was higher in *B. morio*. Geography and biological characteristics such as mating behaviour, dispersal and bee size are likely responsible for these differences in population genetic structure. We conclude that *T. angustula* and *B. morio* are unlikely to suffer extinction die to land clearing, mainly because of their capacity to survive in urban environments and their high rates of male dispersal.

OR292

The ants of Hispaniola: past, present and future.

David Lubertazzi

The Caribbean region is notable for being a biodiversity hotspot. The majority of this diversity is found on the larger islands of the Greater Antilles. Hispaniola, the second largest island, has garnered attention from numerous myrmecologists yet still harbors a poorly characterized ant fauna. There also exists intriguing evidence regarding the insular evolution of some local ant taxa but it is currently not possible to do much more than speculate about taxon specific patterns and potential evolutionary processes due to inadequate sampling. Properly characterizing the island's ant fauna and better understanding its development requires a critical examination of historical specimens and, where possible, additional sampling. Past collections will necessarily have to suffice for some areas. Haiti, occupying the western third of the island, is ecologically decimated. A large majority of the country's natural habitat, and presumably the ant communities they once contained, has been destroyed. The Dominican Republic comprises the remaining two-thirds of Hispaniola. It nominally contains one of the largest and most comprehensive national park systems in the Caribbean. Unfortunately these lands are being steadily degraded by unmanaged anthropogenic conversion. My talk will summarize what is presently known about the Hispaniola ant fauna. This will include a history of ant sampling on the island, what is being learned from the databasing of previously collected specimens, and the results of recent Dominican Republic ant surveys. Despite a summary of our current knowledge showing the potential for promising future studies of Hispaniola's ants, I will close by showing how this research is increasingly imperiled by ongoing threats to the island's existing conservation lands.

OR293

Rediscovery of New Caledonian bulldog ant. Insights into island disharmony

Herve Jourdan, Christian Rabelling, Maureen Cateine, Edward O. Wilson

Because of their remoteness and isolation, islands are dreamed territories for biologists and ecologists where the unexpected is expected, including for ant community organization patterns. New Caledonia archipelago, in the south west pacific, make not an exception. It appears to have been colonized and occupied by a highly endemic and unusual ant fauna, and represent a true ant diversity hotspot in respect to Australian, Indo-Australian and Oriental regions. Following the rediscovery of a population of the rare bulldog ant *Myrmecia apicalis*, an endemic of New Caledonia and the only species of *Myrmecia* known outside of Australia, 70 years after the last sight, we shed light on a taxon which appears unusual for the genus. Its small colonies build cryptic nests in the forest soil and forage arboreally and diurnally. Also, this ant seems to survive in small remnants of coastal forest which appear as unique last refuge facing spread of invasives. Then *M. apicalis* appears as a 'missing link' in the context of biogrography of Australasia and island colonisation. At the light of this original species rediscovery, New Caledonian ant community appears unique and original as adaptative shifts have occur in the context of colonization by ants, resulting in the constitution of a real disharmonic fauna by comparison of others tropical ant communities. It illustrates the interplay of ecological opportunities and constraint which result in the lack of some widely co-evolved patterns observed elsewhere for forest ant communities. The recent spread of numerous exotic ants also appears as a new challenge for these communities and the survival of these communities and associated biogeographic patterns would not be guarantee. Then the conservation of such communities appears as a patrimonial priority according to high value for the understanding of diversification of social insects.

OR294

Diversification and dispersal of Australasian ants, from populations to species.

Milan Janda, Jan Zima, Michaela Borovanska, Pavel Matos Maravi

Oriental and Australasian islands represent one of the most complex biogeographic systems on Earth and are often called ‘evolutionary laboratories’, hinting at their potential to generate many diverse cases of population diversification and speciation. We thus compare diversification and dispersal patterns of different ant lineages in this region. We used ddRADSeq-generated SNPS, microsatellites and mitochondrial and nuclear sequences from over 100 populations of the weaver ant *Oecophylla smaragdina* and the trap jaw ant *Odontomachus simillimus* across their distribution range. For *Oecophylla*, biogeographic reconstructions suggest that the ants dispersed into Australasia from mainland Asia via Sulawesi. Most of the populations on SE Asian islands form unique clusters suggesting single colonization events. New Guinea and Australia show a multipart dispersal pattern with geographically distant populations from over 1000 km along the coast more closely related than inland populations within New Guinea. In *Odontomachus*, both mtDNA and nDNA markers suggest genetically homogeneous clusters correspond with geographical structuring (archipelagos), an exception being Fiji, which shows multiple colonization events from different source populations. In New Guinea, mtDNA suggests the occurence of three different lineages, while microsatellites suggest one homogeneous cluster. To study diversification and dispersal patterns at the species level, we reconstructed the phylogeny of Melanesian members of *Prenolepis* genus-group. Distributional range and habitat association analyses in a phylogenetic context show that New Guinea is an important source for colonization of Melanesia and the South Pacific, and that levels of endemism are linked to island size. Although most South Pacific islands are inhabited by wide ranging Prenilepidini species, several species previously considered widespread actually constitute several cryptic species.

OR295

The ants of Fiji: systematics, biogeography and ecology of an island arc fauna

Eli Sarnat, Evan Economo

Since the time of Darwin, biologists have sought to document and understand the unique evolution and ecology of island biotas. Oceanic archipelagos are often adorned with spectacular evolutionary radiations and unique ecosystems. These distinctive faunas, however, are highly vulnerable to human activities, climate change, and introduction of exotic species. Among island ant faunas, perhaps nowhere are these themes on display as prominently as in the Fijian archipelago. The Fijian terrestrial biota was assembled during approximately 20 million years of over-water colonization, in situ evolution and speciation, and more recently through the arrival of species as stowaways on canoes, galleys, and battleships. Today's Fijian ant fauna is characterized by extreme geographic isolation from source areas, differentiation and pattern formation among islands, and contemporary invasions. The ant fauna of Fiji, clustered together on an oceanic archipelago abutting the eastern extent of Old World ants' native range, is a diverse assemblage of endemic radiations, pan-Pacific species, and exotics introduced from around the world. Here we provide a taxonomic synopsis of the entire Fijian ant fauna by incorporating previously published information with the results of a recently completed, archipelago-wide biodiversity inventory. This synopsis updates the first and only other treatment of the fauna, W. M. Mann's 1921 monograph, The Ants of the Fiji Islands. A total of 187 ant species representing 43 genera are recognized here. Of these species, 70% are endemic to Fiji, 18% are native to the Pacific region, and 12% are introduced into the Pacific region. The monograph has established the ants of Fiji as a model system for testing ideas about eco-evolutionary community assembly and biological invasion, and will serve as a foundation for future work on Pacific island insects.

OR296

Convergent evolution of Stage-I species in Camponotus

Ronald Clouse

In his description of the taxon-cycle hypothesis, Wilson (1961) included *Camponotus* (*Tanaemyrmex*) in the set of genera and subgenera that produce 'Stage-I' species, which are those that disperse into outlying regions from large landmasses. What has been called *C. chloroticus* is a member of this subgenus and is indeed a widespread form throughout the Pacific. A recent collaborative effort on its systematics has shown it actually consists of two distantly related species. One lives predominantly in Micronesia, the other in Polynesia, and they overlap in Melanesia. The Micronesian species is closely related to *C. humilior* and *C. novaehollandiae* in Australia and New Guinea, and the Polynesian species is related single-island endemics in Micronesia, including *C. eperiamorum* on Pohnpei and what has been called *C. irritans kubaryi* on Palau. Both species favor marginal habitats, have large ranges, and are the most recently evolved species in their respective lineages (originating < 5 Ma). These features are consistent with those predicted for species engaged in the expansion phase (Stage I) of the taxon cycle. The species are not, however, consistent with the idea that expanding lineages originate on large landmasses, as the Polynesian species appears to have diversified down from Micronesia, and the Micronesian species up from Vanuatu. In addition, some Pacific members of the subgenus are clearly not Stage I, and some appear to have colonized from the New World, not nearby large landmasses in Southeast Asia. The remarkable morphological convergence of these two forms is described and future attention is directed to the connections between morphology, behavior, and ecology in this group. This result also raises questions about resource partitioning between the two forms in Melanesia, where they overlap.

OR297

Island communities in the context of global hyperdiversification

Evan Economo, Eli Sarnat, Benoit Guenard, Beatrice Lecroq, Lacey Knowles

The analysis of how island communities assemble through ecological and evolutionary dynamics has long shed light on broader theoretical questions in ecology and evolutionary biology. Here we examine the assembly of Pacific island faunas of the hyperdiverse ant genus *Pheidole*, placed in context of a new global phylogenetic and morphological analysis of the genus. We examine the extent to which communities were formed through dispersal and colonization versus in situ evolution of species and morphotypes, the origins and genetic structure of widespread species, and the role of ecological niches in promoting dispersal. These pieces, taken together, shed light on broader integrated theories for biogeographic dynamics in the region such as Wilson's taxon cycle hypothesis.

OR298

Supergenes mediate alternative social organization in ants

Laurent Keller, John Wang, Yannick Wurm, Oksana Riba-Grognuz, DeWayne Shoemaker, Mingkwan Nipitwattanaphon

In this talk I will present data showing how a supergene underlies important variation in social organization and individuals phenotypes in fire ants. I will then argue why one should expect to find many such cases in other social insects

OR299

Royalactin induces queen differentiation in honeybees

Masaki Kamakura

The honeybee (*Apis mellifera*) forms two female castes, the queen and the worker. This dimorphism depends not on genetic differences, but on ingestion of royal jelly (RJ). So far, I found that a 57-kDa protein in RJ, designated as royalactin, induces differentiation of honeybee larvae into queens. Royalactin increased body size and ovary development and shortened developmental time in honeybees. Surprisingly, it also showed similar effects in fruit fly (*Drosophila melanogaster*). Mechanistic studies revealed that royalactin activated p70 S6 kinase, which was responsible for the increase of body size, increased the activity of mitogen-activated protein kinase, which was involved in the decrease of developmental time, and increased the titer of juvenile hormone (JH), an essential hormone for ovary development. These actions were mediated by epidermal growth factor receptor (Egfr) in fat body (FB) of both insects, because knockdown of Egfr expression resulted in a defect of all phenotypes induced by royalactin. These findings indicated that a specific factor in RJ, royalactin, drives queen development through an Egfr-mediated signaling pathway. Furthermore, I investigated the factors involved in increase of fecundity by royalactin. I found that Egfr signaling in FB, which is activated by royalactin, leading to induction of JH synthesis and a consequent increase of yp expression, thereby increasing fecundity. Methoprene tolerant (Met) is a specific receptor of JH. The mutation of Met suppressed the increase of gene expression of yp and fecundity by RJ, but did not affect the changes of other phenotypes in flies reared with RJ. Inhibition of *Drosophila* Met in FB caused suppression of increased yp expression and fecundity in flies reared with RJ, suggesting that increase of JH titer by royalactin might induce upregulation of yp expression via Met in FB, leading to the increase of fecundity.

OR300

Juvenile hormone signaling pathways and the social physiology of the bumblebee Bombus terrestris

Guy Bloch, Hagai Shpigler, Yang Li, Adam Siegel, Zachary Huang, Gene Robinson, Mark Band

The evolution of advanced sociality in bees was apparently associated with significant modifications in juvenile hormone (JH) functions. By contrast to most insects in which JH is a gonadotropin regulating female fertility, in the highly eusocial honeybee (*Apis mellifera*) JH has lost its gonadotropic function in adult females and instead it regulates age-related division of labor among worker bees. This variation in JH function provides an excellent model system for understanding major evolutionary changes that are mediated by modifications in endocrine signaling pathways. We manipulated JH levels in workers of the 'primitively eusocial' bumblebee *Bombus terrestris* by removing the sole JH producing glands, the corpora allata (CA). Allatectomized bees showed strong reduction in several behavioral, physiological, and molecular systems: egg laying, egg-cell construction, ovarian development, hemolymph vitellogenin protein abundance, wax secretion, and vitellogenin and the transcription factor krüppel homolog 1 fat body transcript levels. These effects were reversed, at least partially, by treating the allatectomized bees with JH-III, the natural JH of bees. By combining these manipulations with RNA-seq transcriptomic analyses we identified hundreds of brain transcripts that are regulated by JH. These include krüppel homolog 1 and many genes of the insulin/ insulin-like signaling pathway. JH also influenced the splicing pattern of several dozen transcripts. By contrast, manipulations of JH levels by the CA inhibitor precocene and replacement therapy treatments, which did inhibit ovaries, had no effect on foraging or nursing activities. These results provide the strongest available support for the hypothesis that JH is a gonadotropin in *B. terrestris* and start to reveal the molecular processes it regulates in the bumblebee brain. Our study lends credence to the hypothesis that the evolution of eusociality was associated with major modifications in JH signaling in honeybees but not in bumblebees.

OR301

Transcriptomics on social interactions in termites: effects of soldier presence

Dai Watanabe, Masatoshi Matsunami, Yoshinobu Hayashi, Hajime Yaguchi, Shuji Shigenobu, Toru Miura, Kiyoto Maekawa

The organization of social-insect colonies requires sophisticated mechanisms to regulate caste composition according to colony demands. In termites, although it has been known that soldier is responsible for inhibiting workers to differentiate into soldiers, the mechanism underlying the regulation of soldier differentiation is still unclear. Since genome-wide approaches for the identification of genes involved in social interactions are expected to clarify this issue, we performed transcriptome analyses by Next Generation Sequencer to identify genes expressed in workers that are up-regulated by the soldier existence in the subterranean termite *Reticulitermes speratus*. In our analyses, we applied the artificial induction of soldier differentiation by juvenile hormone (JH), where the inhibitory effects of soldier differentiation by the soldier presence were evaluated. By comparing transcriptomes prepared from workers that were kept with or without soldiers under the JH induction, the 181,244 contigs (i.e. potential transcripts) that covered 70% of the protein-coding genes in *Drosophila melanogaster*, were obtained by transcriptome sequencing and de novo assembly. Using the contigs, expression analyses were performed to identify genes differentially expressed in each experimental treatment, showing that only a small number of genes were up-regulated by soldier presence. Gene Ontology (GO) analysis showed that some of those are responsible for the negative regulation of cell cycle and transportation of xenobiotic substances, suggesting that they could be involved in the developmental arrest and pheromonal regulations in workers. It was also found that many genes were up-regulated by JH application and actually included factors for JH regulations and insulin signaling pathways. By identifying the candidate genes underlying the regulatory mechanisms of social interactions, it is suggested that the suppression of soldier differentiation by the soldier presence could be accomplished by the expressions of a small number of genes that repress the majority of genes required for soldier differentiation.

OR302

Linking development with worker behaviors using RNAseq and gene manipulations

Christina Schulte

Honeybee workers show altruistic behaviors in contrast to queens and drones, which show behaviors that are related to reproduction. The collective behaviors of the worker bees produce group phenotypes that allow them to remain well-adapted in a changing environment. These worker specific behaviors have been largely described but we have little understanding of the molecular control that specifies these behaviors in the brain during development, and of its evolution that gave rise to social behaviors during the last 60 million years. Differentiation of the worker brain is specified by female- and caste-determining signals. The sex-determining signal is implemented by Feminizer protein (Fem) that regulates female splicing of gene transcripts downstream in the sex-determining cascade. On the other hand the worker specific brain is determined by caste development and differential feeding of royal jelly that induces an increase of the Egfr expression level in queens and a reduction in workers. The caste and sexual signals must be integrated which determines the hard-wiring of the worker bee brain during development. I will present data of an approach that seeks to identify genes, which specify the altruistic worker behaviors by hard-wiring the neuronal substrate during brain differentiation. These genes are essential for worker-specific behavior, but not behavior in general. We identified sexual spliced and caste-specific expressed genes via RNAseq approaches and molecularly verified the splicing or expression pattern of these genes to identify good candidate genes. Finally, I will present data of our honeybee expression system, which will enable for the first time the conditional manipulation of gene functions through genetic transformation. The high efficient integration and expression of this system encourage to knockdown our candidate genes to dissect the gene's role in specifying behavior.

OR303

Genomics of caste determination and social parasitism in harvester ants

Chris Smith, Alexander Mikheyev, Juergen Gadau, Sara Helms-Cahan, Andrew Suarez

Reproductive division of labor is the core element of eusociality and it evolved via the canalization of two (at least) female developmental pathways, one for queens and one for workers. The genes that regulate the bifurcation of these developmental pathways are key regulators of social organization, and potentially involved in the evolution of eusociality. We use the *Pogonomyrmex* genetic caste determination system to map out the timing of developmental differentiation of queens and workers during larval development. We couple development with comparative genomics on the hybridizing species from which the genetic caste determination system evolved in order to look for developmental genes that associate with caste determination. Lastly, we use comparative genomics to examine the consequences of losing the worker caste, which has occurred in two social parasites that are part of the same species complex. Using the social parasites we are able to ask whether genes involved primarily in worker development are lost as part of the evolution of parasitic specialization and the loss of the worker developmental pathway? In summary, our goal is to find genes that are essential for eusociality, at least in harvester ants, and then see what happens to these genes when social parasitism causes a reversion from eusociality. We suspect our findings will have broad relevance to the study of phenotypic plasticity and social evolution.

OR304

Anarchy in the honeybee colony: genetic basis of worker sterility

Isobel Ronai, Vanina Vergoz, Julianne Lim, Benjamin P. Oldroyd

In honeybee (*Apis mellifera*) colonies the queen monopolises female reproduction, while the workers are 'altruistically' sterile. However, even within the highly cooperative system of the social insects reproductive cheating occurs. A mutant 'anarchistic' strain of honeybee has been selected in which workers activate their ovaries and lay eggs, despite the presence of a queen. This compelling example of intra-specific social parasitism enables the investigation of mechanisms that must have evolved to enforce worker sterility. Mapping and gene expression studies of the anarchistic strain have yielded a short list of candidate genes for worker sterility. Anarchy (GB13621), a peroxisomal membrane protein, is the strongest candidate gene based on map location and differential expression between anarchistic and wildtype workers; and workers with activated and non-activated ovaries. To determine whether there is a causal relationship between expression of the candidate genes and ovary activation we are experimentally manipulating gene expression using RNA interference and observing the effect on reproductive phenotype. This will help establish the molecular pathway that regulates functional sterility in honeybee workers.

OR305

Larval transcriptomes and developmental plasticity in a tetraphenic ant

Lukas Schrader, Robert Knüppel, Tobias Platschek, Jürgen Heinze, Jan Oettler

One of the most enigmatic examples of developmental plasticity is found in eusocial insects where distinct castes and subcastes arise from the same genotype. In addition to queens and workers, the ant *Cardiocondyla obscurior* also evolved a male diphenism with winged and ergatoid (worker-like) individuals, rendering this species an ideal model to unravel principles of developmental plasticity. Using immunohistology and in situ hybridisation we describe caste-specific development of brain and imaginal discs of early to late 3rd instar larvae. Furthermore, by comparing individual transcriptomes of 28 early 3rd instar larvae, we assess differences in developmental pathways after caste determination in late 2nd instar. We describe caste specific, sex specific and winged/wingless-specific genes and pathways, shedding light on the genetic basis of developmental and phenotypic plasticity in eusocial insects. Finally, we show that these gene sets evolve under significantly different efficiency of purifying and positive selection as a consequence of their caste specificity.

OR306

Genetic regulation of behavior in the clonal raider ant

Peter Oxley, Daniel Kronauer

Mary Jane West-Eberhard's 'Ovarian Ground Plan Hypothesis' states that during the transition from subsocial to eusocial, the physiology and behavior expressed during the reproductive phase of the subsocial lifecycle became robustly expressed in the queen caste, while the physiology and behavior expressed during the brood care phase became expressed in the worker caste. Colonies of the clonal raider ant (*Cerpachys biroi*) consistently cycle between reproductive and brood care phases, which are synchronised to the development of the brood. Larvae hatch at the end of the reproductive phase and trigger the transition to brood care by inducing foraging behavior and suppressing ovarian activity in the adults. The reproductive phase, during which workers lay eggs and no foraging occurs, begins when the cohort of larvae pupates. The colony cycle of *C. biroi* thereby recapitulates the phasic lifecycle of the ancient subsocial ancestor, providing a unique opportunity to study the molecular regulation of reproductive physiology and worker behaviour. Using the newly sequenced genome of *C. biroi*, we performed qPCR of candidate genes previously shown to influence division of labour in social insects and found them to be dynamically regulated during the colony cycle of *C. biroi*. Using brood manipulation, it is possible to precisely regulate the transition between reproductive and brood care phases in discrete age cohorts of clonally related *C. biroi* workers. By collecting and RNA-sequencing individuals at specific intervals during this transition, we are creating transcriptional profiles of *C. biroi* brains associated with their phenotypic response to a well-defined social cue. So far, we have identified 100 genes that are differentially expressed between individuals in the reproductive vs. brood care phase. These transcriptional profiles will allow us to identify the molecular pathways that modulate behaviours that are fundamental to the division of labour in eusocial insects.

OR307

Molecular adaptations to advanced fungus farming in leaf-cutting ant symbiosis

Morten Schiott, Henrik H. de Fine Licht, Adelina Rogowska-Wrzesinska, Pepijn W. Kooij, Peter Roepstorff, Jacobus J. Boomsma

About 50 million years ago a single ancestor of today's more than 230 species of fungus-growing ants committed herself irreversibly to farming fungi for food instead of being a hunter-gatherer. The most derived genera within this group, the *Atta* and *Acromyrmex* leaf-cutting ants, have realized the key innovation that they use fresh leaves as substrate for their fungus gardens, which allowed them to tap into tremendously abundant resources and to evolve enormous societies. Assumingly this innovation was accompanied by specific adaptations in the ants and their fungal symbionts to be able to ameliorate the effects of defence compounds found in live plant tissues. A promising venue for identifying such adaptations at the molecular level is the fecal fluid of the ants, which contains many fungal enzymes that pass through the ant digestive system unharmed to end up in the fecal fluid that the ants mix with the fresh leaves and deposit on top of their gardens. To understand the adaptive value of this phenomenon, we have used state of the art proteomics and high-throughput genome and transcriptome sequencing to identify the proteins found in the ant fecal droplets. Enzyme assays for selected fecal proteins showed that they functionally disappeared from the fecal droplets when the ants were deprived of their fungal symbiont. We further used qPCR to establish that many of these proteins are more highly expressed in the hyphal tips (gongylidia) of the fungus on which the ants feed, than in normal mycelium, suggesting that they have been actively selected to be ingested by the ants. Of particular interest is the finding of a polyphenol-oxidizing laccase enzyme that shows signs of positive selection in the higher attine ant symbionts, and may be an important prerequisite for the ability to cope with the polyphenols present in living plant tissues.

OR308

The developmental basis of caste evolution in ants

Ehab Abouhe

For over 100 years, social insect biologists, from Darwin, to William Morton Wheeler and E.O Wilson, have been fascinated by the remarkable morphological diversity of the worker castes of ants. However, the origin and evolution of worker caste diversity remains a fundamental, yet unresolved, question. Here, I describe the last 20 years of research in my lab showing that developmental processes are key understanding this question and for connecting genes to societal regulation.

OR309

Stress and aging in honeybee workers

Olav Rueppell

The Western honeybee, *Apis mellifera*, is emerging as an important model in biogerontological research due to its social organization, high phenotypic plasticity, and experimental accessibility. Life expectancy of workers can be influenced by several experimental manipulations, such as restriction of foraging opportunity and changes to colony size and demography. These manipulations affect the workers' behavior, physiology, and life history. In most organisms, these variables are also influenced by different forms of stress but the connections of stress to social life history and life expectancy of honeybees have not been sufficiently studied. I will discuss a series of experiments to investigate survival of acute oxidative stress and its heritable consequences, the effects of early life stress on later life social behavior and life expectancy, and the influence of honeybee management practices on levels of oxidative stress and life expectancy. Characterizing the connections of different stressors to honeybee life history and mortality is of fundamental importance to understanding social life history evolution, but it also has significant implications for applied management practices to halt the honeybee health crisis.

OR310

Inducing rapid and slowed brain aging through manipulation of social tasks in honeybees

Daniel Munch

The honeybee model features extremely diverse longevity patterns among castes of queens and workers. The phenotypic disparities between the longest-lived caste (queens) and shorter-lived workers are relatively fixed in early development. In contrast, flexible longevity patterns are found in the different worker types. In brief, workers pass through a sequence of worker-type specific social behaviors. These include nursing the brood (nurse bees), foraging for nectar and pollen (forager bees), and temperature regulation in winter (winter bees). Here we exemplify the dramatic consequences of worker-type differentiation for behavioral and cellular aging: rapid senescence in foragers is contrasted by a slowed aging progression in nurses, and by an apparent absence of aging symptoms (negligible senescence) in winter bees. By manipulating the social environment of bees we show how individuals with short and extremely long lifespan can be transformed into one another. Our behavioral, anatomical, proteomic and epigenomic screening data support that worker type transformations affect common symptoms of behavioral and cellular senescence in the brain. Among these symptoms are reduced learning function as well as changed lipofuscin and protein abundance, e.g. of synapse- and glia-specific proteins. We also found that cellular senescence differs between different tissues and brain regions. On the colony-level we identify brood load as a key regulator of aging. On the cellular level, we argue that the alternative utilization of a common yolk precursor protein (vitellogenin) in nursing and somatic maintenance can link social resource transfers with slowed aging. Such a role in somatic maintenance is supported by the presence of vitellogenin in unexpected cellular locations, for example in certain brain cells.

OR311

The effect of colony size on lifespan in social insects

Boris Kramer

Social insects become more and more recognized as important model organisms for aging research especially because different female aging phenotypes emerge from a similar genetic background as a result of division of labor. Several recent findings point to a weakness of the evolutionary theories of aging to explain the lifespan patterns in social insects either between or within different castes. A variety of factors such as caste, social environment, body size and task have significant influence on the lifespan of members of a colony, especially workers. Therefore disentangling the factors that affect worker and queen lifespan is a next step to understand the evolution of aging patterns in the social insects and especially which mechanisms regulate the found lifespan patterns. Colony size is an important variable in the life history and ontogeny of insect colonies and is also known to affect worker lifespan. Here I want to present the results of two studies on the effect of colony size on lifespan in social insects: 1) an interspecies comparison of queen and worker lifespan in relation to average colony size and 2) a case study on worker lifespan in differently sized *Lasius niger* colonies. Our comparative study (1) revealed no relationship between colony size and worker or queen lifespan, but we find a general trend that queen and worker lifespan diverge with increasing colony size across species. Throughout the ontogeny of *L. niger* colonies (2) we find a similar trend. Worker that hatch in small colonies live longer that worker that hatch in large colonies, even if they are kept in a similar social environment.

OR312

Gene expression analysis of somatic maintenance in ants.

Eric Lucas, Oksana Riba-Grognuz, Miguel Corona, Yannick Wurm, Laurent Keller

The theory of somatic maintenance argues that a trade-off exists between longevity and reproduction, and therefore that long-lived phenotypes will be those that invest more into escaping mortality and senescence. Ants are typically characterised by a massive natural variation in lifespan between castes, with queens living as much as 15 times longer than workers while being genetically identical. Furthermore, ant queens are amongst the longest living insects, with queens of the ant *Lasius niger* living for as long as 29 years in laboratory conditions. The challenge is therefore to identify the key features of queen physiology and life history that underlie this drastic difference in aging. We address the theory of somatic maintenance by asking whether queens invest more heavily than workers into molecular systems of maintenance such as DNA repair, using tissue-specific high-throughput sequencing of age-controlled individuals of each caste. We found that 2-month-old queens do indeed have higher expression of maintenance genes than workers of the same age, but that this difference does not exist between 1-day-old queens and workers. Queens therefore appear to be investing more into maintaining their soma, but do not do so consistently across all life stages.

OR313

A decision theory approach to explaining aging in honeybee workers

Natalie Lemanski

Honeybees are an ideal model system for understanding the evolutionary basis of aging because of their extraordinary ability to adjust the aging process of workers according to colony needs. The lifespans of workers change according to the seasonal pattern of colony activity; while summer workers live only two to three weeks, winter workers may live up to 10 months. Workers can also extend their lifespans by postponing foraging. The prevailing evolutionary theory for aging in social insects suggests that senescence is a result of selection to optimize a trade-off between investing resources in somatic maintenance and reproduction. This ‘disposable soma’ theory predicts that colonies should invest more heavily in workers that have a lower risk of being lost to extrinsic mortality. In addition, the reallocation of resources from brood rearing to somatic maintenance explains the greater lifespans of winter compared to summer bees. Current theory fails, however, to explain worker lifespan during reproductive swarming. Analysis of colony needs and constraints during swarming reveal that, for colonies to remain of viable size, workers must exhibit extended lifespans despite experiencing equal or greater extrinsic mortality and working as hard as non-swarming workers in direct contradiction to the current disposable soma theory. Using a decision theory model, we can expand on the disposable soma theory of aging to explain this apparent contradiction and predict the optimal level of investment in somatic maintenance of workers under a variety of colony conditions.

OR314

Vitellogenin binds to cells for shielding effects in the honeybee

Heli Havukainen

A dramatic increase in protein called vitellogenin is a hallmark of changes that prepare the honeybee for wintertime or drought. Under such stressful periods, the honeybee workers must extend their lifespan until favorable conditions return. Vitellogenin is associated with increased resistance against oxidative stress and a slowdown of immunosenescence. What are vitellogenin's molecular mechanisms in supporting longevity in the honeybee? We show vitellogenin can shield insect cells against oxidative damage via binding directly to their membrane, and the protein recognizes damaged cells with increased phosphatidyl serine lipid on their surface in a putatively anti-inflammatory manner. We also introduce honeybee Vg as a pathogen pattern recognition receptor that binds to bacterial material, which adds to the current knowledge of honeybee immunity. In a wider evolutionary perspective, vitellogenin is not a honeybee specific protein, but proteins homologous to it are found in most animals ranging from the very primitive ones to humans. Accumulating evidence associates vitellogenin family proteins with immunological and aging-related processes in a range of species. Thus, vitellogenin is one of the proximate molecules in the regulation of lifespan in the honeybee, and potentially in other species.

OR315

Reproduction and longevity in Cardiocondyla *ants*

Jürgen Heinze, Alexandra Schrempf

Perennial social insects are characterized by the extraordinarily long lifespan of their reproductive females, which may be tens or hundreds of times larger than that of non-social insects of similar body size and also greatly surpasses that of conspecific non-reproductives. Evolutionary theories of aging explain this phenomenon from the low extrinsic mortality queens experience once they have successfully established their colony. In my talk I will summarize recent findings from our studies on *Cardiocondyla* ants, which indicate that both mating and egg laying have a positive effect on queen life span. Furthermore, our studies show that individual life span is greatly affected by the queen’s social environment without any changes in external mortality risks. I will also present preliminary data on the consequences of mating with viable or sterilized males on gene expression in queens.

OR316

Exploring queen longevity by RNA-Seq

Katharina von Wyschetzki, Jan Oettler, Jürgen Heinze

It is generally assumed that aging is genetically controlled. Experiments with short-lived organisms (flies, worms, mice) have revealed the involvement of different candidate genetic pathways. However, many of these studies have disregarded the enormous intraspecific variation in longevity among and within species. In social insects, males and the different female castes (queens, workers) differ in many life history traits, including different aging rates. Ant queens are famous for their exceptionally long life spans compared to workers and males, making them a good model for the study of aging. The myrmicine tramp ant *Cardiocondyla obscurior* lacks the 'reproductive senescence' that characterizes most other organisms (Heinze & Schrempf 2012). In addition, queen-male co-evolution affects the life span of the queen (Schrempf et al. 2005, Schrempf & Heinze 2008, Schrempf et al. 2011). Similar to mated queens, sham-mated queens, which mated with a sterilized male, live considerably longer and start laying eggs earlier than virgin queens. In order to gain insights into the regulation of queen longevity, we conducted RNA-Seq of eighteen-week old queens that were subjected to different mating regimes. By comparing the transcriptomes of mated, sham-mated and virgin queens it is possible to disentangle the effects of mating and reproduction on gene expression patterns. We could identify several genes presumably involved in the variation of longevity.

OR317

Conflict, longevity and ageing in social insects

Edward Almond, Gabrielle Lockett, Tim Huggins, Joel Parker, **Andrew Bourke**

Social evolution and the evolution of ageing interact, but the relationship between them remains incompletely understood. Social insects provide valuable model systems to clarify the interaction of sociality and ageing, and specifically the effect on longevity and ageing of the within-group conflicts engendered by social living. An example of such conflict occurs in systems with resource inheritance, in which inclusive fitness theory predicts that decreasing parental fecundity leads to a zone of kin-selected conflict in which future reproductive offspring are selected to enforce resource inheritance before the parent is selected to cede the resource to them. This conflict potentially accelerates parental ageing and reduces parental longevity. I will discuss results from our work in the bumblebee *Bombus terrestris* to test key elements of this model. Resource inheritance occurs in *B. terrestris* when, on the queen's death, workers inherit the nest and some reproductive individuals produce their own male offspring within it. Worker matricide of queens provides evidence that the timing of inheritance is subject to queen-worker conflict as the model predicts. In our current study, we have used experimental manipulations of queens and colonies to test further the assumptions and predictions of the model. For the same reason, we have used quantitative real-time PCR, and transcriptomic profiling by next-generation sequencing (RNA-Seq), to investigate the expression of genes associated with ageing pathways as a function queens' age and social environment.

OR318

Honeybee scent memories regulate olfactory receptor expression

Judith Reinhard, Richard Newcomb, Julianne Lim, Shanzhi Yan, Charles Claudianos

Olfactory learning and memory formation has been well investigated in honeybees, including the neural and molecular changes that occur in the antennal lobes and mushroom bodies of the honeybee brain as a function of odour learning. One potential area of learning-induced plasticity has received little attention to date, namely the sensory neurons in the honeybee antennae where odours are first detected. Here, we present evidence that odour learning triggers molecular changes in the expression of 7-transmembrane olfactory receptor proteins (ORs) on dendrites of olfactory neurons in the honeybee antennae. The expression of six honeybee ORs that were shown to bind common floral odorants such as linalool and nerol, varies significantly with transition from hive nurse bee to outdoor foraging bees, and with exposure to different flowering plants in the four seasons. When bees were conditioned to these specific floral odorants using the PER-assay, the respective ORs in the antennae were down-regulated. The physiological response of the corresponding olfactory neurons in the antennae was also reduced after scent conditioning. Importantly, OR down-regulation only occurred in context of scent learning; mere exposure to the same scents induced no changes in OR expression, suggesting that molecular mechanisms involved in memory formation also regulate OR expression. Our research demonstrates that the olfactory system of honeybees is highly plastic, constantly adapting via differential gene expression to scent experiences, which in turn affects odour preferences. We propose that this plasticity enables the olfactory system to be optimally tuned to process familiar odorants as well as detect novel ones.

OR319

Micro-RNA regulation of olfactory learning and memory in honeybees

Charles Claudianos, Alexandre S. Cristino, Stephanie D. Biergans, Flavia Freitas, Judith Reinhard

Honeybees forage in an ever-changing olfactory environment, which requires constant learning and re-learning of floral odours associated with food sources. Plastic olfactory memories hence are crucial to a honeybee's foraging success, however the molecular mechanisms regulating olfactory learning and memory are not well understood. Here, we investigated how learning and long-term memory formation affects gene expression in the honeybee brain using an olfactory conditioning paradigm (proboscis extension reflex, PER). A microarray gene expression analysis comparing groups of conditioned and control bees found 77 genes differentially expressed between these two groups. Most of these genes were down regulated in trained bees, with only a few non-coding RNAs upregulated. Many of the down-regulated genes were enriched with binding sites for microRNAs (miRNAs), that putatively dysregulate these genes during memory formation. Indeed, qRT-PCR analysis validated that seven miRNAs were upregulated in trained bees. Of these seven miRNAs, we further investigated miR-210, which is associated with foraging, and miR-932, which is embedded in a key neurological gene: the synaptic adhesion molecule neuroligin 2 (Nlg2). We suggest that modules of miRNAs may regulate synapse development during learning and memory processes. To test this hypothesis we used small interference RNAs to inhibit miR210 and miR932. Feeding cholesterol-conjugated antisense RNA to bees resulted in the inhibition of miR-210 and of miR-932. Loss of miR-932 impaired long-term memory formation but not memory acquisition. Functional analyses showed miR-932 interacts with Act5C, providing first evidence how a miRNA directly targets actin. An activity-dependent increase in miR-932 expression may therefore control actin-related plasticity mechanisms and affect memory formation in the brain.

OR320

DNA methylation and demethylation in honeybee long-term memory formation

Stephanie Biergans, C. Giovanni Galizia, Judith Reinhard, Charles Claudianos

It has been shown that changes in DNA methylation are crucial for long-term memory formation, however the specific role and dynamics of DNA methylation and its targets are still unknown. DNA methyltransferases (Dnmts) and ten-eleven-translocation enzym (Tet) are catalysing methylation and demethylation processes respectively. Here, we first examined the role of Dnmts during long-term memory formation using the honeybee as a model. We trained bees to associate a specific odour with a sugar reward and inhibited Dnmts using two functionally different drugs (Zebularine and RG108). During the memory retention test bees were presented with the learned and a new odour to test for odour-specific memory, which is the ability of bees to form a memory of a specific odour and not generalize to other odours. We showed that associative odour-specific long-term memory is impaired if Dnmts were inhibited. We therefore next investigated the expression of memory related target genes 24 hours after training, and found several of them upregulated after Dnmt inhibition in trained bees. Interestingly, Dnmt3 was upregulated in response to Dnmt inhibition after training, suggesting that Dnmt3 is auto-regulated. Using Sequencing and Sequenome mass spectrometry, we identified differentially methylated regions (DMRs) after long-term memory formation. Because memory formation and its regulation is a temporally dynamic process, we also examined the expression of Dnmts over time for the first 5 hours and at 24 hours after training. Dnmt1a and Tet (ten-eleven-translocation gene involved in demethylation) were upregulated at 1 hour and downregulated 24 hours after training. In contrast Dnmt3 was upregulated 5 hours after training returning to baseline level 24 hours after. Our study provides the first molecular evidence for how DNA methylation regulates long-term memory formation and which genes are targeted by Dnmts and Tet.

OR321

Knockdown of CaMKII impairs long-term-memory in honeybees

Christina Scholl, Wolfgang Roessler

Honeybees possess a highly flexible repertoire of cognitive abilities and are able to perform complex learning tasks. The neuronal and molecular bases underlying these behaviors are key to understanding higher brain functions and social organization, but are still not fully understood. The calcium/calmodulin-dependent protein kinase II (CaMKII) is a highly abundant protein in the brain of vertebrates and initiates biochemical cascades associated with memory formation. In adult honeybees CaMKII preferentially occurs in the mushroom bodies (Kamikouchi et al., 2000 JCN 417:501; Pasch et al., 2011 JCN 519:3700), centers for sensory integration and memory processes. To close the gap between the molecular level and honeybee behavioral plasticity, knockdown of CaMKII in the mushroom bodies was induced and followed by classical olfactory conditioning. siRNA against CaMKII (siCaMKII) or GFP (control) was injected via the occelar tract to cause RNA interference and create a loss of function phenotype. Furthermore, pharmacological inhibition was used as a parallel approach (KN93: active inhibitor of CaMKII, KN92: ineffective analog). Injections of Cy-3-labeled siRNA visualized the distribution in the mushroom bodies. Quantitative RT-PCR confirmed the reduction of CaMKII mRNA 2 hours after the injection, and quantitative western blots showed successful downregulation of the protein 8 hours after injection. Based on these results honeybees were subjected to olfactory conditioning using the proboscis extension response 8 hours after siRNA injection (18 hours after injection of pharmaca). All bees showed normal performances during memory acquisition and during memory retention 1 hour later. However, memory retention 24 hours and 72 hours after learning was significantly impaired in bees injected with siCaMKII and KN93 compared to the control groups indicating that CaMKII is an important mediator for the formation of long term memory in the honeybee brain. Funded by the Graduate School of Life Sciences (GSLS), University of Würzburg.

OR322

The role of the mushroom body in honeybee learning and memory

Randolf Menzel, Ina Filla, Paul Szyszka

The memory trace at the input side of the mushroom body (MB) is characterized by high order combinatorial integration across multiple sensory inputs. Presynaptic boutons of olfactory projection neurons are modulated in their responses to the rewarded olfactory stimuli in a behavioral learning related fashion. Intrinsic neurons of the MB code sensory stimuli in a sparse way both in the temporal domain and on the population level. Learning leads to enhancement, reduction and recruitment of intrinsic neurons changing the sensory code at the individual neuron and population level. MB extrinsic neurons lose their sensory coding properties and appear to represent the acquired values of the learned stimuli. Specifically, extrinsic neurons of the MB may encode learned cues and contexts differently. Memory processing is exemplified by rate changes in an inhibitory recurrent pathway that peak at discrete time windows over three days. Thus MB extrinsic neurons provide neural commands for goal directed behavior and decision making. Furthermore, information stored in the MB and/or extrinsic neurons depends on consolidation processes over the range of days. A model will be presented that aims to capture the multi-faceted and distributed nature of the engram and may help to guide our future search of the engram at a neural systems level.

OR323

Side differences during odour processing in the honeybee brain

Elisa Rigosi, Albrecht Haase, Lisa Rath, Gianfranco Anfora, Giorgio Vallortigara, Paul Szyszka

Left-right asymmetric information processing is a common property of nervous systems. It is thought that lateralization serves to avoid functional incompatibilities between sensory representations and to increase coding capacity by parallel processing. Lateralized sensory processing has mainly been described at behavioral or anatomical level while asymmetric neuronal coding is less studied. We identified a left-right asymmetry in the honeybee's antennal lobes during odour processing. When both antennae were stimulated via a frontal odour source the neurophysiological distances between odours were higher in the right antennal lobe than in the left one. Moreover, the processing of odour mixtures differed between sides, as responses to mixtures were significantly stronger than responses to the strongest component in the right but not in the left antennal lobe. Our data suggest a functional left-right difference in the antennal lobe networks, with the right being tuned for fine odour discrimination. Behavioural data support this hypothesis: bees with only the left antenna in use failed in an olfactory foreground-background discrimination task, while bees with only the right antenna in use succeeded. The implementation of different neuronal coding strategies in the left and right brain side may serve to process opposing tasks at the same time, such as discrimination and generalization of similar odorants.

OR324

Honeybee Kenyon cells are regulated by a tonic GABA conductance

Mary Palmer

The higher cognitive functions of insects are dependent on their mushroom bodies (MBs), which are particularly large in social insects such as honeybees. MB Kenyon cells (KCs) receive multisensory input and are involved in associative learning and memory formation. In addition to receiving sensory input via excitatory nicotinic synapses, KCs receive inhibitory GABAergic input from MB feedback neurons. Cultured honeybee KCs exhibit ionotropic GABA receptor currents, but the properties of GABA-mediated inhibition in intact MBs are currently unknown. Here, using whole-cell recordings from KCs in acutely-isolated honeybee brain, we show that KCs express a spontaneous tonic current that is inhibited by picrotoxin but not by bicuculline. The tonic current is reduced by inhibition of Ca^{2+} channels with Cd^{2+} or nifedipine, but is unaffected by the GABA uptake inhibitor nipecotic acid or by the GABA transaminase inhibitor vigabatrin. Bath application of GABA (5 mM) and taurine (1 mM) activate a tonic current in KCs, but L-glutamate (0.1-0.5 mM) has no effect. The spontaneous tonic current is strongly potentiated by the allosteric GABA(A) receptor modulator pentobarbital. Noise analysis of the GABA-evoked current gives a single-channel conductance value for the underlying receptors of 27 ± 3 pS, similar to that of RDL receptors. Using recordings of KC membrane potential, it was found that action potential firing was evoked by less injected current following inhibition of the tonic current with picrotoxin. Thus, the tonic GABA receptor conductance in KCs acts to reduce neuronal excitability, a property that is likely to contribute to the sparse coding of sensory information in insect MBs.

OR325

Olfactory coding and plasticity in parallel olfactory pathways of the honeybee

Jean-Christophe Sandoz, Antoine Couto, Julie Carcaud

Honeybees are a traditional model for addressing the neural basis of olfactory perception and learning, as olfaction is a crucial sensory modality used within the colony for intraspecific communication but also outside the colony during foraging. In honeybees, odors are detected by sensory neurons on the antennae, which project to a primary processing centre, the antennal lobe (AL). Then two main tracts of projection neurons convey odor information to higher-order brain centers, the mushroom bodies (MB) and the lateral horn (LH). Strikingly, the honeybee brain contains a dual olfactory system, with a clear dichotomy from the periphery up to higher-order centres, subtended by two main neuronal tracts (median and lateral Antenno-Protocerebral Tract, m-APT and l-APT). Such dual system may be the basis for parallel processing of olfactory stimuli, but the exact function of this system is still unclear. We are thus performing a functional study of olfactory coding and plasticity in both subsystems, using in vivo calcium imaging to reveal neuronal activity. Olfactory coding of general odorants appears mostly redundant in both subsystems, but still shows some specificities according to the odorants' chain length and/or functional group information. Concerning pheromonal odorants, a dissociation between queen and brood pheromones was found. Here, we addressed possible differences in learning-induced plasticity and in olfactory mixture processing in both subsystems. Honeybees were first subjected to a differential conditioning procedure (A+B-) using the appetitive conditioning of the proboscis extension response (PER). Then they were subjected to in vivo calcium imaging recordings, either in the standard preparation to record l-APT projection neurons, or in the inverted preparation to record m-APT neurons (Carcaud et al. 2012). In these tests, a range of AB mixtures with gradual changes in component ratios was presented. Analysis of the data is currently under way.

OR326

Navigating in turbulent worlds: High speed smelling in honeybees

C Giovanni Galizia, Jacob Stierle, Rick Gerkin, Brian Smith, Paul Szyszka

Bees memorize odors of visited flowers. These odors are mixtures of different chemicals. However, odor-driven behaviors require perceiving such odor mixtures as unitary odor-objects. This task is difficult in a turbulent world: airborne odors are transported by wind and form erratic and overlapping plumes, similar to cigarette smoke. Consequently, odors from different sources intermingle in temporally unpredictable mixtures. The question arises how bees disentangle such overlapping stimuli. We hypothesize that minute time differences between plumes are exploited by bees to recognize the relevant odors. Here we probe the limits of insects' olfactory temporal resolution by delivering high frequency odor pulses and measuring sensory responses in the antennae. We show that transduction times and pulse tracking capabilities of olfactory receptor neurons (ORNs) are 10 to 30 times faster than previously thought. Once an odorant arrives at the boundary layer of the antenna, signal transduction can start as fast as in 1.1 milliseconds. The temporal resolution increased with increasing stimulus duration. Thus, insect ORNs can track very short stimulus durations as their antennae encounter narrow filaments in an odor plume. These results provide a new upper bound to the kinetics of odor tracking in animals and to the latency of initial transduction events in olfaction. Rapid tracking is likely necessary for odor-background segregation and odor source location in insects. Using in-vivo calcium imaging in the honeybee brain, we show that bees can exploit short temporal asynchronies between odor stimulus onsets to segregate odors from different sources. Consistent with bee's behavioral performance, projection neuron responses in the antennal lobe are sensitive to millisecond stimulus asynchrony: Compared to synchronous mixtures, the responses to asynchronous mixtures contain more information about the components. A detailed analysis shows that the antennal lobe creates a 'slow motion' effect, aiding memory retrieval and possibly encoding.

OR327

Neuroanatomical bases of absolute and differential color learning in honeybees

Frank Sommerlandt, Adrian Dyer, Wolfgang Roessler, Johannes Spaethe

Color vision is important in honeybees to identify flowers as potential food sources. To enable highly efficient foraging, bees have to learn, memorize and retrieve color information. The color discrimination performance of bees is highly dependent on how stimuli are perceived. Discrimination of perceptually similar colors requires differential conditioning with target and distracter stimuli, but fails when target colors are learnt in isolation with absolute conditioning (Dyer and Chittka, 2004; Giurfa, 2004). An enduring question is where in a bee brain is such information processed and stored. Here we present data on fine color discrimination in free-flying honeybees on a rotating screen apparatus (Dyer et al., 2005). Bees were trained with either absolute or differential conditioning considering two similar 'blue' color stimuli. A control group was confronted with an unpaired presentation of the grey background color and a sucrose solution reward. After 50 conditioning trials, the bees were tested with a non-rewarded touch test which simultaneously presented both colors. Bees trained with differential conditioning performed significantly better in the final test than bees of the alternative groups (absolute, grey). For the neuroanatomical analyses, the bees from the three groups were kept for three days in individual chambers excluding light to avoid new stimulus input, but to enable long-term memory formation. Subsequently, these animals were analyzed for differences in the neuronal network of the mushroom bodies by whole-mount immunostaining to search for variations in the number of synaptic boutons in the olfactory lip region and the visually innervated collar. With this work we aim to provide insights into the neuronal mechanisms behind complex color learning and memory in insects. Dyer, A. G. and Chittka, L. (2004). Naturwissenschaften 91(5): 224-227. Dyer, A. G., Neumeyer, C. and Chittka, L. (2005). J Exp Biol 208(Pt 24): 4709-4714. Giurfa, M. (2004). Naturwissenschaften 91(5): 228-231.

OR328

Reinstatement in honeybees is context-dependent

Jenny Aino Plath, Johannes Felsenberg, Dorothea Eisenhardt

During extinction animals experience that the previously learned association between a conditioned stimulus (CS) and an unconditioned stimulus (US) no longer holds true. Accordingly, the conditioned response (CR) to the CS decreases. This decrease of the CR can be reversed by presentation of the US alone following extinction, a phenomenon termed reinstatement. Reinstatement and two additional phenomena, spontaneous recovery and renewal, indicate that the original CS-US association is not lost through extinction but can be reactivated through different processes. In honeybees (Apis mellifera), spontaneous recovery, i.e., the time-dependent return of the CR, has been demonstrated, suggesting that also in these insects the original CS-US association is not lost during extinction. To support this notion, we ask whether honeybees show reinstatement after extinction. In vertebrates reinstatement is context-dependent. Thus we examined whether the same holds true for honeybees. We demonstrate reinstatement in restrained honeybees and show that reinstatement is context-dependent. In addition, we show that an alteration of the wavelength of light, i.e. its colour, illuminating the experimental setup suffices to indicate a contextual change. We conclude that in honeybees the initially formed CS-US memory is not lost after extinction. Rather, honeybees might learn about the context during extinction. This enables them to adequately retrieve one of the two opposing memories about the CS that have been formed after extinction.

OR329

Non-elemental learning in honeybees: how specific?

Jean-Marc Devaud, Martin Giurfa

Learning allows individuals to make reliable predictions about connected events in their environment. Simple and unambiguous links between specific stimuli characterize elemental learning[1] (e.g. a stimulus A is associated with a reinforcement while stimulus B is not : A+ vs. B-). By contrast, ambiguity and non-linearity characterize non-elemental learning, thus making discriminations difficult. Despite the likelihood of foraging honeybees to encounter ambiguous learning situations, non-elemental learning has been poorly investigated under control laboratory conditions. Here we used the ofactory appetitive conditioning of the proboscis extension response in honeybees to determine the neural substrates of non-elemental olfactory learning and the impact of stress on this capacity. First, we asked whether specific brain centres, the mushroom bodies (MBs), are required for non-elemental olfactory learning. Following a study showing impaired reversal learning after inactivating of MB function by a local anesthetic[2], we asked whether the same applies to negative patterning, a form of configural learning where bees must discriminate a non-reinforced mixture of two odorants A and B from its reinforced elements (i.e. AB- vs. A+ and B+). Our results show that the function of MBs is required for the resolution of negative patterning. Second, we studied the impact of stress on non-elemental learning; our preliminary results suggest that, while stress impairs the capacity to solve elemental tasks without suppressing it[3], it has a more dramatic effect on non-elemental learning. We are currently performing experiments to confirm whether this is a general property of different forms of non-elemental learning. Altogether, our results indicate that non-elemental learning tasks differ from elemental ones, not only by their level of difficulty, but also by their neural substrates and their sensitivity to stress. [1]Giurfa (2003) Curr.Opin.Neurobiol. 13:1-10 [2]Devaud et al. (2007) Eur.J.Neurosci. 26: 3193-206 [3]Urlacher et al (2010) Front.Behav.Neurosci. 4:157. doi: 10.3389/fnbeh.2010.00157

OR330

The social side of honeybee learning: what it tells us

Alison Mercer

Chemical communication in honeybees (*Apis mellifera*) is remarkably sophisticated and plays a central role in the success and survival of honeybee colonies. It was discovered relatively recently that some of the chemical signals (pheromones) used by honeybees modulate the learning behaviour of this highly social insect. Aversive learning in young worker bees, for example, can be suppressed by pheromone released by the honeybee queen (Vergoz V, Schreurs HA, Mercer AR. (2007) Science 317:384-386), and alarm pheromone released by guard bees has been shown to inhibit appetitive learning in foragers (Urlacher E, Francés B, Giurfa M, Devaud J-M (2010) Front Behav Neurosci 4:157). These studies highlight the need for a better understanding of honeybee learning in a social context. This talk will outline recent attempts to explain why the effects of pheromones on learning performance in honeybees are age- and state-dependent. It will outline what is known about mechanisms that support pheromone modulation of learning behaviour in the honeybee, and it will discuss the possible adaptive value of social modulation of learning in this insect. Finally, it will consider whether a better understanding of pheromone communication can offer novel insights into cellular and molecular mechanisms that underpin learning and memory formation in the honeybee. Supported by grants from the Royal Society of New Zealand Marsden Fund (UOO0910 and UOO1207)

OR331

The effect of cocaine on the social behavior of bees

Nicholas Naeger, Tim Gernat, Matthew McNeill, Gene Robinson

Cocaine and other addictive drugs ‘hijack’ the reward system of the brain, leading to profound changes in many different behaviors. In honeybees cocaine is already known to cause changes in reward-related behaviors including food responsiveness, learning and memory, and the dance language. To investigate the effect of cocaine on social behavior in bees, we used an optical barcode and camera system to track and monitor the social interactions of all honeybees in a small observation colony. Groups of bees reared in cages for four days with either normal food or food spiked with cocaine were then introduced into the observation hive. We report on the effects of cocaine feeding on the social network formed by the bees and the rates of interactions between individuals within it, including interactions with the queen. In addition, we analyzed patterns of behavior to look for the effects of chronic cocaine feeding on division of labor, circadian rhythms and other activity patterns, and reaction to disturbance.

OR332

Why acquiesce? Worker reproductive parasitism in Apis

Michael Holmes, Ken Tan, Zhengwei Wang, Benjamin P. Oldroyd, Madeleine Beekman

Reproductive acquiescence theory is a popular explanation for the absence of worker reproduction in social insects. This theory posits that in the presence of an effective policing system, the incentive for worker reproduction is removed, and therefore workers should not attempt reproduction. This theory is supported by empirical studies of a variety of Hymenopteran taxa suggesting that when policing is efficient, workers are less likely to attempt to reproduce. The Asian hive bee *Apis cerana* presents a unique opportunity to test reproductive acquiescence theory as it has high levels of ovary activation even when a queen is present, but very few adult males are the sons of workers. We regularly sampled male eggs and pupae, which were genotyped to determine maternity, and adult workers which we dissected to check for levels of ovary activation. Interestingly, we found that 3.8% of male eggs and 1.1% of male pupae were worker-produced. We also found high rates of ovary activation (11.7% overall). Similarly, in a long-term study the well-known Western honeybee *A. mellifera*, we regularly sampled adult workers (for dissection) and male pupae (for genotyping) throughout the course of a season. We found that, contrary to previous studies, workers are actually mothers of 4.2% of males and up to 1.2% of workers have active ovaries. We used this new data to conduct a meta-analysis of reproductive acquiescence theory. When our new data were included, the strong negative correlation between policing efficiency and proportion of reproductive workers was weakened considerably. So far most studies have been performed on species in which the queen mates multiply. What we need now is data on monandrous species. Only then can we properly assess the value of reproductive acquiescence theory as a general explanation for reproductive division of labour in social insects.

OR333

Genetic, maternal and social effects on brood resistance in ants

Michel Chapuisat, Jessica Purcell

The phenotype of social animals can be influenced by genetic, maternal and environmental effects. In our long-term research program, we are investigating the genetic and environmental factors affecting social structure variation in ants. On the genetic side, we recently identified a large, non-recombining ‘social chromosome’ associated with social structure (the presence of one or multiple queens per nest) in *Formica selysi*. Nevertheless, many phenotypic traits that differ between individuals from the two social backgrounds also depend on the ontogeny and environment of individuals. We used cross-fostering experiments to investigate if the maternal origin of eggs and/or the social environment during development affected the survival and fungal resistance of ant brood. We found that the survival of newly eclosed, cross-fostered *F. selysi* workers varied greatly according to their maternal origin. However, the origin of care-giving workers significantly influenced the ability of cross-fostered workers to resist to a fungal entomopathogen, *Beauveria bassiana*. In particular, care-givers that were more resistant to the fungal pathogen reared more resistant workers, which indicates that social interactions during development influence individual immunity. Together, our research indicates a strong genetic control of social structure, coupled with indirect effects of the rearing group on the phenotype of emerging individuals.

OR334

Adaptive evolution among socially parasitic allodapine bees

Simon Tierney, Michael Schwarz

Inquilinism is a form of social parasitism, where parasites have evolved to live largely within their host's colony. Among bees, the tribe Allodapini (Apidae; Xylocopinae) contains the greatest number of independent origins (> 10) of social parasitism, which is only otherwise known from *Bombus* which displays far fewer origins comprising the subgenus Psithyrus. In all allodapine cases, social parasites are phylogenetically related to their hosts, but origins vary widely in their age, from less than 1 Mya to approximately 15 Mya. Most species show convergence in parasitic traits (reduced scopae, proboscis, mandibles, eyes, wing venation), but some facultative parasites can nest independently, suggestive of incipient phases. A key question regarding inquilinism is how it manages to persist over evolutionary time, given that hosts should have high rates of adaptive evolution? Most molecular studies of adaptive evolution have focused on nuclear genes, but a there is a growing focus on mitochondrial DNA, with some studies indicating key adaptive changes associated with changes in life-history traits, including convergent evolution in some gene regions. It has been argued that a higher rate of molecular evolution in social parasites is due to their smaller effective population size (with increasing rates of genetic drift), but this hypothesis conflicts with theoretical predictions. Here we show that rates of mitochondrial evolution in allodapine bees tend to be higher in social parasites than their hosts. Results suggest that mitochondrial genes can play a critical role in social evolution, but the lack of recombination in these maternally inherited genes indicates a very different kind of evolutionary dynamic from nuclear genes. We hypothesize that these higher mitochondrial rates are due to common selective pressures arising from adaptations to life history strategies that entail low metabolic activity.

OR335

Social genotype distribution and discrimination in a socially polymorphic ant

Amaury Avril, Jessica Purcell, Michel Chapuisat

In ants, the number of reproductive queens per nest varies greatly among and within species. The mechanisms maintaining this variation remain little understood. In the fire ant Solenopsis invicta, social organization is under strong genetic control, being determined by the genotypes of queens and workers at a ‘social chromosome’. We have recently discovered an equivalent chromosome in *Formica selysi*, present in two haplotypes, sM and sP. In polygynous colonies, each individual bears at least one sP haplotype. Given that heterozygous queens in polygynous colonies may mate with either sP or sM male, the lack of sM/sM females and sM males in polygynous colonies is unexpected. Here, we investigate why these genotypes are absent from polygynous colonies. We tested for a selective elimination of brood by workers and for an intrinsic incompatibility in sM/sM eggs. We collected mature queens from polygynous colonies and maintained them in separate containers either with or without workers. We compared the presence and proportion of sM/sM eggs produced by those queens in the two conditions. Overall, the results suggest that the workers in polygynous colonies discriminate and selectively eliminate individuals that are not bearing the sP haplotype. These results contribute to the understanding of the genomic mechanisms underlying the two types of social organization.

OR336

Intraspecific parasitism by workers in a highly eusocial bee

Denise Araujo Alves, Julia Pinheiro Figueiredo, Fabio Santos Nascimento

Insect societies are well known for their high degree of cooperation, but the nonclonal structure of their colonies can potentially be exploited by reproductive workers who lay unfertilized, male eggs, rather than work for the good of the colony. Over the past decade, it has also been discovered that workers in bumblebees and honeybees can succeed in entering and parasitizing unrelated colonies to produce their own male offspring. The aim of this study was to test whether such intraspecific worker parasitism might also occur in stingless bees, another group of eusocial bees, with perennial colonies headed by a single once-mated queen. Given that 13% of all the males are workers' sons, *Scaptotrigona depilis* is a good candidate species for investigating the vulnerability to parasitism by workers of conspecific nests. Choice experiments, in which queenright and queenless workers had the choice to remain in their natal nests or to join a queenright or queenless colony nearby, showed that very few workers enter unrelated, conspecific nests. The drifters were between ten and twenty days old and half of them had active ovaries. Although there was no preference to drift into queenright or queenless colonies, we detected a slightly tendency of workers to drift out of queenless colonies. Thus, our results suggest that the best option for a worker with active ovaries is to stay where she is, since she has opportunities of reproducing in her natal colony.

OR337

Fertility signalling with long-chained hydrocarbons in solitary and eusocial insects

Juergen Liebig

The crucial feature of eusocial insects is reproductive division of labor between the members of a colony. It is generally accepted that pheromones play an important role in this context. In many eusocial insects, reproductive individuals differ in their cuticular hydrocarbon (CHC) profile from that of infertile workers, and their CHC profiles correlate with fertility. Similar differences can be found in some species between the eggs laid by queens and eggs laid by workers, and these differences are used for egg discrimination. This suggests that these hydrocarbons are used to signal the fertility and presence of the primary reproductives to the nestmates (Seeley 1985, Keller and Nonacs 1993). In fact, recent studies provide direct evidence for the role of cuticular hydrocarbons in this context. Long-chained hydrocarbons are also used by solitary insects as fertility signals in the context of mate attraction. This suggests that the basis for fertility signalling was already present in the ancestors of eusocial insects. In my presentation, I will explore similarities and differences in fertility signalling between solitary and eusocial insects.

OR338

Conserved class of queen pheromones stops social insect worker reproduction

R Caliari Oliveira, A Van Oystaeyen, L Holman, J.S Van Zweden, C Romero, C.A Oi, P D'Ettorre, M Khalesi, J Billen, F Wäckers, J.G Millar, T Wenseleers

A major evolutionary transition to eusociality with reproductive division of labor between queens and workers has arisen independently at least 10 times in the ants, bees, and wasps. Pheromones produced by queens are thought to play a key role in regulating this complex social system. However, their evolutionary history remains unknown. Here, we sought to identify sterility-inducing queen pheromones in three extant species of social insects, by testing queen-characteristic compounds in bio-assays, and found that in all cases hydrocarbons could reduce worker ovary activation (*Vespula vulgaris*: n-C_{27}; n-C_{28}; n-C_{29}, 3-MeC_{29}; *Bombus terrestris*: n-C_{25}; *Cataglyphis iberica*: n-C_{27}; n-C_{29}, 3-MeC_{29}). Furthermore, we synthesized existing data on compounds that characterize female fecundity in 64 species of social insects, and found that queen pheromones are strikingly conserved across at least three independent origins of eusociality, with wasps, ants, and some bees all appearing to use nonvolatile, saturated hydrocarbons to advertise fecundity and/or suppress worker reproduction. These results suggest that queen pheromones likely evolved from conserved signals that were already present in solitary ancestors, such as fertility cues used by females during courtship.

OR339

Multifaceted roles of a termite queen pheromone

Kenji Matsuura

Pheromones are likely involved in all social activities of social insects including foraging, sexual behavior, defense, nestmate recognition, and caste regulation. Regulation of the number of fertile queens requires communication between reproductive and non-reproductive individuals. Queen-produced pheromones have long been believed to be the main factor inhibiting the differentiation of new reproductive individuals. However, the active compounds of the inhibitory queen pheromone had not been identified in termites until recently. In 2010, we first identified n-butyl-n-butyrate and 2-methyl-1-butanol as the active components of the queen pheromone, which suppress the differentiation of new neotenic queens, in the termite *Reticulitermes speratus*. Identification of the chemical compounds made it possible to study various features of the queen pheromone. Interestingly, the same two volatiles are also emitted by eggs. This dual production of an inhibitory pheromone by female reproductives and eggs provides a mechanism ensuring honest signaling of reproductive status with a tight coupling between fertility and inhibitory power. Exposure to the pheromone resulted in a significant decrease in egg production by each queen, suggesting the role of queen pheromones as a signal regulating colony-level egg production in multiple queen colonies. In addition to the inhibitory functions, these volatiles also act as a promoter of the production of salivary lysozyme, which is an essential antibacterial agent in egg grooming by workers. Because the pheromone compounds suppress the growth of an egg-mimicking parasite fungus ‘termite balls’ and some entomopathogenic fungus, it is likely that the termite utilized antimicrobial volatiles for the defense of eggs and queens to function secondarily as a communication signal informing queen fertility. This is in line with the context of evolutionary parsimony that pheromones are originally utilized as defensive compounds and their communicative function is a secondary development.

OR340

*Cuticular compounds mediate queen recognition in subterranean termites (*Reticulitermes flavipes*)*

Colin Funaro, Edward Vargo

Chemical mediation of reproductive caste is common in many eusocial insects. Functionally sterile workers identify and tend queens or kings within the colony using unique volatile or contact based chemicals. Many of these signals in ants, bees and wasps consist of reproductive-specific cuticular hydrocarbons. In termites, recognition and tending behavior towards queens and kings is not well-studied and no recognition pheromones have been identified to date. Egg recognition pheromones and cuticular hydrocarbons indicating fertility have been identified in a few termites, but there is little information regarding queen and king tending behavior. We investigated the recognition and tending behavior of reproductive individuals in the eastern subterranean termite *Reticulitermes flavipes*. In many termites, including subterranean species, individuals will sometimes shake violently while remaining in place. Although this behavior sometimes occurs in response to various stimuli, it occurs quite conspicuously and frequently in close proximity to reproductively active individuals. Using behavioral assays and classical chemical ecology techniques, we documented the strong behavioral response of termites towards neotenic (secondary) queens, kings, workers, and soldiers, and investigated the potential chemical sources for queen recognition. Results of these ongoing analyses will be presented.

OR341

Caste specific pheromone-related gene expression in honeybee mandibular glands

Abraham Hefetz, Osnat Malka, Elina Lastro-Nino, Christina Grozinger

Social context is often a primary regulator of social behavior, but genes that affect or are affected by it have rarely been investigated. In the honeybee the queen mandibular gland (MG) pheromone mediates reproductive dominance, its absence prompting ovary activation and queen pheromone production in workers. It was therefore interesting to investigate the effect of caste and social environment on genome-wide expression patterns in the MG. We used microarrays to examine virgin and mated queens, queenright (QR) and queenless (QL) workers, with and without activated ovaries. Approximately 2554 transcripts were significantly differentially expressed among these groups, with caste and social context being the main regulators of gene expression patterns. Among these, 27 genes involved in production of the fatty-acid derived MG pheromone were differentially expressed in a caste selective manner, revealing 4 regulatory gene-expression steps that distinguish the queen from worker pheromone biosynthesis pathways. The first step involves stearic acid that although is a common precursor for these pheromones in queens and workers, its production and activation seems nonetheless to be controlled by different, caste specific genes. The second step involved the ω-1 fatty acid hydroxylation via caste selective Cyp 450 genes. The next regulatory step is the expression of caste specific genes that are responsible for chain shortening via β-oxidation. The final regulatory step, the oxidation of 9-hydroxy-2-decenoic acid to 9-oxo-2-decenoic acid is entirely queen specific. These differential gene expressions emphasize the occurrence of disparate pheromone biosynthetic pathways for queens and workers, adding another dimension regarding the regulation of these important pheromones. Gene ontology analysis also revealed genes of different functional categories whose expression was impacted by caste (oxidation-reduction activity, immune response, and several metabolic and biosynthetic processes) or by the social environment (protein catabolism), suggesting that the MG serve more than being a pheromone source in honeybee biology.

OR342

Regulation of brain dopamine by nutrition in female honeybees

Ken Sasaki, Syuhei Matsuyama, Takashi Nagao

Dopamine plays an important role in promotion of the reproduction in primitively and highly eusocial hymenopterans. In female honeybees, the brain levels of dopamine and dopamine-related substances are higher in queens and reproductive workers than in normal workers. However, the mechanisms underlying regulation of dopamine in the brain are still largely unknown. We firstly tested the possibility of different enzymatic activities for dopamine synthesis in the brains between castes. The in vitro enzymatic activities of DOPA decarboxylase (DDC) in brains were not significantly different between castes. The DDC activity in queens was mainly found in the brain, but with lower levels of activity detected in the mandibular glands, salivary glands and ovaries. Gene expression of DDC (*Amddc*) and tyrosine hydroxylase (*Amth*) in the brains were not different between castes. We then considered the possibility of different supply of tyrosine that is a precursor of dopamine and is contained in food of honeybees, and tested the hypothesis by the oral treatments of tyrosine and royal jelly. Oral application of tyrosine and royal jelly to queenless workers led to significantly higher levels of dopamine and dopamine-related substances in the brains than in control workers. The queenless workers fed tyrosine have more developed ovaries than control workers fed sucrose. These results suggest that the higher levels of brain dopamine in the reproductive individuals compared with normal workers can be explained by the tyrosine supply from royal jelly. Such a nutritional supply could drive not only the dopamine signaling, but also other signaling systems to promote the reproduction in females in honeybees.

OR343

The queen, her pheromone & reproductive hegemony in honeybees

Vanina Vergoz

The queen has the reproductive hegemony over the colony. Workers are refrained from developing their ovaries and this inhibition is in part due to the secretion of the queen mandibular pheromone by the queen. Numerous studies showed that dopamine has a strong implication in the regulation of worker sterility. Removing the queen from the colony induces an increase of dopamine levels in the brain and enhance the number of worker with developed ovaries. Feeding of HVA (component of the queen pheromone) not only reduces dopamine level in the brain but also impairs young worker to make negative associations which are in part dopamine dependant. Involvement of dopamine in the regulation worker reproduction and its sensibility to queen pheromone is still a mystery. However, using molecular and behavioural techniques studies are enhancing the understanding of the intricate relationship between dopamine the queen and the reproductive status of workers.

OR344

The organisation of a queenless honeybee colony

Marianne Peso, Naïla Even, Nicholas Naeger, Gene Robinson, Andrew Barron

In queenless colonies honeybee workers activate their ovaries and begin to reproduce. Kin selection theory predicts that because of the altered relatedness structure of a queenless honeybee colony compared to a queenright colony the level of altruism displayed by workers is expected to decrease while reproductive conflict should increase. A common assumption is that reproductive workers focus on personal reproduction over colony tasks. In a study of the behaviour and physiology of workers raised in hopelessly queenless honeybee colonies, we found that reproductive workers foraged (early), had developed brood-food producing glands, had wax scales, and defended the hive. In other words, they were simultaneously reproductive while nursing, maintaining and defending the hive, and foraging. In a hopelessly queenless colony, most colony functions were maintained but both the reproductive division of labour and task specialisation that exemplify queenright colonies had broken down. Reproductive queenless workers are task generalists. To examine the physiology of this novel bee behavioural state we examined their brain biogenic amine levels. Biogenic amines are strongly associated with temporal polyethism (age, and task speciality) in queenright colonies. However, queenless bees did not display the biogenic amine profiles typical of queenright bees, and did not show the changes in amine levels typically associated with age and foraging status in queenright colonies. In queenless colonies brain amine levels correspond most strongly with degree of ovary activation. We showed that in hopelessly queenless colonies, individuals, while reproductive, lose their behavioural specialisation, which was reflected in their brain neurochemistry. The generalist behaviour of queenless workers results in a social structure may reveal an atavistic state, as it resembles the behaviour of primitively eusocial bees, in which individuals cooperate to maintain and defend a nest, while also engaging in personal reproduction.

OR345

Larval inhibition of worker egg-laying in the ant Aphaenogaster senilis

Raphael Boulay, Xim Cerda, Irene Villalta

The workers of many ant species have functional ovaries and are able to lay unfertilized (haploid) eggs though they generally do not do so, at least when a reproductive queen is present in the colony. Theory states that workers may refrain from laying eggs if they have a mutual interest in rearing brothers (the queen's sons) rather than nephews (other workers' sons) with which they are less genetically related. In addition, workers may punish reproductive workers if worker reproduction has a cost for the entire colony. The queen of *Aphaenogaster senilis*, a monogynous and monandrous species inhibits worker reproduction. Here we show that this is also the case of larvae: in queenless condition, worker-derived eggs appear later when the workers are provided with first instar larvae than when they are not. A mesh experiment suggests a contact pheromone emitted by the larvae is partially responsible for this inhibition. In this species, the first instar larvae are exclusively fed with trophic eggs; The larvae may, therefore, signal themselves in order to maintain the production of trophic eggs by the workers and by so doing inhibit the production of parthenogenetic eggs. From an evolutionary perspective, it may not be in the workers' interest to switch to parthenogenetic egg-laying until larvae are present in the colony since, in queenless condition, one of them may develop into queen. This also raises the question whether workers also refrain from laying eggs in the presence of other worker's sons.

OR346

Life strategy of honeybee larvae orphaned at different age

Karolina Kuszewska, Michal Woyciechowski

In honeybees, it is a rule that workers are sterile in the presence of the queen but start laying male-determined eggs in queenless colonies. However, recent studies showed that the larvae fed in an orphaned colony can switch from their typical altruistic role to a more selfish one and develop into so-called rebel workers which are more engaged in laying their own male-determined eggs than in rearing the queen's offspring. Unknown is whether larvae orphaned at any time of their feeding period can develop into the rebel workers. To answer this question we conducted our experiment using five colonies (replicates), where worker larvae were reared in seven different conditions (groups 0-6). The workers from group 0 were raised in queenright conditions during the entire feeding period, while the workers from groups 1 to 6 were raised in queenless conditions, respectively from 1 to 6 of the last days of their open larval life. Newly emerged workers from all 7 groups were weighed and dissected. Our results showed that workers reared as larvae in different conditions did not differ in body mass ($p = 0.284$). Whereas, the workers reared as larvae during 4 or more days in queenless conditions (groups 4-6) have more ovarioles in their ovaries ($p < 0.001$), smaller hypopharyngeal glands ($p < 0.001$), bigger mandibular glands ($p < 0.001$) and bigger Dufour's gland ($p < 0.001$) compared to the workers from groups 0-3. This means that only the workers, which are orphaned during the first 4 or more days of the feeding life develop into rebel workers while larvae orphaned later in their life develop into typical sterile workers.

OR347

Honeybee worker larvae perceive queen pheromones in their food

Michal Woyciechowski, Karolina Kuszewska, Jedrzej Pitorak, Justyna Kierat

If honeybee larvae are reared in a queenless colony they develop into rebel workers, with more ovarioles in their ovaries and less developed hypopharyngeal glands. In natural conditions, rebel workers appear after swarming because some larvae complete their feeding in the absence of a queen after the old-queen leaves her colony with the swarm and before new-queen hatches. The drop in relatedness between the old-generation workers and the new-queen's offspring justifies the rebel strategy of workers, which are more prone to attend to their own reproduction than to rearing the new-queen's offspring. How the larvae recognize the lack of a queen in the colony is unknown. We hypothesized that lack of queen pheromones in the larvae's food is what triggers their development into rebel workers. To test that we used two colonies divided into queenright and queenless subunits, each with an experimental frame containing eggs of similar age. When the larvae appeared in these frames, each day we added a drop of distilled water to the food of some larvae, and the others were left untreated. Additionally, only in the orphaned subunits each day we added a drop of water with macerated mandibular glands of egg-laying queens from independent colonies to the food of some larvae. We dissected bees newly emerged from the experimental frames and found that the workers developing in queenright subunits did not differ from those developing in queenless subunits fed with macerated mandibular glands. Only workers emerging in queenless subunits, those whose food had pure water added and those not treated, developed into rebels. They had more ovarioles per ovary, smaller hypopharyngeal glands, and bigger mandibular and Dufour's glands than the workers from all other compared groups. This suggests that the presence of queen pheromones from mandibular glands in larvae's food prevents larvae from becoming rebels.

OR348

Host-parasite interactions: from phenotype to genome and back

Paul Schmid-Hempel

The interaction between bumblebee hosts, *Bombus* spp, and its trypanosome parasite, *Crithidia bombi*, is a model case for social insects. The interaction has number of consequences for the host, and is highly specific at the level of the involved genotypes. Here, I will discuss how the synthesis of different approaches help to elucidate the underlying mechanisms, such as multiple infections, parasite genetic exchange or host immune and other defences. The ecological and evolutionary implications of these elements will be scrutinized.

OR349

Parasite-induced changes in host behavior and gene expression after infection

Hannes Lerp, Johanna Mazur, Harald Binder, Sara Beros, Susanne Foitzik, **Barbara Feldmeyer**

Parasite infections may affect host organisms on several levels from physiology, morphology, life history to behavior, and thus may have great impact on the hosts' fitness. The observed changes in the host can either be attributed to host defenses to reduce the costs of infection, they can be by-products of infection, or they can be the result of manipulation by the parasite in order to increase its transmission or reproduction. In social insects not only the infected individual itself but the whole colony is affected by the parasites presence and has to buffer the consequences as community. We could show that the cestode parasite *Anomotaenia brevis* greatly affects the behavior, life history and morphology of individual *Temnothorax nylanderi* host ants. In addition parasitized colonies show lower aggression levels than healthy colonies, or colonies after removal of infected individuals. To understand the proximate mechanisms of parasite-induced behavioral changes, we compared brain gene expression patterns between infected individuals, their healthy sisters and individuals from uninfected colonies. We will report on the functional gene categories overrepresented in parasitized individuals compared to the others and specifically investigate genes known to mediate aggressive behavior and immunity in insects.

OR350

Escaping parasite manipulation: Apoptosis and host-parasite co-evolution in Apis mellifera

Christoph Kurze, Yves Le Conte, Claudia Dussaubat, Thomas Mueller, Per Kryger, Silvio Erler, Robin Moritz

Programmed cell death (apoptosis) does not only play an important role in the development of multicellular organisms, but also in the protection against pathogens. Nevertheless, numerous intracellular pathogens have evolved diverse strategies to interfere with and overcome the apoptotic machinery of their hosts. Yet, little is known about the actual mechanisms and how hosts might counter act. We here study the interaction of the intestinal microsporidian parasite *Nosema ceranae* in a susceptible and tolerant honeybee host under laboratory controlled conditions, to understand the importance of apoptosis in this case of host-parasite co-evolution. We visualize apoptotic processes in the gut epithelium using TUNEL assays; relate this to the expression levels of key genes in the apoptotic cascade over the course of the infection, and consequences for metabolic energetics affecting honeybee performance.

OR351

Fire ant viruses: host-parasite dynamics and transcriptomics of the infection

Fabio Manfredini, DeWayne Shoemaker, Christina Grozinger

The fire ant *Solenopsis invicta* is an invasive pest at a global scale. In most recent invasive ranges such as South-East Asia and Australia, fire ant colonies are not affected by natural parasites, as predicted by the Enemy Release Hypothesis. Conversely, in less recent areas of expansion such as North America, fire ants are commonly infected by microsporidia and viruses. These parasites can have a negative impact on fire ant colonies, in particular after translocation from the field to the lab. In this study, we characterized the association between two RNA viruses (SINV-1 and SINV-2) and S. invicta founding queens at multiple levels, by examining the dynamics of the infection and correlating this with changes in the physiology and transcriptome of infected queens. We collected newly mated queens and we reared them in the lab for two months. The two viruses showed different patterns of infection, with SINV-1 infecting more queens than SINV-2 at time 0 while SINV-2 was detected more frequently after one and two months. SINV-2 infection was also associated with lower fertility in founding queens (fewer eggs produced) while SINV-1 infection had no visible effect on queens fitness. Finally, transcriptomic analysis with whole-genome microarrays on a subset of queens revealed that viral infection altered the expression of ~4000 genes, many of which related to reproductive functions and immune processes. Interestingly, infection by SINV-2 alone or co-infection by both viruses altered the expression of these genes more than infection by SINV-1, despite the fact that SINV-1 was more prevalent than SINV-2 as revealed by subsequent quantification of viral titres with QPCR. We conclude therefore that the two viruses follow different dynamics of infection: SINV-1 is more prevalent but less virulent than SINV-2, which may indicate a longer history of coevolution of SINV-1 with its fire ant host.

OR352

Molecular evolution of immune genes in the invasive Argentine ant

Jenni Paviala, Heikki Helanterä, Jes S. Pedersen, Lumi Viljakainen

Ants are among the most abundant and influential social insects in terrestrial ecosystems. Most ant populations are kin structured meaning that the nestmates are highly related to each other. High relatedness between nestmates assures that natural selection is working efficiently. Some ant species, however, possess an unusual form of social organization called unicoloniality. The nests of unicolonial populations contain many reproducing queens which together with the free movement of individuals between the nests lead to extremely low relatedness between the nestmates creating a potential problem for efficacy of selection on worker traits. The introduced populations of the Argentine ant (*Linepithema humile*) represent one of the most extreme examples of unicoloniality. The species has invaded almost all continents in the world. In the introduced range the relatedness within nests and supercolonies is extremely low. In contrast, nestmates in the native range are more closely related, and local genetic differentiation is evident. Introduced populations may have a competitive advantage over the native arthropod fauna, but at the same time the high population densities combined with exposure to new pathogens and reduced genetic diversity pose a threat to the survival of Argentine ants in its introduced range creating potential novel selection pressures on the immune genes. We will compare evolutionary rates of immune genes in invasive and native *L. humile* populations. To separate the effects of changes in population demography and genetic structure from adaptive evolution specific to immune genes, also control genes are included in the study. Our data will provide quantitative estimates of the rate of adaptive evolution in immune system genes relative to the rest of the genome, associated with invasions and changes in the social colony structure.

OR353

Horizontal transfer of RNAi between honeybees and Varroa destructor

Yael Garbian, Eyal Maori, Haim Kalev, Sharoni Shafir, Ilan Sela

RNAi is an RNA-mediated sequence specific gene-silencing mechanism. RNAi has been demonstrated to moderate gene expression in a wide variety of organisms including plants, mammals, insects and ticks. Acquisition of RNAi components (dsRNA, siRNA) by ingestion and their spread within the recipient organism has been previously reported by us and others. Here we extend such observations, demonstrating that RNAi is transferred within the same species and across species which, upon transmission from one organism to another still retains its biological activity. The mite *Varroa destructor* is an obligatory ectoparasite of the honeybee (*Apis mellifera*) and is one of the major threats to apiculture worldwide. Recently, *Varroa* resistance to acaricides has become a global concern and alternative means of control are needed. In recent years, plant-to-parasite RNAi transfer has been documented, suggesting a potential strategy for next-generation pest control. We previously reported that honeybees fed on double-stranded RNA (dsRNA) with a sequence homologous to that of the Israeli acute paralysis virus are protected from the viral disease. Here we show that dsRNA ingested by bees is detected in the bee hemolymph. We also show that bees that were fed with dsRNA transferred it to larval food that they produced (both worker and royal jelly). Finally, we show that dsRNA is transferred from bees to the *Varroa* mite and from mite on to parasitized bee. This cross-species, reciprocal exchange of dsRNA between bee and *Varroa* engendered targeted gene silencing in the latter, and resulted in an over 60% decrease in the mite population. Thus, transfer of gene-silencing-triggering molecules between this invertebrate host and its ectoparasite could lead to a conceptually novel approach to *Varroa* control.

OR354

Constitutive protection, mismatch, and the role of small RNA in transgenerational immune memory in the bumblebee Bombus terrestris

Seth Barribeau, Paul Schmid-Hempel, Ben Sadd

A number of invertebrates show evidence of immune memory or priming. Individuals given cues of a parasite either as a clearable dose or an inactivated inoculum are better able to resist a subsequent exposure than naive individuals or those given a different priming immune exposure. This immune history can also be transferred across generations with offspring having increased resistance to parasites that their parents were exposed to. Since invertebrates lack the antibodies and T-cells of the adaptive immune system, the mechanisms behind such memory remain mysterious. Here we describe the full transcriptome expression profile of transgenerational immune memory in the European bumblebee *Bombus terrestris*. We found that daughters of mothers exposed to an inactivated inoculum of the Gram-positive bacterium *Arthrobacter globiformis* constitutively express all known antimicrobial peptides at much higher quantities than daughters that have no immune history. We also describe how a mismatch between immune history and exposure results in a fundamentally different expression pattern. Finally, we explore the role of small RNA in moderating these expression patterns.

OR355

Immunity, sanitary behaviour and social structure of diseased ant colonies

Sylvia Cremer

Pathogen exposure induces performance of sanitary actions and upregulation of physiological immune defences in host individuals. In social groups, like the societies of ants, sick individuals are further taken care off by group members, for example by removal of infectious particles during allogrooming. These collective defences often beneficially influence the course of disease of the pathogen-exposed individuals, but involve the risk of contracting the disease by previously healthy colony members. We analyse how singular and repeated exposure to entomopathogenic fungi alters immune gene expression and performance of sanitary tasks in both directly exposed ants and their nestmates. We further study the long and short term effect of disease on the social organisation of colonies, by determining immediate changes in the behavioural interaction frequencies upon pathogen entry into the colony and persisting changes in colony structure and the performance of sanitary tasks.

OR356

The impact of social immunity on fungal pathogen adaptation

Miriam Stock, Anna V. Grasse, Sylvia Cremer

The armsrace between hosts and pathogens is characterized by continuous adaptations of both players in a co-evolving system. In social insect hosts, these adaptations do not only include the hygiene behaviour and physiological immune system of the individual group members, but also their collectively performed, social disease defences ranging from sanitary behaviours, use of antimicrobials and organisational adaptations. We performed an experimental evolution using the Argentine Ant, *Linepithema humile*, as a host and the general insect pathogenic fungus *Metarhizium* as a pathogen. We allowed pathogen adaptation over 10 serial host passages to two different evolution regimes: (1) only individual host immunity in a single ant treatment, and (2) simultaneously acting individual and social immunity in a social treatment, in which the exposed ant was accompanied by two healthy nestmates. We found that the killing rate of the pathogen increased under both evolution regimes, yet at different dynamics. Testing the evolved strains that evolved under either the single or the social regime under both single and social current rearing conditions in a full factorial design revealed that the additional collective defences in insect societies add new selection pressures for their coevolving pathogens that compromise their ability to adapt to its host at the group level. To our knowledge, this is the first study directly measuring the influence of social immunity on pathogen adaptation.

OR357

Pharmacophagy: self-medication in bumblebees

David Baracchi, Mark J.F. Brown, Lars Chittka

In the wild, *Bombus spp*. may contract infections of the trypanosome parasite *Crithidia bombi* from their nestmates or contaminated flowers while foraging. The prevalence of colonies infected with *C. bombi* rises during the season and *C. bombi* often infects the majority of foragers within a hive. *C. bombi* has a wide range of impacts on worker bumblebees, inducing a costly immune response, increasing mortality under food stress, and reducing learning and foraging efficiency. The severity of foraging impairments increases with parasite load and any mechanisms that reduce infection intensity in a forager would therefore improve its foraging efficiency and the health of its colony. Evidence that secondary metabolites in nectar have antimicrobial activity suggests that bumblebees may derive a benefit from the consumption of nectars rich in these metabolites. While some insects are known to shift their diet to toxin-rich food to counteract infections, this phenomenon is understudied and there are no reports of active self-medication (pharmacophagy) in pollinators. We tested whether nicotine, a nectar secondary metabolite common in Solenaceae and several other bee-pollinated plants, could reduce pathogen load in bumblebees infected by *C. bombi*. We first studied whether the consumption of nicotine by bumblebees reduced their parasite load, and then, whether nicotine directly reduced the infectivity of the protozoans. Finally, we allowed healthy and infected bees to choose their diet from plastic flowers in an artificial arena to test for a nicotine preference among infected individuals. Our results demonstrated that artificial nectar containing a natural nectar alkaloid reduced the severity of a gut infection in bumblebees. More interestingly, our behavioural test showed that infected bees actively self-medicate themselves by shifting their diet towards non-nutritive secondary metabolites. This finding raises the possibility that pollinators play an important role in maintaining secondary metabolites in plants.

OR358

Are social-immunity biofilms of attine ants monocultures or polycultures?

Tabitha Innocent, Sandra B Andersen, Morten Schiott, Jacobus Boomsma

The association between fungus-growing attine ants and antibiotic-producing cuticular actinobacteria has created some controversy. Are these bacteria really social immunity mutualists? And if so, what pathogens do they suppress? Is it the case that actinobacteria are frequently acquired from the local environment and, consequently, do not co-adapt to their ant hosts? Or does the original idea of strict vertical transmission and substantial coevolution remain largely correct, but with some interesting modifications?

Recent modeling (Scheuring and Yu, 2013) predicted that a single bacterial strain acquired vertically by callow ant workers could maintain a monopoly if the primary function of the antibiotics produced is to suppress competing strains - meaning that the benefits to ant hosts, in terms of controlling fungus garden pests, are indirect. A simultaneous empirical study by Andersen et al. (Molecular Ecology, 2013) showed that *Acromyrmex* workers start their lives with a monoculture of one of only two 'native' *Pseudonocardia symbionts*. Second, results showed that workers only pick up additional strains once they become foragers - i.e. from the point in time when their main role is no longer restricted to tasks within the fungus garden that help suppress *Escovopsis* infections.

We are now extending this empirical approach. We are comparing the biofilms of callow and mature workers in multiple attine genera, using 454 metagenome sequencing; and testing the inhibition efficiency of native and secondarily acquired actinomycete strains against an array of fungus-garden and insect pathogens. We are also initiating collaborative work to test the potency of antibiotics produced by native and secondary strains. Results so far indicate that environmentally acquired secondary strains play little role in *Escovopsis* control, and that native strains are associated with host lineages for sufficient time that at least some co-adaptations have evolved.

OR359

The termite microbiome and its role in disease resistance

Rebeca Rosengaus

Microorganisms, which are ubiquitous, are known to span the entire range of symbiotic associations, from pathogenic to mutualistic interactions. As such, they likely act as important selection forces and ultimately, are responsible for key evolutionary innovations. Because termites exploit microbial rich environments and have had a long co-evolutionary, we hypothesize that some mutualistic microbes help in the control of termite infectious diseases. From the environmentally acquired microorganisms that colonize the insect's cuticle and outer surface of the eggs to the diverse and abundant hindgut microbial community, termite hosts appear to derive protection against pathogens from their mutualistic microbes. Over one hundred strains of actinomycetes have been isolated from *Zootermopsis angusticollis* nest material and egg washes. Among the isolates several strains produced highly diffusible compounds with high activity against *Metarhizium anisopliae*, a common termite pathogen. Additionally, through a combination of *in vitro*, *in vivo* and *ex vivo* experiments, we show that protozoa and/or their associated bacteria colonizing the hindgut of *Z. angusticollis*, are responsible for the synthesis of multiple active beta-1,3-glucanases, enzymes known for their fungistatic activity. These microbial-derived enzymes are also present in the termite's liquid feces which are commonly used in nest building and proctodeal feedings. By externalizing, depositing and sharing these mutualist-derived enzymes, termites can potentially expand individual-level antifungal defenses to encompass colony-wide protection. This research establishes a novel role for the mutualistic association between termites and some of their microbial consortia and provides new insights into factors that could have influenced the origin and/or maintenance of complex mutualisms, the evolution of termite sociality and the host's individual and social immunity.

OR361

The mating biology of social insects

Susanne den Boer, Tamara Hartke, Boris Baer

The reproductive biology of social insects is truly spectacular, as a number of traits have evolved that are either rare or completely absent in other animals. Because high relatedness among workers increases their helping incentive, social insect colonies are closed genetic systems where mate choice and paternity contributions are typically determined once very early in life. Such "marriages for life" influenced the evolution of social insect mating systems. For example, the high demand on queen fertility over prolonged periods of time required males to produce large, high quality ejaculates, and queens to be able to store these ejaculates for years and to use sperm in highly economized ways. Furthermore, polyandry evolved independently in a number of social insect lineages. As ejaculates of competing males co-occur within the sexual tract of queens in these lineages, post-copulatory sexual selection (sperm competition and cryptic female choice) can be expected to evolve, for example to reduce male contributions to the most competitive or preferred male. Research conducted over the past few years has provided novel insights into the evolution of abovementioned traits, but a number of key questions remain to be studied.

The symposium organizers will start the session by providing a brief summary of advances made in the field of mating biology within the past years and discuss exciting opportunities for future research that arise as a result. We will also briefly introduce the contributions that will be presented in this symposium.

OR362

The ant Cataglyphis cursor*, a model to study sexual selection*

Florence Helft, Thibaud Monnin, Claudie Doums

Few studies on sexual selection have been carried out in social insects as it is generally believed that it should not play a major role in social insects. Here, we argue that this might not be the case in all species. In some ant species, mating takes place at the entrance of the nest where young virgin queens (gynes) perform sexual calling and mate with foreign males coming from surrounding colonies. Contrary to species where mating occurs during a nuptial flight, workers are present when mating takes place on the ground and they can have two main effects. First, workers may protect gynes so that mortality during mating is very low. Second, they may interfere with the mating process. For instance, in the ant *Cataglyphis cursor*, workers chase foreign males seeking to mate with gynes, and this may be a form of sexual selection where workers select for fittest mates for their sister gynes (inclusive mate choice). In this species, both males and gynes can mate multiply, which also opens the possibility of post-copulatory sexual selection. In this study, we analyse the effect of worker aggressions on male mating attempts by comparing the mating behaviour of three to four virgin males and one unrelated gyne placed together in a box, with or without workers under laboratory conditions. We also investigate the role of workers under natural conditions by experimentally removing workers at the nest entrance during the mating period. Our data reveals for the first time the involvement of non-reproductive individuals in sexual selection processes.

OR363

Ants in flight: the Found or Fly tradeoff in queens

Jackson Helms, Mike Kaspari

Flight is crucial for most social insect life histories. In ants, queens and males fly to mate and disperse. The queens then locate a nest site, shed their wings and begin laying eggs. Flight is both the deadliest part of the colony life cycle and vital for reproduction, suggesting that flight related selection may drive queen evolution. Because ant flight is brief and difficult to observe, however, we know little about how queens fly. We propose a model, the Found or Fly (FoF) Hypothesis, which links queen morphology to the competing demands of colony founding and flight. It posits a tradeoff between colony founding success and flight ability mediated through abdominal nutrient investment. Heavier abdomens help a queen survive through colony founding and rear her first workers. At the same time, they decrease a queen's ability to mate, disperse and survive the flight phase by adversely impacting metrics of flight ability, namely flight muscle ratio, wing loading and abdomen drag. We evaluate the assumptions and predictions of FoF through comparative studies and live flight experiments. In doing so, we discover a previously unrecognized cost in the evolution of a common reproductive strategy - claustral founding. Claustrally founding queens don't feed during colony founding and require large abdominal energy reserves, resulting in lower flight muscle ratios. Several claustral species are on the verge of flightlessness, with flight muscle ratios among the lowest of any flying insect. This suggests a simple mechanism for the ubiquitous evolution of queen flightlessness. In addition, reproductive strategy may drive evolutionary changes in wing size and lead to dispersal polymorphisms within species. By emphasizing function, FoF links reproduction and dispersal, underscores the role of flight in queen evolution, and provides a quantitative framework for comparing ant life histories.

OR364

Mating biology of the ant Myrmica ruginodis

Jana Wolf, Perttu Seppä

Workerless social parasites have been suggested to evolve sympatrically from their hosts, with miniaturization of queens promoting reproductive isolation of the parasite from its host. The aim of this project was to study various aspects of the mating biology to assess whether behaviour of larger (macrogyne) and smaller (microgyne) queens in the red ant *Myrmica ruginodis* has diverged and potentially promote reproductive isolation between them. Dispersing alates were collected from the nests by trapping them in nets spread over the colonies during nuptial flights. Their size was measured and the difference in the propensity to disperse was compared according to their size. Finally duration of matings in the nuptial flight was recorded, and individual males as well as males and females engaged in copula were collected and measured. Thus, potential spatial and temporal isolation in *M. ruginodis* were analysed, including the propensity of sexuals to join a nuptial flight in the first place vs. staying and mating in the nest, size-assortative mating and whether mating behaviour is associated with the size of the mating individuals. This study will provide insight into the composition of nuptial flights in *M. ruginodis*, and will show whether the mating behaviour is connected to size, therefore promoting possible reproductive isolation in this species. This work will show whether the microgynes of *M. ruginodis* have potential to evolve sympatrically from their host, or whether it is just a consequence of alternative dispersal and reproductive strategies, as in some other ants.

OR365

Queen sex pheromones of two sympatric species of Polyergus

Les Greenberg, Christine Johnson, James Trager, Steven McElfresh, Jocelyn Millar

We have previously described the queen sex pheromone of a western North American ant, *Polyergus breviceps.* Males fly to lures containing the pheromone. We now describe the queen sex pheromone of an eastern species, *P. lucidus*. Both species share the major component of their queen sex pheromones, methyl 6-methylsalicylate. The minor component in *P. breviceps* is (R)-3-ethyl-4-methylpentanol, while in *P. lucidus* the minor component is (R)-3-ethyl-4-methylpentanoate. When lures of these blends are placed in a field in Missouri that contains both species, males fly almost exclusively to their own pheromone blend. This is the first demonstration of a possible chemical mating isolation mechanism between two sympatric ant species.

OR366

Solenopsis invicta*: Chemical communication in mating flights and colony development*

Robert Vander Meer, Tappey Jones, Mon-Yeon Choi

Fire ant nuptial flights are characterized by frenzied activity by workers, and male and female alates that results in the opening of the normally closed nest tumulus, followed by male and female alates taking flight. Males fly first and form a population-wide horizontal lek at an altitude of 100 to 150 m. Females then fly into the male lek, mate, and fly to the ground. These are huge population-wide events with no evidence for localized use of prominent landmarks. Thus, semiochemicals are likely involved in the several defined mating flight events, e.g., flight initiation, male lek formation, female and/or male sex pheromones. During these mating flights the female alates mate only once, in spite of being in the vicinity of large numbers of males. We will discuss recent results that answer some of the questions surrounding semiochemical involvement in fire ant mating flights. In addition, we will address the role of semiochemcals in colony foundation and development.

OR367

Reproductive interference between honeybee species in artificial sympatry

Emily Remnant, Anna Koetz, Ken Tan, Eloise Hinson, Madeleine Beekman, Benjamin Oldroyd

Reproductive isolation between closely related species is often incomplete. The Western honeybee, *Apis mellifera*, and the Eastern hive bee, *A. cerana* have been allopatric for millions of years, but are nonetheless similar in morphology and behaviour. During the last century the two species were brought into contact anthropogenically, providing potential opportunities for interspecific matings. Hybrids between *A. mellifera* and *A. cerana* are inviable, so natural interspecific matings are of concern because they may reduce the viability of *A. cerana* and *A. mellifera* populations - two of the world's most important pollinators. We examined the mating behaviour of *A. mellifera* and *A. cerana* queens and drones from Caoba Basin, China and Cairns, Australia. Drone mating flight times overlap in both areas. Analysis of the spermathecal contents of queens with species-specific genetic markers indicated that in Caoba Basin, 14% of *A. mellifera* queens mated with at least one *A. cerana* male, but we detected no *A. cerana* queens that had mated with *A. mellifera* males. Similarly, in Cairns, no *A. cerana* queens carried *A. mellifera* sperm, but one third of *A. mellifera* queens had mated with at least one *A. cerana* male. No hybrid embryos were detected in eggs laid by interspecifically-mated *A. mellifera* queens in either location. However *A. mellifera* queens artificially inseminated with *A. cerana* sperm produced inviable hybrid eggs, or unfertilised drones. This suggests that reproductive interference will impact the viability of honeybee populations wherever *A. cerana* and *A. mellifera* are in contact.

OR368

Energy metabolism of honeybee sperm

Ellen Paynter, Boris Baer, Harvey Millar

The queens of the social ants, bees and wasps mate only at the beginning of their life, and afterwards store sperm in their spermatheca. As queens are ultimately sperm limited, they use very few sperm per egg fertilisation, selecting for sperm of maximal quality and viability. However, little is known about the physiological processes that allow social insect sperm to achieve these traits. For example, it remains unclear how chemical energy is generated to sustain sperm function during the very different timeframes governing post ejaculation traits or long-term storage traits. We have conducted a detailed metabolite profiling of seminal fluid and combined it with sperm proteome data and measurements of metabolic rate to enhance our understanding of honeybee sperm metabolism. We have identified the specific pathways of energy metabolism that are abundant in the sperm proteome and the chemical components of seminal fluid that change after ejaculation to determine which are most important. Measurements of metabolic rates (glycolysis and respiration) within single bee ejaculates provide quantitative assessment of ATP synthesis rates in sperm. This has pinpointed the subset of metabolites in seminal fluid that are ideal substrates for energy generation in honeybee sperm, and are key in determining sperm activity.

OR369

Sperm competition in Cataglyphis *desert ants.*

Serge Aron, Claire Baudoux, Denis Fournier

In species whose females mate promiscuously, competition of ejaculates from different males for the fertilization of a given set of ova can drive the evolution of sperm traits, such as ejaculate size or sperm length, that determine fertilization success and, ultimately, likelihood of paternity. Males that produce more sperm may often secure more fertilizations, with increased risk of sperm competition selecting for larger numbers of sperm in ejaculates. Sperm competition is also thought to promote the evolution of longer sperm, since sperm length is assumed to be positively associated with sperm swimming velocity. We examined relationships between sperm competition levels, sperm quantity and sperm lengths, in a comparative study of 14 species of *Cataglyphis* desert ants using phylogenetically controlled analyses. The genus displays large contrasts in sperm competition levels with queen mating frequency greatly varying across species, from strict monandry to obligate polyandry. Our results show that males of polyandrous species are significantly larger than males of monandrous species, consistent with positive selection on male size under sexual competition. Also, they produce a higher number of spermatozoa and longer sperm cells than males of monandrous species. Furthermore, across species comparison shows that sperm quantity and, in a lesser extent, sperm length are positively correlated with the average number of matings achieved by queens, independently of male size. Overall, these results show that sperm competition affects the characteristics of males ejaculates in *Cataglyphis* desert ants: they are consistent with the 'fair raffle principle', with increased risk of sperm competition selecting for production of larger numbers of spermatozoa. They also indicate that sperm competition affects sperm size, possibly to enhance sperm swimming abilities.

OR370

Team swimming in ant spermatozoa

Morgan Pearcy, Noemie Delescaille, Pascale Lybaert, Serge Aron

Eusocial bees, wasps and ants are unique in many respects, including their mating biology. Reproductive activity is typically concentrated into a brief mating flight early at sexual maturation. Males die after mating, and their reproductive success is ultimately limited by the amount of sperm effectively stored in the queen's spermatheca. In species where queens mate promiscuously, competition between ejaculates from different males is expected to impose intense selective pressure on males to optimize the transfer of sperm to the storage organ. While investigating the effect of sperm competition on sperm traits in the ant *Cataglyphis savignyi*, we found that males ejaculate highly motile sperm bundles. In these aggregates, 50-100 sperm cells are oriented in the same direction and bound together with their acrosome stuck in an agglutinative cap of extracellular material. Flagella remain completely free and propel the bundles forward in a characteristic helical pattern. Sperm motility trials showed that sperm aggregates swim 51% faster on average than isolate sperm cells. The difference was particularly marked at high viscosity, where bundles move 2 times faster than solitary cells. By forming cooperative groups, sperm increase their swimming efficiency and, hence, potentially benefit from a significant advantage in a context of sperm competition for access to the spermatheca.

OR371

Sperm precedence in polyandrous, resource-defending wool-carder bees

Thomas Eltz, Kathrin Lampert, Vanessa Pasternak, Philipp Brand, Florian Leese, Ralph Tollrian

Polyandry is rare in aculeate Hymenoptera (bees, wasps, ants), with some exceptions in both solitary and social groups. Females of polyandrous European Wool-carder Bees, *Anthidium manicatum*, mate continuously over the course of their reproductive life, while males defend patches of food plants where copulations occur. To better understand the evolution of this mating system we investigated offspring paternity using cage experiments and microsatellite genotyping of brood. We found that, although females possess a spermatheca for long-term sperm storage, most brood was fathered by males that had only recently mated with the breeding female, indicating pronounced last (or at least 'late') male sperm precedence. In the absence of males a large proportion of eggs remained unfertilized (resulting in haploid male offspring), but some daughters arose from fathers that had been removed up to two weeks earlier. It appears that most *A. manicatum* eggs are fertilized with sperm from the bursa copulatrix, while the spermatheca serves only as a back-up reservoir. To our knowledge this is the first demonstration of last male sperm precedence in aculeate Hymenoptera. We suggest that it has co-evolved with and promoted both male resource defence and polyandry in anthidiine bees. Multiple mating combined with 'late male' sperm precedence creates an initiative for males to defend resources, which on the other hand will benefit females through an enhanced resource base. Thus, polyandry may have arisen in these bees exclusively through direct (non-genetic) benefits for males and females.

OR372

Sperm storage and immunity in leaf-cutting ants

Sarah Cherasse, Morten Schiott, Francisco Davila, Serge Aron, Jacobus J Boomsma

Leaf-cutter ant queens mate on a single day and store the sperm for the rest of their life, which may be up to 20 years. Sperm transits through the bursa copulatrix before entering the highly specialized spermatheca organ. Sperm storage creates a possible conflict with immune defences that generally target non-self cells, but queens also need to make sure that sexually transmitted bacteria and fungal spores are killed before sperm is stored. We therefore hypothesized that stored sperm is protected by immune mechanisms that target infecting microorganisms specifically, rather than by general innate non-self-recognizing immune defences. We used tissue-specific differential gene expression of two antimicrobial peptides (AMPs), abaecin and defensin, and we determined enzymatic activity levels and gene expression of phenoloxidase (PO) and its zymogen prophenoloxidase (proPO). The latter have a crucial role in the melanogenesis cascade that mediates a variety of non-specific insect immune responses. We investigated virgin and mated queens of both *Atta colombica*, a species where the bursa copulatrix has been reduced and sperm transfers directly to the spermatheca, and virgin queens and males of *Acromyrmex echinatior*, where the bursa is large and suitable for pre-storing sperm for several hours in between insemination and final storage. Queens of both species mate with multiple males, but the number of inseminations per *Acromyrmex* queen is ca. twice as high as in *Atta* queens. We found that PO/proPO responses were silenced in the spermathecae and bursae of both species, while abaecin was up-regulated in these organs and more so in *Acromyrmex* where queens mate with more males. Ant spermathecae thus appear to be analogous to sulphite-treated boxed wine that can be left alone for years because infections can never reach the interior of the bag, whereas normal body tissues require constant monitoring by standard non-self-recognizing immune defences.

OR374

Dynamics of DNA methylation in honeybees: development and environment

Sylvain Foret

Numerous species of social insects are able to produce castes that are phenotypically distinct but that do not present any systematic difference at the genome level. In the Western honeybee (*Apis mellifera*), DNA methylation has been identified as a central epigenetic mechanism responsible for the developmental plasticity of females. In this species, different nutritional environments trigger specific epigenetic programs that will ultimately give rise to distinct castes (queens and workers). In order to understand the dynamics of epigenetic landscapes and how these changes influence development and behaviour, it is important to disentangle the various causes that can alter the epigenetic state. The factors that shape epigenetic signatures include various interplays between genetic background, development and environment. The honeybee haplo-diploid sex-determination system and its nutritionally-driven caste-determination system provide an attractive system to start disentangling the forces govern DNA methylation profiles. In this talk, three determinants of the honeybee DNA methylation landscapes will be contrasted: 1) the different ploidy of males and females, 2) the nutritional environment of queens and workers, and 3) short term differences through a conditioning experiment. Some of the challenges pertaining to the analysis and interpretation of DNA methylation data from complex heterogeneous samples containing a mixture of tissues and cell types will also be discussed.

OR375

The function of DNA methylation in social insects

Brendan Hunt, Karl Glastad, Soojin Yi, **Michael Goodisman**

Phenotypic plasticity is a fundamentally important process that allows organisms to develop phenotypes well suited to the environment. The production of alternate phenotypes in insects relies on epigenetic inheritance, which plays a key role in regulating gene expression. DNA methylation is one of the most widely conserved forms of epigenetic information. However, the function of DNA methylation in insects remains poorly understood. The goal of our research program is to gain a greater understanding of the importance of DNA methylation and its role in the epigenetic landscape of social insects. We use computational and empirical methods to determine the function and evolution of DNA methylation. We have found that DNA methylation is targeted to genes in insect genomes. In addition, methylated genes tend to be uniformly and highly expressed. Thus, methylation is strongly associated with patterns of gene expression. We have also found that DNA methylation is associated with ploidy variation. Phenotypically-dissimilar diploid males, queens, and workers are more similar to one another in terms of DNA methylation than are phenotypically-similar haploid and diploid males in fire ants. These results indicate that DNA methylation may be involved in ploidy compensation. Evolutionary analysis indicates that targets of DNA methylation are largely conserved in fire ants and honeybees. Thus patterns of DNA methylation do not differ dramatically among social insect species. Finally, we have discovered that DNA methylation is associated with several active histone modifications in social insect taxa. Thus, overall, our research indicates that DNA methylation is a single part of a larger, conserved, integrated epigenetic landscape in social insect genomes.

OR376

Socially-mediated changes in brain epigenome in the fire ant

Oksana Riba-Grognuz, David DeWayne Shoemaker, Laurent Keller

Ants exhibit complex social behavior and organization, but little is known about how individuals respond to their social environment. The dimorphic social organization of the fire ant *Solenopsis invicta* provides a unique opportunity to study the effects of social environment on gene expression and social behavior. In the monogyne (single-queen colonies) social form all females have two copies of the SB variant of the social chromosome. By contrast, polygyne (multiple queen) colonies contain a mix of SB/SB and SB/Sb workers (i.e., workers which also have a copy of the b variant of the social chromosome). Experiments showed that SB/SB shift behavior and accept several queens when colonies contain more than 5% of SB/Sb workers. This change in behavior is associated with changes in gene expression. To investigate the role of epigenetic mechanisms, such as DNA methylation and histone modifications, in modulating gene expression we performed RNA-seq, BS-seq and CHIP-seq assays on the brains of two week-old SB/SB and SB/Sb workers introduced at the late pupal stage in host colonies of both social forms. These results should reveal the implication of DNA methylation and chromatin modifications in brain behavioral response to changes in the social environment, and uncover the contribution of these mechanisms to transcriptional regulation in a neural context.

OR377

Variation in behaviour plasticity by distinct regulation of the brain methylome

Solenn Patalano, Phil Ewels, Tomasz Jurkowski, Anna Vlasova, Claire Asher, Roderic Guigo, Simon Andrews, Seirian Sumner, Wolf Reik

Studies in several social insect species reveal a potential involvement of epigenetic mechanisms, especially DNA methylation, in caste determination. However, few studies have investigated primitively Eusocial insects which have caste determination later in development and show greater behaviour plasticity at adult stage. To understand the underlying epigenetic mechanisms we determined the adult brain methylome dynamics and associated transcriptomes of various individuals of two primitively social species: the paper wasp *Polistes canadensis* and the dinosaur ant *Dinoponera quadriceps*. DNA methylation in gene bodies, particularly at their 5' end, was positively associated with expression of housekeeping genes, a conserved feature in all insect species suggesting an ancestral role of methylation in constitutive gene expression. Notably, DNA methylation was not associated with caste specific gene expression. Key components of the methylation and demethylation machinery including a TET hydroxylase are conserved in primitively eusocial insects, but *Polistes canadensis* lacks DNMT3, which may account for its strikingly unpatterned methylome. Given the flexibility of adult *Polistes* to change caste, we manipulate the social structure of the nest to understand if unpatterned epigenomes reflect greater reprogramming potency.

OR378

Epigenetic regulation of reproduction and behavior in a clonal ant

Romain Libbrecht, Laurent Keller, Daniel Kronauer

Division of labor is at the root of the ecological success of insect societies, yet the mechanisms regulating reproduction and behavior are not fully understood. The clonal raider ant *Cerapachys biroi* has no distinct queen and worker castes, and is characterized by an alternation between reproductive phases (ants lay eggs inside the nest) and brood care phases (ants do not lay eggs but nurse the brood and forage for food). The opportunity to compare queen-like (reproductive phase) and worker-like (brood care phase) individuals, combined with the possibility to control for age, experience and genetic background (all known to influence reproduction and behavior), makes *C. biroi* a great model system to study division of labor. In the past few years, there has been a growing interest in the role of epigenetic mechanisms (e.g. DNA methylation) in division of labor. Our aim is to investigate the role of DNA methylation in regulating reproduction and behavior in *C. biroi*, by comparing whole-genome DNA methylation patterns between brains of individuals collected in reproductive and brood care phases. Preliminary experiments using methylation-sensitive AFLP suggest that the global level of DNA methylation differs between these two types of individuals. We are currently using whole-genome bisulfite sequencing to determine where DNA methylation occurs in the genome, and which genes are differentially methylated between reproductive and brood care phases. Finally, by revealing how DNA methylation affects gene expression and alternative splicing in *C. biroi*, this project will also provide important insights into the function of DNA methylation in insects.

OR379

Allele-specific methylation in the honeybee, Apis mellifera

Laura Wedd, Ryszard Maleszka, Robert Kucharski

Nearly a third of all genes in *A.mellifera* have been found to be methylated, with the occurrence of differentially methylated genes associated with the establishment of distinct developmental and behavioural phenotypes of queens and workers. It is unclear whether DNA methylation drives changes in key regulatory pathways leading to these alternate phenotypes, or if these changes are driven by other mechanisms. The functional characterisation of methylated genes involved in directing development and behavioural maturation will help define the exact role that DNA methylation plays in modulating phenotypic outcomes in *A. mellifera.* One gene in which this is presently being carried out is the gene encoding lysosomal-alpha-mannosidase (alpha-LAM), a conserved enzyme involved in carbohydrate metabolism. alpha-LAM displays a high level of polymorphism, with sequence variation being linked to dramatic changes in DNA methylation. Significantly, this is an indication that the underlying DNA sequence itself may direct changes in methylation. Utilising single drone-inseminated queens we have genotyped individuals, and bisulfite sequencing and gene expression analyses are being performed. Analysis of this allele-specific methylation will help elucidate the mechanisms driving context-dependent expression of alpha-LAM, and will provide a framework from which broader conclusions about the role of DNA methylation in *A. mellifera* can be drawn.

OR380

Parent-of-origin effects on gene expression in honeybees.

Greg Hunt, Sarah Kocher, Jennifer Tsuruda, Miguel Arechavaleta-Velasco, Christina Grozinger

In mammals and plants, genomic imprints silence parental alleles through methylation of intergenic CpG islands or histone modifications. In theory, parent-of-origin effects (POEs) on gene expression are the result of intragenomic conflict. The kin-conflict theory of genomic imprinting predicts more pronounced selective pressure in a highly polyandrous, haplodiploid insect society and some POEs that influence adult worker behaviors. To our knowledge the only invertebrate examples of genomic imprinting involve heterochromatization of entire parental genomes or position effects caused by chromosomal abberations (e.g., in *Drosophila*). These considerations and the prior discovery of a phenotypic paternal effect on stinging behavior in honeybees motivated us to look for POEs on transcription. We sequenced RNA transcripts from reciprocal hybrid workers derived from crosses between one European and one Africanized honeybee colony, as well as genomic DNA from the parents. We identified single-nucleotide polymorphisms (SNPs) within transcripts in each family and used read counts of alternative alleles to determine whether either the maternal allele or paternal allele was overexpressed in the heterozygous F_1 workers. About 1,000 to 2,400 transcripts contained informative SNPs in larvae, adults or individual brains. Our results and validation tests suggest that parental effects on expression are at least as abundant in honeybees as they are in mammals and higher plants. Results also are in accordance with the kinship theory because (in contrast to mammals and plants) parental effects were just as frequent in whole adults and individual brain samples as they were in first instar larvae, and overlap between lifestages and tissues was much higher than expected by chance. In agreement with theory, several transcripts in larval stages with maternal expression bias are involved in IIS signaling and may be negative regulators of growth, including neural lazarillo.

OR381

Investigating genomic imprinting: an epigenetic cycle in the honeybee methylome

Robert Drewell, Eliot Bush, Emily Remnant, Garrett Wong, Suzannah Beeler, **Benjamin Oldroyd**

In honeybees (*Apis mellifera*) the epigenetic mark of DNA methylation is central to the developmental regulation of caste differentiation, but may also be involved in additional biological functions. In this study, we examine the whole genome methylation profiles of three stages of the haploid honeybee genome: unfertilised eggs, the adult drones that develop from these eggs, and the sperm produced by these drones. These methylomes reveal distinct patterns of methylation. Eggs and sperm show 381 genes with significantly different CpG methylation patterns, with the vast majority being more methylated in egg. Adult drones show greatly reduced levels of methylation across the genome when compared to both gamete samples, suggesting a dynamic cycle of methylation loss and gain through the development of the drone and during spermatogenesis. The distinct methylation patterns at these genes suggest parent-specific epigenetic marking in the gametes. The potential developmental and evolutionary implications of methylation in eggs and sperm and how this may relate to predictions of genomic imprinting in this eusocial insect will be discussed.

OR382

Non-coding RNAs in honeybee caste determination

Regan Ashby, Sylvain Foret, Iain Searle, Ryszard Maleszka

In honeybees (*Apis mellifera*), three contrasting adult castes (phenotypes) are produced from the same genome; two diploid females (a sterile worker and the highly reproductive queen) and the haploid male (drone). While sex determination is genetically controlled, phenotypic differentiation between the two female castes has recently been shown to be associated with changes in the epigenome, specifically changes in DNA methylation patterns, in response to dietary composition (royal jelly). In more recent times, the role of another class of epigenetic modifiers, that of small non-coding RNAs, has become an area of major interest in regards to the regulation of phenotypic and developmental plasticity. This talk will discuss resent findings from our laboratory concerning the expression profile of microRNAs between the three major castes using next-generation high throughput sequencing, and how differences in these profiles may relate to phenotypic output. Mature miRNAs are 20-24 nucleotide long molecules which form part of a larger RNA-induced silencing complex (RISC), providing sequence-specific targeting of mRNA molecules. Most commonly, the binding of a RISC-complex to a target mRNA sequence induces translational repression or mRNA degradation. Increasing evidence suggests that small RNAs help confer genetic robustness by reinforcing transcriptional programs.

OR383

Caste-specific RNA-editomes in the leaf-cutting ant Acromyrmex echinatior

Qiye Li, Zongji Wang, Jinmin Lian, Morten Schiott, Sanne Nygaard, Jacobus J. Boomsma, Guojie Zhang

Eusocial insects have evolved the capacity to generate adults with contrasting morphological, reproductive and behavioral phenotypes from the same genome. Recent studies have suggested that RNA-editing might be a mechanism to enhance the diversity of gene products at the post-transcriptional level, particularly to induce relatively rapid functional changes in the nervous system. Using head samples from the leaf-cutting ant *Acromyrmex echinatior*, we have obtained the first comparative RNA-editomes across eusocial castes. We identified an average of 12,000 RNA editing sites per caste (small workers, large workers, gynes). About 60% of these sites were found across three sympatric colonies, targeting >1,000 genes that are enriched in functions for neurotransmission, circadian rhythm, stimulus response and RNA splicing. Although most A. echinatior editing sites were species-specific, it is intriguing that 10-24% were conserved across subfamilies and thus likely to have been generally important for the evolution of eusociality in ants. Editing levels for some sites differed consistently between castes in each of the three colonies that we sampled, suggesting that RNA editing may be a novel and general mechanism for shaping caste morphology and behavior in ants and other eusocial insects.

OR384

Differential seasonal honeybee immune genes expression exposed to deformed wing virus

Nadja Steinmann, Miguel Corona, Peter Neumann, **Benjamin Dainat**

In temperate regions, honeybees, *Apis mellifera*, are characterized by physiologically distinct summer and winter bees. One feature of winter bees is their long life expectancy (~6-7 months), which may render them more prone to pathogen infections, especially in the close spatial proximity of winter clusters. Their immune system is characterized by cellular and humoral immune responses at individual level, and by defense mechanisms involving specific behaviors at the colony level. Most of the colony losses occur during the winter, suggesting that winter bees may have compromised immune function. Here, we evaluated whether the immune system differs between these two seasonal types of bees. We took advantage of natural infections with the ubiquitous deformed wing virus (DWV), which has been shown to interact with bee immunity and to contribute to a reduced longevity of winter bees. Individual workers were regularly collected during summer or winter and individually analyzed for DWV infections and gene expression of defensin-1, dorsal, eater, hymenoptaecin, juvenile hormone epoxidase (JHE), prophenoloxidase (PPO), prophenoloxidase activator (PPOa) and vitellogenin using qPCR. Our data show that summer and winter workers can display significant differences in gene expression patterns associated with DWV infection. Although higher DWV loads were found in winter, genes were actually downregulated in winter bees. In contrast, summer bees showed a significant positive correlation between DWV loads and immune gene expression. The results suggest that winter bees show a specific immune response, consistent with the hypothesis that downregulation is a mechanism to save energy even at the expense to increased risk of pathogen infection in the context of adult longevity. Sampling for future research on bee health should take into account the observed differences in immune responses.

OR385

SNP identification of Africanized honeybees

Nadine C Chapman, Julie Lim, Amro Zayed, Thomas E Rinderer, Benjamin P Oldroyd

Australia is currently *Varroa* free, but testing has shown that Australian commercial honeybees are highly susceptible to *Varroa*. Curently, Africanized ‘killer’ honeybees cannot be differentiated genetically from non-Africanized bees. Australia has banned honeybee imports from areas where Africanized bees are extant and this constrains beekeepeer's ability to import improved genetic stock, in particular strains with resistance to *Varroa*. The ability to differentiate Africanized bees from other strains would enable Australian beekeepers to safely import improved genetic stock from overseas. We are testing a panel of 144 SNPs on African bees from South Africa (*Apis mellifera scutellata*), Cape bees from South Africa (*A. mellifera capensis*) and hybrids of the two, Africanized bees from Brazil and Texas, European honeybees from Italy (*A. mellifera ligustica*) and Yugoslavia (*A. mellifera carnica*), European-derived American honeybees, Varroa-resistant stocks from America and Australian commercial and feral honeybees. We hope to identify a set of diagnostic SNPs that can be used to certify that honeybee queens and semen are free of ‘Africanization'. The technology is likely to have applications for population genetic studies world wide.

OR386

Genomic signatures of social evolution in bees

Karen Kapheim, Cai Li, Hailin Pan, Guojie Zhang, Gene Robinson

Variation in social behavior among bees provides a unique opportunity for understanding the molecular basis of social diversity within an evolutionary framework. Eusociality has repeatedly evolved from a solitary lifestyle in two families of bees - Apidae and Halictidae. More elaborate forms of eusociality, characterized by colony-level reproduction and very large colony sizes, have independently evolved from basic forms of eusociality twice within the Apidae. We used comparative genomics to identify molecular targets of selection, investigate patterns of genomic plasticity through methylation and gene regulation, and describe patterns of gene family evolution, as they relate to this repeated evolution of multiple convergent phenotypes among bees. We compared whole genomes of ten species from three families of bees that vary in social organization, with both solitary and eusocial species represented in Apidae and Halictidae. This involved *de novo* whole genome sequencing of five species, the draft genomes of which we present here. We included an additional five species with independently sequenced genomes for a comprehensive study of sociogenomics. Our analyses identify genes for which evolutionary rate is associated with social complexity, and reveal some of the evolutionary mechanisms likely to be driving these changes, including natural selection, variation in pan-genome distribution of GC content, and changes in cis-regulation. We discuss resulting insights into the mechanisms underlying convergent eusocial evolution.

OR387

Convergent phenotypes but non-convergent genomes in simple social insect societies

Seirian Sumner, Solenn Patalano, Anna Vlasova, Pedro Ferreira, Claire Asher, Simon Andrews, Heinz Himmelbaur, Roderic Guigo,Wolf Reik

Convergent evolution generates remarkable phenotypic similarities in organisms with contrasting evolutionary histories. Does evolution use the same molecular pathways to generate the same phenotypes in different lineages? Social insect castes include some the most impressive examples of convergent phenotypic evolution. We address this question using two sympatric species of social insect: the dinosaur ant *Dinoponera quadriceps* and the paper wasp *Polistes canadensis*. At the behavioural level, both species exhibit similar within-caste behaviours, and a simple social structure. At the evolutionary level, however, they have contrasting histories: *Dinoponera* evolved from a highly eusocial ancestor, representing a reversion to simple sociality. *Polistes* are ancestrally primitively eusocial, representing the early stages of caste evolution. Here we present the first genome sequences for these species, and compare individual-level caste-specific transcription, methylation and microRNAs at the gene, functional-group, and modular (network) level. At the broad-scale, behavioural phenotypes in both species show convergent genomic properties: less than 5% of the genome is caste-biased; there is an extreme asymmetry in caste-biased transcriptional investment, but very little functional enrichment. Conversely, gene identities, functional enrichment, methylation and microRNAs associated with castes in the two species were largely non-convergent. Exceptions included a small number of 'toolkit' genes, and some shared functional enrichment among the reproductive phenotypes. In each species, a substantial proportion of differentially expressed genes were novel, putatively non-coding, genes. These data reveal broad-scale molecular hallmarks underlying simple behavioural castes that are common to phenotypes at the origin of sociality (*Polistes*) and secondarily derived (*Dinoponera*). However, they also suggest that the role of conserved genes and regulatory processes in convergent phenotypes is not as important as previously thought. It appears there has been substantial molecular re-wiring in eusocial evolution, as expected if non-social traits are lost, and new social traits evolved in the evolution of complex eusociality.

OR388

Seek and ye shall find: Seeking selection for sociality

Clement Kent, Amro Zayed, Karl Glastad, Karen Kapheim

Sequence analysis across species is an attractive way to look for genes in a hypothetical 'toolkit for sociality'. Social insects are ideal for this due to the large number of species with varying levels of sociality. However, sociality exerts powerful effects on the genome, some of which are not due to directed natural selection. 'Seek and ye shall find' in this context poses a problem: among genes in which we find sequence changes correlated with level of sociality, which ones change due to selection for mutations advantageous for social function, and which ones change for other reasons? We applied methods that distinguish between selective and neutral changes and sought for examples of functional categories of genes in both groups, drawing upon studies in Hymenoptera. The results were surprising. For example, among genes with dN/dS positively correlated with sociality, certain normally highly conserved transcription factors were overrepresented - is this evidence of a toolkit? Conversely among genes with dN/dS negatively correlated with sociality, 'brain development' was overrepresented - is this evidence that the more social you are the dumber you get? I present reasons for doubting both of these ideas, plus preliminary evidence for which functional gene groups may be positively associated with selection for sociality.

OR389

The genetic basis of mutualism in Pseudomyrmex *plant-ants*

Benjamin Rubin, Corrie Moreau

The obligate mutualism between ants in the genus *Pseudomyrmex* and acacia trees is one of the most well known of any ant-plant relationship. In this particular mutualism, ants nest in and feed on the hollow thorns, food bodies, and extra-floral nectar provided by the acacia. In exchange for these resources, resident ants aggressively protect their hosts by attacking herbivores, trimming encroaching plants, and removing pathogenic fungi. This ant defense is incredibly effective and their absence ensures plant death. In addition to the acacia specialists, the *Pseudomyrmex* ant genus includes two other obligate mutualistic clades that nest in trees from the genera *Triplaris* and *Tachigali*. These trees have hollow trunks and leaf petioles, respectively, instead of thorns and do not provide direct food rewards, as do acacias. Instead, ants feed on honeydew from plant-sucking insects cultivated inside the plants. Despite the differences in diet and nest structure, these ants behave in the same way as acacia-nesters, aggressively biting and stinging any intruders. Amazingly, these three groups of plant-ants evolved mutualistic behavior convergently. We present three newly sequenced *Pseudomyrmex* genomes representing species from each mutualistic clade as well as five genomes of closely related non-mutualistic *Pseudomyrmex* species. Using these genome sequences, we find a set of approximately 60 genes with consistent signatures of positive selection in all mutualists and no non-mutualists, suggesting their involvement in the evolution of mutualistic behavior. Several of these genes are involved in venom synthesis and carbohydrate digestion, reflecting the differences in life histories between mutualists and non-mutualists. On the whole, mutualistic genomes appear to evolve at a faster rate than closely related non-mutualists, an evolutionary difference possibly associated with their highly active lifestyles. Our findings provide a detailed understanding of the genetic features involved in the evolution of this complex behavior.

OR390

Comparative genomics of the attine fungus-growing ants

Sanne Nygaard, Cai Li, Haofu Hu, Zhensheng Chen, Zhikai Yang, Morten Schiott, Ted R. Schultz, Sean Brady, William Wcislo, Guojie Zhang, Jacobus J. Boomsma

The attine fungus farming ants evolved in the Amazon Basin, spread across the (sub)tropical Americas and radiated into 15 extant genera with >250 currently described species. The attine lineage represents three major irreversible evolutionary transitions: (1) The ants becoming farmers ca. 50 MYA, (2) The mutual domestication of the ancestor of the higher attine ants and a single lineage of gongylidia-bearing crop fungus ca. 20 MYA and (3) The irreversibly committed symbiosis jointly adopting leaf-cutting herbivory and multiple queen mating, which resulted in extended worker caste differentiation, large colony size and substantial ecological footprints. After draft genomes of the leafcutting ants *Acromyrmex echinatior* and *Atta cephalotes* were published in 2011, we decided to also obtain high-coverage reference genomes for *Trachymyrmex septentrionalis*, *T. cornetzi*, *T. zeteki* and *Cyphomyrmex costatus*, representing all genus-level branches of the higher attine ants, and a close lower attine outgroup. We also obtained an assembled reference genome of the dikaryotic fungal symbiont of *C. costatus* and transcriptomes of the functionally polyploid symbionts of the other species. I will present an update of the ongoing comparative analyses which so far indicate that: (1) Most significant genome-wise changes occurred when parties irreversibly committed to the mutualistic symbiosis, i.e. 50 MYA for the ants and 20 MYA for the fungal symbionts, (2) Significant additional change happened in the common ancestor of the leaf-cutting ants and their fungal symbiont and we confirm that the latter is a secondary later acquisition, (3) Signatures of selective change across the final two major evolutionary transitions in fungus farming indicate connections to metabolic pathways and mitochondrial function.

OR391

Multiple putative recent horizontal transfers of a transposon in ants

Chih-Chi Lee, John Wang

Transposable elements (TEs) occupy significant fractions of the genomes of metazoans, including 44.8% in humans and about 1.4 ~ 8.8% in ants. They are selfish elements that can increase their copy number via inserting into new locations in the host genome. TEs can cross species boundary through horizontal transfer of TEs (HTT). HTT is well documented in prokaryotes and is increasingly recognized among eukaryotes. In ants, however, there have been only a few studies examining TEs and the frequency of HTT in social organisms remains unclear. The mechanisms or vectors bridging species boundary are also unknown in social insects. To identify putative HTT events, we are investigating TEs in the red imported fire ant (*Solenopsis invicta*) genome. We found a DNA TE sequence having high identity (97.4%) to a mariner family transposon also found in some *Drosophila* species, possibly suggesting independent HTT events into fruit flies and ants. We used a PCR assay to survey the phylogenetic range of this transposon and found it in many different ant species including *Tapinoma melanocephalum*, *Camponotus albosparsus*, *Camponotus formosensis*, *Pristomyrmex pungens*, *Monomorium floricola*, *Tetramorium* sp., *Crematogaster* sp., and *Solenopsis geminata*. However, this mariner transposon was not detected in all species we tested: 11 of 20 ant species did not have amplification products of this mariner TE. Our data may suggest multiple independent HTT events of this mariner TE into ants. We further tried to determine potential vectors of HTT. Using the same PCR assay, we detected the mariner TE from an ectoparasitic mite of *S. invicta*, raising the possibility that this ectoparasitic mite might be a potential vector for HTT among ants.

OR392

Accelerated evolution in transposable element islands in invasive Cardiocondyla obscurior

Jan Oettler, Lukas Schrader, Jürgen Gadau, Jürgen Heinze

Trait variation between populations is a result of selection, genetic drift or a combination of both. Adaptive evolution is based on selection of alternative phenotypes, implying heritable variation at the genotypic or epigenetic level. Genetic drift on the other hand results in a random distribution of genotypes drawn from a source population. We studied phenotypic differences and compared the genomes of two introduced populations of *C. obscurior* with very low effective population sizes to understand how variation is generated. In comparison to the other sequenced ants several subclasses of transposable elements (TEs) have selectively proliferated in *C. obscurior*. The genome is structured into low-density regions (LDR) and distinct TE islands that comprise 12.7% of the genome and range between 0.19 and 1.46 Mb. TE islands evolve faster than LDRs with regard to SNVs, gene/exon duplications/deletions, and degree of orthology. A non-random distribution of gene families, larvae/adult specific expression of genes located in TE islands and signs of stronger past methylation in TE islands compared to LDRs indicate different degrees of evolutionary rates and effective population sizes within the genome. We argue that TE islands have formed through genetic drift and have subsequently become adaptive because they generate a substantial proportion of the genetic diversity and thus provide additional leverage for selection. Our approach allows studying evolutionary genomic dynamics in response to drift and inbreeding in a species that successfully inhabits novel habitats.

OR393

Analysis of a complete high-density recombination map of Apis mellifera

Katelyn Miller, Olav Rueppell, Corbin Jones, Caitlin Ross

The Western honeybee (*Apis mellifera*) is well known among social insects and other model organisms to have an exceptionally high recombination rate. Although recombination maps have been in existence since the mid 1990s our understanding of the high recombination in honeybees and other social insects is incomplete. A population of 192 brother drones was analyzed by multiplexed Illumina HiSeq 2000 re-sequencing. Single nucleotide polymorphisms (SNPs) were identified by alignment with BWA and variant calling with SAMtools. Informative SNPs will be selected and analyzed to construct a high-density, genome-wide map of recombination events. This map will be examined for putative hotspots and specific recombination events will be correlated with sequence motifs and structural properties of the genome. Our results will be compared to several other recent studies to contribute to our understanding of recombination patterns in *A. mellifera* and the evolution of high recombination rates in general.

OR394

Mapping a novel sex determination gene in ants

Yu-Ching Huang, Mingkwan Nipitwattanaphon, Chih-Chi Lee, Laurent Keller, John Wang

Sex determination is a fundamental biological process that has evolved in many organisms and functions through diverse mechanisms. In haplodiploid insects, such as Hymenoptera (which includes ants and bees), sex is determined by the ploidy of the individual. Males are haploid and develop from unfertilized eggs with one set of chromosomes. Females are diploid and obtain two sets of chromosomes from fertilization. In these species, the mechanism to ascertain ploidy has been proposed to be complementary sex determination (csd): individuals heterozygous at the sex locus develop into females, whereas hemizygous haploid or homozygous diploid individuals develop into males. Studies in the honeybee, *Apis mellifera*, have identified a single locus, csd, as the master sex determination gene. Although ants and honeybees share many putative homologs in the sex determination pathway, evidence for their functional conservation is still lacking in ants. We are using the fire ant *Solenopsis invicta* as a model organism to study sex determination. Our genetic analyses have revealed that the fire ants have evolved a novel master sex determination gene. We have mapped the sex locus to a 175 Kb region of chromosome 3, which contains fourteen predicted genes, none with homology to any known sex genes. A hypervariable region was further identified by genome comparisons among different individuals, which is consistent with balancing selection acting on the sex determination locus. Thus far, we have identified 10 sex alleles from individuals sampled from Taiwan and USA in the invasive range. We are currently conducting gene expression analyses and functional tests to identify and characterize this novel sex determination gene in ants.

OR395

Gene co-citation networks associated with worker sterility in honeybees

Emma Mullen, **Graham Thompson**

The evolution of reproductive self-sacrifice is understood from kin theory, yet our understanding of how actual genes influence the expression of reproductive altruism is only beginning to take shape. As a model in the study of social behaviour, the honeybee *Apis mellifera* has yielded hundreds of genes associated in their expression with differences in reproductive status of females, including genes associated with sterility, yet there has not been an attempt to link these candidates into functional networks that explain how workers regulate sterility in the presence of queen pheromone. In this study we use available microarray data and a co-citation analysis to describe what gene interactions might regulate a worker’s response to ovary suppressing queen pheromone. We reconstructed a total of nine gene networks that vary in size and gene composition, but that are significantly enriched for genes of reproductive function. The networks identify, for the first time, which candidate microarray genes are of functional importance, as evidenced by their degree of connectivity to other genes within each of the inferred networks. Our study identifies single genes of interest related to oogenesis, including eggless, and further implicates pathways related to insulin and dopamine signaling as potentially important to reproductive decision making in honeybees. The networks derived here appear to be variable in gene composition, hub gene identity, and the overall interactions they describe. One interpretation is that workers use different networks to control personal reproduction via ovary activation, perhaps as a function of age or environmental circumstance. Alternatively, the multiple networks inferred here may represent segments of the larger, single network that remains unknown in its entirety. The networks generated here are provisional but do offer a new multi-gene framework for understanding how honeybees regulate personal reproduction within their highly social breeding system.

OR396

Reference transcriptome for a facultatively eusocial bee, Megalopta genalis

Beryl M. Jones, William T. Wcislo, Gene E. Robinson

One remarkable feature of the eusocial insects is their extreme phenotypic plasticity, with reproductive division of labor between morphologically distinct queen and worker castes. This plasticity occurs as a result of environmental influences during development, with the same genotype leading to multiple phenotypes. While much is known regarding the developmental influences underlying queen-worker differentiation in highly eusocial insects, we know little about how reproductive division of labor evolved across the multiple independent origins of eusociality. Here we report a new reference transcriptome for *Megalopta genalis*, a facultatively eusocial neotropical sweat bee (Halictidae) which displays a wide range of social behaviors within a single population. Phylogenetic studies of bees point to a solitary ancestral lifestyle, suggesting that mechanisms underpinning social behavior in *M. genalis* may represent some of the ones important in the evolutionary transitions from solitary to social reproduction that occurred in bee lineages. We present life stage- and sex-specific gene expression across 20 adults and 20 pre-adult individuals of *M. genalis*, using Illumina HiSeq sequencing technology and Trinity for assembly. We describe great variation in gene expression across individuals, and evidence for caste-specific gene expression in the brains and abdomens of adult females despite their behavioral flexibility. The phylogenetic position and facultative sociality of *M. genalis* makes them ideal for studies of eusocial evolution at the molecular level, and the availability of this reference transcriptome allows for further comparative questions regarding social evolution in bees.

OR397

Foraging gene expression patterns in eusocial sweat bees using qRT-PCR

David Awde, Adonis Skandalis, **Miriam Richards**

The foraging gene (for) is associated with variation in foraging behaviour in insects, including honeybees and ants. Variation in foraging behaviour is one of the major hallmarks of caste differentiation in primitively eusocial sweat bees, such as *Lasioglossum laevissimum*. We hypothesize that in *L. laevissimum*, actively foraging individuals have high expression levels of for, while non-foragers have low levels. To investigate this hypothesis, we designed a quantitative RT-PCR (qRT-PCR) study ultimately aimed at comparing expression patterns in queens and workers. A critical step in designing qRT-PCR studies that will generate biologically meaningful expression patterns for target genes is to find a set of appropriate control loci, because relative gene expression is measured in terms of ratios of target to control genes (reported as Delta-Delta-Ct values). It is also important to establish whether gene expression varies among tissues, developmental stages, males and females, etc. As a baseline, we evaluated for expression in newly eclosed males not yet capable of flight, calculating Delta-Delta-Ct values with respect to eight control genes (actin, arginine kinase, EF-1alpha, GAPDH, RpL13, Rpl32, PrP2, and RpS5); for gene expression levels were similar in heads, thoraces, and abdomens. We then analysed spring queens caught on the wing during the first brood provisioning period; for expression levels were highest in thoraces and lowest in abdomens. These preliminary results suggest that increased for expression is indeed associated with flight activity in *L. laevissimum*, so comparisons between queens and workers are likely to reveal caste-specific patterns in for gene expression. Methodologically, analyses based on multiple versus single control genes demonstrated that robust inferences require comparisons based on multiple control genes. We will present further analyses comparing for gene expression levels in queens and workers, specifically, foraging (spring) and nonforaging (summer) queens, foraging and non-foraging workers, and young gynes prior to hibernation.

OR398

Brain-phenotypes and pheromone communication in leaf-cutting ants.

Christoph Kleineidam

Huge colonies with millions of polymorphic workers and fungus cultivation on collected leaf fragments impressively illustrate how evolutionary derived leaf-cutting ants are. We utilize the polymorphism and trait variations of brain structures to study olfaction and chemical communication in *Atta vollenweideri*. Within the worker caste, we described neuroanatomical phenotypes: Large workers that cut leaves and forage have an extremely large glomerulus in the first olfactory neuropil, the antennal lobe (AL). Many olfactory receptor neurons, which are specific and extremely sensitive to the releaser component of the trail pheromone, all converge in this glomerulus. Small workers have glomeruli all of the same size and the smallest workers show miniaturization and different connectivity within the AL. While the trail pheromone components are well known for many species of the genus *Atta*, very little is known about sex pheromones. Collecting odors in the field before and during mating flights allows us to identify chemicals that may play important roles during the multi-step process of aggregation and mating flight of alates. In order to combine pheromone communication, sensory physiology and the molecular basis of it, we analyzed the antennal transcriptome and identified members of different chemosensory related gene families (IRs, GRs, ORs, SNMPs, CSPs, OBPs). Across AL-phenotypes, several genes are differentially expressed: For example, two odorant receptor (OR) and four ionotropic receptor (IR) genes are highly expressed in males, and one OR gene is highly expressed in large workers. While the highly expressed OR genes are good candidates for sex and trail pheromone receptors, the role of the IRs is still elusive. Our aim is to reconstruct the possible evolution of pheromone communication and associated chemosensory related genes, and our preliminary phylogenetic analysis of the candidate pheromone receptor genes indicates common ancestry of these two pheromone systems.

OR399

Queen pheromone modulates stress responsiveness in young adult worker honeybees

Elodie Urlacher, Ingrid Tarr, Alison Mercer

In response to intruders into a honeybee (*Apis mellifera*) colony, guard bees located at the hive entrance sound the alarm by producing pheromones that recruit nest mates and elicit aggressive behaviors in these workers. Recently, alarm pheromone was shown in addition to inhibit appetitive learning in bees of foraging age [Urlacher et al. 2010]; an outcome that parallels effects of stress on learning behavior in vertebrates. As older bees in the colony are more likely to take part in colony defense than young bees, we examined whether the effect of alarm pheromone on learning was also age-dependent. Alarm pheromone had no significant effect on the appetitive learning behavior of young (< 1 week old) worker bees from queen-right colonies. Nor did it inhibit learning in young bees maintained from the time of adult emergence in cages supplied with synthetic queen mandibular pheromone (QMP). However, young bees maintained in cages without exposure to QMP showed impaired learning in response to alarm pheromone. Our results show that both age and exposure to QMP are important factors modulating the bee's responsiveness to stressful stimuli. Young bees are more likely to come into contact with the queen and they are exposed to higher levels of her pheromones than are older bees. Blocking the responsiveness of young bees to alarm pheromone might help to ensure that the queen has constant care, even when bees are recruited to defend the colony. Urlacher E, Francés B, Giurfa M and Devaud JM. An alarm pheromone modulates appetitive olfactory learning in the honeybee (*Apis mellifera*). Frontiers in Behavioral Neuroscience 2010 ; 4

OR400

Revisit me: Flower colour evolution and bee discrimination

Zoe Bukovac, Mani Shrestha, Alan Dorin, Martin Burd, Adrian Dyer

Flower colour is valuable information to pollinators, like trichromatic bees, as it is an effective signal allowing a pollinator to distinguish profitable from unprofitable flowers. However, understanding the evolution of flower colouration in response to pollinator selection pressure is confounded by animal species (e.g. bees, butterflies, birds) having different visual capabilities. To compare different flower species' spectral signatures we have previously used spectral reflectance marker point frequencies as keys to interpreting the perceptual responses of different model pollinators (Shrestha et al. 2014). A marker point is a sudden change in reflectance likely to differentially modulate photoreceptors in a colour opponent process, and can be directly compared to delta-lambda/lambda wavelength discrimination data. Here we investigate the application of marker point analysis for comparing discrimination of similar flower colours by insect pollinators. We plot spectral loci of a dataset of Australian flowers in a Hexagon colour space, and correlate the spectral location and frequencies of respective marker points. All of our flower spectra can be conveniently classified by one, two or three marker points. Analyses considering individual flowers show an asymmetric distribution such that flowers with a single marker are most frequently confused with others having a single marker; whilst flowers with three marker points are more frequently confused with other flowers also having three. We also consider the phylogenetic structure of marker point patterns among the species in our data set to reveal how evolutionary history may affect adaptation to different pollinators. Shrestha et al. (2014) J Ecol 102, 126-13

OR401

Comparing aversive and appetitive learning performances in individual honeybees

Pierre Junca, Lionel Garnery, Jean-Christophe Sandoz

Animals learn to associate initially neutral sensory stimuli (color, odor, etc.) with positive (food) or negative events (danger), based on appetitive and aversive learning respectively. The honeybee (*Apis mellifera*) is an influential invertebrate model for studying both types of olfactory learning. Appetitive learning is studied using the protocol for conditioning the proboscis extension response (PER), in which bees learn to associate an odor (conditioned stimulus, CS) with a sucrose reward (unconditioned stimulus, US). Aversive learning is studied using the sting extension response (SER) conditioning, in which an odor CS is associated with an aversive US, in our case a thermal stimulation to the mouthparts. We investigated the relationships between appetitive and aversive learning capacity at the individual level, and the potential influence of bee genotype on both learning types. As learning performance was shown to depend on individuals sensitivity to the US, we measured four different traits systematically in each individual bee: sensitivity to sucrose, PER learning with sucrose US, sensitivity to temperature, SER learning with temperature US. First, we confirmed for both types of learning that learning performance correlates with US responsiveness. Second, we found a trend for a possible trade-off between appetitive and aversive learning performances: bees that were better appetitive learners (and had a lower sucrose US threshold) tended to learn less efficiently (and to show a higher temperature US threshold). This result suggests a potential differential specialization of workers within the hive. We then investigated the influence of genotype on bee's performances. A queen bee usually mates with 15-20 drones, so that within the hive, her worker offspring belong to as many different patrilines. Using microsatellite analysis, we identified the patriline origin of all tested workers. Analyses are still under way but should tell whether aversive and appetitive learning performances are under a genetic influence.

OR402

Larval learning affects adult nest-mate recognition in the ant Aphaenogaster senilis

Lisa Signorotti, Pierre Jaisson, **Patrizia d'Ettorre**

Prenatal olfactory learning has been demonstrated in a wide variety of animals, where it affects development and behaviour. Young ants learn the chemical signature of their colony. This cue-learning process allows the formation of a template used for nest-mate recognition in order to distinguish alien individuals from nest-mates, thus ensuring that cooperation is directed towards group members and aliens are kept outside the colony. To date, no study has investigated the possible effect of cue learning during early developmental stages on adult nest-mate recognition. Here, we show that odour familiarization during pre-imaginal life affects recognition abilities of adult *Aphaenogaster senilis* ants, particularly when the learning process occurs during the first larval stages. Ants eclosed from larvae exposed to the odour of an adoptive colony showed reduced aggression towards familiar, adoptive individuals belonging to this colony compared with alien individuals (true unfamiliar), but they remained non-aggressive towards adult individuals of their natal colony. Moreover, we found that the chemical similarity between the colony of origin and the adoptive colony does not influence the degree of aggression, meaning that the observed effect is likely to be due only to pre-imaginal learning experience. These results help understanding the cognitive processes underlying efficient recognition systems and point to the neglected importance of pre-imaginal learning in social insects.

OR403

A computational modelling approach to understanding honeybee vision and cognition

Alexander Cope, Chelsea Sabo, Esin Yavuz, Eleni Vasiliki, Kevin Gurney, Thomas Nowotny, James Marshall

Apis mellifera, the Western honeybee, has a brain consisting of one million neurons, yet is capable of a range of sophisticated social and individual behaviours. These include communication using the 'waggle dance' and choice behaviour based on 'sameness' and 'difference'. This makes the honeybee an important resource for understanding the neural basis of such sophisticated behaviours. Computational modelling is a valuable tool for understanding neural structure and function, and we can use it to provide constraints on neural mechanisms given experimental data, as well as hypotheses about possible anatomy and function where data are sparse. The honeybee presents a challenge to computational modelling, as while some parts of the brain are well characterised (e.g. the olfactory system), others are poorly understood (e.g. the visual system). There are a wealth of behavioural and ecological experimental data, however, for many aspects of honeybee behaviour. To address the sparseness of the anatomical and physiological information it is important that we add other constraints to the model, and these must come from the behavioural and ecological experimental literature. To connect to this literature we are required to model complete sensori-motor loops, where the model takes sensory stimulation, processes it, and generates motor output. For this approach we must model large parts of the honeybee brain, then compare the hypotheses generated against behavioural experiments or ecological observation. In this talk we present recent progress in graphical modelling of neural systems, along with the use of simplified neuron models and parallel graphics processing technology, which allow us to easily develop, visualise, and rapidly simulate large models. These tools allow us to manage large sensori-motor loop models of the honeybee visual system and connect these models to both software and real world visual environments, allowing us to reproduce behavioural and ecological experimental paradigms.

OR404

Ant navigation under constraints of size and photons

Ajay Narendra, Fiorella Ramirez Esquivel, Chloe Raderschall, Jochen Zeil

Visually guided navigation is crucial for central place foraging insects, an ability that allows them to exploit the resources in their habitat and to reliably return home. The size of the sensory structures and the available light limits the quality of navigational information that is available to animals. Ants, with their dramatic size range coupled with the variety of temporal niches they occupy, provide a unique opportunity to identify the behavioural and sensory adaptations for efficient visual navigation under such constraints. We show in a comparative study across ants from different subfamilies how eye structures scale with body size and with time of activity. More so, by tracking individual ants at different ambient light levels, we demonstrate that navigating at low light is costly, because foragers have to move more slowly, stop more frequently and are less accurate in pinpointing their nest. Using panoramic imagers we quantify the navigational information content of ant habitats and show how much information is available to animals that work at the limits of size and light and discuss the implications for successful visually guided navigation.

OR405

Desert ants' use of green-ultraviolet contrast for directional information

Ken Cheng, Patrick Schultheiss, Sebastian Schwarz, Antoine Wystrach, Jonathan Ives

Ants are known to use the terrestrial panorama as a source of directional information. The skyline - where the tops of terrestrial objects meet the sky - is a major cue in this terrestrial visual compass. Theoretically, a good way for an insect's brain to define the skyline is to use the maximal green-ultraviolet contrast as the boundary between the sky and ground objects. The sky contains relatively more ultraviolet wavelengths, while ground objects reflect relatively more green (longer) wavelengths. A brain using a ratio of different wavelengths gains constancy in the face of changing light levels. We began to test this hypothesis by using a transparent plastic that blocked most of the ultraviolet wavelengths of light. Central Australian desert ants *Melophorus bagoti* were trained in their natural habitat to visit a feeder provisioning food. The feeder was located either in the natural terrain, or else inside a uniformly green-coloured circular arena with a skyline created by distinctive variations in the height of the arena's wall. On tests, an ant was allowed to run home with food from the feeder, but captured just before she entered the nest, and brought back to the feeder location for a test. Such zero-vector ants are forced to rely on the terrestrial panorama for homing. On crucial tests in the natural panorama or arena, the ultraviolet-blocking plastic of uniform height stood between the homing ant and the panorama. This manipulation reduced homing accuracy in the natural panorama, and abolished significant orientation in the artificial arena. We conclude that green-ultraviolet contrast is a major component in the ant's use of the terrestrial panorama.

OR406

Investigating neural mechanisms underlying division of labor in Temnothorax *ants.*

Nicole Fischer, Wulfila Gronenberg, Anna Dornhaus

In social insects, behavioral variation among nestmates is a fundamental feature of division of labor. However, the extent to which worker behavioral variation is regulated by peripheral versus central nervous processes remains poorly understood. Using a response threshold framework, we tested the hypothesis that behavioral variation arises from differences in the peripheral sense organs. We found considerable variation in the number of antennal sensory hairs (sensilla) among individuals in *Temnothorax rugatulus* ants. Yet, variation was not observed in other peripheral sensory structures, such as the ommatidia of the compound eye, suggesting that sensilla variation is not random, but rather has a functional consequence for sensory thresholds. However, we found no correlation between sensilla number and likelihood of task performance or overall activity level in the colony, suggesting that response thresholds do not arise from the peripheral nervous system. As such, the central nervous system may play a more important role in establishing response thresholds, implying that thresholds are potentially flexible, capable of modulation via neuroplasticity. In addition, using a controlled behavioral assay we found that workers that were mostly inactive within a colony context were significantly more responsive to 2-heptanone, an alarm pheromone component, than highly active workers. This suggests the possibility that these inactive workers may serve as a defensive reserve, only becoming active in response to threat. Our results raise questions about the functional consequence of variable sensory organ number in the periphery and suggest a potential role for higher cognitive processes of the central nervous system in creating response thresholds and thus behavioral variation among nestmates.

OR407

Neuroanatomical correlations to social organization and foraging habits in bees

Christina Kelber, Thomas Schmitt, Wolfgang Roessler

Among Hymenoptera, many ecologically successful species evolved a eusocial lifestyle. Several studies emphasize that eusocial honeybees and ants possess a complex brain including a high number of functional units (glomeruli) in the antennal lobe and large mushroom body calyces. Brain complexity may have evolved as the result of eusociality or in response to other selective pressures and therefore be a precondition for the evolution of eusociality. We analyzed the antennal lobe structure in selected wild-bee species and correlated the results with both social lifestyle and foraging habits. We investigated halictid bee species (Halictidae) with different grades of sociality, but polylectic foraging habit and different solitary polylectic and oligolectic bee genera (*Osmia*, *Hylaeus* and others). In halictid bees, we found no significant differences between solitary and eusocial species in terms of number of glomeruli within the same genus. In all investigated solitary bee genera, we found a large interspecies variance in glomerular numbers, correlated to floral preference. Specialized (oligolectic) species showed a significantly lower glomerular number than bee species which forage at a broad range of flower species. Additionally, we also investigated other neuroanatomical traits like main neuropile volume and synaptic density in higher integration centers. Our results give first hints that a complex olfactory system is not the result of eusocial lifestyle in Hymenoptera. Furthermore, the results indicate that the floral preference seems to have a larger impact on the complexity of the antennal lobe structure than social lifestyle. Chemical analyzes of host-plant odors and behavioral tests will be next steps to understand the large impact of floral preference on the complexity of the olfactory system in bees. Funding: DFG KE-1701 1/1.

OR408

Cognitive differences across sex and caste in Polistes *paper wasps*

Elizabeth Tibbetts, Allison Injaian, Michael Sheehan

An animal's cognitive abilities are thought to be shaped by the biotic and abiotic environment it experiences. Social insects provide good models for studying the causes and consequences of variation in cognition given the substantial behavioral and developmental differences that occur across castes and sexes. Here, we compare associative visual learning in foundresses, workers, gynes, and males of the paper wasp *Polistes fuscatus. Polistes* are a primitively eusocial group that lacks discrete pre-imaginal castes, though there are behavioral differences across castes. *P. fuscatus* are a particularly interesting group for studying variation in visual learning, as foundresses and workers use facial patterns for individual recognition. Foundresses are also specialized for face learning, as they learn conspecific faces faster and more accurately than other visual stimuli. We found that associative learning varied significantly across caste and sex. In particular, males learn visual images less quickly and accurately than females. Workers learned all types of images less quickly and accurately than foundresses. Workers also lacked specialization for face learning, as they learned faces and non-face images similarly. Cognitive differences across sexes and castes may reflect different selective pressures, particularly differences in social complexity between foundresses, workers, and males.

OR409

The active role of confidence in ant colonies

Ofer Feinerman, Amos Korman, Efrat Greenwald

Animals that live in groups sense their surroundings by direct environmental cues and indirect social interactions. Social information has the capacity to improve both the sensitivity ('many eyes principle') and the accuracy ('many wrongs principle') of the acquired information and lead to increased adaptivity. These advantages become even more pronounced in the case of eusocial insects where lack of conflict can be expected to lead to an increased motivation for information sharing. Interaction rates within a dense insect society can be huge. Although the information conveyed in such interactions is advantageous, its sheer amounts could lead to excessive cognitive loads. We are interested in identifying communication schemes that balance the advantages of sharing large amounts of information with the required conciseness of both memory and messaging. We approach this issue from two very different perspectives: Theoretically, we borrow techniques from the field of distributed computing to rigorously compare between the performances of different communication strategies employed by interacting agents. Experimentally, we track ants that share information regarding their environment during recruitment to a food source and collective load transport. We find that these two, very different, perspectives point to a similar direction: efficient collective performance can be achieved despite huge compression of memory and communication. This is accomplished by individuals that remember and communicate their opinion and a related confidence measure. We therefore suggest that for strongly cooperative groups, confidence expands its classical definition as a passive, internal state: ants actively share their confidence to enhance group performance.

Poster Presentations

P001

The demography of worker and male size in bumblebee colonies

Heng-Yi Lin, **Matthias, A. Fuerst**, Mark, J.F. Brown

Worker polymorphism in highly advanced social insect societies can take very different shapes. In some ant societies, size polymorphisms are strongly correlated with the tasks performed by the respective workers. This demography may change across the development of colonies. For example, during the very early stages of colony foundation, when a single queen or multiple queens together produce the first offspring without the aid of workers, much smaller workers, nanitics, are produced than in later colony stages. Much less is known about size demography in social insects other than ants. In bumblebees, queens found new colonies each spring completely on their own, and therefore we might expect worker size demography to change similarly over the development of a bumblebee colony. We studied two closely related bumble species, *Bombus terrestris* and *Bombus lucorum*, over the course of the colony cycle. Interestingly we found marked differences between the two species. *Bombus terrestris* workers increased in size over time, whilst the size of *Bombus lucorum* workers decreased over the lifespan of a colony. We also, for the first time, examined male size demography. In *Bombus terrestris* we found no tradeoff in male production, however in *Bombus lucorum* the number of males produced was traded off against male size; the more they produce, the smaller they get. We discuss these results in the light of the different life histories of the two bumblebee species.

P002

Role of queen promiscuity in reproductive swarming by honeybees

Heather Mattila, **Madeline Girard,** Thomas Seeley

Whenever hymenopteran queens mate multiply and create work forces with multiple patrilines of workers, the benefits of unusually high levels of genetic diversity for division of labor and colony efficiency have been of interest to researchers. Evidence shows that multiple-patriline honeybee colonies (the derived state for *Apis*) have a more responsive foraging effort, maintain more stable nest conditions, and better resist disease than colonies with only a single patriline of workers (the ancestral state for Hymenoptera). These enhanced colony-level traits are important but indirect proxies of probable increased fitness. We examined the role that genetic diversity plays in the swarming process, a colony-level phenomenon that is linked directly to reproductive success for *Apis*. We created honeybee (*A. mellifera*) swarms that were composed either of multiple or single patrilines of workers, then we monitored swarms in pairs as they selected new nest sites. Across nine pairs of such swarms, we measured the number of dances that were performed by house-hunting workers as they examined potential nest sites (per capita and per swarm), the number of sites that were reported by workers in each swarm, and the time that it took for swarms to select and depart for a new home. Although workers in honeybee colonies with multiple patrilines did not report more potential nest sites or produce more dances throughout the decision-making process, they did dance at higher rates than workers in colonies with only a single patriline, which resulted in swifter decisions and earlier liftoffs on average. Thus, multiple-patriline swarms reduced the time they spent in a vulnerable 'homeless' state without sacrificing the amount of information that they used to choose a new home. We provide insight into some of the ways that queen promiscuity and patriline diversity directly benefit reproduction in a social insect.

P003

Attachment of Laboulbeniales fungi *on their ant hosts: ultrastructural study*

András Tartally, Simon Tragust, Xavier Espadaler, Johan Billen

Laboulbeniales (Ascomycetes) are ectoparasitic fungi growing on the cuticle of arthropod hosts. Among social insects only termites and ants are known to harbour some species of Laboulbeniales. Although the existence of Laboulbeniales on ants has been known for more than a century, the interactions with their hosts, especially their mode of attachment to the cuticle and their mode of gaining nutrients from their hosts remain understudied. In Europe four of the only six ant-parasitizing Laboulbeniales species have been reported. Aim of the present study is to morphologically compare these four fungi, namely *Laboulbenia camponoti* on its *Camponotus sylvaticus* host ant, *Laboulbenia formicarum* on *Lasius neglectus*, *Rickia wasmannii* on *Myrmica scabrinodis* and *Rickia n.sp.* on *Messor wasmannii*. With the use of Scanning and Transmission Electron Microscopy we elucidate similarities and differences in the attachment of these fungi to their ant hosts. Observations so far have not given any indication of fungus penetration into the host tissues. Insights gained from this comparative approach will provide a better understanding of Laboulbeniales-host relationships in ants.

P004

The influence of diet in microbiota acquisition of Apis mellifera

Pedro A P Rodrigues, Patrick Maes, Brendon M Mott, Kirk E Anderson

In the last decade, High throughput ‘next generation’ sequencing technology has become increasingly popular in studies involving bacterial symbionts of insects. In the honeybee *Apis mellifera* for instance, microbes found in their guts were shown to belong to groups able to produce antibiotics, participate in nitrogen recycling and improve digestibility of pollen. While descriptive work on the identity of gut bacteria has been abundant, we still know very little about the costs and benefits of harboring these microbes, their mechanisms of inheritance and their effects in the health of the hive, both in the individual and colony levels. The urgency in clarifying these areas has increased since the diet and management of honeybees have been implied to be involved in causing Colony Collapse Disorder. We hypothesize that the honeybee microbiota is changed when the colony is fed food supplements, which can be especially detrimental for newly emerged bees. On the other hand, both honey and bee bread contain the core microbiota found in adult workers. We tested this hypothesis by feeding colonies with either food supplement or a natural diet (bee bread and honey). At the same time, we attempted to clarify the route of bacterial transmission by manipulating the presence and absence of nurse bees. In this talk we present the results of this experiment, with an emphasis on the role of diet on the colonization of the digestive tract of newly emerged bees.

P005

Coverage of Rickia wasmannii *(Ascomycota: Laboulbeniales) on* Myrmica scabrinodis *(Formicidae)*

Bence Tánczos, László Tálas, Gábor Nagy, **András Tartally**

In our research we are investigating the interactions between *Myrmica scabrinordis* (Hymenoptera: Formicidae) and its ectoparasitic fungus, *Rickia wasmannii* (Ascomycota: Laboulbeniales). Previous studies suggest that interactions between insects and Laboulbeniales fungi are mainly parasitism, but in the terms of *Myrmica* spp., results remain controversial. According to recent results, interaction can negatively affect the fitness of the ants, but details of the mechanism remain unclear. In our current research we are using scanning electron microscope techniques to reveal the morphological and structural details of this fungus. Based on the analysis on 20 specimens of one colony collected from NE-Hungary, the following morphological and fungus distributional properties were revealed. i. Severely infected ants are trying to get rid of the bodies of the fungus on their antennae while other anatomically inaccessible regions remain highly infected. ii. Low percentage of broken fungus filaments on the ants suggests that they do not clean each other from fungi. iii. 90 percent of the ants examined were severely affected by the fungal infection on the ocular region. iv. Scanning electron microscopy revealed that the fungus penetrated the cuticula from the internal direction in the eyes. v. Post-petiolar region of the ants examined often holds a high percentage of broken fungal bodies, possibly because of the movement of the abdomen.

P006

Rickia wasmannii *(Laboulbeniales) influences the water consumption of* Myrmica scabrinodis

Eniko Csata, Ferenc Bathori, **András Tartally**

Ant colonies are highly rewarding targets for parasites. Several fungi exploit ants, most of them are from the tropics, but some fungi are known from Europe. *Rickia wasmannii* (Ascomycetes: Laboulbeniales) is an ectoparasitic fungus living on different species of the ant genus *Myrmica* in Europe. In the Carpathian Basin (Central Europe), the most frequent host species is *Myrmica scabrinodis*. The thallus consists of a multiseriate receptacle which is only one layer of cells thick. The fungal thalli of the fungus penetrate the outer layer of the cuticle of ants, which could increase evaporation. Little is known about the effect of *R. wasmannii* on their hosts. We know that the infected ants die faster than the uninfected ones. In our research the water consumption of infected (240) and uninfected (240) *M. scabrinodis* workers were tested under laboratory conditions. The time one individual spent with water consumption after 12 hours thirsting was measured in 24 ant nests, collected from two regions. The analysis showed that infected ants spent significantly more time with consuming water than the uninfected ones which suggests that the ants have to replace the loss of water. These results support the possibility that the fungus absorbs water from the tissue, or it can cause increased evaporation through penetration of the cuticle with the haustoria. We found therefore new evidence that the fungus has a negative effect on the host ant. Supported by: the scholarship of Collegium Talentum; a ‘Bolyai János' scholarship (MTA); the ‘AntLab’ Marie Curie CIG and TÁMOP-4.2.4.A/2-11/1-2012-0001 National Excellence Program.

P007

Antifungal effect of silver nanoparticles on Rickia wasmannii *infected ants*

László Tálas, Bence Tánczos, **András Tartally**, Gábor Nagy

The aim of our research was to examine the possible antifungal effects of silver nanoparticles against *Rickia wasmannii* (Laboulbeniales, Ascomycetes) infection on *Myrmica scabrinordis*. Laboulbeniales is a widely spread and diverse fungi class, with more than 2000 (sub)species, there are some agriculturally important insects amongst the possible infected host. According to our recent research this fungus negatively affects the survivability and overall fitness of infected host ants. Heavily infected *M. scabrinordis* workers were separated from each other in special containers to avoid the possible cross-contamination and the experiments were carried out under the same environmental conditions. The measurements of silver nanoparticles effects and image acquisition were made in the same time of the day for 7 days. During the experiments infected ants were treated with different concentrations of silver nanoparticle solution in aerosol form. In our pretrial we found that the 20 ppm concentration of the silver nanoparticles is ineffective against the fungus, because it has created a gelatinous coating around the ant, and also caused a bluish coloration in the cuticle (argiria). In conclusion we decreased the concentration of the silver nanoparticles, and used multiple treating solutions in 0.1 ppm; 0.5 ppm; 1 ppm; 5 ppm; 10 ppm; 20 ppm concentrations. Our results show that high concentrations (10-20 ppm) cause argiria and death of the ant. The lowest concentrations (0.1-1 ppm) have no visible effect on the ants or fungi. The ideal concentrations (1-5 ppm) caused quantitative changes in the infection rate, as lowered the number of fungus hyphae in the infected regions against control. In the future we would like to develop a protocol to clean the Laboulbeniales caused fungal infection from the agriculturally important insects. Furthermore we are planning the examination of cleaned ant behavioral properties and reinfection by releasing it back into an infected colony.

P008

Behavioural effect of Rickia wasmannii *(Laboulbeniales) on* Myrmica scabrinodis *workers*

Ferenc Bathori, Eniko Csata, **Andras Tártally**

The interactions of parasitic Laboulbeniales (Ascomycetes) fungi and their hosts are understudied. *Rickia wasmannii* is the commonest ant parasitic Laboulbeniales species in Europe. Workers of *Myrmica scabrinodis* (Hymenoptera: Formicidae), the commonest host in Hungary, are heavily infected with this fungus and can be easily collected in high numbers. *Rickia wasmannii* is therefore a quite available model species of Laboulbeniales to study the effect of such fungi on their hosts. Thalli of this fungus can form a strong surface on the ant body and we supposed this structural cuticular change could influence the behaviour of the infected ants. The behaviour of infected and uninfected *M. scabrinodis* workers were studied individually under lab conditions, by using hundreds of infected and uninfected specimens in two different experiments. The time of leaving a dark tube was measured in the bravery test and the number of aggressive behaviour patterns was registered in the one-to-one aggression test. Based on our results the infected individuals were significantly less aggressive and brave than the uninfected ones. According to these, we can conclude that *R. wasmannii* has an effect on the behaviour of *M. scabrinodis*. Reduced aggression and braveness suggest this effect to be negative as these can cause disadvantage against the competitors of the host ants. Further research would be necessary to explore the level of this negative effect and it also would be important to do similar research with other Laboulbeniales species. Supported by: the scholarship of Collegium Talentum; aa ‘Bolyai János' scholarship (MTA); the ‘AntLab’ Marie Curie CIG.

P009

Withdrawn resources: Rickia wasmannii *shortens the lifespan of* Myrmica scabrinodis

Ferenc Bathori, Eniko Csata, **András Tartally**

Rickia wasmannii is a common ectoparasitic fungus occurring in Europe and is currently known as a parasite of at least eight *Myrmica* species. Laboulbeniales is a poorly researched taxonomic group in Europe and effects of species within this group on their hosts are rather unknown. *Rickia wasmannii* can be a good model organism, compared with the hosts of other European Laboulbeniales species, of this question as this Laboulbeniales species covers the host in very high density and infected host individuals can be easily collected in high numbers. The effect of *Rickia wasmanni* on the survival rate of its most common host species, *Myrmica scabrinodis*, was investigated in a laboratory experiment at the individual level. To enhance the results, environmental stresses were simulated by depriving water and food from infected and uninfected workers. The survival of individuals was recorded every hour until the death of the last individual. Infected specimens were significantly more sensitive to the withdrawal of resources than the uninfected ones. Based on these results, R. wasmannii substantially decreases the survival chances of infected individuals, at least in resource limited environments, which proves that R. wasmannii has a negative effect on its host. Supported by: the scholarship of Collegium Talentum a 'Bolyai János' scholarship (MTA); the 'AntLab' Marie Curie CIG.

P010

Shifts from intracellular to extracellular symbiosis in attine gut symbionts

Mariya Zhukova, Elena Kiseleva, Panagiotis Sapountzis, Morten Schiott, Jacobus J. Boomsma

Metagenome sequencing has revolutionized our understanding of symbiotic bacteria of ants and other social insects, but almost nothing is known about the interaction mechanisms with host cells and the interaction dynamics during ontogeny. Recent work has revealed that both *Wolbachia* and specific Entomoplasmatales are obligate symbionts of *Acromyrmex* leaf-cutting ants, associated with the cytoplasm but also free-living in the gut lumen. We used confocal and electron microscopy to analyze the spatial localization and detailed ultrastructure of these bacteria in *Acromyrmex echinatior* and discovered that both symbionts are present in many tissues of adult workers (brain, muscles, digestive system, fat body), except for the midgut. Both *Wolbachia* and the Entomoplasmatales species change their ultrastructural organization when they shift between an intracellular and extracellular lifestyle. Bacteria with dense cytoplasm and without an outer membrane of host origin were observed in the intestinal lumen of workers and they were also found by confocal microscopy in several tissues of larvae. As the epithelium of *Drosophila* larval guts is known to completely degenerate and be replaced by imaginal cells during the prepupal stage, we expect that pupal metamorphosis is a critical transition stage towards extracellular colonization of the intestinal lumen of workers. We assume that further advanced electron microscopy will allow us to gain better understanding of the mechanisms that allow these transitions in bacterial life-style. Both *Wolbachia* and Entomoplasmatales are likely to be mutualists of leaf-cutting ants, so that electron microscopy may also offer further insight in the adaptive function of these bacteria and in possible constraints in expressing these functions. For example, the presence of a thick (ca. 1 µm) cuticle layer at the luminal side of the hindgut epithelium suggests that the bacterial shift from an intracellular to an extracellular niche may be inhibited in some parts of the digestive system.

P011

Is there a coordination cost to cooperative transport?

Aurelie Buffin, Takao Sasaki, Stephen Pratt

The coordination of social insects, despite their relatively simple cognitive capacities and decentralized organization, has drawn much attention from biologists, as well as from engineers seeking design inspiration for collective robotics. Group work can lead to synergy, or social facilitation, but several studies instead show that groups perform worse than expected from a simple sum of their members' abilities. Cooperative transport in ants is an excellent system to investigate how the performance of the group is influenced by its size. We investigated this in the desert ant *Aphaenogaster cockerelli*, which is particularly adept at group retrieval of large food items. These ants normally encircle objects to carry them, making it difficult to identify the specific contribution of each transporter. To simplify the process, we presented ants with artificial loads designed to make all ants pull in a similar orientation. We examined teams of two, three, and four ants, with loads designed so that all individuals pulled equal weight (i.e., load weight was proportional to team size). The results showed a social facilitation effect in retaining individuals in a team, with ants less likely to cease pulling as team size increased. Individual ants transported the dummy faster than ants working in teams but all teams travelled at the same speed. These results imply that, when working in teams, individuals either spent energy to coordinate with teammates or reduced their effort. We discuss these results in relation to earlier findings of enhanced efficiency through teamwork in collective transport by army ants.

P012

The ant GMap project: Turning outreach into basic research

Donald H. Feener Jr., Pat Lambrose, Robert Rooley, Adrian T. Feener, Katie Luecke, James Ehleringer

GMap was initiated in 2010 as an outreach project to train Utah K-12 teachers in GIS technology that they could then use in their classrooms. In a series of annual workshops, Utah teachers used GPS devices to record colony locations of two co-occurring species of seed-harvesting ants, *Pogonomyrmex occidentalis* and *Pogonomyrmex rugosus* at two localities in southeastern Utah, the Rio Mesa Center (RMC) and the Canyonlands Research Center (CRC). Teachers then uploaded their data to ArcGIS and produced visual maps of the colony locations. At RMC locations of 279 P. occidentalis colonies and 384 P. rugosus colonies have been mapped in a ~27 ha plot. At CRC locations of 278 *P. occidentalis* colonies and 123 *P. rugosus* colonies have been mapped in an 18 ha plot. Data and maps provided by teachers were used to analyze spatial point patterns for evidence of intraspecific and interspecific competition and microhabitat sorting among colonies of the two ant species. Maps were also used to selectively sample workers from geo-referenced colonies for dietary analysis using stable isotope analysis. We were particularly interested in whether species identity or spatial location was the primary determinant of colony-level diets as reflected in carbon and nitrogen isotope ratios. Future studies will look at the genetic structure of these populations. K-12 teachers will continue to follow mapped colonies through time to obtain data on the demography of these populations. This study represents an unusual instance in which a project designed primarily as outreach has led to a new avenue of basic research in a university laboratory. We encourage other laboratories take advantage of such synergistic opportunities.

P013

The effects of rearing environment on physiological development of Polistes *wasps*

Jennifer Jandt, Amy Toth

Artificial rearing environments are designed to mimic natural environments while conditions remain controlled and constant. A benefit to artificial environments is that the effects of isolated stimuli can be tested under controlled conditions. The disadvantage is that studies performed under controlled conditions leave unknown how those same stimuli would affect the organism under fluctuating, natural conditions. Here we compared the physiological development of social wasp larvae (*Polistes fuscatus*) reared in an artificial environment with a constant prey and sugar feeding regime, with those reared in a natural environment, where weather conditions naturally affected prey and nectar abundance. We focused on brood that had been monitored from egg to pupa construction after the colony had been established in either the artificial or natural environment. We collected half of the focal brood when they reached the ‘pre-pupal’ stage (5th instar with a recently constructed silken cocoon), and quantified their %lipid content. The other half were allowed to pupate, and we monitored their behavior as adults for 10-days. We found that those larvae reared under lab conditions were significantly fattier (had higher %lipid) than those reared in the field. The adults reared in the lab were found more often sitting off the nest on a cube of sugar, whereas those reared in the field were found sitting on the nest, resting. In social insects, we would describe the phenotype of those individuals collected from the field as more ‘worker-like’, and those collected from the lab as more ‘queen-like’ even though both groups were collected at the time of year when they all should have been developing into new queens. These results may have important implications on how data, collected from individuals reared in lab environments and compared to those reared in field environments, are interpreted.

P014

*Pollen nutrition on honeybee (*Apis mellifera *L.) health*

Bruna Frias, Juliana Veloso, **Anete Lourenco**

The environmental nutrients available to honeybees are essential to their development and survival. Pollen is the main source of protein for honeybees, and its quality and digestibility are important factors for bee health. We have compared the effect of fourteen different mixed pollen diets on the development and survival of caged adults and larvae. In the adult queenless workers, mixtures containing Asteraceae as the main pollen type provoked more mortality, maybe due to its morphology that causes low digestibility. On the other hand, bees fed diet with Myrtaceae and Moraceae as the most abundant pollen type showed higher survival rates, suggesting that these pollen types offer better digestibility and nutritional assimilation to the bees. Ovary activation was also investigated and all pollen diets promoted ovary activation in the queenless workers. Although bees fed on diets with higher protein content accumulated Vitelogenin and Hexamerin at higher levels, they did not show higher rates of ovary activation, suggesting that ovary activation may depend on other nutrients present on the pollen such as lipids and carbohydrates. Larvae reared in vitro on distinct dietary regimes did not show clear changes in their survival or development as observed in adults. Nonetheless, more mortality and delay in development were observed in larvae fed diet with Asteraceae as the main pollen type. On the other hand, Cyperaceae pollen type was the most appropriate for larval development. Our results support the idea that quality and morphology of the pollen grains may be detrimental to bee health. Financial Support: FAPEMIG, CNPq

P015

Begging signals in ant larvae

Matilde Sauvaget, Aurelie Guion, Fabrice Savarit, Dominique Fresneau, Renee Feneron

Animals sometimes use more than one signal to convey a message, although the multiple displays are supposed to be costly for the signaler. Theories on multiple signaling predict a possible modification or redundancy of the initial message [1], for instance to add new information, to reinforce the message or to reach different receivers. Offspring begging for parental care is widely spread in subsocial and social insects. Larval begging has been described in some wasps [2], bees [3,4] and ants [5]. For each studied species, researches have only focused on one modality of communication, such as acoustic, chemical or behavioral communication. In the ant *Ectatomma tuberculatum* (Ectatomminae), larvae may rely on odors and behaviors through movements, to modulate both larval care and foraging activities. So larvae in this species would use multiple signals to communicate their needs. Thus, we study if both larval signals are related to the hunger state of the larvae and if they induce feeding from the workers. The role of those multiple signals will be discussed. [1] Bro-Jørgensen, J. (2010). Trends in Ecology & Evolution 25 (5). [2] Ishay, J., Landau, E. M. (1972). Nature 237 (5353). [3] Boer, S. P. A. den, Duchateau, M. J. H. M. (2006). Insectes Sociaux 53 (3). [4] Huang, Z.-Y., et Otis, G. W. (1991). Journal of Insect Behavior 4 (2). [5] Kaptein, N., Billen, J., Gobin, B. (2005). Animal Behaviour 69 (2).

P016

The levels of (accumulated) pesticides detected within honeybee comb wax

Kris Wisniewski, William Kirk, **Falko Drijfhout**

Pesticide contamination of hives can originate from a range of agricultural sources, i.e. contaminated pollen from crop management (foliar spray or seed dressings) or from treatments of honeybee pests. Pesticides are also easily spread through the hive and can transfer to and from comb wax, leading to potential exposure of developing brood and honey stores which can result in increased brood mortality. Comb wax is often considered a ‘chemical sink’ and therefore a good indicator of pesticide exposure. Pesticides are very stable and soluble in wax and as a result they can remain within the comb for over 5 years. QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) is often employed as a suitable pesticide extraction method and has been applied to multiple matrices, including beeswax. In this study we adopted a modified QuEChERS technique, followed by analysis (using liquid chromatography-mass spectrometry) of 150 honeybee comb wax samples to identify any pesticides present. We also designed an experiment to monitor the potential accumulation of pesticides over a two year period by providing beekeepers with clean foundation wax. This presentation highlights the levels of pesticides detected within samples of UK honeybee comb wax, as well as those accumulated over a two year period. Potential risk to bees with regards to the levels of pesticides will be discussed.

P017

Bee diversity in non-dependent crops in Brazil

Rafael Ferreira, Roberta Nocelli, Osmar Malaspina

Bees are the main pollinators of forests and crops. The dependence of a crop in relation to pollinator varies from little until totally dependent. However, the fact that a culture is not dependent does not mean that it does not bring benefits when present. Studies show that the presence of bees improves fruit quality and quantity, but there are few data regarding the presence of bees in crops not dependent in Brazil. The presence of bees exposes them to various pesticides used, it is important to assess the diversity to ensure a management that permits the association of production with conservation. Thus, the objective of this study was to evaluate the diversity of bees in coffee, citrus, cotton and soybean in different Brazilian regions. Two areas distant 1000 Km were evaluated and divided into 16 plots of 250m^2 each. Three collections per day were carried out from beginning, middle and end of flowering. In coffee and citrus were found 4 species of Apidae family. In cotton crop 7 species of Apidae, 2 of Halicitidae, 1 of Andrenidae and 1 of Colletidae and in soybean, considered autogamous plant, were found 9 species of Apidae and 1 each of the other families of bees. This survey demonstrates the fact that this insect is not a mandatory pollinator these cultures does not mean they do not visit the area and not collect pollen and nectar. The visit could signal an important route of exposure of bees to pesticides, since there, just talking about products with insecticidal action, there are 43 products registered for coffee, 52 for citrus, 72 for cotton and 50 for soybeans. This demonstrates that the discussions regarding the protection of pollinators and the reversal of environmental services deficit should be extended to all cultures, not just those dependent on bees.

P018

Floral resource competition between honeybees and bumblebees along land-use gradients

Wiebke Kamper, Nico Bluthgen, Thomas Eltz

For the past decades abundance and diversity of wild pollinator species was declining for reasons mostly unknown. Intensification of land use and competition with domestic honeybees are suspected stressors that have the potential to influence the resource availability for a wide range of flower visitors. We investigated how these two stressors may affect wild bumblebees, Bombus, on 46 grassland plots (Swabian Alb, Germany) in the Biodiversity Exploratories project. Plots were chosen to represent a land-use gradient and differed in honeybee abundance. Abundance and floral resource use of bumblebees were measured via two different methods. We conventionally conducted censuses of foraging bees along flower transects. Additionally, we extracted (with hexane) and quantified (by GC/MS) long-chain cuticular hydrocarbon (CHC) deposits (‘footprints’) of bumblebees on flowers. Such CHCs remain on flowers in near-unchanged quantities for up to 48 hours and thus present a cumulative measure of bumblebee visitation. This method allows collecting large numbers of samples quickly at the end of a day. A first analysis of census data showed a negative correlation between honeybee abundance or land-use intensity and the proportion of visits by bumblebees to bumblebee-attractive plant species. Currently, chemical footprint samples are being analysed, and we will investigate whether CHC footprints lead to similar answers concerning honeybee abundance and land-use intensity. Using both methods will allow us to evaluate and compare the two methods. This will help us to assess the influence the two stressors have on wild pollinators.

P019

Study of methodologies for evaluating effectiveness of the grooming behavior

Igor De Mattos, David De Jong, Marco Aurelio Prata, Jairo Souza, Ademilson Espencer E.

The Africanized honeybee is well known for its resistance against pathogens and parasites. The grooming behavior has been pointed as one of the mechanisms of resistance displayed by *Apis* bees against *Varroa destructor*. This study aimed to identify suitable methods for evaluate efficient grooming performance by correlating some behavioral data with the infestation rates (IR) of the studied colonies. The method developed by Aumeier (2000) as well as the technique of the bottom-board trap was evaluated. None of the parameters analyzed through the bottom-board trap (Idiossoma damaged mites, appendix damaged mites and total number of trapped mites) showed significant statistical correlation to IR. Some behavioral parameters evaluated by the Aumeier technique presented high linear correlation to IR as well as statistical relevance. The time takes by the worker bee to notice the presence of *Varroa* on its body and starts grooming behavior was the parameter that presented the highest linear correlation (0.832) to IR. The time spent displaying intense grooming behavior has shown as well a significant linear correlation to the IR (-0.816). Behavioral experiments have also shown that the dorsal surface of the thorax is the area that most exposes the mite to be touched or removed through legs grooming movements. This research showed that the efficiency of the grooming behavior is correlated to quick starting and long lasting grooming behavior. The former concept that the ability to chew mite as a main promoter to control mite populations has proved to be not completely correct. This study also describes a significant influence of the mite’s behavior on the success of grooming as promoter of mite population control. The methodology developed by Aumeier (2000) is highly recommended as diagnostic method to evaluate the grooming behavior efficiency.

P020

Influence of Varroa *infestation on viruses of the honeybee*

Fanny Mondet, Joachim De Miranda, **Alison Mercer,** Yves Le Conte

Over the past fifty years, annual honeybee (*Apis mellifera*) colony losses have been steadily increasing worldwide. These losses have occurred in parallel with the global spread of the honeybee parasite *Varroa destructor.* Indeed, *Varroa* mite infestations are considered to be a key explanatory factor for the widespread increase in annual honeybee colony mortality. The host-parasite relationship between honeybees and *Varroa* is complicated by the mite's close association with a range of honeybee viral pathogens. The 10-year history of the expanding front of *Varroa* infestation in New Zealand offered a unique opportunity to assess the dynamic quantitative and qualitative changes in honeybee viral landscapes in response to the arrival, spread and level of *Varroa* infestation. We studied the impact of *de novo* infestation of bee colonies by *Varroa* on the prevalence and titres of seven well-characterised honeybee viruses in both bees and mites, using a large-scale molecular ecology approach. We also examined the effect of the number of years since *Varroa* arrival on honeybee and mite viral loads. The dynamic shifts in the viral loads of black queen cell virus and Kashmir bee virus mirrored the patterns of change in *Varroa* infestation rates along the *Varroa* expansion front. Strikingly, this was not the case for deformed wing virus (DWV). Our results suggest that the observed link between *Varroa* and DWV may be governed by DWV viral loads in mites, rather than by the level of mite infestation. These findings provide important new insights into the role of the parasitic mite *Varroa destructor* in influencing the viral landscape that affect honeybee colonies.

P021

Thiamethoxan in cyst of spermatozoa in Apis mellifera *black-eyed pupae*

Jane Moreira, Maria Izabel Camargo Mathias, Vinicius Araujo, Jose Lino-Neto

Applications of insecticides to control insect pests in Brazilian agriculture affect directly bees. In these social insects, spermatozoa are transferred from the testes to the seminal vesicles in bundles containing spermatozoa involved in glycoprotein capsule. In the seminal vesicle, spermatozoa are individualized during the sexual maturation process and are transferred during copulation to the female. In *Apis mellifera* the bundles are composed of 64 spermatozoa and in this work we performed tests bioassays to analyze if sublethal doses of thiatometoxan has affects on bundles in black-eye pupae. Twenty black-eye pupae were taken from healthy hives in apiary of the Department of Biology, UNESP, Rio Claro, São Paulo, Brasil had been their abdomen immersed for 5 minutes in the insecticide thiamethoxam diluted in water in concentration of 0.025% of LD50 established by [1] and methodology of contact according to [2]. Ten male individuals are used how control and his abdomen are dissected and had been immersed in water for 5 minutes. After, they were transferred to sterile polystyrene plates and were kept in BOD for 24 hours and their testes dissected according to histology routine technique. Cysts containing 64 sperm were unaffected by the insecticide application. The glycoprotein capsule that holds them together cysts also did not change for the thiametoxan concentration. Other insecticides are being tested in bees at the Department of Biology, UNESP to evaluate possible changes in spermatogenesis. According studies with natural product (like neem) results showed that this product not promote the morphological changes in testis cells in male of stingless bees [1] D.A. Tavares, (2011). Magister scients thesis UNESP, Rio Claro [2] R.O. Drummond et al., J Econ Entomol, v.66 (1973).

P022

Spatial genetic structure and behaviour of common and declining bumblebees

Seirian Sumner, Stephanie Dreier, Jinliang Wang, Andrew Bourke, John Redhead, Matt Heard, Claire Carvell

Bumblebees are important pollinators of food crops and wild plants, but their populations are in decline. Although bumblebees have been well-studied in some respects, fundamental aspects of their ecology remain unknown. We used a novel combination of molecular microsatellite markers, intensive field studies and landscape modelling to determine the spatial genetic structure of five species across an agricultural landscape, as a tool for predicting the impact of habitat structure on foraging range and queen dispersal. We sampled the study populations at a fine spatial scale to maximise the likelihood of detecting sister workers at multiple sites, allowing us to estimate the locations of large numbers of wild colonies. Worker foraging ranges differed between species and were significantly related to the amount and spatial distribution of foraging resources across the c. 20km2 landscape, depending on their colony locations. Using the reconstructed queen genotypes at these estimated nest locations, we determined whether isolation by distance was present at this scale. We therefore present the first detailed analysis based on queen genotypes of whether or not, within bumblebee species, related queens tend to nest near one another. This project is funded under the UK Insect Pollinators Initiative.

P023

Sub-lethal effects of a neonicotinoid pesticide on honeybee flight performances

Simone Tosi, James C. Nieh

Pesticide residues found in pollen and nectar are usually not lethal for pollinators, but can have complex sub-lethal effects that decrease the fitness and health of bee pollinators. Multiple studies have demonstrated sub-lethal behavioral effects of pesticides on honeybee foraging and orientation, but no studies have yet examined the detailed effects of pesticides on the flight ability of honeybees. Our goal was thus to investigate how acute and chronic sub-lethal field-realistic doses of thiamethoxam (a neonicotinoid pesticide) influence honeybee flight performances. We conducted two experiments. In the acute exposure experiment, honeybees received a single sub-lethal dose of pesticide (1.34 ng/bee) while in the chronic exposure experiment, bees were fed with 1.8 M sucrose solution containing either 0 ppb (control), 33 ppb, or 45 ppb of thiamethoxam for the 2 days before the test flight. Honeybee flight performances were evaluated using a flight mill that measured duration, distance, average velocity and maximum velocity. Each bee performed two flights. We show that an acute exposure to thiamethoxam causes temporary excitation, a significant increase of the duration and distance flown by the bees. In contrast, chronic exposure significantly decreases of duration, distance and velocity (both average and maximum) of the flights. These results provide the first demonstration that a sub-lethal pesticide dose can alter bee flight performances. The effect depends upon the duration of exposure to thiamethoxam.

P024

BeeNet and BEST: the Italian 'beekeeping and environment' projects

Claudio Porrini, **Simone Tosi,** Teresa Renzi, Fabio Sgolastra, Laura Bortolotti, Piotr Medrzycki, Angelo Libertà, Franco Mutinelli, Marco Lodesani

The BeeNet 'beekeeping and environment' monitoring network has been working from September 2011 and up to now 303 apiaries distributed in all Italian regions (i.e. 3,000 colonies) are involved. Each colony is checked 4 times per year (early Spring, late Spring-early Summer, late Summer-early Autumn, before Winter). In the first and third visit, samples of living bees are collected for pathological analysis as well as beebread for crude protein content analysis and pesticide residues. The first results (2012) show that winter mortality is negatively correlated to the crude protein content found in beebread and that more than 50% of beebread samples were positive to at least one active ingredient. In addition, *Varroa* infection was directly correlated to ABPV and *Nosema ceranae* was present in all Italian regions while neither *N. apis* nor *N. apis/N. ceranae* co-infection were detected. DWV was present in 96.7% of the samples and in 40% of cases exceeded 10 million viral copies per bee. The Bee Emergency Service Team (BEST) was recently developed within the framework of BeeNet. Its main goal is to study honeybee and colony losses events when the causes are difficult to identify and the phenomenon is still in action. BEST works all over Italy and field intervention, samples and data collection are scheduled in case of bee mortality reported by beekeepers. In 2013, 72 reports have been received from the North (42), South (20) and Centre (10) of Italy. Preliminary results show that the main cause of mortality were pesticides (15), while diseases and pathogens-pesticides interaction caused bee/colony losses in 2 and 3 cases, respectively. To conclude, BeeNet and BEST together provide the rare opportunity to compare by time and space all the main parameters connected to honeybee health, and provide insights on the actual causes of bee losses.

P025

The chemistry of competition: exploitation of heterospecific cues in ants

Hellena Binz, Susanne Foitzik, Franziska Staab, **Florian Menzel**

Most organisms try to avoid interspecific competition since it can reduce individual fitness and, consequently, population growth. Less competitive species benefit from circumventing direct encounters with aggressive dominants, whereas dominant species could use cues left by subordinates to exploit their resources or to chase them off. Here, we studied competitive interactions among five common and syntopic ant species in central Europe (*Formica polyctena*, *F. rufibarbis*, *Lasius niger*, *Myrmica rubra* and *Tetramorium caespitum*) and investigated their reaction towards heterospecific chemical cues. In aggression assays, we established a clear dominance hierarchy of these species, with *L. niger* and *F. polyctena* as the most dominant species. Using Y-mazes, we then tested whether the ants avoid or prefer areas with cues of either dominant or subordinate species. These cues included trail pheromones, cuticular hydrocarbons, and chemical footprints. Ants of all species ignored heterospecific trail pheromone extracts, but two out of three subordinate species avoided cuticular hydrocarbons of the dominant species. In contrast, dominants either ignored or approached cuticular hydrocarbon extracts of subordinates. The avoidance behavior of the subordinate might be dose-dependent, as footprints of the dominant *L. niger* attracted two subordinate species. The lowest-ranking species, *M. rubra*, ignored cues of heterospecifics but avoided following the traces of its own colony members. Our study shows that ants exploit heterospecific cues either to avoid or seek competition and that their reaction depends on their dominance rank in the local community.

P026

Floral ddours are learnt and processed via key odorants

Judith Reinhard, Charles Claudianos

Pollinating insects such as honeybees have to detect, process and learn numerous floral scents from their environment during their daily foraging trips. Floral scents are complex mixtures of different odorants. How the bee brain unravels the complex information contained in scent mixtures and reliably discriminates different floral scents is unclear. Here, we investigated learning of complex odour mixtures in honeybees using a simple olfactory conditioning procedure, the Proboscis-Extension-Reflex (PER) paradigm. Restrained honeybees were trained to three scent mixtures composed of 14 floral odorants each, and then tested with the individual odorants of each mixture. Bees did not respond to all odorants of a mixture equally: They responded well to a selection of key odorants, which were unique for each of the three scent mixtures. Bees showed less or very little response to the other odorants of the mixtures. The bees' response to mixtures composed of only the key odorants was as good as to the original mixtures of 14 odorants. A mixture composed of the non-key-odorants elicited a significantly lower response. Neither an odorant's volatility or molecular structure, nor learning efficiencies for individual odorants affected whether an odorant became a key odorant for a particular mixture. Odorant concentration had a positive effect, with odorants at high concentration likely to become key odorants. Our study suggests that the bee brain processes complex scent mixtures by predominantly learning information from selected key odorants. We propose that the 'selection' of key odorants occurs via inhibitory neural mechanisms in the glomeruli of the antennal lobes.

P027

Ant and poison dart frog interactions

Robert Van der Meer, Paul Weldon, Yasmin Cardoza, Thomas Spande

Studies of alkaloids isolated from the skins of neotropical poison frogs (Dendrobatidae) have led to the discovery of nearly 500 compounds, representing over 20 alkaloid structural classes. The skin alkaloids of dendrobatids, are acquired from the ants mites, and other leaf-litter arthropods that these frogs eat, generally are believed to deter vertebrate predators, e.g., snakes and birds. The biological activities of alkaloids from dendrobatids and other anurans, however, are known primarily from neuromuscular preparations designed to uncover pharmacological modes of action. We test the hypotheses that: 1) poison dart frog skin alkaloids, acquired in part from ants, are an effective deterrent to ant predation and 2) poison dart frog skin alkaloids deter predatory ants even if the alkaloids are derived from that predatory ant. To do this we developed a very sensitive contact bioassay using *Solenopsis invicta* as the test ant. We challenged the ants in this bioassay with 20 alkaloids (12 structural classes) identified from dendrobatids or other anurans. Results clearly demonstrate that not all frog alkaloids deter fire ants at concentrations that would be found in nature, but others were highly active. We also found that the ant, *Solenopsis geminata*, originates in Central America and is sympatric with a poison dart frog, *Oophaga pumilio*, that sequesters the main piperidine alkaloid produced by *S. geminata*. The contact bioassay showed that this piperidine alkaloid was not effective against *S. invicta*. How this impacts the relationship between the ant and poison dart frog will be discussed.

P028

How temporally stable are polydomous wood ant networks?

Samuel Ellis, Elva Robinson

The location a social insect colony nests has a profound effect on how that colony can interact with their environment. Many ant species change this relationship by one colony spreading into several spatially separated, but still socially connected, nests. This dispersed nesting strategy is called polydomy, and is found in a number of phylogenetically and ecologically diverse ant species. Polydomy is a particularly interesting life-history strategy in the red wood ant (*Formica rufa*) group because nesting system is flexible both within and between species. Insights into the benefits polydomy may bring can be provided by examining the structure of the polydomous colonies, and how this structure relates to the environment. In particular it is unknown how stable the colony nest-structure is, and how a colony expands and founds new nests. We investigated the temporal stability of red wood ant *F. lugubris* colonies by regularly remapping thirteen polydomous colonies over a three years period, collecting information on the number, size and status of nests in the colonies and the layout of internest and foraging trails. This has given valuable insights into how polydomous wood ant colonies change both within and between years. Specifically we found that polydomous wood ant colonies have a higher nest turnover than might be expected for an ecologically dominant species in a stable environment. We also investigate the conditions under which new nests are in the colony are formed and the properties favour the survival of a particular new nests in a colony. This study demonstrates the insights which can be gained by examining colonies through time, and the importance of temporal factors in the fitness benefits of particular life-history strategies.

P029

Interference of insecticides in the testes of Apis mellifera

Maria Izabel Camargo-Mathias, Jane Moreira, Karim Furquim, Jose Lino-Neto

Recent research is uncovering diverse sub-lethal effects of pesticides on bees. *Apis mellifera* is one of the most important pollinators of agricultural crops and despite the large amount of papers describing various aspects of the biology and behavior of this species, the role of males has been neglected, since they did not participate in the daily tasks of the colony. In order to analyze if sublethal doses of thiatometoxan have affect on testes and geminative cells of *A. mellifera*, white-eye pupae were treated with the insecticide thiamethoxam 0.025% of DL50 established by Tavares D. (2011). Twenty white-eyed pupae taken from healthy hives in Department of Biology apiary, UNESP, Rio Claro, São Paulo, Brasil. They were transferred to sterile polystyrene plates, containing thiamethoxan diluted in destilled water. Ten pupae of white-eyed males had their abdomen immersed for 5 minutes in the thiamethoxan according to R.O. Drummond et al., 1973 and 10 males controls had his abdomen immersed in water for 5 minutes. After exposure pupae were kept in B.O.D for 24 hours and their testes dissected according to histology technique. The results indicate that thiametoxan penetrated the testes capsule causing tissue disorganization and changing the thickening between cysts, disruption of testicular capsule tissue and showing the presence of pyknotic nucleus in spermatocytes. This insecticide can alter spermatogenesis in bees, which would lead to alterations in the processes of reproduction of these insects. Other products with insecticide function are the subject of studies in the Biology Department of UNESP/ Rio Claro, SP, Brasil and the results will be compared. Studies in general showed different results in males of stingless bees when they are treated with natural product (like neem), since these products do not promote morphological changes in testis testes cells.

P030

Urban ant assemblages found in three different microhabitats

Sean Menke, Jeremy Boeing, Kaya Cuper

Human populations continue to grow, and an increasing number of people are moving from rural environments into urban centers. As a consequence, our urban habitat continues to grow and shape the surrounding environment. As cities increase in size, they disturb and destroy the surrounding natural environment. Traditionally, it was thought that cities were a homogenized environment supporting relatively few widespread species throughout the urban matrix. However, recent work has demonstrated that variation in urban environments plays a major role in biodiversity and species assemblages. Cities are composed of their own microhabitats including street medians, green roof tops, parks, and the indoors. Using ants as a representative taxon, we compare and contrast the diversity found in this ecologically important group of insects inhabiting the extreme microhabitats in urban environments; street medians, parks, and green rooftops. During the summers of 2011-2013 pitfall traps were placed in 45 street medians, 12 urban parks, and 8 green rooftops spanning more than 20 miles in Chicago, IL USA were placed to determine ant community composition. We collected and identified over 11,000 individual ants from 27 different species. The most species rich site with 12 species was found in a street median while the lowest richness sites with only one species were found in a street median and green rooftop. Significantly greater species richness was found in parks compared to street medians and green rooftops. The differences found in species richness and composition between these three environmental extremes supports the idea that different microhabitats in the same urban area have distinct ant communities.

P031

Inter-individual variation of dance activity in honeybee foraging groups

Ebi George, Divya R, Axel Brockmann F

Honeybee foragers communicate information about the profitability of a food source as well as its direction and distance to nest mates via dance behaviour. The food reward affects the probability and intensity of the dance behaviour which in turn regulates the recruitment to that food source. Previous experiments indicated a large variation in the dance responses of individuals to different sugar concentrations and suggested that most of the dances within a foraging group are done by a few foragers (Seeley, 1994). We recently started studying this inter-individual variation in dance behaviour with a focus on three questions: (1) Does an individual's dance activity remain stable over time? (2) Does the composition of the foraging group affect individual dance activity? (3) Are differences in dance responses correlated with differences in neuro-modulatory systems? In line with previous experiment, we found strong differences in dance activity between individuals in a foraging group (5-10 foragers). Some foragers danced for all sugar-water concentrations, whereas others did not dance at any concentration. In addition, differences between foragers mostly remained consistent over 3-4 days. We are currently performing manipulation experiments in which we remove foragers, which show the highest dance activity, from the foraging group. Preliminary results suggest that changes in the composition of the foraging group may have an effect on an individual's dance activity. Our results raise the question whether honeybee foragers visiting the same food source show a division of labor with respect to food collection and recruitment behaviour. Seeley, T. (1994). Honeybee foragers as sensory units of their colonies. Behavioral Ecology and Sociobiology, 51: 62.

P032

Honeybee foragers adjust crop contents before leaving the hive

Ken-Ichi Harano, Akiko Mitsuhata-Asai, Takayuki Konishi, Takashi Suzuki, Masami Sasaki

Honeybee foragers leave the nest after loading a small amount of honey in the crop. The honey serves as fuel during a foraging trip. The present study demonstrated that both nectar and pollen foragers of *Apis mellifera* finely regulate crop contents upon departing the hive. When waggle dancers advertizing nectar sources and their followers were examined, their crop contents upon departing the hive increased with food-source distances expressed in waggle-run durations. It was also found that the crop contents were generally smaller in dancers than in followers. Another experiment showed that they significantly reduced crop contents at departure as repeatedly visited a feeding site. Bees with limited information for food sources such as followers and inexperienced bees may carry more fuel to increase likelihood to reach an unfamiliar food source. Pollen foragers had larger crop contents upon leaving the hive than nectar foragers. This can be explained by additional honey to be mixed with collected pollen (called ‘glue’ honey). When bees collected pollen from a single pollen-source species, their crop contents at departing the hive increased with food-source distances as in nectar foragers. However, this tendency disappeared when analyzing a group of waggle-dancers visiting various pollen-sources. Interestingly, their followers still showed a positive correlation between crop contents at departure and food-source distances expressed in waggle dances. These results suggest that experienced pollen foragers learn an actual need of honey for glue which may vary among pollen-source species and subsequently adjust amounts of honey carried from the hive but such information was not transmitted to nestmates through waggle dances.

P033

Dance communication about a location in honeybees: Vector or location?

Randolf Menzel, Andreas Kirbach, Uwe Greggers

A human observer can read the flight vector of a recruited bee from the dance performance it had followed. It has been concluded that vector information is transmitted from the dancing bee to the recruit. An alternative view posits that the dance informs a recruit about the location of an interesting site. Such a view requires an integration of the ego centric vector information from the dance and the learned spatial relations of compass and landmark cues. We used harmonic radar tracking to address this question and conclude from our data that recruits integrate own spatial experience with information from dance communication. Additional experiments exclude the possibility of vector addition between learned flight vectors and dance communicated vectors. Therefore, we conclude that bees integrate information from dance communication in a learned common spatial reference system that stores the spatial relations of landmarks.

P034

How do foraging honeybees decide to quit in natural conditions?

Michael Rivera, Matina Donaldson-Matasci, Anna Dornhaus

Honeybees (*Apis mellifera*) are well known for their ability to efficiently find and exploit resources. However, the factors surrounding individuals' decisions to stop foraging at a site are not well understood. Two hypotheses exist in the literature: (1) a decline in reward or (2) an imbalance in task allocation within the colony may lead foragers to abandon a resource. When a bee returns to the hive with nectar, she needs a food storer, to receive and store the nectar. If many foragers are successful, receivers may be in short supply. This could indicate that enough bees are foraging and other tasks may be more important. Alternatively, receiver bees may prefer to unload other bees, indicating that the forager's nectar is of low quality compared to that being collected by others. Thus the amount of time that an individual forager spends looking for a food storer bee is indicative of the colony's need for this forager's nectar. In such a natural setting, bees visit many types of resources, which change quality at different timescales. Using marked bees in an observation hive, we construct foraging histories for individual bees and identify when an individual stops foraging at a particular resource. Using unload time to represent the balance of foragers and receiver bees, and trophallaxis duration to estimate amount of nectar collected and thus quality of the resource, we ask which of the two factors seems to influence the decision to stop foraging most strongly under natural conditions. Surprisingly, neither our estimate of resource quality nor the delay to unload predicted a forager's decision to abandon a resource in our data. Our study indicates that the collective foraging process of honeybees is a mosaic of individual decisions, and its outcome in a natural setting is not predictable based on a few simple factors.

P035

*Foraging activation in the common wasp (*Vespula vulgaris*)*

Davide Santoro, Philip J Lester, Stephen Hartley

The absence of intra-nest signals and communication about food resources (recruitment) among social wasps is a brainteaser for biologists. In the present study, we tested the hypothesis that the common wasp (*Vespula vulgaris*) shares food-related information inside the nest and shows foraging activation (an increase in the probability of an individual leaving the nest as a result of information received from successful foragers). We controlled for local enhancement, eventual chemical trails at the food source and climatic variation. We find evidence that food choice and localization of resources in the field by naive foragers (newcomers) was assisted by information previously or simultaneously provided by experienced nestmates. This information was related to chemical cues associated to food and possibly to its location. Pilot flights might explain the observed patterns. At the trained nest, there was an information-mediated variation in the foraging effort at colony level when known resources were available. Reactivated, experienced foragers were the main responsible for the foraging traffic rate increase recorded at the nest. To our knowledge, this is one of the first studies demonstrating foraging activation in social wasps. Our data are excitingly consistent with the possibility of recruitment in this group of social insects.

P036

The mechanisms of resin use in wood ants

Timothee Brusch, Geoffrey Jaffuel, Michel Chapuisat

An advantage of social life is the emergence of collective defences to combat pathogens. The wood ant *Formica paralugubris* disinfects the nest by collecting conifer resin, which confers protection against fungal and bacterial pathogens. So far, we know little about the mechanisms of resin use. We recently investigated i) whether workers processed the resin to increase its antibiotic effect and ii) the factors eliciting resin collection and placement within nests. We found that resin that had been in contact with workers had significantly increased inhibitory activity against the fungal pathogen *Metarhizium brunneum*, as compared to control resin untouched by workers. Additionally, we showed that workers bring more resin to their nest when brood is present, and preferentially place the resin near the brood. These findings indicate that wood ants process the resin and use it strategically for brood protection.

P039

Differential antiseptic brood care in the ant Lasius niger

Christopher D. Pull, Mark J. F. Brown, Sylvia Cremer

Ants are efficient at detecting and responding to pathogen-exposed nestmates, both at the adult and brood stage. Yet, not all colony members may experience the same level of hazard to a pathogen, with the different brood stages, for example, likely having different levels of exposure (some brood stages are often moved to the surface of the nest to speed up development), and different physiologies, which may make them more or less susceptible to infections. Further, some nest members represent a higher level of investment to the colony than others, such as the pupae, which have consumed more resources and worker care than, for example, the eggs. As a result, we might expect tending ants to invest more care into colony members that 1) have a greater susceptibility to a pathogen, and 2) those that represent a larger colony investment. To test these hypotheses, we studied the sanitary brood care of garden ants (*Lasius niger*) towards fungal pathogen-exposed brood, which comprises the eggs, early-instar larvae, late-instar larvae and the pupae. Using behavioural assays to determine the frequency of worker-brood interactions, we report on how antiseptic worker behaviour differs according to brood age and pathogen treatment. We also investigated the role of the pupal cocoon with regards to the process of infection, as the cocoon appears to act as a barrier, both delaying pathogen infection processes but also likely interfering with the ability of the tending ants to effectively sanitise pathogen-exposed pupae.

P040

Social interaction networks and pathogen-induced behavioural defences in ants

Nathalie Stroeymeyt, Bahram Kheradmand, Anna Grasse, Sylvia Cremer, Laurent Keller

Social insects live in dense, highly interactive groups of closely related individuals. They are therefore particularly vulnerable to infection by pathogens and have evolved numerous collective defences to prevent both entry and spread of pathogenic agents into colonies. For example, there is increasing evidence that the spatial and behavioural compartmentalisation arising from division of labour in many social insect colonies also serves to decrease the risk of pathogen transmission between functional units that interact little with one another thus ultimately protecting the queen and the brood from infections originating from outside ('organisational immunity'). Beyond the prophylactic aspects of organisational immunity, social insects could also benefit from modifying their interaction patterns upon entry of a pathogen into a colony to further reduce its transmission, e.g. by increasing compartmentalisation even more. We investigated the possible existence of such pathogen-induced organisational immunity in the ant *Lasius niger*. Using an automated tracking system allowing the simultaneous monitoring of all individuals in a colony for extensive periods of time, we recorded the movement and interactions of all workers before and after infection of certain colony members with the generalist entomopathogenic fungus *Metarhizium brunneum*. To relate observed interactions to actual pathogen transmission, we quantified the pathogen load of all workers, the queen and the brood at the end of the experiment (i.e. 9 days after infection) using real-time polymerase chain reaction. We will describe how individual behaviour and social interaction networks changed upon introduction of the pathogen into colonies, and how this might affect pathogen spread and the final outcome of infection events.

P042

Seasonal changes in the immune defense in the ant Formica exsecta

Dimitri Stucki, Dalial Freitak, Nick Bos, Liselotte Sundström

Eusocial insects are favorable targets for parasites owing to high population density and high relatedness within colonies. Consequently, eusocial insects have evolved defenses against parasites both at the individual and the social level. Often colonies face different demands in energy allocation to different life history functions during active season or before hibernation. These seasonal differences create potential changes in trade-offs in e.g. resource allocation between energy saving for hibernation and immune defense, depending on the progress of the season. Especially when resources are scarce, individuals and colonies may be weakened, which may allow infection by opportunistic pathogens. This further accentuates these trade-offs. Here we investigate seasonal differences in the regulation of immune defenses under starvation during different times of the season (summer and fall) in the wood ant *Formica exsecta*. We used bioassays with the analysis of immune-, stress- and storage-related gene expression, and survival analysis, to investigate the seasonal differences in the ants' response to oral infection with the entomopathogenic bacteria Serratia marcescens and *Pseudomonas entomophila* under starvation and unlimited food conditions.

P043

Diet subsidies and climate may contribute to Vespula *invasion impacts*

Erin Wilson Rankin

Vespula (yellowjacket wasps) includes some of the most ecologically damaging invasive insects. Native to western North America, *Vespula pensylvanica* (western yellowjacket) became established over 30 years ago in Hawaii, where it reduces endemic arthropod densities. Similar to some other *Vespula*, these wasps exhibit plasticity in colony structure. While annual colonies are most typical of their native geographic range, up to 20% of colonies in the introduced range overwinter, producing perennial colonies that can reach sizes orders of magnitude larger than annual colonies. Such a shift in life history may greatly amplify net ecological effects. Given the geographic distribution of *V. pensylvanica* perenniality in Hawaii and, very rarely, southern California, factors associated with such climates may favor the expression of a perennial life history. Other invaders, such as the Western honeybee (*Apis mellifera*), may promote yellowjacket survival by serving as diet subsidies. Here we report how the effects of diet subsidies (in the form of honeybee colonies) and experimentally manipulated soil temperature are linked to changes in yellowjacket foraging and colony phenology. Close proximity to honeybee hives was correlated with increased daily activity rates and prolonged seasonal activity of yellowjackets. Increasing minimum soil and yellowjacket nest temperatures led to increased wasp activity and to a lesser degree prolonged seasonal activity. These data suggest that diet subsidies and mild climate contribute to invader life history shifts and ultimately will modify ecological impacts. Understanding how localized warming affects life history will help predict how trait evolution or plasticity may respond to future climate change.

P044

Recent range expansion and population genetics of the Argentine ant

Maki Inoue, Satoshi Koyama, Carolina Paris, Koichi Goka, Fuminori Ito

The Argentine ant, *Linepithema humile*, has successfully spread from its native range in South America across much of the globe. A form of social structure, supercoloniality, whereby individuals from separate nests cooperate, is attributed to its successful worldwide expansion. The Argentine ant, first noted in 1993, is now found in several regions of Japan. Here, we used mitochondrial DNA to examine the genetic structure of introduced *L. humile* populations in Japan and how they relate to other introduced and native populations to understand the species' invasion history in Japan. Sequencing revealed six haplotypes distributed across its introduced ranges, of which five were present in Japan. One supercolony that extends across Europe, North America, Australasia, and Japan comprises the dominant supercolony (i.e., the largest colony) recorded on each continent or island where it is found. Whereas several minor supercolonies with different haplotypes are locally distributed within their introduced ranges. We also examined the potential gene flow among the four adjacent supercolonies to improve our understanding of the mechanism of supercolony formation. Our behavioral assay revealed that the workers did not show strong aggressiveness toward foreign males early in the reproductive season, suggesting the possibility of male-mediated gene flow. However, genetic analyses using microsatellite markers revealed significant genetic differentiation and restricted gene flow among the supercolonies. Each supercolony is therefore likely to function as a reproductive unit, from an evolutionary perspective. We also found that workers from two supercolonies with broad distributions tended to be more aggressive than the other colonies toward foreign males. If these two supercolonies are both more aggressive and superior competitors, then restricted gene flow among the supercolonies may be responsible for the maintenance of specific behavioral traits that help these supercolonies expand into new habitats, resulting in successful worldwide expansion of *L. humile*.

P045

Size of individuals and the duration of development in ants

Elena Lopatina

Based on literature and own data altogether 43 ant species were compared by their development times at 25 ± 1 °C. For the purpose of comparison the prepupal stage was combined with the pupal one because in Myrmeciinae, Ponerinae and Formicinae they both were normally enclosed within a cocoon. Immature development was 2 - 3 times longer in "primitive" ants *Dinoponera quadriceps, D. grandis*, *Myrmecia forficata*, *Tetraponera anthracina*, *Cryptopone gilva* in comparison with species from Dolichoderinae, Formicinae and Myrmicinae.

Significant positive correlations were found between the development times of egg and larval stages ($r = 0.33$, $p = 0.03$, $n = 39$), the egg and combined prepupal+pupal stages ($r = 0.69$, $p = 0.00011$, $n = 22$), and the larval and combined prepupal+pupal ($r = 0.59$, $p < 0.0016$, $n = 22$) stages. The development times of all stages were strongly correlated with individual sizes of workers. It means that the larger was the species the longer was the development.

We calculated the parameters of the linear regression of the development rate on temperature ($R = a + bT$) and compared the values of the regression coefficient b for species of different sizes. The coefficient of linear regression of the development rate on temperature b (or the thermal sensitivity coefficient) determines how the development rate changes when temperature increases or decreases by 1 degree.

There was no correlation between the size of individual and the value the thermal sensitivity coefficient in general for all ant species, but negative correlations were found for Myrmicinae and Formicinae examined separately. It means that in these two subfamilies the temperature dependence of the development was slightly greater for small species with faster development than for large species with slower development. The temperature dependence of larval development demonstrated much more complicated relationships.

P046

Morphological analysis of the Dolichoderine ants of Madagascar (Hymenoptera: Formicidae)

Brett Morgan, Brian Fisher

Although systematics research is increasingly reliant on molecular data to define relationships, morphological analysis remains essential for identifying characters to diagnose and identify lineages. In ants, mouthpart and petiole structures are useful for these purposes. Ant mouthpart structures are highly variable because they reflect adaptations to different diets for different ant species, and may provide reliable characters for differentiating between genera and species. Petiole shape and structure are also variable among species and genera, and may be informative in the identification of lineages. However, little research has involved creating a permanent, online accessible record of ant mouthpart and petiole morphology. The main goals of this project were to create such a record, and to identify mouthpart and petiole characters to differentiate between the genera of Malagasy ants within the subfamily Dolichoderinae. The Dolichoderinae of Madagascar include 12 species of Technomyrmex, 8 Tapinoma, 2 Aptinoma, and 2 Ravavy. Petioles and mouthparts from multiple individuals of the 24 species were dissected and imaged using an auto montage camera. Mouthparts were first removed from the ant, stained using aqueous 3% eosin Y, and permanently fixed on slides with Euparal mounting medium. Characters scored include maxillary and labial palp segment counts, mandibular denticle pattern, mandible shape, labrum shape, and petiole shape. Images of the mouthparts and petioles are publicly accessible online at AntWeb.org. These characters were used to create a key to the Dolichoderine genera of Madagascar.

P047

Double-origin of swarm-founding in the genus Ropalidia *(Polistinae).*

Fuki Morooka

The wasps in the vespid subfamily Polistinae fall into two groups in terms of their modes of colony foundation. One is independent founding, in which a colony is founded by one to several inseminated females. The other is swarm founding, where a colony is founded by a swarm of workers associated with one to many queens. Swarm founding is considered to be more advanced mode of colony foundation than independent founding. *Ropalidia*, one of the largest genera in the Polistinae, consists of about 200 extant species and is widely distributed in Old World tropics and subtropics. They have the two way of these colony founding. Swarm-founding species of *Ropalidia* are divided into two groups based on their distribution ranges: Oriental species distributed from India in the west to Borneo, the Philippines and western part of the Lesser Sunda Islands in the east; and Papuan and Australian species. No swarm-founding species are distributed in Sulawesi, Moluccas and eastern part of the Lesser Sunda Islands. Phylogenetic analysis of *Ropalidia* wasps using molecular data shows dual origin of the swarm founding in *Ropalidia* in accordance with their distribution.

P048

Transport of vitellogenin in the ovary of social Hymenoptera

Jose Eduardo Serrao, Milton Ronnau, Dihego Azevedo, Maria do Carmo Fialho

Vitellogenins are the major yolk proteins, synthesized in the fat body, released into the hemolymph and transferred to developing oocytes. To be endocytosed for oocytes, vitellogenin should cross the follicular epithelium barrier surrounding the oocyte. However, the mechanisms by which this protein crosses through the follicular cell layer are still poorly understood. This study compared the routes for transporting vitellogenin into the oocyte in social Hymenoptera represented by bees *Apis mellifera* and *Melipona quadrifasciata*, wasp *Mischocyttarus cassununga* and ant *Pachycondila curvinodis*. Ovaries were submitted to immunocytochemistry with antibody against vitellogenin. In the vitellogenic oocytes of all species, vitellogenin was detected into follicle cells, perivitellinic space and oocytes, indicating a transcelular route until the perivitellinic space. Actin filaments undergo rearrangement in response to vitellogenin transport in the vitellogenic follicles. In vitellogenic follicles of *A. mellifera* and *M. cassununga* there is accumulation of granular material between the peritoneal sheath and the base of the follicular epithelium. Septate junctions occur near to the apical region of follicle cells, resulting in a barrier to the movement of substances in the intercellular space. The vitellogenin in the follicular cells occurs into a membrane reticular system. In the ovary of social Hymenoptera, vitellogenin is initially carried by intercellular spaces, which dilate due to the rearrangement of actin filaments, until the median apical portion, then, following a transcellular route to the perivitellinic space. Supported by FAPEMIG, CNPq and CAPES.

P049

Group-living results in higher foraging success for a kleptoparasitic spider

Deborah Smith, Yong-Chao Su

Nearly all of the > 200 species in the spider subfamily Argyrodinae (Araneae: Theridiidae) live in association with other spiders: as predators, solitary kleptoparasites, or group-living kleptoparasites. About 20 out of 238 argyrodine species are group-living in the webs of their hosts. In these species, multiple individuals forage in one host web and show strong conspecific tolerance, especially when they share prey items directly with hosts. We aimed tested whether the group-living behavior of *Argyrodes miniaceus* increases their *per capita* foraging benefit. *Argyrodes miniaceus* and their host, *Nephila pilipes*, were housed in 60 x 60 x 60 cm cages in the lab. Trials were conducted on groups of 1 to 7 unrelated adult female kleptoparasites. For each experimental trial, a ~0.3 g domestic cricket (*Acheta* sp) was provided to the host spider. We used time to reach the prey item, mean time spent feeding per individual, and proportion of time spent feeding per individual as indicators of foraging benefits. Mean time spent feeding (in seconds) and proportion of time spent feeding (seconds feeding/total length of interaction with host) was highest when groups consisted of two or three individuals. Correspondence analysis showed that individuals in a group of two or three were more likely to reach the prey and feed. A time series analysis showed that when a group size was larger than four kleptoparasites, *A. miniaceus* individuals took turns feeding and kept two to three kleptoparasites searching around the host for the duration of each experimental feeding trial. We concluded that group-living behavior in *A. miniaceus* is not due simply to aggregation at a rich resource; instead, it has the function of social foraging.

P050

Nest architecture and colony composition of a Malaysian sphecid wasp

Stefano Turillazzi, David Baracchi, Duccio Pradella, Fabio Meucci

Social life in the sphecid wasp presents various degrees of organization in different species especially in the tribe Pemphredonini. The eusocial stage is reached in the genus *Microstigmus* by *M. comes*, a neotropical species (Matthews 1968). The genus *Spilomena* belongs to the same group of *Microstigmus* and is also characterized by species where one can find colonies with various females at the same time. We found colonies of *Spilomena sp*. on the walls of buildings at Bukit Fraser, a mountain resort on the Central Range of Peninsular Malaysia at a height of about 1500 m a.s.l.. The nest consists of cells attached to a vertical plane substratum. Construction material is formed by tiny pieces of vegetal and mineral origin, parts of insects and fungal hyphae kept together by silk threads secreted by the wasps' glands. Cells are placed vertically side by side and form 'batteries' of up to 5 and more units. Nests composed by several batteries of cells are common. The upper part of a cell presents the entrance protected on one side by a sort of 'roof'. When various cells form a battery their roofs are transformed in a long tube which connects all the cell entrances and furnishes a shelter for the adults of the colony. Nests can contain up to 47 cells (average 10.39 cells, N=48). Colonies may be inhabited by several females (range=1-13, average= 4.3 females per nest, N=20) and some males. Females with developed ovaries increase significantly with the total number of females present. In 6 out of 24 colonies with more than 3 females only one female had developed ovaries. This species presents a relatively high degree of social organization.

P051

Male genitalia and ant evolution

Masashi Yoshimura

Our recent studies show that the morphological information discovered from male ants is a valuable to clarify their evolution and to find a proper taxonomic position for a taxon. Wings, flight sclerites, and genitalia in male ants are particular characters that differ from the characters present in the workers, which are mainly targeted for establishing the taxonomic system of ants. Among these useful characters, especially the male genitalia are expected as one of the most efficient sources to gain new morphological insights into ant evolution. However, the information is not yet sufficient and standardized enough to evaluate the actual value of the genital characters. Most data about the genital characters of male ants is scattered in many sources and mostly as part of species descriptions. Only few of the characters have been proposed to organize a taxonomic hierarchy. Although intra-taxon variation should be assessed before a comparison can be made across groups of higher taxonomic rank, few studies include enough material to estimate the variation. This study reviews and reorganizes the data of male-genital characters collected throughout our taxonomic and comparative studies of male ants in order to discuss the actual usefulness of the genital characters in ant systematics. Dracula ants, the subfamily Amblyoponinae is targeted to examine the differences in species and genus rank. The material examined covers most species found in the Malagasy region, and the covered range of variation seems sufficient to estimate intra-generic variation and inter-generic differences among the major genera in this group. Dolichoderinae and Formicinae are focused on for a comparison across subfamilies.

P052

Novel partners support two-way by-product mutualism in a converted ecosystem

Kalsum Yusah, Tom Fayle, David Edwards, William Foster, Edgar Turner

Human disturbance disrupts networks of interactions, but also creates novel connections between species. Non-native species may play an important role in maintaining cooperative interactions, particularly when relationships are non-specific. We investigate this possibility by assessing how the benefits of the by-product mutualism between litter-trapping epiphytic ferns and their ant inhabitants changes when primary forest is logged and converted to oil palm plantation in the global biodiversity hotspot of Southeast Asia. We find that despite high turnover of ant species, both the level of ant protection from herbivory and the fern provision of nesting space are robust to disturbance. Indeed, as nesting space increases with fern size, the potential for partner fidelity feedbacks is enhanced for particular ant species in disturbed habitats. However, the specificity of the interaction is lower in oil palm than in forest habitats. Non-native ant species, which are widespread in oil palm, are at least as important as native ones in protecting ferns from herbivory, and are more important in driving the increase in ant abundance and species richness with fern size. We conclude that novel connections with functionally equivalent species to those that are lost after disturbance can endow by-product mutualisms with resilience to land-use change.

P053

Histological study of the venom gland in the wasp Apoica thoracica*, Acre, Brazil.*

Isisdoris Rodrigues de Souza, **Rusleyd Maria Magalhaes de Abreu**

The wasps belong the order Hymenoptera, group Aculeata, have functional sting. The wasps sting is used primarily as defense, there is a strong dependence of the animal on this structure. The objective of this study was to analyze the histology of the venom gland *Apoica thoracica* wasp, as well as to observe the formation of the poison. The specimens were dissected to remove the venom gland, after fixed in Carnoy, dehydrated and embedded in Leica resin and sectioned with microtome to application histological techniques with hematoxylin and eosin (HE). The venom gland is formed by two secretory filaments, an convoluted gland and an reservoir, connected with sting. Histologically, the epithelium of the venom gland filaments consists of cylindrical cells, with spherical and large nuclei, containing evident nucleoli; the reservoir epithelium is formed by cubic cells with spherical nuclei and small nucleoli. The convoluted gland cells showed spherical, enormou, central nuclei, containing nucleoli. In general, nuclei and nucleoli were strongly stained with hematoxylin, reflecting intense activity of RNA synthesis, more pronounced in cells of convoluted gland. The cytoplasm of secretory filaments cells, convoluted gland as well as in the lumen of the secretory filaments and of the reservoir has basic pH secretion, reflecting protein nature. The secretion present in reservoir was observed homogeneou and granulated. The results show the filaments epithelium, reservoir and convoluted gland are secretory. The secretion is basic when present in the cytoplasm of secretory filaments cells, reservoir and convoluted gland, as well as in the lumen of the secretory filament and reservoir.

P054

Cytochemical evaluation the venom gland of the wasp Apoica thoracica*, Acre, Brazil.*

Marcelo Augusto de Oliveira da Silva, **Rusleyd Maria Magalhaes de Abreu**

The Insecta are the most diverse and most abundant group of arthropods. The venom apparatus of Hymenoptera is directly derived from the oviduct and functions as an effective weapon of defense in the Aculeata. In social wasps the venom gland is formed by two free secretory tubules and a reservoir, which is connected to the stinger, wrapped externally by a muscular layer. The objective of this study was to analyze the contents of the wasp venom gland of *Apoica thoracica*. Twelve adult worker wasps were collected, the venom gland removed, submitted to a process of fixation, dehydration and sectioning before application the three cytochemical techniques: toluidine blue (DNA and RNA), bromophenol blue (total proteins) and PAS+Blue Alcian (polysaccharides). Toluidine blue stained the nuclei and nucleolar regions in the epithelium of the secretory filaments, reservoir and convoluted gland, indicating synthesis of RNAs, and higher levels of secretory cells and filaments in the convoluted gland. Bromophenol blue staining was observed in the nuclei and nucleoli epithelium secretory filaments, reservoir, convoluted gland and cell lumens of the secretory filaments and reservoir. The technique of PAS+Blue Alcian showed the presence of basic polysaccharides in the constitution of the epithelium of the secretory filaments, reservoir and in the cytoplasm secretion in cells of the convoluted gland. By these results we conclude that the secretory cells of the filaments, reservoir and convoluted gland secrete proteins nature and basic polysaccharides, constituents of the venom.

P055

Morphology as a tool to understand ant physiology

Maria Izabel Camargo-Mathias

The new era of the studies of insect makes use of tools that allow to perform the morphological analysis telling a story which contained many facts to be reported through new information obtained mainly by the application of histological and histochemical techniques as well as by the improvement of the researchers knowledge. Exemplifying the use of the morphohistological tools in studies with insects, we mention those which the internal organs like: exocrine glands, reproductive, nervous and digestive systems. Specifically with ants, many knowledge acquired were obtained from the application of the morphohistological techniques, which provided data those when associated with another ones like taxonomic, behavioral and molecular allowed to draw a more accurate profile of the biology of the animals. In the last decade, such studies have been carried out by researchers such: Ortiz et al, 2012; Vieira et al, 2012; Roma et al, 2008, 2010, Pavon, 2009, under the supervision of Camargo-Mathias, M.I. leader of the group, which showed different aspects of the morphology and physiology of internal and external ants organs. Specific studies with ants and using morphology techniques, have shown the fat body, like responsible for the synthesis and storage of proteins, lipids and carbohydrates specially of the vitellogenin, important in the reproduction. Roma et al (2009) performed a study of the fat body and showed results that helped in better understanding of the phylogenetic relations between basal and derived species of the Attini ants using ultrastructural cytochemistry. Vieira et al (2012) showed important differences of the metapleural glands among several species of ants, making use of morphological tools like histology and ultrastructure. Like this another studies have been performed using the morphology as a main tool to understand the physiology and behavior of the ants, by the analyses of tissues organization and cell specialization.

P056

Primary survey for pathogen infection in bees in Thailand

Chanpen Chanchao, Pawornrat Nonthapa

Apis cerana and *A. mellifera* are economically important honeybees in Thailand. It is important to understand infection and pathogen distributions in these species. For *A. cerana,* 10 and 45 hives were collected from Samut songkram (the Central) and Chumphon (the South) provinces respectively. For *A. mellifera*, 50 hives were collected from Nan (the North) province. In addition, 16 hives of stingless bees (*Tetragonula larviceps*) were collected from Chantabuti (the East) province. In *A. cerana* only, *Varroa* mites were 5x more frequent on pupae than on larvae. For multiplex PCR, we focussed on bees without any disease symptoms. 16S rRNA of target organisms and pol of the Sac Brood Virus (SBV) were used for primer design. Bees in Samut songkram were infected with *Nosema api*s (30%), *N. ceranae* (80%), and *Paenibacillus larvae* (10%). Also, 16%, 2%, and 20% of bees from Chumphon were infected by *P. larvae*, *N. apis*, and *Ascosphaera apis*, respectively. For *A. mellifera*, no infection by *N. apis* was reported but 2.5% was infected by *N. ceranae* and 25% were infected by *P. larvae*. However, we found no evidence of infection in stingless bees. No SBV infection was found in any bee samples. Life stage was an important predictor of infection. By morphology observation, abnormal morphology (brown and black) were seen 8x more in larvae than in pupae. By multiplex PCR, in *A. cerana* only, it seemed that any life stages could be infected by *P. larvae* and *N. ceranae*. In contrast, *N. apis* was found in adults only. In addition, *A. apis* was found in larvae and pupae more than adults. In the future, outbreak prevention in the country should be concerned. Keywords: *Apis*, bee disease, bee stage, infection, multiplex PCR, Thailand

P057

Effects of cardanol from Apis mellifera *propolis on breast cancer BT- 474 cell line*

Sureerat Buahorm, Chanpen Chanchao, Preecha Phuwapraisirisan, Kriengsak Lirdprapamongkol, Songchan Puthong

The number of patients sick with breast cancer keeps increasing in many countries. Since many cancer types can be resistant to recent chemotherapeutic drugs or those drugs still cause side effect, searching an active compound, especially from natural products, is very interesting. As known, a bioactivity of natural products depends on biogeography, thus, in this research, *Apis mellifera* propolis from Nan province (the North of Thailand) was focused. Propolis was sequentially partitioned with methanol, dichloromethane, and hexane. Starting with 90 g of propolis, after evaporation, crude methanol extract (CME) at 660 mg, crude dichloromethane extract (CDE) at 1,540 mg, and crude hexane extract (CHE) at 21,600 mg could be obtained. By MTT assay, CME, CDE, and CHE showed the inhibitory activity against breast cancer cell line (BT474) with the IC50 values of 41.46, 29.31 and 34.06 ug/ml, respectively. Therefore, CDE was the most active. It was further purified by quick column chromatography, adsorption chromatography. The expected band containing cardanol was proved by thin layer chromatography (TLC). The chemical structure of cardanol was verified by Nuclear Magnetic Resonance (NMR). Also, by MTT assay, cardanol could inhibit the growth of BT474 at the IC50 value of 15.57 ug/ml. Also, the growth curves between cardanol-treated and untreated BT474 could be drawn. In the future, the program cell death and cell arrest will be detected on cardanol treated BT474 by flow cytometry. The expression change of genes relating to cancer will be determined by quantitative reverse transcriptase polymerase chain reaction. Keywords: breast cancer BT 474 cell line, cardanol, cytotoxicity assay, IC50 value, propolis

P058

Work or rest? Resting of bumblebees in a social context

Amber Dodd, Mark, J.F. Brown, **Matthias, A. Fuerst**

Sleep in vertebrates is essential and has accordingly received a lot of attention. Much less is known about resting behaviours in invertebrates. Social insects constitute an especially interesting case as there should be a trade-off between the optimal individual resting time at the individual and at the colony level. Whilst the individual should optimize its regeneration the colony should optimize its efficiency. We established 4 different states of resting behaviour in the bumblebee *Bombus terrestris* and monitored resting and working behaviours of workers in three different social contexts (isolated solitary bees, groups of 5 bees without brood and groups of 5 bees with brood) under laboratory conditions. The individual, non-social bees rested significantly longer than bees in a social context. In the social context bees with brood rested significantly longer than bees without brood. We will discuss these novel results on the so far neglected resting behaviour of social insects in the light of conflicts of interest in a bumblebee colony.

P059

Aquaporin in the salivary glands of the worker termites

Kohei Kambara, Masaaki Azuma, Wakako Ohmura

Subterranean termites favor higher humidity and the workers seek and carry water for their colony, utilizing it as a solvent for nest construction and gallery building and to maintain wetness for their nestmates. Such water management in a social organization relies largely upon the function of the workers in the colony, and on the individuals that control the location and movement of water inside their bodies. The movement of water via aquaporins (AQPs; water channels) into and out of cells is a key feature of the numerous physiological functions related to whole-insect water balance. We have characterized the homologue of the water-specific *Drosophila* AQP [*Drosophila* integral protein (DRIP)] in workers of the Formosan subterranean termite *Coptotermes formosanus* (Isoptera: Rhinotermitidae). Immunoblot analysis of DRIP-type AQP reveals that the Coptotermes DRIP with a molecular mass of approx. 25.7 kDa is expressed predominantly in the salivary (labial) gland of the workers. As evidenced by immunocytochemistry, the *Coptotermes* DRIP is present at deep invaginated basal plasma membrane of the parietal cells, opening to the duct of each acinar lumen. By contrast, there is no DRIP detected within the salivary glands of soldier termites, and neither caste expresses DRIP in their labial gland reservoir (water sac), a tissue which has been suggested to have a function as a water sink. A worker termite is an efficient lignocellulose decomposer. The AQP present in the salivary glands is of physiological importance in regards to salivation, aiding in the secretion of cellulolytic enzymes for wood ingestion by the workers of the subterranean termite. Furthermore, we found another type of AQP in the salivary gland of workers. It has been currently underway how the drywood termites regulate water under less wetness of their living environment.

P060

Investment vs vulnerability tradeoff during brood transport in Diacamma indicum

Rajbir Kaur, Sumana Annagiri

Animals relocate their dwelling places for various reasons. During colony relocation, unlike any other animal, ants need to transport their brood in addition to the adults from their old nest to their new nest. As almost no information is available on the mechanism of brood transport, we conducted a set of relocation experiments with 50 *Diacamma indicum* colonies. We found that there was a complete overlap between transport of adult and brood and there were no brood transport specialists. An interesting tradeoff exits between vulnerability and investment among the different kinds of brood items. The larvae are more vulnerable and represent smaller investment of the colony while pupa due to their covering is less vulnerable but represents bigger investment of the colony. We examined the preference of individual ants to larva and pupa in choice experiments (n=295) during relocation. This provided us another platform to investigate the solution that these superorganism have evolved to balance an important trade-off in their social life.

P061

Co-evolution is the primary force shaping termite gut microbial communities

Nurdyana Abdul Rahman, Dana Willner, Donovan Parks, Rudolf Scheffrahn, Mark Morrison, Philip Hugenholtz

Termites provide an appealing model system to explore the relative effects of co-evolution and environmental factors on symbiotic gut microbiota as, unlike most insects, their gut communities are relatively complex. Lignocellulosic biomass is digested through an obligate symbiosis with specialized gut microbiota comprising bacteria and protists in lower termites and bacteria only in higher termites. Accordingly, transmission of gut microorganisms between termites is more strictly regulated than in mammals via trophallaxis (oral transmission) or coprophagy and co-speciation with the host has been observed in selected members of the gut community. Despite the successful application of culture-independent techniques on termite gut, very little molecular data exist for Australian termite species. Here, we surveyed the gut microbiomes of 42 Australian and 24 North American termite samples, representing 16 genera, using 16S rRNA amplicon pyrosequencing. These data represent the first gut microbial community profiles for three higher (*Tenuirostristermes, Drepanotermes, Gnathamitermes*) and two lower (*Marginitermes, Porotermes*) termite genera. Microbial community profiles were compared between termite genera, diet and geographical locations. Our molecular survey revealed that while all termite genera shared a distinctive core set of microbial phyla (Bacteroidetes, Firmicutes, Spirochaetes, Proteobacteria), each termite genus had a characteristic set of microbial populations consistent with vertical inheritance. Secondarily, relative abundance of these populations reflected dietary preferences. Our findings suggest that co-evolution is the primary force shaping gut communities in termites.

P062

Transcriptomic analysis on physiological roles in the mixed segment

Rohitha Kumara, Seikoh Saitoh, Hiroaki Aoyama, Gaku Tokuda

Nasutitermes takasagoensis is a wood-feeding higher termite, which digests cellulose using cellulases secreted from the midgut tissue. The adjacent gut region is called the mixed segment, consisting of the mesenteric and the proctodeal epithelial tissues. It harbors bacteria in the ectoperitrophic space. So far, the physiological function of the mixed segment has yet to be clarified. Here, we carried out 454 pyrosequencing to profile the transcriptome of the mixed segment and compared with those of the midgut and the proctodeal segment (P1). In total, 76197, 45283, and 24603 reads were obtained by sequencing mRNAs from the midgut, the mixed segment and P1 respectively. These were assembled to obtain 4813, 4563 and 3629 transcripts, for the midgut, the mixed segment and P1 respectively. Assembled transcripts were annotated with Gene Ontology , KEGG, Pfam and dbCAN databases. Of the identified genes, those encoding lignocellulolytic enzymes such as, β-glucosidases (GH1) and endo-β-1,4-glucanases (GH9) were mainly identified from the midgut and almost absent in the mixed segment. On the other hand, these analyses identified the genes involved in glycolysis, TCA cycle, oxidative phosphorylation, phagosome, lysosome and synaptic vesicle cycle pathways, which were dominantly detected in the mixed segment. More abundant detection of genes encoding V-ATPase subunits was also characteristic in the mixed segment than in the other tissues. V-ATPases are known to be shared among oxidative phosphorylation, phagosome, lysosome and synaptic vesicle cycle pathways. The dominant expressions of all V-ATPase subunits in the mixed segment were further confirmed with qPCR analyses. Thus, we propose that V-ATPases play a key role in the mixed segment. To our knowledge, this study is the first to illuminate tissue-specific gene expression to elucidate the physiological roles of the mixed segment in the N. takasagoensis. Key words: cellulase, digestion, transcriptome

P063

Insemination and fertilization success of individual males in the honeybee

Madlen Kratze, Mat Welch, Kristina Tabur, Susanne den Boer, Boris Baer

The queens of honeybees and leaf cutter ants are highly polyandrous and store sperm for years to decades, which allows them to fertilize millions of eggs. As queens never remate later in their life to replenish sperm, they have become highly efficient in storing sperm and keeping them alive as well as in their sperm usage during egg fertilizations. However, the factors that determine male insemination and fertilization success have not been studied in great detail. Obviously, both natural selection for long-term sperm storage and economy as well as sexual selection such as sperm competition and/or cryptic female choice could have influenced the evolution of these spectacular reproductive traits.

Here we developed a method that allows us to distinguish the sperm of different males using a number of fluorescence dyes. We used these dyes to study both the insemination success of different males using artificial insemination as well as the usage of sperm on freshly laid eggs. We find that the different dyes used had no effect on semen viability and honeybee queens inseminated with fluorescence dyed sperm store them for several months and use them to lay eggs. We will present an overview of ongoing experimental work that quantified sperm storage and usage in both honeybees and leaf cutter ants. Our data indicate that queens of both species are highly economic in their sperm usage and typically use only 2-3 sperm for each egg fertilization. However, sperm use patterns differ substantially between species, revealing some insights into the mechanistic of sperm usage of queens as well as differential contributions of different males.

P064

Diversity of the chemical signature in the invasive hornet Vespa velutina

Jeremy Gevar, Jean-Philippe Christides, **Anne-Genevieve Bagneres**, Eric Darrouzet

The yellow-legged hornet, *Vespa velutina nigrithorax*, was accidentally introduced to southwestern France in 2004, probably as a result of ceramic pottery being imported from China by boat. The species subsequently successfully established itself in France and Europe. The hornet now occurs across more than 60% of France and is currently colonizing neighboring countries (Spain, Portugal, Belgium, and Italy). It is predicted that the species will continue to spread along the Mediterranean coast and will invade northern Europe. Since the species preys on several insect and arthropod taxa, it can have a significant effect on biodiversity. *V. velutina* is a pest in France because it preys upon domestic honeybees, *Apis mellifera*. Beekeeping operations are directly affected by *V. velutina* predation, with some beekeepers reporting colony losses. The species presents also a risk to human health. Accidents have occurred, some of which have resulted in death, when people have accidentally approached the hornet nests.

Among the different subjects we analyze (biology and ecology of the species, selective traping), we study the chemical signature (cuticular hydrocarbons or CHCs) of the invasive hornet. Preliminary study shows that few insects were introduced in France, probably only one queen. In this condition, we wanted to show whether the chemical signature is different among colonies or not. First, cuticular hydrocarbons were identified in GC-MS. Second, we analyzed the CHCs according to individuals. Hornets have specific CHCs according to their gender, their caste and their colony. These differences in the chemical signatures are linked to the relative quantity of each compound. Moreover, in each caste from the same colony, it is possible to separate different groups of individuals with their CHCs. This plasticity of the chemical signature could be linked to the age or the function of each individual.

P065

Colony breeding structure of the invasive termite Reticulitermes urbis

Elfie Perdereau, Alessandro Velona, Simon Dupont, Marjorie Labedan, Andrea Luchetti, Barbara Mantovani, **Anne-Genevieve Bagneres**

The invasive success of social insects is related to their ability to adapt to new environments. To gain a better understanding of the biology of invasive termites, the social organization of the subterranean termite, *R. urbis*, was investigated by analyzing the breeding structure and number of reproductives within colonies from three introduced populations. Using eight microsatellite loci to determine the genetic structure, it was found that all the colonies from the three populations were headed by both primary reproductives (kings and queens) and secondary reproductives (neotenics) to form extended-family colonies. *R. urbis* appears to be the only *Reticulitermes* species with a social organization based solely on extended families in both native and introduced populations, suggesting that there is no change in their social organization on introduction. F-statistics analyses indicated that there were few neotenics within the colonies from urban areas, which did not agree with results from previous studies and field observations. This suggests that, while several neotenics may be produced, only a few become active reproductives. The factors that may cause an individual to differentiate into a neotenic in Reticulitermes species are discussed. The invasive success *of R. urbis* may be due to different reproductive strategies in urban and semi-urbanized areas.

P066

Spatial genetic structure analyses of an invasive termite in France

Zoe Chevalier, Elfie Perdereau, Simon Dupont, Stephanie Bankhead-Dronnet, **Anne-Genevieve Bagneres**

Among social insects, termites are characterized as major pests which cause considerable economic and biological damage. This is the case of *Reticulitermes flavipes*, a subterranean species that causes significant damage to human-built structures. This termite may have been introduced into Europe from Louisiana during the 18th century and has become invasive in several areas of France. This invasive success is attributable to a particular social structure, the permanent presence of numerous active secondary reproductives and a high degree of colony fusion associated with a lack of intraspecific aggression, and to a territorial advantage. To determine the extent to which termites had spread within the Central Region of France, the population and social structure of this invasive species was studied at regional and local scale in urban areas. The genetic variability was determined using mitochondrial (COII, COI, 16S) and nuclear markers (microsatellites). At regional scales, preliminary mtDNA analyses showed that there were four main centers of infestation in this region originating from the west coast of France and north east USA. At local scale, the initial analyses of the population genetics show that all colonies contained a large number of reproductives with a high degree of colony fusion. By integrating geographical data, analyses of landscape genetics should show whether urban environment plays a significant role in shaping the genetic structure of local populations of this termite species.

P067

Social organization in source and introduced populations of an invasive termite

Guillaume Baudouin, Elfie Perdereau, Simon Dupont, Franck Dedeine, Ed Vargo, **Anne-Genevieve Bagneres**

The social organization in colonies of the termite *Reticulitermes flavipes*, an invasive species in France, is mainly composed of extended families (colony headed by multiple related reproductives with their offspring) or mixed (colony headed by multiple unrelated reproductives with their offspring). It was recently found that *R. flavipes* populations introduced in France probably originated from Louisiana (USA). This study was carried out to determine whether the social organization of invasive populations differed from the source population. The genetic structure of *R. flavipes* in introduced (Olonne sur Mer, France) and source (New Orleans, Louisiana, USA) populations was compared by microsatellite genotyping. Analyses showed that the French population comprised two extremely large colonies, which both had a mixed-family structure, whereas for twenty small colonies identified in Louisiana, half had a mixed-family structure and half had an extended-family structure. F-statistics indicated that all colonies in both source and introduced populations contained a large number of secondary reproductive (neotenics). The social organization in the Louisiana population differed from that of most other North American populations, which usually consist of simple families with few neotenics. The social organization did not appear to differ significantly between source and introduced populations. The differences observed in termite populations native to North America are discussed.

P068

Using a native ant to control the Argentine ant

Alok Bang, Gloria Luque, Franck Courchamp

Biological invasion is one of the foremost drivers of loss of biodiversity around the globe. Identification and investigation of native species as effective competitors against the invasive species is a relatively underutilised line of inquiry. Argentine ant, *Linepithema humile*, is 'one of the 100 of the worst invasive species' on the planet at present as identified by IUCN, and is gradually increasing its range in temperate regions around the world. In this study, we investigated changes in behaviour, survival and productivity of *L. humile* by pairing it with the native Mediterranean ant *Tapinoma nigerrimum*. We found baseline differences between the two species as well as differences in behaviour, survival and productivity arising due to the presence of a competing species. 1. *L. humile* outcompeted *T. nigerrimum* when in equal numbers. For *T. nigerrimum* to pose as an effective competitor, it had to be at least ten times more abundant than *L. humile*. 2. *L. humile* showed behavioural plasticity with change in group size; displaying more aggressive and group-based behaviours when group size was larger, and more submissive and escape behaviours when group size was smaller. 3. In a surprising discovery, we found a third strategy when *L. humile* colonies were smaller. In some replicates, we observed a close living-in between *L. humile* and *T. nigerrimum* colonies, wherein *L. humile* individuals were neither aggressive nor were aggressed. This could be an indication of a less commonly exercised yet an effective survival strategy. *L. humile* is a highly adaptive species, but *T. nigerrimum* was found to impact *L. humile* survival, productivity and behaviour. Studies such as these should pave way for field trials and if successful, should be incorporated in broader conservation policy.

P069

Pesticide changes odds of interactions between native and invasive ants

Rafael Barbieri, Phil Lester

Neurotoxic pesticides, such as neonicotinoids, are reported to negatively affect the behaviour and fitness of non-target organisms. Such changes are probably most relevant when they involve interactions between native and invasive species. We have been examining the effects of sublethal exposure to a neonicotinoid on the (1) foraging activities, (2) colony fitness, and (3) outcome of interspecific interactions of two ants that are known to coexist in New Zealand - the native *Monomorium antarcticum* and the invasive Argentine ant (*Linepithema humile*). Neonicotinoid exposure did not affect the probability of both ant species to locate a food source. However, exposed *M. antarcticum* colonies had lower probability to explore new foraging territories. Brood production was significantly lower in Argentine ant colonies exposed to the neonicotinoid. Importantly, pesticide exposure reduced interspecific aggressive behaviour of *M. antarcticum* and increased aggression of the Argentine ant. Such changes in behaviour significantly increased the survival probability of the Argentine ant after interspecific interactions. The success of the invasive Argentine ants is often linked to their high aggressive behaviour and their ability to displace native communities and manipulate food sources. Thus, our results suggest that non-target exposure of native ants to neonicotinoids could potentially increase the probability that this invader survives in invaded settings. Given that, in any community, different species have different food preferences and thus different exposure to pesticides, non-target exposure could potentially change the dynamics of communities and influence invasion success.

P070

Individual, group and colony dominance among invasive ants

Cleo Bertelsmeier, Amaury Avril, Olivier Blight, Herve Jourdan, Sebastien Ollier, Franck Courchamp

Ants figure prominently among the worst invasive species because of their enormous ecological and economic impacts. Here, we explored interactions among several highly invasive ant species, which have been shown to have overlapping suitable areas. Each of the species included in this study is capable of becoming numerically and behaviourally dominant and of displacing native species. However, it remains to be investigated which species would be behaviourally dominant when confronted with another invasive ant species, should two species be introduced in the same area. The aim of this study was to evaluate the performance in interference competition of the 7 of the world's worst invasive ant species (*Anoplolepis gracilipes*, *Paratrechina longicornis*, *Myrmica rubra*, *Linepithema humile*, *Lasius neglectus*, *Wasmannia auropunctata* and *P. megacephala*). We conducted pairwise confrontations, testing the behaviour of each species against each of the 6 other species (in total 21 dyadic confrontations). We used single worker confrontations and group interactions of 10 versus 10 individuals to establish a dominance hierarchy among these invasive ant species. We discovered two different behavioural strategies among invasive ants: Three species displayed evasive behaviour when individuals or groups were confronted, while the four remaining species were highly aggressive during encounters and formed a linear dominance hierarchy. Among these four, interactions at the colony level led to a similar hierarchy, although with more complex extinction processes and more variability.

P071

Parasites and genetic diversity in an invasive bumblebee

Catherine Jones, **Mark Brown**

Biological invasions pose one of the biggest threats to biodiversity, with climate change and the global transportation of species are likely to increase the ecological and economic damage caused by biological invasions. Therefore understanding the mechanisms behind invasion success is essential. Both the release of non-native populations from natural enemies, such as parasites, and the genetic diversity of these populations may play key roles in their invasion success. We investigated the roles of parasite communities, through enemy release and parasite acquisition, and genetic diversity in the invasion success of the non-native bumblebee, *Bombus hypnorum*, in the United Kingdom. The invasive *B. hypnorum* had higher parasite abundance than native congeners, probably due to higher susceptibility and parasite acquisition. Consequently parasites had a higher impact on the invader's fitness than on native species. *B. hypnorum* also had lower functional genetic diversity than native species. Higher parasite abundance and lower genetic diversity have not prevented the rapid invasion of the UK by *B. hypnorum*. These data may inform our understanding of similar invasions by commercial bumblebees around the world. In addition, this study has important implications for biodiversity and species conservation, such as introduction and translocation programs, where small founding populations, with low parasite loads, are required to establish, produce self-sustaining populations and expand their range

P072

A pathogen reduces yellow crazy ant reproductive ability in Australia

Meghan Cooling, Monica Gruber, Ben Hoffmann, Phil Lester

The yellow crazy ant (*Anoplolepis gracilipes*) is a widespread invasive species which can have severe ecological impacts on native ant and invertebrate communities. In some areas of their invaded range, yellow crazy ant populations have been observed to fluctuate strongly. In Arnhem Land, Australia, populations of these ants fluctuate spatially and temporally and sometimes disappear altogether. The mechanisms responsible for such declines have yet to be investigated. Yellow crazy ant populations in Arnhem Land represent a unique opportunity to investigate mechanisms by which a globally significant invader declines. It is my hypothesis that microorganisms affect fitness of yellow crazy ants. In order to test this hypothesis, I will compare pathogen and bacterial endosymbiont load of ants from sites with declining and expanding populations, I will also evaluate the effects of these microorganisms on colony fitness. Initial findings suggest pathogens may affect egg production in queens. By identifying the mechanisms behind such population collapses, we aim to improve the management and control of invasive species.

P073

The need for subterranean termite management in New Orleans, Louisiana

Carrie Cottone, Claudia Riegel, Nan-Yao Su, Eric Guidry

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) has become the most economically important structural pest species in New Orleans since its introduction into the city following World War II. In 1998, an area-wide termite population management program, named 'Operation FullStop', was initiated to control populations of Formosan subterranean termites in the city's French Quarter to protect historically significant structures and trees. In 2012, funding for this program ceased, resulting in many historically properties being without termite protection. Two previously established research sites, Louis Armstrong Park and Canal Street, now serve as models to show the effect of termite baiting technology on areas of high termite activity. Both of these sites are located in close proximity to the French Quarter and both exhibit high levels of termite pressure. Previous studies within Louis Armstrong Park have shown that if termite bait is removed from the site, the number of termite colonies and relative termite activity will increase to pre-baiting levels within five years. Currently, bait has been applied again to Louis Armstrong Park and the park exhibits little termite activity. Canal Street, as an untreated site until just recently, has exhibited levels of high termite activity. The contrast between the baited and untreated sites shows the potential for future termite damage in the French Quarter.

P074

Tapinoma nigerrimum *as safeguard for Italian myrmecofauna against Argentine ant*

Dario D'Eustacchio, Alberto Fanfani, Donato Antonio Grasso

The invasive Argentine ant (*Linepithema humile*, Dolichoderinae) spreads easily worldwide from its native range, occurring mainly in Mediterranean regions. A copious literature investigates the impact of its invasive populations, suggesting that Argentine ant dominates ant communities it invades by effective exploitation and interference competition, and displacing most of the native species. In spite of first occurrence in Italy dated at early 20th century, scientific literature lacks of topics concerning *L. humile* for this country. In a preliminary investigation, we verified the occurrence and distribution of Argentine ants in some suitable habitats: Mediterranean coastline segments of Tuscany, Latium, Campania and Sicily. We observed the following pattern of occurrence: a local abundant and patchy distribution of *L. humile* with native *Tapinoma nigerrimum* (Dolichoderinae), with several frontline areas reflecting a systematic exclusion between those two species. In fact, *T. nigerrimum* as *L. humile* often exhibited ‘unicoloniality’, consisting of large colonies that lack boundaries due to absence of intraspecific aggression. We investigated the impact of Argentine ant on indigenous myrmecofauna compared with presence/absence of *T. nigerrimum*. Moreover, we evaluated their ability to discover and monopolize food resources. We selected Casteporziano Reserve (Latium) as a model area for the following reasons: i) high colony density of both *L. humile* and *T. nigerrimum* with several frontline areas; ii) high myrmecological diversity. Pitfall traps and artificial baits were placed along six transects across the frontline ‘hot-spots’, representing various environments with different disturbance degree. First results highlight a clear reduced ant diversity in the *L. humile* patches in respect to the adjacent *T. nigerrimum* patches. Moreover, concerning the access to food resources, the two species do not seem to differ in competitive ability. Our results suggest that *T. nigerrimum*, limiting the spread and impact of the Argentine ant invasion, may act as an efficient ‘safeguard’ for the local myrmecofauna.

P075

Intraspecific aggressive behavior of the subterranean termite Microcerotermes diversus *Silvestri (Isoptera: Termitidae)*

Behzad Habibpour, Marjan Ekhtelat, Farhan Kocheili

Microcerotermes diversus is an extremely destructive structural wood pest in Khuzestan province, Southwest Iran. A better understanding of basic termite ecology is necessary in order to search for alternative termite control methods. The aim of this study was to investigate intraspecific aggressive behaviors among four colonies of the subterranean termite, *M. diversus* collected from two different locations in Ahwaz, Iran. Termite interactions over 24 h were examined in paired combinations of castes (soldiers versus soldiers, soldiers versus workers, and workers versus workers). Highest and lowest mortality were observed in paired combinations of soldiers versus workers and soldiers versus soldiers from different colonies, respectively. Termite agonistic behaviors indicated that interactions between different colonies within a species, as well as between different castes within a colony, are variable. Interactions between colonies of *M. diversus* from the same geographic area are complex, and these interactions could influence termite control strategies and because intra- and interspecific agonistic behavior could influence termite foraging patterns. Therefore, it can be suggested that intercolony aggression can be used to delineate the foraging territories of *M. diversus* colonies. Keywords: Agonistic behavior, Intra-specific, *Microcerotermes diversus*

P076

Assessment of the invasive wasp Vespula germanica *in South Africa*

Karla Haupt, Pia Addison, Ruan Veldtman, Heidi Prozesky

Invasive social insects stand out as successful invaders worldwide. One such example is *Vespula germanica* which has invaded South Africa. It flourishes in newly invaded ecosystems, where it has become a major pest in urban settings. The wasps negatively affect tourism and outdoor activities and are also agricultural pests globally, with negative impacts recorded for viticulture, apiculture and horticulture. There is a gap in the research on the wasps in South Africa, but negative impacts are already prevalent in the core of the wasps' current range. This study aims to fill the existing research gap by gathering the relevant baseline data on the species. Bait preference trials were conducted on eight sites over two seasons, with meat-based lures in yellow delta traps proving to be the most effective method in trapping the wasps. Imported artificial lures also show promising results and are now being further tested. The distribution of *V. germanica* was determined through bait-trapping in combination with public observations. A comprehensive questionnaire was completed by forty farm owners about the wasps on their property with the objective of determining the wasps' current realised negative impacts. A wide variety of subjects are dealt with in the questionnaire, including the farmers' awareness about the wasps; number of nests found; the future management of the wasps and the viability of an eradication programme. These preliminary findings form an integral part of the greater Invasive Wasp Project and will help motivate the need for an immediate eradication programme for the species. It is the first step in assisting relevant stakeholders to make informed decisions in implementing future monitoring (short- or long term), control and possibly an eradication programme for the wasps.

P077

Three tramp Dacetine ants in Taiwan

Chung-Chi Lin, Tung-Yi Huang

The species of Strumigenys of ant tribe Dacetini are very small predatory ants that generally feed on springtails and other tiny soil arthropods. In Taiwan, 28 species *Strumigenys* have been recorded including two exotic species, *S. emmae* and *S. membranifera*. The first record of the tropical tramp ant, *S. rogeri* is reported from Taiwan and East Asia in 2008. *Strumigenys rogeri* is reported as originated in tropical Africa, but has achieved broad distributions in the Old World and New World more than 400 sites. Whereas *S. rogeri* and *S. emmae* are almost exclusively tropical, *S. membranifera* has spread to higher latitudes. The three tramps ants have spread to invaded areas through human commerce of Taiwan, probably spread through transport of soil and potted plants, common in urban lawns, gardens, cultivated fields, and dense forest. In the present study, we investigated the situation of tiny dacetine ants from villages and towns in west Taiwan. Field work was carried out from 2009 to 2012. Between August 2009 and July 2012 surveyed using hand collecting and nest collection. Between March 2011 and May 2012 systematic collecting was carried out along by north-south longitudinal provincial highway (No.1, 460 km-long and No. 438 km-long) to the conducted surveys using winkler bag. The results show *S. emmae* and *S. membranifera* are island-wide distribution and occur disturbed village and urban environments. *S. rogeri* has a central-south distribution and occurs lower disturbed natural park, grassland and forest margins. These tramp ants occur at low-elevation sites (< 300m).

P078

The causes and consequences of a colonising pollinator

Kirsty Lloyd, Mairi Knight, Jon Ellis, Mark Brown, Dave Goulson

Climate change has caused dramatic shifts in species' distributions; many species are expanding in their range while others are becoming more restricted. With species range expansions there is also an increase in the number of colonisation events. Insects are particularly good colonisers as they have high dispersal capabilities and high reproductive rates. Bumblebees are a valuable group of pollinating insects and are the primary pollinators of several agricultural crops. There is evidence for severe declines of many bumblebee species globally. In contrast, *Bombus hypnorum* was first recorded in the UK in 2001 in Wiltshire, South West England. The species has expanded its range and is now well established across much of the UK. The origin, colonisation route and drivers behind its rapid expansion are largely unknown. Using whole genome scans this study aims to investigate the genetic signatures of *B. hypnorum's* colonization, specifically looking for; indicators of a genetic bottleneck, inbreeding, single or multiple founding events, and whether populations at the periphery of the expansion have experienced extreme selective pressures. We are also considering the possibility that parasitic release may have been a contributing factor via molecular screening and investigating the potential for foraging competition with native species. The results will highlight the qualities that have contributed to *B. hypnorum's* success as a coloniser and may indicate whether it has the potential to negatively impact native species or if it will simply fit into an empty niche and have negligible impacts on our current native fauna. In addition, this study will contribute to the current understanding of the ecological and evolutionary factors underpinning changes in distribution and abundance of an important group of pollinators.

P079

*Detecting Argentine ants (*Linepithema humile*) on California's Channel Islands*

Korie Merrill

The Argentine ant, *Linepithema humile* (Mayr), is an extremely invasive ant species that has spread to urban, commercial and natural areas worldwide. This pervasive expansion has quickly led to highly detrimental effects, both ecologically and economically. As a result, vast amounts of resources are already being allocated to the control of this species in urban and agricultural areas, while new efforts are currently being initiated to control them in ecologically sensitive habitats, such as California's Channel Islands. As Argentine ant eradication efforts are implemented in these areas, the need for a standardized detection protocol is essential. If small populations of ants go undetected during pre- or post-treatment stages eradication efforts will likely be ineffective. To aid in the creation of such protocols, we conducted field trials and assessed attractant efficacy for Argentine ant detection throughout the year. Four different attractants were placed in 50ml vials for 12 or 24 hours at designated monitoring points. After their respective durations, the attractants were collected and the number of ants at each was counted. Preliminary results suggest that cooked egg plus sucrose attracts more Argentine ants than a sucrose water solution and that 24 hour exposure captures more Argentine ants than 12 hour exposure. We also investigated other co-variables (i.e. topography, soil moisture, vegetation type and ground disturbance) that may contribute to Argentine ant bait preference in order to better understand how to detect small populations in diverse habitats. In the future, this information could be used to standardize Argentine ant detection protocols in a diversity of ecosystems.

P080

Argentine ants don't like it hot: long-term effects of fire on Argentine ant abundance and distribution

Natasha Palesa Mothapo, Carlien Vorster, Theresa Wossler

Fire potentially plays an important role in minimising the impact of biological invasions through its ability to change habitat structure, particularly resource availability; generating unsuitable environments that may prevent the spread of invasive species. The invasive Argentine ant, *Linepithema humile* has been introduced into the Cape Floristic Region (Fynbos) of South Africa and has negative impacts on this biodiversity hotspot. Understanding both the abiotic and biotic factors that reduce its spread into pristine areas are important. Fires may limit the invasion of *L. humile* and play an important role in controlling its population dynamics, and consequently be a potential mechanism that prevents the spread of *L. humile* into pristine Fynbos environments. Short and long term effects of fire on *L. humile* abundance and distribution were measured using a combination of bait surveys and pitfall trap sampling pre- and four years post-fire that swept through the Jonkershoek Nature Reserve, South Africa in 2009. The fire changed the plant community structure and soil condition along the distribution range of *L. humile* dominated by large stands of nectar producing *Protea nitida* and *Protea repens*. Native ant species, especially the displaced dominant ants *Pheidole capensis* and *Anoplolepis custodiens*, recolonised burnt areas soon after the fire while 80% fewer *L. humile* workers were sampled four years post-fire. The invasion front receded by 92 m after the fire owing most likely to a deficiency in floral carbohydrate resources and drier soil conditions , limiting *L. humile* currently to areas dominated by *P. nitida* stands. More studies are needed to understand the role of fire in regulating invasion success of introduced species.

P081

Why can the fire ant adapt to various environments? Effects of hybridization in invasive fire ant populations

Takahiro Murakami, Carolina Paris, Chifune Sasa, Hironori Sakamoto, Kazuki Sato, Seigo Higashi

To investigate the evolution and adaptation of invasive ant species in various foreign environments, we conducted cytogenetic, karyological, and molecular analyses of the fire ant (*Solenopsis invicta*) in invasive areas, including the southern United States (Florida) and Taiwan (Taoyuan), and compared the results to those obtained from a native (northern Argentina) population. The main results were as follows: (1) the fire ant *Solenopsis invicta* in invasive ranges had different chromosomal morphologies, indicating that invasive populations are intermediate between *S. invicta* and other *Solenopsis* species; (2) the fire ant populations in Florida and Taiwan indicated a high variation in the number of chromosomes, mainly differing in ploidy; (3) fire ant populations in Taiwan exhibited the highest variations in the number of chromosomes; (4) there was a significantly large number of Ag-NOR signals on the chromosomes of the Taiwanese population, indicating a positive regression between the number of Ag-NOR signals and transfer physical distance from the native population; (5) the 18S rDNA and telomere FISH signals differed among each population, and the telomeric signals were only detected on a pair of chromosomes in the Taiwanese population; and (6) the molecular phylogeny of the fire ant revealed a high frequency of introgression and hybridization, even in native populations. These data suggest that high frequencies of cytogenetic and molecular variations in invasive populations might invoke rapid evolution and adaptability. Possible factors influencing cytogenetic diversity include hybridization among different *Solenopsis* species and/or the use of insecticides in invasive areas.

P082

Evaluation of wood preservatives against the drywood termite, Incisitermes minor

Wakako Ohmura, Yuko Itoh, Ikuo Momohara, Akira Makita

Laboratory evaluation of wood preservatives was performed to determine their resistance to the invasive termite, *I. minor.* Wood preservatives listed in JIS K 1570:2010 were impregnated to sugi sapwood samples (20 mm x 20 mm x 10 mm) to K3 and K4 levels in Japanese Agricultural Standard (JAS). Non-fixative waterborne disodium octaborate tetrahydrate (DOT) was impregnated to the same size of sugi sapwood to two levels of the retention at 3 kg/m3 boric acid equivalent (BAE) and 6 kg/m3 BAE. Each treated sample was forced to feed on twenty psudergates of I. minor under 26 oC, 75 % R.H. for 6 weeks. The average mass loss of untreated sugi sapwood reached over 15 %, and those of treated specimens with wood preservatives listed in JIS K 1570 were under 3 % even after leaching procedure. The average mass losses of treated specimens with DOT at 3 kg/m3 BAE and 6kg/m3 BAE were 4 % and 2 %, respectively. Timbers used in interior and dry conditions are recommended to be treated at K1 level in JAS with boron compound at over 1.2 kg/m3 BAE for protecting from biodeterioration by wood-boring beetles and dyrwood termites, considering ISO Use Classification System. Our results suggest that higher retention level of wood preservatives in timbers is necessary for resistance to the drywood termite, *I. minor.*

P083

Fast spread of a fungal parasite in an invasive supercolony

Simon Tragust, Heike Feldhaar, **Jes Søe Pedersen**

Many ant species are highly successful invaders and can dominate vast areas by forming dense networks of connected nests in contrast to the smaller and spatially dispersed colonies of most social insects. However, it was recently proposed that such supercolonies are more vulnerable to infection by parasites and diseases as they would serve as large targets with high rates of transmission from nests to nest. Here we studied the invasive garden ant *Lasius neglectus*, a new pest species which is currently spreading throughout Europe where several populations are infected with the ectoparasitic fungus *Laboulbenia formicarum*. In one population (supercolony) we followed the prevalence and intensity of the infection over 10 years, revealing an epizootic spread of the ectoparasite with the mean annual prevalence increasing from 0.126 to 0.997. The body parts of the ants had markedly different infection intensities, and at low intensities antennae and thorax were free from signs of infection. There were no seasonal differences in infection intensity and no other *Lasius* species in the area was found to be infected. These results give the first direct support to the hypothesis that supercolonies of invasive ants potentially face an important threat from parasites and diseases, implying interesting perspectives for biological control of these species.

P084

*Survey establishing the Formosan subterranean termite (*Coptotermes formosanus*) within Louisiana*

Claudia Riegel, Carrie Cottone, Eric Guidry, Timothy Madere, Barry Yokum

The Formosan subterranean termite, *Coptotermes formosanus*, has become the most economically important structural pest in New Orleans, Louisiana since its introduction into the city. It has displaced indigenous termite species through their aggressive nature and large colony size. Formosan subterranean termites often cause extensive damage to wooden structures and frequently infest and consume living trees. Multiple surveys documenting established colonies have taken place throughout the Southeastern United States. Since the last published known distribution of Formosan subterranean termites in Louisiana, established colonies have been located within eight parishes in which this termite had not previously been observed. Currently, forty-one of the sixty-four parishes have established Formosan subterranean termite colonies. Introduction of this termite into new locations have economic, as well as quarantine implications.

P085

A synopsis of success: honeybees out of Africa

David Roubik

Africanized and pure African *Apis* dominate the *Apis* world. Books and review articles notwithstanding, there are no robust explanations of Africanized honeybees success in the Americas. What distinguishes Africanized, now naturalized, honeybees in the Americas? Relatively high metabolism, toticolony defensive behavior, rapid and evasive queen behavior and colony absconding, predator and parasite deterrence, formation of megaswarms, long-distance dispersal, extreme generalization (equable use of primary resources), limited honey hoarding, and visual and olfactory specialization at the neural level. The colony's opportunistic nest site selection, has, as a counterpart, mechanisms of choosing to maintain a reproductive home or moving on. It is a migratory strategy, similar to its biologically closest relatives, migratory giant honeybees. I suggest the key success of this new honeybees variety stems from a ‘cut and run’ strategy. If a monumental defense is ineffective, the queen and the colony abscond. If flowering is meager, the bees forage as individuals not as a colony. And the colony is small, and migratory. This could be called a ‘sustainable’ honeybee for the Americas, or is it? Most the above traits are the antithesis of managed, hived honeybees [colonies]. They are not aggressive foragers, do not usurp other bee nests, but individual swarms disperse 30 km or more. Early work in French Guiana typified reproduction as semi-monthly, while later work in Panama showed one or two annual swarming peaks, coinciding with floral abundance. Early work showed greater reaction to alarm pheromones, and later work showed a greater proportion of defending colony members. Early work indicated polylecty, while later work showed true generalization’ equal proportional resource use. It is tempting to speculate that behavioral resistance to diseases, anti-honey-rustler ‘attitude' and foraging success in suboptimal conditions outweigh other factors which now guide honeybees management.

P086

Antkey: a web-based tool for the identification and study of invasive, introduced and commonly intercepted ants

Eli Sarnat, Andrew Suarez

Over 15,000 species of ants have been described, and more than 200 have established populations outside of their native ranges. A small subset of these have become highly destructive invaders including five which are currently listed among the world's 100 worst invasive species. Unfortunately, detection of non-native ants is hampered by the taxonomic specialization required for accurate species identification. Antkey seeks to mitigate the spread of established introduced ants and prevent the incursion of new introductions by providing quarantine personnel, inspectors and conservation biologists with a user-friendly identification tool specifically designed for non-specialists. The Antkey taxonomic classification includes 8 subfamilies, 43 genera and 120 valid species. Features include an interactive Lucid key, dynamically generated species pages, a searchable media collection of over 1150 images, over 70 live video clips of introduced ants, a fully illustrated glossary with over 400 terms, a searchable database of introduced ant literature, over 12,000 specimen records of introduced ants imported from Antweb (www.antweb.org), and community features such as blogs, discussion forums and comment options. The interactive Lucid key allows users to start at multiple entry points, skip ambiguous or difficult characters, and keep track of the choices already made. Novice users can use the 'best' feature to determine which available characters will lead to the most parsimonious pathway. More advanced users can skip straight to subfamily or genus. The characters are illustrated with original line drawings and link to glossary definitions and additional specimen photographs. The Scratchpads platform (www.scratchpads.eu) used to build Antkey is based on the Drupal content management system. It allows users to participate in the discussion forums and post comments on nearly every page. The platform also allows multiple authors to create and edit content without using any html code.

P087

Genetic evidence for multiple invasions of subterranean termites into Canada

Graham Thompson

Social insects are among the world's most successful species at invading new environments. Their characteristic division of labor can influence their capacity to colonize new habitats, often with negative ecological or economic impact. The social Hymenoptera (i.e., ants, bees, and wasps), are well studied in this regard, but much less is known about the invasive biology of termites (Isoptera). In this study we use province-wide sampling and a population genetic analysis to infer the minimum number of eastern subterranean termite introductions into Ontario (Canada). Structure analysis of multilocus microsatellite genotypes grouped the 30 collection points into three genetic clusters, suggesting as many as three independent introductions into southern Ontario. Levels of genetic diversity were higher in termites from a remote, semi-natural region than in termites from Toronto and other Ontario cities, suggesting that these non-urban termite populations are potentially older and native to Ontario. A single origin scenario, in which all populations stem from a single source, therefore is not supported by the genetic data. Instead, our analysis suggests multiple independent introductions of this highly social, subterranean termite into Ontario, where the species is now well established as a structural pest of urban habitats.

P088

Galápagos threatened by tropical fire ant invasion

Nina Wauters, Wouter Dekoninck, Maria Luisa Martin Cerezo, Henri Herrera, Denis Fournier

The Galápagos islands (Ecuador) are famous for their highly endemic biota, but are fragile and sensitive to biological invasions. One of the most notorious invasive species on the Archipelago is the tropical fire ant, *Solenopsis geminata* (TFA). Here we provide a combined approach that includes distribution data, ecological aspects and genetic life-history traits of this species. First, we updated the distribution of the TFA by adding 63 new records. Seven main islands and 12 islets are colonized by the TFA. We then investigated the impact of the TFA on local ant communities on the main island of Santa Cruz (155 collection points, spread over 28 sites; 8505 identified individuals). The TFA and the little fire ant *Wasmannia auropunctata,* another worldwide pest, are largely dominant. The presence of both species is negatively correlated with the proportion of native and endemic ants, and leads to a harsh disruption of ant communities by diminishing the regularity of species distribution. Finally, to investigate the life-history traits, the population connectivity and the demographic history of the TFA, we studied the genetic aspects of its invasion in the archipelago. We characterized patterns of genetic variation in populations collected in Galápagos and South America using mitochondrial DNA sequences and nuclear microsatellite loci. Altogether, our results suggest that (i) a significant proportion of mating occurs between relatives, (ii) TFA populations have undergone recent bottleneck, and (iii) introduced populations of the Archipelago might have originated from Venezuela and Costa Rica, two countries that have trade relations with Ecuador and the Galápagos. Our results are discussed in the light of the invasion risk of *S. geminata* in preserved areas of the archipelago and across all continents, and in terms of economic impacts and potential threats to native biota.

P089

Molecular mechanisms of caste-specific cuticular tanning in termites.

Yudai Masuoka, Kiyoto Maekawa

Termites have three distinctive castes (reproductives, workers, soldiers), which are morphologically specialized for their tasks. Especially, cuticular nature is remarkably different among each caste. To know the formation mechanisms of the caste-specific traits is important for understanding of the termite social evolution. The cuticle of each caste is expected to be the result of specific modifications to the cuticular tanning (sclerotization and pigmentation) process, but the origins of those differences have not been resolved. The tyrosine metabolic pathway plays an important role in cuticular tanning in insects. Thus, we hypothesized that this pathway and related hormone signaling were involved in the caste-specific cuticular tanning process. We performed gene expression and functional analyses of some candidate genes during each molt (worker-worker, worker-presoldier, presoldier-soldier and nymph-alate) in *Zootermopsis nevadensis*. First, gene expression analysis revealed that some tyrosine metabolic genes were highly expressed just before and after molts to soldiers and alates. Moreover, suppressing expression of Laccase2 (Lac2), which is known to act on both sclerotization and pigmentation in some model insects, using RNA interference (RNAi) caused the soldier and alate cuticles with light color and soft nature. Next, expression patterns of the ecdysone and juvenile hormone (JH) signaling genes were likely correlated to those of the tyrosine metabolic genes. RNAi of the ecdysone receptor gene (EcR) also produced a soldier cuticle with similar characteristics to Lac2 RNAi, but did not affect alate cuticular formation. Knockdown of methoplene tolerant (Met), which is a candidate receptor gene of JH, did not change the nature of soldier and alate cuticles, but resulted in shorter mandibles and smaller head capsules of soldiers. These results suggest that the caste-specific cuticular tanning requires expression level changes of the tyrosine metabolic genes, probably regulated by the ecdysone signaling.

P090

Developments of termite neotenics regulated by high Juvenile Hormone titers

Ryota Saiki, Kiyoto Maekawa

Termites show the highly flexible caste differentiation and each caste is morphologically specialized in their own tasks including reproduction. There are mainly two types of reproductive caste in termites; the primary reproductives which found a new colony, and the neotenic reproductives (neotenics) which take over reproduction when the primary reproductives are lost. Neotenics have highly developed gonads, whereas other imaginal organ formations are strongly arrested. Therefore, organ formation of neotenics must be different from those of primary reproductives. Juvenile hormone (JH) is the multifunctional hormone playing many important roles, including the gonad development and retaining juvenile characteristics. Although JH might be involved in the neotenic differentiation, developmental mechanisms underlying are still unknown. Especially, it is not clear if JH has an effect on their body-parts specific developments. To clarify this issue, using *Reticulitermes speratus*, we (1) quantified the JH titers of neotenics and compared those of other developmental stages, and (2) analyzed gene expression changes of JH signaling molecules [including the homolog of Methoprene-tolerant (Met) which is a JH receptor gene] in their different body parts (head, thorax and abdomen). Consequently, the JH titers of neotenics, quantified by the high performance liquid chromatography-mass spectrometry, were significantly higher than those of nymphs and workers. The JH titers observed in neotenics were clearly higher than those of primary queens shown in the previously published data. These high JH levels might be related to the greater gonad developments and the possession of the higher fertility in neotenics than primary reproductives of this species. Moreover, expression levels of the Met homolog in the abdomens of neotenics were significantly higher than those of any other developmental stages. These results suggest that the developments of neotenics are caused by the large increase of JH titers and body-parts specific activation of JH signaling pathway.

P091

Sociogenomic studies on soldier differentiation in damp-wood termites

Hajime Yaguchi, Shuji Shigenobu, Kiyoto Maekawa

Termite soldiers are a unique caste among social insects in terms of their morphology and role. Soldier differentiation involves many developmental changes, but proximate mechanisms remain unclear. In the incipient colonies of the damp-wood termite *Zootermopsis nevadensis*, it is revealed that the oldest 3rd instar larva (No. 1 larva) always differentiate into a presoldier. This is the first case showing that a particular individual differentiated into a presoldier before the molt. To elucidate the molecular mechanisms of soldier differentiation, we focused on the presoldier differentiation from No. 1 larva, and compared with the molt into 4th instar from other larva. We performed transcriptome analyses using next-generation sequencing (RNA-seq), and expression and function analyses of the candidate gene during presoldier differentiation. First, RNA-seq analyses showed that there was a significantly highly expressed gene ZnNLaz, the *Drosophila* NLaz homolog, in No. 1 larva. Our *de novo* assembly data and molecular phylogenetic studies indicated that there were at least two ZnNLaz homologs in this species. Next, we performed the RNA interference (RNAi) analysis to clarify the ZnNLaz function in presoldier differentiation. Double-stranded siRNAs or DDW were injected into No. 1 larva, and we observed the resulting molted individual phenotypes. Results showed that ZnNLaz RNAi strongly decreased presoldier differentiation rates, and most individuals molted into 4th instar. In the latter case, proctodeal trophallaxis from reproductives to No. 1 larva was significantly lower than the case for those undergoing presoldier differentiation. Our results suggest that ZnNLaz is the candidate genetic determinant for soldier differentiation in *Z. nevadensis*. Soldier differentiation is known to be regulated by some insect hormones (e.g. juvenile hormone). Based on the gene expression analyses of hormone signalings and the ZnNLaz protein localization in No. 1, we will discuss about the role of ZnNLaz and the proximate mechanisms during soldier differentiation in termites.

P092
Is every female equal? Caste biasing in tropical paper wasps
Emily Bell, Robin Southon, Solenn Patalano, Andy Radford, Seirian Sumner

The division of labour in the form of castes among group members in eusocial societies is a hallmark of social evolution. Differing degrees of caste commitment helps define levels of social complexity from primitive to highly eusocial species. The classical paradigm for species representing the early stages of social evolution, such as *Polsites* paper wasps, proposes that castes differ only in behaviour, with individuals remaining plastic and reversible throughout adulthood. Without caste-biasing, females are equally capable of exploiting reproductive opportunities when they arise. Recent studies on temperate *Polistes* spp. suggest potential biasing of castes during development through mechanisms such as differing larval nutrition and mechanical stressors. However, *Polistes* are thought to have a tropical origin. Thus, it is not clear whether such biasing is a secondary product of selection for over-wintering in temperate environments. Here, we provide the first test of caste-biasing in a tropical primitively eusocial insect, where their continuous life cycle without a diapause phase is likely to represent the ancestral state. Using the wasp *P. canadensis* we investigate individual-level variation in caste expression. Specifically, we test the hypothesis that some females are biased towards particular caste roles, as evidenced by developmental timing and diverging behavioural profiles over the lifetime of individually tracked wasps. We found no evidence of phenotypic divergence among individuals during development, suggesting that females were equivalent on emergence. Developmental caste-biasing is therefore unlikely to be an ancestral trait in *Polistes*, and patterns found in other species may reflect a secondary adaptation to temperate climates. Conversely, we found evidence for two distinct behavioural phenotypes that emerge during adulthood, with individual-level variation in investment into maternal care and reproductive strategies among age-matched females. Variation in female reproductive potential during adulthood may be an important source of trait variation in the evolution of castes and sociality.

P093
Evolution of sociality of ants under optimization-thermodynamic theories
Amlan Das, Carlos A. Navas

In the evolution of sociality of ants we posed the questions: is the energetic cost obeys diel cycle and how and at what extent metabolic rhythm is performed in ants? Is there any correlation between dial energy budget and weight of ants under the working hypothesis of optimization-thermodynamic predictions? To test our questions oxygen consumption rate (sVO_2) among workers of varied body mass of a south American leaf cutting ant, *Atta sexdens rubropilosa*, Forel (Hymenoptera: Formicidae) was monitored. As a whole 843 respiratory measurement data were recorded from 47 individual worker ants at different time-hours over 24 h diel periods. From our experiments we assorted three results and discussed as: 1) correlation of metabolism (sVO_2) in ants with body mass in general 2) correlation of sVO_2 with 24 hour diel repertoire, and 3) correlation of S_VO_2 between three ant groups of different body masses with 24h diel repertoire. *Atta* shows a positive correlation between their respiratory rate and body mass under constant temperature (23 ± 1 °C) ($r = 0.704$, $r^2 = 0.485$, df = 45, $p<0.0001$). Over a 24h diel period *Atta* exhibits a complex, irregular and multifarious energetic cost distribution ($r^2 = 0.183$, $p<0.0001$), although there remains a clear propensity of higher sVO_2 during night than day hours. Key words: *Atta sexdens rubropilosa*, metabolic cost, diel cycle, body mass

P094
Octopamine regulates social behaviors between genetically unrelated ant queens.
Satoshi Koyama, Shingo Mastui, Toshiyuki Satoh, Ken Sasaki

Queens of arborial ant, *Polyrhachis moesta*, facultatively perform multiple colony-founding after nuptial flight, even though queens are usually genetically unrelated to each other. However, only single queens remain in a mature colony. It is known from previous studies, that queens in a founding colony perform trophallaxis (food exchange) with other queens more often than those in mature colonies. The queens in mature colonies show more aggressive behavior to other queens. To reveal the physiological mechanism of this behavioral change in the queen, biogenic amine titer in brain of queens of founding and mature colony were measured. We found thatoctopamine titer in brain of queens in mature colonies was significantly higher than those in queens of founding colonies . Thus, in a second experiment, we orally administrated octopamine to queens in a founding colony and kept two queens together under dark condition. Octopamine treated queens performed trophallaxis and allogrooming significantly less often and for a shorter period than control queens. Conversely, there was no significant difference in avoidance of the other queen. We observed aggression behavior in only one pair of octopamine treated queens, which occurred five times between the pair and lasted 75 min in total; no aggression behavior was observed in control queens. These results suggest that an increase of octopamine in the brain decreases the level of cooperation among queens of *P. moesta*. Our study gives new insight into physiological mechanism of cooperation in social insect.

P095
Local determinants of ant functional diversity in a forest fragment
Rodolfo S. Probst, Carlos Roberto F. Brandao, Rogerio R. Silva

The functional trait approach is a powerful tool for the development of quantitative and predictive models of community assembly rules; patterns of morphological variation in ants can allow us to infer the relationship between morphology and ecology. We evaluated local determinants of ant morphological diversity in three distinctive vegetation types in an Atlantic Forest fragment, southeastern Brazil. We used 120 pitfall traps set for seven days (summer and winter) along two transects of 100 meters per area, sampling points at each 10 meters, recording also thirteen abiotic variables and environmental resources at each collection point. We collected 82 species (seven subfamilies and 29 genera; 65 in summer and 55 in winter). We recorded 3,300 morphological measures of 11 ant worker characters with putative functional role (six per species, when possible) to quantify the morphological diversity in each sample. We described morphological diversity using Petchey & Gaston’s functional diversity (PD), mean pairwise distance (MPD), and mean nearest taxon distance (MNTD) indexes. We quantified the influence of the measured variables on richness and composition of communities and the relationship among resources and environment variables. We used GLMMs, LMEs and GAMMs to test the relationship between species richness, morphological diversity and environmental variables. Temperature, soil pH and saturation, number of herbs and their proximity to traps, trees, DBH and length of twigs were found to be the best predictors of taxonomic and morphological ant diversity. We used fourth-corner analysis to evaluate relationships between environment and ant morphological traits, analyzing matrices simultaneously (sites by environmental variables, sites by species presence/absence and species by morphological traits). Community assembly wide analysis based on traits showed a seasonal environmental filter influencing species composition, suggesting different responses of species richness and morphology to microhabitat structure depending on the time of the year. FAPESP grant: 10/17051-9.

P096

Host range of Myrmecophiles

James Glasier, David Eldridge

Grazing by domestic livestock is known to have substantial effects on ecosystems. Previous studies in arid and semi-arid rangelands have shown that ant community composition is a useful indicator of disturbance and can be a valuable tool for assessing rangeland health. We examined the effects of grazing on community structure of surface-active and subterranean ants centered on water points in nine *Callitrus glaucophylla* woodlands in western New South Wales Australia. We tested the notion that the form of the logistic relationship between distance from water and community composition would vary with grazing history; being strongest at sites with a long history of current grazing and weakest at long-ungrazed sites. We present preliminary results on how ants respond to both the grazing gradient, grazing history and environmental variables at the sites.

P097

Ant differential response to nutrient addition in an Andean forest

Justine Jacquemin, Yves Roisin, Maurice Leponce

Ants in the tropics present a wide variety of diets and degrees of specialization that can be functionally categorized into feeding groups. Carbon (C), nitrogen (N) and phosphorus (P) are limiting in the leaf-litter food web, from microorganisms to arthropods. Our aim was to study the response of ant prey and competitors in terms of density to a nutrient addition, and the response of ants -according to their feeding group- in terms of density, taxonomic richness, taxonomic and feeding group composition. We performed a 6-month nutrient addition experiment (+CN and +CNP) in a premontane tropical forest in Ecuador. We distinguished different ant feeding groups, based on their isotopic signature and literature. Due to an increased microbial activity, litter volume in treated plots decreased significantly compared to control, leading to habitat loss. Among the mesofauna, Collembola density was enhanced by the treatment. The density of predatory ant competitors such as spiders generally increased. Ant taxonomic richness was similar in control and fertilized plots, although the taxonomic composition changed significantly. While the overall feeding group composition remained unchanged, the density of predatory ants decreased and the taxonomic dominance changed within each group. *Solenopsis*, the dominant genus among omnivores in control plots, was numerically replaced by *Pheidole* in nutrient-treated plots. The same trend was observed among fungus, nectar and honeydew-eaters, and predators. Our experiment had an impact on the structure of the whole leaf-litter food web. Our results showed a differential response of ants to fertilization according to their feeding group. Predatory ants seemed to be limited by habitat rather than by prey availability. While the feeding functions were maintained among the ant assemblage, a taxonomic shift occurred within each feeding group, suggesting that the functions are maintained in a changing environment even if they are fulfilled by other taxa.

P098

Seasonal change in phototropic behavior of Formica polyctena

Stepanka Kadochova, Jan Frouz

Red wood ants are an important part of boreal forests, they are considered ecosystem engineers because they affect the nutrition flow and tree growth in the vicinity of their nests. *Formica polyctena* ants build huge nests, which may contain over one million workers. In those nests a precise thermoregulation takes place thanks to unique properties of nest material (heat capacity, isolative properties), microbial heating and trapping of solar radiation. Moreover the ants are capable of active nest thermoregulation via metabolic heating and several behavioral reactions. One of those is sunning on the nest surface in the spring. In my research I combined novel approach (use of thermo camera) with classical field ecology methods to study spring sunning behavior and phototropism of ant workers under different ambient situations. I found out that the phototropism is switched from positive, which occurs mainly in the spring, to negative, which is more common in late summer. This change of phototropic reaction correlates significantly with time of year. In early spring the workers behavior is affected by date, solar income and air temperature, whereas in summer nest surface temperature and nest volume plays a vital role. We can suggest that this seasonal switch of phototropic reaction is an adaptive strategy which helps to establish thermal homeostasis in the nest in the spring and avoids overheating of ant workers later in summer, when the nest surface temperature reaches level which is harmful for the ants.

P099

How termite community assembly changes with anthropogenic disturbance in a tropical forest (Togo, West Africa)

Koami Gbenyedji, Boris Kasseney, Isabelle Glitho, Judith Korb

Termites (Order Isoptera) are one of the most abundant invertebrates in tropical forests and they play ecologically important roles in litter decomposition. This study considers the effects of land use and environmental variables on termite diversity, abundance and assemblage structure. In Togo, (West-Africa), one old natural forests and two secondary forests (teak plantations) aged 4 and 10 years, respectively, were surveyed for termites using a standardized protocol. Environmental conditions and vegetation were assessed at each site. Species were identified using molecular barcoding (COI and COII) and phylogenies were constructed to do phylogenetic community analyses. Generic richness and abundance of termites were highest in old natural forests, and decreased in secondary forest sites. The highest species richness (29 species) occurred in natural forests followed by the 10 year old teak plantation (8 species) and least species were found (6 species) in young plantation. Our data will show how anthropogenic disturbance, environmental variables and random factors interact in structuring local termite assemblage of West-African forests

P100

How do agricultural practices in South China affect ant communities?

Cong Liu, Benoit Guenard, Evan Economo

Understanding the effects of habitat change on species diversity and community structure is fundamental to conservation planning. In South East Asia, one of the primary hotspots of ant biodiversity, natural habitat transformation to agricultural fields such as rubber plantation has tremendously increased over the past decades. In this context, understanding how habitat modification affects species assemblages is crucial for biodiversity conservation. Here, we investigated responses of ant species richness and composition to habitat change in Xishuangbanna, a tropical region of southwest China (21° 55’ N, 101° 15’ E). We studied the response of leaf litter inhabiting ants across different sites of primary (12 sites) and secondary forest (14 sites) and rubber plantation (14 sites), collected with Winkler extraction. In total, over 20,000 specimens from 58 genera and 237 morphospecies were collected. Secondary and primary forests were the most diverse habitats with respectively 143 and 119 species while 103 species were collected in rubber plantation. At the site scale, species richness of secondary forest and primary forests was similar, but the average number of species collected in rubber plantation was significantly lower. The composition of ant community within rubber plantation was also more distinct from the composition of primary and secondary forest. Our findings indicate that habitat transformation strongly affect ant communities, probably due to the frequency of disturbance observed in such habitat. For future directions, we will investigate the effect of anthropogenic disturbance on genetic diversity of ant communities and its implication for biodiversity conservation.

P101

Impact of social parasitism on colony development of Bombus ignitus

Hinako Matsuyama, Masato Ono

There are several reports from Europe and Japan about unrelated bumblebee queens of the same or even different species forming a mixed colony, suggesting that nest takeover occurs in nature, although a foundress queen can build a colony by herself. A possible cause of this phenomenon may be a limited number of suitable nest sites and variation in when queens wake after overwintering. In temperate regions, there may be severe competition for nest sites in spring between foundress queens. Clarification of bumblebee life cycles is important in understanding the adaptive significance of social parasitism, but field observation is very difficult. Therefore, we studied the impact of nest takeover on colony development by *Bombus ignitus* by using laboratory-reared colonies. The number of egg cups built by a foundress queen tended to increase in taken-over nests compared to nests not taken over. Taken-over nests also tended towards earlier production of reproductives. In addition, we examined the number of reproductives and workers in both types of colonies to compare productivity.

P102

Disturbing ant-aphid mutualism for better biological control of aphids

Csaba Nagy, Jerry V. Cross, Viktor Marko

Ants and myrmecophilous aphids have a complex mutualistic relationship where both partners benefit from the association. The rosy apple aphid, *Dysaphis plantaginea* (Passerini) is a serious pest of apple, and is commonly attended by the black garden ant, Lasius niger (L.). To determine whether manipulating the presence of *L. niger* can lead to increased predation on populations of D. plantaginea by natural enemies an orchard experiment was conducted in three organic apple orchards at East Malling Research (UK) in 2011 (cv. Queen Cox and Bramley) and 2012 (cv. Fiesta) and at Research and Extension Centre for Fruit Growing, Újfehértó (Hungary) in 2013 (cv. Topaz). Ants were excluded or distracted from trees by placing either sticky barrier bands around or sucrose solution in bottle feeders at the base of the trunks. Exclusion of ants from trees, as opposed to control trees, resulted in rapid decreases in *D. plantaginea* populations caused by increased activity of natural enemies, even though the sticky barrier prevented the important generalist predator *Forficula auricularia* L. from climbing the trees, too. Provision of sucrose feeders also reduced the activity of *L. niger* in the tree canopies. On most of the trees, ants were distracted from searching for aphids in the tree canopies or abandoned the aphid colonies that they were already tending. The loss of ant protection caused rapid reduction in *D. plantaginea* numbers by the increased activity of aphidophagous predators. Also, this method did not disturb the activity of *F. auricularia*. Occasionally, the ants did not completely stop visiting the colonies, but they stopped or reduced their efforts in protecting them. The implications of these results for supporting better biological control of aphids in apple orchards are discussed.

P103

How do mangrove-living ants survive inundation?

Mogens Gissel Nielsen, Keith Christian, Dorthe Birkmose

Ants are one of the animal groups that inhabit extreme habitats, and one is the mangrove forests. Mangroves are both marine and terrestrial as determined by the fluctuating tide. Information on mangrove living ants is sparse even though ants are one of the most important terrestrial groups in this ecosystem. In the Australian mangrove in Darwin Harbor, the amplitude of the tide is up to 8 meters, which provides a unique opportunity to studying how the mangrove ants have adapted to these extreme conditions. The most 'marine' ant is *Polyrarhcis sokolova*, which have nests in the mud. During high tide, the nests can be inundated for more than three hours, by up to 3.5 meters of seawater over the nest entrance. The ants and brood avoid drowning by residing in small air pockets in complex gallery system. The CO_2 concentration can reach 12 % in the galleries, but during receding tide, the water sinks in the nests and fresh atmospheric air replaces the water. *Camponotus anderseni*, nests in cavities in small twigs of the mangrove tree *Sonneratia alba*, together with scale insects. During high tide a soldier ant 'blocks' the entrance with its head and prevents seawater from intruding. The volume of ants, brood and scales can reach 50 % of the cavity volume, and if 'normal' respiratory rates were maintained, all O_2 would be used after 20-30 minutes. We showed that the ants can reduce their O_2 metabolism down to 1% when the CO_2 concentration reached 30%. We also showed, for the first time, that ants can change from aerobic to anaerobic respiration.

P104

Non-destructive estimation of Oecophylla smaragdina *colony biomass*

Christian Pinkalski, Joachim Offenberg, Karl-Martin Jensen, Rene Gislum, Christian Damgaard, Renkang Peng

In most ecosystems, ants are a dominant part of the arthropod community. However, understanding of their ecological importance has been hampered by limited availability of data on ant abundance. We developed a model to estimate the size (biomass and number of workers) of smaragdina colonies in mango plantations in Darwin, Australia. The total nest volume of *O. smaragdina* colonies in a tree was related to the activity of the ants (R^2=0.85), estimated as the density of ant trails in the tree. Subsequently, the relation between nest volume and ant biomass (R^2=0.70) was added to enable a prediction of ant biomass directly from ant activity. With this combined regression the ant biomass in a tree equaled 244.5 g fresh mass*ant activity. Similarly, the number of workers in trees was estimated using the relationship between nest volume and worker numbers (R^2=0.84). Based on the model, five *O. smaragdina* colonies assessed in a plantation in the dry season, ranged in size from 131,000-562,388 workers and from 0.8-3.3 kg total ant wet biomass for the smallest and largest colony, respectively. Correspondingly, the areal abundance of ants in the plantation was 353 workers/m^2 and 2.1 g ant mass/m^2. With this model, estimates on *O. smaragdina* abundance can be obtained with a minimum of workload and it provides a possibility to scale physiological experiments on per capita rates (e.g. food consumption) to an ecological context. Thus, colonies of *O. smaragdina* can serve as model organism providing valuable information on the impact of ants in ecosystems.

P105

Why do vespine wasp workers commit matricide?

Kevin Loope

Matricide, or the killing of the mother queen by workers, is a dramatic example of intracolony conflict in some species of annual yellowjacket wasps, but the selective pressures that have produced this behavior are poorly understood. Matricide may be very costly because queens cannot be replaced in annual colonies, and following queen death, no new females (workers or reproductives) can be produced. Here we provide the first description of matricide in *Dolichovespula arenaria* from three video-recorded observations. Further, we present the first direct tests of two hypotheses that could explain the intraspecific variation in occurrence of matricide. The male production hypothesis suggests that workers execute queens that are producing primarily (or exclusively) male brood, because workers are more related to worker-produced males than to queen-produced males. An alternative explanation for matricide, the unrelated-worker hypothesis, suggests that workers are unrelated to the queen they kill because they are the daughters of a usurped queen or they are socially parasitic drifters from a foreign colony. We tested the male production hypothesis by experimentally creating male-laying queens via surgical removal of the queen's spermatheca in colonies entering the reproductive stage. Colonies were then observed to determine if male production induces matricide, compared to controls without spermatheca removal. To test the unrelated-workers hypothesis, we looked for a correlation between multiple matrilines and observed or inferred matricides. Our results suggest that queen-killing workers are daughters of the queen but do not respond to queen sex investment, counter to theoretical predictions. We suggest that manipulating queen signals may be a promising next approach to determining the proximate and ultimate triggers for matricide.

P106

Genome discovery of novel RNA viruses infecting Formica exsecta

Kishor Dhaygude, Helena Johansson, Jonna Kulmuni, Kalevi Trontti, Stafva Lindström, Liselotte Sundström

Only a few viruses are currently known to infect ants. Metagenomic and transcriptomic approaches have accelerated the rate of discovery of viruses and other infectious agents, allowing for new studies into biological interactions between a species and its pathogens. Here, we report on two putative new viruses that infect the ant *Formica exsecta*. We used a metatranscriptomic approach to detect sequences with homology to viral pathogens. The viruses, provisionally named *Formica exsecta* virus 1 (FEX-1) and *Formica exsecta* virus 2 (FEX-2), belong to the insect specific viruses family Dicistroviridae and Iflaviridae (respectively). A phylogenomic analysis of these and 17 other Dicistroviruses and seven other Iflaviruses showed that these viruses were phylogenetically distinct from other known viruses in these groups. Domains such as RNA-dependent RNA polymerase (RDRP) and Helicase, responsible for vial replication and genome organization were highly conserved and observing very slow evolution rate with purifying selection. Helicase and RDRP used to know taxonomy of these viruses and for inferring their evolutionary relationships. In contrast structural proteins, which function to build capsid structure of virus were evolving faster. We also predicted the protein structure and performed homology modeling of the aparavirus proteins. These methods showed that helicase domain position shifting gives indication of parallel evolution between *Solenopsis invicta* virus-1 and FEX1,where as other aparavirus showed structual homology with FEX-1 polyprotein. Overall, our study shows the usefulness of metatranscriptomic approaches for virus discovery and also great resources for specific information regarding genome organization, molecular characteristics, virion structure, phylogenetics,host immune response elicited by social insect viruses.

P107

Does pathogen presence and within-colony relatedness affect social contact networks?

Svjetlana Vojvodic, Anna Dornhaus, Timothy Linksvayer

Social insects depend on complex social networks to communicate information among nestmates. These same networks are also hijacked by parasites to invade social insect societies, resulting in high host exposure due to elaborate contact networks and high host densities. Individual susceptibility to parasites is determined by the individual’s genes and by the probability of exposure, as a direct result of the individuals’ location and function within the network. Social insects present an excellent study system for integrating social network dynamism and disease transmission. In this study we used *Temnothorax curvispinosus* ant colonies with artificially manipulated relatedness that were exposed to the entomopathogenic fungus *Metarhizium brunneum*. We used an ant network tracking system with individually color marked ants to produce spatial and temporal contact networks among high and low related colonies and with or without the parasite. From these data we extrapolated the effect of pathogen presence and within colony relatedness on the contact network structure, as well as the specific behaviors toward infected individuals that can lead to parasite transmission and parasite suppression.

P108

Characterization of bifidobacteria in the indigenous honeybee of Saudi Arabia

Mohammad Ansari, Ahmad Al-Ghamdi, Khalid Khan

Bifidobacteria were isolated from the intestinal tract of the indigenous honeybee of Saudi Arabia, *Apis mellifera jemenitica*, and investigated for potential application as a probiotic agent against some drug multi-resistant human pathogen, based on the findings of *in vitro* inhibition assays. A total of 07 bifidobacteria strains (designated as KsuBF1-KsuBF07) were isolated using a culture-dependent method and their 16S rRNA gene sequences were analysed. The KsuBF isolates belonged to three distinct bifidobacterial phylotypes that were similar to those found in the Japanese honeybee, *Apis cerana japonica*. Although the Saudi Arabian and Japanese honeybees are distinct species with different traits and habits, the observation that they share highly similar bifidobacterial phylotypes suggests that bifidobacteria are conserved among honeybee species. Despite having extremely high 16S rRNA gene sequence similarities, the KsuBF isolates had markedly different carbohydrate fermentation profiles. In addition, in vitro growth inhibition assays revealed that the cell-free supernatants of all KsuBF isolates exhibited antagonistic effects on drug multi-resistant *E. coli*, *P. aureginosa*, *B. subtilis* and *S. aureus* growth. These results indicate that the bifidobacteria isolated from the gut of indigenous Saudi Arabian honeybee could potentially be employed to produce some probiotics against some human pathogens. Keywords: Bifidobacterium, Saudi Arabian honeybee, drug multi-resistant human pathogens, probiotics

P109

Gamergate controls dopamine levels of workers in Diacamma sp.

Hiroyuki Shimoji, Hitoshi Aonuma, Masato S. Abe, Kazuki Tsuji, Toru Miura, Yasukazu Okada

Eusocial insects construct complex societies by local interactions among individuals. It has been suggested that the queen signal is a principle cue that can regulate the fertility of workers, establishing a reproductive division of labor. Traditionally, much effort has been devoted to identifying queen pheromones. Recently, some researchers have investigated the dynamics of physiological regulators, such as JH or biogenic amines, to reveal how the queen signal acts on worker's physiological conditions. *Diacamma sp*. is a queenless ant species. Colonies consist of a mated worker, called gamergate, and helper workers. The queen signal is transferred to workers by direct contact with the gamergate. Dominance interactions among workers occur frequently in large colonies or in colonies lacking a gamergate, indicating that the dominance interactions are also controlled by direct contact with the gamergate. Thus, the gamergate equalizes the physiological conditions of workers by physical contact. Although previous research indicated correlations between social rank and amine levels in other ant species, little is known about how such heterogeneity among individuals arises. To address the physiological basis of the heterogeneity of workers, we examined amine dynamics in worker brains in the presence and absence of queen signals. First, our results indicated that dopamine levels in dominants were higher than those in subordinates, whereas octopamine levels showed the opposite pattern in the colonies with the gamergates. Second, the dopamine level and variance increased within only 3 h of isolation from the gamergate, suggesting that the contribution of the gamergate to the regulation of dopamine levels in worker brains may be limited. Together with the network analysis of the dominance hierarchy, we further discuss the role of dopamine as the key factor in constructing the hierarchy.

P110

Genetic differentiation and structure within the Reticulitermes *species complex in Southwestern Europe: a multi-approach study to complete a speciation story*

Thomas Lefebvre, Edward L Vargo, Magdalena Kutnik, Marie Zimmermann, Simon Dupont, **Anne-Genevieve Bagneres**

Reticulitermes subterranean termites spread across South-western Europe for ages, but their distribution and their genetic relationships have evolved though several paleoclimatic and geological variations. Our study proposes to trace genetically and geographically the evolution of the Iberian species complex which has led to the separation of two lineages: *Reticulitermes grassei* and *R. banyulensis*. A large sampling area of 145 sites in Spain, Portugal, France and Morocco was investigated combining the analysis of diverse markers (cuticular hydrocarbon GC profiles, mitochondrial COI and COII sequences, ITS2 nuclear sequences and a panel of 10 microsatellite loci). Considered separately, each marker provided a fragmented picture of the evolutionary history of the two taxa at different time scales. Both mathematical analyses (ACP) of cuticular hydrocarbon profiles and phylogenetic analysis (BY, MP, ML, NJ) of mitochondrial and nuclear genes showed a clear separation between the *R. grassei* and *R. banyulensis*. In comparison to other European subterranean termites, these results would date their divergence back to possible vicariant events in the Late Miocene. However, the detection of intermediate chemical profiles and asymmetric mtDNA introgressions in some Spanish colonies suggests porosity in their genetic relationships. In fact, the current genetic structure of Iberian populations would be better explains by the isolation/dispersion alternations in Quaternary glacial periods. The genetic structure analysis of 15 localities accurately sampled along transects enabled the identification of the post-glacial colonization routes from southern Spain where heterozigosity is high to south-western France resulting in strong genetic bottlenecks after the passage across the Pyrenees. Finally, this study provides a nuanced answer to the question ‘have *R. grassei* and *R. banyulensis* reached species status?’. If the presence of a mosaic of mixed colonies in Spain suggests that speciation was not completed on the Iberian Peninsula, French populations were completely separated according to a parapatric model.

P111

Diversity of termites from the upper Madeira River region, Brazil

Tiago Carrijo, Rafaella Santos, Eliana Cancello

The Santo Antônio and Jirau hydroelectrics plants (HP) were recently built in the Madeira River region, in Porto Velho, Rondônia, Brazil. This region is unique, since the alignment of Amazonas-Madeira- Mamoré Rivers divides the Neotropical region into two areas of endemism for a diversity of taxa. This work aimed to monitor termites for three years in areas near the Jirau HP and two years in areas near the Santo Antônio HP, as well as conduct a community level study and analyze the spatial distributions of termites from the areas influenced by Jirau HP. Twelve modules were marked, seven on the left bank of the river and five on the right bank. Each module had 3 or 4 km transects and perpendicular parcels every 1 km. The sampling was conducted in subparcels of 5x2 m inside each main parcel. During 20 expeditions, 1121 subparcels were investigated and a total of 7875 termite samples were collected and identified to 169 species. For the community study, 20 subparcels were randomized in the six modules of the Jirau HP, with five subparcels marked in relation to distance from the river margin (P1-50m, P2-1km, P3-2km, and P4-3km). The termite species composition was not related to side of the river bank. The beta diversity analysis with termite species composition clustered for modules with the same soil type, suggesting that some termite species may be distributed accordingly to this variable. Parcels closest to the river were the most peculiar, both in terms of termite species composition and abundance patterns, with some species restricted to the P1 and others absent there. This study incorporates a greater sampling effort than ever employed by other published studies to date for a medium scale area, and registered one of the highest local termite diversity in some of the sampled modules.

P112

Molecular phylogeny and character evolution of Crematogaster inflata*-group*

Shingo Hosoishi, Munetoshi Maruyama, Kazuo Ogata

A recent molecular phylogeny of the genus *Crematogaster* reclassified some members of the former subgenera *Paracrema* and *Physocrema* into the *C. inflata*-group, comprising five species: *C. subcircularis*, *C. inflata*, *C. onusta*, *C. ampullaris* and *C. modiglianii*. Molecular analysis of the *Crematogaster inflata*-group and seven other species of former subgenus *Physocrema* (*C. aurita*, *C. difformis*, *C. mucronata*, *C. physothorax*, *C. sewardi*, *C. tanakai*, *C. vacca*) were investigated using a total of 1425 bp of sequence data from four nuclear protein-coding genes (long wave length rhodopsin, arginine kinase, carbamoylphosphate synthase, wingless). Monophyly of the *C. inflata*-group was strongly supported, but rejected for the former subgenus *Physocrema*. These findings suggest that a swollen propodeum and a circular-shaped metapleural gland opening are not synapomorphic characters for all members of the former subgenus *Physocrema*. Two morphologically distinct subgroups, the *C. difformis*-subgroup (C. *ampullaris*, *C. difformis*, *C. sewardi*, *C. tanakai*) and the *C. inflata*-subgroup (*C. aurita*, *C. inflata*, *C. onusta*, *C. physothorax*) were also supported by the molecular phylogeny. The *Crematogaster difformis*-subgroup is distinguished by a moderately swollen propodeum, and the *C. inflata*-subgroup by a strongly swollen propodeum. Phylogenetic analysis thus supports the loss of propodeal spines in these two independent lineages, and also indicated the reduction in the number of antennal club from four to three segments in C. mucronata. In addition, cytochrome oxidase I (COI) sequence data (711 bp) supports the species status of *Crematogaster tanakai*, which is unique in having a swollen yellow-colored head and was first discovered inhabiting the same nest with *C. difformis* (5.5 % COI divergence between the two species).

P113

Delimiting species: Phylogeny and taxonomy of the fungus-growing ant genus Sericomyrmex

Ana Jesovnik

All fungus-growing ants actively cultivate and eat fungus gardens grown on a substrate of organic material brought into their nests. *Sericomyrmex* is a poorly known genus of fungus-growing ants that is closely related to the leaf-cutting genera *Atta* and *Acromyrmex*, the dominant herbivores in Neotropical ecosystems. *Sericomyrmex* includes 22 described species and subspecies and is distributed throughout most of South and Central America, and belongs to ‘higher attine’ agriculture. In most cases, when poorly known taxa are subjected to increased scrutiny, particularly at the genetic level, cryptic diversity is revealed. In the case of genus *Sericomyrmex*, however, extensive field work across its entire range in Central and South America, morphological study of worker variation, and genetic data from 8 genes and three transcriptomes, all indicate that there may be far less species than the 22 described by previous authors. If this hypothesis is correct, it suggests that *Sericomyrmex* has achieved a wide geographic and biotic distribution (in savannahs, cerrados, and rain forests from Argentina to Mexico) with only a small degree of accompanying speciation, in contrast to most other ant species, including those in its similarly distributed sister taxon *Trachymyrmex*. Is the current geographic distribution of *Sericomyrmex* the product of a recent, rapid geographic radiation with minimal accompanying diversification? I describe my current efforts to delimit *Sericomyrmex* species by integrating multiple sources of information, including reconstructing a phylogeny based on >500 Ultraconserved Element (UCE) loci. I am also exploring ecological and behavioral data as sources of information about *Sericomyrmex* species boundaries, particularly nest architecture. Understanding the biology of *Sericomyrmex* species is important for reconstructing the origin and evolution of higher attine agriculture and for explaining the remarkable ecological success of the leaf-cutting genera *Atta* and *Acromyrmex*.

P114

The role of hybridization in shaping evolutionary divergence

Jonna Kulmuni

Speciation is not a linear process, but it can halt, reverse or even speed up by hybridization. Hybridization is not a mere reproductive dead-end, but recent examples show that it can be a major player in the process of speciation and can even introduce adaptive genetic variation. I have studied hybridization in the recently speciated *F. rufa* group wood ants. These species have diverged during the last 500 000 years and several species pairs putatively hybridize. My results show that hybridization between *F. aquilonia* and *F. polyctena* in Southern Finland has resulted in a bizarre situation, where hybridization is favored in the females but selected against in the males. Thus it seems that hybridization has complex consequences in these ants, which at the same time could promote and reverse the early stages of speciation. My current studies aim at identifying those genomic areas and possible genes that have been acquired through hybridization and cause antagonistic selection in haploid and diploid genomes. I have also identified putative hybrid populations between other *F. rufa* group species, which will be used to study the genetic basis of speciation and evolutionary consequences of hybridization. In contrast to their ecological dominance, ants represent little explored systems in the context of speciation. Yet, because of their haplodiploidy and sociality they offer unique advantages to study the genetic basis of species divergence.

P115

Genetic differentiation in populations of Nasutittermes corniger *(Isoptera) from Brazil*

Amanda Santos, Tiago Carrijo, Eliana Cancello, **Adriana Correa e Castro**

Termites are abundant and responsible for soil aeration, drainage and nutrient recycling in tropical and subtropical ecosystems and the genus *Nasutitermes* (Termitidae: Nasutitermitinae) is dominant in the Neotropical primary forests. Thus, the aim this work was to elucidate, using the mitochondrial gene 16S rRNA, the relationship between some populations of *Nasutitermes corniger* from north (upper Rio Madeira, RN), northeast (João Pessoa, PB), central (Corumbá, MS and Cáceres, MT), southeast (Jaboticabal, SP) and south (Castro, PR) Brazil, and compare them with samples from Ecuador, Suriname, Mexico and Central America, whose respective sequences were obtained from the Genbank. The results showed high genetic diversity in studied populations, a shown by the significant number of distinct haplotypes found (20 haplotypes to 62 sequences), plus an haplotypic diversity (Hd) of 0,897. Although shared haplotypes were found between populations from southeast and northeast Brazil and also between the central Brazil populations, broadly speaking, the species is genetically structured. Furthermore, population expansion events were detected in *N. corniger* samples from north Brazil, and also indication of stability through time, detected the bimodal curve Mismatch Distribution found in central Brazil samples and sequences from Genbank. Neighbor joining analysis found two groups: one composed by populations of north, central and south Brazil, and another group composed of the samples from southeast and northeast Brazil, Ecuador, Suriname, Central America and Mexico. In a phylogeographic analysis, restricted gene flow with isolation by distance was detected in the total cladogram analysis. These last results suggest that there are two dispersion corridors for *N. corniger*, mostly evidenced by the small genetic distances between the studied groups, together with the reduced haplotype sharing among their populations.

P116

Molecular characterization of Nasutitermes similis *and* Nasutitermes guayanae *using 16SrRNA*

Rullian Ribeiro, Tiago Carrijo, Eliana Cancello, **Adriana Correa e Castro**

The termite species *N. similis* and *N. guayanae* (Termitidae, Nasutitermitinae) are morphologically very similar, therefore requiring other means of taxonomic identification, like molecular markers. Thus, our aim in this study was to characterize, using the mitochondrial gene 16S rRNA*, N. guayanae* and *N. similis* samples collected in the upper Rio Madeira, in the Brazilian Amazon, and in French Guiana, together with *N. guayanae* samples from Trinidad and Tobago, obtained in Genbank. Six haplotypes were found in 38 sequences belonging to *N. guayanae*, and two haplotypes were found in 49 belonging to *N. similis*. The average genetic distance between the two species haplotypes was 0.017. The phylogenetic reconstruction using Bayesian evidence shown that the *N. guayanae* haplotypes from Brazil share a close relationship, as also the haplotypes from Trinidad and Tobago and French Guiana, pointing to a possible genetic structuration in the species. A similar genetic pattern was not found for *N. similis*, since the two haplotypes found by us belong to the same clade, showing a low genetic distance and a possible genetic homogeneity in the species. The relaxed molecular clock estimate time for the most recent common ancestor of the Brazilian samples of *N. guayanae* and *N. similis* is 1.86 my, a lesser time than the equivalent one found by us in the analysis of the clade formed by the *N. guayanae* samples from Trinidad and Tobago and French Guiana - 2,49 my. In conclusion: although morphologically very similar, *N. guayanae* and *N. similis* are very distinct genetically.

P118

Speciation of Cataglyphis *around the Gibraltar strait: vicariance or dispersal?*

Irene Villalta, Xim Cerda, Fernando Amor, Mike Jowers, Rapahael Boulay

The Strait of Gibraltar is a major geographical barrier separating the Iberian Peninsula from Morocco. After the Messinian salinity crisis and the closure of connection between the Altlantic ocean and the Mediterranean sea (5.9 - 5.3 mya), the strait has remained open, preventing many organisms from crossing. The genus *Cataglyphis* comprises more than 100 species inhabiting hot and desertic areas in Asia, Europe and North Africa. Nine species of *Cataglyphis* have been recorded in the Iberian Peninsula, none of which is known in North Africa. On the other hand, approximately 20 species of *Cataglyphis* are present in Morocco, none of which is present in Spain or Portugal. By means of molecular data (COI and nuclear genes) we examine the phylogenetic relationships between Moroccan and Iberian species in order to test various models of speciation by vicariance or dispersal. Data suggest the existence of cryptic species on both sides of the strait. Moreover, preliminary results suggest Iberian species (with the exception of *C. cursor* which may have dispersed from north-eastern populations) were formed by vicariance and rapid speciation following the closure of the strait.

P119

Seed preferences of the tropical fire ant, Solenopsis geminata *in Taiwan*

Yu-Chen Chen, **Wen-Jer Wu**, **Li-Chuan Lai**

Solenopsis geminata was introduced into Taiwan many years ago, and is now widely distributed in Central and Southern Taiwan. Because of their granivorous habit (dyszoochory), *S. geminata* workers are observed to attempt harvesting seeds from numerous plant species. This study was conducted by excavating fire ant nests to find seeds harvested by fire ants. A total of 37 seed species in 12 plant families were collected from four counties (Taichung, Yunlin, Chiayi and Tainan) in Taiwan. *S. geminata* preferentially harvested herb seeds, especially the Poaceae including 16 species. Three species of seeds from tree and shrub were found in the nests. They are *Broussonetia papyrifera*, *Flueggea virosa* and *Solanum torvum*. A large quantity of the seeds collected in Taichung is *Panicum maximum*, collected in Yunlin are *Chamaesyce hirta*, *Eleusine indica* and *Paspalum conjugatum*, collected in Chiayi are *Pouzolzia zeylanica*, *Chamaesyce hirta* and *Dichanthium annulatum*, and collected in Tainan are *Amaranthus patulus, Dactyloctenium aegyptium* and *Digitaria sanguinalis*. We performed removal experiments to study preferences of *S. geminata* for seeds comprising ten species (seven grasses and three forbs) in Taichung and Chiayi counties. The results showed that grass seeds, such as *Dichanthium annulatum, Panicum maximum,* and *Paspalum orbiculare* were readily removed by fire ants. In contrast, fire ants appear to less prefer seeds from forbs, such as *Chamaesyce hirta*. Seed weight seemed to influence the ant's choices. Seeds above 0.56 mg were harvested preferentially, but 0.07-0.43 mg seeds were rarely retrieved. This study suggests that seed preferences of *S. geminata* may have impacts on the abundance and composition of the plant community, especially grasses in fields infested with *S. geminata* in Taiwan.

P120

Excluding ants reduces herbivory on male Adriana quadripartita plants

Kieren Beaumont, **Molly Whalen,** Duncan Mackay

Here we report the results of an experiment in which ants were excluded from extra-floral nectaries (EFNs) of male and female plants of the dioecious species *Adriana quadripartita* (Euphorbiaceae). The study was conducted on Torrens Island, South Australia and the abundance of ants and herbivores as well as the level of foliar herbivore were assessed. The abundances of ants and invertebrate herbivores were overall greater on male than on female plants. The exclusion of ants from branches resulted in an increase in herbivore abundance on male and female plants, however ant exclusion resulted in an increase in foliar damage only on male plants. While previous research on adrianas has shown that ants can decrease herbivore abundance, these results provide the first evidence that EFNs in adrianas can function to decrease foliar herbivory. More generally, our studies of *Adriana* taxa demonstrate the presence of extensive geographic variation in almost all aspects of the interaction between ants, plants and herbivores. This variation suggests that defensive associations between ants and adrianas are loose and facultative, and that the efficacy of ant defense in *Adriana* populations is likely to be contingent on the particular combination of abiotic conditions and ant and herbivore faunas that are present in a given population.

P121

Correlates of rarity and fitness in UK bumblebees.

Sarah Rustage, Jonathan Ellis, Richard Billington, Mark Brown, Mairi Knight

As rare species tend to exist in more isolated, scattered populations than common species, theory predicts that they may experience reduced gene flow and population size, and as a result be vulnerable to the combined effects of drift and inbreeding. The subsequent reduction in genetic diversity may cause increased susceptibility to disease and parasitism. In recent years, many native UK bumblebee species have suffered significant population declines and reductions in range due to changing land use and habitat loss, with three species becoming extinct. Given their key role as pollinators, particularly in temperate ecosystems, it is vital to understand the possible effects that such declines may have on future population viability. This project aims to assess whether there is evidence of population structuring and reduced genetic diversity in a declining bumblebee species (*Bombus monticola*) when compared with an abundant and stable species (*B. pratorum*). The possible implications for fitness are assessed by measuring two components of the innate immune response; phenoloxidase (PO), an enzyme which plays a crucial role in melanisation, the process responsible for rapidly neutralising pathogens, and antimicrobial peptides (AMPs) which circulate in the haemolymph and provide a long-term antibacterial response. These results are compared with quantification of the parasite load to give an indication of overall fitness. The implications of the results for practical conservation applications are then discussed.

P122

Rapid expansion of range and population in the social-parasitic hornet

Katsuhiko Sayama, Jun-ichi Kojima, Fuki Saito-Morooka, Shun'ichi Makino

Vespa dybowskii is the only socially-parasitic species in the hornet genus *Vespa*, and its known hosts are *V. crabro* and *V. simillima*. Overwintered *V. dybowskii* queens are unable to initiate a nest, but they usurp a host hornet nest just after the emergence of the first brood workers of the host (Matsuura, 1995). Once a *V. dybowskii* queen has succeeded in usurpation, she utilizes host workers to produce her own workers. The host workers are gradually replaced by the *V. dybowskii* workers. *Vespa dybowskii* had been considered a rare hornet species in Japan and was found in mainly mountainous areas in the northern half of Honshu (main island in Japan) and Hokkaido (northern island in Japan), with records from 17 prefectures by the early 1990s (Matsuura, 1995). Since the 2000s, however, reports of the occurrence of *V. dybowskii* have shown a dramatic increase in areas new to *V. dybowskii*, exhibiting marked expansion in the westward range. By 2013, *V. dybowskii* was recorded in nearly all the prefectures in Honshu. While the recorded hosts were nearly always *V. crabro* about 60 years ago (Sakagami & Fukushima, 1957), recent host records show that more than half of the host-identified nests originally belonged to *V. simillima*. The population sizes of *V. simillima* have recently increased in urban areas, whereas those of *V. crabro* have decreased in various regions in Japan. These findings suggest that a possible switch in the principal host from *V. crabro* to *V. simillima* is one of the factors underlying the rapid range expansion and increase in the population size of *V. dybowskii.*

P123

Spatio-temporal pattern of ants (Hymenoptera : Formicidae) in an oil palm ecosystem

Noor Ain Shaari, Wan Zakhir Wan Nazri,Faszly Rahim

This study was conducted to identify the differences in abundance of ants and the spatio-temporal pattern in palm oil ecosystem in Endau Rompin, Pahang. Sampling was carried out for four consecutive months on three types of soil: clay, deep peat and shallow peat. Pitfall trapping was used as it is suitable because most of the Formicidae forage on the ground. A total of 3,968 individual ants were captured consisting 13 genera in four subfamilies. *Anoplolepis* were the most abundant with 1,692 individuals (45.75%) followed by *Pheidole* (862 individuals; 23:31%) and *Paratrechina* (228 individuals; 6.17%). In clay, *Anoplolepis* is the most abundant meanwhile in deep and shallow peat, *Pheidole* have the highest number of individuals compare to other genera. There were significant differences ($p < 0.05$) in the abundance of the eight genera which are *Anoplolepis*, *Oecophylla, Paratrechina, Odontomachus, Ponera, Crematogaster, Pheidole*, and *Tetramorium* in the interaction between soil, months and genus. In general, Subfamily Formicinae showed clustered pattern of distribution ($Ia > 1$) while, Subfamily Ponerinae, Myrmicinae and Aenictinae showed random distribution pattern ($Ia < 1$) in the sampling plots.

P124

Contribution of insect pollination to macadamia and coffee in Hawaii

Jane Tavares, Mark Wright, Ethel Villalobos, Don Drake

Apis mellifera (the honeybee) is accepted to be an important pollinator in many agricultural crop systems in Hawaii; however, its contribution to pollination, along with other insect visitors, has not been determined for macadamia (*Macadamia integrifolia*) and coffee (*Coffea arabica*) orchards in Hawaii. Several aspects to determine pollinator contribution in a macadamia orchard and coffee orchard were measured: 1) species richness and abundance of insects visiting flowers in each orchard, 2) the effects of insect pollination in regards to fruit set, fruit retained, fruit size, and weight, and 3) pollen removal or transfer efficacy based on number of pollen grains an individual insect removed from or transferred to the stigma while foraging on a macadamia or coffee flower. Results concluded that while the order Diptera was highest in richness, *A. mellifera* was the most abundant species vising the macadamia and coffee flowers. Flowers pollinated by insects resulted in higher fruit set, retention, and yield compared to flowers excluded from insect visitation. Coffee bean size and weight was increased with insect pollination. Abundance, coupled with foraging behavior and stigma contact suggested that honeybees were the greatest contributors to macadamia nut and coffee pollination over the other insects observed in the study orchards.

P125

Can transposons drive genomic mosaicism between castes in Solenopsis invicta*?*

Ni-Chen Chang, John Wang

Transposons are expressed highly in neural tissues and drive transcriptomic heterogeneity in the brains of humans and *Drosophila*. The neural diversity in the brain caused by transposon insertions might contribute to behavioral differences between individuals. Whether such behavioral differences are beneficial is hard to evaluate in solitary species. However, testing this in social species may be possible. In ants, females are divided into two castes: queens who reproduce and workers who perform tasks such as nursing larvae, foraging for food, and defending the nest. Because colony efficiency is determined in part by the efficient partitioning of labor among workers, it may be beneficial to have a more behaviorally diverse work force. Thus, we hypothesize that transposon gene expression would be higher in the brains of workers than in queens. We are using the fire ant (*Solenopsis invicta*) to test this possibility. To date, we have compared transposon expression in the brain between virgin queens and workers from one colony using Illumina RNA-seq. Our initial analysis has revealed 2,029 putative transposons expressed out of 95,599 total unique transcripts (2%). Of these, 917 of the transposons potentially exhibited differential expression between castes, although the total expression of all transposons between the two castes was not significantly different. The major expressed classes of transposons in both castes were DNA transposons of the P-element and mariner families. Currently, to obtain more power we are conducting more biological repeats to determine whether transposon expression differences exist between the two castes.

P126

The queen-worker olfactory interaction in the fire ant Solenopsis invicta

Viet Dai Dang, John Wang

The two types of social organization, monogyny and polygyny, in the fire ant (*Solenopsis invicta*) are known to be fully associated with a single gene, Gp-9. In contrast to the genetic homogeneity of all individuals in monogyne colonies (all Gp-9BB genotype), queens of polygyne colonies have the Gp-9Bb genotype while their workers have either the Gp-9BB or the Gp-9Bb genotype. Recently, the Gp-9 gene has been found to be a part of a big supergene. Although the supergene holds the potential for explaining the many characteristics associated with social form in fire ant, the precise relationships between genes and phenotypes are still unclear. One of the interesting fire ant behaviors is the ability of Gp-9Bb workers to distinguish and accept only Gp-9Bb queens into polygyne colonies based on odor cues. We are using a transcriptomics approach to determine what gene products expressed in workers may be responsible for sensing the queen odors. We are currently profiling gene expression in the antennae and brains of different castes and genotypes of the two social forms by using Illumina RNA sequencing. We will present our preliminary results and analyses.

P128

Honeybees' physiological and behavioural immunity deficit induced by DW viruses

David Baracchi, Gennaro Di Prisco, Valentina Gentili, Francesco Pennacchio, Stefano Turillazzi

Honeybee societies are formed by thousands of individuals which incessantly interact in the sheltered nest space. As a consequence they are exposed to risk of epidemics and are often associated with high infection levels of viruses, bacteria and fungi. As a result of co-evolutionary dynamics between parasites and host, honeybees have evolved several adaptations to face the increased risks of epidemic diseases. One of the most important defence is represented by antimicrobial compounds present either in hemolymph and venom. In particular, the application of venom on the body surface and on the wax of the comb as a way of protection against pathogens has been suggested for *Apis mellifera*. Nevertheless, whether the expression of melittin (a strong venom antimicrobial peptide) and its presence on the cuticle is affected by ongoing infections is still under debate. For example, the quantity of melittin on the cuticle might be actively increased by bees to counteract an incipient infection. By contrast, this defence might be weakened or neutralised by the infection itself or it might follow both trends depending on the pathogen load. Here we described the ontogeny of the chemical shield on the cuticle in both healthy and deformed wing virus infected bees and we studied the relationship between the synthesis and the use of antimicrobial peptides, mostly melittin, and the DWV titer. RT-PCR, qRT-PCR and MALDI-TOF Mass Spectrometry analyses were used to quantified the viruses' genome copies, the transcriptional level of melittin and the amount of melittin on the cuticle in healthy, naturally and artificially infected bees. Behavioural experiments showed how the grooming behaviour is responsible for the presence of the venom antimicrobial layer on the cuticles of healthy bees and the way DWVs affect the immune system either at the behavioural and the physiological level.

P129

How do gall-forming social aphids keep their closed nest clean?

Mayako Kutsukake, Xian-Ying Meng, Noboru Katayama, Naruo Nikoh, Harunobu Shibao, Takema Fukatsu

Waste disposal is one of essential tasks for sustaining social insect colonies. In aphids, specialized castes called soldiers perform both colony defense and gall cleaning in open galls. However, some social aphids form completely closed galls, wherein hundreds to thousands of insects grow and reproduce for several months in isolation. Why these social aphids are not drowned by accumulated honeydew has been an enigma. *Nipponaphis monzeni* forms completely closed galls on *Distylium racemosum* trees. The mature galls often contain over 2,000 individuals, but surprisingly, no accumulation of honeydew is observed in galls. The aphids did excrete honeydew, because when they were placed on an artificial feeding system, a number of honeydew droplets appeared around the insects. These observations suggested that the excreted honeydew is removed from the gall cavity in some way. To clarify this mystery, we examined the possibility that honeydew is absorbed by the inner gall wall. Field experiments revealed that water is absorbed by the inner gall wall efficiently and removed via the plant vascular system. On the other hand, in open galls of *Tuberaphis styraci* formed on *Styrax obassia* trees, no water absorption was observed. The inner wall of the closed galls was remarkably different from that of the open galls in hydrophobicity and ultrastructure: hydrophilic inner wall surface covered with a thin, reticular and spongy layer in closed galls, in contrast to hydrophobic inner wall surface covered with a thick wax layer in open galls. These properties were also observed in congenic aphids, *Ceratovacuna japonica* and *C. nekoashi*, which form open and closed galls on the same *Styrax japonicus* trees, respectively. These results indicate that the water-absorbing property is a novel plant phenotype caused by the aphid’s manipulation of plant morphology and physiology, which can be regarded as ‘indirect social behavior’ of the social aphids.

P130

Fortress defense in social aphids

Sarah P. Lawson, Andrew Legan, Patrick Abbot

Defendable nests are a crucial resource for protection of the group and offspring rearing. This form of sociality, referred to as 'fortress defenders' has been convergently found in divergent taxa from thrips to snapping shrimp. Many of these fortress defenders have evolved specialized means of defense, including some aphid species. First-instar nymphs of the social aphid, *Pemphigus obesinymphae*, an aphid that forms galls on the petioles of poplars, do not have special morphological features, but still act as 'soldiers' to defend their maternal group from predators. Here, we first demonstrate that *P. obesinymphae* soldiers exhibit effective defense by using their style to attack and, even, kill natural predators. A closely-related non-social species, *Pemphigus populi-caulis* does not exhibit a comparably defense. This suggests that closely-related species differ substantially in the ability to deter predators. Secondly, because attack by soldiers appears to trigger a melanization response in the surrogate predator (*Drosophila*), we investigated the possibility that aphid soldiers induce an immunological-like response in victims. Using qPCR, we compared expression levels of multiple genes from the IMD, Toll and melanization pathways in *Drosophila* that had been attacked by *P. obes* soldiers, to control *Drosophila* placed in an empty gall. To further quantify the melanization response, we are using a subjective assay to compare the melanization response in multiple *Drosophila* lines, including lines with mutations in either the Toll, IMD or melanization pathway. These results will be the first to quantify this soldier behavior in social aphids and begin to explore how these aphid soldiers are able to kill natural predators.

P131

Role of JH in the sex-biased termite soldier differentiation

Kiyoto Maekawa, Shutaro Hanmoto, Dai Watanabe, Kouhei Toga, Toru Miura

Termite soldier is the peculiar caste among other social insects, in terms of its morphology and social function. Generally in termites, soldiers are differentiated from workers via an intermediate state, presoldier. Soldiers are sterile, and have species-specific defensive morphologies. In the basal lineages, sex ratios of soldiers are either equal or only slightly biased. In the apical lineages (family Termitidae), however, there are many species that have soldiers with strongly biased sex ratio. For example, soldiers are all males in most of the examined species in the Nasutitermitinae, while they are females in most species in the Termitinae and Macrotermitinae. High juvenile hormone (JH) titer is required for the soldier differentiation, so that the strongly biased soldier-sex ratio might be caused by the differences of JH titers between male and female workers. To clarify this hypothesis, we focused on the Japanese nasute termite *Nasutitermes takasagoensis*, in which there are three worker types (male-minor, male/female medium, and female-major workers), and male-minor workers molt into presoldiers/soldiers in natural conditions. First, we tried to artificially induce presoldiers from three worker types, according to the previously performed method. Presoldier molt was induced from all worker types, although the induction rate was significantly higher in male-minor workers than in other worker types. Second, JH titers of each worker type were quantified by high performance liquid chromatography-mass spectrometry. The measurements performed in two different seasons (April and December) showed that JH titers in male-minor workers were consistently higher than those of other worker types. These results revealed that male-minor workers maintain JH titers at a high level throughout a year, and this may cause the male-biased presoldier differentiation. Expression analyses of some JH related genes will also be introduced, and the proximate mechanisms on the sex-biased soldier differentiation will be discussed.

P132

Experimentally evolved trypanosome: infection success and virulence in the bumblebee

Monika Marxer, Paul Schmid-Hempel

The outcome of host-parasite interactions is strongly affected by several factors including host and parasite genotypes. Selection on basic growth properties in parasites may therefore have far reaching consequences for infection outcome and the consequences for host fitness. It is know that strains of the trypanosome *Crithidia bombi* infecting bumblebees have widely varying growth rates when cultured in vitro. We succeeded in experimentally evolving this parasite in vitro, selecting for fast and slow growing sub-lines. This enabled us to investigate the costs, benefits and fitness trade-offs related to parasite growth rate by subsequently measuring in vivo infection profiles, host immune response, and competitive ability under co-infection. These results will help define the fitness consequences for the observed natural variation in the growth of *C. bombi*, which has many ramifications for a social insect host. This will, more generally, also inform important aspects of host-parasite evolution including the evolution of virulence and host-defense mechanisms.

P134

Ant colonies prefer nest sites containing infectious corpses

Luigi Pontieri, Svjetlana Vojvodic, Riley Graham, Jes Pedersen, Timothy Linksvayer

The nesting site of social insect colonies determines local resource availability as well as exposure to competitors, predators, and pathogens. During colony relocation, nest site selection involves exploration and assessment of potential sites followed by colony movement on the basis of a collective decision making process. Nest hygiene and pathogen load are predicted to be factors ant scouts evaluate, given the high risk of epidemics in group-living animals. Some invasive ant species may experience particularly high selection pressure posed by pathogens due to the limited genetic variation and unicolonial social system characterizing their introduced populations. The well-known Pharaoh's ant (*Monomorium pharaonis*) is a tramp species that shows life-history traits and population structure that makes it a good candidate to test the ability of invasive ants to detect and avoid infected nests during colony emigration. Surprisingly, when presented with the choice of a nest containing nestmates overgrown with sporulating mycelium of the entomopathogenic fungus *Metarhizium brunneum* (infected nest) and a nest containing nestmates killed by freezing (uninfected nest), experimental colonies preferentially moved into the infected nest (84%, $P < 0.001$). Colonies did not show a significant preference when presented with the choice of an empty nest with no corpses and an infected nest (38%, $P = 0.229$), neither between an empty and an uninfected nest (63%, $P = 0.143$). While we cannot rule out the possibility that this is a case of pathogen manipulation, we propose that the overall preference for infected nests we observed is an adaptive strategy operated by the host to 'immunize' the colony against future exposure to the same pathogen.

P135

Venom toxicity and deployment method as means of biotic resistance

Fabian L. Westermann, Tappey H. Jones, Lesley Milicich, Philip J. Lester

Venom could play an essential role in invasions and give the invader a substantial advantage against native species. However it might also support biotic resistance against an invader, if native species are venomous. One ant genus that has repeatedly been reported to successfully withstand other competitively strong ant species is *Monomorium*. We tested whether the co-occurrence patterns of four *Monomorium* species with the invasive Argentine ant, could be related to venom toxicity, deployment method or behavioural interactions. We analysed Argentine ant survivability when treated with a range of venom concentrations from four *Monomorium* species and compared them with mortality rates in arena fights between *Monomorium* and Argentine ant workers. Venom toxicity was not directly linked with co-occurrence patterns in the field and survival in arena fights did not meet the expected correlation with venom toxicity. We further investigated how venom is utilized by different *Monomorium* species, since using the venom as a repellent to keep hostile workers at a distance might bear less risk of injury than physically engaging them. We found that three of the tested *Monomorium* species displayed significant variation in venom usage depending on increasing numbers of Argentine ant workers they encountered in the arena fights. Lastly we explored how aggressive interactions influence the survival rate of the species engaging Argentine ants. We found significant differences in worker mortality for *Monomorium* and Argentine ant workers, depending on the *Monomorium* species. High levels of aggression were displayed by the species which have not been found to co-occur in the past. We conclude that the three factors toxicity, venom utilization and aggressiveness towards an invader play a role in forming ant communities and influence the establishment success of an invasive species.

P136

Conditional mutualism emerges from a largely antagonistic species network

Rachelle M. M. Adams, Joanito Liberti, Anders Illum, Tappey Jones, David Nash, Jacobus Boomsma

Collective decision-making characterizes all advanced social insects and is particularly important for the acquisition and defense of nests. The outcomes of such collective decisions are usually adaptive for colonies, even though they are based on many binary individual decisions. Here we report on an unusual conditional decision-making system involving three antagonistic ant species: A *Sericomyrmex* fungus-farming host ant, a parasitic *Megalomyrmex* guest ant producing alkaloid venom, and a raiding *Gnamptogenys* agro-predator. We show experimentally that a complex conditional mutualism is mediated through simple pairwise interactions at both individual and colony levels. We treat the system as a symbiotic species network (host ant-fungus-social parasite), building from interaction trials with few individuals to a colony level choice experiment. The guest ants effectively protect their host colonies against agro-predator raids because their alkaloid venom is more potent than the biting defenses of the host ants. Relatively few guest ants are sufficient to kill raiders that invariably exterminate host nests without guests. We also show that the odor of guest ants discourages raider scouts from recruiting nestmates to host colonies. Our results imply that *Sericomyrmex* fungus-growers obtain a net benefit from their costly guest ants behaving as a functional soldier caste to counter agro-predator raiders (www.megalomyrmex.com/videos). The different life histories of the agro-predators and guest ants can facilitate their coexistence in a negative frequency dependent manner. Because a guest ant colony is committed to exploit a single host colony for life, the guests would harm their own interests by not defending the host. Cooperation is thus enforced by external threat according to the principle that the enemy of my enemy is my friend. This conditional mutualism is analogous to episodes in human history when mercenary city defenders offered net benefits or imposed net costs, depending on the level of threat from invading armies.

P137

Pheromone-based collective navigation in the ant Paratrechina longicornis

Ehud Fonio, Ofer Feinerman

Similar to many other social insects, *Paratrechina longiccornis* ants rely on pheromone scent marking in order to perform collective behaviors such as trail formation and cooperative transport. To date, there were only a few attempts to visualize the dynamics of pheromone marking. Such studies typically rely on the questionable assumption of continuous markings based on the positions of the ants and not on actual marking behavior. We have developed a method that allows us, to our knowledge for the first time, to spatially and temporally pinpoint pheromone scent marks deposited by individual ants over areas that are more than 30,000 fold greater than their size. We measure scent marks from single ant trajectories and use them to assemble a continuously updated scent map. We then analyze the relations between this dynamic map and the kinematics of adjacent ants. We have found that the ants perform highly efficient moment-to-moment collective navigation. In this process, scent marks can be viewed as a means of translating individual information regarding a suggested route into pheromonal messages that are accessible to other ants. This mechanism allows for navigation that, rather than following static predetermined trails, breaks new ground as it advances. This new example of collective navigation is an addition to our growing knowledge on swarm intelligence.

P138

Competing for limited numbers of individuals in quota-driven decisions.

Mary Myerscough, Timothy Schaerf, James Makinson, Madeleine Beekman

Many collective decisions are essentially competitive processes. Politicians compete for voter's ballots; shops compete for customers who may implicitly decide a business should close when they fail to patronize it; individual choices in vertebrates are arrived at as neurons in the brain compete to be active; and honeybees choose a home as scouts that support each potential nest site compete to attract other scouts to become committed to their choice. Each of these potential choices made by voters, customers, neurons or bees are finalized when the number of individuals that support a particular option rises above a particular threshold. The speed and accuracy of a decision may potentially depend on the number of options available and whether the pool of individuals that collectively make the decision is large or small compared to the threshold that the decision requires. We will examine threshold-determined decisions in the context of house-hunting honeybees. We will first explore the effects of the number of available scouts with a simple model before presenting experimental results from large and small swarms. Although these results do not at first sight agree with the predictions of the model, further exploration shows that the idea of 'competing for scouts' is valid for honeybee decision-making and has implications for other systems too.

P139

Worker aggregation leads to collective chamber excavation in leaf-cutting ants

Daniela Romer, Isabel Reuter, Flavio Roces

Leaf-cutting ant nests consist of a complex system of tunnels and chambers that are excavated through a self-organized process. It is an open question, what the excavation rules are that underlie the emergence of either nest chambers, which are rounded and spacious, or tunnels, which are long and narrow. We examined whether worker aggregation at a digging site, i.e. the spatial distribution of workers, guides tunnel and chamber emergence in the leaf-cutting ant *Acromyrmex lundi*. In the laboratory, worker groups could excavate for 48 h in a clay arena. Differences in the extent of aggregation were achieved by presenting workers with either a small or a large available space as the starting point for excavation. The excavated chamber and tunnel volumes were measured, and aggregation was quantified during the excavation process using serial photography. When worker aggregation was dense because of the reduced available space, most of the digging activity was allocated to chamber enlargement, with less and delayed tunnel excavation. When aggregation was sparse because of the larger available space, ants only slightly enlarged the chamber, but excavated more tunnels, which were initiated earlier. The number of workers inside the nest structure was similar despite differences in initial available space, indicating that ant density, which is known to positively influence digging activity, was low when a large space was initially offered. It is suggested that available space determines the magnitude of worker aggregation at an excavation site, which in turn guides the excavation of chambers. These are enlarged until the actual worker density diminishes beyond a given threshold value, resulting in tunnel excavation.

P140

The role of olfactory cues in leaf-cutting ant waste management

Daniela Romer, Flavio Roces

Waste management plays an important role in leaf-cutting ant colonies because of the susceptibility of their symbiotic fungus to contaminants and pathogenic fungi. Workers continuously remove pieces of exhausted and infected mycelium from their fungus chambers. But where to dispose of the colony waste? We investigated whether leaf-cutting ants try to avoid the disposal of refuse near their symbiotic fungus, and whether volatiles arising from both the refuse and the fungus are used as orientation cues for waste disposal. A subcolony of *Atta laevigata*, consisting of a fungus garden and inhabiting workers, had access to a circular arena with a fine-meshed bottom, so that workers could perceive volatiles coming from below. In the middle of the mesh, a small amount of waste was placed. A round container located below the arena was divided into two half-rounds filled with fungus or waste as odour sources. We performed three series offering the following odour sources: fungus vs. empty, waste vs. empty, and waste vs. fungus. After 3 h we measured how much of the waste initially placed in the circular arena was relocated towards one of the sides exposed to the volatiles. Ants did not remove the waste material when exposed to fungus volatiles coming from below as far as the alternative side contained no odour source. However, when waste was presented as odour source, significantly more waste was relocated towards that side. Our results indicate that workers use the waste odor as an orientation cue to decide where to dispose of the refuse, which would lead to massive waste accumulation at an already established place within the nest.

P141

Predator's odor influences aggressive behavior of Reticulitermes termites *in competition tests*

Christophe Lucas, Lauriane Lefloch, Simon Dupont, Jean-Philippe Christides, **Anne-Genevieve Bagneres**

Hydrocarbon compounds (CHCs) are well known to be involved in numerous communication processes in insects and are particularly involved in recognition and social organization of social insects. The presence of CHCs in near environment are clues potentially used to assess predators or competitors. We investigated to know if the presence of the predator's odor (*Lasius niger*) influence several traits like caste ratios, foraging strategies, chemical signatures and survival rates of two species of termites (*Reticulitermes flavipes* and *R. grassei*). To do so, CHCs from 50 *Lasius niger* workers were extracted and added to a filter paper transferred into termite nests, along with nutritive filter paper disks. 350 termite workers per nests were reared during two months with the predator's odor or the control odor (solvent). After 2 months, several social traits were measured and the aggressive behavior was recorded. We showed that the presence of chemical extracts of the predator ant did influence the aggressive behavior of termites in intraspecific competition tests. Individuals submitted to the predator extracts survived more than individuals submitted to controls in both species.

P142

Novel insights into the ontogeny of Polistes *nestmate recognition*

Lisa Signorotti, **Federico Cappa**, Patrizia d'Ettorre, Rita Cervo

Polistes paper wasps have been used as a model for studying nestmate recognition mechanisms. It is generally believed that each wasp learns the olfactory recognition cues from the paper of its natal nest during a strict sensitive window, namely the first few hours after emergence. Indeed, nest material possesses the same chemicals of the colony inhabitants, providing information about colony membership, and it is therefore considered to be the primary source of cues for the acquisition of the referent template. Nonetheless, the ontogeny of nestmate recognition has been experimentally tested only in three American species, out of more than 200 species belonging to the genus, and then generalized for all *Polistes* species, without direct experimental evidences. Different *Polistes* species, however, experience different colony kin structures and different ecological pressures that could somehow affect the nestmate recognition mechanism. To date, there is no evidence that in the Mediterranean species *Polistes dominula* such mechanism follows the same rules. Here, we experimentally show that workers of *P. dominula* are able to develop correct nestmate recognition abilities even in absence of what have been so far considered the necessary cues. *P. dominula* workers were exposed for the first four days of adult life to paper fragments from their natal nest, or from a foreign conspecific nest or to a neutral condition, without nest material. Wasps were then transferred to their original nests where recognition abilities were tested. Our results show that *Polistes* wasps do not necessarily use nest material as a source of recognition cues to be leaned during the early stages of adult life, and therefore provide a novel perspective for the study of ontogeny of nestmate recognition processes in *Polistes* genus and in other social insects.

P143

First evidence of a chemical call-for-help in Cataglyphis cursor *ants*

Elise Nowbahari, Pyrène Rieu, Alain Lenoir, Jean-Luc Durand, Karen L Hollis

Previously we have shown that when *Cataglyphis cursor* ants become trapped and are unable to extricate themselves, nestmates engage in highly sophisticated pro-social behavior, namely rescue behavior, performing two novel behavioral patterns that require them to recognize what, exactly, holds the victim in place. That is, when sand digging and limb pulling, two well-known forms of rescue in ants, did not result in release of victims ensnared with nylon thread and partially buried beneath the sand, rescuers next began to transport sand away from the snare and to direct their behavior to the snare in particular, biting and tugging at the snare itself. To examine the role of chemical signals in victims' call for help, we performed biochemical analyses of released substances by distressed ants, using Solid Phase Micro Extraction (SPME), spectrum-mass chromatography of gland contents, and in vivo application of gland extracts. Here we present the first results of these analyses, which reveal not only the presence of specific volatile components involved in victims' call for help, but also the quantitative and qualitative changes in these substances over the course of two hours, a time period that corresponds to the performance of sustained rescue behavior observed under controlled laboratory conditions.

P144

Ant olfactory receptors underwent dramatic expansion and positive selection

Eyal Privman, Julien Roux, Laurent Keller

One of the gene families that stood out in first analyses of ant genomes was the olfactory receptor (OR) gene family, receptors that bind specific ligands and thereby trigger a response to specific odors. These receptors are responsible for the identification of chemical signals, a major component of communication in ant societies. In contrast with other sequenced insects that contain between 50 to 250 OR genes, ant genomes have around 400. This dramatic expansion is a strong indication of adaptive evolution and evolutionary innovation. Duplicated ORs could evolve specificity to new ligands and expand the olfactory vocabulary of ants (neofunctionalization). Alternatively, an OR that binds several similar compounds could be duplicated to evolve a specific OR for each compound (subfunctionalization). Both processes are expected to create the signature of positive selection if the new functions are adaptive. To look for positive selection in ant OR sequences I applied the branch-site test on each branch of the gene tree. The large numbers of genes in each species is challenging for positive selection inference and required taking several measures: I included only genes of two ant species (*Linepithema humile* and *Pogonomyrmex barbatus*) and one outgroup species (the wasp *Nasonia vitripennis*), a total of 873 genes. I used coding sequences that were manually annotated by Hugh Robertson because automatic annotation tools perform poorly on the rapidly evolving OR genes. I had to split the full gene tree to 16 subtrees of less than 100 sequences each, so that the branch-site test could be applied. The results show extensive positive selection throughout the gene tree. 277 branches (23%) displayed a significant signal of positive selection. Some subfamilies show a significant enrichment of positively selected branches, which makes these ORs good candidates for future studies into the evolution of chemical communication in ant societies.

P145

Heterocolonial interactions in a neotropical ant

Matilde Sauvaget, Chantal Poteaux, Nicolas Châline, Gabriela Perez-Lachaud, Jean-Paul Lachaud

Social recognition in insects is performed by the comparison between a perceived chemical signature and a memorized template. This determines the acceptation of an individual as a nestmate or its aggression as a stranger and so, only nestmates are allowed to get into the nest. Nevertheless, parasits or cleptobionts (thieves) sometimes perform entrance into host nests. Such behavior is frequently observed in the ant Ectatomma ruidum: some workers are performing thieveries of food into neighboring nest (intraspecific cleptobiosis). Moreover, those thieves often gain entrance without any aggressive behavior of the workers from the target nest. Thus, this frequent phenomenon could be explained if a modulation of the nestmate recognition exists in this species. One hypothesis is the ‘dear enemy effect’ in which aggression of non-nestmates depends on the distance between the two nests: close neighbors are less aggressed than distant conspecifics. This effect can rely on different mechanisms such as proximity of colonial odors, related to environmental elements or genetic relations, or habituation by learning. To investigate these mechanisms, we tested the relations between close and distant nests with several methods. We explored the behavioral responses of workers by analyzing dyadic encounters, we measured the chemical distances and the relatedness between nests. These tests were carried out after field observations allowing us to know effective behavioral and cleptobiotic relations between neighboring colonies. All the results give a better insight of nestmate recognition and the impact of neighboring relations.

P146

Co-evolutionary adaptation of Phengaris nausithous *and its host* Myrmica rubra

Gaetano Solazzo, Karsten Seidelmann, Robin F. A. Moritz, Josef Settele

Caterpillars of the lycaenid butterfly *P. nausithous* are obligate myrmecophiles and need to be adopted into *M. rubra* colonies to complete their development. The adoption and the integration of *P. nausithous* larvae into the host nests strongly depends on chemical mimicry. To screen for host-parasite co-evolution, several *M. rubra* populations with and without *P. nausithous* were chemically, behaviourally and genetically assayed. Chemical extracts using four different solvents were tested in behavioural choice assays to identify compounds which the parasite might use to mimic the host larvae. The pheromonal activity of tetracosane, the major compound found in behaviourally active extracts, was subsequently confirmed through behavioural choice assays with synthetic tetracosane. Behavioural assays of *M. rubra* workers towards concolonial and *P. nausithous* larvae were performed to test the efficiency of *P. nausithous* to mimic the host brood and the efficiency of parasitation avoidance of *M. rubra* workers. The local adaptation between *P. nausithous* and *M. rubra* was tested by comparing the adoption rate between infested and uninfested host populations. *M. rubra* colonies with high intracolonial genetic variation had higher infestation rates, suggesting that the variability of odour among colony members may have reduced the scrutinity of the workers when discriminating conspecific and parasitic larvae.

P147

Speed and accuracy in wasp nestmate recognition: vision and olfaction

David Baracchi, Iacopo Petrocelli, Lars Chittka, Giulia Ricciardi, **Stefano Turillazzi**

Social insects evolved highly developed recognition systems to accept nestmates but reject alien conspecifics. Chemical communication plays a crucial role in this ability. Recently it was discovered that visual quality signals and individual recognition also play a role in some species of social wasps. In the primitively eusocial wasp *Liostenogaster flavolineata* (Vespidae: Stenogastrinae), individuals differ in their cuticular hydrocarbon profiles according to colony membership and each female also possesses a unique facial pattern. Since both chemical and visual channels mediate the recognition abilities in these wasps, this species represents a model to understand how these senses are integrated during the perceptual processing and the extent to which wasps prioritize one channel over the other to discriminate aliens and nestmates. Here we reveal that although *L. flavolineata* females are able to discriminate between alien and nestmate females using only visual or chemical cues, the chemical profiles become redundant when the visual and chemical stimuli are presented together. Our findings indicate that resident wasps regulate the level of aggression towards intruders on the basis of the opponent's faces regardless of their cuticular hydrocarbons. Moreover, the visual sensory mode allows faster responses than chemical one either toward intruders and nestmates. However, in our experimental condition, the two sensory modes do not always have the same accuracy. Facial cues, when presented without odour cues, induce an increased number of erroneous attacks on nestmates (false alarms), odour cues, presented in isolation, result in an increased number of misses: erroneous acceptances of outsiders. The cost of incurring in these two different types of errors (i.e. attacking a nestmate or being peaceful to outsiders) might depend on the number of outsider visits experienced by colonies. The possible existence of tradeoffs in using one mode or another to recognise nestmates in diverse ecological contexts merits further investigation.

P148

The role of chemical cues for antiseptic behaviours in ants

Line V. Ugelvig, Sylvia Cremer

Chemical cues are crucial in transmission of information and regulation of processes within social insect societies. The origin of chemical cues is highly diverse, and so is the response they trigger, including both physiological and behavioural changes in individuals. Here we investigate the role of chemical cues - and their perception - for ant workers' performance of collective antiseptic behaviours. Ant workers express a composite behavioural response towards brood exposed to fungal pathogens. This is manifested in a care-kill dichotomy, where workers groom exposed brood to free them for pathogens, yet at the same time remove - and thereby doom - incurable brood to free the colony for pathogens. We focus our investigation on time points that correspond to the onset of these behavioural responses, that is, chemical cues that elicit grooming behaviour and disposal of brood. By combining standard and novel methodology we analyse cuticular hydrocarbons and more volatile compounds, which stem from changes in the ant brood's odour after pathogen exposure and infection or from the pathogen itself.

P149

Chemical signalling efficiency in Tetragonula*, a social Australian bee genus*

Bernadette Wittwer

Within the Australian social bee genus *Tetragonula*, tasks including brood care, nest guarding and foraging are age-dependent. Regulation of these behaviours is likely determined by the production and detection of chemical signals. The relationship between efficiency and sensitivity of chemical signal detection and gross antennal morphology is poorly understood. I examined this relationship by investigating efficacy through cross-fostering experiments, conspecific introductions and floral resource manipulation, compared with antennal morphology including sensilla density and condition. The tests were carried out in wild and laboratory populations of *Tetragonula*. These data are discussed in the context of the capacity of differently aged workers across the genus, to detect and respond to chemical cues and signals and the implications for the ecology of these species.

P150

Nestmate recognition in a ponerine ant Cryptopone sauteri

Yuki Yamaguchi, Kazuyuki Kudo

Eusocial insects discriminate nestmates from non-nestmates by cuticular hydrocarbon cues. When two adult individuals encounter each other in the field, two individuals have proved to cuticular hydrocarbon (CHC) components of each other, and show aggressive behavior toward non-nestmates. Many ant species exhibit polydomy in which the colony occupy in at least two spatially separated nests, but connected socially by the exchange of colony members. Although there are growing evidences on nestmate recognition and the role of CHCs in ants, the very limited number of studies has been known in the polydomous ant species. In this study, we investigated nestmate recognition and the role of CHCs in the polydomous ant *Cryptopone sauteri*. We conducted two designs of laboratory experiments to investigate whether workers showed aggressive behavior against non- nestmates. Firstly, a pair of nestmates and non-nestmates was placed in a glass Petri dish, and behavioral interactions between the pairs were observed. Workers did not show aggressive behavior more frequently against non-nestmate workers. Next, when a single worker was picked up from a donor nest and introduced it into either her or alien nest, the worker was aggressively attacked by workers of the alien nest. We identified 57 hydrocarbons, consisting of n-alkanes, dienes and triene on the cuticle of *C. sauteri* workers and conducted a bioassay whereby a glass bead was used as a surrogate ant. Aggression against non-nestmate compounds was significantly higher than against nestmate compounds. Our results suggest that workers of *C. sauteri* discriminate the nestmates from non-nestmates by the CHCs cues, and show aggressive behavior toward non-nestmates.

P151

A second known swimming ant, Odontomachus malignus *(Hymenoptera: Formicidae)*

Perry Buenavente, Dave General

The swimming ability of *Odontomachus malignus* F. Smith 1859 is reported for the first time. Observations of its swimming stroke show that it propels itself in a similar fashion with *Polyrhachis sokolova* Forel 1902, the first known swimming ant, but steers differently. Observations of its foraging behavior confirm previous reports that this ant forages diurnally as well as nocturnally and performs group transport of prey items. Field experiments show that these ants are not aggressive towards conspecific ants with food. They also dominate other ant species at baits except when the baits are immediately surrounded by other ants. Although the nest entrances seem to be inundated at high tide, the colonies themselves may occupy galleries in the karst above the water line.

P152

Zoogeography of ants on the Aegean Islands

Nikoletta Stylianidi, Chris Georgiadis, Giota Kozompoli, Maria Bibika, Anastasios Legakis, **David Nash**

The myrmecofauna of Greece is one of the most diverse in the Mediterranean area, but little is known about its distribution across the many islands of the Greek archipelago. Recent work on the ant fauna of Greece has provided a general taxonomic framework useful for exploring ant biodiversity, and more general biogeographic work useful for providing a baseline to understanding the distribution of species in general across the Aegean Islands. We carried out a survey of the distribution of ants across these islands, based on specimens collected in pitfall traps at 169 stations across 12 main islands, and covering a range of habitats. These have allowed us to simultaneously examine the island biogeographical principles underlying ant distribution, and how these are modified by anthropogenic disturbance, which varies across the islands. Based on the results of our analysis, we will present conservation and management recommendations for maintaining ant biodiversity across the Aegean islands.

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Energy imbalance causes the mortality in social isolation

Akiko Koto, Danielle Mersch, Eyal Privman, Brian Hollis, Laurent Keller

Ants have the strong social organization with the division of labor. Each worker performs different tasks such as foraging, nest construction and nursing. Recent study revealed that about 30 percent of workers travels outside of the nest to collect the food (Mersch et al., 2013). Foragers transfer the nutrient with their nestmates via trophallaxis. This indicates that the fitness of each ant depends on the colony they belong to. In nature, the separation from the colony is unusual situation for them. However, in the laboratory-reared conditions, it is reported that workers in some species can apparently not survive in social isolation. To date, the reasons for the mortality of isolated ants remain unknown. We used the carpenter ant *Camponotus fellah* to study the systemic mechanisms how social isolation causes the mortality in ants. We found that isolated ants have a shorter life span than grouped ants that were kept with two or ten nestmates. Additionally, we found that brood presence increased survivability of isolated ants. By monitoring the ant behavior with the tracking system, we revealed that the activity of isolated ants was highly upregulated compared with grouped ants, suggesting that they consume more energy than when they are with nestmates. Furthermore, though they showed the foraging behavior and collected the food, they had the impairment of digestive function. It causes the decrease in energy supply. Thus, our study revealed that the social deprivation affect the energy balance, resulting in the shorter life span in ants. We will discuss about the molecular mechanisms how the social environment affects the fitness of ants from the transcriptome analysis.

P154

Gene expressions for the sexually-dimorphic antennae in a ponerine ant

Chifune Sasa, Satoshi Miyazaki, Seigo Higashi, Toru Miura

Many of eusocial hymenopterans show conspicuous sexual dimorphisms due to sex differences in social behaviors. In most species of ants, antennae involved in sophisticated chemical communications showed distinctive sexual differences in the number of segments (12 in females and 13 in males) and in the scape length (longer in female). By understanding the proximate developmental mechanisms of dimorphic traits controlled under the molecular genetic bases, the evolutionary origin of sexual dimorphisms can also be inferred. The expressions of antennal patterning genes (i.e. toolkit genes or morphogenetic factors), regulated downstream of sex-determination genes such as doublesex (dsx), were thought to be different between sexes. In this study, therefore, to identify the responsible genes for the sexually-dimorphic development, the expression patterns of dsx and 18 antennal patterning genes in antennal discs of last (fourth) instar larvae (4L) were investigated in a ponerine ant *Diacamma sp*. The qRT-PCR results showed that 1) escargot (esg, related to dorsoventral patterning), Notch (related to segmentation in insect appendages) and a female-specific isoform of doublesex (dsxF) were highly expressed almost exclusively in females, while a male-specific doublesex isoform (dsxM) were highly expressed in males. Furthermore, in situ hybridization for these genes showed that esg was only expressed in antennal discs of female 4L. On the other hand, the homothorax (related to proximal patterning) expression was restricted in the intermediate region of discs in females while in the proximal-intermediate region in males, although the expression level was not significantly different. Taken together, it is suggested that sex-specific manners in expression levels and localizations of the patterning genes are responsible for the sex differences in antennal morphology in *Diacamma sp*.

P155

Developing transgenics in the clonal raider ant, Cerapachys biroi.

Waring Trible, Leonora Olivos-Cisneros, Benjamin Matthews, Peter Oxley, Daniel

The past few decades have seen genetic information become integrated into all aspects of biological research, from sub-cellular to ecosystem scales. This progress has been mirrored in social insect biology, which will soon have available tens of genome sequences and an increasingly precise phylogeny at all levels of taxonomic diversity. This information, in addition to forthcoming RNA sequence data, proteomic studies, and epigenetic data, is providing powerful and surprising insights into the ecology, behavior and evolution of the social insects. In one crucial aspect, however, social insects have been largely excluded from the progress of molecular biology: functional genetics. Genomes, phylogenies, and gene expression data generate intriguing correlative hypotheses of gene function, but these hypotheses can only be satisfactorily tested via direct experimental intervention. Long generation times, reproductive division of labor, difficulty in establishing controlled breeding programs, and genetically heterogeneous colony populations are among the host of factors that have prevented functional genetic tools from becoming well-established in social insect research. Here we present our recent attempts to create germ-line transgenics in the clonal raider ant *Cerapachys biroi* using the CRISPR/Cas genome engineering platform*. C. biroi* is a promising genetic model organism with colonies comprised of totipotent, clonal individuals (allowing large numbers of F1 reproductives to be screened for germ-line modifications), with a generation time of approximately 50 days, and colonies that are readily maintained in the laboratory. Our preliminary efforts include techniques for generating CRISPR/Cas target sites within the genome, synthesizing injection materials, egg collection, injection, and rearing protocols, and using the Illumina platform to screen for genome modifications. These promising advances hint that functional genetics may soon be an important new experimental tool in the repertoire of social insect biologists.

P156

Aversive learning of tastes in the honeybee

Marie Guiraud, Martin Giurfa, Lucie Hotier, Gabriela de Brito Sanchez

Honeybees seem to have a limited gustatory repertoire in accordance with a reduced number (10) of gustatory receptor genes identified so far in their genome. The bee gustatory system provides therefore an intriguing model for studies of taste discrimination because of its remarkable difference with that of other insects which are endowed with numerous gustatory receptors. Behavioral studies on taste perception in bees have been restricted to an appetitive framework as the distinctive hallmark used to assess taste responsiveness is the spontaneous extension of the proboscis (proboscis extension response or PER) upon antennal, tarsal or buccal contact with sucrose and other sweet tastants. Aversive substances do not elicit PER so that their perception can only be assessed indirectly, via PER inhibition following sucrose stimulation. The study of taste perception and discrimination is limited by the fact that sucrose acts as an unconditioned stimulus (US) eliciting a spontaneous unconditioned reaction. A fundamental step is, therefore, to overcome the traditional US status of tastants and to conceive conditioning forms in which they act as conditioned stimuli (CS). Here we present the first gustatory conditioning protocol in bees in which tastants are used as CSs and in which reinforcement is strictly dissociated from gustatory input. We took advantage of the sting extension response (SER), which can be elicited in harnessed honeybees by a mild electric shock. We developed a new conditioning protocol in which tastants are delivered to the antennae and paired with electric shock in order to induce gustatory conditioning of SER. We thus provide the first study of taste learning and discrimination in which, contrary to other attempts, taste perception is clearly separated from ingestion. Our results reveal principles of within- and between-taste perception in bees and open new perspectives for the study of the biology of taste in insects.

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Experience dependent modification of signalling in the honeybee brain

Christopher Jernigan, Fernando Locatelli, Irina Sinakevitch, Brian Smith

Learning is defined as the modification of behavior based on past experience, however what does this experience convey and how? Presumably these past experience cause changes in neural connections resulting in the physiological mechanism for learning. Learning has been well studied both physiologically and behaviorally in the honeybees, such studies have revealed a large variation in the representation of individual odors within an individual honeybees's olfactory processing centers, the antennal lobes (AL). The antennal lobes are important association areas for olfactory sensory input with reward signaling via octopamine (OA). There is a consistent variation between bees in the density of the AmOA1 (OA) receptor across coding areas (glomeruli) in the AL. Together with these findings we hypothesize that the observed variation within the AL is due to individual variation in olfactory experience between the bees, which has not been controlled for in previous studies. Here we will present that bees with the same experience in a semi-natural foraging situation show less variation in odor signaling in the AL. We hypothesize AmOA1 expression is increased in areas of the AL that are activated by an odor that has been associated with reward, and this change correlates to more similar calcium activity during olfactory activity between bees with similar experience. Here we show evidence of this using immunocytochemistry and calcium imaging of the Honeybees antennal lobe.

P158

Conditioning your bee - in one, two, three!

Nicholas Kirkerud, David Gustav, Giovanni Galizia

The honeybee has served as an important model species in studies of learning and memory for the past decades. A growing concern worldwide that honeybees suffer from a wide range of agrochemicals and diseases has led to increased effort in understanding the impact of these culprits at individual as well as on colony level. Despite the spike in interest, few attempts have been made to develop standardized assays where different aspects of behavior can be quantified. We recently developed a semi-automatic device for conditioning bees in a free-walking paradigm. This system, which we have named APIS (Automatic Performance Index System) is flexible, easy to operate and reliant. APIS consists of a rectangular conditioning chamber where individual bees can walk unhindered (but not fly) from end to end. A metal grid covers the interior surface through which weak electric pulses can be delivered. Airstreams carrying odors are connected to distal ends, and the odor stimuli as well as the electric pulse stimuli can be accurately controlled from a custom written software. The position of the bee is continuously sampled by infrared light sensors arranged along the length of the chamber. The history of the bees' movement is provided by the system readout, from which variables of interest can be extracted and analyzed. In an ongoing experiment we screen the effect of neonicotinoids at sub-lethal concentrations on honeybee behavior. Findings so far indicate that treated bees have difficulties with differentiating in their behavior towards a previously shock-reinforced odor and a neutral odor during a short-term memory test. These initial findings serve as examples of APIS as a powerful tool in screening the impact of chemicals by using measurements of learning and memory as proxies.

P159

PER conditioning of monochromatic light stimuli in bumblebee drones

Leonie Lichtenstein, Frank Sommerlandt, Johannes Spaethe

Learning visual cues constitutes a vital ability of bees to orientate in space and to recognize nest sites, food sources and mating partners. To study learning and memory in bees under controlled environmental conditions, the proboscis extension reaction (PER) assay provides a well-established behavioral approach. While many studies exist about olfactory learning, only little is known about PER conditioning of visual stimuli in bumblebees. Moreover, research using the PER method has primarily focused on workers, whereas males were largely neglected. In the present study, intact *Bombus terrestris* drones were tested in different color learning tasks using classical PER conditioning. Different monochromatic light stimuli (435nm, 455nm, 488nm, 528nm) in combination with different grey filters (transmission: 13%, 51%, 100%) were used to ensure that the bumblebees can only use chromatic but not achromatic (e.g. brightness) information. Drones were trained with absolute (A+) and differential (A+ vs. B-) conditioning and tested for memory recall after two hours (mid-term memory). Furthermore, drones were tested after differential conditioning for information transfer to a novel discrimination task in a Y-maze. The results indicate that drones are capable of discriminating between monochromatic light stimuli and retrieve the learned stimulus after two hours. Drones reach performance levels similar to that of workers. However, in contrast to workers, drones are not able to transfer the learned information to a novel context (Y-maze). The PER approach provides a useful tool to investigate color learning and discrimination abilities of bees and reveal potential differences between sexes under controlled conditions.

P160

Genetic bases of collective decisions in Drosophila

Ireni Clarke, Stephen Simpson, **Mathieu Lihoreau**

Fruit flies (*Drosophila melanogaster*) hatch and feed collectively in a single host fruit during their entire larval development. Larvae exhibit two distinct foraging strategies attributed to variations in a single polymorphic foraging gene: the 'rovers' (forR/R) cover larger distances and are more likely to leave a food patch, whereas the 'sitters' (forR/s) cover smaller distances and stay longer in patches. Sitter-like behaviour can be obtained in mutants lines 'S2' by knocking down the expression of forR in individuals with a rover background. These two behavioural phenotypes occur at stable frequencies of 70% rovers and 30% sitters in wild populations and are maintained through frequency-dependent competitive advantages over food acquisition. Here we investigated whether and how these behavioural differences at the individual level affect higher-level phenomena such as collective foraging decisions. Using different arrangements of artificial food patches in agar-based arenas and automated video tracking systems, we conducted a series of laboratory experiments to compare the foraging behaviour of individuals and groups of sitters, rovers and S2s. At the individual level, rovers were more efficient in finding foods with an optimal ratio of protein to carbohydrate than sitters, but did not stop feeding for long. At the collective level, however, sitters showed higher tendencies to follow each other and to form feeding aggregations than rovers. These preliminary results suggest that variations in the proportions of rovers and sitters within *Drosophila* populations mediate variations in the amplitude, speed and accuracy of collective foraging decisions through a subtle combination of exploration and social attraction. Specific ratios of rovers and sitters might be adaptive in different nutritional environments.

P162

Epigenetics of brain development in workers of the Western honeybee, Apis mellifera*.*

Hironori Sakamoto, Norichika Ogata, Tetsuhiko Sasaki

Worker honeybees change their tasks in the colony with aging from nursing larvae in the hive to foraging in the field. This task transition involves extensive changes of gene expression in the brain and an increase in learning and memory ability. These changes are thought to be regulated by epigenetic mechanisms including DNA methylation. In this study, we compared DNA methylation pattern in brains of nurses and foragers by whole genome bisulfite sequencing to identify differently methylated genes that may be involved in brain development. DNA was extracted from brains of 105 nurses and 195 foragers and subjected to bisulfite treatment. Next generation sequencing were performed using a HiSeq2000 (Illumina), and sequence reads were mapped to the *A. mellifera* genome (Amel 4.5). The *A. mellifera* genome contains 9,498,691 CpG sites, of which more than 80% were covered with at least 5 sequence reads in both nurses and foragers. In these CpG sites, 54,174 sites in nurses and 69,210 sites in foragers were significantly methylated compared with the background level (binomial test with the method of Benjamini and Hochberg, $p<0.05$), suggesting that methylated sites are only approximately 1% of the total CpG sites. Most of the methylated sites were on exons. In comparison between nurses and foragers, 774 CpG sites showed significantly different methylation level: 419 sites were significantly more methylated in nurses than foragers and 355 sites were more methylated in foragers than nurses. More than 85% of these differently methylated sites were placed on genes (exons and introns). We identified 439 genes that include differently methylated CpG sites.

P163

Promoter analysis of the mushroom body-preferential genes of the honeybee

Shota Suenami, Rajib Paul, Tomoko Fujiyuki, Kenichi Shirai, Takekazu Kunieda, Hideaki Takeuchi, Takeo Kubo

Mushroom bodies (MBs) are a higher center of the insect brain, and comprise interneurons called the Kenyon cells (KCs). There are four KC subtypes (class I large-, middle-, small-, and class II KCs) in the honeybee (*Apis mellifera* L.) MBs. Recent study indicated that parasitism, but not sociality, is associated with the evolution of the elaborate MBs in Hymenopteran insect brains. How each KC subtype contributes to the honeybee social behaviors, however, remains largely unknown. We have so far identified many genes expressed in a KC subtype-preferential manner in the honeybee brain. We expect that analyses of the function and regulatory mechanisms of genes expressed in a KC subtype-preferential manner may contribute to our better understanding of the molecular and neural bases underlying the honeybee social behaviors. In the present study, we aimed to clarify regulatory mechanism(s) of the KC subtype-preferential gene expression in the honeybee brain. We previously used cDNA microarray to comprehensively search for candidate genes expressed preferentially in the honeybee MBs. In the present study, we focused on three genes, phospholipase C epsilon (PLCe), Synaptotgmin 14 (Syt14), and discs large 5 (dlg5), whose expression seemed highly enriched in the honeybee MBs, among the candidate genes identified. Quantitative RT-PCR and in situ hybridization revealed that PLCe is expressed almost selectively in all KC subtypes, while Syt14 and dlg5 are expressed almost selectively in the lKCs in the honeybee brain, suggesting that these three genes are most appropriate for the analysis of KC subtype-selective promoters. We used electroporation to introduce and express reporter genes, in which gfp is ligated downstream of the upstream regions of the above three genes, in the honeybee brain. So far, basic but not MB-selective promoter activities were detected in the genomic regions around the transcription start site of each gene.

P164

Transcriptomic differences underlying instinctive and learned behavior in honeybees

Nicholas Naeger, Gene Robinson

Although it has been over half a century since Lorenz first wrote on the differences between instinctive and learned behavior, we still understand little about how instinctive behaviors are hard-wired into the brain. Honeybees drones and queens make their mating flights at a specific window of time during the day, the timing of which is instinctual and species-specific within the genus *Apis*. In this study, we trained *Apis mellifera* workers to fly for food only during a three hour window of time that coincided with the timing of the drones mating flight. Both drones and time-trained workers were collected in the afternoon as they initiated reward seeking behavior to look for mates or food, respectively, and were additionally collected during the morning when they were inactive. The dorsal protocerebrum, including the mushroom bodies and surrounding regions of the brain associated with reward, was dissected out of each bee for RNA sequencing and gene expression analysis. A common set of genes were identified as being important for reward seeking behavior generally, while other genes were identified as being important for either the instinctive or learned context specifically. Coexpression network analysis reveals how the same genes are utilized in different networks to produce different types of behavior.

P165

Genetic variability of captive breeding populations of Tetragonisca angustula*.*

Leandro Santiago, Flavio Francisco, **Maria Arias**

Animal breeding has been a common practice since centuries ago. Honeybees, bumblebees and stingless bees are amongst the most managed bee species, especially by their role as pollinators. Studies have shown that animals breeding leads to a decrease in their genetic diversity. Indeed, managed captive populations of bees have shown low genetic variability. Here we analyzed feral and captive breeding populations of *Tetragonisca angustula*, a highly eusocial stingless bee. We aimed at detecting genetic variability differences by microsatellites genotyping and mitochondrial DNA (mtDNA) sequencing. Ours results showed that nuclear genetic variability was similar between feral and captive breeding populations. Nonetheless genetic variability differences at mtDNA were remarkable between them. Higher differentiation was verified inter-populations but not intra-population. Gene flow by males, artificial transportation and colony split are the most probable explanations for the genetic scenario observed. It is worth noting that meliponaries with different degrees of management showed different results. We concluded that increasing population density by colony split does not affect negatively the nuclear genetic variability. This later seems to be maintained high through male dispersal. Keywords: Management, genetic variability, *Tetragonisca angustula*, meliponaries.

P166

Pictorial key to Indo-Malayan stingless bee genera

Abu Hassan Abdul Jalil, Ibrahim Shuib

This provides a pictorial key to identification of Indo-Malaysian stingless bees. It is designed to be especially useful as a teaching aid for beekeepers, farmers and hobbyists. The Indo-Malayan stingless bee genera key is especially critical to classify those that are dependent on Dipterocarp trees. The conservation of both stingless bees and Dipterocarp trees are reliant on such a pictorial Key.

P167

Steps towards eusociality - lessons from a digger wasp species

Thomas Schmitt, Mareike Wurdack, Heike Feldhaar, Jurgen Liebig, Carlo Polidori

In Hymenoptera, eusociality evolved several times independently and subsequently reached different levels of complexity. In some taxa like honeybees and various ant species, queens and workers live in perennial colonies and display a strong morphological and reproductive dimorphism. In several bee species e.g. from the family Halictidae, however, eusocial colonies have an annual life cycle with colonies consisting of one or a few reproductive females and reproductively totipotent helpers. Within the large group of solely solitary digger wasp species, *Cerceris rubida* shows definite traits of eusociality. A single fertile female is producing male and female offspring early in the season. Emerging daughters stay in the nest and help their mother with provisioning her offspring. All individuals from the same nest share a similar cuticular hydrocarbon (CHC) profile and they are able to recognize each other. Individuals accept only other nestmates and treat foreign intruders aggressively. Furthermore, the founding female exhibits a unique aspect of her CHC profile that may serve as fertility signal as discovered in several other eusocial Hymenoptera. Although all females are mated and thus they are capable of producing offspring of both sexes, most offspring within one nest are daughters of the founding female. All in all, several traits indicating eusociality could be detected for the first time within the digger wasps. Finally, we compare the traits of eusociality of *C. rubida* with those of other eusocial taxa in the Hymenoptera to give insights in the evolution of sociality in the studied species.

P168

Tetragonula *(Hymenoptera: Meliponini): morphology, behavior, genomics and the citizen scientist*

Victor Gonzalez, Claus Rasmussen, **Deborah Smith**

Tetragonula is the most common, widely distributed, and economically important group of Old World stingless bees. This monophyletic genus comprises 32 nominal species of small to very small bees found in the Indian subcontinent, Southeast Asia, and Australia. Though some species are readily recognizable based on morphology, taxonomic boundaries of most *Tetragonula* are poorly defined and many are exceedingly difficult to distinguish, even for specialists. Several are suspected to contain cryptic species, given their broad, discontinuous distributions and morphological variation. There is no identification key encompassing all *Tetragonula* species. These problems hinder our understanding of *Tetragonula* diversity and distribution, limiting opportunities for research, conservation and sustainable use. We outline a plan for study of *Tetragonula* diversity and evolution that will enlist citizen scientists (beekeepers, farmers, amateur entomologists) in collection of data and specimens, and will make results, including species ranges and identification methods publically available on an interactive website for bee conservation and cultivation. The project includes (1) collection specimens and nesting data, drawing on local expertise; (2) screening of colonies using the mitochondrial COI bar-coding sequence; (3) range mapping of mitotypes as first approximation of species distributions; (4) Sanger sequencing of mitochondrial and nuclear genes to determine if putative species suggested by bar-coding are supported; (5) genomic analysis of taxa in the *T. iridipennis* and *T. laeviceps* groups to generate an abundance of single nucleotide polymorphisms (SNPs) to examine species boundaries; (6) morphological and morphometric study of species indicated by genetic and behavioral data; (7) phylogeny of well-defined species, incorporating morphological and genetic data; and (8) production of keys and databases. Steps (1) and (6) are particularly amenable to citizen scientist participation, (2), (3), (4) and (6) to student participation. Here we present examples of bar-coding and wing morphometrics from small samples of *Tetragonula* species from several locations.

P169

Splitting nests: what decides eduction in stingless bees

Preeti Virkar, Shivam Shrotriya, V P Uniyal

Culturing stingless bees (*Triogona spp*.) is a challenging task. Acquiring their colonies from wild by eduction, i.e., natural hive duplication by attaching bee-box, is a slow process taking from three months to over a year. We investigate specific nest building requirements of *Trigona iridipennins* that could affect nest splitting decision. We precisely address the (1) space requirement for eduction and preference for bee-box materials, (2) requirement of nest building materials for splitting and (3) seasonal pattern of bee behaviour with respect to the nest building process. The study was initiated in July 2013 in a suburban human-dominated landscape at Dehradun, Uttarakhand, India, and is at the early stage. We initially identified three natural nests and are experimenting with different shapes, sizes and materials of bee-boxes following adaptive sampling. We used spherical earthen urn of 0.9-litre capacity and cuboidal wooden box of 2 litre capacity to begin with. We will subsequently search for more natural nests and install bee-boxes of variable dimensions. Nest building materials are analysed quantitatively and qualitatively by standard lab techniques. Spatio-temporal availability of nest building materials is to be estimated by plot sampling at fixed radial distances from the nests. Pollen and resin loads brought in by forager bees suggest seasonal availability of nest building resources. We observed the colonies building tubular extensions to join natural nest entrances to the openings provided through bee-boxes. This pre-winter event shows no signs of nest splitting. Nests were completely sealed off during peak winters from mid-December to mid-January. A post-winter scenario is likely to supply the environmental requirements, measuring which would provide the base-line data for our analysis. Precise ecological understanding of the requirements for nest splitting by stingless bees could explain the variable time taken for eduction, which would support a sustainable meliponiculture.

P170

Fertility-related volatiles in higher termites

Klara Dolejsova, Jana Krasulova, Romain Fougeyrollas, David Sillam-Dusses, Robert Hanus, Yves Roisin

Fertility signalling is at the heart of the debate on the evolution and maintenance of social hierarchy and reproductive division of labour in insect societies. While in social Hymenoptera, non-volatile cues on the body surface, the cuticular hydrocarbons, receive an increasing support, the situation in termites is much less clear. In primitive termites, living in small societies with a direct mutual contact among nestmates, the cuticular hydrocarbons are likely to be involved in fertility signalling as well. However, in populous colonies of advanced termites, the queens more probably announce their presence using volatiles, as evidenced by the recent discovery of the volatile queen pheromone in *Reticulitermes speratus* 1. Therefore, it is likely that in higher termites, the signal of queen presence will also be conveyed by volatiles. Here, we report on the identification of a volatile compound emitted by queens of the Neotropical higher termite *Embiratermes neotenicus* (Termitidae: Syntermitinae). Primary and secondary queens produce large amounts of (E)-nerolidol, a sesquiterpene alcohol, which is absent in all other castes. The quantity of the compound emitted by the queens appears to be correlated with their reproductive status; it increases with the level of physogastry and thus fertility of the queens while it is absent in young non-breeding queens. Interestingly, we identified this same compound also in the extract of eggs. Moreover, we observed similar volatiles being produced by the primary and/or secondary queens of two other syntermitine species. Multiple functions can be hypothesized for the observed volatiles, ranging from queen and egg recognition signal to primer pheromone function preventing the nestmates from reproduction. Nevertheless, these putative functions are not mutually exclusive and should all be considered in future research. 1. Matsuura, K., Himuro, C., Yokoi, T., et al. 2010. Identification of a pheromone regulating caste differentiation in termites. PNAS 107: 12963-12968.

P171

Reproductive competition between polygynous parasite queens in ant colonies

Janni Larsen, Terezinha M. Della Lucia, Morten Schiott, **David R. Nash**

One of the most remarkable and complex parasitic interactions is social parasitism, where a parasite exploits a complete society, rather than an individual organism. By integrating into a society the parasite gains protection against predators and diseases, and can redirect resources from the host to increase its own fitness. Among the most specialized social parasites are the inquilines that exploit social insect colonies. Inquilines are usually close relatives of their host and so share ancestral characteristics (Emerys rule). They are dependent on being fully integrated into their host colony throughout their lives in order to reproduce. Most inquiline ants have completely lost their sterile worker caste. Exceptions to this are *Acromyrmex insinuator* and *Acromyrmex ameliae*, parasites of fungus-growing ants. Previous studies have shown that a threshold proportion of parasite workers in the colony is essential for parasite reproduction. Multiple invasions of parasite queens into host colonies suggest that each parasite queen may need to produce fewer parasite workers and that the reproductive phase can be achieved more quickly. Polygyny among parasite queens is expected to select for intraspecific hyperparasitism, where some queens might cheat by only producing sexual offspring, effectively parasitizing the worker force produced by other queens. We investigated hyperparasitism in *A. insinuator* by genotyping parasite offspring, workers and alates in polygynous nests to investigate any bias in the production of reproductive castes relative to workers. We also investigated infiltration techniques and the possible use of queen pheromones to influence brood development by the social parasite and to its host.

P173

Queen signalling in social wasps

Jelle van Zweden, Wim Bonckaert, Tom Wenseleers, Patrizia d'Ettorre

Social Hymenoptera are characterized by a reproductive division of labour, whereby queens perform most of the reproduction and workers help to raise her offspring. A long-lasting debate is whether queens maintain this reproductive dominance by manipulating their daughter workers into remaining sterile (queen control), or if instead queens honestly signal their fertility and workers reproduce according to their own evolutionary incentives (queen signalling). Here, we test these competing hypotheses using data from Vespine wasps. We show that in natural colonies of the Saxon wasp, *Dolichovespula saxonica*, queens emit reliable chemical cues of their true fertility and that these putative queen signals decrease as the colony develops and worker reproduction increases. Moreover, these putative pheromones of *D. saxonica* show significant conservation with those of *Vespula vulgaris* and other Vespinae, thereby arguing against fast evolution of signals as a result of a queen - worker arms race ensuing from queen control. Lastly, levels of worker reproduction in these species correspond well with their average colony kin structures, as predicted by the queen signalling hypothesis but not the queen control hypothesis. Altogether, this correlative yet comprehensive analysis provides compelling evidence that honest signalling explains levels of reproductive division of labour in social wasps.

P174

Leafcutter ant ejaculates; more than just a carrier for sperm

Susanne den Boer, Marlene Sturup, Jacobus J. Boomsma, Boris Baer

Males transfer ejaculates consisting of sperm and other components such as seminal fluid, mating plugs and spermatophores to the female's reproductive tract during copulation. These ejaculate components are shaped by natural and sexual selection and have been shown to influence male fertility and competitiveness. Studying the biology of ejaculates, including their composition, the interaction between components, and their mode of transfer could thus help to understand factors affecting male fitness and paternity. We present a detailed account of the ejaculate biology of the leafcutter ant *Atta colombica*. Sperm is stored in the accessory testes (AT) prior to copulation, which starts with the appearance of clear pre-ejaculatory fluid (PEF) at the tip of the endophallus. This PEF likely originates from the accessory glands (AGs) located posterior to the ATs, as PEF and AG secretion have identical protein banding patterns on 1D gels, whereas those of AT secretion are different. The release of PEF is followed by the joint expulsion of sperm and AT secretions, along with a small mating plug. When studying the biological activity of these fluids, we could show that PEF, AG and AT secretion all have positive effects on sperm viability, but that PEF and AG secretion also reduce the survival of other males' sperm. Because PEF is transferred before sperm and AT secretion, it may have evolved to interact with rival sperm already present in the reproductive tract of potentially multiply mated queens (sperm competition), while at the same time providing a supportive biochemical environment for own transferred sperm. Finally, we could show that the protein fraction of the PEF is responsible for sperm incapacitation, warranting further proteomic studies to unravel their identity and biological function.

P175

Molecular warfare in the leaf cutter ant Atta colombica

Ryan Dosselli, Susanne den Boer, Jacobus J. Boomsma, Boris Baer

Polyandry is phylogenetically widespread in the social bees, ants and wasps where queens mate with multiple males during a single reproductive episode such as a nuptial flight. This results in sperm from multiple males to compete for access to the female's sperm storage organ (spermatheca) prior to egg fertilisation. The resulting sperm competition might however not always be in the interest of the queen, because they never re-mate later in life and therefore depend on sufficient numbers of viable sperm being stored to complete a full colony cycle. This results in adaptations in queens such as for example cryptic female choice, biasing paternity contributions after matings and in the absence of males. Glandular secretions, which males and females provide to sperm in the form of seminal fluid or spermathecal fluid, are now known as major determinants of these sexual conflicts. We used the leaf cutter ant *Atta colombica*, where seminal fluid incapacitates sperm of rival males but spermathecal fluid of queens neutralizes this effect. The molecules in seminal fluid that are able to detect and incapacitate non-self rival sperm are unknown, as are the molecules in the spermathecal fluid that are responsible to neutralise the sperm incapacitation effect. Our data show that proteins seem to be the biologically active molecules and modern proteomics technologies allowed us to quantify and identify those proteins using mass spectrometry. We therefore conducted experiments to understand interactions between seminal fluid and spermathecal fluid in order to test whether spermathecal fluid targets and modifies specific proteins in the seminal fluid. We investigated the presence and abundance of seminal fluid proteins in the spermatheca over time to quantify abundance changes of proteins after insemination. Furthermore we used mass spectrometry to identify those seminal fluid proteins that changed in abundance in the spermatheca.

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Termite reproductive traits as manifestations of conflict and cooperation

Tamara Hartke, Boris Baer

The best interests of a termite queen and king should be to maximise their joint fertility, resulting in traits that do not harm each other or that contribute in a positive way to the partner's reproduction. Traits that suggest termite mating systems have evolved along these generally cooperative lines include lack of sclerotized genitalia and reduction in male accessory glands. However, conflicts should still occur when reproductives are not strictly monogamous. Indeed, lower termites, 25-82% of colonies show genetic evidence of contributions from more than a single unrelated king-queen pair. Further, many termite species can generate multiple secondary reproductives, individuals that reproduce within their parent colony but do not complete the alate developmental pathway. These reproductives may be related (full- or part-siblings), but all else being equal, an individual that has made the transition should still prefer to reproduce itself rather than allow a non-clonal sibling to monopolize reproduction. This suggests that sexual conflicts occur regularly within termite colonies, in spite of a ‘generally monogamous’ mode of colony establishment. These types of conflicts may manifest in structures such as male accessory glands and their secretions, known from other species to produce proteins involved in sexual conflicts and/or produce mating plugs or mating signs to manipulate female mating rates. Reproductive conflicts could also have shaped sperm anatomy and physiology, as sperm competition tends to select for greater sperm motility or more uniform sperm morphology. Little is known about these traits in termites, so we have examined male reproductive structures and sperm across the family tree. Here we provide new data on relative size and morphology of testes and accessory glands and compare variation in sperm morphology within and between kings. This provides a first step toward understanding if and how reproductive conflicts have influenced termite anatomy and physiology in different lineages.

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Transcriptomics of mating and overwintering behaviour in Bombus terrestris

Fabio Manfredini, Inti Pedroso, Seirian Sumner, Mark Brown

Bumblebees queens health and behaviour are key factors for the production of a new colony. Through their lifetime, bumblebees queens undergo major physiological changes that drive different behaviours and mark important life stages. The molecular basis of these major transitions is largely unknown. In this project, we used whole-genome transcriptomics to characterize the dynamics of gene expression in *Bombus terrestris* queens across key periods of their life: from virgin to mated, overwintered and fully reproductive. Mating occurs with only one male at the end of the summer and triggers important physiological changes that prepare the queen for winter diapause: this lasts several months and is spent underground. Successfully overwintered females emerge at the beginning of the spring, actively forage for pollen and nectar and start a new colony alone. Mating and diapause are highly interconnected (non-mated females are not able to overwinter) and both processes are necessary for the successful production of a new colony in the spring. We sampled bumblebees females from commercial colonies and we induced mating and diapause in standard lab conditions. We obtained four experimental groups of bees: virgin, mated, overwintered with undeveloped ovaries (i.e. non-fertile) and overwintered fully reproductive bees. We dissected brains from 33 bees and we processed these samples for TruSeq mRNA sequencing. We used five lanes of an Illumina Sequencer to produce ~50 million reads per sample with 10X mean coverage. RNA sequencing data were analysed with edgeR for R to identify patterns of differential gene expression across the four experimental groups and we performed Gene Ontology analyses to characterize the biological processes involved in mating behaviour and diapause. These findings provide new insights to understand the molecular basis for fundamental behaviors in bumblebees and might help in developing strategies to improve the health of this important pollinator.

P178

Microsatellite analysis of nesting aggregation in Xylocopa nasalis

Natapot Warrit, Watcharapong Hongjamrassilp

Individual discrimination among social insects is one of the most important characteristics of eusociality. In bees (superfamily Apoidea), the ability to discriminate between nest-mates and non nest-mates has paved way to other advance social behaviors that are apparent in some halictine and corbiculate bees. However, little is known regarding other group of primitive social bees such as the carpenter bees (Apidae; Xylocopinini); how individual discrimination and tolerance have evolved. While genetic relatedness among individuals has played a central role in explaining why such traits originated and maintained in nature, ecological constraints and other factors might as well influence social behaviors to evolve. In this study, we attempt to explain our finding of a nesting aggregation of more than 80 bamboo nests of an Oriental carpenter bee, *Xylocopa nasalis* Westwood, 1838, located in the province of Ratchaburi in central Thailand. We are testing whether the genetic factor can contribute to the aggregation behavior of this subsocial bee by collecting 27 nests of *X. nasalis* from the site for microsatellite analyses. Genomic DNA of the founding female of each nest along with other adult nest mates (if present) and her progenies were extracted, whereas 14 microsatellites primers based on *X. frontalis* genome were used to analyse the intra- and internidal genetic relationships among individuals within and among nests. Mating frequencies and paternity analyses were also performed to assess the mating system of *X. nasalis*. Our finding will answer the question whether the aggregation behaviors (including individual discrimination and tolerance repertoires) occur as a result from the genetic perspective or other ecological factors.

P179

Transcriptome assembly for non-model Apinae bees: reference or de novo approach?

Natália Araujo, Maria Cristina Arias

RNA-Seq is a cost-effective method to characterize the gene set of species under interest. Initially it is required the reconstruction of all full-length transcripts based on short nucleotide reads, which represents a substantial computational challenge. Basically there are two strategies for transcriptome assembly, the Mapping-first and the Assembly-first (de novo). The first one is based on the alignment of all reads to a reference genome. It is less intensive computationally and, in principle, provides maximum sensitivity. However it demands an accurate mapped genome as reference. Conversely, the Assembly-first assemble the reads in contigs not using a reference genome as a guide. Herein we compare the success of both methods for assembling the transcriptome of the solitary bee *Tetrapedia diversipes*. This species belongs to the subfamily Apinae and is native of the American Tropics. The total RNA from 9 adults was extracted. These RNA samples were grouped in 3 pools and sequenced by Illumina HiSeq2000. Approximately 90 million paired reads of 100bp were generated. After quality trimming steps the reads were aligned to *Apis mellifera* genome using Bowtie2 (Mapping-first). The Trinity program was used for the normalization and the de novo assembly, the resulting contigs were then aligned to the reference genome using Bowtie2 (Assembly-first). In general, the alignment of the de novo transcriptome against the genome of reference recovered larger similar regions. It is worth to note that the contigs were previously combined (Trinity assembly) whereas for Maping-first the alignment was done with smaller reads. To our understanding the de novo assembly strategy seems to be more effective to study non-model species, here *T. diversipes*. This is the first transcriptome generated for a Neotropical solitary species. The transcripts data will be analyzed under the perspective to better understand fundamental questions about evolution of the social behavior.

P180

Light-inducible transcriptomic and epigenomic changes underlying brain plasticity in honeybees

Nils Becker, Robert Kucharski, Sylvain Foret, Ryszard Maleszka, Wolfgang Roessler

Honeybee workers perform different tasks in the colony throughout their adult life span. This is accomplished via division of labor, whereby young bees progress through a series of tasks inside the hive (cleaning, nursing), and older bees start foraging at ~3 weeks of age. However, division of labor is not exclusively age-regulated, but also context-dependent. Nurses may start precocious foraging when the colony is in need of foragers, and foragers can revert back to nurses in case of shortage of nurses. The honeybee, therefore, is an ideal model to investigate neuronal mechanisms of environmentally induced behavioral plasticity. The shift from in-hive to outside tasks involves adaptations to different environments. One major difference in the outside world is exposure to light as bees leave the dark pheromone-filled hive. Light plays an essential role for foragers, particularly for visual navigation and spotting food sources. Therefore, foragers need to be optimally prepared by adaptive changes in the neuronal circuitry. Transition to foraging is associated with remarkable changes in brain structure and associated synaptic plasticity (Groh et al. 2012, J Comp Neurol). Ongoing work shows that light exposure of adult workers is sufficient to induce structural synaptic plasticity in visual subcompartments of the mushroom bodies (Scholl et al. 2013, Front Behav Neurosci). Our aim is to study how light-inducible neuronal plasticity in visual centers of the brain is controlled at both transcriptional and epigenomic levels. In a broader context we wish to understand how light contributes to the genome-environment interplay that generates strikingly different phenotypes and behaviors with no conventional genetic changes. We will describe our experimental set-up designed to integrate environmental exposure with molecular changes in the brain and present some preliminary data on gene expression in optic lobes. Funded by the Graduate School of Life Sciences (GSLS), University of Würzburg.

P181

Comparative genomic linkage mapping in Apis

Olav Rueppell, Katelyn Miller, Ryan Kuster, Mananya Phiancharoen, Salim Tingek, Nikolaus Koeniger

The exceptionally high genomic recombination rate of the Western honeybees, *Apis mellifera*, has attracted significant scientific attention. A recent linkage map in the closely related *A. cerana* has found similarly high recombination rates and the same conclusion was reached for the more distantly related *A. florea*, based on limited data from two chromosomes. Here, we report on two complete linkage maps in this genus, extending existing genome-wide linkage maps to all three major honeybees clades. Based on genotyped SNPs identified by RAD-tag sequencing in single-family mapping populations, we will present genome-wide recombination maps of *A. florea* and *A. dorsata.* We will compare our results to the published findings in *A. mellifera* and *A. cerana*. Together with the emerging genome sequences of these two species the recombination maps will also be used to assess genomic synteny, recombination rate distribution, and genome correlates of local recombination rate. The combined results will improve our understanding of the evolution of very high genomic recombination rates and genome organization of the advanced eusocial insects.

P183

Age-, experience- and avoidance learning-related synaptic plasticity in leaf-cutting ants

Agustina Falibene, Flavio Roces, Claudia Groh, Wolfgang Roessler

Long-term behavioral changes related to age, experience and learning were shown to be associated with structural remodelling in the brain. Leaf-cutting ants are capable of learning to avoid previously preferred plants after those were experienced to be harmful for the symbiotic fungus. Workers retain this information for prolonged periods of time and recall avoidance memories with the presence of the leaves' odors. We studied the dynamics of brain microarchitectural changes after olfactory long-term memory formation following avoidance learning in *Acromyrmex ambiguus*. Quantification of synaptic complexes (microglomeruli, MG) in olfactory regions of the mushroom bodies (MB) at different times after learning revealed a transient change in MG densities. Two days after learning, the MG density was higher than before learning. At days 4 and 15 after learning - when ants still showed a rejection behavior - MG densities had decreased to finally reach the initial state. Furthermore, we found that enriched experience - like simultaneous collection of multiple non-harmful plant species - resulted in a decrease in MG densities compared to brains from ants that had collected only one kind of leaf. We further investigated age-related changes in the MB calyces and found subregion-specific effects on MG numbers in olfactory MB subregions (lip): MG numbers increased with age in the inner lip, but remained constant in the outer lip, where the overall density was higher. The results indicate that age, sensory experience and learning affect the synaptic architecture of the MB calyces via different processes: aging promoted an increase in MG numbers, sensory experience resulted in a decrease, and formation of long-term associative memory prompted a temporary increase in MG densities. This suggests a high degree of olfactory plasticity in the MBs comprising the formation and elimination of synaptic complexes. Funded by DAAD (A/11/76441) and DFG (SFB 1047)

P184

Monoamines, individual decisions, and collective-organization of pavement ant wars

Andrew Bubak, Kenneth Renner, John Swallow, **Michael Greene**

Wars are hostile conflicts in which members of one society coordinate their efforts to kill or injure members or disrupt the normal functioning of another. Animal wars seem to arise from changes to social context or circumstance instead of primarily from the inherent aggressive tendencies of individuals and are examples of how cooperation within a society can lead to aggression between members of opposing societies. Ants live in societies, called colonies, and it has been argued that they are the only other animal to war like humans. The pavement ant (*Tetramorium caespitum*) is a tramp species well known for its ant wars in which thousands of workers from two colonies fight. Fighting appears to be ritualized; ants fight by grabbing another ant's mandibles with its own and pairs undergo what can be described as a 'push-of-war' while other ants recruit more workers to the battle and few, if any, ants die during the battle. What are the rules that influence the collective-organization of these 'wars' and how are cooperative and agonistic behaviors modulated by brain monoamines? We report that workers discriminate nestmates and non-nestmates by detecting cues coded relative abundance of methyl-alkane and alkene hydrocarbons on the cuticle of ants. Detection of non-nestmate cues is not sufficient to stimulate fighting in and of itself; social context influences aggressive decisions as a recent history of high interaction rate with nestmate ants increases the probability workers will fight. Workers respond to interactions with heterospecific ants using rules that do not depend on density of nestmates. We also report how patterns of monoamines (serotonin 5-HT, dopamine, and octopamine) in pavement ant brains correspond to interactions with live nestmates and non-nestmates as well as cuticular hydrocarbons from nestmates and non-nestmates.

P185

Ants, Tetramorium species E*, learn to avoid predatory antlions' pit traps*

Karen Hollis, Kelsey McNew, Alexandra Bemis, Talisa Sosa, Felicia Harrsch, Elise Nowbahari

Pavement ants, *Tetramorium sp. E*, often inhabit the same sandy soils as a common predator, pit-digging larval antlions (*Myrmeleontidae spp.*). Previous research in our laboratory has shown that pavement ants are able to rescue captured nestmates from antlions' pit traps, thus sabotaging antlions' attempts to capture them. Here we show that pavement ants possess yet another antipredator strategy, namely the ability to learn to avoid antlion pits following a successful escape from a pit trap. In Phase I, an ant was confined to an arena containing an antlion pit and, if it fell into the pit and successfully escaped, which happens in nature as well as in the laboratory, it was tested for its ability to avoid a pit in Phase II. Seven different experimental conditions, including the presence or absence of an actual antlion in the pit during either or both phases, and the opportunity to confront the same or a different pit, allowed us to test various explanations of how, exactly, ants might avoid antlions. Results suggest that ants are able to form a generalizable memory of pit characteristics and that the ability to avoid pits does not depend on cues that the ant, itself, leaves behind in the pit or cues emanating from an antlion's presence.

P186

Colony defense and plasticity of the stingless bee, Tetragonisca angustula

Christopher Jernigan, David Roubik, William Wcislo, Brian Smith

Understanding how animals respond to novel and familiar stimuli in their world is a vital part of understanding their behavior and ecology. Previous studies exploring colony defense behavior by social insects have shown two general outcomes of repeated interactions. In the ant, *Pheidole denata*, repeated exposure to a heterospecific 'enemy' ant caused an increase in defense response to that species. However in a number of ants, including *Pheidole, Acromyrmex, Manica* and *Formica*, decreased aggression with repeated exposure has been observed. We explore the defense plasticity and compare and contrast previous findings to that of the stingless bee defense system in *Tetragonisca angustula* using a natural alarm stimulus, synthetic alarm pheromone mixture, novel odors, and visual threat models in the field in Panama. These bees are unique in that they maintain a force of both standing and hovering guards. Our goal was to explore the role of these two guard castes during controlled aggression events and measure the plasticity of these behaviors with regards to habituation to their own alarm pheromones and novel stimuli. We found during aggression events by *T. angustula*, they express a dramatic increase in the activity and number of hovering guards and a prominent decrease in standing guards, as many of these took to areal defense. Bees often performed 'death bites' on foreign objects during these events. We found in mild events, colony working-memory of the event had impacts on behavior for approximately 24 hours. Bees exhibited habituation, or a decrease in defense behavior, to the same stimuli with repeated stimulation 2-12 hours after exposure. Normal defense responses were recovered after 24 hours, however in mass raid events bees were observed to maintain highly increase guard numbers for up to 3 days.

P187

Searching behaviour of two desert ants from contrasting visual environments

Patrick Schultheiss, Thomas Stannard, Sophia Pereira, Ken Cheng, Rudiger Wehner

Within the navigational toolkit of desert ants, one well-established component is visual navigation using terrestrial objects. With experience, a foraging ant is able to use the visual appearance of an arrangement of objects to navigate along routes, and to pinpoint locations of interest, such as the nest entrance. Should the ant be unable to find the exact location of the nest, her path will loop around the spot of its expected location, thus leading to a systematic search. All navigational mechanisms continue during this search, including visual navigation. Here, we investigated how two ants of similar phylogenetic history but vastly different natural visual ecology cope with large changes to their visual environment. We compared the two Australian species *Melophorus bagoti*, living in visually cluttered arid bushland, and *Melophorus sp*. (an as yet unnamed species), living in visually barren dry salt-pans. Two prominent visual objects ('walls') of black cloth were erected close to the nest entrance, and foragers trained to visit feeding stations. On a test, the ants were hindered from entering the nest, and were tested either with the two prominent walls in place, or removed. Their return paths from the feeder to the nest and the subsequent searches were comparable in the two species. With the walls in place, the search was tightly concentrated around the expected nest location in both species. With the walls removed, the search of both species increased considerably in size, but was nonetheless centred on the nest location. It appears that the habitual visual ecologies of the respective species are of minor importance in this regard, and the two species may have a similar searching strategy for coping with unexpected visual changes.

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Royal jelly of Apis cerana japonica *acts as a bee-attracting pheromone*

Michio Sugahara, Yasuichiro Nishimura, Takafumi Nakatani, Naoki Noda, Ayaka Harada, Midori Sakura

The flower of the oriental orchid *Cymbidium floribundum* is known to attract the Japanese honeybee *Apis cerana japonica* not only workers but also drones and queen, that is, to attract even swarm and absconding-bees. A mixture of 3-hydroxyoctanoic acid (3-HOAA) and 10-hydroxy-(E)-2-decenoic acid (10-HDA) was identified as the active principles from the orchid flower. Both compounds are also mandibular gland components of worker honeybees (*Apis cerana japonica*) with related compounds. In this study, aliphatic compounds in the mandibular gland of nurse and forager workers were analyzed by GC-MS. Aliphatic compounds in royal jelly made in the mandibular gland were also analyzed. In addition, by a bioassay, a queen cell containing royal jelly was demonstrated to attract bees and the royal jelly constituents which have attraction activity were identified. Finally, it was shown that royal jelly acted as a bee-attracting pheromone.

P189

Individual differences in the behaviour of Formica fusca *workers (Formicidae)*

Anna Somogyi, **Andras Tartally**, Zoltan Barta

Animal personality traits, such as activity, aggressiveness, boldness, exploration and sociability have been described for several animal species. Eusocial species, like ants exhibit distinctive division of labour and workers differ in their behaviour in various situations, e.g. in emerging tasks. It is still unclear whether these variations of behaviour are consistent. Colonies of *Formica fusca* (Hymenoptera: Formicidae) was used to study this question. All of the colonies contained fertilized queens, workers, larvae and pupae. The behaviour of workers (activity, boldness and exploration) was investigated on individual level repeatedly. Based on the preliminary results, there are significant differences between the behaviour of *F. fusca* workers in these elements and they behave consistently on the individual level.

P190

The final frontier of honeybee cognition: Can bees learn art?

Judith Reinhard, Wen Wu, Jason Tangen

Honeybees have remarkable visual learning and discrimination abilities that extend beyond learning simple colours, shapes or patterns. They can discriminate landscape scenes, types of flowers, and even human faces. This suggests that in spite of their small brain, honeybees have a highly developed capacity for processing complex visual information, comparable in many respects to vertebrates. Here, we investigated whether this capacity extends to complex images that humans distinguish on the basis of artistic style: Impressionist paintings by Monet and Cubist paintings by Picasso. We show that honeybees learned to simultaneously discriminate between five different Monet and Picasso paintings, and that they do not rely on luminance, colour, or spatial frequency information for discrimination. When presented with novel paintings of the same style, the bees even demonstrated some ability to generalize. This suggests that honeybees are able to discriminate Monet paintings from Picasso ones by extracting and learning the characteristic visual information inherent in each painting style. We propose that like birds and humans, honeybees may use the underlying structural regularities that are consistent across all paintings by the same artist as the visual cue for discrimination and categorization. The cognitive capacity of honeybees to learn and categorize even visually highly complex images may underlie their amazing ability to navigate cluttered landscapes and develop robust navigational maps of their foraging environment.

P191

Antennal cropping in the Asian dry-wood termite, Neotermes koshunensis

Yasushi Miyaguni, Koji Sugio, Kazuki Tsuji

Antennal cropping, a behavior inferred to exist because queens and kings have shorter antennae than fresh alates, is widespread in lower-termites. However, the proximate and ultimate mechanisms underlying this phenomenon remain unclear. We studied the occurrence of antennal cropping in queens and kings of the dry-wood termite *Neotermes koshunensis* (Kalotermitidae). The tip structure of the cropped antennae of queens and kings showed the edges folded inward. The same inward folding was also observed in some antennae cropped using the mandible of a termite. Antennal cropping also occurred in isolated dealates reared in isolation. This implies that self-cropping is an important proximate mechanism of antennal cropping in this termite. Previous studies inferred that antennal cropping may play a key role in the life-history of alates at the colony-founding stage. However, we also found antennal cropping in adultoid reproductives (secondary reproductives) that had not experienced colony founding. We propose a new hypothesis that antennal cropping is important for individuals in regulating their physiology when they change from the non-reproducing to the reproducing phase. The adaptive significance of antennal cropping may be to reduce the inhibitory effect of pheromones released by the reproductives on their own reproduction.

P192

Balancing act: Head stabilisation in Myrmecia *ants during twilight*

Chloe Raderschall, Ajay Narendra, Jochen Zeil

As animals move, they generate image motion in their visual field. Image motion generated by translation contains information about the environment, while image motion generated by rotations does not and therefore needs to be minimised. Locomotion - be it flying or walking - induces rotations of the body and of the head of an animal thereby degrading vision. To reduce these unintended locomotion-induced eye rotations, animals are known to perform compensatory head movements that help stabilise gaze. To date, most studies investigating gaze stabilisation in insects have come from flying species. Yet, walking insects have a special problem in keeping their gaze stable because they are in direct contact with the substrate, which will induce complex and unpredictable body rotations as they walk. Bull ants (*Myrmecia pyriformis*) are crepuscular animals that experience a wide range of light intensities during their foraging trips. These ants rely heavily on visual cues for navigation and for catching prey. To measure their ability to control head orientation in the presence of substrate-induced body roll, we took advantage of the ants' motivation for walking along fallen sticks. We induced ants to walk along a twisted band that gradually induced body roll. Filming ants from the front allowed us to quantify head and body roll in a range of ambient light conditions in the ants' natural habitat, as well as in room light and complete darkness in the laboratory. We find that (1) ants are able to keep their head horizontally aligned against body roll of up to 60 degrees; (2) head stabilisation is partly visually controlled, because there is a small degradation of the response as light levels drop; (3) however, the ants also compensate partially for body roll in complete darkness, suggesting non-visual information contributing to head stabilisation.

P193

Stamping the royal seal with DNA methylation

Mat Welch, O Edwards, H Millar, R Lister, B Baer

Background: Female honeybees have two distinctly separate castes that either work (workers) or reproduce (queens). Phenotypically the two castes are highly divergent and exhibit caste-specific morphological traits, yet they both originate from fertilised eggs. Queen-destined individuals are fed royal jelly and this nutritional stimulus initiates queen development. Epigenomic modifications allow phenotypically plastic shifts in response to external stimuli such as dietary cues, by tethering the genome to the environment. Epigenome dynamics are facilitated in-part by DNA methylation, which is known to be critical for caste determination during early larval development. Our aim is to identify the initial molecular response to royal jelly in larvae that are yet to commit to their developmental fate, in order to unravel the very first and most critical genetic and epigenomic changes that underlie caste determination in honeybees.

Study Design: We collected young larvae prior to the caste determination and provided them with royal jelly to artificially initiate queen development. To determine the initial trigger(s), we placed age-matched (+/-1 hr) 1 day old larvae in either worker comb (control) or artificial queen cells with fresh royal jelly. Pooled larvae (n=6) were then collected 0, 2, 4, 6 and 8 hrs after placement and for a total of 4 biological replicates. Each of these replicates were used to make independent transcriptome and methylome libraries and sequenced on an Illumina Hiseq 2000. Results about the initial molecular responses to royal jelly feeding will be presented and discussed in a broader context of caste determination in social insects.

Notes

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