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COLONY INTEGRATION IN ANTS: NESTMATE RECOGNITION AND THE GESTALT ODOUR

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ABSTRACT

Nestmate recognition is a key mechanism of cohesion in ant colonies, and we present in this paper a review. Recognition cues are constituted by cuticular hydrocarbons which are continuously exchanged between all the members of the colony, forming a "gestalt" colony odour. This gestalt is dynamic, and when the colony is separated in subgroups, the subgroups odours diverge. The individuals which are individually separated from the mother colony are progressively not recognised because they cannot update their visa. Octopamine is involved in the drive to trophallax to update the individual odour.

KEY WORDS: ants, nestmate recognition, cuticular hydrocarbons, gestalt odour, trophallaxis, allogrooming

Social insects have evolved a highly developed recognition system enabling them to behave altruistically towards nestmates but reject alien conspecifics. The discrimination is based on chemical cues (labels) that form a visa – the so-called "colony odour". These cues are used by one individual to classify confronted individuals by comparison to its own representation or template which is generally learned after the emergence of the adult.

The gestalt colony odour (for reviews see LENOIR et al., 1999; LENOIR et al., 2001b)

Many correlative and experimental studies have suggested the role of cuticular hydrocarbons in nestmate recognition at least in ants and wasps. Recently, experiments demonstrated that only the hydrocarbon fraction of the cuticular lipids allows nestmate recognition in *Cataglyphis niger* (LAHAV et al., 1999) and *Iridomyrmex purpureus* (THOMAS et al., 1999). A theoretical model of colony odour was presented by Crozier who postulated that all the individuals within a colony share their own recognition cues forming a gestalt colony odour (CROZIER and DIX, 1979; CROZIER, 1987). Alternatively to the gestalt model, Crozier proposed for small colonies the individualistic model. In this case, the individual labels are not mixed, and if transfer occurs, it is at low levels. In *Cataglyphis niger,* chemical studies by Hefetz's group, using radioactive compounds, have confirmed that individual ants acquire a common uniform colony odour according to the gestalt model (SOROKER et al., 1994; SOROKER et al., 1995a). We are now able to present a synthetic diagram of the establishment of the gestalt odour in ants (for a figure see DAHBI et al., 1998; LENOIR et al., 1999). Hydrocarbons are probably synthesized in the oenocytes of the fat body like in other insects, and are secreted either directly to the epicuticle or transported through the haemolymph by lipophorin (for termites see SEVALA et al., 2000) to the postpharyngeal gland (PPG) where they are stored. The PPG, a gland specific to the ants, appears to be the only place for storage rather than biosynthesis. Hydrocarbons are transferred to other individuals during trophallaxis or allogrooming and physical contacts and incorporated directly into the recipient PPG. They can be reapplied on the cuticle through selfgrooming (HEFETZ et al., 2001), but can also be cleared via the crop in the alimentary canal (SOROKER and HEFETZ, 2000). The cuticle has also an active role of internalisation of hydrocarbons: in *Camponotus vagus,* an insaturated hydrocarbon not normally synthesised by this species is internalised into the PPG (MESKALI et al., 1995). In two species of *Reticulitermes* in mixed colonies, each species acquires some of the allospecific hydrocarbons (VAUCHOT et al., 1998).

Hydrocarbons are also good candidates to be a fertility signal in ponerine queens (see for example LIEBIG et al., 2000), but experimental demonstration is lacking.

Chemical insignificance and chemical integration

Newly eclosed ants (callows) lack external cues, they have only a few hydrocarbons and their PPG is empty (SOROKER et al., 1995b). We called this period the "chemical insignificance" period. In a few days, callows synthesize their own hydrocarbons (genetic components), they receive many trophallaxis and allogrooming and also adsorb chemicals from nestmates or nest material (environmental components), it is the "chemical integration" period (LENOIR et al., 1999). The chemical insignificance of callows probably exists in all ant species and other social insects like wasps, it confirms an old hypothesis of Fielde on the change of the ant's odour with age (FIELDE, 1905). It explains the formation of artificial heterospecific mixed colonies where ants of different species cohabit peacefully in the same nest (ERRARD, 1994), the integration of parasitic species like *Formicoxenus* in the *Myrmica* host colony by chemical mimicry (LENOIR et al., 1997), and at least partly the invading strategy of the newly fecundated queens of the slave-maker *Polyergus rufescens* (D'ETTORRE and

ERRARD, 1998). It will probably explain many other cases of parasitism (LENOIR et al., 2001b).

Dynamics of the gestalt

Since the individual's turnover in hydrocarbon composition, the population and food are changing, the colony odour must also change continuously. Workers have to continually exchange recognition cues to maintain themselves into the gestalt. In *Cataglyphis iberica*, nest separation occurs spontaneously during hibernation inducing also a shift in nest odour, and an increasing of antennations when the ants are reunited (DAHBI and LENOIR, 1998). This increase was confirmed by splitting a colony in two parts during two months. When reunited, the ants of the two daughter groups had more trophallaxis than the ants which were in the same group (DAHBI et al., 1999). In *Cataglyphis niger,* a three months separation period of the subgroups also induced a segregation in the cuticular profile, but was not sufficient to induce aggression (LAHAV et al., 2001). In *Camponotus fellah* and in *Aphaenogaster senilis,* we observed in a few weeks also a spontaneous shift in the colony odour (BOULAY et al., 2000a; LENOIR et al., in press). This shift has been observe in the field (NIELSEN et al., 1999; LIU et al., 2000).

To investigate this dynamic of the gestalt, we followed the radioactive hydrocarbons flow of a donor ant injected with radioactive acetate in a group of 10 ants, during variable durations. This is supposed to be a close approximation of an in-nest situation. We compared hydrocarbon dynamic in two species with populous colonies, *Camponotus fellah* that performs trophallaxis and *Aphaenogaster senilis* that does not (LENOIR et al., in press). We showed that the labelled hydrocarbons were progressively transferred to all the individuals of the group so that the distribution attains homogeneity, i.e., hydrocarbons transfer rates approach a normal distribution. In the trophallactic *C. fellah,* homogeneity was reached more rapidly than in the non-trophallactic *A. senilis.* In *C. fellah* the mean percentage of hydrocarbons transfer into the PPG and cuticle recipient workers significantly elevated when the duration of encounter increased. Transfer to the PPG after 1 and 3 days did not differ significantly and amounted to 4 to 5% but increased significantly to 7% (the maximum amount of possible transfer) after 6 days. Transfer to the cuticle increased significantly between 1 and 3 days but remained unchanged thereafter. The distribution of transfer amounts in *C. fellah* for 1 day in the PPG and cuticle, and the corresponding normal calculated curves for the PPG was non-significantly different from a normal distribution (Kolmogorov-Smirnov test). For the cuticle the homogenisation process was slower, with the 1day curve being significantly different from normality, many ants did not have any labelled hydrocarbon on their cuticle. The data distributions for the PPG after 3 and 6 days were again non significantly different from a normal distribution, with a shift to maximum percentages at 6 days. For the cuticle, the homogenisation process was slower, with the 1day curve being significantly different from normality but reaching normality after 3 or 6 days.

In *A. senilis,* a longer period of 10 days was used as we predicted that the process would be slower. It revealed also a significant increase with time both for the PPG and cuticle. The transfer into the PPG of the recipients increased progressively, reaching 6% at 10 days. Increase in the cuticle followed a similar progression, with a plateau after 6 days. The distribution of transfer amounts showed that after 1 day the curves were significantly different from normal both for the PPG and the cuticle. In this species, the process of homogenisation was considerably slower than in *C. fellah.* After 6 days the distribution was not significantly different from normal distribution and this was more pronounced after 10 days. The process was similar for the cuticle.

The effects of social isolation (BOULAY et al., 2000b; LENOIR et al., 2001a)

If the gestalt is a dynamic process, because the composition of the colony is changing, individuals isolated from their colony will not fit the common odour, and will progressively deviate from the mother colony. In *C. fellah* and in *A. senilis*, a short isolation period (3–5 days) induced minor changes in hydrocarbon profile compared to the spontaneous drift of control ants in the colony, and provoked frequent trophallactic (*Camponotus*) or allogrooming (*Aphaenogaster*) solicitations which have tendency to decrease later. Longer isolation periods 20 - 40 days) induced a greater change in hydrocarbon profile and made the residents intolerant towards their introduced nestmates. They are considered as aliens, because they were not able to update their hydrocarbons profile. On the contrary, the isolated ants apparently recognize their nestmate as they are not aggressive.

Neurobiological basis of the effects of social cohesion in ants (BOULAY et al., 2000b)

Octopamine was chosen because it is the equivalent of norepinephrine of vertebrates and involved in many behaviours in non social insects.

Octopamine reduced trophallaxis-induced in 5 days isolated *C. fellah* workers and consequently reduced hydrocarbon transfer between nestmates. We verified that these effects were physiologic as octopamine did not change locomotor activity and hydrocarbon biosynthesis was not affected.

Serotonine did not have any effect on isolation-induced trophallaxis. Therefore, we hypothesize that octopamine mediates behaviour patterns linked to social bonding, such as trophallaxis. We suggest that the levels of octopamine in the brain of socially deprived ants may decrease, together with a concomitant increase in their urge to perform trophallaxis and to experience social contacts. Octopamine administration may reduce this social deprivation effect, and octopamine could therefore be regarded as being partly responsible for the social cohesion between nestmates in ant colonies. The role of octopamine in social behaviours has been confirmed recently in honeybees and *Bombus*. In bees, octopamine increases nestmate recognition and aggression toward non-nestmates (ROBINSON et al., 1999), and is involved in division of labour (SCHULZ and ROBINSON, in press). In *Bombus terrestris* dominant workers have higher octopamine levels compared to workers of lower status independently of size, age and ovary state (BLOCH et al., 2000).

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