Queen Dominance May Reduce Worker Mushroom Body Size in a Social Bee

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ABSTRACT: **The mushroom body (MB) is an area of the insect brain involved in learning, memory, and sensory integration. Here, we used the sweat bee *Megalopta genalis* (Halictidae) to test for differences between queens and workers in the volume of the MB calyces. We used confocal microscopy to measure the volume of the whole brain, MB calyces, optic lobes, and antennal lobes of queens and workers. Queens had larger brains, larger MB calyces, and a larger MB calyces:whole brain ratio than workers, suggesting an effect of social dominance in brain development. This could result from social interactions leading to smaller worker MBs, or larger queen MBs. It could also result from other factors, such as differences in age or sensory experience. To test these explanations, we next compared queens and workers to other groups. We compared newly emerged bees, bees reared in isolation for 10 days, bees initiating new observation nests, and bees initiating new natural nests collected from the field to queens and workers. Queens did not differ from these other groups. We suggest that the effects of queen dominance over workers, rather than differences in age, experience, or reproductive status, are responsible for the queen–worker differences we observed. Worker MB development may be affected by queen aggression directly and/or manipulation of larval nutrition, which is provisioned by the queen. We found no consistent differences in the size of antennal lobes or optic lobes associated with differences in age, experience, reproductive status, or social caste.** © 2019 Wiley Periodicals, Inc. Develop Neurobiol 0: 1–12, 2019

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INTRODUCTION

Many animals, including humans, exhibit brain plasticity over the course of their lifetime (May, 2011; Nava and Röder, 2011; Harris *et al.*, 2017). Plasticity is widespread even at the adult stage in insects (Fahrbach and Van Nest, 2016; Fahrbach *et al.*, 2017; Simões and Rhiner, 2017; Sugie *et al.*, 2018). In adult insects, one brain region that exhibits plasticity is the mushroom body (MB). The mushroom bodies support cognitive processes such as sensory integration, learning, and memory (Zars, 2000; Fahrbach, 2006). MBs may increase in volume over time due to dendritic growth (Farris *et al.*, 2001; Seid and Wehner, 2008; Muenz *et al.*, 2015). In social insects, patterns of MB development may reflect social roles (Amador-Vargas *et al.*, 2015; O'Donnell and Bulova, 2017; O'Donnell *et al.*, 2017). In many primitively social insects, reproductives must establish dominance over subordinates and the queens or otherwise dominant individuals have larger MBs than workers or other subordinate individuals (Molina and O'Donnell, 2007; 2008; O'Donnell *et al.*, 2007; 2017; Rehan

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et al., 2015). This is not the case in the honeybees and large-colony ant species, where queens use chemical communication to control worker reproduction, and have smaller MBs than workers (Julian and Gronenberg, 2002; Ehmer and Gronenberg, 2004; Fahrbach, 2006). The queen–worker differences in primitively social species may arise from differences in age, reproductive physiology, experience, or social interactions between the two castes, as all of these factors can affect MB plasticity in the adult brain (reviewed in Fahrbach, 2006).

MBs may change with age or reproductive physiology. Honeybee workers exhibit expansion of MB neuropil volume with age during the first week after eclosion (termed "experience-expectant" plasticity) (Withers *et al.*, 1993; 1995; Durst *et al.*, 1994; Fahrbach *et al.*, 1998). Similar patterns have been found in bumblebees (Jones *et al.*, 2013), wasps (O'Donnell *et al.*, 2007) and ants (Gronenberg *et al.*, 1996; Seid and Traniello, 2005; Seid and Wehner, 2008). Because queens are older than workers (who are typically their daughters), queen–worker differences could be related to age. Queens are reproductive, and workers are not, because queens suppress worker ovary development leading to the reproductive division of labor characteristic of eusociality (Michener, 1990; Spradbery, 1991). Reproductive physiology may also underlie MB differences. In the bee *Ceratina australensis* and the paper wasp *Polistes instabilis,* ovary size correlates with MB volume (Molina and O'Donnell, 2007; Rehan *et al.*, 2015). In honeybees, juvenile hormone (JH) affects MB development (Withers *et al.*, 1995), and JH is associated with dominance and reproduction in primitively social insects (West-Eberhard, 1996; Smith *et al.*, 2013; Hamilton *et al.*, 2017). Thus, differences in reproductive physiology between queens and workers may underlie MB differences.

MBs may also show experience-dependent plasticity, increasing in volume in response to complex tasks like foraging or other sensory stimuli (Withers *et al.*, 1993; 1995; 2007; Gronenberg *et al.*, 1996; Fahrbach *et al.*, 1998; Farris *et al.*, 2001; Kühn-Bühlmann and Wehner, 2006; Ismail *et al.*, 2006; Krofczik *et al.*, 2008; Molina and O'Donnell, 2008; Seid and Wehner, 2008; Maleszka *et al.*, 2009; Stieb *et al.*, 2010; Jones *et al.*, 2013; Amador-Vargas *et al.*, 2015; Rehan *et al.*, 2015; Montgomery *et al.*, 2016; Seid and Junge, 2016; van Dijk *et al.*, 2017; Montgomery and Merrill, 2017). Because queens must find and establish a nest, as well as forage for the first brood, they likely have more cumulative sensory experience, and thus larger MBs than workers. Social interactions, rather than sensory experience more generally, may also affect MB development. *Drosophila* reared in social groups had larger MBs than those reared alone (Heisenberg *et al.*, 1995), and socially isolated *Camponotus* ants had smaller MBs than socially integrated ants of the same age (Seid and Junge, 2016). If social interactions are important for MB development, then both queens and workers should have larger MBs than pre-social nest foundresses who are not living with another bee.

The nature of social interactions, rather than just social interaction *per se* may also influence MB development. In primitively social insect societies, queens establish social dominance over workers (Michener 1990; Spradbery, 1991). This dominance behavior itself, cognitive demands associated with dominance (e.g. Tibbetts *et al.*, 2018), or physiological changes associated with dominance, including increased JH titers and brain amine expression (Hamilton *et al.*, 2017) may also affect MB volume. Paper wasps show a correlation between dominance status and MB size (O'Donnell *et al.*, 2007; Molina and O'Donnell, 2007; 2008). In a study controlling for age, Rehan *et al.* (2015) showed that dominant females had larger MBs, and subordinate females smaller MBs, than solitary nest foundreses, although a study of same-generation paper wasp nest co-foundresses found no difference between dominants and subordinates (Ehmer *et al.*, 2001). Lastly, just as queens are dominant, workers are subordinate and bullied by queen aggressive behaviors which leads to suppressed ovarian development and other physiological effects, including lower levels of JH (Smith *et al.*, 2013; Hamilton *et al.*, 2017). Queen manipulation of worker behavior and reproductive physiology extends to the larval stages as well because it is the foundress queen who controls the larval provisions provided to the developing workers (Michener and Brothers, 1974; Kapheim *et al.*, 2011; Kapheim, 2017; Lawson *et al.*, 2017). Thus, workers may have smaller MBs as a result of maternal manipulation of nutrition and/or behavioral aggression. As mentioned above, Rehan *et al.* (2015) showed that subordinate foragers had smaller MBs than solitary nest foundresses; other studies did not distinguish between queens enlarging MBs through dominance versus reducing worker MBs through subordinance. Both the "enlarged dominant" and "reduced subordinate" hypotheses posit that these factors influence MB size in addition to the differences in ovarian development that result from queen dominance.

Here, we use the facultatively eusocial sweat bee *Megalopta genalis* (Halictidae) to test for queen–worker differences in MB size and other measures of brain volume. Next, we test whether these differences arise from workers' MBs being smaller, or queens' being larger. We then use other treatment groups to test the alternative hypotheses outlined above for factors other than social dominance interactions that may lead to queen–worker differences in MB size. *M. genalis* females initiate nests as solitary foundresses by digging tunnels into dead sticks suspended above the ground in vegetation (Wcislo *et al.*, 2004). The first daughter(s) to emerge usually remain in their natal nest as nonreproductive worker(s) (social nests usually have one or two workers). They are smaller than the queen and their younger sisters, which emerge later before dispersing to reproduce, and they are also subject to aggressive dominance from the queen (Smith *et al.*, 2008; 2009; 2019; Kapheim *et al.*, 2011; 2013; 2016). Newly emerged females remain in their natal nest for ~5–7 days before either beginning work as a forager or dispersing to reproduce (Kapheim *et al.*, 2013).

In a previous study on *M. genalis,* Smith *et al.* (2010) found that newly emerged bees had smaller MBs than queens and solitary reproductives (but not workers). Queens and workers did not differ in MB volume. However, this study did not control for age (bees were of unknown age from field-collected nests), nor did it include any other stages of adult development except for newly emerged females. Here we use known age queens and workers, newly emerged females, and three other treatment groups to test six nonexclusive hypotheses for queen–worker differences in MB development. To test these hypotheses, we measured the volumes of the MB calyces and whole brain size using unbiased stereological techniques. We also measured the volumes of two sensory neuropils: the antennal lobes (AL, olfactory input center) and optic lobes (OL, visual input center). This let us test whether plasticity in MB calyces was reflected in other brain areas as well, and also to compare whether the sensory neuropils were more responsive to changes in the sensory environment than the MBs. We used bees from six different groups that differed in age and experience: newly emerged bees, bees kept in sensory and social isolation for 10 days, observation nest foundresses of known age, natural nest foundresses (dispersers) of unknown age who were just beginning a new nest, established queens, and 10-day old workers.

The hypotheses and predictions that we tested are listed below. (1) *Age:* Queens should have larger MBs, and newly emerged bees smaller MBs, than all other groups. (2) *Reproductive status:* Dispersers, observation nest foundresses, and queens are all actively nesting, and thus should have larger MBs than the workers, isolated bees, and newly emerged bees. (3) *Experience:* The queens, which foraged to provision the first worker brood, should have larger MBs, and the newly emerged bees and isolation bees smaller MBs, than the other groups (which have some foraging experience). (4) *Social interactions:* Queens and workers, which are living in a social nest, should have larger MBs than other groups. The predictions are less clear for the dispersers, as they are nesting solitarily, but had social experience with their mother at their natal nest before dispersing. (5) *Enlarged dominant:* Queens should have larger MBs than all other groups if social dominance leads to enlarged MBs. (6) *Reduced subordinate:* If queen aggressive behavior and/or nutritional manipulation of larval nutrition from the queen reduces MB size, the workers should have smaller MBs than all other groups.

METHODS

We collected bees at Barro Colorado Island (BCI), Panama (9.1521° N, 79.8465° W), where this species has been studied in detail (Smith *et al.*, 2003; 2008; 2009; 2013; 2019; Wcislo *et al.*, 2004; Kapheim *et al.*, 2011; 2012; 2013; 2016).

Experimental Groups

For this study, we used six experimental groups, similar to the methods of previous studies on this species (Kapheim *et al.*, 2012; Smith *et al.*, 2013). We reared bees from brood cells that we took from field-collected nests at ambient temperature. Developing immatures were checked daily, and newly emerged adults removed each day. Newly emerged bees ($N = 4$) are females collected upon emergence. Isolated bees ($N = 3$) were females that were moved to cages (round plastic deli containers 13 cm diameter and 10 cm height) the day of their emergence. Bees were kept in social isolation (one bee per cage) and darkness at ambient temperature with ad-lib food (honey:water:soy-protein powder, 45:45:10 by volume). Other females were placed into standardized observation nests on the day of their emergence, and these nests were then placed in the field. Observation nests consist of a piece of balsa wood with a straight tunnel cut into the middle placed between two sheets of opaque Plexiglas; see Kapheim *et al.* (2013) and Smith *et al.* (2013) for more details. Observation nest foundresses ($N = 5$) are females from these observation nests collected when they have completed an entrance collar for their nest (Smith *et al.*, 2003; 2013). We use this as a proxy for beginning to nest, since the typical first step in nesting, constructing the tunnel, is not necessary in our pre-excavated observation nests. This is the first nest construction step performed by females in observation nests, and distinguishes them from other females who may wait for several days and then abandon the observation nest without nesting. The observation nest foundresses averaged 5.80 ± 4.32 SD days old (range: 2–13) when they were collected. Queen ($N = 4$) and worker ($N = 4$) bees were collected from these observation nests 10 days after the emergence of the worker, at which point the worker was foraging to provision the nest. Workers are daughters of the queen who are subject to aggressive dominance from the queen (Kapheim *et al.*, 2016) that suppresses worker ovarian development and reproduction (Smith *et al.*, 2009; 2013; Kapheim *et al.*, 2012). Workers remain in their natal nest, where they forage to provision the queen's offspring. Note that while the workers are similarly aged to the "10-day isolation," "observation nest foundress," and likely the "disperser" (below) treatments, the queens are a generation older and averaged 65.25 ± 3.40 days old at collection (range: 62–70). Dispersers ($N = 5$) are bees collected in the field while initiating a new nest. We first collected sticks that appeared to be suitable nesting substrate, confirmed that they contained no existing nests, and placed them in the freezer (–20 C) for at least 24 h to ensure that no undetected nests were present. We then placed these sticks in the field and checked them every three days for nesting activity. When a new nest was discovered, we collected it. Dispersers are of unknown age, but given that dispersing females typically leave observation nests ~ 5–7 days after emergence (Kapheim *et al.*, 2013), we assume they are approximately 10 days old. Dispersers had social interactions with their mother (the queen of their natal nest). Newly emerged bees are fed by the queen or other nestmates before they disperse to initiate a new nest or begin foraging flights as a worker (Wcislo and Gonzalez, 2006; Kapheim *et al.*, 2016). We assume that dispersers were not subject to aggressive dominance because they left the nest to initiate a new nest and reproduce rather than remain in their natal nest as workers. All bees were collected between 2 and 30 July, 2016.

Ovarian Dissections and Size Measurements

We preserved the abdomen of each bee in 70% ethanol at collection and dissected out the ovaries. Ovaries were photographed at 10x magnification through a dissecting microscope and the area of the entire photographed ovary measured using Image J, following methods of previous studies on this species (Smith *et al.*, 2008; 2009). We measured thorax width (intertegular span) of each bee collected with digital calipers as a measure of body size (Cane, 1987). Thorax width correlates with both head width ($r^2 = 0.89$) and whole body dry weight ($r^2 = 0.83$) in *M. genalis* (Kapheim *et al.*, 2011).

Brain Measurements

We preserved bee heads in 4% paraformaldehyde in phosphate-buffered saline (PBS) at collection and stored them at 4°C until dissection. We dissected head capsules in PBS to remove the brain which was immediately placed in glutaraldehyde (2%) for 48 h, bleached in a formamide solution, and dehydrated in a series of ethanol washes of increasing concentration following McKenzie *et al.* (2016). Because the fixative and histology methods used here differ from Smith *et al.* (2010), volume measures are not comparable between the two studies. Prior to imaging, brains were mounted in methyl salicylate. Brains were imaged using an Olympus Fluoview FV1000 confocal microscope using autofluorescence at 10X magnification and a step size of 10 µm (Fig. 1). We calculated volumes of the brain and different neuropils (MB calyces, AL, and OL, including both the lamina and medulla) through tracing and serial reconstruction using the software program Reconstruct (Fiala, 2005). We chose these neuropils because they were the ones affected by social status and changes in the sensory environment in previous studies (O'Donnell *et al.*, 2007; 2011; 2013; Molina and O'Donnell, 2008; Molina *et al.*, 2009; Rehan *et al.*, 2015). Brain and neuropil volumes were standardized to average body size by calculating a correction factor that was applied to each bee: mean body size of all bees in the study divided by the individual's body size. This correction factor was then multiplied to brain and neuropil volume for each bee, which is referred to as "size-corrected volumes" below. Ratios for each neuropil:whole brain were calculated for each individual.

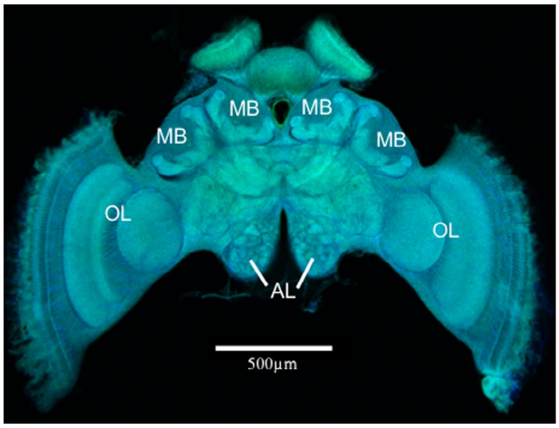

Figure 1 Frontal section of a *Megalopta genalis* brain imaged with autoflorescence showing the brain areas measured for this study. Mushroom body calyces are the cup-shaped structures surrounding the labels "MB." Antennal lobes are labeled "AL" and optic lobes are "OL." The scale bar is 500 µm.

Statistical Analyses

For queen–worker comparisons, we used a paired *t*-test to account for the effect of shared nest and developmental history (Kapheim *et al.*, 2016). We tested for differences across all treatment groups using an ANOVA followed by Tukey's post hoc pairwise comparisons.

RESULTS

Body size did not differ between groups ($F_{5,19} = 0.53$, $P = 0.750$). Body size did not correlate with AL:whole brain ratio ($r = -0.05$, $N = 25$, $P = 0.807$) or MB:whole brain ratio ($r = 0.23$, $N = 25$ $P = 0.260$), but body size did correlate negatively with OL:whole brain ratio ($r = -0.47$, $N = 25$, $P = 0.019$). Large bees invested relatively less tissue in OLs.

Ovary size differed between groups ($F_{5,19} = 15.21$, $P < 0.001$, Fig. 2). Queens and dispersers had enlarged, reproductive ovaries, while the other treatment groups did not (pairwise comparison p values for both queens and dispersers vs. young bees, isolation bees, observation nest bees, and workers all ≤0.001). Thus, observation nest foundresses were not yet reproductive. Ovary size did not correlate with size corrected MB calyx volume ($r = 0.27$, $N = 25$, $P = 0.898$) or the MB calyces:whole brain ratio ($r = -0.14$, $P = 0.519$, $N = 25$).

Brain Differences Between Queens and Workers

Each queen was larger bodied than her worker, but only slightly so (average queen:worker thorax width ratio = 1.040 ± 0.036, range: 1.003–1.076), and this difference was not significant (paired *t*-test $t_4 = 2.15$, $P = 0.121$). Workers had significantly smaller brains than queens (size-corrected volume paired $t_4 = 3.60$, $P = 0.037$, Fig. 3a). Workers had significantly smaller MBs than queens (size-corrected volume paired $t_4 = 4.97$, $P = 0.016$, Fig. 3b) and significantly smaller OLs than queens (size-corrected volume paired $t_4 = 5.99$, $P = 0.009$, Fig. 3c). There was not a significant queen–worker difference in AL size (size-corrected volume paired $t_4 = 2.37$, $P = 0.098$, Fig. 3d).

Queens also had significantly larger MB calyces then workers when measured as neuropil volume:whole brain volume ratio (paired $t_4 = 5.06$, $P = 0.015$, Fig. 4a). There was no difference between either queen and worker OL:whole brain ratio (paired $t_4 = 1.44$, $P = 0.238$, Fig. 4b) or the AL:whole brain ratio (paired $t_4 = 1.44$, $P = 0.556$, Fig. 4c).

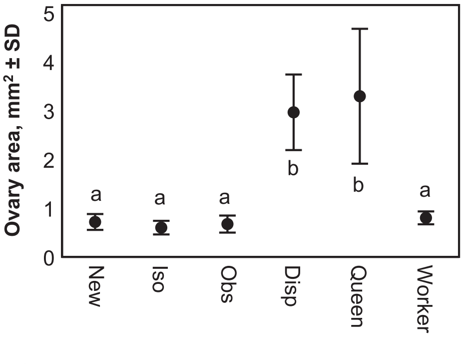

Figure 2 Mean ovary size for each treatment group ± standard deviation (SD): Newly emerged (New), isolated (Iso), observation nest foundresses (Obs), dispersers (Disp), Queens and Workers. Values that do not share a letter were significantly different as calculated from Tukey's post hoc pairwise comparisons following ANOVA.

Comparisons Across All Groups

Whole brain volume corrected for body size was not significantly different across treatment groups ($F_{5,19} = 2.14$, $P = 0.104$, Table 1), although workers were nearly significantly smaller than newly emerged bees in post hoc pairwise tests ($P = 0.053$). Size-corrected MB calyx volume was significantly different across treatment groups ($F_{5,19} = 3.29$, $P = 0.174$, Table 1). Workers had significantly smaller mushroom bodies than newly emerged bees ($P = 0.028$) and isolated bees ($P = 0.037$). The difference between workers and observation nest foundresses was marginally nonsignificant, with workers again having smaller mushroom bodies ($P = 0.072$).

There was an effect of group on size-corrected optic lobe volume ($F_{5,19} = 3.58$, $P = 0.019$, Table 1). Queens' OL volume was significantly larger than observation nest foundress' OL ($P = 0.031$). There was no effect of group on size-corrected AL volume ($F_{5,19} = 1.41$, $P = 0.265$, Table 1).

When measured as a ratio of neuropil to whole brain volume, rather than size-corrected volumes, there were also significant differences in MB calyces, and OLs, but not ALs, between groups. MB calyx neuropil:whole brain ratio showed a significant effect of group ($F_{5,19} = 3.97$, $P = 0.012$, Table 1). Worker MB:whole brain ratios were significantly smaller than isolated ($P = 0.015$) and observation nest foundress ($P = 0.021$) bees, and nearly significantly smaller than young bees ($P = 0.061$). There was an effect of group on OL:whole brain volume ($F_{5,19} = 5.19$, $P = 0.004$, Table 1). Queens had significantly higher ratios than newly emerged bees ($P = 0.018$) and observation nest foundresses ($P = 0.016$). Dispersers also had significantly higher ratios than newly emerged bees ($P = 0.044$) and observation nest foundresses

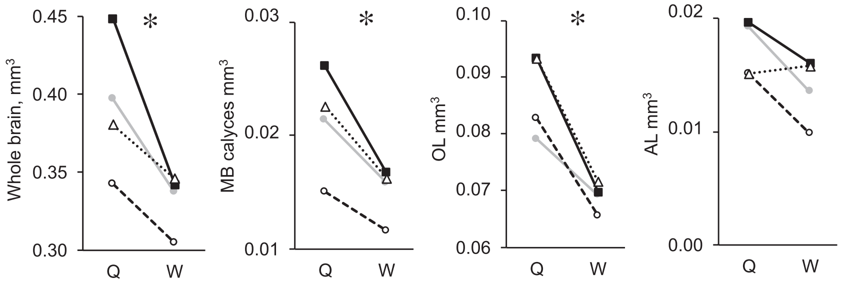

Figure 3 Comparisons between queen (Q) and worker (W) of the size-corrected volumes of (a) whole brain, (b) mushroom body calyces, (c) optic lobes, (d) antennal lobes. Identical symbols connected by a line represent each nestmate queen and worker. Asterisks (*) indicate significant differences between queens and workers in a paired *t*-test.

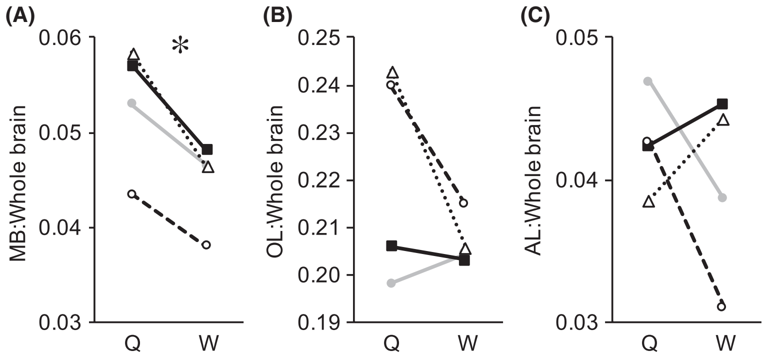

Figure 4 Comparisons between queen (Q) and worker (W) of the ratio of the (a) mushroom body calyces, (c) optic lobes, and (d) antennal lobes volumes to whole brain volume. Identical symbols connected by a line represent each nestmate queen and worker. The asterisk (*) indicate a significant difference between queens and workers in a paired *t*-test.

Table 1 Means and standard deviations (SD) for size corrected (SC) volumes and neuropil:whole brain ratios for each group of bees used in the study

		New	Dispersers	Obs. Nest	Isolated	Queens	Workers
SC Whole brain, mm^3	Mean	0.420	0.384	0.382	0.399	0.392	0.333
	SD	0.041	0.058	0.030	0.019	0.044	0.019
SC MB calyces, mm^3	Mean	0.025	0.021	0.023	0.025	0.021	0.015
	SD	0.006	0.005	0.003	0.002	0.004	0.002
SC Optic lobes, mm^3	Mean	0.075	0.082	0.068	0.076	0.086	0.069
	SD	0.009	0.011	0.005	0.011	0.007	0.002
SC Antennal lobes, mm^3	Mean	0.015	0.015	0.015	0.014	0.017	0.013
	SD	0.001	0.002	0.001	0.001	0.002	0.003
MB:Whole brain ratio	Mean	0.059	0.055	0.060	0.063	0.053	0.045
	SD	0.008	0.006	0.007	0.006	0.007	0.005
OL:Whole brain ratio	Mean	0.178	0.215	0.179	0.189	0.222	0.207
	SD	0.018	0.018	0.011	0.024	0.023	0.005
AL:Whole brain ratio	Mean	0.035	0.039	0.039	0.034	0.043	0.040
	SD	0.004	0.003	0.003	0.002	0.003	0.007

Note. See text for explanations of treatment groups and statistical tests of pairwise comparisons.

(P = 0.040). There were not significant differences in the ratio of AL volume to whole brain volume ($F_{5,19}$ = 2.196, P = 0.098, Table 1).

DISCUSSION

Here, we show that workers invest less neural tissue in MB calyces than queens. Comparisons with other groups suggest that workers' MB calyces are relatively reduced, rather than queens' being enlarged. Comparisons with other groups also show that the queen–worker difference is not a result of differences in body size, age, ovarian development, or sensory experience. This suggests that queen dominance behavior and/or maternal manipulation of larval nutrition may affect worker brain morphology.

Body Size

Body size did not differ between our treatment groups. In previous studies, we have shown that workers are typically, but not always, smaller than their queens and other reproductive foundresses (Smith *et al.*, 2008; 2009; Kapheim *et al.*, 2012; 2013). In this study, each worker was smaller than her queen, but the differences were slight and not statistically significant. Workers were not smaller than queens in general or dispersers, which differs from previous studies of this species and may be a result of the small sample size of this study (Smith *et al.*, 2008; 2009; Kapheim *et al.*, 2012; 2013). Body size correlated negatively with OL:whole brain ratio, suggesting that bees invest relatively less in OL tissue at larger sizes. We do not know what the effect of increased OL tissue on vision would be, but previous studies on the optics of *M. genalis* suggest that they fly at the limit of their visual abilities (Warrant, 2017). Body size did not correlate with the neuropil:whole brain ratios of MB calyx or AL, suggesting that there is not size-based allometry in these brain areas.

Mushroom Bodies

We made six predictions about MB calyx size variation between groups. First, being dominant would enlarge queens MBs. Second, being subordinate may reduce worker MB calyx volume. Next, we tested whether age, reproductive status, sensory experience, or being part of a social group affected MB calyx size. Workers had smaller MB calyces and MB calyx:brain ratios than all other groups (although not all differences were significant; Figs. 3 and 4, Table 1). The other groups, including queens, did not significantly differ from each other. This suggests that workers' MBs are relatively small, but that queens' MBs are not especially large. Our data did not fit the age prediction because newly emerged bees did not have smaller MB calyces, nor did queens have larger MB calyces than the other groups. It did not fit the reproductive status prediction because the two groups with reproductive ovaries, queens and dispersers, did not have larger MB calyces than the nonreproductive groups, except for workers. This did not fit the sensory experience prediction because newly emerged and isolated bees did not have smaller MB calyces, nor did queens have larger MB calyces than other groups. The results also did not fit the social interactions prediction because workers and queens together did not have larger MB calyces than the other groups.

Our MB data suggest that a combination of reduced nutrition during development and/or behavioral dominance after emergence lead to reduced neural investment in worker MB calyces relative to other bees of the same age and size that are either experimentally (the observation nest foundresses and isolated bees) or naturally (the dispersers) free from queen control. High dominance status, rates of aggression, and enlarged ovaries are associated with larger MB calyces in paper wasps (Molina and O'Donnell, 2007; 2008; O'Donnell *et al.*, 2007; 2017), and socially dominant females of the bee *Ceratina australensis* also have larger MB calyces than their subordinate sisters (Rehan *et al.*, 2015). Rehan *et al.* (2015) showed that queens had larger MBs than solitary reproductives, and that workers had smaller MBs than solitary reproductives. The latter result is similar to the queen–worker differences we report here, although in our study queens did not differ from the solitary nest foundress groups. In the related sweat bee *Augochlorella aurata,* early season nest foundresses (collected before the emergence of the worker brood) had larger MB calyces than workers from social nests (S. Pahlke, S. Jaumann, M. A. Seid, and A. R. Smith, in review). Previous authors interpreted these results in terms of increased cognitive demand associated with dominance and/or the older age of the queens (Molina and O'Donnell, 2007; 2008; O'Donnell *et al.*, 2007; 2017; Smith *et al.*, 2010; Rehan *et al.*, 2015) or the increased larval nutrition provided to future queens to survive overwintering diapause in temperate climates (S. Pahlke, S. Jaumann, M. A. Seid, and A. R. Smith, in review). However, our results here suggest that workers' MB development may be suppressed by queen dominance, rather than queen's MBs being enlarged, since workers' MB calyx volume, both absolutely and as a ratio of whole brain volume, was significantly smaller than all other groups except dispersers, while queens' MB calyx volume was not significantly larger than any other group except workers.

How might maternal manipulation affect MB development? We do not know whether it is behavioral dominance, maternal manipulation of nutrition or a combination of the two that leads to the reduction of worker MB calyx size. Previous work on this species shows that caste has morphological and physiological components: workers are smaller than queens (Smith *et al.*, 2008; 2009; Kapheim *et al.*, 2013) due to reduced larval nutrition (Kapheim *et al.*, 2011), although the queen–worker size differences were greater in those studies than we found here. Queens aggressively dominate workers (Kapheim *et al.*, 2016; Smith *et al.*, 2019). This results in physiological changes: workers have reduced ovaries, lower vitellogenin (the egg precursor protein, Vg) titers, and lower juvenile hormone (JH) levels than queens and both observation nest foundresses and natural dispersers (Smith *et al.*, 2009; 2013; Kapheim *et al.*, 2012). JH is associated with, but not required for, MB expansion in honeybees (Withers *et al.*, 1995; Fahrbach *et al.*, 2003). However, in the studies cited above (Kapheim *et al.*, 2012; Smith *et al.*, 2013), newly emerged and socially isolated bees also had reduced ovaries, Vg, and JH, yet those same groups showed greater MB development than workers in this study, which suggests that variation in MB development is not driven directly by these physiological variables. It may be the aggressive dominance behavior of queens toward their workers itself that affects MB calyx size in workers. In the ant *Diacamma,* aggression toward workers reduced brain dopamine levels (Shimoji *et al.*, 2017), which may influence brain volume (Taylor *et al.*, 1992). Larval thermal stress can affect MB development in *Drosphila* (Wang *et al.*, 2007), and stress generally affects brain structure and function across animals (Lupien *et al.*, 2009). The reduced MB investment in workers may be a response to the behavioral stress of queen aggression, a factor to which no other group in the study was subjected.

Another explanation for workers' smaller MB calyces, and whole brains as well, is that larval nutrition may influence neural development. Larval nutrition affects brain morphology in honeybees, with queens having larger and more rapidly growing brains in the larval stage (Moda *et al.*, 2013), and reduced larval nutrition results in smaller MB calyces at emergence in workers (Steijven *et al.*, 2017), but this has not been studied in primitively eusocial groups. Variation in larval nutrition can affect reproductive physiology and behavior in other sweat bee species (Richards and Packer, 1994; Brand and Chapuisat, 2012) as well as other species of primitively eusocial insects (Judd *et al.*, 2015; Lawson *et al.*, 2016; 2017; Kapheim, 2017). Our previous work on this species suggests that queens manipulate larval pollen resources to create small, subordinate worker daughters (Smith *et al.*, 2008; 2009; Kapheim *et al.*, 2011; 2013). However, our study suggests that effects on worker brain morphology result from more subtle nutritional variation than just reduced quantity, as workers were not significantly smaller than the other groups. On the one hand, the lack of queen–worker body size difference is likely an artifact of the small sample size of this study, given the ubiquity of this difference in previous studies (Smith *et al.*, 2008; 2009; Kapheim *et al.*, 2013). On the other hand, it allows us to see that the dramatic MB calyx differences of this study are apparently related to being a subordinate worker, rather than just a small bee. Richards and Packer (1994) showed that offspring of different castes but similar body size differed in the relative amounts of sugar and protein in their larval provisions in the sweat bee *Halictus ligatus*. Future studies coupling nutritional manipulation with brain measurements, and the interaction of larval nutrition and adult experience of aggression, would be productive.

Our study found no effect of age on MB development. The lack of difference between newly emerged bees and other groups contrasts with a previous study on this species which showed that newly emerged bees had significantly smaller MB calyces than queens, but not workers (newly emerged bees' AL and OL volumes were also lower in the previous study) (Smith *et al.*, 2010). Previous studies on honeybees, bumblebees, the bee *C. australensis,* the paper wasp *Mischocyttarus mastigophorus,* and multiple species of ants also showed increases in MB calyx volume during the first week after emergence (Gronenberg *et al.*, 1996; Fahrbach *et al.*, 1998; 2003; Seid *et al.*, 2005; O'Donnell *et al.*, 2007; Seid and Wehner, 2008; Rehan *et al.*, 2015; Seid and Junge, 2016). The solitary bee *Osmia lignaria* did not show such an increase but that is complicated by the fact that *O. lignaria* overwinters in their nest as an adult; MB expansion may have occurred prior to leaving the nest (Withers *et al.*, 2008). Our contrasting results with our previous study on *M. genalis* may also result from the limited sample sizes in each study (Smith *et al.*, 2010).

Many studies have shown experience-dependent plasticity of the MB calyces in Hymenoptera (Withers *et al.*, 1993; 1995; 2008; Gronenberg *et al.*, 1996; Fahrbach *et al.*, 1998; Farris *et al.*, 2001; Kühn-Bühlmann and Wehner, 2006; Molina and O'Donnell, 2008; Maleszka *et al.*, 2009; Stieb *et al.*, 2010; Jones *et al.*, 2013; Amador-Vargas *et al.*, 2015; Rehan *et al.*, 2015), other insects (Montgomery *et al.*, 2016;

van Dijk *et al*., 2017; Montgomery and Merrill, 2017) and even a spider (Stafstrom *et al*., 2017). Fahrbach *et al*. (2003) showed that honeybee foragers that spend the winter in the hive without foraging had similar MB development to other foragers of younger age but similar experience. Seid and Junge (2016) showed that socially isolated ants had smaller MBs than same-age ants in their natural social group. This suggests that age–MB volume associations after the first week or so of adult life result from increased experience rather than age per se. Yet in our study, the one group that was both markedly older and more experienced, the queens, did not have larger MB calyces than any of the younger, less experienced groups, except for their workers. Future studies of known age nests coupled with observations of foraging trips could explicitly measure experience-dependent MB plasticity.

Optic Lobes and Antennal Lobes

We predicted OLs and ALs to increase with sensory experience. Our results were mixed (Figs. 3 and 4), and given the small sample sizes of each group, our data have little power to partition the different influences on sensory neuropil development. However, the sensory neuropils were not as dramatically reduced in workers relative to queens as were the MB calyces, especially when measured relative to whole brain size (Fig. 4). This suggests that the effects of queen manipulation are stronger on the MB calyces than the sensory neuropils.

Conclusions

The most dramatic result of our study was that workers had smaller MB calyces than queens, but other groups in the study did not. This suggests that the queen–worker differences were not a result of age, sensory environment, experience, or reproductive status, because other groups in the study differed from queens in at least one of each of those variables. Workers were the only group subject to queen control. This may have taken the form of manipulation of developmental nutrition (Kapheim *et al*., 2011) and aggressive behavioral dominance (Kapheim *et al*., 2016; Smith *et al*., 2019). Future studies partitioning these two types of manipulation will be useful for understanding social influences on brain development.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in figshare.com at https://figshare.com/s/2071319e35b38ceecb9c, https://doi.org/10.6084/m9.figshare.7927409.

LITERATURE CITED

Amador-Vargas, S., Gronenberg, W., Wcislo, W.T. and Mueller, U. (2015) Specialization and group size: brain and behavioural correlates of colony size in ants lacking morphological castes. *Proceedings of the Royal Society B: Biological Sciences*, 282, 20142502.

Brand, N. and Chapuisat, M. (2012) Born to be bee, fed to be worker? The caste system of a primitively eusocial insect. *Frontiers in Zoology*, 9, 35.

Cane, J.H. (1987) Estimation of bee size using intertegular span (Apoidea). *Journal of the Kansas Entomological Society*, 60, 145–147.

Durst, C., Eichmüller, S. and Menzel, R. (1994) Development and experience lead to increased volume of subcompartments of the honeybee mushroom body. *Behavioral and Neural Biology*, 62, 259–263.

Ehmer, B. and Gronenberg, W. (2004) Mushroom body volumes and visual interneurons in ants: comparison between sexes and castes. *The Journal of Comparative Neurology*, 469, 198–213.

Ehmer, B., Reeve, H.K. and Hoy, R.R. (2001) Comparison of brain volumes between single and multiple foundresses in the paper wasp *Polistes dominulus*. *Brain, Behavior and Evolution*, 57, 161–168.

Fahrbach, S.E. (2006) Structure of the mushroom bodies of the insect brain. *Annual Review of Entomology*, 51, 209–232.

Fahrbach, S.E., Farris, S.M., Sullivan, J.P. and Robinson, G. (2003) Limits on volume changes in the mushroom bodies of the honey bee brain. *Journal of Neurobiology*, 57, 141–151.

Fahrbach, S.E., Moore, D., Capaldi, E.A., Farris, S.M. and Robinson, G.E. (1998) Experience-expectant plasticity in the mushroom bodies of the honeybee. *Learning & Memory*, 5, 115–123.

Fahrbach, S.E., Trawinski, A.M. and Velarde, R.A. (2017) Actions of developmental hormones in adult social insects. In Pfaff, D.W. and Joëls, M. (Eds.) Hormones, Brain and Behavior, 3rd edition. Oxford: Academic Press, pp. 350–365.

Fahrbach, S.E. and Van Nest, B.N. (2016) Synapsin-based approaches to brain plasticity in adult social insects. *Current Opinion in Insect Science*, 18, 27–34.

Farris, S.M., Robinson, G.E. and Fahrbach, S.E. (2001) Experience- and age-related outgrowth of intrinsic neurons in the mushroom bodies of the adult worker honeybee. *Journal of Neuroscience*, 21, 6395–6404.

Fiala, J.C. (2005) Reconstruct: a free editor for serial section microscopy. *Journal of Microscopy*, 218, 52–61.

Gronenberg, W., Heeren, S. and Hölldobler, B. (1996) Age-dependent and task-related morphological changes in the brain and the mushroom bodies of the ant *Camponotus floridanus*. *Journal of Experimental Biology*, 199, 2011–2019.

Hamilton, A.R., Shpigler, H., Bloch, G., Wheeler, D.E. and Robinson, G.E. (2017) Endocrine influences on the organization of insect societies. In: Pfaff, D.W. and Joëls, M. (Eds.) Hormones, Brain and Behavior, 3rd edition. Oxford: Academic Press, pp. 421–452.

Harris, R.M., O'Connell, L.A. and Hofmann, H.A. (2017) Chapter 15: Brain evolution, development, and plasticity. In: Shepherd, S.V. (Ed.) The Wiley Handbook of Evolutionary Neuroscience. 1st edition. West Sussex: John Wiley & Sons Ltd, pp. 422–443.

Heisenberg, M., Heusipp, M. and Wanke, C. (1995) Structural plasticity in the *Drosophila* brain. *Journal of Neuroscience*, 15, 1951–1960.

Ismail, N., Robinson, G.E. and Fahrbach, S.E. (2006) Stimulation of muscarinic receptors mimics experience-dependent plasticity in the honey bee brain. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 207–211.

Jones, B.M., Leonard, A.S., Papaj, D.R. and Gronenberg, W. (2013) Plasticity of the worker bumblebee brain in relation to age and rearing environment. *Brain, Behavior and Evolution*, 82, 250–261.

Judd, T.M., Teal, P.E., Hernandez, E.J., Choudhury, T. and Hunt, J.H. (2015) Quantitative differences in nourishment affect caste-related physiology and development in the paper wasp *Polistes metricus*. *PLoS ONE*, 10, e0116199.

Julian, G.E. and Gronenberg, W. (2002) Reduction of brain volume correlates with behavioral changes in queen ants. *Brain, Behavior and Evolution*, 60, 152–164.

Kapheim, K.M. (2017) Nutritional, endocrine, and social influences on reproductive physiology at the origins of social behavior. *Current Opinion in Insect Science*, 22, 62–70.

Kapheim, K.M., Bernal, S.P., Smith, A.R., Nonacs, P. and Wcislo, W.T. (2011) Support for maternal manipulation of developmental nutrition in a facultatively eusocial bee, *Megalopta genalis* (Halictidae). *Behavioral Ecology and Sociobiology*, 65, 1179–1190.

Kapheim, K.M., Chan, T., Smith, A., Wcislo, W.T. and Nonacs, P. (2016) Ontogeny of division of labor in a facultatively eusocial sweat bee *Megalopta genalis*. *Insectes Sociaux*, 63, 185–191.

Kapheim, K.M., Smith, A.R., Ihle, K.E., Amdam, G.V., Nonacs, P. and Wcislo, W.T. (2012) Physiological variation as a mechanism for developmental caste-biasing in a facultatively eusocial sweat bee. *Proceedings of the Royal Society B: Biological Sciences*, 279, 1437–1446.

Kapheim, K.M., Smith, A.R., Nonacs, P., Wcislo, W.T. and Wayne, R.K. (2013) Foundress polyphenism and the origins of eusociality in a facultatively eusocial sweat bee, *Megalopta genalis* (Halictidae). *Behavioral Ecology and Sociobiology*, 67, 331–340.

Krofczik, S., Khojasteh, U., De Ibarra, N.H. and Menzel, R. (2008) Adaptation of microglomerular complexes in the honeybee mushroom body lip to manipulations of behavioral maturation and sensory experience. *Developmental Neurobiology*, 68, 1007–1017.

Kühn-Bühlmann, S. and Wehner, R. (2006) Age-dependent and task-related volume changes in the mushroom bodies of visually guided desert ants, *Cataglyphis bicolor*. *Journal of Neurobiology*, 66, 511–521.

Lawson, S.P., Ciaccio, K.N. and Rehan, S.M. (2016) Maternal manipulation of pollen provisions affects worker production in a small carpenter bee. *Behavioral Ecology and Sociobiology*, 70, 1891–1900.

Lawson, S.P., Helmreich, S.L. and Rehan, S.M. (2017) Effects of nutritional deprivation on development and behavior in the subsocial bee *Ceratina calcarata* (Hymenoptera: Xylocopinae). *Journal of Experimental Biology*, 220, 4456–4462.

Lupien, S.J., McEwen, B.S., Gunnar, M.R. and Heim, C. (2009) Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*, 10, 434.

Maleszka, J., Barron, A.B., Helliwell, P.G. and Maleszka, R. (2009) Effect of age, behaviour and social environment on honey bee brain plasticity. *Journal of Comparative Physiology A*, 195, 733–740.

May, A. (2011) Experience-dependent structural plasticity in the adult human brain. *Trends in Cognitive Sciences*, 15, 475–482.

McKenzie, S.K., Fetter-Pruneda, I., Ruta, V. and Kronauer, D.J. (2016) Transcriptomics and neuroanatomy of the clonal raider ant implicate an expanded clade of odorant receptors in chemical communication. *Proceedings of the National Academy of Sciences of the United States of America*, 113, 14091–14096.

Michener, C.D. (1990) Reproduction and castes in social halictine bees. In: Engels W. (Ed.) Social Insects. Berlin: Springer, pp 77–121.

Michener, C. and Brothers, D. (1974) Were workers of eusocial hymenoptera initially altruistic or oppressed. *Proceedings of the National Academy of Sciences of the United States of America*, 71, 671–674.

Moda, L.M., Vieira, J., Freire, A.C.G., Bonatti, V., Bomtorin, A.D., Barchuk, A.R. and Simoes, Z.L.P. (2013) Nutritionally driven differential gene expression leads to heterochronic brain development in honeybee castes. *PloS one*, 8, e64815.

Molina, Y., Harris, R.M. and O'Donnell, S. (2009) Brain organization mirrors caste differences, colony founding and nest architecture in paper wasps (Hymenoptera: Vespidae). *Proceedings of the Royal Society B: Biological Sciences*, 276, 3345–3351.

Molina, Y. and O'Donnell, S. (2007) Mushroom body volume is related to social aggression and ovary development in the paperwasp *Polistes instabilis*. *Brain, Behavior and Evolution*, 70, 137–144.

Molina, Y. and O'Donnell, S. (2008) Age, sex, and dominance-related mushroom body plasticity in the paperwasp *Mischocyttarus mastigophorus*. *Developmental Neurobiology*, 68, 950–959.

Montgomery, S.H. and Merrill, R.M. (2017) Divergence in brain composition during the early stages of ecological specialization in *Heliconius* butterflies. *Journal of Evolutionary Biology*, 30, 571–582.

Montgomery, S.H., Merrill, R.M. and Ott, S.R. (2016) Brain composition in *Heliconius* butterflies, posteclosion growth and experience-dependent neuropil plasticity. *The Journal of Comparative Neurology*, 524, 1747–1769.

Muenz, T.S., Groh, C., Maisonnasse, A., Le Conte, Y., Plettner, E. and Rössler, W. (2015) Neuronal plasticity in the mushroom body calyx during adult maturation in the honeybee and possible pheromonal influences. *Developmental Neurobiology*, 75, 1368–1384.

Nava, E. and Röder, B. (2011) Adaptation and maladaptation: insights from brain plasticity. In: Lepore F., Green A., Chapman C.E., Kalaska J.F. (Eds.) Progress in Brain Research, Vol. 191. Amsterdam: Elsevier, pp. 177–194.

O'Donnell, S. and Bulova, S. (2017) Development and evolution of brain allometry in wasps (Vespidae): size, ecology and sociality. *Current Opinion in Insect Science*, 22, 54–61.

O'Donnell, S., Bulova, S.J., DeLeon, S., Barrett, M. and Fiocca, K. (2017) Caste differences in the mushroom bodies of swarm-founding paper wasps: implications for brain plasticity and brain evolution (Vespidae, Epiponini). *Behavioral Ecology and Sociobiology*, 71, 116.

O'Donnell, S., Clifford, M.R., DeLeon, S., Papa, C., Zahedi, N. and Bulova, S.J. (2013) Brain size and visual environment predict species differences in paper wasp sensory processing brain regions (Hymenoptera: vespidae, polistinae). *Brain, Behavior and Evolution*, 82, 177–184.

O'Donnell, S., Clifford, M. and Molina, Y. (2011) Comparative analysis of constraints and caste differences in brain investment among social paper wasps. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 7107–7112.

O'Donnell, S., Donlan, N. and Jones, T. (2007) Developmental and dominance-associated differences in mushroom body structure in the paper wasp *Mischocyttarus mastigophorus*. *Developmental Neurobiology*, 67, 39–46.

Rehan, S.M., Bulova, S.J. and O'Donnell, S. (2015) Cumulative effects of foraging behavior and social dominance on brain development in a facultatively social bee (*Ceratina australensis*). *Brain, Behavior and Evolution*, 85, 117–124.

Richards, M.H. and Packer, L. (1994) Trophic aspects of caste determination in Halictus ligatus, a primitively eusocial sweat bee. *Behavioral Ecology and Sociobiology*, 34, 385–391.

Seid, M.A. and Junge, E. (2016) Social isolation and brain development in the ant *Camponotus floridanus*. *The Science of Nature*, 103, 1–6.

Seid, M.A. and Traniello, J.F. (2005) Age-related changes in biogenic amines in individual brains of the ant *Pheidole dentata*. *Naturwissenschaften*, 92, 198–201.

Seid, M.A. and Wehner, R. (2008) Ultrastructure and synaptic differences of the boutons of the projection neurons between the lip and collar regions of the mushroom bodies in the ant, *Cataglyphis albicans*. *The Journal of Comparative Neurology*, 507, 1102–1108.

Shimoji, H., Aonuma, H., Miura, T., Tsuji, K., Sasaki, K. and Okada, Y. (2017) Queen contact and among-worker interactions dually suppress worker brain dopamine as a potential regulator of reproduction in an ant. *Behavioral Ecology and Sociobiology*, 71, 35.

Simões, A.R. and Rhiner, C. (2017) A cold-blooded view on adult neurogenesis. *Frontiers in Neuroscience*, 11, 327.

Smith, A.R., Kapheim, K.M., O'Donnell, S. and Wcislo, W.T. (2009) Social competition but not subfertility leads to a division of labour in the facultatively social sweat bee *Megalopta genalis* (Hymenoptera: Halictidae). *Animal Behavior*, 78, 1043–1050.

Smith, A.R., Kapheim, K.M., Pérez-Ortega, B., Brent, C.S. and Wcislo, W.T. (2013) Juvenile hormone levels reflect social opportunities in the facultatively eusocial sweat bee *Megalopta genalis* (Hymenoptera: Halictidae). *Hormones and Behavior*, 63, 1–4.

Smith, A.R., Seid, M.A., Jimenez, L.C. and Wcislo, W.T. (2010) Socially induced brain development in a facultatively eusocial sweat bee *Megalopta genalis* (Halictidae). *Proceedings of the Royal Society B: Biological Sciences*, 277, 2157–2163.

Smith, A.R., Simons, M., Bazarko, V., Harach, J. and Seid, M.A. (2019) Queen-worker aggression in the facultatively eusocial bee *Megalopta genalis*. *Insectes Sociaux*. https://doi.org/10.1007/s00040-019-00712-0

Smith, A.R., Wcislo, W.T. and O'Donnell, S. (2003) Assured fitness returns favor sociality in a mass-provisioning sweat bee, *Megalopta genalis* (Hymenoptera: Halictidae). *Behavioral Ecology and Sociobiology*, 54, 14–21.

Smith, A.R., Wcislo, W.T. and O'Donnell, S. (2008) Body size shapes caste expression, and cleptoparasitism reduces body size in the facultatively eusocial bees *Megalopta* (Hymenoptera: Halictidae). *Journal of Insect Behavior*, 21, 394–406.

Spradbery, J. (1991) Evolution of queen number and queen control. In: Ross, K. and Matthews, R. (Eds.) The Social Biology of Wasps. Ithaca, NY: Cornell University Press, pp. 336–388.

Stafstrom, J.A., Michalik, P. and Hebets, E.A. (2017) Sensory system plasticity in a visually specialized, nocturnal spider. *Scientific Reports*, 7, 46627.

Steijven, K., Spaethe, J., Steffan-Dewenter, I. and Härtel, S. (2017) Learning performance and brain structure of artificially-reared honey bees fed with different quantities of food. *PeerJ*, 5, e3858.

Stieb, S.M., Muenz, T.S., Wehner, R. and Rössler, W. (2010) Visual experience and age affect synaptic organization in the mushroom bodies of the desert ant *Cataglyphis fortis*. *Developmental Neurobiology*, 70, 408–423.

Sugie, A., Marchetti, G. and Tavosanis, G. (2018) Structural aspects of plasticity in the nervous system of Drosophila. *Neural Development*, 13, 14.

Taylor, D.J., Robinson, G.E., Logan, B.J., Laverty, R. and Mercer, A.R. (1992) Changes in brain amine levels associated with the morphological and behavioural development of the worker honeybee. *Journal of Comparative Physiology A*, 170, 715–721.

Tibbetts, E.A., Injaian, A., Sheehan, M.J. and Desjardins, N. (2018) Intraspecific variation in learning: worker wasps are less able to learn and remember individual conspecific faces than queen wasps. *American Naturalist*, 191, 595–603.

Van Dijk, L.J., Janz, N., Schäpers, A., Gamberale-Stille, G. and Carlsson, M.A. (2017) Experience-dependent mushroom body plasticity in butterflies: consequences of search complexity and host range. *Proceedings of the Royal Society B: Biological Sciences*, 284, 20171594.

Wang, X., Green, D.S., Roberts, S.P. and de Belle, J.S. (2007) Thermal disruption of mushroom body development and odor learning in Drosophila. *PLoS ONE*, 2, e1125.

Warrant, E.J. (2017) The remarkable visual capacities of nocturnal insects: vision at the limits with small eyes and tiny brains. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372, 20160063.

Wcislo, W.T., Arneson, L., Roesch, K., Gonzalez, V., Smith, A. and Fernández, H. (2004) The evolution of nocturnal behaviour in sweat bees, *Megalopta genalis* and *M. ecuadoria* (Hymenoptera: Halictidae): an escape from competitors and enemies? *Biological Journal of the Linnean Society*, 83, 377–387.

Wcislo, W. and Gonzalez, V. (2006) Social and ecological contexts of trophallaxis in facultatively social sweat bees, *Megalopta genalis* and *M. ecuadoria* (Hymenoptera, Halictidae). *Insectes Sociaux*, 53, 220–225.

West-Eberhard, M.J. (1996) Wasp societies as microcosoms for the study of development and evolution. In: West-Eberhard, M.J. and Turillazzi, S. (Eds.) Natural History and Evolution of Paper Wasps. Oxford: Oxford University Press, pp. 290–317.

Withers, G.S., Day, N.F., Talbot, E.F., Dobson, H.E. and Wallace, C.S. (2008) Experience-dependent plasticity in the mushroom bodies of the solitary bee *Osmia lignaria*. *Developmental Neurobiology*, 73–82.

Withers, G.S., Fahrbach, S.E. and Robinson, G.E. (1993) Selective neuroanatomical plasticity and division of labour in the honeybee. *Nature*, 364, 238–240.

Withers, G., Fahrbach, S. and Robinson, G. (1995) Effects of experience and juvenile hormone on the organization of the mushroom bodies of honey bees. *Journal of Neurobiology*, 26, 130–144.

Zars, T. (2000) Behavioral functions of the insect mushroom bodies. *Current Opinion in Neurobiology*, 10, 790–795.