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ORIGINAL PAPER

Biogenic amines are associated with worker task but not patriline in the leaf-cutting ant *Acromyrmex echinatior*

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Abstract Division of labor among eusocial insect workers is a hallmark of advanced social organization, but its underlying neural mechanisms are not well understood. We investigated whether differences in whole-brain levels of the biogenic amines dopamine (DA), serotonin (5HT), and octopamine (OA) are associated with task specialization and genotype in similarly sized and aged workers of the leaf-cutting ant *Acromyrmex echinatior*, a polyandrous species in which genotype correlates with worker task specialization. We compared amine levels of foragers and waste management workers to test for an association with worker task, and young in-nest workers across patrilines to test for a genetic influence on brain amine levels. Foragers had higher levels of DA and OA and a higher OA:5HT ratio than waste management workers. Patrilines did not significantly differ in amine levels or their ratios, although patriline affected worker body size, which correlated with amine levels despite the small size range sampled. Levels of all three amines were correlated within individuals in both studies. Among patrilines, mean levels of DA and OA, and OA and 5HT were also correlated. Our results suggest that differences in biogenic amines could regulate worker task specialization, but may be not be significantly affected by genotype.

Keywords Division of labor · Biogenic amines · Response threshold · Social insect · Task specialization

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Introduction

Division of labor is one of the diagnostic features of eusociality, and understanding how work in insect societies is efficiently organized among thousands of interacting nestmates is a major topic in the evolution of biological complexity (Wilson 1971; Maynard Smith and Szathmáry 1995). Innate or experience-related neurobiological variation among workers likely underlies worker motivational states, sensory thresholds, and/or task-related aptitudes that can serve as mechanisms for polyethism and thus division of labor. The response threshold model of social insect polyethism, for example, posits that stimulus–response thresholds vary among individuals, and that their interaction influences worker task specialization and division of labor (Detrain and Pasteels 1992; Robinson 1992; Theraulaz et al. 1998; Beshers and Fewell 2001). Response thresholds may vary systematically among workers of different ages, sizes, and genetic backgrounds, producing behaviorally differentiated workers. However, while variation in worker behavior associated with size, age and genotype have been documented (Robinson and Page 1989; Detrain and Pasteels 1992; Hölldobler and Wilson 1990; Oldroyd et al. 1994; Huang and Robinson 1996; Naug and Gadagkar 1999; Arathi and Spivak 2001; Seid and Traniello 2006; Chapman et al. 2007; Ravary et al. 2007; Hughes and Boomsma 2007), the neural mechanisms underlying subcaste- and age-related task performance and the genesis of inter-individual differences in responsiveness to task stimuli are only beginning to be understood (Withers et al. 1995; Ben-Shahar et al. 2002; Molina and O'Donnell 2008; Arenas et al. 2009; Kelber et al. 2009; Lucas and Sokolowski 2009; Maleszka et al. 2009; Riveros and Gronenberg 2010; Stieb et al. 2010, 2012; Muscedere and Traniello 2012; Giraldo et al. 2012; Kamhi and Traniello 2013).

Accumulating evidence suggests that the actions of biogenic amines such as dopamine (DA), serotonin (5HT), and octopamine (OA), which function as neurotransmitters, neuromodulators, and neurohormones, can mediate behavioral plasticity in insects (Kravitz 2000; Libersat and Pflueger 2004; Anstey et al. 2009). In ant and honey bee (*Apis mellifera*) workers, amine levels have been associated with task specialization and sensory sensitivity (Schulz and Robinson 2001; Barron et al. 2002, 2007; Schulz et al. 2003; Barron and Robinson 2005; Seid and Traniello 2005; Page et al. 2006; Scheiner et al. 2006; Seid et al. 2008; Wnuk et al. 2011; Muscedere et al. 2012). However, in most cases, the association between amine levels and task performance in social insects correlates with age: workers typically remain in the nest while young, and forage outside when older, thus confounding age and task specialization (reviewed by Page et al. 2006; Scheiner et al. 2006; Bloch et al. 2009). Only in honey bees have differences in amine levels been shown among same-age workers with different task specializations (Taylor et al. 1992; Božic and Woodring 1998; Schulz and Robinson 1999). Many ants exhibit well-developed task specialization among workers, and amines appear to influence several aspects of ant behavior (e.g. Boulay et al. 2000; Hoyer et al. 2005; Cuvillier-Hot and Lenoir 2006; Falibene et al. 2012; Muscedere et al. 2012; reviewed in Kamhi and Traniello 2013), but the aminergic regulation of worker polyethism has specifically been investigated in only *Pheidole dentata* (Seid and Traniello 2005; Seid et al. 2008; Muscedere et al. 2012, 2013) and *Formica polyctena*: (Wnuk et al. 2011), largely in the context of age-related behavioral development. Moreover, in many ant species, division of labor among workers is associated with variation in body size and morphology, further confounding physiological comparisons (Hölldobler and Wilson 1990).

Leaf-cutting ants (Attini: *Atta* and *Acromyrmex*) are a paradigm of size-related polyethism (alloethism), in which colony tasks are finely subdivided among workers of distinct size ranges (Wilson 1980). However, not all tasks are performed by workers of particular size classes: in *Acromyrmex echinator*, foragers—ants that leave the nest to search for leaf material on which to grow their fungal crop—and waste management workers—ants that remove waste from the nest and/or tend a refuse pile—are well-suited for understanding genetic influences on behavioral physiology because workers typically are of similar size and age (Waddington and Hughes 2010). *Acromyrmex echinator* queens are polyandrous (Sumner et al. 2004) and patriline differences between half-sister workers affect caste development, disease resistance, and task performance (Hughes et al. 2003, 2010, Armitage and Boomsma 2010; Constant et al. 2012). Specifically, genotype influences whether workers engage in waste management or forage (Waddington et al. 2010). The advantages of this division of labor have been used to explain selection for polyandry: a more genetically diverse workforce consisting of half-sisters from multiple patrilines may have a greater range of response thresholds to perceive environmental stimuli (Crozier and Page 1985; Oldroyd and Fewell 2007; Jones et al. 2004; Mattila and Seeley 2007). The proximate basis of patriline-associated differences in responsiveness to task-related stimuli, however, remains unknown. One possibility is that genotype influences brain levels of biogenic amines, which are known to modulate behavior and could mechanistically translate genetic variation into variation in task performance by affecting sensory thresholds.

Here we investigate the influence of genetic variation on the aminergic regulation of division of labor by testing for differences in brain biogenic amine levels between patrilines and task groups (foragers and waste managers) of *Acromyrmex echinator*. Foraging and waste management are developmental endpoints for leaf-cutting workers, which have broadly overlapping size and age distributions, and individuals do not typically switch between these specializations (Hart and Ratnieks 2001, 2002; Bot et al. 2001; Ballari et al. 2007; Camargo et al. 2007; Waddington and Hughes 2010). *A. echinator* thus offer an excellent system to test the effect of brain amines on worker task specialization and the association of genetic differences and neurochemistry independent of worker size and age. No previous study has examined differences in amine levels between patrilines of a polyandrous social insect, nor tested for a difference in biogenic amines independent of age in any species other than the honey bee. We first tested whether levels of the amines DA, 5HT, and OA differ between *A. echinator* foragers and waste workers by quantifying whole-brain levels of DA, 5HT, and OA in individuals of both groups across two colonies. We then determined whether amines differ between genotypes by measuring amine levels in young, in-nest workers and using microsatellite markers to designate patriline. Differences between patrilines would indicate a genetic influence on amine levels. We used in-nest workers because it is unclear to what extent amine differences between older groups of workers are a cause or a consequence of different task specialization (e.g. Muscedere et al. 2013), thereby avoiding potentially confounding effects of experience. Amine level differences identified among young in-nest workers that have not yet developed into either foragers or waste workers would be consistent with genetic differences in amine levels influencing task specialization by mature workers.

Materials and methods

Two mature monogynous colonies of *A. echinatior* (Ae088 and Ae07P4) collected in Gamboa, Panama, in 2007 and 2008 were used. At the time of the study, Ae088 contained ~900 ml of fungus garden and Ae07P4 ~1,000 ml. Colonies were kept in plastic boxes (17 × 36 × 54 cm), provided with inverted plastic beakers to contain the fungus gardens and a 10-cm diameter pot in which the ants deposited waste. Colonies were maintained on a diet of privet leaves (*Ligustrum* spp.) provided in a separate 10-cm diameter pot, and cultured at 80 ± 5 % relative humidity and 26 ± 2 °C.

Sampling

On average, foragers are larger and slightly older than waste workers, but there is broad overlap between the groups (Waddington and Hughes 2010). To minimize the influence of these differences, we collected only workers of intermediate size (~1.6 mm head width) and medium-brown cuticular coloration, excluding both young light-colored individuals and dark individuals which occur more frequently among foragers (Waddington and Hughes 2010). Cuticle color was used to estimate age: workers darken predictably with maturation (Armitage and Boomsma 2010). For analysis of task groups, individuals were removed from either the leaf pot (foragers) or the waste pot (waste workers) with foreceps and placed immediately into chilled containers on ice. Under normal conditions, waste workers and foragers do not switch between tasks (Waddington and Hughes 2010), so extensive behavioral observation to confirm task specialization was not necessary. For patriline analyses, we collected within-nest workers by placing a portion of the fungus garden into a chilled container on ice and removing workers from the garden with forceps. We chose individuals of approximately the same size as foragers and waste workers already sampled, but with lighter brown cuticle coloration, as within-nest workers are generally younger than outside-nest foragers and midden workers. We did not sample callow workers. The total number of workers measured for task specialization analysis was 17 for Ae088 (9 foragers, 8 waste workers) and 20 for Ae07P4 (10 foragers, 10 waste workers), while 53 workers from Ae07P4 and 33 from Ae088 were analyzed for the patriline comparisons. After brain dissection (see below), we measured the length of one rear femur from each worker using Image J (Rasband 1997) from digital photographs taken through a microscope at 13× magnification as a measure of body size. Femur length was used as a proxy for body size: workers from a separate sample show that femur length correlates significantly with head width (Pearson $r = 0.99$, $n = 42$, $P < 0.001$). The same digital photographs were also used to measure cuticle color as a grayscale value in Image J, which divides grayscale into 256 bins (0 = black, 256 = white) following Armitage and Boomsma (2010) to estimate relative age.

Preparation of samples for HPLC

Worker brains were dissected in ice-cold saline in a petri dish on ice. Each brain was removed from the head capsule and placed into a cold 0.5 ml plastic centrifuge tube, which was immediately submerged in liquid nitrogen. Samples were then kept on dry ice for 1–2 days before HPLC analysis.

Measurement of biogenic amine levels

Brain amine levels were quantified by high-performance liquid chromatography with electrochemical detection (HPLC-ED) as described previously (Muscedere et al. 2012, 2013), with modifications to allow the simultaneous detection of OA in addition to DA and 5HT. The HPLC-ED system used consisted of a Coulochem III electrochemical detector, a model 5014B dual channel coulometric microdialysis cell, a model 584 pump, a model MD-150 (3 × 150 mm) reversed-phase analytical column (all components manufactured by Dionex), and custom mobile phase (50 mM citrate/acetate buffer, 1.5 mM sodium dodecyl sulfate, 0.01 % triethylamine, 22 % acetonitrile in MilliQ water). Individual brains were immediately homogenized in mobile phase after removal from dry ice. Each homogenate was then centrifuged and 20 µl of supernatant injected onto the HPLC column. 5HT and DA were detected on the first electrode channel (set to 375 mV), and OA was detected on the second (set to 600 mV). Amine levels were quantified with reference to serial dilutions of external standards containing all three amines, run daily.

Molecular analysis

DNA was extracted from ant thoraces using 5 % Chelex (Bio Rad, Hercules, CA) and amplified at microsatellite loci *Ech1390*, *Ech3385*, *Ech4126*, and *Ech4225* (Ortius-Lechner et al. 2000). Polymerase chain reaction (PCR) amplification was performed using 10 µl mixtures containing 40 µM deoxyribonucleotide triphosphates, 3 mM $MgCl_2$, 0.5 µM primers, 0.5 U of *Taq* DNA polymerase, 1× buffer, and 1 µl DNA. Amplifications were run in GeneAmp 9700 PCR Systems with an initial denaturing step of 94 °C for 2 min followed by 35 cycles of 94 °C for 2 min, 55 °C (*Ech3385* and *Ech4225*) or 60 °C (*Ech1390* and *Ech4126*) for 45 s, and 72 °C for 2 min, and finally 72 °C for 7 min. Products were genotyped using an ABI 3130*xl* capillary sequencer, and allele sizes determined by comparison with internal size standards. The genotypes of the mother queen and her multiple mates were deduced from the multilocus worker genotypes and individuals assigned to patrilines. Individuals that could not be assigned to patrilines due to failed PCR amplification or sharing the same genotype at a diagnostic locus as a heterozygous mother queen were excluded from the analysis.

Statistical analyses

Amine levels (pg/brain) were square-root transformed to fit the assumptions of parametric statistics (for summary statistics of the untransformed values, see ESM Tables 1, 2). To test for an effect of task specialization on amine levels, we used the General Linear Model (GLM) function in SPSS 18 with square-root transformed amine level as the dependent variable, task group (nested within colony) as a fixed effect, and both leg length and cuticle color as covariates. To test for an effect of patriline, we used a similar model, but used patriline (nested within colony) in place of task group. We also performed GLMs using the ratio of each pair of amines, which may be important in neurochemical control (Kravitz 2000; Seid and Traniello 2005), computed from the square root transformed values, as dependent variables.

Results

Task specialization and brain amine levels

Foragers had significantly higher levels of DA and OA, but not 5HT, than waste workers (Fig. 1; Table 1). For all amines, the effect of body size (measured as femur length) was significant (Table 1), and body size correlated strongly with amine levels (Fig. 2a; DA Pearson's $r = 0.72$, $n = 37$, $P < 0.001$; 5HT Pearson's $r = 0.71$, $n = 37$, $P < 0.001$; OA Pearson's $r = 0.50$, $n = 35$, $P = 0.002$). There was no significant effect of relative age on amine levels (Table 1). Waste workers and foragers did not differ in femur length ($F = 0.12$, $df = 1$, LR χ^2 $P = 0.73$) or cuticle color ($F = 0.62$, $df = 1$, LR χ^2 $P = 0.44$), as expected from our sampling methodology. Levels of the three amines were correlated among individuals based on residuals of the GLM (summarized in Table 1; Fig. 3a–c; DA–5HT: Pearson's $r = 0.42$, $n = 37$, $P = 0.01$; DA–OA: Pearson's $r = 0.48$, $n = 35$, $P = 0.003$; 5HT–OA: Pearson's $r = 0.74$, $n = 35$, $P < 0.001$; Fig. 3a–c). There was no significant effect of task group (nested within colony) for the ratios of DA:5HT or DA:OA, but there was for OA:5HT (DA:5HT: GLM $F_{3,31} = 1.95$, LR χ^2 $P = 0.14$; DA:OA: $F_{3,29} = 0.84$, LR χ^2 $P = 0.48$; OA:5HT: $F_{3,29} = 3.75$, LR χ^2 $P = 0.02$).

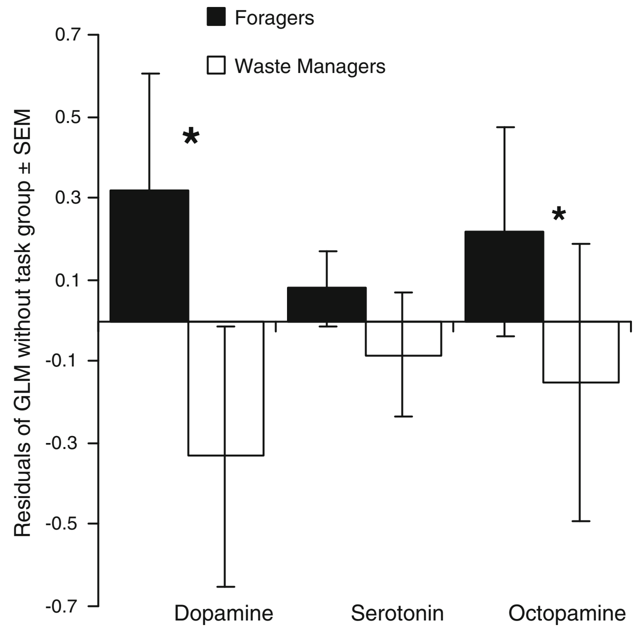


Fig. 1 Levels of the amines DA, 5HT and OA in the brains of medium-sized and medium age leaf-cutting ant workers engaged in foraging (*gray*) and waste management (*white*). Amine levels are presented as the mean ± SEM residuals controlling for the size and age of the ants. *Asterisks* above an amine pair indicate a significant difference between the task groups

Patriline and brain amine levels

There were no significant effects of patriline on the levels of any of the three amines (Table 2; Fig. 4). For all amines, the effect of body size was significant (Table 2), and body size correlated strongly with amine levels (Fig. 2b; DA Pearson's $r = 0.63$, $n = 88$; 5HT Pearson's $r = 0.79$, $n = 88$; OA Pearson's $r = 0.66$, $n = 87$, all P values <0.001). There was no significant effect of age (Table 2). There were correlations across patrilines in the mean

Table 1 General Linear Model results for task specialization

Variable	Dopamine			Serotonin			Octopamine		
	df	F	P	df	F	P	df	F	P
Task (colony)	3	3.83	**0.02**	3	0.94	0.43	3	3.24	**0.04**
Tibia length	1	37.46	**<0.001**	1	32.39	**<0.001**	1	11.11	**0.002**
Cuticle color	1	0.47	0.50	1	0.62	0.44	1	0.02	0.89
Error	31			31			29		

"Task (colony)" refers to task specialization (waste workers or foragers) nested within colony; *P* values are likelihood ratio Chi square. *P* values <0.05 are in bold font

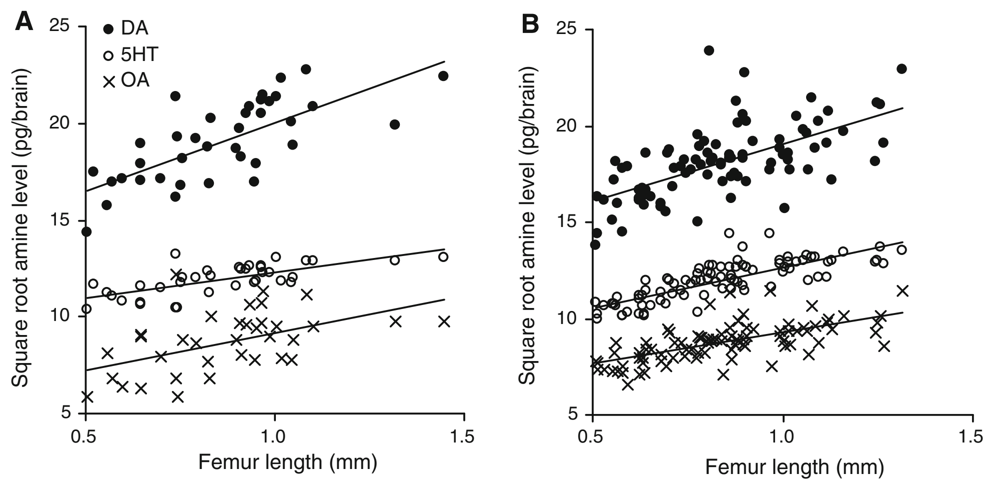


Fig. 2 Correlations between body size (measured as femur length) and square-root transformed whole-brain amine levels in leaf-cutting ant waste managers and foragers (**a**), and in-nest workers (**b**). Equations for the trend *lines* shown are **a** DA: $y = 7.05x + 12.99$, 5HT: $y = 2.70x + 9.59$, OA: $y = 3.86x + 5.34$. **b** DA: $y = 5.93x + 13.11$, 5HT: $y = 4.14x + 8.49$, OA: $y = 3.30x + 5.98$

residual levels of the three amines (Fig. 5; DA–OA: Pearson's $r = 0.69$, $P = 0.04$; DA–5HT: Pearson's $r = 0.34$, $P = 0.38$; 5HT–OA: Pearson's $r = 0.88$, $P = 0.002$; $n = 9$ for all correlations as Patriline 5 of Ae07P4 was excluded because of small sample size (3 individuals). Individual amine residuals also correlated across the three amines (Fig. 3d–f; DA–5HT: Pearson's $r = 0.29$, $n = 85$, $P = 0.007$; DA–OA: Pearson's $r = 0.46$, $n = 84$, $P < 0.001$; 5HT–OA: Pearson's $r = 0.77$, $n = 84$, $P < 0.001$). There was no significant effect of patriline (nested within colony) for the ratios of DA:5HT, DA:OA, or OA:5HT (DA:5HT: GLM $F_{9,73} = 0.63$, LR χ^2 $P = 0.77$; DA:OA: $F_{9,70} = 0.91$, LR χ^2 $P = 0.53$; OA:5HT: $F_{9,70} = 0.57$, LR χ^2 $P = 0.82$). There was no significant effect of patriline (nested within colony) on age ($F_{9,74} = 3.74$, LR χ^2 $P = 0.10$), but there was on body size ($F_{9,74} = 2.42$, LR χ^2 $P = 0.02$).

Discussion

Our results suggest that brain amine levels are associated with *A. echinatior* worker task specializations: foragers had higher levels of DA and OA than waste management workers. This supports previous studies demonstrating differences in amine levels between ant workers performing different age-related tasks (Seid and Traniello 2005; Seid et al. 2008; Wnuk et al. 2011), as well as a larger body of similar work in honey bees (reviewed in Scheiner et al. 2006; Page et al. 2006), including demonstrations of task-related differences independent of age (Taylor et al. 1992; Božic and Woodring 1998; Schulz and Robinson 1999). Our finding that body size strongly influenced amine levels is at one level unsurprising because we measured whole-brain amine levels, and larger individuals have larger brains (Seid et al. 2011; Riveros et al. 2012). Indeed, in *Atta cephalotes*, brain amine levels scale linearly to brain mass across polymorphic workers (Kamhi and Traniello 2013). However, the strength of the effect that we found is notable, in spite of analyzing individuals from a relatively narrow size range. Our data demonstrate that even across a small fraction of the size range of workers found in an *A. echinatior* colony (ca. 16 %), allometric influences of brain scaling on amine levels are very strong. In contrast, our finding that age does not affect amine levels suggests that fine-scale differences in relative age do not strongly affect amine titers. However, as we intentionally selected

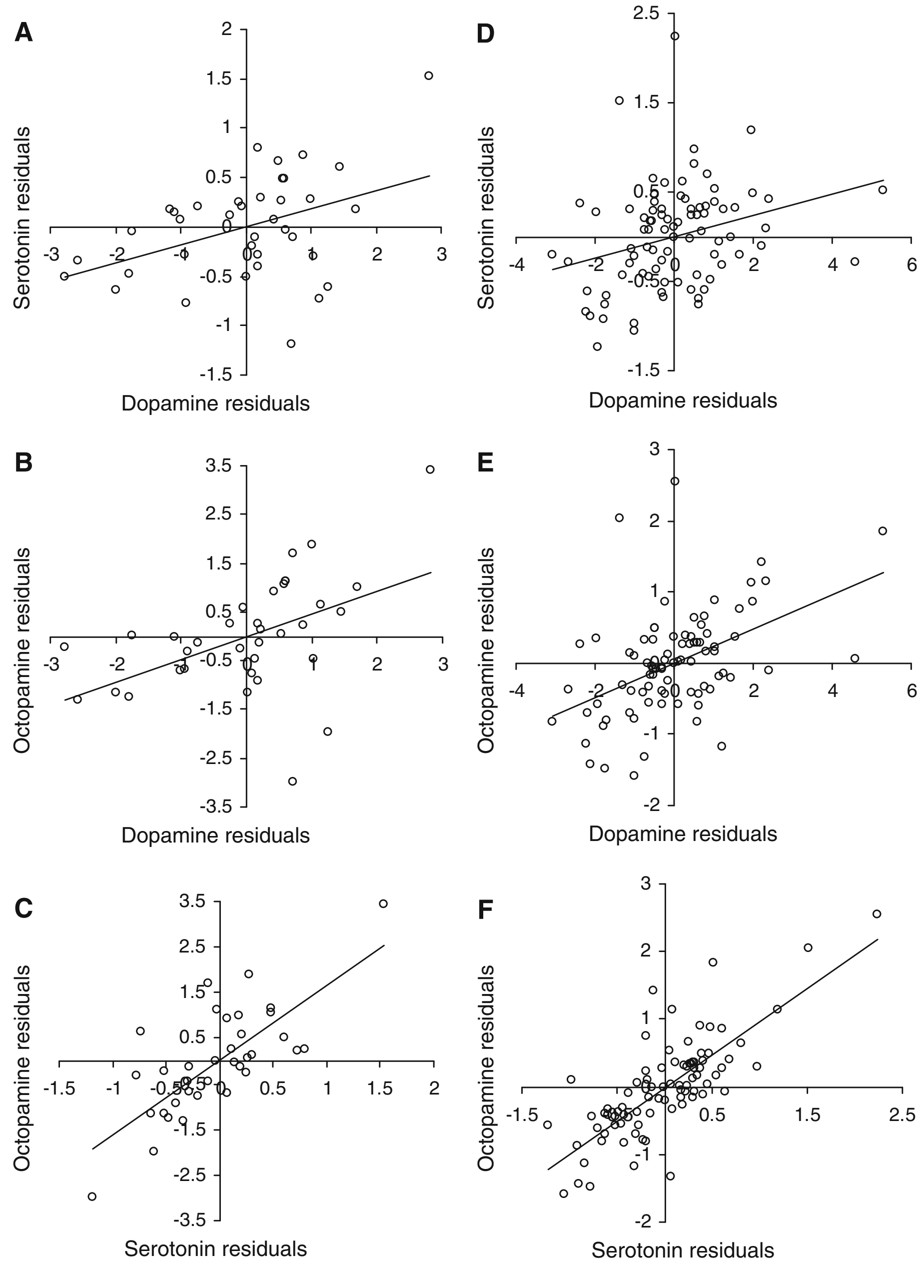


Fig. 3 Correlations among levels of the amines DA, 5HT and OA in the brains of leaf-cutting ant waste managers and foragers (**a–c**) and in-nest workers (**d–f**). Amine levels are presented as the residuals controlling for the size, age, and either task group (**a–c**) or patriline (**d–f**) of the ants. Equations for the trend *lines* shown are **a** $y = 0.18x + 0$. **b** $y = 0.46x - 0.01$. **c** $y = 1.64x + 0.04$. **d** $y = 0.12x + 0$. **e** $y = 0.241x - 0.01$. **f** $y = 0.98x - 0.02$

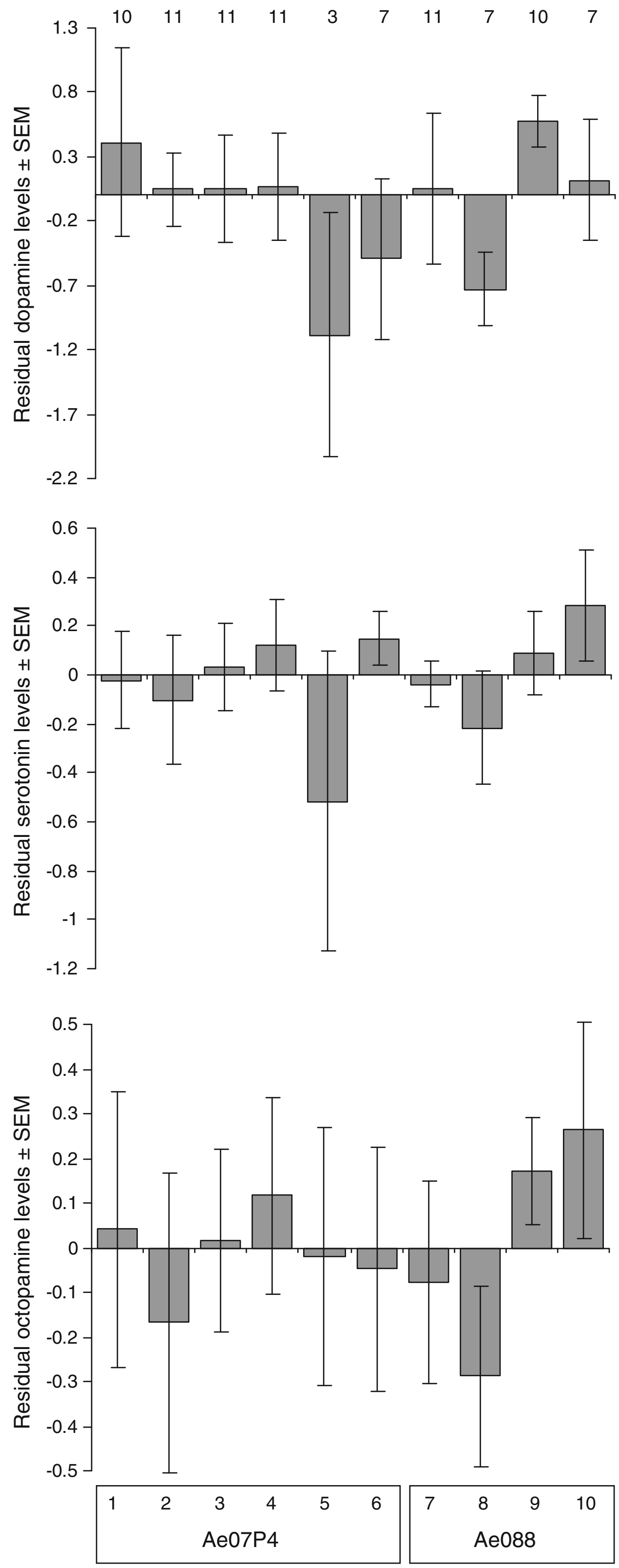
Table 2 General Linear Model results for patriline effects

Variable	Dopamine			Serotonin			Octopamine		
	df	F	P	df	F	P	df	F	P
Patriline (colony)	9	0.68	0.73	9	1.55	0.15	9	0.61	0.78
Tibia length	1	22.38	**<0.001**	1	50.49	**<0.001**	1	21.43	**<0.001**
Cuticle color	1	0.03	0.92	1	0.82	0.37	1	0.04	0.84
Error	73			73			72		

"Patriline (colony)" refers to patriline nested within colony; *P* values are likelihood ratio Chi square. *P* values <0.05 are in bold font

similarly aged individuals for each comparison, our data do not necessarily imply that amine levels do not differ across a wider age range, as seen in other ants (Seid and Traniello 2005; Seid et al. 2008; Wnuk et al. 2011), but only that any fine-scale effects of age are relatively small. We also found that there were strong correlations between

Fig. 4 Levels of the DA, 5HT and OA amines in the brains of young, ▶ medium-sized leaf-cutting ant workers from the different patrilines in two colonies. Amine levels are presented as the mean ± SEM residuals controlling for the size and age of the ants. Sample size for each patriline is listed above the bars, and the patrilines and source colonies are listed below the *bars* with patrilines from each colony in a separate *box*



OA, DA, and 5HT in *A. echinatior*, even after controlling for age and size, suggesting that factors influencing their fine-scale levels have at least the same direction of effect on the three amines. One such factor may be residual variation among workers in brain size even after correction for body size differences; however, this remains to be tested.

OA is well established as a modulator of olfactory coding and mediator of appetitive associative learning in insects (Hammer and Menzel 1998; Schwaerzel et al. 2003; Rein et al. 2013; Riffell et al. 2013). In honey bees, OA titer also regulates the transition from in-nest to foraging behavior and modulates foraging related activities such as dance language communication (Barron et al. 2002, 2007; Barron and Robinson 2005; reviewed in Page et al. 2006; Scheiner et al. 2006). In ants, the role of OA is largely unknown. In *Pheidole dentata* OA levels were not clearly associated with age in minor workers (Seid and Traniello 2005), while in *Formica polyctena*, forager brains contained significantly less OA than those of in-nest workers (Wnuk et al. 2011). However, in neither of these cases were similarly aged older workers with different task specializations compared. In honey bees, the transition to foraging is associated with an increase in sensory stimuli and cognitive demand: foraging bees are presented with visual and chemical stimuli and navigational challenges not present inside the nest (Capaldi et al. 1999). In nature, leaf-cutting ant foragers travel long distances outside the nest, and must incorporate olfactory information encoded in trail pheromones, distinguish between multiple potential plant species as food sources, and likely use landmark or compass navigation to supplement the chemical trails (Cherrett 1972; Vilela et al. 1987; Wetterer et al. 1992; Do Nascimento et al. 1994; Kleineidam et al. 2007a, b; Howard et al. 1996; Riveros and Srygley 2008; Saverschek et al. 2010). In contrast, waste dumps are located underground or at a short distance from the nest, so the sensory and cognitive requirements of workers engaged in waste management may be qualitatively different and substantially less demanding than those of foragers. Our finding of higher OA levels in foragers than in waste management workers is, therefore, in keeping with the apparent increased cognitive demands of foraging being associated with raised OA levels, as has been found in honey bees. In our study, the colony environments were unnaturally limited, with a single type of food located very close to the fungus garden. Therefore, it is possible that the differences in amine levels between foragers and waste management workers may be even greater in natural colonies.

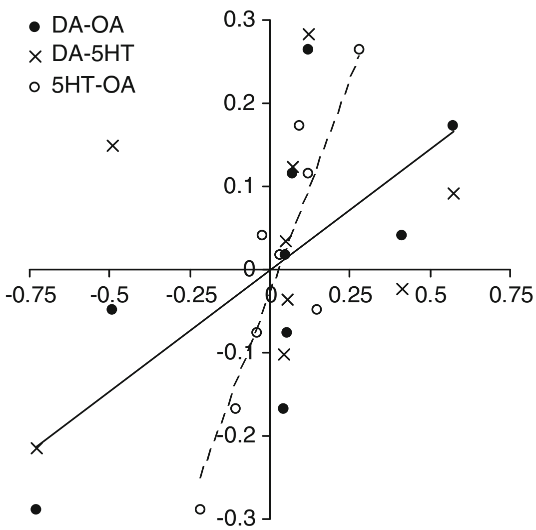


Fig. 5 Correlations of patriline mean residual amine levels. For each pair, the value for the first amine is plotted on the *X*-axis and the second on the *Y*-axis. DA–OA are represented by *closed circles* and *solid regression line* (equation: $y = 0.29x - 0$), DA–5HT by crosses and no line as the correlation was not significant, and 5HT–OA by open circles and a dashed line (equation: $y = 1.02x - 0.03$)

Both DA and 5HT are generally higher in foragers of ants and honey bees than in within-nest workers, and both generally increase with age (Page et al. 2006; Scheiner et al. 2006; Seid and Traniello 2005; Seid et al. 2008; Wnuk et al. 2011). The role of DA in detecting olfactory stimuli is unclear, and the lack of DA-immunoreactivity in the antennal lobes of the ant *Harpegnathos saltator* (Hoyer et al. 2005) suggests this amine is unlikely to directly modulate olfactory sensitivity in ants. However, DA-immunoreactive processes innervate all mushroom body subcompartments in *H. saltator* (Hoyer et al. 2005), suggesting DA could still affect behaviors mediated by olfaction through higher-order effects on olfactory memory or sensory integration. Such neuromodulation might be relevant to the behavioral specializations of midden workers and foragers by altering learned avoidance of aversive stimuli (Schwaerzel et al. 2003; Agarwal et al. 2011). Leaf-cutter ant middens may have significant pathogen loads and high risk of infection; they are tended by a dedicated subset of a colony's workforce (e.g. Hart and Ratnieks 2001; Waddington and Hughes 2010), and generally avoided by other workers. Higher DA levels in foragers may, therefore, be consistent with stronger aversive responses to midden odors by these workers. Honey bee workers, for example, learn to avoid the color of a noxious physical location better if they are fed exogenous DA (Agarwal et al. 2011).

5HT was not directly associated with behavioral role in our sampled *A. echinatior* workers. However, the significantly higher ratio of OA:5HT in foragers suggests that 5HT may nevertheless be involved in task specialization in more subtle ways. 5HT is present in ant antennal lobes (Hoyer et al. 2005) and enhances responsiveness to trail pheromone and the ability to follow chemical trails in the ant *Pheidole dentata* (Muscedere et al. 2012), suggesting it may modulate olfactory sensitivity. Interestingly, Seid and Traniello (2005) found that OA:5HT ratios decreased with age among *Pheidole dentata* minor workers, and Wnuk et al. (2011) found that *Formica polyctena* foragers had lower OA:5HT ratios than young, in-nest workers. It may, therefore, be that the OA:5HT ratio decreases with age in *A. echinatior* as well, but to a greater extent in waste management workers than foragers, or the relationships between amines and behavior could be quite different between ant species.

Our study is the first to examine patriline effects on brain amine titers and task performance in a polyandrous and polymorphic ant. Our data show no patriline effect on amine levels, despite a demonstrated genetic influence on worker task specialization (Waddington et al. 2010). However, our sampling was modest. Patriline influenced body size, consistent with previous studies (Hughes et al. 2003; Hughes and Boomsma 2007; Waddington et al. 2010), suggesting that genetic variation may indirectly modulate amine levels in association with worker size variation. Patrilines with a relatively high mean level of one amine tended to also have relatively high mean levels of the other two, and vice versa after controlling for age and size. As with the individual correlations, this may reflect patriline influences on brain size not captured by measuring body size. Also, amine levels may differ between certain regions of the brains of different worker groups, even when whole-brain amine levels remain similar (Schulz and Robinson 1999). It is also possible that differences between genotypes are not expressed until the ants are older or that responses vary with age, and amine levels or receptor activity may respond to individual experience (Behrends and Scheiner 2012; Rein et al. 2013).

Genetic variation in amines has been investigated in the context of task specializations among adult workers in honey bees: foragers from colonies that were artificially selected to hoard pollen had lower response thresholds to sucrose and a resulting higher tendency to forage for pollen rather than nectar, than those from colonies selected not to hoard pollen (Pankiw and Page 1999; Pankiw et al. 2001; Page et al. 2006; Page and Amdam 2007). Scheiner and Arnold (2009) showed that sucrose response thresholds vary between honey bee patrilines, but Schulz et al. (2004) found no differences in amine levels between foragers from the high- and low-pollen strains, although amines and sensory sensitivities were not measured in the same individuals. The finding that even honey bees selected for dramatic differences in sensory thresholds and associated task specialization do not differ in levels of amines that have been shown to directly affect those thresholds suggests that the role of amines in division of labor may be complex and highlights the need for studies in species other than the honey bee. Ants provide diverse species for comparative analyses to improve our understanding of the neuromodulation of social behavior.

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