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SHORT COMMUNICATION

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Biogenic amine levels, reproduction and social dominance in the queenless ant *Streblognathus peetersi*

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Abstract Social harmony often relies on ritualised dominance interactions between society members, particularly in queenless ant societies, where colony members do not have developmentally predetermined castes but have to fight for their status in the reproductive and work hierarchy. In this behavioural plasticity, their social organisation resembles more that of vertebrates than that of the "classic" social insects. The present study investigates the neurochemistry of the queenless ant species, *Streblognathus peetersi*, to better understand the neural basis of the high behavioural plasticity observed in queenless ants. We report measurements of brain biogenic amines [octopamine, dopamine, serotonin] of *S. peetersi* ants; they reveal a new set of biogenic amine influences on social organisation with no common features with other "primitively organised societies" (bumble bees) and some common features with "highly eusocial" species (honey bees). This similarity to honey bees may either confirm the heritage of queenless species from their probably highly eusocial ancestors or highlight independent patterns of biogenic amine influences on the social organisation of these highly derived species.

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Introduction

Social dominance refers to an asymmetry of relationship among individuals, as defined by the outcomes of dyadic encounters of a competitive or agonistic nature. Recent studies on vertebrate and invertebrate species suggest that differences in social status may reflect variations in monoaminergic activity in the central nervous system (Bloch et al. 2000; Huber et al. 2001; Kaplan et al. 2002; Reisner et al. 1996). The neurophysiological basis of social status, already extensively examined in vertebrates, has recently begun to be investigated in social insects. Some studies have shown that biogenic amine levels are linked to the social role of infertile *Apis* workers: foragers have higher levels of octopamine (OA) compared to nurses of the same age (Schulz et al. 2002; Wagener-Hulme et al. 1999), and dancers have higher levels of dopamine than other foragers (Bozic and Woodring 1998). Bloch and colleagues (Bloch et al. 2000) investigated the brain biogenic amine levels according to reproductive dominance in bumble bee workers. They found that, in queen-right colonies, workers with higher levels of octopamine had more developed ovaries and were more dominant than sisters of the same age, and that in queenless colonies, high dopamine levels were associated with the final stages of ovarian development in workers.

Most of the highly social insect species considered so far, have morphological castes that are determined by a specific developmental pathway that fixes the reproductive role and associated behavioural repertoire of each individual in the colony. Queenless ant species, on the other hand, have colonies composed exclusively of monomorphic workers, each one capable of mating and laying eggs, forming societies with a more plastic mode of organisation (Peters 1993). In these colonies, the division of labour depends on interindividual behavioural interactions that determine not only gathering tasks but also more importantly, reproductive roles. As in most vertebrate societies, the potential reproducers in a queenless ant colony form a dominance hierarchy through confrontations. Because castes are determined through behavioural interactions, the social position of individual workers changes frequently and rapidly. At each change in status, the ant must quickly adapt its behaviour to its new position. Such behavioural plasticity is unique among social insects. Queenless ants could prove useful in investigating the neural bases of behavioural plasticity. Unlike wasps, workers in queenless ant species are restricted by ecological constraints from forming new, independent colonies; their access to reproduction is entirely dependent on their rank in the dominance hierarchy. The strict behavioural castes created by this social system make queenless ants a particularly suitable model for studying the interactions between social environment and individual behaviour in a context of high relatedness and developmental similarity among colony members. Indeed, unlike queen-right ants, queenless ants all experience the same developmental program—that of a worker. For this reason, we will compare our results mainly to bibliographic data that concern workers, rather than queens of other social species.

The clear behavioural interactions displayed in queenless societies allow one to determine the "behavioural state" of each worker through behavioural observation. As emphasized by Page and Erber (2002), this is an important prerequisite in studies on the effect of the biogenic amine levels on social behaviour. We chose to investigate the relationship between social dominance and brain levels of octopamine, dopamine and serotonin in a queenless ant whose behavioural castes are well-defined. *Streblognathus peetersi* is a monogynous queenless ant with colonies organised in three distinct groups: the alpha ant is the most dominant worker and the only one to mate and lay eggs; workers of high social ranks are behaviourally dominant (while submissive to the alpha), are rather young and do not mate or lay eggs; workers of low social ranks are subordinate to the other two groups, can be of any age and also remain infertile. This species is particularly useful for two reasons: alpha and high-ranked workers (ranks beta to delta) each show clear behavioural displays that allow their immediate and unequivocal identification (Cuvillier-Hot et al. 2004). In addition, the individuals are very large (~2 cm long), making behavioural observation easier and allowing amine dosage at the individual level.

Materials and methods

Ant collection and housing

Entire colonies of *S. peetersi* were collected near Magoebaskloof, Limpopo province, South Africa: three in January 2000, two in February 2001 and seven in April 2001. The ants were kept in plaster nests in the laboratory, at 25°C and under 12 h light : 12 h dark cycles. Colonies were fed daily with cockroach nymphs and crickets. Each ant was labelled with a plastic number glued onto the thorax. The dominance hierarchy was established by careful behavioural observation as already described in Cuvillier-Hot et al. (2004). Alpha ants, workers of high rank (beta to delta), and clearly subordinate workers (foragers) were selected for brain biogenic amine level measurements.

Sample preparation

Ants were quickly frozen in liquid N_2; the heads were dissected in dried ice and the brains without the optic lobes were kept at −80°C until amine analysis. Each brain was treated separately. The brain was placed in an Eppendorf tube on ice with 100 µl of cold perchloric acid (0.1 M) mixed with an internal standard (DHBA, 50 pg/µl). Samples were sonicated (Ultra Turax T25, Bioblock Scientific, Vernon Hills, IL), chilled for 20 min, and then centrifuged at 13.000 rpm for 10 min (4°C). The supernatant was collected and filtered through a syringe-connected filter (Durapore membrane, 0.45 µm, SIGMA), and 20-µl volume of this solution was injected in the chromatography circuit.

HPLC dosage of brain octopamine, dopamine and serotonin

Biogenic amines were dosed by high-pressure liquid chromatography (HPLC) coupled with electrochemical detection (Antec, electrode potential fixed at 800 mV). Samples were injected using a Rheodyne 7725i injector valve with a 20-µl injection loop. Brain extracts were separated on a reversed phase C18 column (3.2×100 mm, 5-µm particle size, LC-22C, BAS, West Lafayette, IN). The mobile phase (pH 3) was composed of 3% methanol, 7% acetonitrile and 90% citric acid 20 mM, monobasic phosphate sodium 10 mM, octanesulfonic acid 3 mM, heptanesulfonic acid 3.25 mM, EDTA 0.1 mM, KCl 2 mM, *o*-phosphoric acid 6%, and diethylamine 2%. The flow rate was adjusted at 0.3 ml/min with a Gold 118 system (Beckman, Fullerton, CA), and temperature was set at 35°C (technical set-up from (Kodas et al. 2002)). External standards (octopamine, dopamine, serotonin) were run before and after each set of biological samples.

Statistical analysis

Kruskal–Wallis and Mann–Whitney tests were used to test the significance of the observed differences (XLStatistics). Differences were considered significant at $\alpha \leq 0.05$.

Results

Figure 1 compares the levels of octopamine, dopamine and serotonin for each social category. Octopamine levels were

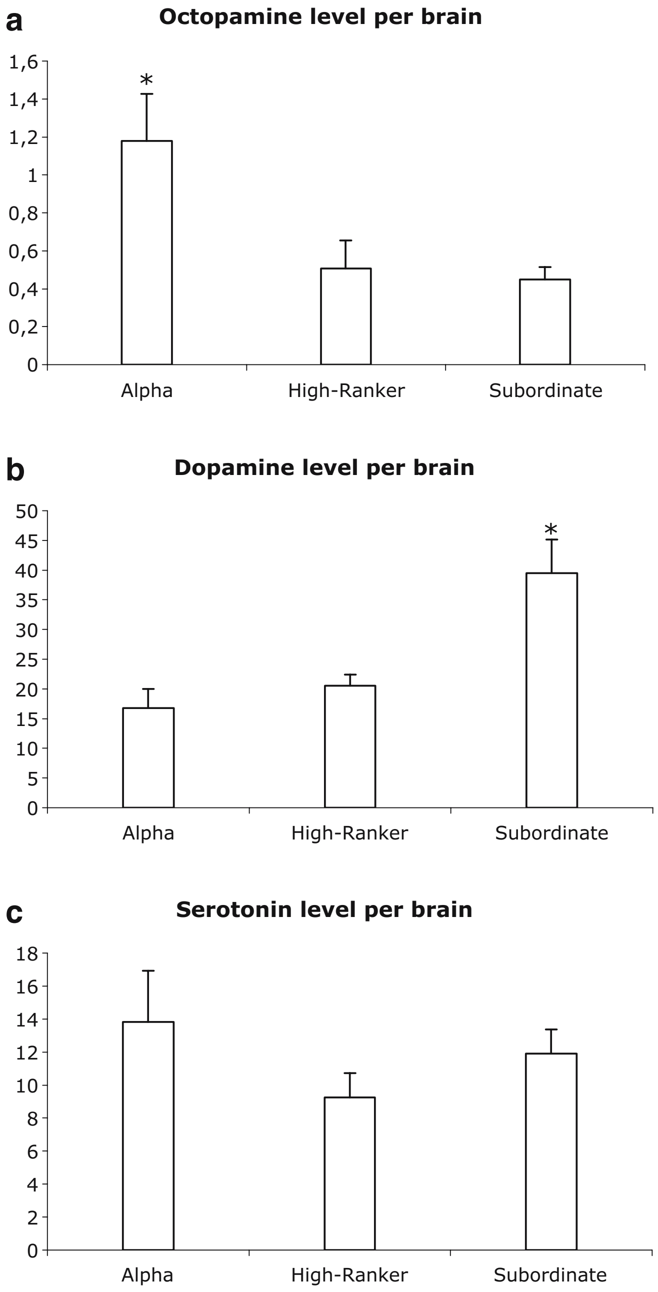

Fig. 1 Mean levels (in ng) and standard errors of **a** octopamine (*OA*), **b** dopamine (*DA*) and **c** serotonin, dosed in individual brains of ants of three different social status: alpha rank ($n=14$), high rank ($n=19$) and subordinate rank ($n=28$). *Asterisks* mark the amine dosages that significantly differ for a social group ($p<0.05$)

significantly higher in alpha workers (Kruskal–Wallis test: $H=15.7$, $df=2$, $p=0.0004$; Mann–Whitney tests: alpha/high-rank, $p=0.0009$; alpha/subordinate, $p=0.0003$; high-rank/subordinate, $p=0.3$). Dopamine levels were significantly higher in subordinate workers (Kruskal–Wallis test: $H=15.5$, $df=2$, $p=0.0004$; Mann–Whitney tests: alpha/high-rank, $p=0.07$; alpha/subordinate, $p=0.0006$; high-rank/subordinate, $p=0.006$). No significant difference was found in serotonin levels among social ranks (Kruskal–Wallis test: $H=5.7$, $df=2$, $p>0.05$).

Discussion

Individual dosage of brain monoamine levels revealed that fertile alpha workers are clearly characterised by higher levels of octopamine (Fig. 1a); this monoamine, thus, seems to correlate with reproductive activity in *Streblognathus*. In *Bombus* however (Bloch et al. 2000), brain OA dosages correlate with dominance status and not with ovarian development, playing the role of a social regulator that contributes to the whole physiological and behavioural package associated with high social ranking. It is well known that OA raises the general level of arousal, as extensively investigated in locust, for example (Stern 1999). OA treatment also was found to enhance the ability of guarding bees to distinguish between nestmates and non-nestmates (Robinson et al. 1999). Additionally, OA specifically increases the response of *Bombyx mori* olfactory neurons to the sexual pheromone (Pophof 2002). In view of these studies, it is very tempting to hypothesize that OA may be involved in the ability of the alpha workers to be the first to detect any change in the social status of their nest mates. Indeed, it has been shown that queenless ants can detect changes in the reproductive ability of other ants in their colony through a contact pheromone (Tsuji et al. 1999), most probably a blend of cuticular hydrocarbons (Cuvillier-Hot 2001; Peeters et al. 1999). Behavioural observations revealed that the alpha ant is the first to react when another reproductive worker is experimentally introduced in the nest (Cuvillier-Hot et al. 2005).

In *S. peetersi*, dopamine levels are inversely associated with social position: the fertile alphas show the lowest dopamine (DA) titres, while foragers show the highest, and high-ranked workers have intermediate values not significantly different from the alphas' levels ($p=0.07$; Fig. 1b). Thus, it is not likely that DA is involved in the stimulation of ovarian activity, as it is in other 'primitively organised' societies such as bumble bees (in *Bombus*, DA titre is correlated with ovarian development in workers, (Bloch et al. 2000)) or honey bee queenless workers (Harris and Woodring 1995; Sasaki and Nagao 2001). In honey bee queen-right workers, on the other hand, DA levels appear to be linked with age polyethism; older foragers display higher DA levels than younger nurses (Taylor et al. 1992; Wagener-Hulme et al. 1999). In *Pheidole dentata* workers, the DA level is also correlated with age (Seid and Traniello 2005). It is difficult to interpret results from workers of different tasks and ages, as the DA levels could depend on age, social activity, or both. Similarly, the DA levels in *S. peetersi* may simply reflect the age structure of a typical colony. Indeed, the alpha and the high-ranking workers are often the youngest individuals of the nest and are mainly involved in brood care, whereas, subordinate workers can be of any age. In this study, we specifically chose foragers as subordinate workers; they are generally the oldest individuals in the colony (documented in a close species, *Dinoponera quadriceps*, (Monnin and Peeters 1999)). The phylogenetic history of queenless ants may explain why, despite extremely different social organisations, the high levels of DA in both honeybees (Schulz and Robinson

1999) and queenless ants may be a result of age rather than social status. Phylogenetic studies have shown that the queenless ants had queen-right ancestors; the queen caste was replaced by reproductive workers. The neuro-physiological background of queenless ants is therefore that of "highly eusocial" species, closer to honeybees' than to bumble bees' physiological properties. The present results suggest a shift through which DA no longer regulates reproduction but has been co-opted for polyethism regulation. This major physiological transition of the role of DA is similar to the well-documented change in juvenile hormone activity in honey bees: juvenile hormone (JH) has a clear gonadotropic role in "primitively eusocial" species (*Bombus* (Bloch et al. 2000), *Ropalidia* (Agrahari and Gadagkar 2003)), whereas, it seems to have lost its reproductive function in honey bees, and instead, regulates colony polyethism (Hartfelder 2000; Robinson and Vargo 1997). As opposed to the pattern of JH levels in *Bombus*, JH levels in both honey bees and queenless ants are low in reproductive castes (and in honeybee nurses) and high in foragers in both cases (Robinson et al. 1991; Sommer et al. 1993). This double inversion of JH and DA roles suggests that DA in queenless ants plays an allatotropic role, as it does in several species (e.g., *Locusta migratoria* (Lafon-Cazal and Baehr 1988), *Apis mellifera* (Kaatz et al. 1994; Rachinsky 1994)).

The loss of the queen caste in queenless species occurred quite recently and independently in several branches of the Ponerini tribe, generating derived societies composed only of workers (Peeters 1993). Based on this phylogenetic history, we conclude that it would be better to compare queenless reproductive workers to reproductive workers observed in queenless colonies of normally queen-right species than to queens of queen-right species or to reproductive workers of other "primitively organised" species (bumble bees or wasps). For instance, the low DA and JH levels in reproductive queenless ants are very different from what has been observed in fire ant queens, which show a rise in DA with the onset of reproduction (Boulay et al. 2001) and have high JH levels when mature (Brent and Vargo 2003).

No significant differences in serotonin dosages were found between the three social groups in *S. peetersi* (Fig. 1c). Serotonin has often been associated with agonistic behaviour and dominance status both in vertebrates and invertebrates (reviewed in Lesch and Merschdorf (2000) for vertebrates; for invertebrates, e.g. (Huber 2005; Kravitz 2000)). It is somewhat surprising that serotonin brain levels were similar among alpha ants, high-ranked workers and subordinate workers, three behavioural castes that have markedly different standard aggressive states. However, it is possible that the castes experience differences in serotonin bursts at the very moment of a confrontation. The hypothesis that short bursts of serotonin may influence behaviour was suggested by the relationship established in crickets and lobsters between the serotoninergic activity and the incentive to fight (Dyakonova et al. 1999; Huber et al. 1997).

The results presented in this paper reveal a new combination of neurochemical influences on social structure: in *Streblognathus*, OA would regulate ovarian activity and DA seems involved in the control of age polyethism, as in honeybees. This combined pattern may testify to the inheritance of main regulatory pathways typical of highly eusocial species, as may be the case for DA. On the other hand, the new role for OA described in *Streblognathus* may reflect a highly labile character of the biochemical tools involved in the CNS control of social interactions. The social insect species previously studied are highly derived and are under very different ecological constraints. These differences may be sufficient, and may have existed long enough, to have relaxed the selective constraints on neuromodulators independently in several taxa. Each neurochemical actor would then have been recruited independently to control, in a specific manner, one or another aspect of the social framework of each species.

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