

# Effect of past and present behavioural specialization on brain levels of biogenic amines in workers of the red wood ant *Formica polyctena*

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**Abstract.** Social insect workers usually participate first in intranidal tasks (i.e. act as nurses within the nest) and then switch to extranidal tasks and become foragers. However, foragers sometimes switch back again to brood care and become reverted nurses. Behavioural and physiological correlates of the transition nurse–forager (behavioural maturation) and forager–reverted nurse (behavioural reversion) are relatively well known in the honeybee, although they are less explored in ants. To understand better the role of biogenic amines in ant behavioural maturation and behavioural reversion, the levels of octopamine (OA), dopamine (DA) and serotonin (5-HT) are examined in the brains of nurses, foragers and reverted nurses of the red wood ant *Formica polyctena* Först. (Hymenoptera: Formicidae). Brain OA levels and the ratios OA : DA and OA : 5-HT are higher in nurses than in foragers and reverted nurses. Reverted nurses and foragers do not differ significantly with respect to brain biogenic amine levels and amine ratios. Biogenic amine levels in brains of workers of *F. polyctena* are thus maturation-related rather than task-related. This is one of the first studies of neurochemical correlates of ant behavioural maturation and the first attempt to identify neurobiological correlates of ant behavioural reversion. The data obtained provide further evidence that neurobiological processes underlying honeybee and ant behavioural maturation and behavioural reversion reveal important differences.

**Key words.** Behavioural maturation, behavioural reversion, biogenic amines, dopamine, Formicidae, *Formica polyctena*, octopamine, ontogeny, polyethism, serotonin.

## Introduction

Workers of social Hymenoptera usually show strong behavioural polymorphism related largely to their specialization in various tasks (Page & Amdam, 2007; Smith *et al.*, 2008; Robinson, 2009; Hölldobler & Wilson, 2009). The term ‘behavioural specialization’ is now often reserved for relatively narrow specializations such as pollen or nectar foraging (Page & Amdam, 2007; Smith *et al.*, 2008). However, it is still

used in its broader sense to denote general specialization in brood care or foraging (Robinson, 2009). Such general specialization in intranidal/extranidal tasks depends strongly on worker age and/or attained stage of physiological maturation: young workers usually act first as nurses and then become foragers (Hölldobler & Wilson, 1990, 2009; Robinson, 2002, 2009; Smith *et al.*, 2008; Robinson *et al.*, 2009). However, organization of work in social insect colonies is determined not only by internal factors, but also by external ones. Among others, various environmental cues, including those generated by other colony members, may induce the expression of behaviour patterns atypical for a given developmental stage (Lenoir, 1987; Gordon, 1996). In particular, the transition nurse–forager may be delayed, accelerated and even reversed

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in response to changes of the social context (e.g. the creation of colony fragments composed solely of individuals of the same age). Foragers that switch again to brood care are termed reverted nurses (Free, 1965; Lenoir, 1979, 1987; Hölldobler & Wilson, 1990, 2009; Robinson, 2002, 2009; Page & Amdam, 2007; Smith *et al.*, 2008).

Behavioural and physiological correlates of the transitions nurse–forager and forager–reverted nurse are studied mostly in the honeybee and, to a lesser degree, also in other social Hymenoptera. In the honeybee, the transition nurse–forager is accompanied by changes in the expression of many thousands of genes and involves not only modifications of worker behaviour, but also profound modifications of many other phenotypic traits, such as morphology and activity of various exocrine glands, neuroanatomy and neurochemistry, hemolymph titres of juvenile hormone and vitellogenin, flight metabolism, abdominal lipid stores, relative concentrations of various proteins and functioning of the immune system. The majority (but not all) of these modifications are reversible (Free, 1965; Robinson, 2002, 2009; Page *et al.*, 2006; Page & Amdam, 2007; Smith *et al.*, 2008; Hölldobler & Wilson, 2009).

Ant behavioural maturation and behavioural reversion are less known (Robinson, 2009; Hölldobler & Wilson, 2009). The ability of ant foragers to revert to nursing behaviour was reported already by Ehrhardt (1931) and then confirmed subsequently in other studies (Dobrzańska, 1959; Lenoir, 1979; Sorensen *et al.*, 1984; McDonald & Topoff, 1985; Hölldobler & Wilson, 2009; Robinson, 2009), although physiological correlates of that process are so far little known. However, the fact that induction of brood care in ant foragers may occur very rapidly, within 24 h, argues against exocrine control of that process, and suggests that ant and honeybee behavioural reversion may represent fundamentally different phenomena (Lenoir, 1979; Sorensen *et al.*, 1984; McDonald & Topoff, 1985). Recent research demonstrates that the ability of older ant workers to perform tasks usually associated with an earlier developmental stage may not necessarily represent true reversion of behavioural ontogeny. In the myrmicine ant *Pheidole dentata*, worker behavioural development involves the expansion of behavioural repertoire rather than switching to new tasks coupled with the suppression of the ability to carry out formerly performed ones (Seid & Traniello, 2006). In particular, older workers of that species retain the ability to care for the brood and, in some respects, they are even more efficient as nurses than young individuals (Seid & Traniello, 2006; Muscedere *et al.*, 2009a).

Neurobiological correlates of behavioural maturation and behavioural reversion are of crucial importance for unravelling of proximate causation of these phenomena. Neuroanatomical modifications adapting workers to extranidal activities are well documented in the honeybee (Withers *et al.*, 1993; Farris *et al.*, 2001), wasps (O'Donnell *et al.*, 2004) and various species of ants (Gronenberg *et al.*, 1996; Seid *et al.*, 2005; Stieb *et al.*, 2010). Research on neurochemical correlates of behavioural specialization of social insect workers focuses mostly on the analysis of brain levels and patterns of brain localization of various biogenic amines (Taylor *et al.*, 1992; Kirchof *et al.*,

1999; Schulz & Robinson, 1999; Wagener-Hulme *et al.*, 1999; Schulz *et al.*, 2002, 2003; Seid & Traniello, 2005).

In the present study, attention is focused on three biogenic amines implicated in the mediation of numerous physiological and behavioural processes in invertebrates: octopamine (OA), dopamine (DA) and serotonin (5-hydroxytryptamine; 5-HT). Brain levels and/or localization of these three amines are often investigated jointly in the honeybee (Mercer *et al.*, 1983; Harris & Woodring, 1992; Taylor *et al.*, 1992; Schulz & Robinson, 1999; Wagener-Hulme *et al.*, 1999; Schulz *et al.*, 2003), bumblebees (Bloch *et al.*, 2000) and ants (Seid & Traniello, 2005; Cuvillier-Hot & Lenoir, 2006; Vander Meer *et al.*, 2008).

In the present study, brain levels of OA, DA and 5-HT and the ratios OA : DA, OA : 5-HT and 5-HT : DA are compared in three classes of workers of the red wood ant *Formica polyctena* Först. (Hymenoptera: Formicidae): nurses, foragers and reverted nurses. The study aims to determine whether and to what degree biogenic amine levels and/or amine ratios in the brains of ant workers depend on their past and present behavioural specialization, or, in other words, whether and to what degree they are maturation- and/or task-related.

## Materials and methods

### *Ant collection and housing*

Two groups of workers of *F. polyctena* were collected at the site Rzeszotków (central-eastern Poland) on 10 May 2006. The first group (approximately 10 000–15 000 workers) was collected from the mound together with the brood, mostly worker pupae. The second group (approximately 4500 foragers) was collected from the trails at approximately 2–5 m from the mound. The ants collected from the mound were housed in an artificial nest containing several large test tubes filled partly with water trapped in with a cotton plug to provide humidity gradient and covered with a sheet of aluminum foil to provide darkness. Already on the next day, these tubes were almost completely filled with brood and contained numerous nurses engaged in brood care behaviour. The ants collected from the trails were divided into two groups. The first group (approximately 500 individuals) was kept in a similar laboratory nest without contact with the brood. The second group (approximately 4000 individuals) was placed in a separate nest and exposed to the brood in the absence of nurses to induce the process of behavioural reversion. All the nests were maintained under an LD 12 : 12 h photocycle at a constant temperature (24°C). Carbohydrate food (honey mixed with crushed apple and sand added to make the mixture less sticky) and protein (pieces of dead house crickets) were provided three times a week.

### *Induction of behavioural reversion*

Behavioural reversion was induced by introducing a large amount of homocolonial brood (consisting mostly of several hundreds of worker pupae) into the nest housing the second

group of foragers. The brood was first introduced into that nest on 15 May 2006, 5 days after the collection of the ants. After 24 h, the brood had been moved by the adults into the test tubes with water reservoirs provided to serve as brood chambers. The contents of these tubes were then gently transferred on a small tray. During the next 15 min, workers were collected when engaged in interactions with the brood (i.e. sitting on the brood pile, making antennal contacts with the brood, licking and carrying it). The selected ants were then put together with the brood in a separate nest, and a new brood was introduced into the nest housing the second group of foragers to induce behavioural reversion in the next part of them. The procedure was repeated daily. The reverted nurses used in the study were taken from among former foragers (approximately 300 individuals) that began contact with the brood on 15 May 2006 but reverted to brood care in response to the brood introduced to their nest on 19 May 2006. They were placed in a separate nest on 20 May 2010 and, during the subsequent 7 days, they continued to be exposed to the brood. Newly-eclosed adult workers, the so-called callows, were removed daily to exclude the possibility that their presence close to the brood would increase the probability that the reverted nurses would switch again to the behavioural specialization of a forager. To counterbalance brood loss resulting from eclosures of callows, new brood taken from the nest housing the ants collected from the mound was added whenever necessary.

#### Experimental groups

Brains of three classes of workers were analyzed: nurses, foragers and reverted nurses. Workers belonging to each class were identified by means of a multistep procedure based on both spatial and behavioural criteria.

Nurses were selected from among individuals that, successively, were collected in the field from the nest mound, during the subsequent 16 days were kept in a laboratory nest together with the brood, and, finally, 1 h before being killed, stayed on brood piles inside the brood chambers of their nest.

Foragers were selected from among individuals that, successively, were collected in the field from ant trails at 2–5 m from the nest, during the subsequent 16 days were kept in a separate laboratory nest without contact with the brood and other ants, and, finally, 1 h before killing, were active in the foraging area of their nest.

Reverted nurses were selected from among individuals that, successively, were collected in the field from ant trails at 2–5 m from the nest, were housed in a separate nest without contact with other workers, after 5 days from their capture started to be exposed to the brood in the absence of nurses, after 5 days from the beginning of exposure of foragers to the brood were found on brood piles in the brood chambers of their nest, remained on brood piles when the tubes serving as brood chambers were taken out of the nest, engaged in interactions with the brood when the contents of these tubes were transferred to a tray, during the subsequent 7 days were kept in a separate nest in which they continued to be exposed to the brood in the absence of other workers, and, finally, 1 h

before killing, stayed on brood piles inside the brood chambers of their nest.

#### Brain dissection

To reduce the possible effects of disturbance caused by removal from the nest, before killing, several hundreds of workers from each experimental group were gently transferred into small plastic containers provided with small pieces of folded aluminium foil under which they could settle. Nurses and reverted nurses were transferred together with the whole contents of the test tubes acting as brood chambers in their nests. Foragers were captured by forceps in the foraging area. The ants were left for approximately 1 h and then were killed instantly by submersion in liquid nitrogen ( $-196\text{ }^{\circ}\text{C}$ ) poured rapidly into each container. Workers used in the further stages of the experiment were then stored in Eppendorf tubes at  $-80\text{ }^{\circ}\text{C}$  until dissection. Dissections were performed on dry ice to minimize the degradation of biogenic amines. Because amine levels had to be expressed in fmol/brain, even slightly damaged brains were discarded. Extracted brains were placed in 1.5-mL Eppendorf tubes (five brains per sample) and stored at  $-80\text{ }^{\circ}\text{C}$  until high-performance liquid chromatography (HPLC) analysis.

#### Sample preparation

Chilled bufor (75  $\mu\text{L}$ ; 25% methanol, 15% acetonitrile, 60%  $3 \times \text{dH}_2\text{O}$ ) was added to each sample, sonicated in ice for 1 min (0.85 cycle, 75% amplitude; sonicator: U50 control; IKA Labortechnik, Wilmington, North Carolina), and centrifuged at  $4\text{ }^{\circ}\text{C}$  at 11 525 g for 10 min (MPW 60; MPW Medical Instruments, Poland). The supernatant was collected and filtered (Spartan 3 Syringe Filter, Nylon Membrane 0.2  $\mu\text{m}$ ; Sigma, St Louis, Missouri). Twenty microlitres of filtered sample was then injected into the chromatography circuit by means of a Hamilton syringe. For each experimental group (nurses, foragers and reverted nurses), ten samples were analyzed, with five brains per sample.

#### HPLC analysis of octopamine, dopamine and 5-hydroxytryptamine

The HPLC system consisted of a L-6200A Intelligent Pump (Merck-Hitachi, Japan), L-7360 Column Oven (Merck, Germany), Li-ChroCART 250-4 column (Purospher RP18, 5  $\mu\text{m}$ ; Merck) and L-3500A Amperometric Detector (Merck-Recipe, Germany). Electrochemical detector electrode potential was set at 800 mV. The mobile phase (pH = 4.17) was composed of 1.2% methanol, 6.1% acetonitrile, monobasic phosphate sodium 75 mM, octanesulfonic acid 0.525 M and EDTA 1.25 mM. The flow rate was adjusted at  $0.7\text{ mL min}^{-1}$ , and the temperature was set at  $35\text{ }^{\circ}\text{C}$ . The samples were injected in sets of three. Each set included samples from all three analyzed groups. Within each set, samples were injected

in random order. External standards (OA, DA and 5-HT) were run before and after each set of experimental samples. Data were collected and analyzed using MULTI HPLC SYSTEM MANAGER software, version 3.1.1 (Merck-Hitachi).

### Statistical analysis

The data obtained for three experimental groups were compared by analyzing six neurochemical variables: brain levels of OA, DA and 5-HT expressed in fmol/brain and the ratios OA : DA, OA : 5-HT and 5-HT : DA calculated separately for each tested sample. Because the distributions of the analyzed variables were mostly not normal, nonparametric tests were used [Kruskal–Wallis analysis of variance (ANOVA) followed by Siegel & Castellan post-hoc tests for pairwise comparisons of independent data taking into account the inherent error rates accompanying multiple comparisons] (Siegel & Castellan, 1988).

### Results

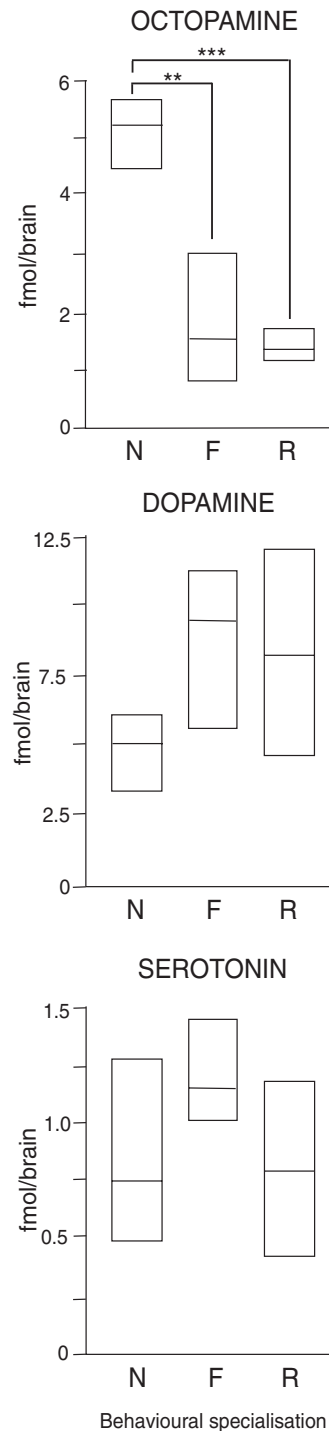
Brain levels of OA, DA and 5-HT obtained for nurses, foragers and reverted nurses of the red wood ant *Formica polyctena* are shown in Fig. 1. The ratios of various biogenic amine levels (OA : DA, OA : 5-HT and 5-HT : DA) obtained for these groups are shown in Fig. 2.

As shown in Fig. 1, significant differences between the tested groups were found only in the case of OA (Kruskal–Wallis ANOVA:  $P < 0.005$ ). As revealed by Siegel & Castellan post-hoc tests, brain OA levels were significantly higher in nurses than in foragers and reverted nurses (Fig. 1). Foragers and reverted nurses did not differ significantly either with respect to OA levels, nor to any of the remaining analyzed amine levels (Fig. 1). Brain DA levels tended to be higher in foragers and reverted nurses than in nurses (Fig. 1) and 5-HT levels tended to be higher in foragers than in the remaining worker groups, and to take intermediate values in reverted nurses (Fig. 1). However, none of these trends was significant.

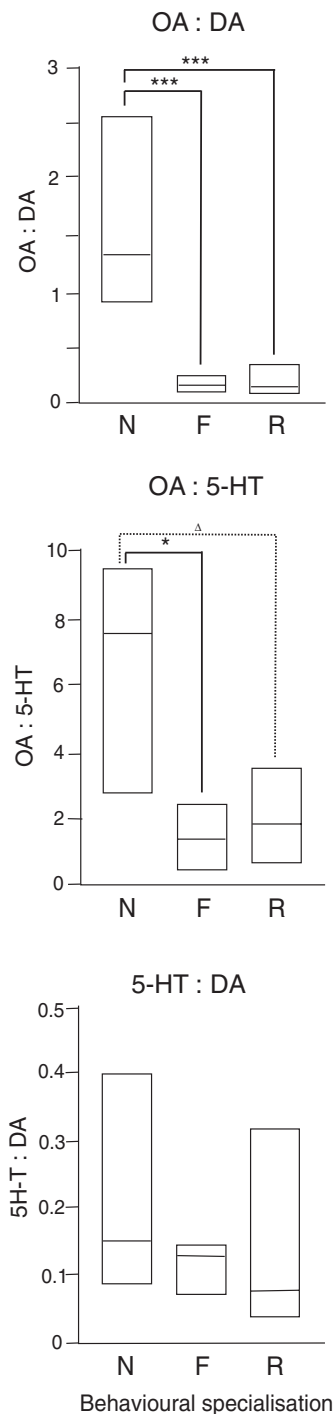
The analysis of brain biogenic amine ratios yielded significant intergroup differences in the case of the ratios OA : DA (Kruskal–Wallis ANOVA:  $P < 0.001$ ) and OA : 5-HT (Kruskal–Wallis ANOVA:  $P < 0.02$ ). Both these ratios took significantly higher values in nurses than in other ant groups (Fig. 2). Foragers and reverted nurses did not differ with respect to any of the analyzed biogenic amine ratios (Fig. 2).

### Discussion

The present study provides one of the first reports of significant differences in brain amine levels and amine ratios related to age-based behavioural specializations in ants. So far, among social Hymenoptera, such differences are documented mostly in the case of the honeybee (Harris & Woodring, 1992; Taylor *et al.*, 1992; Wagener-Hulme *et al.*, 1999; Schulz & Robinson, 1999; Schulz *et al.*, 2003). In ants, age- and task-related



**Fig. 1.** Brain levels of octopamine (OA), dopamine (DA) and serotonin (5-HT) in nurses (N), foragers (F) and reverted nurses (R) of the red wood ant *Formica polyctena* (medians and quartiles,  $n = 10$  samples in each group). Kruskal–Wallis analysis of variance:  $P < 0.005$  (OA), NS (DA), NS (5-HT). Siegel & Castellan post-hoc tests for pairwise comparisons of independent data: \*\* $P < 0.01$ , \*\*\* $P < 0.005$ .



**Fig. 2.** Ratios OA : DA, OA : 5-HT and 5-HT : DA in brains of nurses (N), foragers (F) and reverted nurses (R) of the red wood ant *Formica polycтена* (medians and quartiles,  $n = 10$  samples in each group) (OA, octopamine; DA, dopamine; 5-HT, serotonin). Kruskal–Wallis analysis of variance:  $P < 0.001$  (OA : DA),  $P < 0.02$  (OA : 5-HT), NS (5-HT : DA). Siegel & Castellan post-hoc tests for pairwise comparisons of independent data:  $\Delta$ :  $P = 0.05$ , \* $P < 0.05$ , \*\*\* $P < 0.005$ .

differences of biogenic amine brain levels and/or patterns of brain localization are reported in few studies (Punzo & Williams, 1994; Seid & Traniello, 2005; Seid *et al.*, 2008).

The present study shows many parallels with the study of Seid & Traniello (2005) on age- and task-related differences in brain biogenic amine levels and amine ratios of minor workers of the myrmicine ant *P. dentata*. However, whereas Seid & Traniello (2005) investigate workers from four age classes selected on the basis of their pigmentation and behaviour, in the present study, the criteria are not based solely on worker age, but also on two factors influencing worker behaviour: the attained stage of age-related behavioural maturation (nurses versus foragers and reverted nurses) and current behavioural specialization (ordinary and reverted nurses versus foragers).

The results of the present study and Seid & Traniello (2005) show both differences and similarities. In *P. dentata*, brain 5-HT levels are higher in foragers than in nurses from all age classes, DA levels rise steadily with worker age, and OA levels do not differ between the tested groups (Seid & Traniello, 2005). By contrast, in *F. polycтена*, brain levels of 5-HT and DA do not differ significantly between the tested groups. However, it should be noted that DA levels tend to be higher in foragers and reverted nurses than in ordinary nurses (presumably younger than workers from the other two classes) and 5-HT levels tend to be higher in foragers than in both ordinary and reverted nurses. Hence, it cannot be excluded that age- and task-dependence of brain DA and 5-HT levels may follow the same pattern in the myrmicine species *P. dentata* and in the formicine species *F. polycтена* but, in *F. polycтена*, these effects are relatively less strongly expressed. Relatively small sample sizes cannot be held fully responsible for a lack of statistical significance of the discussed effects in *F. polycтена* because Seid & Traniello (2005) employ the same sample size and, nevertheless, obtain significant intergroup differences in brain 5-HT and DA levels.

Although, in *P. dentata*, brain OA levels do not show significant intergroup differences (Seid & Traniello, 2005), in *F. polycтена*, they are significantly higher in nurses than in foragers and reverted nurses. The compared studies show, however, striking concordance with respect to maturation-related differences in brain ratios of OA : 5-HT and OA : DA. In *P. dentata*, the ratio OA : 5-HT is significantly higher in the youngest nurses than in the oldest workers acting as foragers, and the ratio OA : DA is significantly higher in the youngest nurses than in the nurses belonging to the next age class. In the present study, both these ratios are higher in nurses than in both classes of worker that had already acted as foragers. This concordance is particularly worthy of note because *P. dentata* and *F. polycтена* belong to different ant subfamilies. However, the same effect may result from different mechanisms in the case of each tested species. Although, in *P. dentata*, OA levels appear to remain stable during behavioural maturation, whereas DA and 5-HT levels rise (Seid & Traniello, 2005), in *F. polycтена*, OA levels drop, whereas DA and 5-HT levels remain relatively more stable.

The present study provides one of the first reports of maturation-related differences in brain amine levels and amine ratios in ants and the first attempt to shed light on

neurobiological correlates of ant behavioural reversion. Similar to Seid & Traniello (2005), age- and task-related changes in brain biogenic amine levels show important differences between ants and honeybees. In the honeybee, the transition nurse–forager is associated with increased levels of brain OA, in particular in the antennal lobes (Schulz & Robinson, 1999; Wagener-Hulme *et al.*, 1999; Schulz *et al.*, 2003). The oral administration of OA also may accelerate the onset of foraging behaviour (Schulz & Robinson, 2001; Schulz *et al.*, 2002). In ants, the mediation of the transition nurse–forager does not appear to be associated with the elevation of brain OA levels, which remain stable (Seid & Traniello, 2005) or even drop (present study). Data from studies by Seid & Traniello (2005) and Seid *et al.* (2008) indicate the role of the maturation of brain 5-HT networks, in particular those involved in the processing of visual information and in the mediation of agonistic behaviour. The role of 5-HT in the mediation of the transition nurse–forager in myrmicine ants is confirmed by Muscedere *et al.* (2009b), who report that oral administration of the 5-HT precursor 5-hydroxytryptophan has an enhancing effect on the probability of leaving the nest and on trail-following behaviour of workers of *P. dentata* compared with controls and workers whose 5-HT levels are reduced by a 5-HT synthesis blocker. As already stated, in the present study, brain levels of 5-HT also tend to be highest in foragers, although the effect is not statistically significant.

In the present study, brain 5-HT levels also tend to take intermediate values in reverted nurses in relation to nurses and foragers (Fig. 1), exactly as might be expected in the case of incomplete reversion from the physiological state of a forager to that of a nurse. However, there are no significant effects providing evidence of the reversibility of neurochemical modifications accompanying the transition nurse–forager. Significant differences in brain amine levels and amine ratios are seen solely between nurses and two groups of older workers that have already acted as foragers (Figs 1 and 2). Biogenic amine levels and/or amine ratios in the brains of workers of *F. polyctena* are thus maturation-related rather than task-related, and significant maturation-related modifications appear to be irreversible.

In the honeybee, the question of reversibility of neurochemical modification accompanying the transition nurse–forager is difficult to answer in an unequivocal way. Wagener-Hulme *et al.* (1999) report that although nurses from control colonies have lower brain levels of OA, DA and 5-HT than foragers from the same colonies, reverted nurses do not differ from the foragers from reversion colonies with respect to brain OA and 5-HT levels and, surprisingly, they have significantly higher brain DA levels. A similar result is obtained when only mushroom bodies of worker honeybees are examined (Schulz & Robinson, 1999). However, reverted nurses and foragers from the reversion colonies differ significantly with respect to biogenic amine levels in the antennal lobes and in the parts of the brain that remain after the removal of the mushroom bodies and the antennal lobes. These differences show almost exactly the same pattern as differences between control nurses and control foragers, which suggests strongly that behavioural reversion is accompanied by the reversal of neurochemical

changes taking place in these brain structures during the transition nurse–forager.

The question arises as to what degree neurochemical similarities and differences between various classes of ants investigated in the present study are indeed maturation- and/or task-related, and to what degree they may result from the impact of other factors, such as differences in social environment and/or group demography, or disturbance experienced before killing. Although all ants are collected from the same maternal colony in the present study, workers belonging to various experimental groups are housed in separate nests during the approximately 2 weeks separating their collection and death. Nurses live in a larger group than foragers and reverted nurses, and have contacts with older ants acting as foragers, whereas foragers and reverted nurses have no contacts with younger workers acting as nurses. Moreover, whereas both nurses and reverted nurses have contacts with the brood and newly-eclosing callows, foragers are brood-deprived.

The possible impact of differences in group demography on brain amine levels of the compared ant groups is difficult to evaluate. The available literature on that topic is scarce. Kostowski *et al.* (1975) report a significant increase in the brain level of 5-HT in workers of *Formica rufa* after 1 h of isolation in a small group (10–12 individuals) and Vander Meer *et al.* (2008) report a significant decrease in OA levels in the brains of minor workers of a myrmicine species *Solenopsis invicta* in response to queen removal. However, Boulay (1999) fails to find significant differences in brain levels of OA, DA and 5-HT in workers of the formicine species *Camponotus fellah* after 5 days of complete social isolation, although the data show high variability and, as noted by Boulay (1999), any conclusions made cannot be considered robust. Interestingly, brain levels of 5-HT tend to rise as a consequence of social isolation (Boulay, 1999), similar to the findings of the study of Kostowski *et al.* (1975), whereas brain levels of OA and DA tend to drop. In light of these data, it cannot be entirely ruled out that significantly higher brain levels of OA detected in nurses of *F. polyctena* might be related to larger group size. Such an eventuality is unlikely, however, because no significant concurrent rise in 5-HT levels is observed in brains of ants reared in smaller groups. Moreover, even if foragers and reverted nurses are kept in smaller groups, these groups comprise several hundred individuals, and provide a social environment profoundly different from the conditions encountered in a small worker group or in complete social isolation.

It is also important that reverted nurses and foragers are selected on the basis of behaviour, and not simply by taking any worker from the respective experimental nests. An absence of significant neurochemical differences between these two classes of ants is thus observed despite profound behavioural differences, which further supports the conclusion that significant neurochemical differences detected in the present study are maturation-related rather than task-related. Significant differences in brain OA levels of nurses and reverted nurses, two classes of workers that currently specialize in nursing behaviour, also imply that the expression of nursing behaviour is not determined solely by brain amine levels.

It is also possible that the differences in brain OA levels of nurses and the remaining two worker groups might be at least partly generated by differences in disturbance encountered shortly before death. 'Stress' and the activation of the octopaminergic system are closely interlinked in insects (Davenport & Evans, 1984; Harris & Woodring, 1992; Hirashima *et al.*, 2000). The period during which the ants are kept outside their nest before killing may be more 'stressful' for nurses, which, up to that point, remain inside the nest, than for foragers and reverted nurses, which are already well accustomed to outside-nest conditions. However, Harris & Woodring (1992) describe a rapid increase of brain levels not only for OA, but also for 5-HT in honeybee foragers with clamped legs. In the present study, brain 5-HT levels of nurses are not elevated in comparison to the remaining worker groups, which argues against the role of disturbance in the causation of the observed intergroup differences in OA levels. The final determination of the effects of age, physiological maturation, present behavioural specialization, social environment, and disturbance before death on brain levels of biogenic amines in ants from various phyla remains the subject for future research.

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