

Inter and Intraspecific Differences in Neural Investment in Social and Solitary Sweat Bees

by Sarah Pahlke

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Adam Smith
Assistant Professor of Biology

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Abstract

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The social brain hypothesis predicts that the cognitive demands that accompany the evolution of social behavior will be met with corresponding changes in neural investment. In Chapter 1, we use a bee species that has lost social behavior to test for an accompanying loss in neural investment in cognition. We compared relative brain investment in a social (*Augochlorella aurata*) and a closely related, derived solitary species (*Augochlora pura*). We compared females at the nest foundress stage so that brain development would not be influenced by social interactions. In support of the social brain hypothesis, our data show that the loss of sociality was accompanied by a 32.9% reduction in relative neural investment in the mushroom bodies, an area of the insect brain responsible for sensory integration and learning. However, the solitary species had a larger whole brain size relative to body size that was not explained by increases in other sensory neuropil. This is the first study to demonstrate differences in mosaic brain evolution between social and solitary species.

At the intraspecific level, differences in neural investment have been observed between different castes of social bees, with MBs being larger in queens than workers. This suggests a strategy of increased cognitive investment in individuals who will need to exercise social dominance, consistent with the social brain hypothesis. However, larger MBs in queens may be a plastic response to social interactions in the nest. In Chapter 2, we show that nest foundresses—the reproductive females who will become queens but are solitary until their first workers are born—have MBs that are 15.8% larger and

antennal lobes that were 28.3% larger relative to whole brain than workers in the primitively eusocial sweat bee *Augochlorella aurata*. Whole brain size, body size, and optic lobe size did not differ between the two groups, however. This suggests that increased cognitive investment is part of the broader queen phenotype, supporting the social brain hypothesis. Larger MBs among foundresses may reflect the increased larval nutrition provisioned to reproductive females to enable diapause and/or an absence of social aggression from a dominant queen upon adult emergence.

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Chapter 1: Loss of sociality is accompanied by loss of neural investment in cognition: support for the social brain hypothesis

Introduction

The social brain hypothesis predicts that social behavior imposes additional cognitive challenges on animals not present in their solitary relatives, and that as a result, the evolution of sociality should lead to the evolution of increased investment in brain tissue (Dunbar 2009). Insects, especially halictid bees, offer an excellent opportunity to test the social brain hypothesis because they have multiple evolutionary gains and losses of sociality and a wide range of group sizes (reviewed in [Schwarz et al. 2007, Feinerman & Traniello 2016, O'Donnell & Bolova 2017]).

Brains evolve to match the selective challenges in an animal's environment, but due to the numerous physiological processes involved in neural circuits (transduction, synaptic transmission, and electric signal transmission), neural tissue is costly. Animals should therefore only increase neural investment when needed (Niven & Laughlin 2008). Moreover, the brain is compartmentalized into discrete anatomical regions with different cognitive functions (Barton & Harvey 2000), thus increases in neural investment are predicted to occur in specific neuropil to meet the increased demand.

In insects, the brain areas predicted to respond to selection for increased cognitive capacity are the mushroom bodies (MBs). MBs are paired neuropils associated with learning, memory, sensory integration, and social behavior (Farbach 2006). MBs also show neural plasticity in response to adult experience. This has been shown in honeybees (Szyszka 2008), bumblebees (Riveros & Gronenberg 2010), drosophila (Heisenberg et al.

1995), ants (Milder et al. 2017, Seid & Junge 2016), and butterflies (Montgomery et al. 2015, Dijk et al. 2017) . Thus, social interactions themselves may affect MB morphology. For instance, *Drosophila melanogaster* kept in communal groups had larger MBs than those kept in isolation, and ants kept in isolation had smaller MBs than their social nestmates (Heisenberg et al. 1995, Seid & Junge 2016).

Previous studies have shown relationships between MB development and social structure in bees and wasps (Smith et al. 2019, Rehan et al. 2015, O'Donnell & Bulova 2017). However, the only study that has directly compared related social and solitary species found the opposite relationship as predicted: social wasps had *smaller* MBs than their solitary relatives. This suggested that social species could alternatively benefit from 'distributed cognition', or the spreading of cognitive capacity across the group through cooperation and task specialization (O'Donnell et al. 2015); under this hypothesis, such specialization would result in reduced constitutive investment in brain tissue compared to non-social taxa, where individuals must engage in a wider array of tasks.

Here we test the contrasting predictions of the social brain and distributed cognition hypotheses by measuring MB size in a social bee, *Augochlorella aurata*, and the closely related solitary species *Augochlora pura*. The genera *Augochlora* and *Augochlorella* share a common ancestor that was social, but *Augochlora. pura* has lost sociality (Danforth & Eickwort 1997, Gonclaves 2016). We compared both species at the nest foundress stage in early summer, when they are solitary because the workers of *A. aurata* are not yet born (Mueller 1996). This ensures that any differences in neural investment are not in response to the interactions that an *A. aurata* queen has with her worker daughters. Differences in MB investment should thus reflect evolved species-

specific differences rather than differences in adult experience. This study allows us to test whether the loss of sociality is associated with changes in neural investment, and to our knowledge, is the first study to compare neural investment in a social and solitary species before individuals are part of a social group.

Methods

Augochlorella aurata (= *A. striata*) and *Augochlora pura* (Halictidae, Augochlorini) are generalist foragers sympatric in the eastern United States (Figure 1). Females initiate nests in the late spring and early summer (Mueller 1996, Stockhammer 1966). The first brood of 7-9 *A. aurata* offspring remain in the nest as non-reproductive workers with undeveloped ovaries, whereas *A. pura* offspring disperse and initiate their own nests (Stockhammer 1966). *A. aurata* queens do not leave the nest to forage after their workers are born, thus *A. aurata* caught at flowers with enlarged reproductive ovaries represent foundresses whose first (worker) offspring have not yet matured (Mueller 1996).

We collected female foundresses of *A. pura* and *A. aurata* foraging on flowers in Montgomery Co. MD, Fairfax Co. VA, and Washington, D.C., USA, from 4-14 June, 2017; one individual of *A. pura* was collected on 29 June. We immediately placed bees into 4% paraformaldehyde in phosphate buffered saline (PBS) and stored them at 4°C until species identification. We dissected away the tergites to photograph the ovaries dorsally at 10x magnification. We measured ovary size by tracing the outline of each

photograph using ImageJ software following Smith et al. (2010). As an allometric measure of body size, we used thorax width, measured with calipers (Cane 1987).

Heads were dissected in PBS to remove brains and fixed in 4% PFA. Brains were then placed in a post-fix 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 10x magnification. Images were optically sectioned at 4.27 μ m until the entire brain was imaged in series. The PC-based software Reconstruct was used to quantify volumes of the mushroom body calyces, optic lobes, antennal lobes and the whole brain by tracing one side of each brain and extrapolating the total volumes for each brain section (Figure 2) (Fiala 2005).

We corrected brain size for body size variation by calculating an average head width for all the bees in the study and dividing this average by the actual head width size for each bee to create a size correction factor which was then multiplied by the volumes measured in Reconstruct. Ratios for each sub region of the brain relative to the whole brain were calculated to compare neural investment. We used a Welch's two sample t-test for difference in mean proportions to compare the two species.

Results

Analysis of ovaries showed that none of the *A. aurata* females collected were workers. There was no correlation between body size and MB:Whole brain ratio ($p = -$

0.116, $r = -0.60$). Both species were similarly sized: *A. pura* mean head width = 2.15 mm \pm 0.13 SD, *A. aurata* mean head width = 2.00 mm \pm 0.08.

The solitary *A. pura* showed decreased MB investment relative to the social *A. aurata*. MB calyx : total brain volume ratio for solitary *A. pura* was 32.9% smaller than that of social *A. aurata* ($n = 8$; 4 of each species, $t = 4.63$, $df = 4.52$, $p = 0.007$; Figure 3a). All *A. aurata* individuals had larger MB calyx : total brain volume ratios than all *A. pura* individuals.

However, while they invested proportionally less neural tissue in MBs, the size-corrected whole brains of solitary *A. pura* were larger than those of *A. aurata* ($t = 4.67$, $df = 3.75$, $p = 0.01$; Figure 3b). This was not explained by proportionally larger investments in the primary sensory neuropils, the optic lobes and antennal lobes. In fact, *A. pura* optic lobes were significantly smaller than those of *A. aurata* (antennal lobes: $t = 0.69$, $df = 6$, $p = 0.52$; optic lobes: $t = 4.79$, $df = 6$, $p = 0.003$; Figure 3c).

Discussion

Here we show that two closely related bee species that differ in the presence of sociality also differ in MB investment. We show that these brain differences do not result from differences in social experience, suggesting that they reflect species-specific patterns of neural investment which may be due to any number of uncontrolled differences in the biology and evolutionary history of these two species. This is consistent with previous studies of socially polymorphic bees (those that can nest socially and solitarily) which also found smaller relative MB size in solitary reproductives relative to

social queens, but these did not control for potential effects of social interactions on MB size (Smith et al. 2010, Rehan et al. 2015).

However, the patterns we document are more complicated than we predicted. The solitary *A. pura* had larger brains relative to body size than the social *A. aurata*, but this difference was not explained by greater investment in the other sensory neuropil. Relative to total brain size, *A. pura* antennal lobes were equal to, and optic lobes were smaller than in the social *A. aurata*. Previous studies of social wasps found no differences in antennal or optic lobes related to social behavior (O'Donnell & Bulova 2017), although in some ant groups antennal lobe volume tracks colony size (Riveros et al. 2012). Differences in visual ecology can lead to differential investment in sensory neuropils (e.g. [Stöckl et al. 2016]) but we know of no such differences between *A. aurata* and *A. pura*. Both are similarly sized, diurnal, generalist foragers with widely overlapping distributions (Mueller 1996, Stockhammer 1966)

Our results contradict the only other study to directly compare related social and solitary species (O'Donnell et al 2015). This may be due to the small size of *A. aurata* colonies; O'Donnell et al. (2015) studied wasps with colonies of 20-4000 workers. Perhaps the benefits of distributed cognition do not emerge until colony size is larger. Halictid bees show multiple evolutionary gains and losses of sociality (Danforth & Eickwort 1997, Gibbs et al. 2012), and further studies of MB size across these multiple transitions can reveal whether social behavior is generally associated with increased or decreased neural investment.

Chapter 2: Queen-worker brain differences precede social group formation in the primitively eusocial bee *Augochlorella aurata*

Introduction

Reproductive division of labor is one of the defining characteristics of eusociality: queens reproduce and their sterile worker daughters gain indirect fitness by helping to provision the queen's offspring, who are their sisters (Michener 1974). Physiological differences between queens and workers also extend to the brain (Molina and O'Donnell 2007, 2008, O'Donnell et al. 2008, Smith et al. 2010, Rehan et al. 2015, reviewed in O'Donnell et al. 2017). Because neural tissue is costly, animals' investment in brain tissue should reflect increased demands for the functions of that brain area (Barton et al. 1995, Niven and Laughlin 2008). In insects, a brain area responsible for sensory integration, learning, and memory is the mushroom body (MB; Farbach 2006). In the social bees and wasps studied to date, queens have larger MBs than workers, which may reflect increased investment to meet the cognitive challenge of maintaining social dominance (Rehan et al. 2015, O'Donnell et al. 2017). If so, this would support the social brain hypothesis, which posits that the cognitive demands of social interactions select for increased brain size (Dunbar 2009). However current data cannot distinguish whether queens invest more in MB tissue *before* establishing a social colony, or if increased MB size is a plastic response to subsequent social interactions.

There is no neurogenesis after eclosion in Hymenoptera. It has been shown that changes in MB volume result from axonal pruning of the projection neurons that

commensurate with outgrowth of dendrites of the Kenyon cells. This process is responsible for the enlargement of the MB calyces that is associated with aging and experience (Farris et al. 2001).

Adult foraging experience and social interactions can lead to enlargement of the MBs (Gronenberg et al. 1996, Withers et al. 2007, Heisenberg et al. 1995, Rehan et al. 2015, Seid & Junge 2016). Age may also play a role. In some, but not all, species of bees, ants, and wasps studied, MBs continued enlarging throughout adult life (Withers et al. 1993, Withers et al. 2007, Molina and O'Donnell 2008, Seid and Junge 2016). Thus, queen-worker MB differences may reflect queens' increased age and experience rather than social status *per se*. In one previously studied species, the bee *Ceratina australensis*, social groups are comprised of same-age sister pairs, rather than mother-daughter associations. Dominant females had larger MBs than subordinate ones, although it is not clear if size differences preceded or resulted from social dominance interactions (Rehan et al. 2015).

Here we compare early season foundresses of a primitively eusocial bee, *Augochlorella aurata*, with late season workers. A foundress is a solitary female who builds a nest and forages for her first generation of daughters. When these daughters mature, they become sterile workers, taking over foraging duties. The foundress, now a queen, then remains in the nest and produces more offspring to be provisioned by the foraging efforts of her subordinate workers (Michener 1974, Mueller 1996). Thus, a foundress and worker both have similar foraging experience. Indeed, a study of brain gene expression in paper wasps (*Polistes metricus*) showed that foundresses and workers

were more similar to each other than to queens or newly emerged females (Toth et al. 2007).

We predict that if the queen-worker differences of previous studies result from accumulated experience, there should be no differences between worker and foundress MBs because both are engaged in similar foraging and provisioning behavior. If social interactions *per se* influence MB development, then workers, who are part of a social group, should have larger MBs than solitary foundresses. If queen-worker MB differences reflect a strategy of increased cognitive investment in individuals who will need to exercise social dominance, then foundresses should have larger MBs than workers.

A confounding factor in our analysis is that while foundresses and workers each have similar foraging experience, foundresses are much older, having overwintered underground as diapause adults from the previous summer. However, previous studies of overwintering bees showed no increase in MB development (Fahrbach et al. 2003), although the effect of diapause has never been explicitly tested (Withers et al. 2007). This study allows us to compare the brain morphology of foundresses, rather than active queens, to workers.

Methods

Augochlorella aurata (= *striata*) (Halictidae) is a generalist bee common in eastern North America. Reproductive females emerge, mated, from diapause in late spring and early summer. Worker daughters (7-9 individuals) emerge in mid-summer.

Thus, females captured foraging at flowers early in the season with developed ovaries are foundresses, as queens with a worker brood would not be found outside their nest.

Females captured foraging at flowers with undeveloped ovaries later in the summer are workers. (Mueller 1996)

We collected *A. aurata* females foraging at flowers near the Potomac River in Montgomery Co., MD and Washington, D.C., USA between 4-14 June, 2017 and 12 April- 19 August, 2018. Foundresses were collected between 12 April and 15 June and workers were collected between 27 July and 19 August. Bees were preserved in 4% paraformaldehyde in phosphate buffered saline (PBS) and stored at 4°C. We dissected the abdomens to observe ovaries and measured thorax width with digital calipers as an allometric measure of body size (Cane 1987). Ovaries were assigned scores following Michener (1974): 5-both ovaries plump and opaque with nearly-developed eggs, 4-one ovary plump and opaque, 3-both ovaries containing moderately developed eggs and somewhat opaque, 2-both ovaries translucent, containing barely developed eggs, 1-both ovaries slender and translucent without developing eggs visible.

We dissected heads in PBS to remove the brain which was immediately placed in glutaraldehyde (2%) for 48 hours, bleached in a formamide solution, and dehydrated in a series of ethanol washes. 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 4.27 μ m (Figure 4). Volumes of the brain and different neuropil (MBs, optic lobes (OL), and antennal lobes (AL)) were calculated using the software program Reconstruct (Fiala 2005).

Brain size was corrected for variation in body size by taking the mean thorax width for all bees and dividing this number by the individual thorax measurements to create a correction factor for each bee. Each of the volume measurements calculated in Reconstruct were then multiplied by this correction factor. Ratios for each neuropil: whole brain were calculated for each individual. Bees were divided into foundresses and workers based on the date of collection, confirmed by ovary dissections. We compared the ratios between groups using a Welch's two sample t-test for proportions. We used non-parametric statistics to compare ovary scores, and a generalized linear model to test for an effect of ovary development independent of caste.

Results

Body size was similar between the two groups ($n=16$; 8 for each group, $t=0.86$, $df=11.30$, $p=0.41$; foundress thorax width mean = $2.23 \text{ mm} \pm 0.10 \text{ SD}$, worker = $2.16 \text{ mm} \pm 0.18$). All early season bees (foundresses) had reproductive ovaries (scores 3-5) except for one individual collected 12 April with undeveloped ovaries (score = 1). All later season bees (workers) had undeveloped ovaries (scores 1-2) except for one individual collected 3 August with developed ovaries (score = 4). Ovary development score significantly differed between foundresses (4.13 ± 1.46) and workers (1.75 ± 1.04 ; Mann-Whitney $W = 7$, $p = 0.008$).

Whole brain size was similar between foundresses and workers ($n=16$; 8 for each group, $t=-0.45$, $p=0.66$; Figure 5a). Foundresses of *A. aurata* had a MB:whole brain that was 15.8% larger than that of workers ($n=16$; 8 for each group, $t=1.78$, $df=12$, $p = 0.0007$;

Figure 5b). There was no correlation between body size and MB:Whole brain ratio ($p = 0.52$, $r = 0.18$). There was no effect of ovary development on MB size after accounting for caste (foundress or worker) in a generalized linear model (caste LR $\chi^2 = 7.22$, $df = 1$, $p = 0.007$; ovary LR $\chi^2 = 0.40$, $df = 1$, $p = 0.53$). The foundress collected with undeveloped ovaries did not have exceptionally small MBs (rank = 8, out of 16), nor did the worker with developed ovaries have exceptionally large MBs (rank = 16 out of 16). Foundresses showed a 28.3% increase in antennal lobe investment as well (AL:Whole brain, $n=16$; 8 for each group, $t=3.50$, $p=0.008$; Figure 5c). There was no difference in OL:Whole brain between foundresses and workers ($n=16$; 8 for each group, $t=1.15$, $p=0.27$; Figure 5d)

Discussion

Our results show that foundresses of *A. aurata* invest more neural tissue in MBs compared to workers. Similar queen-worker differences in MB size have been shown in other bees and wasps, but in all previous cases, queens and workers were collected from existing social groups (reviewed in O'Donnell et al. 2017). However, in our study, foundresses were collected prior to worker emergence. This means that those individuals had highly reduced social interactions influencing their MB development. This suggests that queens invest more in MB development as part of the general queen reproductive phenotype, rather than in response to social interactions in their colony.

It is not clear how to interpret our antennal lobe results. O'Donnell et al. (2014) found increased AL:OL ratios in paper wasp queens relative to workers—consistent with

our findings—but this was driven by differences in OL volume, which is not consistent with our data (Figure 2). Perhaps queens need increased chemosensory ability in order to keep track of nestmates and maintain dominance in addition to the demands of foraging, but this remains to be tested.

One foundress had undeveloped ovaries. She may have recently emerged from diapause and not yet enlarged her ovaries. One worker had developed ovaries. She may have been a replacement queen from a colony with a weak or deceased foundress queen (Mueller 1996). Excluding these individuals from our comparisons did not alter our conclusions.

We assume that foraging experience is approximately equal for each group (they each provision one brood of offspring), but we have not quantified this potentially important difference. If foundresses had more foraging experience than the workers, this may have contributed to the observed differences in neuropil size. Also, queens were much older than the foragers, because they had experienced diapause. No study has tested whether diapause time affects MB size (Withers et al. 2007), although overwintered honeybee workers showed no MB size differences relative to younger nestmates with similar foraging experience (Fahrbach et al. 2003).

The developmental history of foundresses and workers is quite different. Although we do not know the details for *A. aurata*, in other sweat bees, worker females are reared on significantly smaller pollen provisions compared to reproductives that are provisioned with extra nutrition for diapause (Brand and Chapuisat 2012). Larval nutrition affects brain morphology in honeybees, with queens having larger and more rapidly growing brains in the larval stage (Moda et al. 2013), but this has not been

investigated in primitively eusocial groups. Moreover, workers may be subject to aggressive dominance from the queen during their first days of adult life (Michener and Brothers 1974, Kapheim et al. 2016). Differences in nutrition and social aggression dramatically affect physiology, producing the reproductive queen and sterile worker phenotypes (Kapheim et al. 2017). Our results suggest that differential brain development, particularly of the MBs and ALs, may also be part of this phenotype. Future studies of the effects of larval nutrition on brain development and foundress-worker comparisons in other primitively eusocial species are needed to test the generality of our findings of caste-based developmental differences in the brains of *A. aurata*.

Literature Cited

- Barton, R. A., Harvey, P. H. 2000 Mosaic evolution of brain structure in mammals. *Nature*. 405, 1055.
- Brand, N., & Chapuisat, M. 2012 Born to be bee, fed to be worker? The caste system of a primitively eusocial insect. *Frontiers in Zoology*,9(1), 35. doi:10.1186/1742-9994-9-35
- Cane, J. 1987 Estimation of Bee Size Using Intertegular Span (Apoidea). *Journal of the Kansas Entomological Society*, 60(1), 145-147. Retrieved from <http://www.jstor.org/stable/25084877>
- Cayre, M., Scotto-Lomassese, S., Malaterre, J., Strambi, C., & Strambi, A. 2007 Understanding the Regulation and Function of Adult Neurogenesis: Contribution

- from an Insect Model, the House Cricket. *Chemical Senses*, 32(4), 385-395.
doi:10.1093/chemse/bjm010
- Danforth, B. N., Eickwort, G. C. 1997 *The evolution of social behavior in the augochlorine sweat bees (Hymenoptera: Halictidae) based on a phylogenetic analysis of the genera*. In *Social behavior in insects and arachnids* (eds J. C. Choe, B. J. Crespi), pp. 270-292. Cambridge: Cambridge University Press.
- Dijk, L. J., Janz, N., Schäpers, A., Gamberale-Stille, G., & 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(1866), 20171594. doi:10.1098/rspb.2017.1594
- Dunbar, R. I. 2009 The social brain hypothesis and its implications for social evolution. *Ann. Hum. Biol.* **36**, 562-572.
- 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. *J. Neurobiol.*, 57: 141-151.
- Fahrbach, S. E. 2006 Structure of the mushroom bodies of the insect brain. *Annu. Rev. Entomol.* **51**, 209-232.
- Farris, S.M., Robinson, G.E., Fahrbach, S.E., 2001 Experience- and age-related outgrowth of intrinsic neurons in the mushroom bodies of the worker honeybee. *J. Neurosci.* 21, 6395–6404.

- Feinerman, O., Traniello, J. F. 2015 Social complexity, diet, and brain evolution: modeling the effects of colony size, worker size, brain size, and foraging behavior on colony fitness in ants. *Behav. Ecol. Sociobiol.*, 1-12.
- Fiala, J. C. 2005 Reconstruct: a free editor for serial section microscopy. *J. Microsc.* **218**, 52-61.
- Gibbs, J., Brady, S. G., Kanda, K., Danforth, B. N. 2012 Phylogeny of halictine bees supports a shared origin of eusociality for *Halictus* and *Lasioglossum* (Apoidea: Anthophila: Halictidae). *Mol. Phylogenet. Evol.* **65**, 926-939.
- Gonçalves, R. 2016 A molecular and morphological phylogeny of the extant Augochlorini (Hymenoptera, Apoidea) with comments on implications for biogeography. *Systematic Entomology*, *41*(2), 430–440.
<https://doi.org/10.1111/syen.12166>
- Gronenberg, W., Heeren, S., Hölldobler, B. 1996 Age-dependent and task-related morphological changes in the brain and the mushroom bodies of the ant *Camponotus floridanus*. *J Exp Biol* *199*:201 1- 2019
- Heisenberg, M., Heusipp, M., Wanke, C. 1995 Structural plasticity in the *Drosophila* brain. *J Neurosci* *15*:1951–1960
- Kapheim, K., Chan, T., Smith, A., Weislo, W., & Nonacs, P. 2015. Ontogeny of division of labor in a facultatively eusocial sweat bee *Megalopta genalis*. *Insectes Sociaux*, *63*(1), 185–191. <https://doi.org/10.1007/s00040-015-0454-y>
- 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.

- McKenzie, S. K., Fetter-Pruneda, I., Ruta, V., Kronauer, D. J. 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. (DOI 1610800113 [pii]).
- Michener, C. 1974. *The social behavior of the bees; a comparative study*. Cambridge, Mass: Belknap Press of Harvard University Press.
- Michener, C. D., & Brothers, D. J. 1974. Were workers of eusocial Hymenoptera initially altruistic or oppressed?. *Proceedings of the National Academy of Sciences*, 71(3), 671-674.
- Mildner, Stephanie & Roces, Flavio. 2017 Plasticity of Daily Behavioral Rhythms in Foragers and Nurses of the Ant *Camponotus rufipes*: Influence of Social Context and Feeding Times. *PLOS ONE*. 12. e0169244. 10.1371/journal.pone.0169244.
- Moda, L., Vieira, J., Guimarães Freire, A., Bonatti, V., Bomtorin, A., & Barchuk, A. (n.d.). Nutritionally Driven Differential Gene Expression Leads to Heterochronic Brain Development in Honeybee Castes. *PLoS One*, 8(5), e64815. <https://doi.org/10.1371/journal.pone.0064815>
- Molina Y., O'Donnell S. 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., O'Donnell S. 2008 Age, sex, and dominance-related mushroom body plasticity in the paperwasp *Mischocyttarus mastigophorus*. *Dev Neurobiol* 68:950–959.

- Montgomery, S.H., Merrill, R.M. & Ott, S.R. 2015 Brain composition in Heliconius butterflies, post-eclosion growth and experience dependent neuropil plasticity. *J. Comp. Neurol.* 524: 1747-1769.
- Mueller, U. G. 1996 Life history and social evolution of the primitively eusocial bee *Augochlorella striata* (Hymenoptera: Halictidae). *J. Kans. Entomol. Soc.*, 116-138.
- Niven, J.E., Laughlin S.B. 2008 Energy limitation as a selective pressure on the evolution of sensory systems. *J Exp Biol* 211:1792–1804
- O'Donnell S., M.R. Clifford, S. DeLeon, C. Papa, N. Zahedi & S.J. Bulova. 2014 A test of neuroecological predictions using paperwasp caste differences in brain structure (Hymenoptera: Vespidae). *Behavioral Ecology & Sociobiology* 68: 529-536.
- O'Donnell, S., Bulova, S. J., DeLeon, S., Khodak, P., Miller, S., Sulger, E. 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. (DOI 10.1098/rspb.2015.0791 [doi]).
- O'Donnell, S., Bulova, S.J., DeLeon, S., Barrett, M. 2017 Caste Differences in the mushroom bodies of swarm founding paper wasps: implications for brain plasticity and brain evolution (Vespidae, Epiponin). *Behav Ecol Sociobiol* 71:116
- O'Donnell, S., 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., Donlan, N., Jones, T. 2007 Developmental and dominance associated differences in mushroom body structure in the paperwasp *Mischocyttarus mastigophorus*. *Dev Neurobiol* 67:39–46
- Rehan, S. M., Bulova, S. J., O'Donnell, S. 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. (DOI 10.1159/000381414 [doi]).
- Riveros A, J, Gronenberg W. 2010 Brain Allometry and Neural Plasticity in the Bumblebee *Bombus occidentalis*. *Brain Behav Evol* 2010;75:138-148. doi: 10.1159/000306506
- Riveros, A. J., Seid, M. A., Wcislo, W. T. 2012 Evolution of brain size in class-based societies of fungus-growing ants (Attini). *Anim. Behav.* 83, 1043-1049.
- Schwarz, M. P., Richards, M. H., Danforth, B. N. 2007 Changing paradigms in insect social evolution: insights from halictine and allodapine bees. *Annu. Rev. Entomol.* 52, 127-150.
- Szyszka, P. 2008 Associative and non-associative plasticity in Kenyon cells of the honeybee mushroom body. *Frontiers in Systems Neuroscience*, 2. doi:10.3389/neuro.06.003.2008
- Seid, M.A., Junge, E. 2016 Social isolation and brain development in the ant *Camponotus floridanus*. *Sci Nat* 103(1-6):42
- Smith, A. R., Seid, M. A., Jimenez, L. C., Wcislo, W. T. 2010 Socially induced brain development in a facultatively eusocial sweat bee *Megalopta genalis* (Halictidae). *Proc. Biol. Sci.* **277**, 2157-2163. (DOI 10.1098/rspb.2010.0269 [doi]).

- Stockhammer, K. A. 1966 Nesting habits and life cycle of a sweat bee, *Augochlora pura* (Hymenoptera: Halictidae). *J. Kans. Entomol. Soc.*, 157-192.
- Stöckl, A., Heinze, S., Charalabidis, A., El Jundi, B., Warrant, E., Kelber, A. 2016 Differential investment in visual and olfactory brain areas reflects behavioural choices in hawk moths. *Scientific Reports*. **6**, 26041.
- Toth, A.L., Varala, K., Newman, T.C., Miguez, F.E., Hutchinson, S.K., Willoughby, D.A., Simons, J.F., Egholm, M., Hunt, J.H., Hudson, M.E., Robinson, G.E. 2007 Wasp gene expression supports an evolutionary link between maternal behavior and eusociality. *Science*, 318:441-444.
- Withers, G. S., Fahrbach, S. E. & Robinson, G. E. 1993 Selective neuroanatomical plasticity and division of labour in the honeybee. *Nature* 364, 238–240.
- Withers, G.S., Day, N.F., Talbot, E.F., Dobson, H.E., Wallace, C.S. 2007 Experience-dependent plasticity in the mushroom bodies of the solitary bee *Osmia lignaria* (Megachilidae). *Dev Neurobiol* 68:73–82

Figure Legends

Figure 1. Geographic ranges of *A. aurata* (top) and *A. pura* (bottom) from DiscoverLife.

Figure 2. Confocal image of *A. pura* (left) and *A. aurata* (right) showing the brain areas used for analyses, the optic lobes, antennal lobes (AL) and mushroom bodies (MB). Scale bars = 200 μ m.

Figure 3. A) Mean MB calyces volume relative to whole brain volume. B) Mean whole brain volume, corrected for body size. C) Volume of the sensory neuropils relative to whole brain volume. Social *A. aurata* is shown in green bars, solitary *A. pura* in white bars. *A. aurata* photo by Sam Droege, *A. pura* by Benjamin Smith.

Figure 4. Confocal image of *A. aurata* showing the brain areas used for analyses, the optic lobes, antennal lobes (AL) and mushroom bodies (MB). Scale bars = 200 μ m.

Figure 5. A) Size corrected mean whole brain volume for *A. aurata* foundresses and workers. B) Mean MB calyces volume relative to whole brain volume. C) Mean volume of the antennal lobes relative to whole brain volume. D) Mean volume of optic lobes relative to whole brain volume. *A. aurata* foundress is shown in green bars, *A. aurata* worker in white.

Figure 1

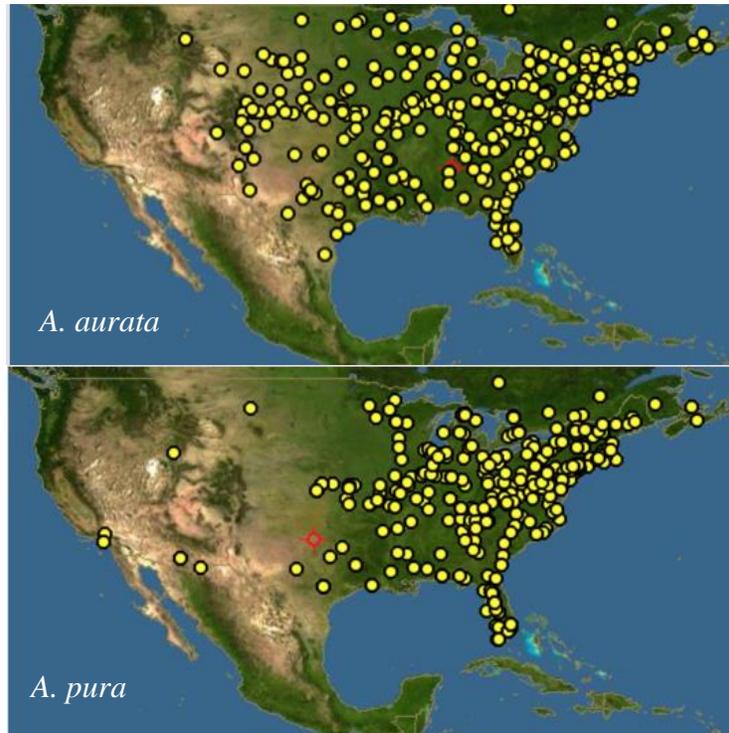


Figure 2

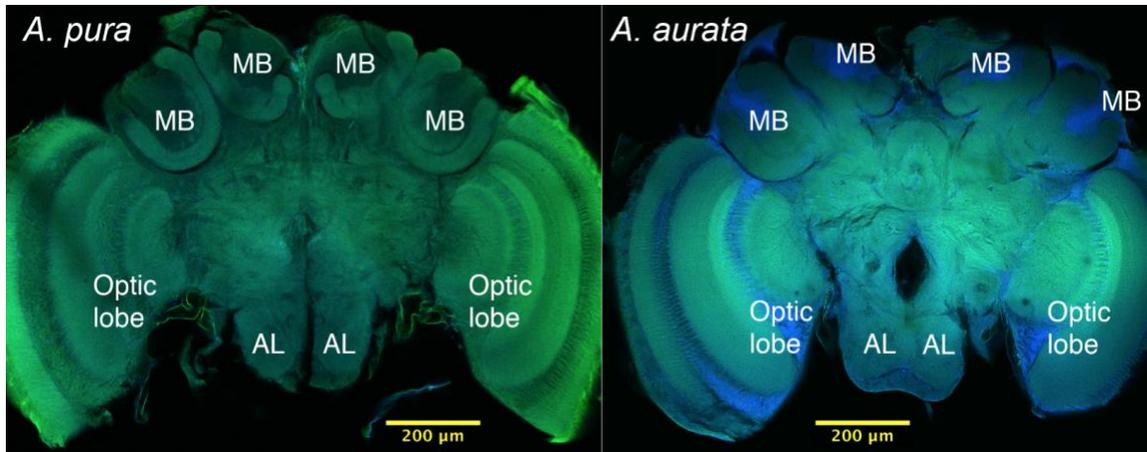


Figure 3

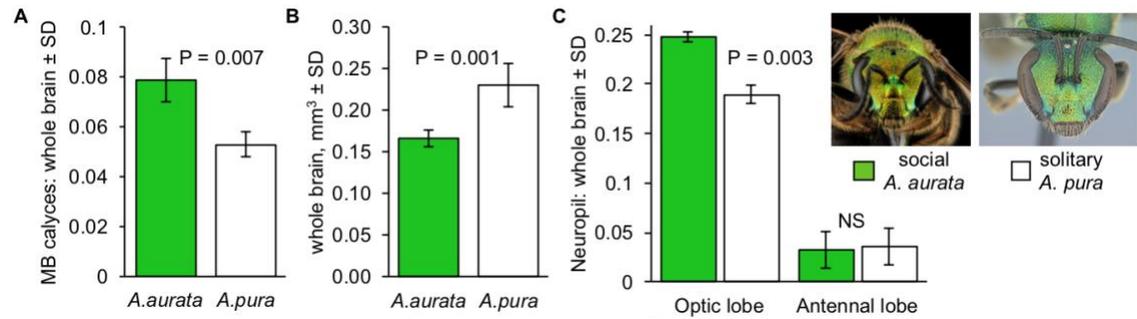


Figure 4

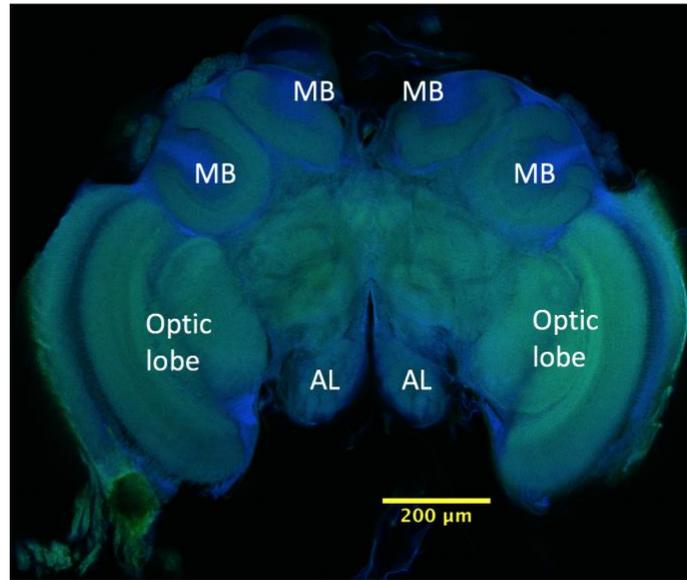


Figure 5

