

The Loss of Sociality Is Accompanied by Reduced Neural Investment in Mushroom Body Volume in the Sweat Bee *Augochlora Pura* (Hymenoptera: Halictidae)

Sarah Pahlke,¹ Marc A. Seid,² Sarah Jaumann,¹ and Adam Smith^{1,3}

¹Department of Biological Sciences, George Washington University, Washington, DC, ²Department of Biology and Program in Neurobiology, University of Scranton, Scranton, PA, and ³Corresponding author, e-mail: adam_smith@gwu.edu

Subject Editor: Qian "Karen" Sun

Received 5 February 2020; Editorial decision 29 June 2020

Abstract

Social behavior has been predicted to select for increased neural investment (the social brain hypothesis) and also to select for decreased neural investment (the distributed cognition hypothesis). Here, we use two related bees, the social *Augochlorella aurata* (Smith) (Hymenoptera: Halictidae) and the related *Augochlora pura* (Say), which has lost social behavior, to test the contrasting predictions of these two hypotheses in these taxa. We measured the volumes of the mushroom body (MB) calyces, a brain area shown to be important for cognition in previous studies, as well as the optic lobes and antennal lobes. We compared females at the nest foundress stage when both species are solitary so that brain development would not be influenced by social interactions. We show that the loss of sociality was accompanied by a loss in relative neural investment in the MB calyces. This is consistent with the predictions of the social brain hypothesis. Ovary size did not correlate with MB calyx volume. This is the first study to demonstrate changes in mosaic brain evolution in response to the loss of sociality.

Key words: Augochlorini, bees, behavioral evolution, Halictidae, mushroom bodies

Because of their diversity of societies, social insects are excellent subjects for understanding how social behavior affects brain evolution (reviewed in Lihoreau et al. 2012, Farris 2016, O'Donnell and Boulva 2017, Godfrey and Gronenberg 2019). The 'social brain hypothesis' posits that the complexities of social interactions select for increased neural investment in cognition (Dunbar 1992, 2009; Dunbar and Schultz 2007; but see DeCasien et al. 2017; Kverková et al. 2018). In applying the social brain hypothesis to the Hymenoptera (the bees, ants, and wasps), Gronenberg and Riveros (2009) predicted that in the initial stages of social evolution characterized by small groups, cognitive demand would increase, as individuals had to contend with the same challenges as solitary species in addition to the complexities of social interactions. However, in larger-colony species with specialized division of labor, cognitive demand would decrease, as each individual would only be required to perform a subset of tasks (Gronenberg and Riveros 2009, Riveros et al. 2012, Godfrey and Gronenberg 2019).

The area of the insect brain that has received the most focus in studies of social insect brain evolution is the mushroom bodies (MB), which are paired neuropils involved in learning, memory, and sensory integration (Fahrbach 2006). The evolutionary origin of elaborate

MBs in the Hymenoptera was apparently driven by the demands of foraging for a host that arose with the evolution of parasitoid wasps, which predates the evolution of nesting behavior and sociality in the aculeate Hymenoptera (Farris and Schulmeister 2011, Farris 2016). However, recent intraspecific comparisons in social insects suggest that while the evolutionary origins of large MBs may predate sociality, differences in social behavior can still lead to differences in MB investment. For example, differences in MB volume associated with dominance status within social species demonstrate a social influence on MB size (Molina et al. 2007, 2008; O'Donnell et al. 2008, 2017; Smith et al. 2010; Rehan et al. 2015; Jaumann et al. 2019; Pahlke et al. 2019). A direct test of whether social behavior leads to increased MB investment relative to solitary living should compare closely related social and solitary species. While many studies have compared the MBs of individuals within social insect species (reviewed in Fahrbach 2006, Lihoreau et al. 2012, Farris 2016, O'Donnell and Boulva 2017, Godfrey and Gronenberg 2019), only one study to date directly compared a social group (the paper wasps, Vespidae, Polistinae) with the most closely related solitary subfamily (the potter wasps, Vespidae, Eumeninae) (O'Donnell et al. 2015). O'Donnell et al. (2015) found the opposite pattern to that predicted by the social brain hypothesis:

the solitary potter wasps had larger MBs than the social paper wasps, although the differences were not statistically significant after controlling for phylogeny because their sample included only one evolutionary origin of sociality. O'Donnell et al. (2015) proposed that the social species benefit from 'distributed cognition' (Zhang and Norman 1994): the spreading of cognitive effort across the group through cooperation and task specialization. O'Donnell et al. (2015) argued that the cognitive benefits of task specialization were present even in small social groups, rather than only larger social insect colonies with more specialized division of labor, and that because early insect societies were family groups, kin selection reduced the conflict associated with sociality. Because neural tissue is expensive (Niven and Laughlin 2008), social insects should be selected to reduce MB size if not necessary due to distributed cognition.

A complicating factor in comparing social and solitary insect MBs is that social interactions themselves, as well as general foraging experience, may affect MB size. *Drosophila* reared in groups had larger MBs than those raised alone (Heisenberg et al. 1995), and *Camponotus* ants reared in isolation had smaller MBs than those left in their nest (Seid and Junge 2016). MBs may also show experience-dependent plasticity, increasing in volume in response to foraging experience (Withers et al. 1993, 1995, 2008; Gronenberg et al. 1996; Farris et al. 2001; Kuhn-Buhlman and Wehner 2006; Maleszka et al. 2009; Molina and O'Donnell 2008; Seid and Wehner 2008; Stieb et al. 2010; Jones et al. 2013; Amador-Vargas et al. 2015; Rehan et al. 2015).

Here, we use an evolutionary loss of sociality to test whether there is a difference in the size of the MB calyces, the area of the brain found to differ between social and solitary paper wasps (O'Donnell et al. 2015), between a social species and its derived solitary relative. We use a social bee, *Augochlorella aurata*, and the closely related, sympatric solitary species from its sister genus (Goncalves 2016) *Augochlora pura*, to test if differences between these taxa are consistent with the contrasting predictions of the social brain or distributed cognition hypotheses. *Augochlora pura* and *Augochlorella aurata* share a common social ancestor, but *A. pura* has lost sociality (Stockhammer 1966, Ordway 1966, Mueller 1996, Danforth and Eickwort 1997, Dalmazzo and Roig-Alsina 2015). We compared the two species at the nest foundress stage in early summer, when they both exhibit solitary behavior because the workers of *A. aurata* have not yet eclosed. At this time, solitary foundresses of both species are foraging to provision their first generation of offspring (Ordway 1966, Mueller 1996). This ensures that any differences in neural investment are not in response to differences in foraging experience or the interactions that an *A. aurata* queen has with her workers. Differences in MB investment should thus reflect species-specific differences rather than adult experience. The social brain hypothesis predicts that the social species, *A. aurata*, should have larger MB calyces than the solitary *A. pura*, whereas the distributed cognition hypothesis predicts that the loss of sociality in *A. pura* will be accompanied by an increase in MB calyx volume. This is the first study to test whether the loss of sociality influences neural investment, and the first to compare neural investment in a social and solitary species before individuals are part of a social group.

Materials and Methods

Life History of Study Species

Augochlorella aurata (= *A. striata*) and *Augochlora pura* are both generalist foragers sympatric in the eastern United States. Females initiate nests in the late spring and early summer after emerging from winter diapause (Ordway 1966, Stockhammer 1966, Mueller

1996). In both species, the foundress females provision first brood offspring. The first brood *A. aurata* daughters remain in the nest as nonreproductive workers (average of four workers; Mueller 1991) with undeveloped ovaries, whereas *A. pura* offspring disperse and initiate their own nests. *Augochlorella aurata* queens do not leave the nest to forage after their workers have emerged; thus, *A. aurata* caught at flowers with enlarged reproductive ovaries represent foundresses whose (worker) offspring have not yet matured (Mueller 1996). Beyond their social behavior, the only notable difference in their ecology is that *A. pura* excavate nests in the rotting wood of fallen logs, whereas *A. aurata* excavate nests in the ground.

Collections

We collected seven female foundresses each of *A. pura* and *A. aurata* foraging on flowers in Montgomery Co. MD, Fairfax Co. VA, and Washington, DC, from 4 to 14 June 2017; one individual of *A. pura* was collected on 29 June 2017, and from 12 April to 15 June 2018. We immediately placed bees into 4% paraformaldehyde in phosphate buffered saline (PBS) and stored them at 4°C until species identification. We measured ovary size because this correlated with MB volume in some previous studies (Molina et al. 2007, Rehan et al. 2015). We dissected away the tergites to photograph the ovaries dorsally at 10× magnification. We measured ovary size by tracing the outline of their photograph using ImageJ software following Smith et al. (2010). We used head width, measured with digital calipers, as a measure of body size.

Brain Analyses

Head capsules were dissected in PBS to remove brains and fixed in 4% PFA. Brains were then placed in a postfix of glutaraldehyde (2%), and dehydrated in a series of ethanol washes. Brains were mounted in methyl salicylate and visualized with autofluorescence following McKenzie et al. (2016) using an Olympus Fluoview FV1000 laser confocal microscope at 10× magnification (Fig. 1). The brain visualization used the 405-nm laser to enhance contrast (seen as blue) and the 488-nm (seen as green) to autofluoresce the glutaraldehyde. Images were optically sectioned at 4.27 μm until the entire brain was imaged in series. The PC-based software Reconstruct was used to quantify volumes of the MB calyces, optic lobes (OL), antennal lobes (AL), and the whole brain by tracing one side of each brain and extrapolating the total volumes for each brain section (Fiala 2005). All traces were done by MAS.

We measured volumes for the whole brain, MB calyces, the OL (including the lamina and medulla), and AL. We calculated ratios for each subregion of the brain relative to the whole brain to compare neural investment. To test whether brain size relative to body size differed between species, we standardized individual whole brain volume to individual 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 volume for each bee to calculate size-corrected whole brain volume (Jaumann et al. 2019). We used an independent samples *t*-test with equal variances not assumed to compare the two species; all variables fit a normal distribution with Shapiro-Wilk test *P*-values >0.05.

Results

Morphological Measurements

Augochlora pura (mean head width \pm SD = 2.26 \pm 0.17 mm) were larger than *A. aurata* (2.04 \pm 0.08 mm; $t_{8,41} = 3.00$, *P* = 0.016; Fig. 2a). Mean ovary size did not differ between species (*A. pura*: 0.59 \pm 0.34

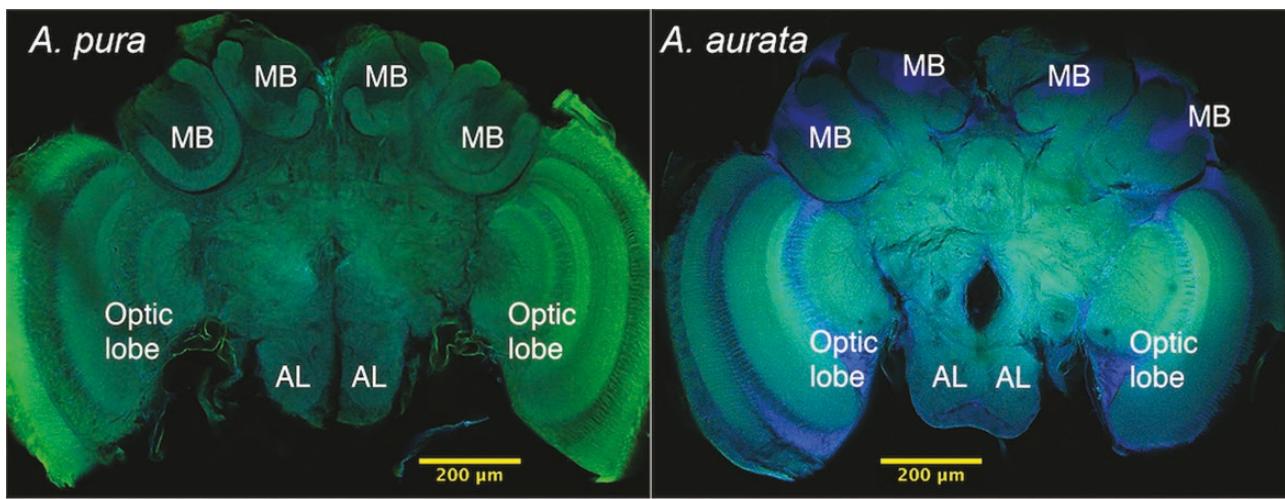


Fig. 1. Confocal image of *Augochlora pura* (left) and *Augochlorella aurata* (right) showing the brain areas used for analyses, the optic lobes, AL and MB calyces. Scale bars = 200 μ m. The brain visualization used the 405 nm laser to enhance contrast (seen as blue) and the 488 nm (seen as green) to autofluoresce the glutaraldehyde.

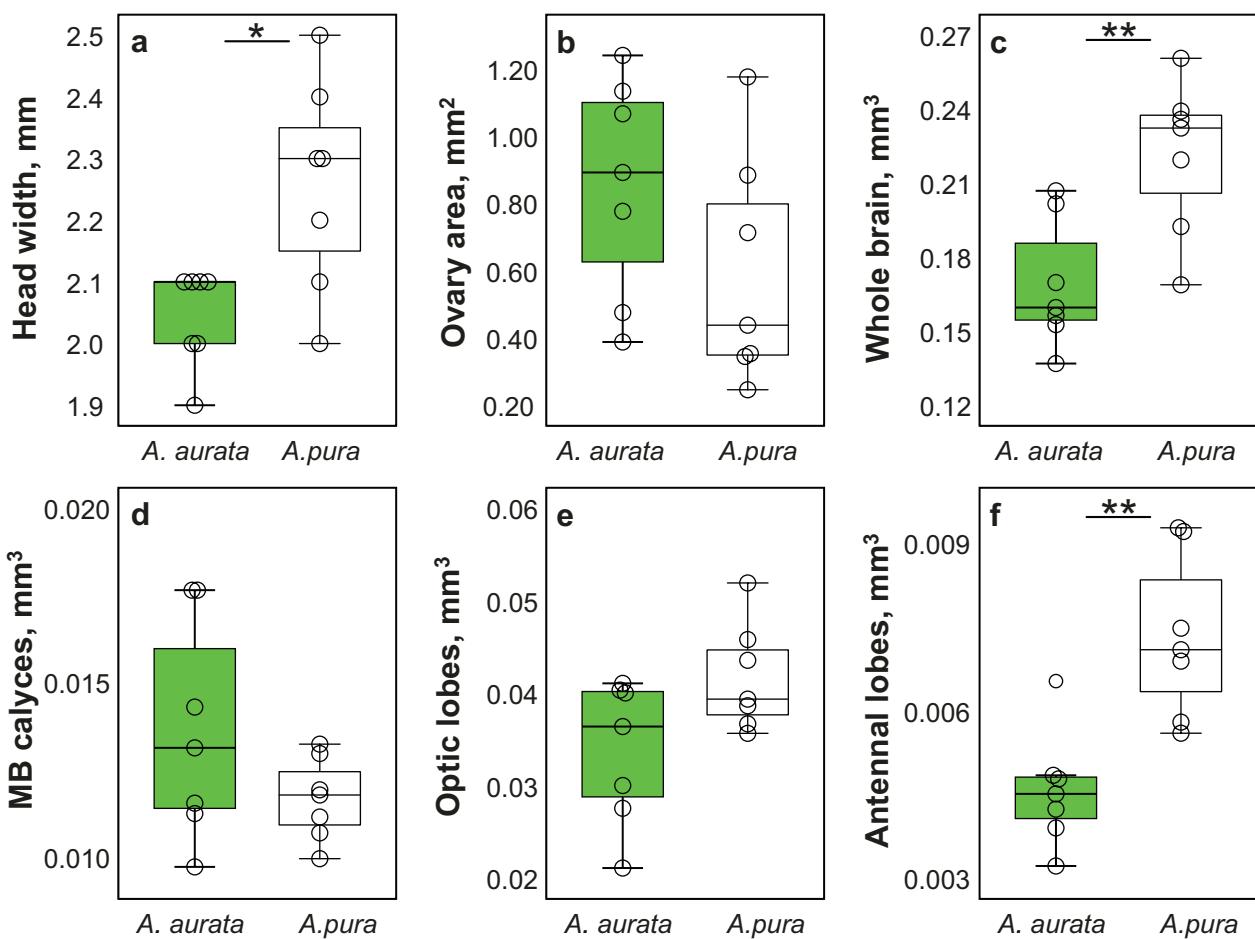


Fig. 2. Morphology data for the bees in our study, including head width (a), ovary area (b), whole brain volume (c), MB calyces volume (d), optic lobes volume, (e) and AL volume (f). Filled boxes represent *Augochlorella aurata*, open boxes *Augochlora pura*. Open circles are individual data points. Upper and lower bounds of boxes are one interquartile range (IQR) above and below the median. Error bars represent data within 1.5 (IQR) of the median. Asterisks indicate statistically significant differences of $P < 0.05$ (*) or $P < 0.01$ (**).

mm^2 , *A. aurata*: $0.85 \pm 0.33 \text{ mm}^2$, $t_{11.97} = 1.45$, $P = 0.172$; Fig. 2b), nor did it correlate with body size either across species ($r = 0.30$, $n = 14$, $P = 0.30$) or within species (*A. aurata*: $r = -0.63$, $n = 7$, $P = 0.13$; *A. pura*: $r = 0.18$, $n = 7$, $P = 0.71$).

Brain Measurements

Without accounting for body size, the larger species, *A. pura*, had larger brains than *A. aurata* (*A. pura*: $0.22 \pm 0.03 \text{ mm}^3$, *A. aurata*: $0.17 \pm 0.03 \text{ mm}^3$, $t_{11.63} = 3.41$, $P = 0.005$; Fig. 2c), although MB calyx volume did not differ (*A. pura*: $0.012 \pm 0.001 \text{ mm}^3$, *A. aurata*: $0.014 \pm 0.003 \text{ mm}^3$, $t_{7.69} = 1.53$, $P = 0.165$; Fig. 2d). Both AL and OL were larger in *A. pura*, but the difference in OL was not significant (AL: *A. pura*: $0.007 \pm 0.001 \text{ mm}^3$, *A. aurata*: $0.005 \pm 0.001 \text{ mm}^3$, $t_{10.74} = 4.07$, $P = 0.002$; OL: *A. pura*: $0.042 \pm 0.005 \text{ mm}^3$, *A. aurata*: $0.034 \pm 0.008 \text{ mm}^3$, $t_{11.14} = 2.163$, $P = 0.053$; Fig. 2e and f).

Body size-corrected whole brain volume was larger in the solitary *A. pura* than the social *A. aurata*, but not significantly so (*A. pura*: $0.206 \pm 0.04 \text{ mm}^3$, *A. aurata*: $0.172 \pm 0.024 \text{ mm}^3$, $t_{9.90} = 1.92$, $P = 0.084$; Fig. 3d). *Augochlorella aurata* showed more investment in MB calyces than *A. pura* (*A. aurata* MB calyces:whole brain ratio = 0.080 ± 0.008 , *A. pura* = 0.053 ± 0.006 ; $t_{11.14} = 6.88$, $P < 0.001$; Fig. 3a). All of the *A. aurata* individuals had larger MB calyces,

relative to whole brain volume, than all of the *A. pura* individuals. The ratio of AL: whole brain volume did not differ between species (*A. aurata* = 0.028 ± 0.008 , *A. pura* = 0.033 ± 0.005 ; $t_{10.93} = 1.62$, $P = 0.133$; Fig. 3b). Nor did the ratio of OL: whole brain volume differ between species (*A. aurata* = 0.204 ± 0.056 , *A. pura* = 0.189 ± 0.019 ; $t_{7.34} = 1.62$, $P = 0.538$; Fig. 3c). Neither body size nor ovary size correlated with any of the neuropil: whole brain ratios in either of the two species.

Discussion

Here we show that a social species, *A. aurata*, has larger MB calyces relative to brain size than the closely related *A. aurata*, which has lost sociality. This is consistent with predictions of the social brain hypothesis. These brain differences do not result from differences in social experience, because all individuals were solitary at the time they were collected, which suggests that the MB calyx size differences reflect species-specific patterns of neural investment. This is consistent with previous studies of socially polymorphic bees (those that can nest socially and solitarily) which also found smaller relative MB calyx size in solitary reproductives relative to social queens, but these did not control for potential effects of social interactions

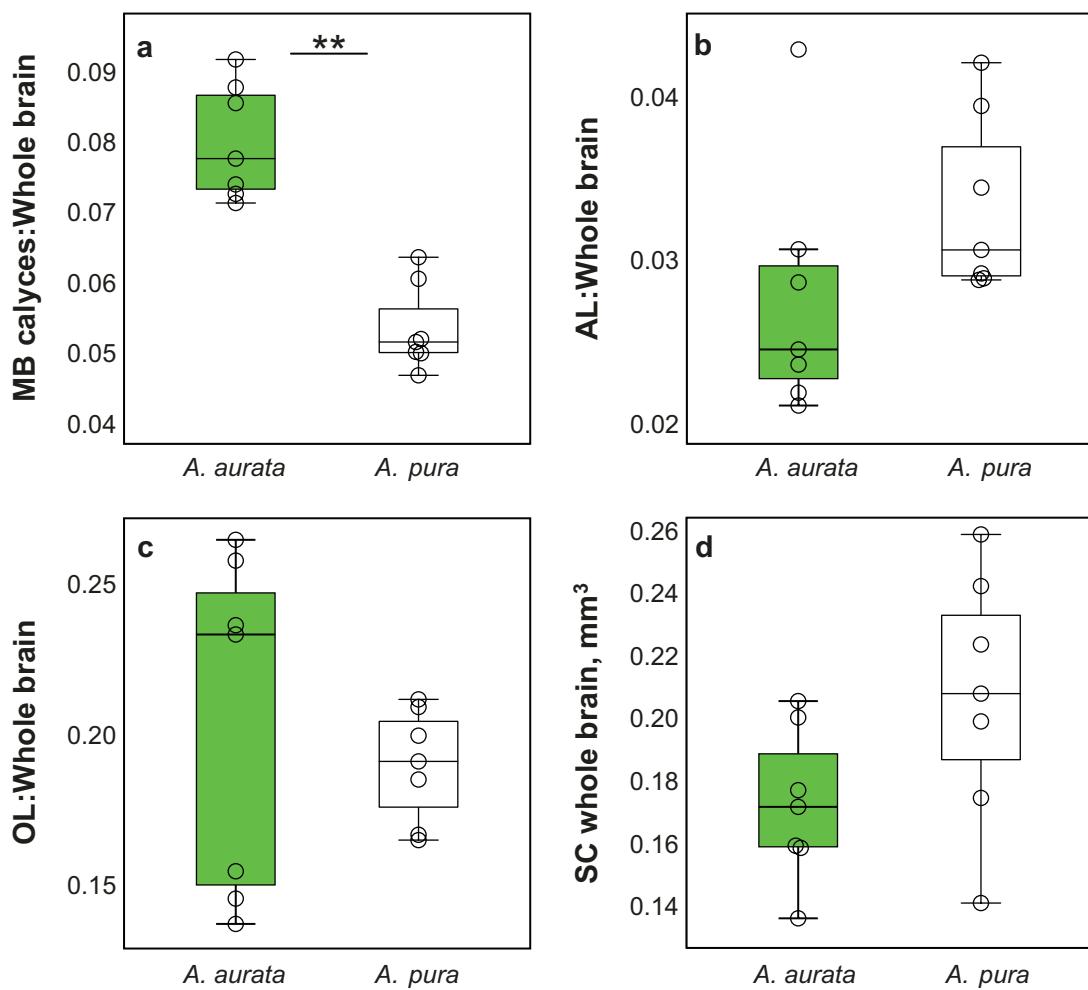


Fig. 3. Ratios of MB calyces to whole brain volume (a), AL to whole brain volume (b), and OL to whole brain volume (c) for the social species *Augochlorella aurata* (filled boxes) and the solitary species *Augochlora pura* (open boxes). Panel (d) shows body size-corrected (SC) whole brain volume for each species. Open circles are individual data points. Upper and lower bounds of boxes are one interquartile range (IQR) above and below the median. Error bars represent data within 1.5(IQR) of the median. Asterisks (**) indicate statistically significant differences of $P < 0.01$.

on MB size (Smith et al. 2010, Rehan et al. 2015; but see Jaumann et al. 2019).

Our MB calyx volume results show the opposite trend as the only other study to directly compare social species with closely related solitary species (O'Donnell et al. 2015). This may be due to the small size of *A. aurata* colonies, as O'Donnell et al. (2015) studied wasps with colonies of 20–4,000 workers, which even at the lower end is substantially larger than a typical *A. aurata* colony comprised of a queen and approximately four workers (Mueller et al. 1991). Perhaps the benefits of distributed cognition do not emerge until colony size is larger. Also, both our study and O'Donnell et al. (2015) examined only a single evolutionary transition between solitary and social behavior. Comparisons of multiple transitions are required before it will be clear if there is a general pattern of greater or less MB investment in social species. Halictid bees show multiple evolutionary gains and especially losses of sociality (Wcislo and Danforth 1997; Danforth et al. 1999, 2002; Danforth 2003; Gibbs et al. 2012) among species that are otherwise generally ecologically similar. Further studies of MB size across these multiple transitions can reveal whether social behavior is generally associated with increased or decreased neural investment in MBs or if differences result from lineage-specific factors unrelated to social behavior.

Acknowledgments

Christopher Day and Stephanie Keer assisted with confocal microscopy. This work was supported by National Science Foundation grant #17-1028536545 to A. R. S. and M. A. S. S. P. was supported by the Harlan Family Foundation and the Washington Biologists Field Club.

References Cited

- Amador-Vargas, S., W. Gronenberg, W. T. Wcislo, and U. Mueller. 2015. Specialization and group size: brain and behavioural correlates of colony size in ants lacking morphological castes. *Proc. Biol. Sci.* 282: 20142502.
- Dalmazzo, M., and A. Roig-Alsina. 2015. Social biology of Augochlora (Augochlora) phoenomenon (Hymenoptera, Halictidae) reared in laboratory nests. *Insectes Soc.* 62: 315–323.
- Danforth, B. N. 2002. Evolution of sociality in a primitively eusocial lineage of bees. *Proc. Natl. Acad. Sci. U.S.A.* 99: 286–290.
- Danforth, B. N., and G. C. Eickwort. 1997. The evolution of social behavior in the Augochlorinae sweat bees (Hymenoptera: Halictidae) based on a phylogenetic analysis of the genera, pp. 270–292. In J. C. Choe and B. J. Crespi (eds.), *Social behavior in insects and arachnids*. Cambridge University Press, Cambridge, United Kingdom.
- Danforth, B. N., H. Sauquet, and L. Packer. 1999. Phylogeny of the bee genus *Halictus* (Hymenoptera: Halictidae) based on parsimony and likelihood analyses of nuclear EF-1alpha sequence data. *Mol. Phylogen. Evol.* 13: 605–618.
- Danforth, B. N., L. Conway, and S. Ji. 2003. Phylogeny of eusocial *Lasioglossum* reveals multiple losses of eusociality within a primitively eusocial clade of bees (Hymenoptera: Halictidae). *Syst. Biol.* 52: 23–36.
- DeCasien, A. R., S. A. Williams, and J. P. Higham. 2017. Primate brain size is predicted by diet but not sociality. *Nat. Ecol. Evol.* 1: 0112.
- Dunbar, R. I. 1992. Neocortex size as a constraint on group size in primates. *J. Hum. Evol.* 22: 469–493.
- Dunbar, R. I. 2009. The social brain hypothesis and its implications for social evolution. *Ann. Hum. Biol.* 36: 562–572.
- Dunbar, R. I., and S. Shultz. 2007. Understanding primate brain evolution. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 362: 649–658.
- Fahrbach, S. E. 2006. Structure of the mushroom bodies of the insect brain. *Annu. Rev. Entomol.* 51: 209–232.
- Farris, S. M. 2016. Insect societies and the social brain. *Curr. Opin. Insect Sci.* 15: 1–8.
- Farris, S. M., and S. Schulmeister. 2011. Parasitoidism, not sociality, is associated with the evolution of elaborate mushroom bodies in the brains of hymenopteran insects. *Proc. Biol. Sci.* 278: 940–951.
- Farris, S. M., G. E. Robinson, and S. E. Fahrbach. 2001. Experience- and age-related outgrowth of intrinsic neurons in the mushroom bodies of the adult worker honeybee. *J. Neurosci.* 21: 6395–6404.
- Fiala, J. C. 2005. Reconstruct: a free editor for serial section microscopy. *J. Microsc.* 218: 52–61.
- Gibbs, J., S. G. Brady, K. Kanda, and B. N. Danforth. 2012. Phylogeny of halictine bees supports a shared origin of eusociality for *Halictus* and *Lasioglossum* (Apoidea: Anthophila: Halictidae). *Mol. Phylogen. Evol.* 65: 926–939.
- Godfrey, R. K., and W. Gronenberg. 2019. Brain evolution in social insects: advocating for the comparative approach. *J. Compar. Physiol. A* 205: 13–32.
- Goncalves, R. B. 2016. A molecular and morphological phylogeny of the extant Augochlorini (Hymenoptera, Apoidea) with comments on implications for biogeography. *Syst. Entomol.* 41: 430–440.
- Gronenberg, W., and A. J. Riveros. 2009. Social brains and behavior: past and present. *Organization of insect societies: from genome to sociocomplexity*. Harvard University Press, Cambridge, MA, pp. 377–401.
- Gronenberg, W., S. Heeren, and B. Hölldobler. 1996. Age-dependent and task-related morphological changes in the brain and the mushroom bodies of the ant *Camponotus floridanus*. *J. Exp. Biol.* 199: 2011–2019.
- Heisenberg, M., M. Heusipp, and C. Wanke. 1995. Structural plasticity in the *Drosophila* brain. *J. Neurosci.* 15: 1951–1960.
- Jaumann, S., M. A. Seid, M. Simons, and A. R. Smith. 2019. Queen dominance may reduce worker mushroom body size in a social bee. *Dev. Neurobiol.* 79: 596–607.
- Jones, B. M., A. S. Leonard, D. R. Papaj, and W. Gronenberg. 2013. Plasticity of the worker bumblebee brain in relation to age and rearing environment. *Brain. Behav. Evol.* 82: 250–261.
- Kühn-Bühlmann, S., and R. Wehner. 2006. Age-dependent and task-related volume changes in the mushroom bodies of visually guided desert ants, *Cataglyphis bicolor*. *J. Neurobiol.* 66: 511–521.
- Kverková, K., T. Běliková, S. Olkowicz, Z. Pavelková, M. J. O'Riain, R. Šumbera, H. Burda, N. C. Bennett, and P. Němc. 2018. Sociality does not drive the evolution of large brains in eusocial African mole-rats. *Sci. Rep.* 8: 9203.
- Lihoreau, M., T. Latty, and L. Chittka. 2012. An exploration of the social brain hypothesis in insects. *Front. Physiol.* 3: 442.
- Maleszka, J., A. B. Barron, P. G. Hellwell, and R. Maleszka. 2009. Effect of age, behaviour and social environment on honey bee brain plasticity. *J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol.* 195: 733–740.
- McKenzie, S. K., I. Fetter-Pruneda, V. Ruta, and D. J. Kronauer. 2016. Transcriptomics and neuroanatomy of the clonal raider ant implicate an expanded clade of odorant receptors in chemical communication. *Proc. Natl. Acad. Sci. U.S.A.* 113: 14091–14096.
- Molina, Y., and S. O'Donnell. 2007. Mushroom body volume is related to social aggression and ovary development in the paperwasp *Polistes instabilis*. *Brain. Behav. Evol.* 70: 137–144.
- Molina, Y., and S. O'Donnell. 2008. Age, sex, and dominance-related mushroom body plasticity in the paperwasp *Mischocyttarus mastigophorus*. *Dev. Neurobiol.* 68: 950–959.
- Mueller, U. G. 1991. Haplodiploidy and the evolution of facultative sex ratios in a primitively eusocial bee. *Science*. 254: 442–444.
- Mueller, U. G. 1996. Life history and social evolution of the primitively eusocial bee *Augochlorella striata* (Hymenoptera: Halictidae). *J. Kans. Entomol. Soc.* 69: 116–138.
- Niven, J. E., and S. B. Laughlin. 2008. Energy limitation as a selective pressure on the evolution of sensory systems. *J. Exp. Biol.* 211: 1792–1804.
- O'Donnell, S., and S. Bulova. 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., N. Donlan, and T. Jones. 2007. Developmental and dominance-associated differences in mushroom body structure in the paper wasp *Mischocyttarus mastigophorus*. *Dev. Neurobiol.* 67: 39–46.

- O'Donnell, S., S. J. Bulova, S. DeLeon, P. Khodak, S. Miller, and E. Sulger. 2015. Distributed cognition and social brains: reductions in mushroom body investment accompanied the origins of sociality in wasps (Hymenoptera: Vespidae). *Proc. Biol. Sci.* 282: 10.1098/rspb.2015.0791.
- O'Donnell, S., S. J. Bulova, S. DeLeon, M. Barrett, and K. Fiocca. 2017. Caste differences in the mushroom bodies of swarm-founding paper wasps: implications for brain plasticity and brain evolution (Vespidae, Epiponini). *Behav. Ecol. Sociobiol.* 71: 116.
- Ordway, E. 1966. The bionomics of *Augochlorella striata* and *A. persimilis* in eastern Kansas (Hymenoptera: Halictidae). *J. Kans. Entomol. Soc.* 39: 270–313.
- Pahlke, S., S. Jaumann, M. A. Seid, and A. R. Smith. 2019. Brain differences between social castes precede group formation in a primitively eusocial bee. *Naturwissenschaften*. 106: 49.
- Rehan, S. M., S. J. Bulova, and S. O'Donnell. 2015. Cumulative Effects of Foraging Behavior and Social Dominance on Brain Development in a Facultatively Social Bee (*Ceratina australensis*). *Brain. Behav. Evol.* 85: 117–124.
- Riveros, A. J., M. A. Seid, and W. T. Wcislo. 2012. Evolution of brain size in class-based societies of fungus-growing ants (Attini). *Anim. Behav.* 83: 1043–1049.
- Seid, M. A., and E. Junge. 2016. Social isolation and brain development in the ant *Camponotus floridanus*. *Sci. Nat.* 103: 1–6.
- Seid, M. A., and R. Wehner. 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*. *J. Comp. Neurol.* 507: 1102–1108.
- Smith, A. R., M. A. Seid, L. C. Jiménez, and W. T. Wcislo. 2010. Socially induced brain development in a facultatively eusocial sweat bee *Megalopta genalis* (Halictidae). *Proc. Biol. Sci.* 277: 2157–2163.
- Stieb, S. M., T. S. Muenz, R. Wehner, and W. Rössler. 2010. Visual experience and age affect synaptic organization in the mushroom bodies of the desert ant *Cataglyphis fortis*. *Dev. Neurobiol.* 70: 408–423.
- Stockhammer, K. A. 1966. Nesting habits and life cycle of a sweat bee, *Augochlora pura* (Hymenoptera: Halictidae). *J. Kans. Entomol. Soc.* 39: 157–192.
- Withers, G. S., N. F. Day, E. F. Talbot, H. E. Dobson, and C. S. Wallace. 2008. Experience-dependent plasticity in the mushroom bodies of the solitary bee *Osmia lignaria*. *Devol. Neurobiol.* 73–82.
- Withers, G. S., S. E. Fahrbach, and G. E. Robinson. 1993. Selective neuroanatomical plasticity and division of labour in the honeybee. *Nature*. 364: 68: 238–240.
- Withers, G. S., S. E. Fahrbach, and G. E. Robinson. 1995. Effects of experience and juvenile hormone on the organization of the mushroom bodies of honey bees. *J. Neurobiol.* 26: 130–144.
- Zhang, J., and D. A. Norman. 1994. Representations in distributed cognitive tasks. *Cogn. Sci.* 18: 87–122.