J Comp Physiol A (2000) 186: 261–268

ORIGINAL PAPER

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Brain biogenic amines and reproductive dominance in bumble bees (*Bombus terrestris*)

Accepted: 10 December 1999

Abstract To begin to explore the role of biogenic amines in reproductive division of labor in social insects, brain levels of dopamine, serotonin, and octopamine were measured in bumble bee (*Bombus terrestris)* workers and queens that differ in behavioral and reproductive state. Levels of all three amines were similar for mated and virgin queens. Young workers that developed with or without a queen had similar amine levels, but in queenright colonies differences in biogenic amine levels were associated with differences in behavior and reproductive physiology. Dominant workers had significantly higher octopamine levels compared with workers of lower dominance status but of similar size, age, and ovary state. High dopamine levels were associated with the last stages of oocyte development irrespective of worker social status and behavior. These results suggest that biogenic amines are involved in behavioral and physiological aspects of regulation of reproduction in bumble bees.

Key words Dominance · Reproduction · Biogenic amines · Bumble bees · Social insects

Abbreviations *5-HT* serotonin · *DA* dopamine · *JH* juvenile hormone · *OA* octopamine

Introduction

Reproductive division of labor is believed to be fundamental to the evolution and organization of insect societies. In social insects, reproduction is strongly biased toward queens with high reproductive potential, relative to workers that are either sterile or have low reproductive potential. In different species, the differences in reproductive potential may be the product of caste-specific developmental pathways, or because the reproductive potential of a worker is diminished by behavioral and pheromonal signals from the queen, or both (reviewed in Wilson 1971; Michener 1974; Fletcher and Ross 1985). Worker-worker interactions also influence the reproductive potential of a worker (Ratnieks and Visscher 1989; Bloch and Hefetz 1999), adding even more complexity to the situation. How these social cues are integrated to influence worker reproduction is an important question in sociobiology that remains poorly understood. In the current report we explore the involvement of biogenic amines, a group of neurochemicals with well-established roles in the modulation of behavior and physiology.

Biogenic amines modulate diverse behavioral phenomena in both vertebrates and invertebrates. These include behaviors such as locomotion, feeding, aggression, sexual behavior, learning, and memory acquisition (e.g., Kravitz 1988; Bicker and Menzel 1989; Winberg and Nilsson 1993; Erber et al. 1993; Melis and Argiolas 1995; Steckler and Sahgal 1995; Hasselmo 1995; Menzel et al. 1996; Izquierdo and Medina 1997). Biogenic amines also influence diverse physiological processes in insects (e.g., Orchard 1982; Dedecker et al. 1994; Roeder 1994) including the regulation of reproduction. In several insect species biogenic amines regulate the synthesis of juvenile hormone (JH), a gonadotropic hormone in many insects (e.g., Lafon-Cazal and Baehr 1986; Thompson et al. 1990; Pastor et al. 1991b; Woodring and Hoffmann 1994), and biogenic amines appear to be involved in follicle development (e.g., Grillot and Raabe 1989; Pastor et al. 1991a; Harris and Woodring 1995). For example, *Drosophila melanogaster* females with experimentally lower levels of dopamine (DA) had abnormal oocytes (Neckameyer 1996; Pendleton et al. 1996). Flies with lowered octopamine (OA) due to a

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mutation in the tyramine beta-hydroxylase gene were sterile because they retained eggs in their ovaries (Monastirioti et al. 1996).

Studies on the regulation of reproduction in bumble bees such as *Bombus terrestris* showed that workers possess significant reproductive potential, which is best expressed under queenless conditions (reviewed in Röseler and Van Honk 1990). Worker bumble bees also reproduce in the presence of the queen toward the end of the annual colony cycle. This "competition phase" is characterized by overt reproductive conflict among workers and between the queen and workers (Van der Blom 1986; Van Doorn and Heringa 1986; Duchateau and Velthuis 1988; Bloch 1999; Bloch and Hefetz 1999). The relatively small size of bumble bee colonies enables detailed behavioral observations on individually identified bees, showing that reproductive success is correlated with high social status. Accordingly, dominant individuals can readily be seen to perform more oviposition and egg cup construction (Röseler and Röseler 1977; Van Honk and Hogeweg 1981; Van Doorn 1987, 1989).

As a first step to explore the possible involvement of biogenic amines in the regulation of reproductive dominance in bumble bees, we compared brain levels of DA, OA, and serotonin (5-hydroxytryptamine, 5-HT), in bumble bees that differ in social status, reproductive state, and after manipulations of the social environment. We chose to examine levels in the brain, and not in the hemolymph, because biogenic amines act as both neuromodulators and neurohormones, and we were particularly interested in gathering correlative data to explore whether the influences of the social environment on reproduction might be mediated by amine neuromodulation in the brain. In experiment 1, we compared young workers that developed with or without a queen. In experiment 2, we investigated the association of biogenic amines with behavior and social status in normally developing laboratory colonies. In experiment 3, we compared mated queens heading colonies with young virgin queens. We further used our data to explore age-related changes in biogenic amine levels and the association of biogenic amines with follicle development.

Materials and methods

Bees

Colonies of *B. terrestris* were obtained from Bio-Bee Sde-Eliahu Industry, Bet Shean, Israel, a few days after the first worker in each colony had emerged. Colonies contained a queen, one to ten workers, and brood at different stages of development. They were reared in Styrofoam nesting boxes (18 cm × 27 cm × 12 cm) with a cardboard bottom in an environmental chamber at 29 ± 2 °C and constant darkness, except for a few minutes of light during feeding or experimental manipulations. Sugar syrup (Bee Happy, obtained from Bio-Bee Sde-Eliahu Industry) and fresh pollen (collected by honey bees) were supplied to all colonies ad libitum. Observations were performed under dim red light through a glass lid placed on top of the nest box.

The onset of the competition phase was identified when one or more of the following events were observed: (1) worker oviposition, (2) egg eating (oophagy), (3) clear signs of egg cup destruction, and (4) two or more open egg cups over two or more successive days (Duchateau and Velthuis 1988, Bloch 1999; Bloch and Hefetz 1999).

The length of the media vein of the front wings was used as an index for body size because the need to make dissections on dry ice (see below) precluded using direct measurements of brain size, and because wings are commonly used as index for body size in bumble bees (e.g., Van Honk et al. 1981; Duchateau 1989; Van Doorn 1989). Wings were removed from each bee, mounted on a glass slide, and the length of the media vein was measured with an ocular ruler under a dissecting microscope (× 20– × 40 magnification).

Evaluation of ovary status

Bees were dissected in "bee saline" with an ionic ratio of 1.5Na:1K, as found in *B. terrestris* worker hemolymph (Bloch et al. 1996). Ovaries were removed and the length of each terminal (basal) oocyte was measured with an ocular ruler under a dissecting microscope (×20 – ×50). The length of the largest terminal oocyte was used as an index for ovary status.

Sample collection

All bees were collected with long forceps (approximately 25 cm) that enabled them to be removed with minimal disturbance to the colony. Bees were immediately placed into a container with liquid N_2. The bees were frozen within ca. 3 s, minimizing the possibility of stress-induced changes in biogenic amines (Davenport and Evans 1984; Woodring et al. 1988; Harris and Woodring 1992). Bees were then stored at −80 °C to prevent amine degradation.

Brain dissection

Brains were dissected on a frozen dissecting dish in dry ice and remained frozen during the entire dissection procedure. The hypopharyngeal glands, optic lobes and subesophageal ganglion were removed during dissection. Because amine levels are expressed on a per brain basis, brains in which pieces of tissue were lost were discarded and only intact brains were analyzed. Each brain was stored individually at −80 °C until amine analysis.

Sample preparation

Sample preparation was as previously described (Wagener-Hulme et al. 1999). Fifty microlitres of chilled perchloric acid (0.2 mol l^{-1}) was added to each tube while on ice (4 °C). Dihydroxybenzylamine (DHBA, 50 pg μl^{-1}) and synephrine (25 pg μl^{-1}) were added as internal standards. DHBA was used to quantify DA and 5-HT, and synephrine was used to quantify OA. Each sample was sonicated, chilled for 20 min and then centrifuged at 13,000 rpm for 10 min at 4 °C (Hermle 2360 K centrifuge). The supernatant was then transferred to a 0.22-µm nylon membrane filter and centrifuged again for 6 min. The filtered sample was then transferred to a microvial, precooled to 2 °C, for analysis by high-performance liquid chromatography (HPLC).

Measurements of biogenic amine levels

Measurements were made as in Wagener-Hulme et al. (1999). Brain extracts were separated by a HPLC system which consisted of a refrigerated Kontron automatic injector, a Shimadzu (LC-10AD) pump, an 80 mm × 4.6 mm high-efficiency reverse-phase ESA Catecholamine HR-80 column (no. 316 stainless steel, packed with 3-µm spherical octadecylsilane) and a Coulochem II electrochemical detector coupled to a model 5014 microdialysis analytical cell. Channel 1 of the detector was set at 425 mV for DA and 5-HT and channel 2 was set at 650 mV for OA. The mobile phase (pH = 5.6) was composed of 15% methanol, 15% acetonitrile, 1.5 mmol l^{-1} sodium dodecyl sulfate, 75 mmol l^{-1} sodium phosphate monobasic and trace amounts of triethylamine and EDTA (Sigma Chemical, St. Louis, Mo.), with water purified by a Nanopure II system (Millipore). The flow rate of the mobile phase was 1 ml min^{-1}. The HPLC system was connected to an EZChrom Chromatography Data System version 6.5 (Scientific Software) for analysis of peak areas using both internal and external standards. External standards were run before and after each set of biological samples. Sample runs always contained 24 bees, one to three samples from each behavioral group. This was done to minimize the effect of any possible variation in system performance on intergroup comparisons.

Experiment 1: effect of the queen on brain biogenic amine levels in young workers

Callow workers (< 12 h after emergence and before full coloration) collected from different colonies were pooled, marked with colored tags, and re-introduced randomly into queenright colonies or kept as groups of three without a queen (queenless group). Queenless groups were placed in small plastic cages (95 mm × 107 mm × 107 mm) with a cardboard bottom. Measurements of brain biogenic amine levels were made for 0-, 1-, and 3-day-old bees for both queenright colonies and queenless groups.

Experiment 2: association of social status with brain levels of biogenic amines in queenright workers

A worker was assumed to have high social rank if seen performing one of the following behaviors: (1) oviposition behavior, i.e., inserting her abdomen into egg cup for approximately 2 min while moving her hind legs (Bloch and Hefetz 1999), (2) construction or destruction of an egg cup, or (3) overt agonistic behavior (e.g., aggression or threatening) toward another bee. Previous studies indicated that these behaviors are performed mainly by workers with high social rank (Van Honk and Hogeweg 1981; Van Doorn and Heringa 1986; Van Doorn 1987; Duchateau 1989). Workers of similar size and age that did not exhibit these behaviors during a 5-to 10-min observation period were also collected from the same colony for comparative purposes. Typically, these workers were engaged in activities such as brood nursing and brood incubating, or were walking through the comb with no obvious function. They were assumed to be non-egg-laying workers of lower social rank. Previous observations (Bloch and Hefetz 1999; Bloch et al. 2000a) indicate that this observation period is sufficient for the assessment of worker social rank.

Two replicates were performed. In the first, dominant workers of unknown age were collected from colonies that were a week or more after the initiation of the competition phase. In the second, all callow workers in four colonies were marked shortly after emergence with colored numbered plastic tags and collected for analysis 2–3 weeks after the onset of the competition phase. Age was calculated as number of days from the day of marking to the day of collection. We compared pairs of high- and low-ranked bees from the same colony and of approximately the same age and size. All workers were examined for ovary status, brain biogenic amine levels, and body size.

Experiment 3: brain biogenic amine levels in queens

Mated and virgin queens were collected from normally developing colonies. Mated queens were functionally egg-laying queens heading colonies with normal pattern of development. To assure that the queens were virgins, they were removed from the nest as pupae or immediately after emergence (before pigmentation occurred) and kept in small cages without males. Measurements of brain biogenic amine levels, body size, and ovary status were made for 12 virgin queens (3–4 days of age), and for 11 mated queens.

The relationship between biogenic amine levels in the brain and ovary status

Correlations between biogenic amine levels and ovary status (length of largest terminal oocyte length) were explored in a pooled analysis that included all the bees in experiments 1 and 2. Only data obtained from the same bees were used to examine these relationships. The only amine that was correlated with ovary status was DA (see below). To further explore the relationship between DA and ovary status, we divided oocyte development into four stages (see also Duchateau and Velthuis 1989): stage 1, initial oocyte (largest terminal oocyte length ≤0.5 mm); stage 2, moderately developed oocyte (0.5 mm < largest terminal oocyte length ≤1.0 mm); stage 3, developed oocyte (1.0 mm < largest terminal oocyte length ≤2.5 mm); stage 4, highly developed oocyte (2.5 mm < largest terminal oocyte length).

Results

Experiment 1: effect of the queen on brain biogenic amine levels in young workers

Queenright and queenless workers did not differ in brain levels of DA, OA, and 5-HT (Fig. 1, unpaired *t*-test, $P > 0.05$). A trend of higher DA levels for queenless bees on day 1 is suggestive (*t*-test, $P = 0.14$), and variance is significantly larger (*F*-test for equality of variance, $F = 6.5$, $P < 0.01$; Sokal and Rohlf 1995) compared to queenright workers of similar age. OA

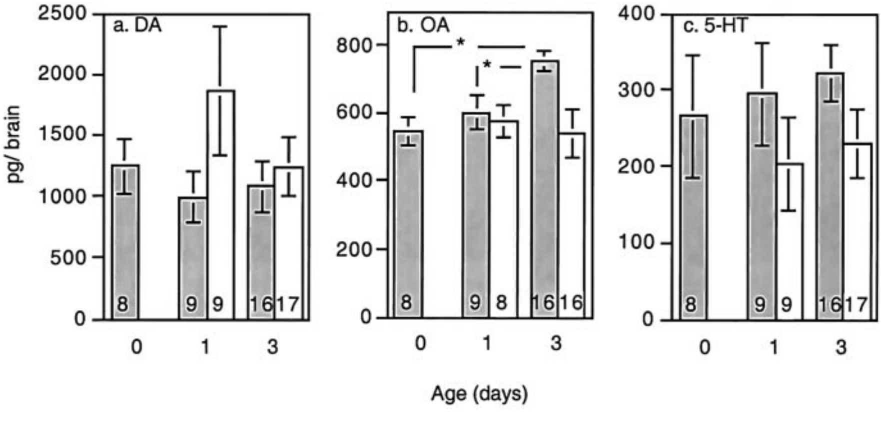

Fig. 1a–c Mean (± SE) brain biogenic amine levels for 0- to 3-day-old workers maintained with (*shaded bars*), or without (*open bars*) a queen. Sample sizes within bars. **a**. Dopamine (DA); **b** octopamine (OA); **c** serotonin (5-HT). Differences between queenless and queenright workers were not significant. *$P < 0.05$

brain levels in 3-day-old queenright workers were significantly higher than in 0- or 1-day-old workers from the same colonies (Fig. 1b, ANOVA, $P = 0.002$; Scheffe post hoc test, $P = 0.004$, $P = 0.033$, respectively). A similar age-related increase was not detected in DA and 5-HT in queenright bees, or in levels of any amine in queenless workers. Regression analyses did not detect any significant effect of body size on brain amine levels (linear regression analysis, $r^2 < 0.001$, $P > 0.8$ for all three amines analyzed).

Experiment 2: association of social status with brain levels of biogenic amines in queenright workers

In the first repetition, we compared high and low ranked nestmates of similar size but unknown age. There were no detectable differences in DA or 5-HT levels between the two worker types (Fig. 2). In contrast, workers with presumed high social status had significantly higher OA levels, and oocytes in their ovaries were larger (Table 1). In the second repetition, when high and low ranked workers were also of similar age, there were no significant differences in ovary status (Table 1, Wilcoxon signed test, $P = 0.32$) but OA levels were still significantly higher in high ranked workers (Fig. 2). In the second repetition, there also were no detectable differences in either DA or 5-HT.

Experiment 3: brain biogenic amine levels in queens

Mated queens had highly developed oocytes [mean (±SE) largest oocyte length = 3.55 ± 0.11 mm, $n = 10$], whereas the ovaries of virgin queens contained only follicles at initial stages of development (0.09 ± 0.09 mm, $n = 11$; unpaired t-test, $t = 24.4$, $P < 0.0001$). Brain biogenic amine levels were not significantly different between the two types of queens (unpaired t-test, $P > 0.05$; Fig. 3). Regression analyses did not detect any significant effect of body size on brain amine levels in queens (linear regression analysis; $r^2 < 0.01$, $P > 0.7$ for all three amines examined).

Queens (Fig. 3) had higher levels of all three biogenic amines relative to workers (Figs. 1, 2), (unpaired t-test; DA, $t = 6.7$, $df = 134$, $P < 0.0001$; 5-HT, $t = 1.7$, $df = 130$, $P = 0.08$; OA, $t = 8.1$, $df = 135$, $P < 0.0001$). However, because queens are bigger than workers (media cell length = 2.8–3.0 mm, mean = 2.96 mm, $n = 23$, versus 1.5–2.5 mm, mean = 2.03 mm, $n = 108$ for workers, unpaired t-test, $t = 28.8$, $df = 129$,

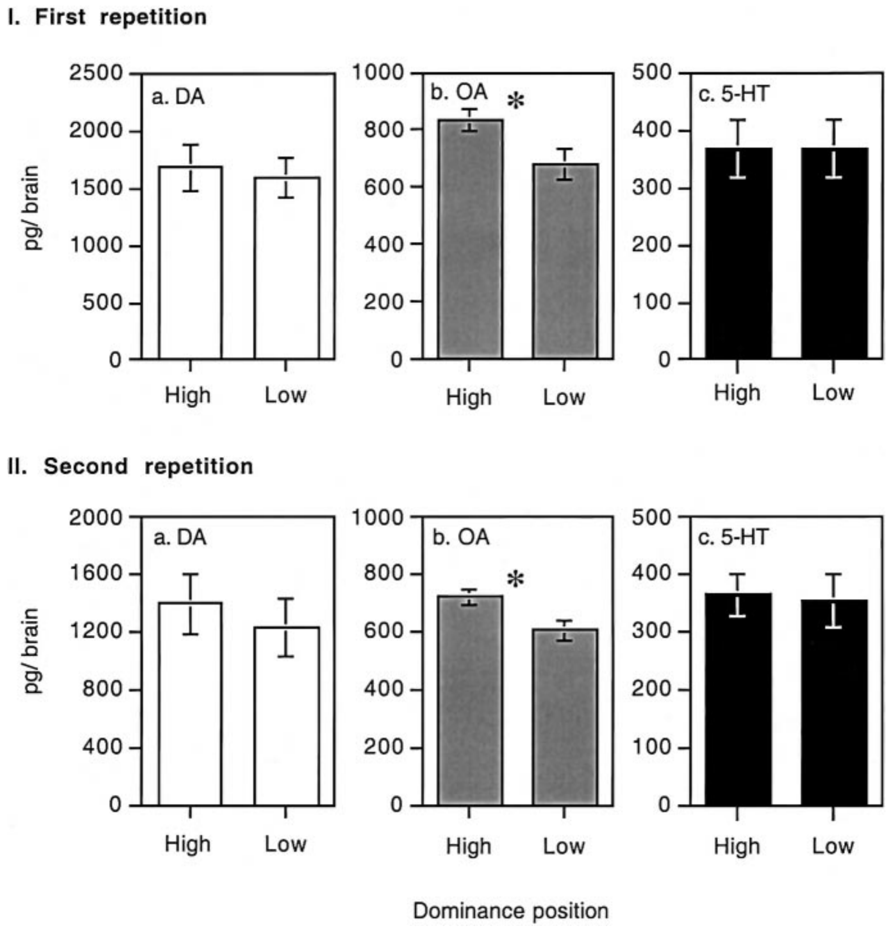

Fig. 2 Mean (±SE) brain biogenic amine levels in high- and low-ranked queenright workers. *I. First repetition*: worker age unknown ($n = 15$, for each group). *II. Second repetition*: worker age known ($n = 15$, for each group). *$P < 0.05$; unpaired t-tests in the first repetition and paired t-test in the second repetition. Measurements of size, age and ovary status for these bees are given in Table 1

Table 1 Size, age and ovary status in high- and low-ranked queenright workers (mean ± SE, sample size in parentheses). First repetition: worker age unknown; second repetition: worker age known. Differences between high- and low-ranked workers: Biogenic amine levels for these bees are in Fig. 2. Statistical analysis: first repetition, unpaired *t*-tests, Mann-Whitney test for oocyte length; second repetition, paired *t*-test, Wilcoxon sign test for oocyte length. *ns* not significant

Dominance position	Length of largest oocyte (mm)	Wing media vein length (mm)	Age (days)
First repetition			
High	2.85 ± 0.22 (15)	2.15 ± 0.5 (14)	Unknown
Low	1.53 ± 0.28 (9)**	2.10 ± 0.2 (9) ns	Unknown
Second repetition			
High	3.02 ± 0.11 (12)	1.94 ± 0.36 (14)	19.2 ± 2.4 (13)
Low	2.38 ± 0.32 (12) ns	1.98 ± 0.42 (14) ns	19.6 ± 2.7 (13) ns

**$P < 0.01$, ns-not significant

$P < 0.0001$), perhaps these differences are due to queens having larger brain size.

Age-related changes in biogenic amine levels in the brain

In a pooled data set that included queenright workers in experiment 1 (0–3 days of age) and queenright workers in the second repetition of experiment 2 (8–39 days of age) we found that age accounted for only 7% of the variance in 5-HT levels (linear regression analysis; $r^2 = 0.07$, $P = 0.04$), and was not a significant variable for DA or OA ($r^2 = 0.01$, $P = 0.38$; $r^2 = 0.002$, $P = 0.7$, respectively).

The relationship between biogenic amine levels in the brain and ovary status in workers

There was no significant correlation between terminal oocyte length and OA or 5-HT levels in the brain. In contrast, DA levels were significantly correlated with oocyte length (Spearman rank correlation, first repetition: Rho = 0.41, $n = 24$, $P < 0.05$; second repetition, $n = 27$, Rho = 0.42, $P < 0.05$). Finer analysis (Fig. 4) showed that this correlation was due mainly to a significant increase in DA levels associated specifically with the last stage of oocyte development (ANOVA, $P = 0.001$; Fisher's PLSD post hoc test, $P < 0.05$). The difference in DA levels cannot be attributed to differences among workers in size (ANOVA, $P = 0.64$), or age ($P = 0.38$).

Discussion

Reproductive division of labor is mediated by the effects of complex social interactions on plasticity in individual behavior and reproductive physiology. Our study shows that aspects of dominance and reproductive division of labor in bumble bees are associated with differences in biogenic amine levels in the brain. Differences in social rank are associated with differences in biogenic amine levels in both vertebrates and other invertebrates (e.g., Kostowski and Tarchalska 1972; Kostowski et al. 1975; Kravitz 1988; Brain and Haug 1992; Winberg and Nilsson 1993; Huber et al. 1997). We showed that dominant, egg-laying workers have higher OA levels in the brain, and previous detailed behavioral observations showed that dominant bumble bee workers have higher reproductive success and are more aggressive relative to workers with lower social status (Van Honk and Hogeweg 1981; Van Honk et al. 1981; Van Doorn and Heringa 1986). We suggest that the increase in OA levels is not due to differences in ovary state, because in one experiment dominant workers had higher OA levels but similar ovary status relative to low-ranked workers. This finding is consistent with earlier studies that showed that developed ovaries are not required for a bumble bee to acquire high social rank. Of specific relevance are the reports that bees who were ovariectomized upon emer-

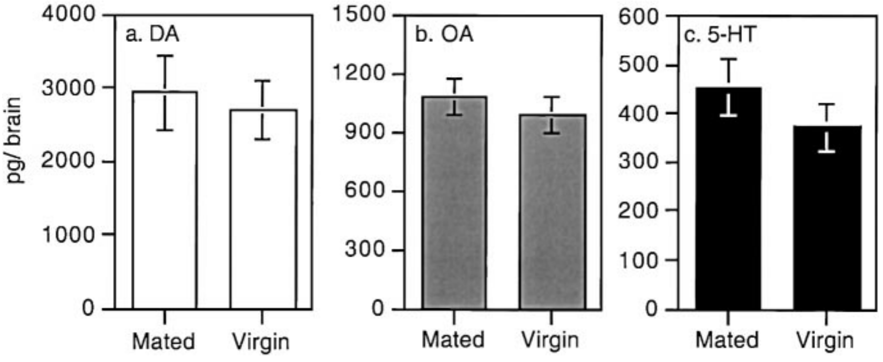

Fig. 3a–c Mean (±SE) brain biogenic amine levels in mated ($n = 11$), and in young virgin ($n = 12$) queens. Differences between queens were not statistically significant ($P > 0.05$)

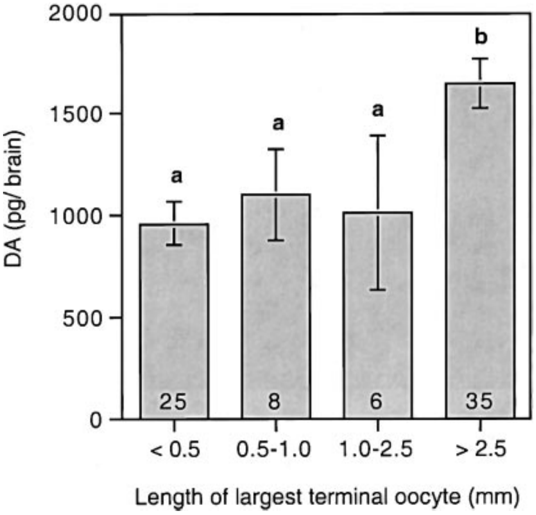

Fig. 4 Mean (± SE) brain DA levels and ovary status in queenright workers (sample sizes within bars). Bars with different letters are significantly different ($P < 0.05$)

gence were still able to achieve high dominance position and to express most of the behavioral repertoire of intact dominant bees (Van Doorn 1987, 1989).

High brain OA level may be associated with the aggressive behavior characteristic of dominant individuals, or their heightened oviposition activity, or both. In insects, OA commonly has a releasing or enhancing effect (Bicker and Menzel 1989). Elevation in OA levels is often associated with changes such as increased locomotor activity, stress and arousal (e.g., David and Verron 1982; Orchard 1982; Davenport and Evans 1984; Woodring et al. 1988; Harris and Woodring 1992; Adamo et al. 1995). In this context, the high OA levels we detected may be related to the frequent agonistic encounters of dominant bees (Free 1955; Röseler and Röseler 1977; Van Doorn and Heringa 1986; Duchateau 1989). However, 3-day-old queenless workers that are typically more aggressive than queenright workers of similar age (Free 1955; Röseler and Röseler 1977; Van Doorn 1989; Bloch et al. 1996), did not have higher OA levels. Perhaps the OA-aggression link, if extant, is context dependent.

Several lines of evidence link OA to oviposition. In crickets (*Acheta dometicus*) injection of OA resulted in significantly increased egg laying (Adamo 1999). OA modulates contractions of visceral muscle of the oviduct in the locust *Locusta migratoria*, and in the stable fly *Stomoxys calcitrans* (Orchard and Lange 1985; Lange and Orchard 1986; Cook and Peterson 1989). Furthermore, female fruit flies (*Drosophila melanogaster*) lacking OA due to a null mutation at the tyramine β-hydroxylase gene mated and had normally developed ovaries, but were sterile because they retained mature eggs. When transferred onto OA-supplemented food, these mutant flies initiated egg laying (Monastirioti et al. 1996). Although most of these studies suggest a neurohormonal role for OA, changes in hemolymph levels of OA also may reflect changes in the brain. Likewise, brain levels may correspond to circulating levels or the brain maybe the source of OA secretion (Davenport and Evans 1984; Woodring et al. 1988).

Differences in brain biogenic amine levels between bees are also associated with differences in reproductive physiology. Among the three biogenic amines analyzed in the current study, only DA levels were correlated with ovary status. This positive correlation appears to be related to a significant increase in workers with ovaries containing oocytes at the last stages of development (Fig. 4). In *B. terrestris*, oocyte development depends on the social environment, and is more rapid in queenless workers. The accelerated oocyte growth is preceded by a significantly higher titer and biosynthesis rate of JH, the presumed gonadotropic hormone in this species (Röseler and Van Honk 1990; Bloch et al. 1996, 2000a). Therefore, if biogenic amines are involved in the regulation of JH biosynthesis this might be reflected as an early peak before the peak in JH biosynthesis (Pastor et al. 1991a). Such a peak is expected to occur earlier and to be more notable in queenless workers. DA levels were indeed high in 1-day-old queenless workers, but this peak was not significantly higher compared to queenright workers at the same age, perhaps because of the high variance (Fig. 1). Queenless bees also showed higher variance than queenright bees for ovary state, JH titer, and JH biosynthesis rate (Bloch et al. 1996, 2000a). It is possible that a larger sample size or a finer analysis of brain regions (e.g., Schulz and Robinson 1999), or both, are necessary to properly evaluate the role of DA in young queenless bees.

Age did not account for the differences in biogenic amine levels between *B. terrestris* workers. This contrasts with robust age-related increases of the same amines in the honey bee brain (Harris and Woodring 1992, 1995; Taylor et al. 1992; Wagener-Hulme et al. 1999). However, honey bee division of labor is based on strong age polyethism (reviewed in Winston 1987; Robinson 1992), and it appears that amines, OA in particular, are involved in the regulation of this behavioral development in honey bees (Schulz and Robinson 1999; Wagener-Hulme et al. 1999). Bumble bees division of labor is not strongly age-dependent (reviewed in Michener 1974; Heinrich 1979).

Finally, our results hint at several caste-specific differences in amine-behavior and amine-reproduction relationships. First, dominant workers had higher OA brain levels than lower-ranked workers, but mated dominant queens did not have higher levels than virgin queens. However, it is not clear how differences in social status between mated and virgin queens correspond to differences among workers. Second, DA levels in queens were not associated with oocyte stage, but in workers they were. We cannot exclude the possibility that a social hierarchy is formed in groups of caged virgin queens, which might mask the influence of reproductive status on amines. However, similar caste-specific differences have been also shown for the honey bee: high DA levels are associated with the last stage of oocyte development in workers (Harris and Woodring 1995), but not in queens (Brandes et al. 1990). We speculate that these caste-specific differences may be related to some other function of DA in queens, because the association of high brain levels with the last stages of oocyte development in workers is consistent with results from other insects. For example, a late peak of DA corresponding to the time of chorionation, ovulation and oviposition was reported for the cockroach *Blattella germanica* (Pastor et al. 1991a). In *Drosophila* depletion of DA by inhibition of the enzyme tyrosine hydroxylase resulted in abnormally developed oocytes, demonstrating that DA is necessary for normal oocyte development. These abnormalities were reversed by co-administration of L-Dopa, the precursor of DA (Neckameyer 1996; Pendleton et al. 1996). Third, queens had higher brain amine levels relative to workers. It is not clear whether these discrepancies represent caste-specific physiological differences, or are a consequence of analyzing biogenic amines on a per-brain basis (with queens larger than workers). It would be interesting to see whether these differences between queens and workers hold in an analysis that accounts for size differences.

This is the first study of biogenic amines and reproductive division of labor in an insect society with a well-developed dominance hierarchy. To better understand the relationships emerging in bumble bees, it is necessary to experimentally manipulate the different aminergic systems. We expect that studies that explore the interplay between biogenic amines and endocrine systems that are implicated in the regulation of reproduction (Röseler and Van Honk 1990; Bloch et al. 2000b) will be especially fruitful.

Acknowledgements This research has been supported by a research grant funded by the Binational Agriculture Research and Development (BARD) grant No. IS-2306-93 (to AH and GER) and NIH grant DCO3008 (to GER) and by Fulbright and BARD (FI-256-97) postdoctoral fellowships (to GB). We thank Jack C. Kuehn for expert assistance in HPLC analyses and David J. Schulz for critically reading and commenting on a previous version of this manuscript.

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