Brain Allometry in Bumblebee and Honey Bee Workers

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Abstract

Within a particular animal taxon, larger bodied species generally have larger brains. Increased brain size usually correlates with increased behavioral repertoires and often with superior cognitive abilities. Bumblebees are eusocial insects that show pronounced size polymorphism among workers, whereas in honey bees size variation is much less pronounced. Recent studies suggest that within a given colony, large bumblebee workers are more efficient foragers and are better learners than their smaller sisters. Here we examine the allometric relationship between brain and body size of worker bumblebees and honey bees. We find that larger bees have larger brains and that most brain components show a similar size increase as the overall brain. One particular brain structure, the central body, is relatively smaller in large bumblebees compared to small bees. The same is true for the mushroom body lobes, whereas the mushroom body calyces, which receive sensory input, are not reduced in larger bumblebees or honey bees. Honey bees have relatively smaller brains, as well as smaller mushroom bodies, than bumblebee workers. We discuss why brain or mushroom body size does not necessarily correlate with the degree of a species' social organization.

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Introduction

In many social insect species, different groups of individuals (castes) perform distinctly different tasks. In some species, division of labor can be quite advanced, with several morphologically distinct worker types that perform different tasks [e.g., leafcutting ants *Atta*; Hölldobler and Wilson, 1990]. Such polymorphism is common (although usually less pronounced) in other species of ants, whereas workers of most social bees and wasps are much more uniform in size. Division of labor is best studied in honey bees *(Apis mellifera)*, where all the workers are of similar size, slightly smaller than their queen. In honey bees, workers perform different tasks depending on their age: young workers care for the brood, older ones guard the nest entrance and the oldest workers forage [temporal polyethism; reviewed by Wilson, 1971; Winston, 1987; Seeley, 1996]. In contrast, bumblebees do not show pronounced temporal polyethism as some workers start foraging on their first day whereas others may not

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forage before they are more than 30 days old [O'Donnell et al., 2000].

Temporal polyethism correlates with brain development in honey bees [Withers et al., 1993; Durst et al., 1994] and in carpenter ants [*Camponotus floridanus*; Gronenberg et al., 1996]. In both species, foragers navigate in the environment and remember the nest position or the location of food sources etc. and are exposed to different kinds of sensory input whereas brood-care workers live in the nest where it is dark and many fewer odors prevail than in the open. Importantly, in honey bee *(A. mellifera)* and ant *(C. floridanus)* foragers as well as in foragers of wasps [*Polybia*; O'Donnell et al., 2004], a particular brain neuropil is increased in size compared to nest workers, suggesting that it is involved in the advanced behavioral capabilities of foragers. This brain structure is the mushroom body, a multimodal integration center that underlies learning and memory [Erber et al., 1980; Kaulen et al., 1984; Heisenberg et al., 1985; de-Belle and Heisenberg, 1994; Heisenberg, 1998; Strausfeld et al., 1998; Dubnau et al., 2001; Pascual and Préat, 2001]. Given its known function, it makes intuitive sense that the mushroom body should increase in size as the insects need to integrate more sensory information and learn multimodal cues from the world outside the nest.

Unlike honey bees, in bumblebees the workers within a colony differ significantly in size [Garófalo, 1978] and the division of labor depends in part on body size. Larger workers (as determined by wing length) forage more than smaller workers [Foster et al., 2004]. Other studies suggest that larger workers are more efficient foragers whereas the smallest workers do not forage at all [Morse, 1978; Goulson et al., 2002; Spaethe and Weidenmüller, 2002]. With respect to sensory integration and memory capacity, foraging behavior is presumably more demanding for bumblebees than is work inside the nest. This coincides with findings that larger bumblebees have better visual resolution [Spaethe and Chittka, 2003] and are faster learners than their smaller sisters in a color-learning paradigm [Worden et al., 2005]. In analogy with honey bees and ants, one might therefore expect certain brain components, such as the mushroom bodies, to be larger in large bumblebees who are more efficient foragers and learners. In this study we address the question of whether (as expected) in bumblebees brain size increases with body size and, if so, whether particular brain regions increase disproportionately compared to the rest of the brain.

Although much is known about the design and development of honey bee brains [Kenyon, 1896; Mobbs,

1982; Withers et al., 1993; Durst et al., 1994; Fahrbach et al., 1995b; Strausfeld, 2002], there are only a few accounts on bumblebee brains. They show that bumblebee brains are anatomically similar to honey bee brains [v. Alten, 1910; Burling-Thompson, 1914; Barendrecht, 1931]. We compare brain morphology of bumblebee workers of different body size with that of honey bee workers. This comparative study of brain anatomy is timely because of the recent surge of interest in bumblebee foraging and learning capacities [Goulson et al., 2002; Spaethe and Weidenmüller, 2002; Spaethe and Chittka, 2003; Worden et al., 2005].

Materials and Methods

Bumblebee colonies *(Bombus impatiens)* were purchased from Koppert Biological Systems (Romulus, MI, USA) and kept in the laboratory. Hive boxes were connected to a flight cage (1 × 1.2 × 0.8 m; 50% rel. humidity) allowing bees to forage for pollen, sugar water and water. Different sized workers were caught from the flight cage or, in case of the smallest workers that did not leave the nest, directly from the nest box. Male bumblebees were readily discriminated from workers by their tufts of light hair on the frons of their heads and their longer antennae (fig. 1d, e) and could thus be excluded from our bumblebee samples. The age of the colonies was 10–20 weeks, but we did not determine the age of individual bumblebees.

Honey bee foragers *(Apis mellifera)* were caught on flowers or at water sources on the campus of the University of Arizona. These foragers were of unknown age, but because honey bee colonies are not known to exist on the campus, the bees had to fly a considerable distance to reach the flowers or water sources, suggesting that our honey bee sample was comprised of experienced foragers.

Bees were cold anesthetized, weighed, and their maximum head width (distance between outer margins of left and right eye) measured using calipers under a stereomicroscope. Bees were then decapitated, the head held in place in a small dish using a warm mixture of wax and rosin, the head capsule cut open frontally, and the brain dissected out under fixative (4% formaldehyde and 0.1% picric acid in phosphate buffer, pH 6.8) and fixed for 3 h. Brains were rinsed in four repeated changes of buffer and then stained in 1% aqueous osmium tetroxide solution for 2 h at 4°C and for one additional hour at room temperature. Next, brains were rinsed in water for 4 h, dehydrated in acidified 2,2-dimethoxypropane [Thorpe and Harvey, 1979], plastic-embedded (Fluka, Durcupan) and polymerized at 65°C. Brains were sectioned on a sliding microtome at 10–20 μm thickness, mounted and cover-slipped.

Outlines of the brains and brain components were traced from the sections using a camera lucida attachment to the microscope and drawings were scanned into the computer. Alternatively, in some brains in which the staining was insufficiently light, sections were photographed using Nomarski interference contrast and a digital camera (Diagnostic Instruments, SPOT 2). In either case, respective areas of the digitized images were measured using the Photoshop (Adobe) pixel counting routine. As a control, the sections of one brain were digitized both ways (scanned drawings and
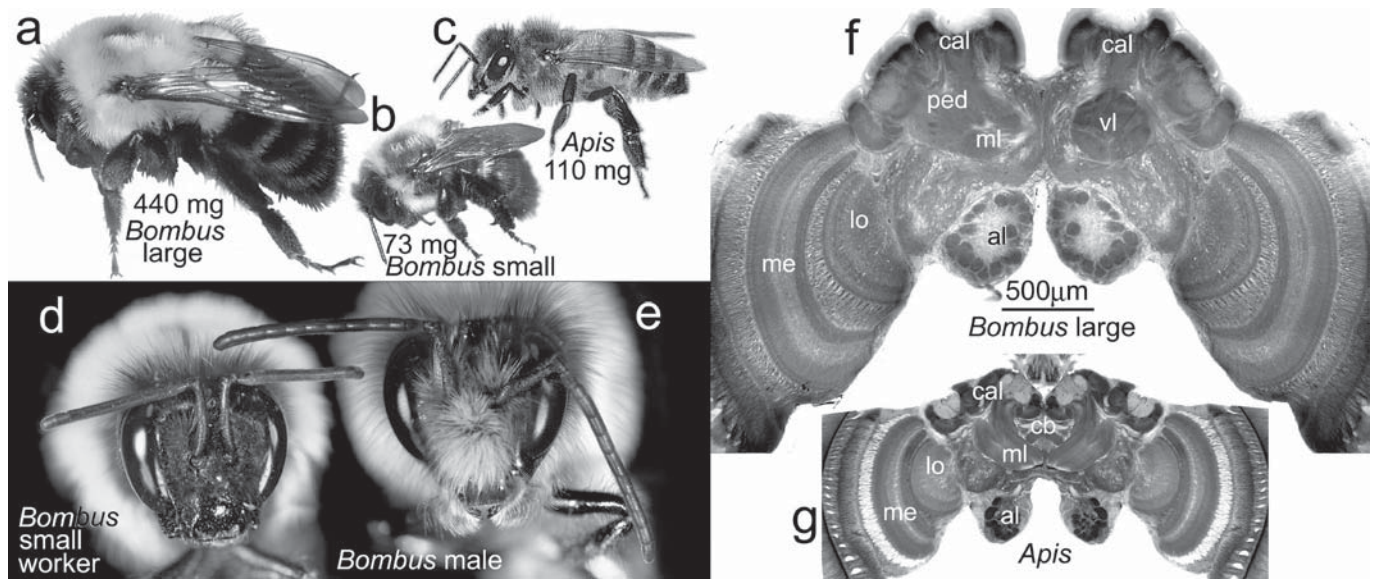
Fig. 1. Photographs of bees and their brains. Bumblebee workers [large (**a**) and small (**b**)] differ considerably in body size; honey bee workers (**c**) overlap with smaller bumblebees; respective body weight indicated. Bumblebee males (**e**) can be discriminated from workers (**d**) by the presence of long and prominent hairs on the head's frons and mandibles. The brains of bumblebee workers (**f**) can be considerably larger than those of honey bee workers (**g**), but the overall shape and composition is similar. The following brain components were measured in this study: optic lobes medulla me and lobula lo, antennal lobes al, central body cb and the mushroom bodies comprising the calyx cal, the peduncle ped, the vertical vl and the medial lobe ml.

digital photomicrographs). The calculated areas differed less than 4% between the two different methods. The volumes of each brain (including the suboesophageal ganglion, but without the retina and lamina) and its components (antennal lobes, medullae, lobulae, mushroom bodies, central body; see fig. 1g) were calculated from the area measurements multiplied by the section thickness and number of sections.

Every second section (20-μm sections) or every third section (10-μm sections) was thus traced and measured. This protocol slightly differs from the 'Cavalieri' method, a standard morphometric technique in which the sequence of analyzed sections is randomized [Gundersen and Jensen, 1987; Michael and Cruz-Orive, 1988]. In control experiments we measured all sections (one brain sectioned at 10 μm, one at 20 μm) and estimated the overall volume by including in the calculation every second section up to including only every eighth section and starting the sequence with the first, second, third section, etc., thus testing all possible permutations. We found differences of more than 5% from the 'exact' value (derived from using every section) when using only every fifth section (10-μm sections) or every fourth section (20-μm sections), and these errors occurred only for the smallest structure, the central body. For larger structures (medulla, mushroom body, overall brain volume) it would have been sufficient to sample only every 100 μm. These controls showed that our method was well below the error mark of 5%, deemed acceptable in other studies on honey bee brains [Fahrbach et al., 1995a; Withers et al., 1995].

Relative volumes of brain components were calculated dividing the respective volume (e.g., the medulla volume) by the overall brain volume. Correlations between brain volumes and body weight or head width were compared using linear regressions, and the means of brain volumes of different groups (bumblebee or honey bee workers) were compared using a t test for independent samples (Statistica software, StatSoft Inc.). Head width and body weight were sampled for 46 bumblebee and 77 honey bee foragers, respectively, and the brain volume was calculated for 45 bumblebee workers, and 25 honey bee foragers, respectively.

Results

Bumblebee Workers

Workers of *Bombus impatiens* show substantial differences in body size. Figure 1a–c show a large and a small bumblebee and a large honey bee worker, respectively. The head of a relatively small bumblebee worker is shown in figure 1d. As is generally the case in insects, head width tightly correlates with body size and body weight in bumblebees (fig. 2a; linear regression: $y = 4.70x + 2.82$; $R^2 = 0.76$; $p < 0.00001$; $n = 38$). The maximum weight of bumblebee workers in our sample was 503 mg and the minimum weight was 56 mg, hence body weight varied almost by a factor of ten, as reported by Cumber [1949].
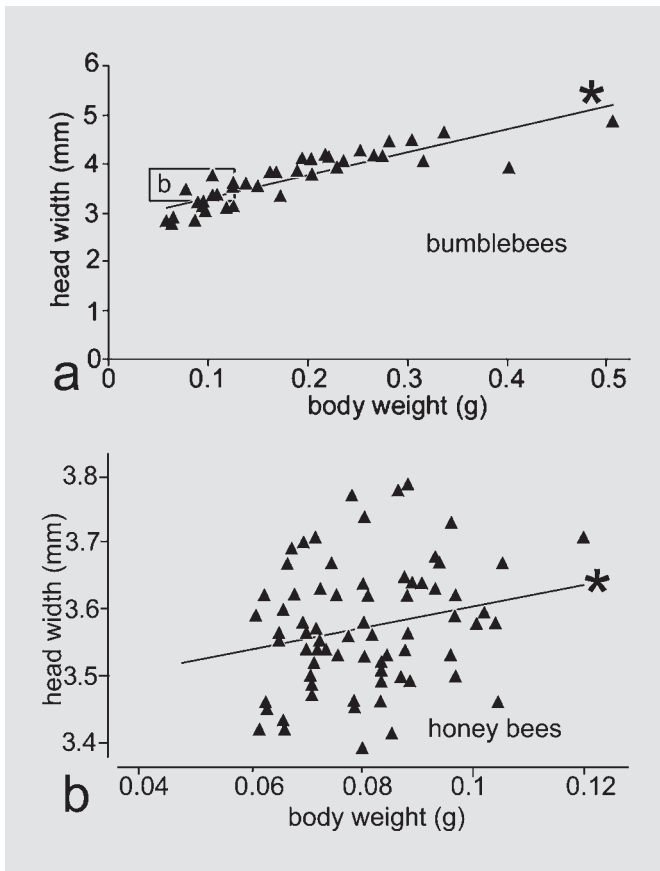
Fig. 2. Correlation between body weight and head width of bumblebee workers (**a**) and honey bee workers (**b**). Asterisk indicates statistical significance (see text for further statistical data). Note different scales for **a** and **b** (range of honey bee data in **b** indicated by box in **a**).

As expected, brain size increases significantly with body weight in worker bumblebees (fig. 3a; linear regression: $y = 4x + 0.6$; $p < 0.00002$; $R^2 = 0.34$; $n = 46$). The same is true for the correlation between brain volume and head width, another measure representing body size (fig. 3c; linear regression: $y = 0.4x + 0.4$; $p < 0.002$; $R^2 = 0.22$; $n = 41$). The mean brain volume was about 1.2 mm³ (table 1); the smallest brain was less than 0.25 mm³ and the largest was more than 3 mm³ in our bumblebee worker sample. Assuming the density of the brain tissue to be 1 g/ml, bumblebee worker brains weighed about 0.4% of the overall body weight on average (table 1).

The brains of bumblebee workers have large optic lobes (lamina, medulla and lobula; fig. 1f; the lamina was not considered in our volumetric measurements as it was sometimes damaged during dissection) and antennal lobes, the primary olfactory processing centers (fig. 1f). The mushroom bodies are prominent structures in the central brain and are composed of the calyces, the peduncle and the medial and the vertical lobe (fig. 1f). Another important, although smaller, brain component volumetrically analyzed in this study is the central body, which cannot be seen in the particular section of a bumblebee brain depicted in figure 1f, but which is visible in the slightly more posterior section of a honey bee brain (fig. 1g). It looks almost identical in bumblebees.

Brain Composition in Large and Small Bumblebees

In order to examine differences in the relative size of particular brain structures, we compared these brain components to the respective brain size rather than to the body weight or head size of the bees (figs. 4, 5). Mean relative sizes of these structures are given in table 1 (column 2). We did not find any statistically significant correlation between overall brain size and the relative size of the optic lobes (medulla and lobula) or antennal lobes in bumblebee workers (fig. 4a, c, e), suggesting that the relative size of these brain components is the same in large and small bees.

In contrast, a strong and highly significant negative correlation was found between the relative size of the central body and the brain size of bumblebees (fig. 4g), indicating that larger bumblebees have relatively smaller central bodies (linear regression: $y = -0.001x + 0.004$; $R^2 = 0.26$; $p < 0.0003$; $n = 45$). However, the reader needs to keep in mind that in absolute terms the central body is small compared to the other brain components (fig. 4g; table 1), hence the data are confounded by a larger measurement error. Despite the negative correlation, there is no evidence that the central body is of an approximately constant size in small and large bumblebees: the mean central body volume was twice as large in the largest 10 bees of the sample, compared to the smallest 10 bees, whereas the mean total brain volume was more than three times larger in the 10 largest bumblebees.

Although the relative volume of the mushroom bodies appears constant in large and small bumblebee workers (fig. 5a), the relative volume of the mushroom body lobes and peduncle decreases significantly in larger bumblebee workers (fig. 5e; linear regression : $y = -0.005x + 0.06$; $R^2 = 0.13$; $p < 0.16$; $n = 45$). This leads to an increased calyx-to-mushroom body lobes ratio in larger bumblebee workers (fig. 5g; linear regression: $y = 0.5x + 3.07$; $R^2 = 0.13$; $p < 0.015$; $n = 45$), which may have consequences for the foraging behavior of larger bumblebees (see Discussion).
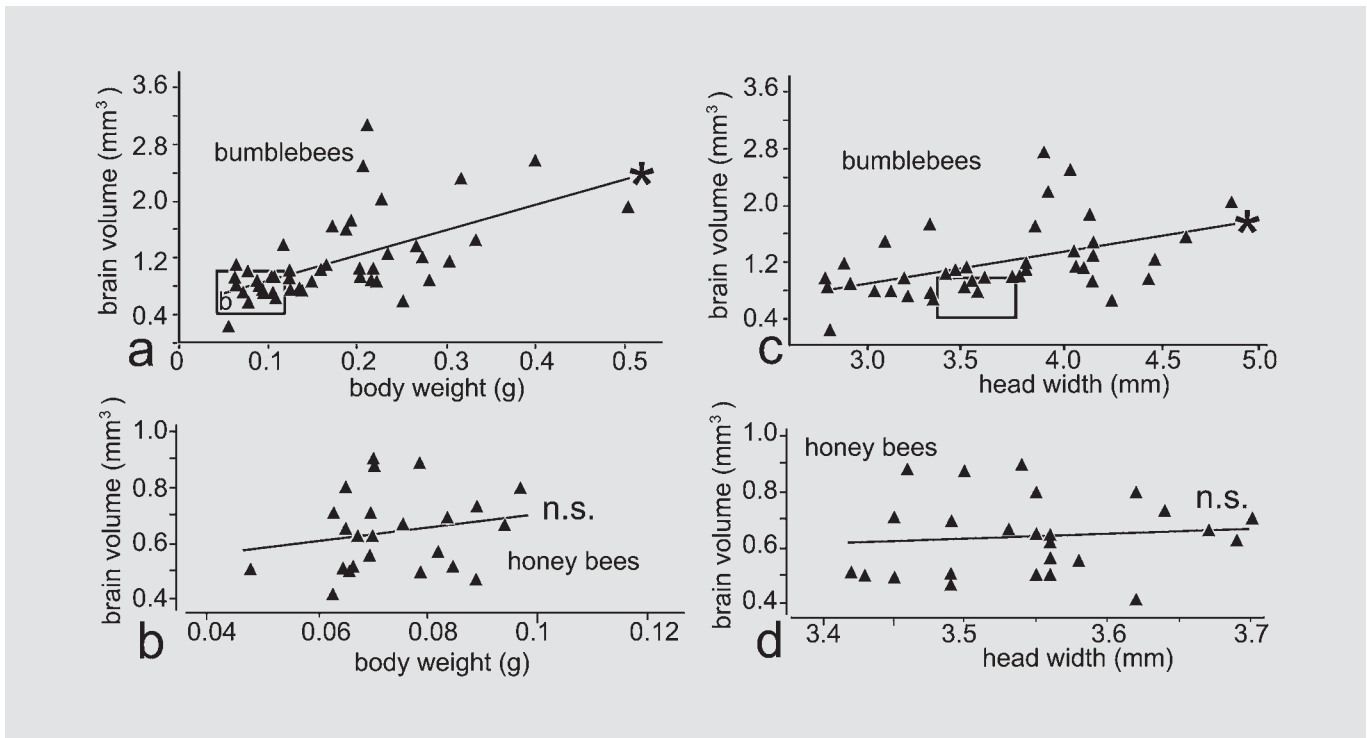
Fig. 3. Correlation between body weight (**a**, **b**) or head width (**c**, **d**) and brain volume of bumblebee (**a**, **c**) and honey bee workers (**b**, **d**). Range of honey bee data in **b** and **d** indicated by box in **a** and **c**, respectively; n.s. not statistically significant; asterisk indicates statistical significance ; see text for further statistical data.

Table 1. Mean absolute brain volume and relative volumes of the brain and brain components of all bumblebee (n = 46) and honey bee workers (n = 25) examined (columns 2 and 3, respectively).

	B. impatiens (all workers)	*A. mellifera* (workers)	*B. impatiens* (small workers)
Total brain volume, mm^3	1.16 ± 0.6	0.64 ± 0.12[a,b]	0.72 ± 0.16 (n = 12)
Medulla/brain	0.17 ± 0.02	0.19 ± 0.03[c]	0.17 ± 0.015 (n = 18)
Lobula/brain	0.05 ± 0.01	0.05 ± 0.005	0.05 ± 0.01 (n = 18)
mb lobes/brain	0.05 ± 0.01	0.05 ± 0.008[d]	0.06 ± 0.01 (n = 18)
mb calyx/brain	0.19 ± 0.02	0.14 ± 0.01[e]	0.18 ± 0.02 (n = 18)
mb total/brain	0.24 ± 0.02	0.19 ± 0.02[f]	0.24 ± 0.02 (n = 18)
Antennal lobe/brain	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01 (n = 12)
Central body/brain	0.003 ± 0.001	0.004 ± 0.001	0.004 ± 0.001 (n = 18)

For comparison, column 4 shows data of only those (small) bumblebee workers that matched the brain sizes of the honeybees (n = 18). For comparing the absolute brain volume (row 2), honey bees are compared to those bumblebee workers (n = 12; column 4) that matched the range of body weights, rather than brain volumes, found in the honey bee workers. Significant differences were found between honey bee workers and the respective small bumblebee workers.

[a–f] Statistically significant differences (t test).

[b] Indicates that significant differences were also found between honeybees and all bumblebee workers, including the larger ones. See text for further statistical data.
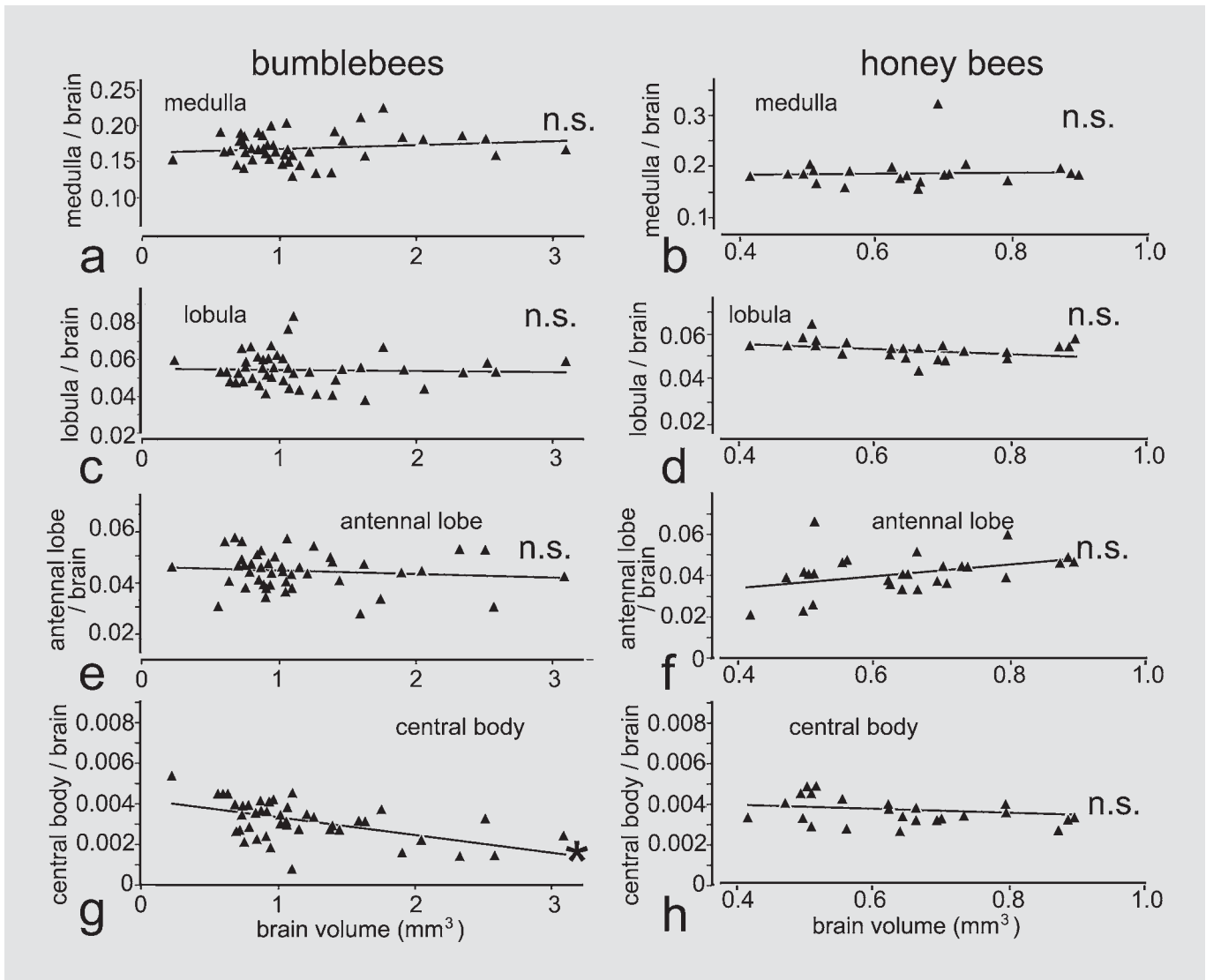
Fig. 4. Size dependence of the medulla (**a**, **b**), lobula (**c**, **d**), antennal lobe (**e**, **f**) and central body (**g**, **h**) in bumblebee (**a**, **c**, **e**, **g**) and honey bee workers (**b**, **d**, **f**, **h**). Correlation of the respective structure's relative volume with respect to overall brain volume (ordinate) and brain volume (abscissa). n.s. not statistically significant; asterisk indicates statistical significance; see text for further statistical data.

Honey Bee Workers – General Differences

Honey bee workers differ much less in body size than do bumblebees. In our sample, their wet body weight ranged from 48 to 120 mg and was positively correlated with their head width (fig. 2b; linear regression: y = 1.63x + 3.44, R^2 = 0.0539, p < 0.019). Part of the body weight variation probably resulted from combining satiated water foragers and 'empty' pollen foragers and because the bees came from different natural colonies. As we did not find any statistically significant differences (t test) in head

width, body weight and overall brain size between the water foragers and the nectar/pollen foragers in our sample, we combined the data of all honey bees for the comparison with bumblebees (table 1). Honey bee workers' body weight and head width completely overlap with those of the smaller bumblebee workers (fig. 2). This finding statistically factors out body size.

The brains of honey bee and bumblebee workers are similar in their overall appearance and composition (fig. 1f, g). Both have large optic lobes, antennal lobes, and
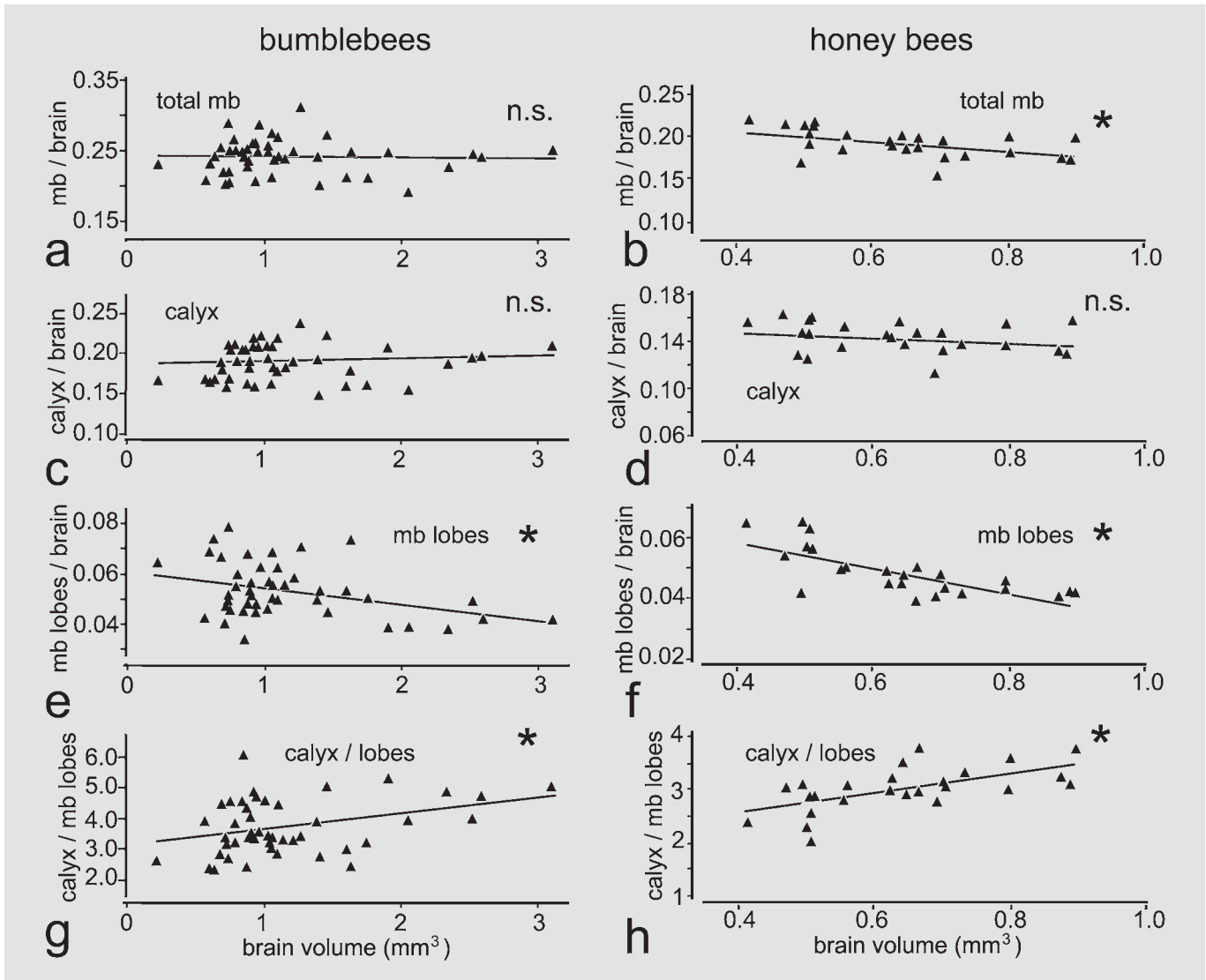
Fig. 5. Size dependence of mushroom bodies and their components in bumblebee (left; **a**, **c**, **e**, **g**) and honey bee workers (right; **b**, **d**, **f**, **h**). Correlation between overall brain volume and relative volume of the entire mushroom body (total mb; **a**, **b**), the calyx (**c**, **d**), the mushroom body lobes and peduncle (mb lobes; **e**, **f**), and relative calyx volume with respect to the mushroom body lobes (calyx/lobes; **g**, **h**). n.s. not statistically significant; asterisk indicates statistical significance; see text for further statistical data.

central bodies (table 1; figs. 4, 5) and their mushroom bodies look similar (fig. 1f, g) [see v. Alten, 1910; Barendrecht, 1931]. The overall pronounced similarity between the brains and brain components of bumblebees and honey bees justifies the attempt to compare the brains of these groups. The superficial similarity of comparable brain sections in the two species is corroborated by the finding that the mean volumes of the antennal lobes, central body and lobula do not differ significantly between honey bee and bumblebee workers (table 1). However, there are also significant differences between worker honey bee and bumblebee brains:

i) The average honey bee brain is smaller not only compared to the average bumblebee brain (table 1, row 2; $p < 0.00004$; $t = 4.37$; d.f. = 69), but also compared to the brains of those small bumblebee workers that match the body size range of honey bees (table 1, row 2; $p < 0.037$; $t = 2.17$; d.f. = 35), suggesting that this is a species-specific trait.

ii) Honey bee workers have larger medullae than bumblebees of the same body size (table 1, row 3; p < 0.013; t = 2.60; d.f. = 41), which is, surprisingly, not accompanied by an increased size of the lobula (table 1, row 4). This would have been expected considering that these two neuropils are closely interconnected and that the lobula receives most of its input from the medulla.

iii) Although the mushroom bodies look similar overall, the mushroom body lobes appear a little more slender in honey bees compared to bumblebee workers (fig. 1f, 1g). Morphometric measurements confirm that honey bee mushroom body lobes are slightly smaller than they are in those (small) bumblebees that have the same brain volume as the honey bee workers in this study (table 1; p < 0.046; t = 2.06; d.f. = 41). More importantly, the mushroom body calyx is significantly smaller in honey bee compared to bumblebee workers (table 1; p < 0.00001; t = 7.97; d.f. = 41). Therefore, the total relative mushroom body volume is about 25% smaller in honey bee workers than it is in bumblebee workers of the same body size (table 1, row 7; p < 0.00001; t = 7.75; d.f. = 41).

Honey Bees – Differences Related to Body Size

As stated above, body size varies much less in honey bees than it does in bumblebees, although there is a significant correlation between head size and body weight (fig. 2b; linear regression: y = 1.63x + 3.44; R^2 = 0.05; p < 0.019; n = 52). The tendency of the brain to increase in size with increasing body weight or head width is much less pronounced and not statistically significant in honey bees (b.w.: fig. 3b; linear regression: y = 2x + 0.4; p < 0.33; R^2 = 0.04; n = 25; head width: fig. 3d; linear regression: y = 0.2x – 0.04; p < 0.6; R^2 = 0.01; n = 25).

As in bumblebees, we did not find any statistically significant correlation between overall brain size and the relative size of the optic lobes of honey bee workers (fig. 4b, d). For the antennal lobes, a slight trend existed towards relatively larger antennal lobes in larger honey bee workers (fig. 4f; linear regression: y = 0.025x + 0.023; R^2 = 0.13; p < 0.07; n = 25). We did not find a significant correlation between the relative size of the central body and the brain size of honey bee workers (fig. 4h; linear correlation: y = –0.001x + 0.005; p < 0.55; R^2 = 0.016; n = 25). This is in contrast to bumblebees, where larger workers had a significantly smaller relative central body volume (fig. 4g).

In honey bees, the only significant body size-dependent correlation was found with respect to the mushroom bodies (fig. 5): overall mushroom body volume decreased significantly in larger bees (fig. 5b; linear regression: y =

–0.05x + 0.23; R^2 = 0.29; p < 0.006). This decrease in mushroom body volume in larger bees mainly results from a pronounced, significant decrease in the volume of the mushroom body lobes and peduncle (fig. 5f; linear regression y = –0.04x + 0.08; R^2 = 0.54; p < 0.00003; n = 25). This leads to an increased relative calyx size (calyx volume divided by mushroom body lobe volume) in larger honey bee workers (fig. 5h; y = 2x + 1.78; R^2 = 0.385; p < 0.001; n = 25). Hence, in large honey bee as well as bumblebee workers (fig. 5g), the mushroom bodies comprise relatively more calyx than lobe neuropil compared to smaller bees. In absolute terms, the calyces are always 2–3 times as large as the mushroom body lobes (compare calyx and lobes in table 1).

Discussion

Brain Size and Behavior in Social Insects

Comparative data are best interpreted in terms of relative brain sizes, minimizing biases resulting from differences in body size, but not related to a species' behavioral capacities. Here we relate sets of brain volumes to the respective body weights (fig. 3) or compare the size of particular brain components to the respective brain volumes (fig. 4, 5). Besides body size, individual age and experience are also known to correlate with the size of particular brain components. In honey bee workers, age and foraging experience affect not only their behavior, but also the size of their mushroom bodies: calyces are smaller in (younger) nurse bees and larger in older and more experienced foragers [Withers et al., 1993, 1995; Durst et al., 1994]. As we did not determine the exact age of individual bees in our samples, some of the variability in our data might reflect differences in the age or experience of the bees sampled although all our honey bees were probably experienced foragers. We are assuming the same for our bumblebee workers, but we cannot be sure as in our laboratory setting bumblebees did not have to fly over longer distances and our sample might have contained an occasional younger bee starting exploratory flights. Variation in worker age could have affected our data on mushroom body volume, and the volume of the calyces in particular.

Nevertheless, we found that overall brain volume correlates with body size in *Bombus impatiens* (fig. 3). This was expected, as it is a general rule across the animal kingdom that brains, similar to other organs, increase with body size: within a given group (e.g., primates or rodents), larger animals have larger brains [reviewed by Gould,

1966; Harvey, 1988]. Trivial as it might seem, this general trend bears on the discussion of intelligence in humans [Jerison, 1973; Peters, 1993].

More importantly in the current context, the finding conforms to the idea that larger brains allow larger animals to generate more elaborate behavioral repertoires or more advanced behaviors, including social behaviors [vertebrates: Reader and Laland, 2002; Seyfarth and Cheney, 2002; social insects: Howse, 1974; Cole, 1985; Jaffé and Perez, 1989]. In bumblebees, body size differences have been assumed to underlie task specialization and the efficiency of foraging [Morse, 1978; Heinrich, 1979; Goulson et al., 2002]. Based on these studies, our findings suggest that the larger brains might help to improve foraging performance of larger foragers. Larger brains could also underlie the increased learning abilities that have recently been found in larger bumblebees [Worden et al., 2005]. A comparable significant correlation between brain and body size was not found in honey bees (fig. 3), perhaps because body sizes are much more homogeneous in honey bees.

As large brains (and large mushroom bodies) have repeatedly been described for social Hymenoptera [honey bees, paper wasps and ants; Flögel, 1878; Jonescu, 1909; v. Alten, 1910], the general notion exists that large brains, and large mushroom bodies in particular, might be prerequisites for sociality. However, this idea has never been rigorously tested. It would require comparing closely related social and solitary species of similar body size that feature a comparable range of behaviors. A study comparing the brains of vespid wasps did not find a close correlation between size and morphological features of the mushroom bodies and the level of the respective species' social organization [Ehmer and Hoy, 2000]. This suggests that advanced behavioral repertoires, but not the social lifestyle per se, might require large and sophisticated mushroom bodies.

The same could be true for the brain plasticity associated with behavioral transitions in social insects [Withers et al., 1993; Durst et al., 1994; Fahrbach et al., 1995a; Gronenberg et al., 1996; O'Donnell et al., 2004]: it might be necessary for an advanced social life-style, but it might not be restricted to the adults of social insects (in all insects, larval forms rely on brain plasticity as they develop towards their adult form). In solitary insects that perform their entire behavioral repertoire throughout their lifetime, there should be no need for brain plasticity. However, at least in solitary rove beetles *(Aleochara curtula)* [Bieber and Fuldner, 1979] and weevils [Rossbach, 1962], the brain and the mushroom body size increase consider-

ably during the adult lifetime of the beetles, presumably to support mating and brood-care behavior (weevils) or host-finding behavior (rove beetles), which only are required later in the beetle's life once they have acquired enough metabolic reserves to be able to engage in reproduction.

In beetles, the increased brain size is based on adult neurogenesis. By contrast, in adult Hymenoptera, increases in mushroom body volume result from dendritic outgrowth [Farris et al., 2001], but not from cell proliferation, which is completed at adult eclosion [Fahrbach et al., 1995b]. As in overall brain size or morphological brain complexity, brain plasticity might be required by social insects, but it is not a trait distinguishing them from solitary insects.

Correlation between Brain Component Size and Body Size

In primates the frontal cortex increases more dramatically than does the rest of the neocortex with increasing brain or body size, and frontal cortex also increases more strongly in primates than it does in carnivores [Bush and Allman, 2004]. A volume increase of the brain or one of its components generally corresponds to an increased number of neurons, resulting in more sophisticated computational capacities of that respective part of the brain [Stevens, 2001]. Here we have examined whether disproportionate relative volumes of particular brain regions exist in bumblebees of different size, similar to the example given above for mammalian frontal cortices.

We found the central body to deviate particularly strongly from the overall expansion of brain volume accompanying body size increase; the central body is much smaller (in relative terms) in larger bumblebees compared to smaller ones (fig. 4g). This supports the assumed function of the central body in leg coordination and motor control [Strauss and Heisenberg, 1993; Strausfeld, 1999] for the following reason: the brains of larger insects in general and bumblebees in particular must accommodate increased sensory input and processing, e.g., more chemosensory receptors on the antennae or more ommatidia, hence photoreceptors, in the eyes [Spaethe and Chittka, 2003]. However, the motor output stays basically the same, as larger muscles do not require more motor neurons to control them. It is therefore to be expected that in larger insects motor centers do not increase in size as much as sensory brain centers. Thus, the relatively small size of the central body in larger bees suggests a motor function role.

The same, although to a lesser degree, appears to be the case for the mushroom body lobes: they are relatively smaller in large bumblebee and honey bee workers than in small ones (fig. 5e, f). This trend is also reflected in figure 5g and h, where the proportion of mushroom body calyx-to-lobe neuropil increases in larger bee workers. This suggests that the larger mushroom bodies of larger bumblebees and honey bees integrate more sensory information (in the calyces) compared to the less strongly increased volume, hence processing capacity, of the mushroom body lobes.

The calyx is known as a prominent sensory input region in many insects and in particular the Hymenoptera where the conversion of olfactory, visual and probably mechanosensory and gustatory input has been shown [Jawlowski, 1958; Mobbs, 1982; Homberg, 1984; Gronenberg, 1999; Schröter and Menzel, 2003; reviewed by Strausfeld et al., 1998]. In contrast, output from the mushroom body lobes serves premotor functions or the coordination of behavioral programs, as suggested by lesion, stimulation and recording experiments and comparative brain anatomy [van der Kloot and Williams, 1954; Huber, 1959, 1960; Maynard, 1967; Howse, 1974; Wadepuhl, 1983; Mizunami et al., 1998]. The trend of increasing relative calyx volume with increasing overall brain size (fig. 5g, h) thus supports the general idea that larger bumblebees and honey bees are able to integrate more sensory cues (as are important for navigation, learning and memory). This, in turn, might contribute to the superior foraging performance shown for larger bumblebee foragers [Morse, 1978; Heinrich, 1979; Goulson et al., 2002; Spaethe and Weidenmüller, 2002] and learning abilities [Worden et al., 2005]. This increased performance of larger bees would not require significant changes in motor processing as the individual movements and motor programs should be essentially the same in larger and smaller bees. Hence, the mushroom body lobes and the central body in particular would not be expected to expand as much as the calyx does in larger bumblebees.

Brain Size and Sociality

The advantage of bumblebees as subjects for the study of brain allometry over many other animals is their pronounced size polymorphism. In bumblebees, size differences can be analyzed within a single species or even colony, which makes it easier to interpret the data as they are not confounded by species-specific idiosyncrasies and adaptations. However, the results reported here also lend themselves to interspecific comparison. Comparing bumblebees and honey bees is facilitated by the finding that both brain volumes and body sizes of the latter completely overlap with those of smaller bumblebees (figs. 1–3).

Intuitively, and by comparison to brain size correlations in primates and other vertebrates, one would assume that honey bees with their apparently more complex behavioral repertoire and more advanced social lifestyle should have relatively larger brains and larger mushroom bodies than bumblebees. The finding that for the same body size honey bees have slightly smaller brains and about 25% smaller mushroom bodies than bumblebees (table 1) is therefore unexpected and appears counter-intuitive based on the general assumption that honey bees (genus *Apis*) are socially more advanced than bumblebees: i) honey bee colonies are much larger (up to more than 40,000 workers) than bumblebee colonies (usually 50–250 workers); ii) division of labor is more elaborate and communication is much more evolved in honey bees [sophisticated dance language; v. Frisch, 1967] than it is in bumblebees who communicate the presence of food sources only by a general arousal of nestmates [Dornhaus and Chittka, 2001]; iii) honey bee queens form new colonies by budding together with a large number of workers, whereas at least in temperate zones, bumblebee queens found a new colony by themselves after over-wintering; iv) honey bee queens live for up to five years, whereas bumblebee queens live for just a little over a year [honey bee biology reviewed by Wilson, 1971; Winston, 1987; Seeley, 1996; bumblebee biology reviewed by Heinrich, 1979; Kearns and Thompson, 2001].

The finding that honey bees have relatively smaller brains and mushroom bodies than expected (fig. 4, table 1) corroborates an earlier qualitative study [Howse, 1974] that found relatively larger mushroom bodies in a bumblebee *(Bombus sp.)* and a social wasp (*Vespa sp.*). It might be explained by the idea that increased division of labor may require less, rather than more, neuronal capacity. Individuals of highly social insect species with advanced division of labor and task specialization might not need to perform the entire behavioral repertoire of the species. The general idea that in more advanced social insects, individuals are less pluripotent and more behaviorally restricted, has been reviewed by Anderson and McShea [2001]. For instance, honey bee queens, unlike bumblebee queens, cannot survive or found a colony by themselves, and they do not need to forage. In that respect, bumblebees are more pluripotent and might therefore require larger overall brain capacities than their more specialized relatives.

Very little is known about brain specializations in social insect workers of any species, except for the differ-

ences between nurses and foragers in honey bees [Withers et al., 1993; Durst et al., 1994] and carpenter ants [Gronenberg et al., 1996]. The present study suggests that more advanced division of labor could coincide with a reduction in relative brain size. It would be interesting to examine in more detail the correlation between brain morphology and the degree of specialization in different social insect taxa (social bees, social wasps, ants, and termites) to find out whether this concept holds across social insects in general.

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