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The Impacts of Maturation and Experience on Volumetric Neuroplasticity in Solitary and Social Bees

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THE IMPACTS OF MATURATION AND EXPERIENCE ON VOLUMETRIC NEUROPLASTICITY IN SOLITARY AND SOCIAL BEES

by

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ABSTRACT

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Brains are dynamic, often changing dramatically in structure and function over the lifetime of an organism. This neuroplasticity is particularly well-documented among social Hymenoptera (e.g., ants, bees, and wasps). Fascinatingly, this neural plasticity also accompanies substantial changes in behavior, as with the behavioral maturation and task specialization that occurs among highly social species. Therefore, understanding sociality and how it evolved requires evaluating the plasticity of the neural systems underpinning these elaborate phenotypes. Yet, after decades of research, we are still limited in our understanding of relationships between the brain and eusociality. My dissertation research aims to redress this issue by investigating the role of neuroplasticity in facilitating sociality in bees that vary in their degree of social behavior. I started by exploring solitary bees that are closely related to social bees for two forms of neuroplasticity—age-related and experience-dependent—that characterize brain plasticity patterns in the females of highly-social taxa. Specifically, I tested whether age-related plasticity is a preadaptation of, or an adaptive response to, eusociality and how the brains of solitary bees respond to social interactions. I found evidence that age-related plasticity is likely an adaptive response to social life and that the ancestors of social bees may have had brains predisposed to

responding to social cues. Second, I investigated if similar age-related patterns of change occur in the brains of male bees. We found investment in brain tissue increases in males as they age for both facultatively eusocial and obligately eusocial species, something previously undocumented, but shared with females of many highly eusocial species. Lastly, I wanted to explore how two of the behaviors that are fundamental to defining sociality (e.g., reproduction and helping behaviors) influence neuroplasticity. Here, I characterized how the brain responds to reproductive (queens) and non-reproductive (helping worker) roles in a social colony. We determined that the brains of queens and workers respond differently to characteristic queen-like behaviors (i.e., laying eggs without caring for them). Together, these studies add depth to our understanding of how neuroplasticity impacts and responds to social life and how these relationships may have evolved.

(161 pages)

PUBLIC ABSTRACT

The impacts of maturation and experience on volumetric neuroplasticity in solitary and social bees

Mallory A. Hagadorn

Some animals are incredibly social, living and working together as one cohesive group. Alternatively, many animals are solitary, never living with and rarely interacting with others. A large body of biological research has focused on understanding the role that brains play in promoting these behavioral differences across species. Even so, it remains unclear why some brains facilitate social behavior while others do not. My dissertation aims to advance our understanding of this concept by characterizing bees' brains and how they change over a lifetime. Bees are beneficial for investigating relationships between the brain and social behavior because some species are solitary while others are highly social. However, sociality in bees is more dynamic than that; a blending of these two extremes can also occur. This enables us to explore how brains change with social context within a single group of organisms. My first chapter uses a solitary bee to understand how simple social interactions can impact the brain. I found that—even in a solitary bee—certain brain regions grow in size in response to the presence of other bees. This trait may have been important in the evolutionary origins of social behavior. My second chapter investigated the effects of aging in the brains of two bee species, one that is sometimes social while the other is always social. I found that the brains of these species naturally change over time, a feature common to highly social species, e.g., honey bees. This suggests that having brains that change with age may be an important feature of sociality. My final research chapter made comparisons between queen and worker bees to investigate if their colony roles and behaviors dictated the relative size of different regions of their brains. I found that queen and worker brains respond differently to removing offspring care, a trait fundamental to defining their role in

the colony. This highlights a potentially unique relationship between the brain and social life. Collectively, my dissertation used bees to enhance our understanding of what it means to have a social brain.

This dissertation is dedicated to everyone who has loved, motivated, and supported me along the way. It took a village; without you all, I'd still be a ***hot*** *mess.*

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Mallory A. Hagadorn

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CHAPTER 1

INTRODUCTION

General overview

Social insects have intrigued biologists for over a century (Darwin, 1859). On one hand, they are among the most ecologically successful and important organisms on earth (Wilson et al., 1990). Collectively, they encompass over half of the earth's terrestrial animal biomass (Holldobler et al., 2009) and provide various ecosystem services, including pollination, nutrient cycling, and biological control (Elizalde et al., 2020). Some social insects even serve as important indicators of habitat health (Elizalde et al., 2020).

On the other hand, transitions between solitary and advanced social life present a fascinating evolutionary conundrum (Darwin, 1859). Specifically, the cooperative behaviors that define eusocial life lead to an inevitable asymmetry of reproduction among individuals in the group (Batra, 1966; Michener, 1969; Wilson, 1971); some individuals reproduce, while others provide care for offspring. Understanding the maintenance of such disparity in reproductive potential has driven decades of social insect research. Yet, even so, the factors facilitating cooperative behaviors among social insects are still not fully understood. Moreover, the role of the brain in facilitating social behavior and how these roles may have shifted with sociality is even less well understood. My dissertation begins redressing this gap by exploring how sociality influences neuroplasticity and drives brain investment patterns in bees.

Eusociality, social Hymenoptera, and neuroplasticity

Eusociality is an elaboration of social living (Wilson and Hölldobler, 2005) and, while relatively rare (Smith et al., 2008), marks a major transition in evolutionary history (Maynard Smith and Szathmary, 1997). Reproductive division of labor and cooperative

brood rearing are among the defining features of this phenomenon (Batra, 1966). In social Hymenoptera (e.g., ants, bees, and wasps), reproductive dominance is typified by the majority of colony females foregoing their own direct fitness benefits (e.g., workers) in favor of supporting their mother, the queen, by caring for their siblings (Michener, 1969; Queller and Strassmann, 1998; Wilson, 1971). This task specialization increases the direct reproductive success of the queen and yields indirect fitness benefits to the workers (Queller and Strassmann, 1998; Smith et al., 2008). Therefore, understanding eusocial evolution requires investigating how and why the worker caste evolved.

The potential for a relationship between sociality and brain evolution in insects has long been recognized (Dujardin, 1850; Lihoreau et al., 2012). Early on, a connection was made between social taxa and enlarged mushroom bodies (Dujardin, 1850). Mushroom bodies are paired, higher-order cognitive processing regions in the insect brain that are often associated with learning and memory (Gronenberg, 2001; Strausfeld et al., 2009). These structures incorporate multiple smaller subsections, including the Kenyon cells, calyces, and lobes (Fahrbach, 2006). Dendrites from the Kenyon cells (i.e., the neural cell bodies) innervate functionally-distinct subregions of the calyces, called the lip (olfactory input) and the collar (visual input) (Fahrbach, 2006; Gronenberg, 2001). Meanwhile, the axons of the Kenyon cells become peduncles that branch into the specific lobes (Fahrbach, 2006). Interestingly, mushroom body tissues are also known to be structurally and functionally plastic throughout an organism's lifetime (Heisenberg, 1998). Phenotypic plasticity is the ability of a single genotype to produce multiple distinct phenotypes in response to environmental variation (DeWitt and Scheiner, 2004; Pigliucci, 2001; Schlichting and Pigliucci, 1998; West-Eberhard, 2003; Whitman et al., 2009). Amazingly, this biological process extends to the brain, facilitating dynamic, not static, nervous systems. It is this neuroplasticity, in addition to the perceived increases in investment in social taxa, that made the mushroom bodies prime targets for exploring relationships between the brain and social behavior.

The social environment dramatically shapes the sensory system of insects (Jernigan

and Uy, 2023). In fact, insect brains show extraordinary neurological plasticity in response to social life (Gandia et al., 2022; Gronenberg et al., 1996; Jaumann et al., 2019; Molina and O'Donnell, 2007, 2008; O'Donnell et al., 2007; Rehan et al., 2015; Smith et al., 2010; Valadares et al., 2022; Withers et al., 1995, 1993). This includes distinct changes in brain morphology associated with age (i.e., 'experience-expectant') and experience (i.e., 'experience-dependent'). Among social insects, experience-expectant neuroplasticity is associated with routine large-scale shifts in individual behavior (Fahrbach, 2006; Fahrbach et al., 1998; Withers et al., 1995, 2008, 1993). Many workers of highly eusocial insect species undergo age-polyethism, whereby, as they age, they progress through a systematic process of behavioral maturation (Robinson, 1992). Amazingly, substantial brain architecture changes (e.g., mushroom body expansion) coincide with this behavioral development (Fahrbach, 2006; Fahrbach et al., 1998; Withers et al., 1995, 2008, 1993). Since these changes occur prior to these elaborate behavioral shifts, they are considered a potential priming mechanism for the onset of new behaviors by individuals in a social colony (Durst et al., 1994; Withers et al., 1995, 1993). Experiences also induce brain plasticity. For instance, differences in neural investment are associated with behavioral specialization in stingless bees (Valadares et al., 2022) and with the specific sensory needs of wasps (Gandia et al., 2022). Furthermore, mushroom body plasticity has been documented across numerous species in response to social dominance, including bees (Jaumann et al., 2019; Rehan et al., 2015; Smith et al., 2010) and wasps (Molina and O'Donnell, 2007, 2008; O'Donnell et al., 2007). Isolation in social species also impacts brain plasticity. Isolated ants (Seid and Junge, 2016) and wasps (Jernigan et al., 2021) invest less in certain neural tissues relative to socialized counterparts. Moreover, in sweat bees, mushroom body investment decreases with the evolutionary loss of sociality (Pahlke et al., 2021). Together, these studies highlight potential underlying patterns between insect sociality and neuroplasticity.

Using bees to investigate the role of neuroplasticity in social life

Where specifically neuroplasticity fits into the evolution of eusociality is less clear.

First, whether experience-expectant neuroplasticity evolved prior to, or along with, eusocial colonies is still an open question. To date, only one study has explored experience-expectant plasticity in a solitary Hymenopteran species (Withers et al., 2008), and those results suggest that age-related plasticity is likely absent in solitary bees. However, species limitations hinder our ability to draw definitive conclusions about the emergence of experience-expectant neuroplasticity within social evolution. Second, we know that social interactions, or lack thereof, have distinct impacts on the brains of social insect species (Jaumann et al., 2019; Jernigan et al., 2021; Molina and O'Donnell, 2007, 2008; O'Donnell et al., 2007; Rehan et al., 2015; Seid and Junge, 2016; Smith et al., 2010). However, whether solitary species with social experience are predisposed to exhibiting similar shifts in neural investment is undetermined. This ambiguity stalls our understanding of if and how brain plasticity may contribute to social transitions. Next, while experience-expectant neuroplasticity has been linked to behavioral maturation in females (Durst et al., 1994; Fahrbach et al., 1998; Gronenberg et al., 1996; O'Donnell et al., 2004; Tomé et al., 2014; Withers et al., 1995, 1993), we know next to nothing about age-related plasticity in social insect males. This female-biased focus impedes our ability to discern how and why age-related plasticity evolves and, thus, any role it may have played in the evolution of sociality. Finally, the asymmetric distribution of reproduction and caregiving behaviors among the queen and worker caste is fundamental to defining eusociality. Yet, how these experiences alter and are influenced by plasticity in the brain remains largely unexplored, thwarting forward progress regarding the role of neurodevelopment in the origin and maintenance of alternative female castes. Without answers to these questions, relationships between eusociality and neuroplasticity remain inconclusive.

My dissertation aims to resolve these long-standing questions using bees. Bees are useful for exploring questions about social behavior and the evolution of sociality (Wcislo and Fewell, 2017) because they exhibit a range of social forms in nature. While the majority of bees are solitary, they also include species that are facultatively social through obligately complex eusocial, social parasites, and even lineages where sociality has been lost

(Michener, 1969; Michener, 1974; Wcislo and Fewell, 2017, see for review; Wilson, 1971). My second chapter explored experience-expectant and experience-dependent neuroplasticity in the alkali bee, *Nomia melanderi*. Alkali bees are ancestrally solitary, but closely related to a subfamily where eusociality has evolved multiple times (Brady et al., 2006; Gibbs et al., 2012). Here, I used volumetric neuroplasticity to draw conclusions about the likelihood of experience-expectant plasticity occurring prior to the transition to social life, as well as how social stimuli impact the brain of a solitary species. My third chapter investigated brain morphology in facultatively eusocial sweat bees and obligately eusocial bumble bees to determine if the patterns of age-related plasticity observed in social species are consistent across sexes. My fourth chapter characterized brain architecture changes associated with egg-laying and brood care behaviors in bumble bees. I make comparisons both within and across castes to understand how the brains of female bees respond to reproduction and offspring care and whether patterns are consistent across queens and workers. My final chapter summarizes the results of my dissertation and synthesizes overarching conclusions from the findings. Together, these chapters enhance our understanding of how maturation and social experiences impact neuroplasticity in solitary and social bees.

REFERENCES

Batra, S. W. (1966). Social behavior and nests of some nomiine bees in india (hymenoptera, halictidae). *Insectes Sociaux*, 13:145–153.

Brady, S. G., Sipes, S., Pearson, A., and Danforth, B. N. (2006). Recent and simultaneous origins of eusociality in halictid bees. *Proceedings of the Royal Society B: Biological Sciences*, 273(1594):1643–1649.

Darwin, C. (1859). *On the Origin of Species by Means of Natural Selection*. Murray, London.

DeWitt, T. J. and Scheiner, S. M. (2004). *Phenotypic plasticity: functional and conceptual approaches*. Oxford University Press.

Dujardin, F. (1850). Mémoire sur le système nerveux des insectes. *Ann Sci Nat Zool*, 14:195–206.

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(3):259–263.

Elizalde, L., Arbetman, M., Arnan, X., Eggleton, P., Leal, I. R., Lescano, M. N., Saez, A., Werenkraut, V., and Pirk, G. I. (2020). The ecosystem services provided by social insects: traits, management tools and knowledge gaps. *Biological Reviews*, 95(5):1418–1441.

Fahrbach, S. E. (2006). Structure of the mushroom bodies of the insect brain. *Annu. Rev. Entomol.*, 51:209–232.

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(1):115–123.

Gandia, K. M., Cappa, F., Baracchi, D., Hauber, M., Beani, L., Uy, F. M., et al. (2022). Caste, sex, and parasitism influence brain plasticity in a social wasp. *Frontiers in Ecology and Evolution*, 10:2.

Gibbs, J., Brady, S. G., Kanda, K., and Danforth, B. N. (2012). Phylogeny of halictine bees supports a shared origin of eusociality for *Halictus* and *Lasioglossum* (apoidea: Anthophila: Halictidae). *Molecular phylogenetics and evolution*, 65(3):926–939.

Gronenberg, W. (2001). Subdivisions of hymenopteran mushroom body calyces by their afferent supply. *Journal of Comparative Neurology*, 435(4):474–489.

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(9):2011–2019.

Heisenberg, M. (1998). What do the mushroom bodies do for the insect brain? an introduction. *Learning & memory*, 5(1):1–10.

Holldobler, B., Wilson, E. O., et al. (2009). *The superorganism: the beauty elegance and strangeness of insect societies*. WW Norton & Company.

Jaumann, S., Seid, M. A., Simons, M., and Smith, A. R. (2019). Queen dominance may reduce worker mushroom body size in a social bee. *Developmental neurobiology*, 79(6):596–607.

Jernigan, C. M. and Uy, F. M. (2023). Impact of the social environment in insect sensory systems. *Current Opinion in Insect Science*, page 101083.

Jernigan, C. M., Zaba, N. C., and Sheehan, M. J. (2021). Age and social experience induced plasticity across brain regions of the paper wasp polistes fuscatus. *Biology Letters*, 17(4):20210073.

Lihoreau, M., Latty, T., and Chittka, L. (2012). An exploration of the social brain hypothesis in insects. *Frontiers in physiology*, 3:442.

Maynard Smith, J. and Szathmary, E. (1997). *The major transitions in evolution*. OUP Oxford.

Michener, C. D. (1969). Comparative social behavior of bees. *Annual review of entomology*, 14(1):299–342.

Michener, C. D. (1974). *The Social Behavior of the Bees: a comparative study*. Harvard University Press, Cambridge, MA.

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(2):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(7):950–959.

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(1):39–46.

O'Donnell, S., Donlan, N. A., and Jones, T. A. (2004). Mushroom body structural change is associated with division of labor in eusocial wasp workers (polybia aequatorialis, hymenoptera: Vespidae). *Neuroscience letters*, 356(3):159–162.

Pahlke, S., Seid, M. A., Jaumann, S., and Smith, A. (2021). The loss of sociality is accompanied by reduced neural investment in mushroom body volume in the sweat bee *Augochlora pura* (hymenoptera: Halictidae). *Annals of the Entomological Society of America*, 114(5):637–642.

Pigliucci, M. (2001). *Phenotypic plasticity: beyond nature and nurture*. JHU Press.

Queller, D. C. and Strassmann, J. E. (1998). Kin selection and social insects. *Bioscience*, 48(3):165–175.

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(2):117–124.

Robinson, G. E. (1992). Regulation of division of labor in insect societies. *Annual review of entomology*, 37(1):637–665.

Schlichting, C. D. and Pigliucci, M. (1998). *Phenotypic evolution: a reaction norm perspective.* Sinauer associates incorporated.

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

Smith, A. R., Seid, M. A., Jiménez, 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(1691):2157–2163.

Smith, C. R., Toth, A. L., Suarez, A. V., and Robinson, G. E. (2008). Genetic and genomic analyses of the division of labour in insect societies. *Nature Reviews Genetics*, 9(10):735–748.

Strausfeld, N. J., Sinakevitch, I., Brown, S. M., and Farris, S. M. (2009). Ground plan of the insect mushroom body: Functional and evolutionary implications. *Journal of Comparative Neurology*, 513(3):265–291.

Tomé, H. V. V., Rosi-Denadai, C. A., Pimenta, J. F. N., Guedes, R. N. C., and Martins, G. F. (2014). Age-mediated and environmentally mediated brain and behavior plasticity in the stingless bee *Melipona quadrifasciata anthidioides*. *Apidologie*, 45(5):557–567.

Valadares, L., Vieira, B. G., Santos do Nascimento, F., and Sandoz, J.-C. (2022). Brain size and behavioral specialization in the jataí stingless bee (tetragonisca angustula). *Journal of Comparative Neurology*, 530(13):2304–2314.

Wcislo, W. and Fewell, J. H. (2017). Sociality in bees. In Rubenstein, D. R. and Abbot, P., editors, *Comparative social evolution*, pages 50–83. Cambridge University Press Cambridge.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution.* Oxford University Press.

Whitman, D. W., Ananthakrishnan, T. N., et al. (2009). *Phenotypic plasticity of insects: mechanisms and consequences.* Science Publishers, Inc.

Wilson, E. O. (1971). *The insect societies*. Cambridge, MA: Belknap.

Wilson, E. O. et al. (1990). Success and dominance in ecosystems: the case of the social insects. *Success and dominance in ecosystems: the case of the social insects.*

Wilson, E. O. and Hölldobler, B. (2005). Eusociality: origin and consequences. *Proceedings of the National Academy of Sciences*, 102(38):13367–13371.

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(1):130–144.

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* (megachilidae). *Developmental neurobiology*, 68(1):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(6434):238–240.

CHAPTER 2

EXPERIENCE, BUT NOT AGE, IS ASSOCIATED WITH VOLUMETRIC MUSHROOM BODY EXPANSION IN SOLITARY ALKALI BEES [1]

Abstract

In social insects, changes in behavior are often accompanied by structural changes in the brain. This neuroplasticity may come with experience (experience-dependent) or age (experience-expectant). Yet, the evolutionary relationship between neuroplasticity and sociality is unclear, because we know little about neuroplasticity in the solitary relatives of social species. We used confocal microscopy to measure brain changes in response to age and experience in a solitary halictid bee (Nomia melanderi). First, we compared the volume of individual brain regions among newly emerged females, laboratory females deprived of reproductive and foraging experience, and free-flying, nesting females. Experience, but not age, led to significant expansion of the mushroom bodies—higher-order processing centers associated with learning and memory. Next, we investigated how social experience influences neuroplasticity by comparing the brains of females kept in the laboratory either alone or paired with another female. Paired females had significantly larger olfactory regions of the mushroom bodies. Together, these experimental results indicate that experience-dependent neuroplasticity is common to both solitary and social taxa, whereas experience-expectant neuroplasticity may be an adaptation to life in a social colony. Further, neuroplasticity in response to social chemical signals may have facilitated the evolution of sociality.

Introduction

Insect species living in cooperative societies have brains capable of changing with colony

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needs and in response to features of social life (Gronenberg et al., 1996; Jaumann et al., 2019; Molina and O'Donnell, 2007, 2008; O'Donnell et al., 2007; Rehan et al., 2015; Smith et al., 2010; Withers et al., 1995, 1993). This neuroplasticity, i.e. changes in neural structure and function over a lifetime (Kolb and Gibb, 2008), comes in two forms—experience-dependent and experience-expectant. Whether each form evolved prior to or in response to sociality is unknown.

Experience-dependent plasticity involves changes in brain architecture driven by experience. Mushroom bodies, paired cognitive processing centers in the insect brain, are associated with learning, memory, and sensory integration (Gronenberg, 2001; Strausfeld et al., 2009). Previous studies have shown experience-based increases in mushroom body volume among insects in response to oviposition (Van Dijk et al., 2017), foraging (Durst et al., 1994; Farris et al., 2001; Gronenberg et al., 1996; Ismail et al., 2006; Maleszka et al., 2009; Rehan et al., 2015; Withers et al., 1995, 2008, 1993), and social interactions (Jaumann et al., 2019; Molina and O'Donnell, 2007; O'Donnell et al., 2007; Rehan et al., 2015; Smith et al., 2010).

Experience-expectant plasticity occurs independent of experience, in anticipation of neural response to the environment. For instance, many workers in highly eusocial colonies progress through distinct behavioral phases with age (Robinson, 1992). This behavioral maturation includes an age-related shift from nest-oriented tasks, such as brood care, to work outside of the nest, such as foraging (Wilson, 1971). In various species of social bees (Durst et al., 1994; Fahrbach et al., 1998; Tomé et al., 2014; Withers et al., 1995, 1993), ants (Gronenberg et al., 1996), and wasps (O'Donnell et al., 2004), behavioral maturation is supported by changes in neural organization (e.g., mushroom body expansion) (Fahrbach, 2006; Fahrbach et al., 1998; Withers et al., 1995, 2008, 1993). These neuroanatomical changes are considered 'experience-expectant' because they occur before behavioral shifts, and can be induced in response to colony need (Durst et al., 1994; Withers et al., 1993). This type of neuroplasticity is thus considered a priming mechanism for the onset of new task performance associated with division of labor within eusocial colonies (Withers et al.,

1995, 1993).

Both types of neuroplasticity occur in eusocial insects with large colonies and behavioral maturation (i.e., honey bees, stingless bees, ants, and highly social paper wasps) (Durst et al., 1994; Fahrbach et al., 2003; Farris et al., 2001; Gronenberg et al., 1996; O'Donnell et al., 2004; Seid et al., 2005; Tomé et al., 2014; Withers et al., 1995, 1993), but the evolutionary relationship between experience-dependent, experience-expected plasticity, and social organization is unclear. Earlier studies suggest that experience-dependent plasticity may be a common feature of all bees and wasps, whether solitary or social. Central-place foraging, a complex task (Avarguès-Weber and Giurfa, 2013; Menzel et al., 1996; Menzel and Giurfa, 2001), is associated with mushroom body plasticity across the Hymenoptera (ants, bees, wasps) (Durst et al., 1994; Farris et al., 2001; Gronenberg et al., 1996; Ismail et al., 2006; Maleszka et al., 2009; Rehan et al., 2015; Withers et al., 1995, 2008, 1993). Honey bees exhibit mushroom body plasticity in response to foraging experience, which occurs independent of age (Durst et al., 1994; Farris et al., 2001; Withers et al., 1993) and persists after foraging ceases (Fahrbach et al., 2003). Additionally, foraging leads to mushroom body expansion in solitary (Withers et al., 2008) and facultatively social species (Rehan et al., 2015). This suggests that experience-dependent plasticity evolved prior to the evolution of eusociality.

However, life in a social colony provides unique experiences, and it is unknown whether neuronal sensitivity to social cues preceded or followed the evolution of eusociality. One of the most important types of experience in social insects is that coming from social interactions (Dunbar, 1998; Lihoreau et al., 2012; O'Donnell et al., 2011), whereby the need for flexible information processing may be necessary for adapting to the social environment (Menzel and Giurfa, 2001). Isolation leads to a lack of mushroom body development and behavioral impairment in carpenter ants (*Camponotus floridanus*) (Seid and Junge, 2016), and the evolutionary loss of sociality accompanies decreased mushroom body investment in the sweat bee *Augochlora pura* (Pahlke et al., 2021). Mushroom body plasticity is also associated with the maintenance of dominance in social wasps (Molina and O'Donnell, 2007;

O'Donnell et al., 2007), facultatively eusocial sweat bees (Jaumann et al., 2019; Smith et al., 2010), and facultatively social small carpenter bees (Rehan et al., 2015). There is some evidence to suggest that solitary species may also exhibit neuronal sensitivity to social stimuli. For example, mushroom body plasticity accompanies high density larval rearing in *Drosophila melanogaster* (Heisenberg et al., 1995). Elucidating the relationship between neural sensitivity to social cues and social organization is important for identifying features that may have facilitated social evolution, yet this is unexplored in solitary Hymenoptera.

It is also unclear whether experience-expectant neuroplasticity is a developmental feature common across Hymenoptera or whether it is unique to species that exhibit advanced eusociality. In highly social species with large colonies and age-related task specialization, neuroanatomical changes precede the transition to foraging (Gronenberg et al., 1996; Seid and Wehner, 2009; Tomé et al., 2014; Withers et al., 1995, 2008, 1993). In honey bees, mushroom body enlargement coincides with the transition to work outside of the hive (Fahrbach, 2006; Farris et al., 2001; Ismail et al., 2006; Withers et al., 1993). However, in stingless bee workers (*Melipona quadrifasciata*), similar age-related changes occur very early in adult life, well before the behavioral transition to foraging (Tomé et al., 2014). Further, where division of labor is size-based (e.g., bumble bees), age-related plasticity happens within the first few days of life (Jones et al., 2013; Kraft et al., 2019; Riveros and Gronenberg, 2010). This indicates that experience-expectant neuroplasticity may be a common developmental feature of social insects (Withers et al., 2008), though the specific time scales vary with socioecological traits. Yet, research with facultatively social species have shown either no effect of age (Jaumann et al., 2019), have not controlled for age (Smith et al., 2010), or leave doubt as to what extent changes are age-related (Rehan et al., 2015). Moreover, only one study has investigated experience-expectant neuroplasticity in a solitary Hymenoptera. In the orchard bee *Osmia lignaria*, age does not significantly impact mushroom body plasticity (Withers et al., 2008). However, because *O. lignaria* overwinter as adults, it is possible that experience-expectant change occurred before spring emergence (Withers et al., 2008). Therefore, the evolutionary relationship between eusociality and

neuroplasticity remains inconclusive.

We investigated the effects of age, social environment, and nesting experience on neuroplasticity in the solitary alkali bee *Nomia melanderi* (Halictidae). Several features of alkali bee biology make them well-suited for investigating the relationship between neuroplasticity and social evolution. First, they overwinter as prepupa (Bohart, 1955), eliminating the possibility that age-related neuroplasticity occurs undetected in overwintering adults. Second, alkali bees belong to the subfamily Nomiinae (Wcislo and Engel, 1996), which is sister to the Halictinae, in which eusociality has evolved two or three times (Brady et al., 2006; Gibbs et al., 2012). The common ancestor of these clades was likely solitary (Danforth, 2002), thus alkali bees may harbor traits shared with the ancestor that gave rise to sociality. Alkali bees also exhibit characteristics considered to be pre-adaptations of sociality, including nesting gregariously (when individual nests are clustered close together) (Cane, 2008) and extended maternal care (tending to eggs in the nest) (Batra, 1970; Batra and Bohart, 1969). As such, alkali bees are a useful solitary model for testing hypotheses regarding the origins of eusociality (Kapheim, 2017; Kapheim and Johnson, 2017a,b).

To explore relationships between social evolution and neuroplasticity, we tested for experience-dependent and experience-expectant neuroplasticity in *N. melanderi*. If *N. melanderi* have experience-dependent plasticity, mushroom body volume will increase with foraging. Therefore, we predicted mushroom body investment to increase with foraging and nesting experience in alkali bees relative to females kept in the laboratory. Additionally, in the absence of foraging, if social stimuli influence mushroom body plasticity, we predicted that alkali bees sharing a cage with another female should have significantly increased mushroom body investment relative to bees kept alone. Finally, if *N. melanderi* exhibit experience-expectant plasticity, they should have increases in mushroom body volume with age, even when kept alone without foraging. If experience-expectant neuroplasticity is absent in alkali bees it could indicate that this feature is unique to social taxa, and potentially evolved as an adaptive response to social complexity. We found that experience,

but not age, led to increases in mushroom body volume, suggesting that neuronal sensitivity to experience preceded the evolution of sociality in bees.

Materials and Methods

Study sites

We conducted this study in Touchet, WA, USA, between 27 May and 19 June 2016 (Experiment 1) and 29 May and 27 June 2018 (Experiment 2). In the Touchet Valley, alkali bees [*Nomia melanderi* (Cockerell 1906)] nest in large, salty soil beds near alfalfa seed fields (Cane, 2008). For Experiment 1, we collected adult female alkali bees from three previously established bee beds, all within 5 km of one another. For Experiment 2, we used two of these three bee beds (3 km apart).

Field Collections

The collection and rearing methods for both experiments are identical to that of Kapheim and Johnson (2017a), but repeated here briefly. We captured newly emerged females (< 24-h old) leaving their natal nests for the first time after emerging from diapause by placing traps over bee beds known to host large nesting aggregations. Nesting, reproductive females were collected in nets and identified as those carrying pollen on their hind legs, which indicates that they were provisioning offspring. After collection, we transported bees back to the laboratory in 15 ml conical tubes placed in a cooler between single layers of cardboard flanked by ice packs to keep bees cool, but not anesthetized. In the laboratory, we kept bees in cages constructed from cylindrical, perforated plastic containers [72 mm x 113 mm (upper diameter) and x 90 mm (lower diameter)]. We provided ad libitum sugar water with pollen mixture [2.5 g of finely ground honey bee pollen (Betterbee, Greenwich, NY, USA) homogenized in 30 ml of 35% (w/v) sucrose solution] that we changed every other day. We maintained cages between 22 and 28°C at 40–85% relative humidity under a 13 h:11 h light:dark cycle (Kapheim and Johnson, 2017b).

Experiment 1: experience-expectant and experience-dependent neuroplasticity

Experiment 1 samples were a subset of those used in a previous study (Kapheim and Johnson, 2017a). We randomly assigned newly emerged bees to one of two treatment groups: (1) newly emerged or (2) laboratory-reared. We killed newly emerged bees (N=7) upon return to the laboratory, whereas laboratory-reared bees (N=7) were kept in individual cages with ad libitum food for 10 days. We collected data on two additional laboratory-reared females (a total of nine). These two females, however, were given alfalfa flowers in addition to sugar water and pollen. Because this stimulus was absent in other laboratory-reared bees, we removed these samples (A18.01 and H4.03) prior to analyses to eliminate the potential for this as a confounding factor. We compared newly emerged and laboratory-reared females with nesting, reproductive females (N=7) of unknown age.

We used newly emerged bees and laboratory-reared females to assess age-related neuroplasticity. These females lacked foraging and nest-construction experience, and were unmated, as confirmed by the absence of sperm in their spermathecae. Newly emerged bees served as a baseline for volumetric measurements. Nesting females had mating (sperm present in their spermathecae), foraging, and nest-construction experience. We compared nesting females with newly emerged and laboratory-reared females to explore experience-dependent neuroplasticity.

Experiment 2: socially induced experience-dependent neuroplasticity

In this experiment, we tested whether living with another bee affected neuroanatomical plasticity. We randomly assigned newly emerged bees to either the solitary (solo) or paired treatment group. Solo females (N=17) were kept alone for 10 days, and paired females (N=23) were given a nesting female cage-mate for the same duration. For paired bees, we measured neuroplasticity only in the focal (10 days old) female, and did not dissect the brains of the female cage-mates. Rearing conditions were as for Experiment 1, except that we provided bees with a daily sprig of fresh alfalfa flowers to avoid an olfactorily barren environment. We paint-marked the dorsal thorax of all bees with enamel paints (Testors Corporation, Rockford, IL, USA) for identification, including solo bees to control for the

effects of paint-marking (Packer, 2005).

Sample preparation, microscopy, and volumetric measurements

We chilled individuals at 4°C for 5 min prior to decapitation. For Experiment 1, we removed the mouthparts after bees were immobilized, whereas for Experiment 2 we also removed eye capsules. We preserved head capsules in 4% paraformaldehyde (PFA) (Alfa Aesar, Ward Hill, MA, USA) in 1X phosphate-buffered saline (PBS) (Ambion, Austin, TX, USA) at 4°C until dissection. We rinsed head capsules in 1X PBS (3x10 min) after removal from PFA and conducted dissections in 1X PBS using a Leica EZ4 HD stereomicroscope (Leica Microsystems, Buffalo Grove, IL, USA). Using 2% glutaraldehyde (Sigma-Aldrich Inc., St. Louis, MO, USA), we post-fixed dissected brains at room temperature for 48 h. Next, we rinsed brains in 1X PBS (3x10 min) and then bleached them in a formamide solution [1x PBS, 3% formamide (Thermo Fisher Scientific, Rockford, IL, USA), 1% Triton-X (Sigma-Aldrich), and 20% hydrogen peroxide (Fisher Scientific, Fair Lawn, NJ, USA)] (modified protocol from Zukor et al., 2010), which decreased the effects of shadowing during autofluorescence owing to residual pigment. We bleached brains from Experiments 1 and 2 for an average of 75 and 35 min, respectively. Post-bleaching, we rinsed brains in 1X PBS (3x10 min) prior to serial dehydration through a series of ascending ethanol concentrations (30%, 50%, 70%, 90%, 95%, and 3x100%, 10 min each). We cleared and stored brains in methyl salicylate (Fisher Scientific) at -20°C until imaging.

We imaged whole brains using autofluorescence and laser scanning confocal microscopy at 10x magnification (Zeiss LMS 710, Jena, Germany) while mounted in methyl salicylate (Fig 2.1A). Scanning included 5 μm intervals, with steps imaged in 3x2 tile scans (2867x1946 pixels) ultimately combined to form image stacks ranging from approximately 700 to 900 μm. We imaged brains simultaneously using two lasers, the first of which had a wavelength, laser power, and a range of gains designated as 410–485 nm, 4.0–3.5, and between 510 and 557, respectively. The second used a wavelength of 495–538 nm, 3.5–3.0 laser power, and a gain range including 500–548. The pinhole was maintained between 6.00 and 7.00 airy units.

Mushroom bodies include Kenyon cells, calyces, and lobes (Fahrbach, 2006). Kenyon cell dendrites innervate the lip and collar, calyx subregions associated with olfactory and visual input, respectively (Fahrbach, 2006; Gronenberg, 2001), and their axons form peduncles that branch into the distinct lobes (Fahrbach, 2006).

We generated volumetric measurements for the whole brain and five neuroanatomical structures, including the lip and collar, mushroom body lobes (basal ring, peduncle, and lobes as one structure), Kenyon cells, and antennal lobes (Fig. 2.1) using serial reconstruction [Reconstruct software (Fiala, 2005), Version 1.1.0.0, available at http://synapses.clm.utexas.e The basal ring is a structure of the calyx; however, owing to image quality, and to promote consistency, this structure was traced with the peduncle and included as a component of the mushroom body lobes. Additionally, because of occasional damage to the outer edge of optic lobes, whole-brain traces always exclude the lamina and retina. Experience-expectant neuroplasticity is associated with increases in neuropil relative to Kenyon cell (N:K) volume (Fahrbach, 2006; Withers et al., 1995, 2008, 1993). Therefore, we also calculated N:K ratios.

Experiment 1 and 2 trace intervals were every 5 μm and 10 μm optical slice, respectively. We randomized samples and traced them blind to treatment group. For each sample, we standardized structure volumes to the whole brain by calculating structure:whole brain ratios, which is referred to as 'relative volumes'.

Statistical analyses

We used R version 3.6.1 (https://www.r-project.org/) to conduct all statistical analyses. We assessed relative volumes for each structure (lip, collar, mushroom body lobes, Kenyon cells, and antennal lobes) and N:K ratios. We used Anderson–Darling normality tests (Nortest, version 1.0-4; https://CRAN.R-project.org/package=nortest) and visual inspections of qq-plots (car, version 3.0-3; Fox and Weisberg 2019) to detect significant departures from normality. One variable—relative lip volume for Experiment 1—failed to meet normality assumptions. Therefore, we applied a Box-Cox transformation (MASS, version 7.3-51.4; Venables and Ripley 2002) based on the optimal value λ=-.364.

We verified homogeneity of variance using R package car (version 3.0-3; Fox and Weisberg 2019).

For Experiment 1, we used ANOVAs followed by Tukey *post hoc* tests (multcomp, version 1.4-10; Hothorn et al. 2008) to evaluate the effects of treatment on relative volumes of brain regions and N:K (stats, version 3.6.1). For Experiment 2, we conducted Student's t-tests (stats, version 3.6.1) to compare sample means between solo and paired bees for each brain regions and N:K. We assessed significance at α=0.05.

Results

Experiment 1: experience-expectant and experience-dependent neuroplasticity

We found evidence for experience-dependent, but not experience-expectant, neuroplasticity in female alkali bees. Relative volume of the mushroom body neuropil ($F_{2,18}$=20.50, P=2.29x10^{-05}; Fig. 2.2A) and N:K ($F_{2,18}$=15.83, P=1.08x10^{-04}; Fig. 2.2B) was significantly different among groups. In both cases, nesting females had significantly larger values than newly emerged and laboratory-reared bees, but newly emerged and laboratory-reared females were not significantly different (Fig. 2.2). We did not find significant differences in Kenyon cell ($F_{2,18}$=0.64, P=0.54; Fig. 2.2A), antennal lobe ($F_{2,18}$=3.01, P=0.07; Fig. 2A), or whole brain ($F_{2,18}$=0.13, P= 0.88) relative volumes across treatment groups.

Experience also had a significant effect on the relative volumes of calyx substructures and mushroom body lobes (includes basal ring, peduncle, ventral lobe, and medial lobe). Relative lip ($F_{2,18}$=30.52, P=1.65x10^{-06}), collar ($F_{2,18}$=12.82, P=3.46x10^{-04}), and total calyx ($F_{2,18}$=24.86, P=6.63x10^{-06}) volumes were significantly different among treatment groups (Fig. 2.3). Tukey's pairwise comparisons showed no significant differences in these structures between newly emerged and laboratory-reared bees, but nesting females had significantly larger relative volumes compared with the other two groups (Fig. 2.3). Additionally, there was a significant effect of treatment on mushroom body lobe relative volume ($F_{2,18}$=10.81, P=8.24x10^{-04}; Fig. 2.3). Nesting females had significantly larger

mushroom body lobes than newly emerged and laboratory-reared bees, but the latter two groups were not significantly different (Fig. 2.3).

Experiment 2: socially-induced neuroplasticity

We found that social environment significantly impacts brain investment in female alkali bees. Relative volumes of total neuropil (t=-1.23, df=38, P=0.23; Fig. 2.4A), Kenyon cells (t=-0.30, df=38, P= 0.77; Fig. 2.4A), antennal lobes (t=-0.79, df=38, P= 0.44; Fig. 2.4A), and N:K (t=-1.01, df=38, P=0.32; Fig. 2.4B) did not differ significantly between solo and paired bees. Social environment did not significantly affect relative volume of the collar (t=0.22, df=38, P=0.82; Fig. 2.5), total calyces (t=-1.73, df=38, P=0.09; Fig. 2.5), or mushroom body lobes (t=0.33, df=38, P=0.74; Fig. 2.5). However, relative volume of the mushroom body lip was significantly larger in females housed with a cage-mate than those reared alone (t=-2.90, df=38, P=0.01; Fig. 2.5). Mean whole brain volumes did not differ significantly between the two groups (t=-1.32, df=38, P=0.19).

Discussion

We found that in solitary alkali bees, as with social bees, mushroom bodies expand in response to adult experience. Remarkably, this includes social experience, which suggests that the ancestors of social bees may have been pre-wired to respond to the cues of social partners—a critical component of sociality. Females with foraging experience also had significantly enlarged mushroom bodies, a finding consistent with other bee species studied. Lastly, our results suggest that solitary bees do not have experience-expectant neuroplasticity, indicating that this phenomenon may have evolved as an adaptive response to age-related changes in task performance among highly eusocial species.

Unlike in eusocial species, where tasks are distributed across castes (Michener, 1974; Wilson, 1971), reproductively mature female solitary bees must manage multiple tasks simultaneously. This includes mating, nest construction, navigation, and foraging activities, all of which may be cognitively demanding. We found that these experiences led to brain changes in alkali bees, such that free-flying, nesting females had significantly

enlarged mushroom bodies relative to females with limited experiences. Our findings corroborate those of a study with solitary orchard bees (*Osmia lignaria*), which found that foraging experience significantly influenced mushroom body investment (Withers et al., 2008). Together, these results suggest that adult experience is an important driver of neuroplasticity in both solitary and social species.

Social experience also leads to neuroanatomical changes in alkali bees. We found that *N. melanderi* individuals paired with a cage-mate had significantly greater lip volume—the calyx subregion primarily associated with olfactory input (Gronenberg, 2001)—relative to those reared alone. Alkali bees from both our solo and paired treatment groups were exposed to olfactory stimuli, including natal nest odors and alfalfa in their housing containers, indicating that the increased calyx lip volume was associated specifically with stimuli present in the social environment. Though we cannot determine whether the lip expansion was driven by enhanced olfactory stimulus in general or was specific to social signals, this result does suggest that the common ancestor of solitary and social bees may have been capable of responding at the neurological level to olfactory cues from conspecifics.

While our study was not designed to differentiate between specific social stimuli, viewing the results in light of socially-relevant tasks, such as communication, is intriguing. Communication is critical for coordinating social behaviors in a colony (Blum, 1996; Leonhardt et al., 2016), and social insects must be able to discriminate various recognition cues, some of which are olfactory (Leonhardt et al., 2016). Therefore, sensory systems that could recognize nestmate from non-nestmate may have been particularly important for facilitating the earliest stages of social life(d'Ettorre et al., 2017). But, as social complexity increases, communication requirements expand to include information from the social environment, such as task allocation, defense, and food acquisition (Blum, 1996; Leonhardt et al., 2016). Social bees invest more in their peripheral olfactory nervous system (antennal sensilla) than their solitary relatives (Wittwer et al., 2017), presumably to facilitate chemical recognition and communication. It is thus unsurprising that social experience in our experiment led to enlargement in the mushroom body region dedicated

to processing chemosensory input.

Solitary bees are similar to many other insects in that they rely on chemical cues to recognize their nests, prospective mates, and potential resources (Anzenberger, 1986; Cane, 1997; Falibene et al., 2015; Guédot et al., 2006; Leonhardt et al., 2016; Shimron et al., 1985; Wcislo, 1992; Wenseleers and van Zweden, 2017). It is therefore possible that the neurological response to conspecifics we observed could represent selection on cognitive sensitivity to novel resources associated with mating, nesting, foraging, or other cognitive tasks unrelated to sociality. However, alkali bees routinely encounter conspecifics. While they are non-social in that each female provisions her own nest, alkali bees live in dense aggregations up to 100 nests per m2 (Cane, 2008; Johansen et al., 1978). Hence, nesting females must be able to recognize their nest among a dense collection of others. Alkali bees use vision for nest recognition (Hackwell, 1967), but may use olfaction as well, since olfactory cues are important for nest recognition in other densely aggregated, solitary ground-nesting bees (Shimron et al., 1985; Wcislo, 1992). Therefore, neuroplasticity in the lip region of the mushroom bodies likely represents functionally relevant neurological responses to socially associated stimuli. Thus, an interpretation of our results that emphasizes selection for response to novel olfactory cues is consistent with the hypothesis that neurological sensitivity to olfactory cues from the social environment is a pre-adaptation for the evolution of sociality.

Dominance or aggressive interactions between our paired females may have also contributed to alkali bee calyx plasticity. Social dominance induces brain plasticity across social insects (Jaumann et al., 2019; Molina and O'Donnell, 2007, 2008; O'Donnell et al., 2007; O'Donnell et al., 2017; Rehan et al., 2015; Smith et al., 2010), and calyx enlargement is associated with high dominance rank and increased aggression in wasps (Molina and O'Donnell, 2007, 2008; O'Donnell et al., 2007; O'Donnell et al., 2017). Aggressive behaviors have been reported in alkali bees in laboratory tests (Smith et al., 2019) and while observing nesting conflict (Batra, 1970; Hackwell, 1967). However, there is no evidence that *Nomia* form dominance hierarchies, and we did not explicitly measure dominance or aggressive

behaviors among our caged bees. Moreover, aggressive interactions were relatively rare in forced associations of *N. melanderi* (26% of all behaviors) (Smith et al., 2019). Thus, dominance and aggression are unlikely to be the primary drivers of the lip expansion observed in socially caged bees.

Experience-expectant neuroplasticity is likely an adaptive response to age-related systems of division of labor and task allocation, which is not possible in solitary bees. Our results complement previous research to suggest that this is a phenomenon unique to social species. Age-related plasticity was not detected in the solitary bee *O. lignaria*, suggesting that the brain may be 'pre-wired' at emergence (Withers et al., 2008). These results were inconclusive, however, because this species overwinters as adults (Bosch and Kemp, 2000), during which time neuronal reorganization could occur undetected (Withers et al., 2008). Alkali bees diapause as prepupa (Bohart, 1955; Hackwell, 1967), making them a useful species for investigating experience-expectant neuroplasticity, while facilitating robust comparisons across differing life history strategies. In our study, mushroom body neuropil volume and N:K ratio increased with age, but the differences between newly emerged and laboratory-reared bees were not statistically significant, potentially owing to a large amount of within-group variance. Interestingly, N:K ratios in alkali (2.35:1) and orchard bees (2.31:1) at emergence were similar to those of behaviorally mature honey bee foragers (2.1:1) (Withers et al., 2008, 1993). This could indicate that solitary bees emerge with brains ready for navigation and foraging, tasks that both orchard and alkali bees perform almost immediately upon emergence (Bohart, 1955; Hackwell, 1967; Withers et al., 2008).

An alternative explanation for our findings is that experience-expectant plasticity occurs after 10 days post-emergence. However, 10 days is approximately 25% of the alkali bee 5 week adult lifespan, and encompasses the period during which most females begin nest building and provisioning (Hackwell, 1967; Pitts-Singer, 2008). This suggests that experience-expectant neuroplasticity is unlikely to occur beyond 10 days.

It is also possible that brain development may occur in the nest prior to emergence above ground (Withers et al., 2008). Rapid calyx plasticity is observed in *Drosophila* 6 h

post-eclosion (Barth and Heisenberg, 1997). Adult alkali bees can spend 3–4 days hardening their exoskeleton prior to emerging from their brood cells (Bohart, 1955). Thus, this may be a period of age-related plasticity undetectable by our methods. If solitary bees do undergo intrinsically driven neuroplasticity before emergence, this would suggest that the evolution of age-related division of labor is accompanied by a shift in timing of experience-expectant plasticity.

Overall, our results suggest that experience-expectant plasticity, as seen in extant eusocial insects, may not have been present in the solitary ancestor of social halictid bees, but it may be an adaptive response to social life. It is not clear whether closely related social halictine bees exhibit experience-expectant neuroplasticity, or even the age polyethism with which it is typically associated. There is mixed evidence for age-associated neuroplasticity in the facultatively eusocial sweat bee *Megalopta genalis*, where females nest either solitarily or in a small social colony, but do not exhibit age-related task specialization (Smith et al., 2007; Wcislo et al., 2004). Young *M. genalis* females had smaller mushroom bodies relative to social queens and solitary reproductives, but age was not explicitly controlled for (Smith et al., 2010). However, a follow-up study found no effect of age on mushroom body development, contrasting the prior finding (Jaumann et al., 2019). A brain ready to engage in all tasks at emergence may be more adaptive for species that maintain totipotency, as is often associated with sociality in halictine bees (Michener, 1974, 1990). Additional research in other social halictines is needed to clarify the evolutionary relationship between sociality and experience-expectant neuroplasticity.

Conclusion

Neuroplasticity in insects is associated with foraging and many aspects of social behavior, including task specialization and dominance interactions. Because most studies have focused on understanding these relationships in social taxa, it is unknown whether neural plasticity is a pre-adaptation or adaptive response to social evolution. We did not find evidence of experience-expectant neuroplasticity in solitary alkali bees, suggesting that this form of plasticity may have evolved with sociality. Conversely, nesting and foraging

experience, as well as social interactions, induce neuroplasticity in both solitary and social species. This suggests that experience-dependent plasticity is a conserved trait in bees, and that mushroom body plasticity in the area responsible for processing chemosensory stimuli may have been an important pre-adaptation to sociality.

Authors' contributions

Conceptualization: M.A.H., K.M.K., A.R.S., M.A.S.; Methodology: M.A.H., A.R.S., M.A.S., K.M.K.; Formal analysis: M.A.H., K.M.K.; Investigation: M.A.H., M.M.J., K.M.K.; Resources: A.R.S., M.A.S., K.M.K.; Data curation: M.A.H.; Writing-original draft: M.A.H., K.M.K.; Writing-review & editing: M.M.J., A.R.S., M.A.S., K.M.K.; Visualization: M.A.H.; Supervision: K.M.K.; Project administration: M.A.H.; Funding acquisition: M.A.H., A.R.S., M.A.S., K.M.K.

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Competing interests

The authors declare no competing or financial interests.

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Data availability

All volumetric data are available from Dryad (Hagadorn et al., 2021a): dryad.xksn02vfc. Relevant code is stored in GitHub: www.github.com/kapheimlab/nomia_neuroplasticity.

REFERENCES

Anzenberger, G. (1986). How do carpenter bees recognize the entrance of their nests?: An experimental investigation in a natural habitat. *Ethology*, 71(1):54–62.

Avarguès-Weber, A. and Giurfa, M. (2013). Conceptual learning by miniature brains. *Proceedings of the Royal Society B: Biological Sciences*, 280(1772):20131907.

Barth, M. and Heisenberg, M. (1997). Vision affects mushroom bodies and central complex in *Drosophila melanogaster*. *Learning & Memory*, 4(2):219–229.

Batra, S. W. (1970). Behavior of the alkali bee, *Nomia melanderi*, within the nest (hymenoptera: Halictidae). *Annals of the Entomological Society of America*, 63(2):400–406.

Batra, S. W. and Bohart, G. E. (1969). Alkali bees: response of adults to pathogenic fungi in brood cells. *Science*, 165(3893):607–608.

Blum, M. S. (1996). Semiochemical parsimony in the arthropoda. *Annual review of entomology*, 41(1):353–374.

Bohart, G. E. (1955). Time relationships in the nest construction and life cycle of the alkali be. *Annals of the Entomological Society of America*, 48(5):403–406.

Bosch, J. and Kemp, W. (2000). Development and emergence of the orchard pollinator *Osmia lignaria* (hymenoptera: Megachilidae). *Environmental Entomology*, 29(1):8–13.

Brady, S. G., Sipes, S., Pearson, A., and Danforth, B. N. (2006). Recent and simultaneous origins of eusociality in halictid bees. *Proceedings of the Royal Society B: Biological Sciences*, 273(1594):1643–1649.

Cane, J. H. (1997). Ground-nesting bees: the neglected pollinator resource for agriculture. *Acta Horticulturae*, 437:309–324.

Cane, J. H. (2008). A native ground-nesting bee (*Nomia melanderi*) sustainably managed to pollinate alfalfa across an intensively agricultural landscape. *Apidologie*, 39(3):315–323.

Danforth, B. N. (2002). Evolution of sociality in a primitively eusocial lineage of bees. *Proceedings of the National Academy of Sciences*, 99(1):286–290.

Dunbar, R. I. (1998). The social brain hypothesis. *Evolutionary Anthropology: Issues, News, and Reviews: Issues, News, and Reviews*, 6(5):178–190.

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(3):259–263.

d'Ettorre, P., Deisig, N., and Sandoz, J.-C. (2017). Decoding ants' olfactory system sheds light on the evolution of social communication. *Proceedings of the National Academy of Sciences*, 114(34):8911–8913.

Fahrbach, S. E. (2006). Structure of the mushroom bodies of the insect brain. *Annu. Rev. Entomol.*, 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(2):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(1):115–123.

Falibene, A., Roces, F., and Rössler, W. (2015). Long-term avoidance memory formation is associated with a transient increase in mushroom body synaptic complexes in leaf-cutting ants. *Frontiers in behavioral neuroscience*, 9:84.

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(16):6395–6404.

Fiala, J. C. (2005). Reconstruct: a free editor for serial section microscopy. *Journal of microscopy*, 218(1):52–61.

Fox, J. and Weisberg, S. (2019). *An R Companion to Applied Regression*. Sage, Thousand Oaks CA, third edition.

Gibbs, J., Brady, S. G., Kanda, K., and Danforth, B. N. (2012). Phylogeny of halictine bees supports a shared origin of eusociality for *Halictus* and *Lasioglossum* (apoidea: Anthophila: Halictidae). *Molecular phylogenetics and evolution*, 65(3):926–939.

Gronenberg, W. (2001). Subdivisions of hymenopteran mushroom body calyces by their afferent supply. *Journal of Comparative Neurology*, 435(4):474–489.

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(9):2011–2019.

Guédot, C., Pitts-Singer, T. L., Buckner, J. S., Bosch, J., and Kemp, W. P. (2006). Olfactory cues and nest recognition in the solitary bee *Osmia lignaria*. *Physiological Entomology*, 31(2):110–119.

Hackwell, G. A. (1967). *The biology and behavior of the alkali bee Nomia melanderi Cockerell (Hymnoptera: Apoidea)*. PhD thesis, University of Oregon, Corvallis,OR.

Hagadorn, M. A., Johnson, M. M., Smith, A. R., Seid, M. A., and Kapheim, K. M. (2021a). Data from experience, but not age is, associated with volumetric mushroom body expansion in solitary alkali bees.

Hagadorn, M. A., Johnson, M. M., Smith, A. R., Seid, M. A., and Kapheim, K. M. (2021b). Experience, but not age, is associated with volumetric mushroom body expansion in solitary alkali bees. *Journal of Experimental Biology*, 224(6). jeb238899.

Heisenberg, M., Heusipp, M., and Wanke, C. (1995). Structural plasticity in the *Drosophila* brain. *Journal of Neuroscience*, 15(3):1951–1960.

Hothorn, T., Bretz, F., and Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, 50(3):346–363.

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*, 103(1):207–211.

Jaumann, S., Seid, M. A., Simons, M., and Smith, A. R. (2019). Queen dominance may reduce worker mushroom body size in a social bee. *Developmental neurobiology*, 79(6):596–607.

Johansen, C. A., Mayer, D., and Eves, J. (1978). Biology and management of the alkali bee, *Nomia melanderi* cockerell (hymenoptera: Halictidae). *Melanderia*, 28:23–46.

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(4):250–261.

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. and Johnson, M. M. (2017a). Juvenile hormone, but not nutrition or social cues, affects reproductive maturation in solitary alkali bees (nomia melanderi). *Journal of Experimental Biology*, 220(20):3794–3801.

Kapheim, K. M. and Johnson, M. M. (2017b). Support for the reproductive ground plan hypothesis in a solitary bee: links between sucrose response and reproductive status. *Proceedings of the Royal Society B: Biological Sciences*, 284(1847):20162406.

Kolb, B. and Gibb, R. (2008). Principles of neuroplasticity and behavior. In Stuss, D., Winocur, G., and Robertson, I., editors, *Cognitive neurorehabilitation: Evidence and application*, pages 6–21. Cambridge University Press, NY, USA, Cambridge.

Kraft, N., Spaethe, J., Rössler, W., and Groh, C. (2019). Neuronal plasticity in the mushroom-body calyx of bumble bee workers during early adult development. *Developmental Neurobiology*, 79(4):287–302.

Leonhardt, S. D., Menzel, F., Nehring, V., and Schmitt, T. (2016). Ecology and evolution of communication in social insects. *Cell*, 164(6):1277–1287.

Lihoreau, M., Latty, T., and Chittka, L. (2012). An exploration of the social brain hypothesis in insects. *Frontiers in physiology*, 3:442.

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(8):733–740.

Menzel, R., Geiger, K., Chittka, L., Joerges, J., Kunze, J., and Müller, U. (1996). The knowledge base of bee navigation. *The Journal of Experimental Biology*, 199(1):141–146.

Menzel, R. and Giurfa, M. (2001). Cognitive architecture of a mini-brain: the honeybee. *Trends in cognitive sciences*, 5(2):62–71.

Michener, C. D. (1974). *The Social Behavior of the Bees: a comparative study*. Harvard University Press, Cambridge, MA.

Michener, C. D. (1990). Reproduction and castes in social halictine bees. In Engels, W., editor, *Social insects: an evolutionary approach to castes and reproduction*, volume 77, page 122. Berlin: Springer.

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(2):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(7):950–959.

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*, 108(17):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(1):39–46.

O'Donnell, S., Donlan, N. A., and Jones, T. A. (2004). Mushroom body structural change is associated with division of labor in eusocial wasp workers (polybia aequatorialis, hymenoptera: Vespidae). *Neuroscience letters*, 356(3):159–162.

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(8):1–9.

Packer, L. (2005). The influence of marking upon bee behaviour in circle tube experiments with a methodological comparison among studies. *Insectes sociaux*, 52:139–146.

Pahlke, S., Seid, M. A., Jaumann, S., and Smith, A. (2021). The loss of sociality is accompanied by reduced neural investment in mushroom body volume in the sweat bee *Augochlora pura* (hymenoptera: Halictidae). *Annals of the Entomological Society of America*, 114(5):637–642.

Pitts-Singer, T. L. (2008). Past and present management of alfalfa bees. In James, R. R. and Pitts-Singer, T. L., editors, *Bee pollination in agricultural ecosystems*, pages 105–123. New York: Oxford University Press.

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(2):117–124.

Riveros, A. J. and Gronenberg, W. (2010). Brain allometry and neural plasticity in the bumblebee bombus occidentalis. *Brain, behavior and evolution*, 75(2):138–148.

Robinson, G. E. (1992). Regulation of division of labor in insect societies. *Annual review of entomology*, 37(1):637–665.

Seid, M. A., Harris, K. M., and Traniello, J. F. (2005). Age-related changes in the number and structure of synapses in the lip region of the mushroom bodies in the ant *Pheidole dentata*. *Journal of Comparative Neurology*, 488(3):269–277.

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 Wehner, R. (2009). Delayed axonal pruning in the ant brain: a study of developmental trajectories. *Developmental Neurobiology*, 69(6):350–364.

Shimron, O., Hefetz, A., and Tengo, J. (1985). Structural and communicative functions of dufour's gland secretion in *Eucera palestinae* (hymenoptera; anthophoridae). *Insect biochemistry*, 15(5):635–638.

Smith, A. R., DeLory, T., Johnson, M. M., Figgins, A. C., Hagadorn, M. A., and Kapheim, K. M. (2019). Small body size is associated with increased aggression in the solitary sweat bee *Nomia melanderi* (hymenoptera, halictidae). *Journal of Insect Behavior*, 32:306–314.

Smith, A. R., Seid, M. A., Jiménez, 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(1691):2157–2163.

Smith, A. R., Wcislo, W. T., and O'Donnell, S. (2007). Survival and productivity benefits to social nesting in the sweat bee *Megalopta genalis* (hymenoptera: Halictidae). *Behavioral Ecology and Sociobiology*, 61:1111–1120.

Strausfeld, N. J., Sinakevitch, I., Brown, S. M., and Farris, S. M. (2009). Ground plan of the insect mushroom body: Functional and evolutionary implications. *Journal of Comparative Neurology*, 513(3):265–291.

Tomé, H. V. V., Rosi-Denadai, C. A., Pimenta, J. F. N., Guedes, R. N. C., and Martins, G. F. (2014). Age-mediated and environmentally mediated brain and behavior plasticity in the stingless bee *Melipona quadrifasciata anthidioides*. *Apidologie*, 45(5):557–567.

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(1866):20171594.

Venables, W. N. and Ripley, B. D. (2002). *Modern Applied Statistics with S*. Springer, New York, fourth edition.

Wcislo, W. T. (1992). Nest localization and recognition in a solitary bee, *Lasioglossum (Dialictus) figueresi* wcislo (hymenoptera: Halictidae), in relation to sociality. *Ethology*, 92(2):108–123.

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(3):377–387.

Wcislo, W. T. and Engel, M. S. (1996). Social behavior and nest architecture of nomiine bees (hymenoptera: Halictidae; nomiinae). *Journal of the Kansas entomological Society*, pages 158–167.

Wenseleers, T. and van Zweden, J. S. (2017). Sensory and cognitive adaptations to social living in insect societies. *Proceedings of the National Academy of Sciences*, 114(25):6424–6426.

Wilson, E. O. (1971). *The insect societies*. Cambridge, MA: Belknap.

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(1):130–144.

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* (megachilidae). *Developmental neurobiology*, 68(1):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(6434):238–240.

Wittwer, B., Hefetz, A., Simon, T., Murphy, L. E., Elgar, M. A., Pierce, N. E., and Kocher, S. D. (2017). Solitary bees reduce investment in communication compared with their social relatives. *Proceedings of the National Academy of Sciences*, 114(25):6569–6574.

Zukor, K. A., Kent, D. T., and Odelberg, S. J. (2010). Fluorescent whole-mount method for visualizing three-dimensional relationships in intact and regenerating adult newt spinal cords. *Developmental dynamics*, 239(11):3048–3057.

Figures

Fig. 2.1: ***Nomia melanderi* confocal microscopy brain image**. (A) A 5 μm optical slice taken in the frontal plane. Mushroom bodies (mb) and antennal lobes (al) are visible. The dotted line represents the mushroom body region enlarged in B to show the Kenyon cells (kc), lip (l), collar (c), basal ring (br) and pedunculus (p). (C) Three-dimensional serial reconstruction of individual traces. Volumetric measurements between treatments were compared for the Kenyon cells (pink), lips (gray), collar (orange), mushroom body lobes (basal ring+peduncle+ventral lobe+medial lobe; purple) and antennal lobes (green). D, dorsal; V, ventral.

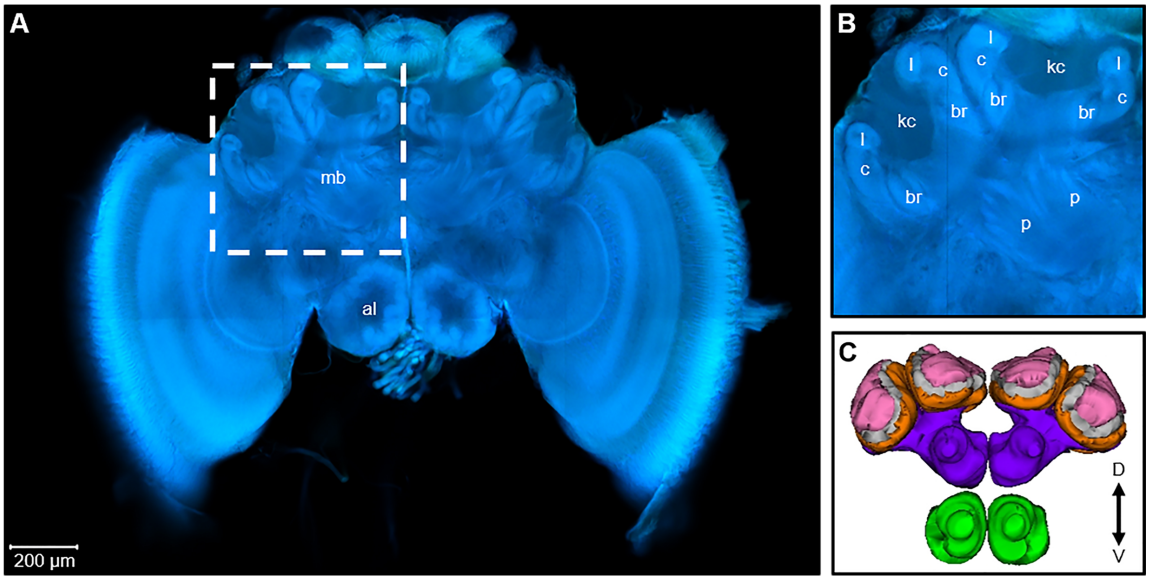


Fig. 2.2: **Effects of age and nesting experience on alkali bee neuroplasticity.** (A) Mushroom body neuropils ($F_{2,18}$=20.50, P=2.29×10^{-05}) were larger in females with nesting experience, whereas no significant differences were found in the Kenyon cells (neural cell bodies) ($F_{2,18}$=0.64, P=0.54) or antennal lobes ($F_{2,18}$=3.01, P=0.07). Volumes are reported as proportions of the whole brain. (B) Neuropil:Kenyon cell ratio also varied with treatment group ($F_{2,18}$=15.83, P=1.08×10^{-04}). Different letters indicate significant differences between groups (P<0.05 in Tukey *post hoc* tests). Boxes represent the interquartile range, with the lines as medians. Whiskers extend to 1.5 times the interquartile range. Filled circles are individual data points. Treatment groups included newly emerged (NE; white boxes; blue circles; N=7), 10 days laboratory-reared (LR; gray boxes; dark red circles; N=7), and nesting (NS; dark gray boxes; orange circles, N=7) females.

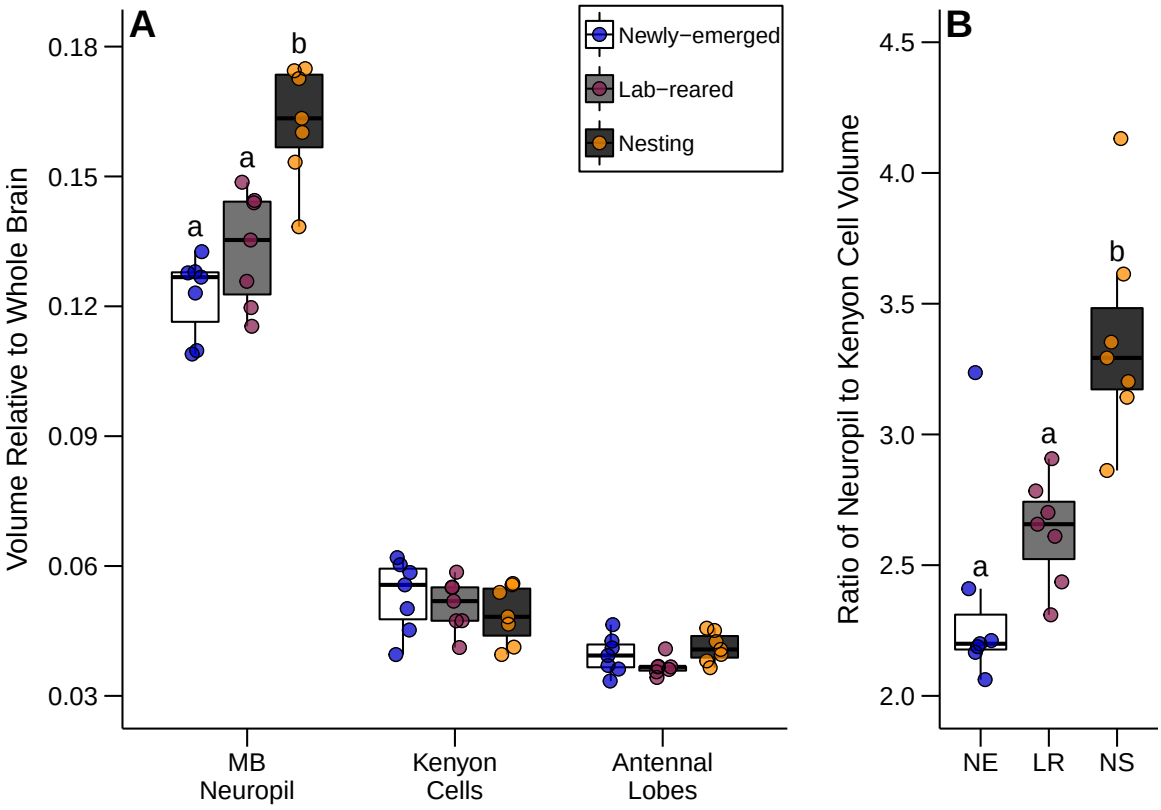


Fig. 2.3: Effects of age and nesting experience on alkali bee mushroom body subregions. Relative volume of the lip ($F_{2,18}$=30.52, P=1.65×10^{-06}), collar ($F_{2,18}$=12.82, P=3.46×10^{-04}), calyx (lip+collar; $F_{2,18}$=24.86, P=6.63×10^{-06}) and mushroom body lobes (basal ring+peduncle+ventral lobe+medial lobe; $F_{2,18}$=10.81, P=8.24×10^{-04}) were significantly larger in nesting (dark gray boxes, orange circles; N=7) compared with 10 days laboratory-reared (gray boxes, dark red circles; N=7) and newly emerged females (white boxes, blue circles; N=7). Volumes are reported as proportion of the whole brain. Different letters indicate significant differences between groups (P<0.05 in Tukey *post hoc* tests). Boxes represent the interquartile range, with the lines as medians. Whiskers extend to 1.5 times the interquartile range. Filled circles are individual data points.

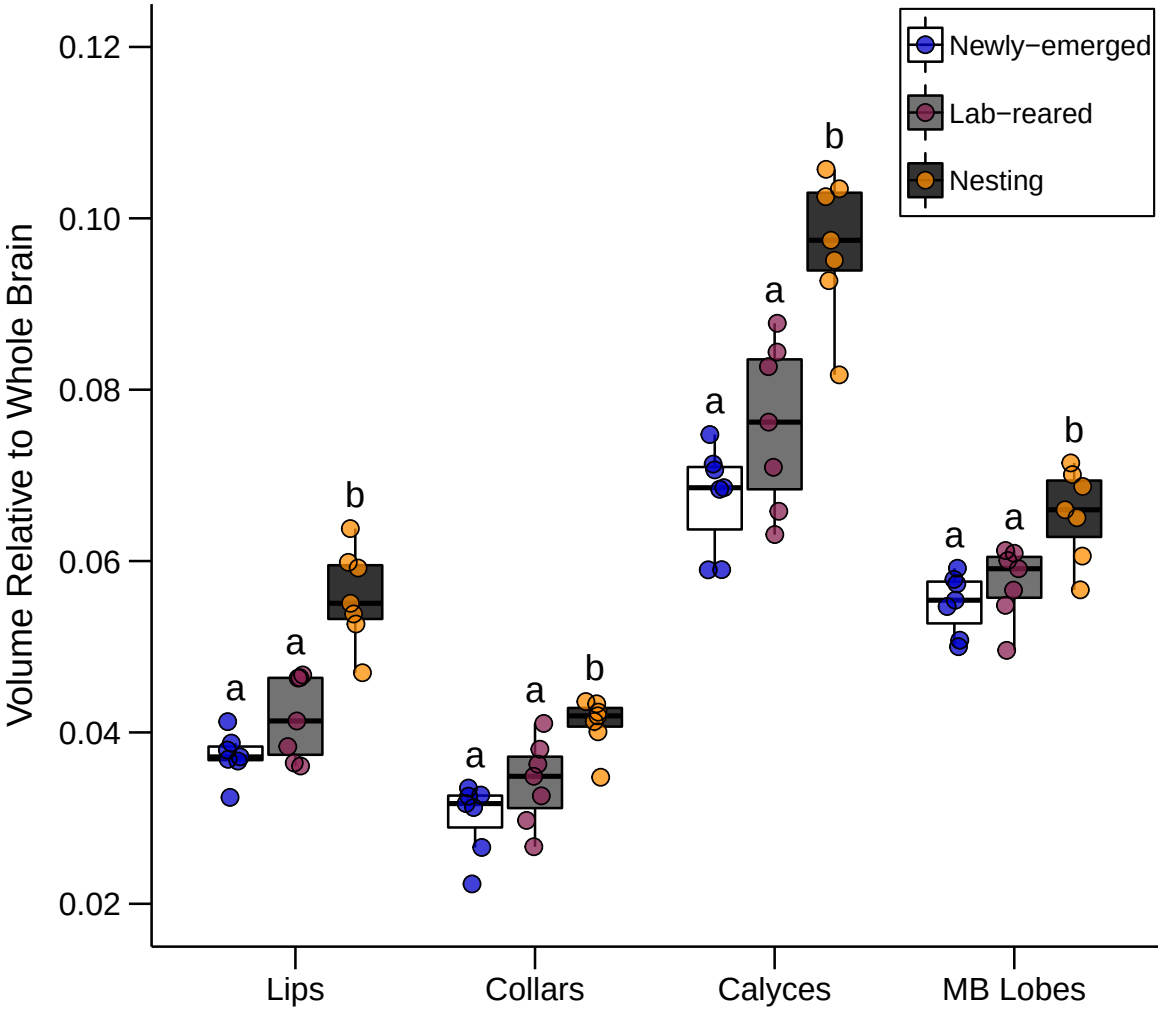


Fig. 2.4: **Effects of social environment on alkali bee neuroplasticity**. (A) Relative volumes of mushroom body neuropil (t=-1.23, d.f.=38, P=0.23), Kenyon cells (t=-0.30, d.f.=38, P=0.77) and antennal lobes (t=-0.79, d.f.=38, P=0.44) were not significantly different between females reared alone ('solo') and with a cage-mate ('paired'). Volumes are reported as proportion of the whole brain. (B) Neuropil:Kenyon cell ratio also did not differ significantly between groups (t=-1.01, d.f.=38, P=0.32). Boxes represent the interquartile range, with the lines as medians. Whiskers extend to 1.5 times the interquartile range. Filled circles are individual data points. Treatment groups included solo (white boxes, green circles; N=17) and paired (gray boxes, purple circles; N=23) females.

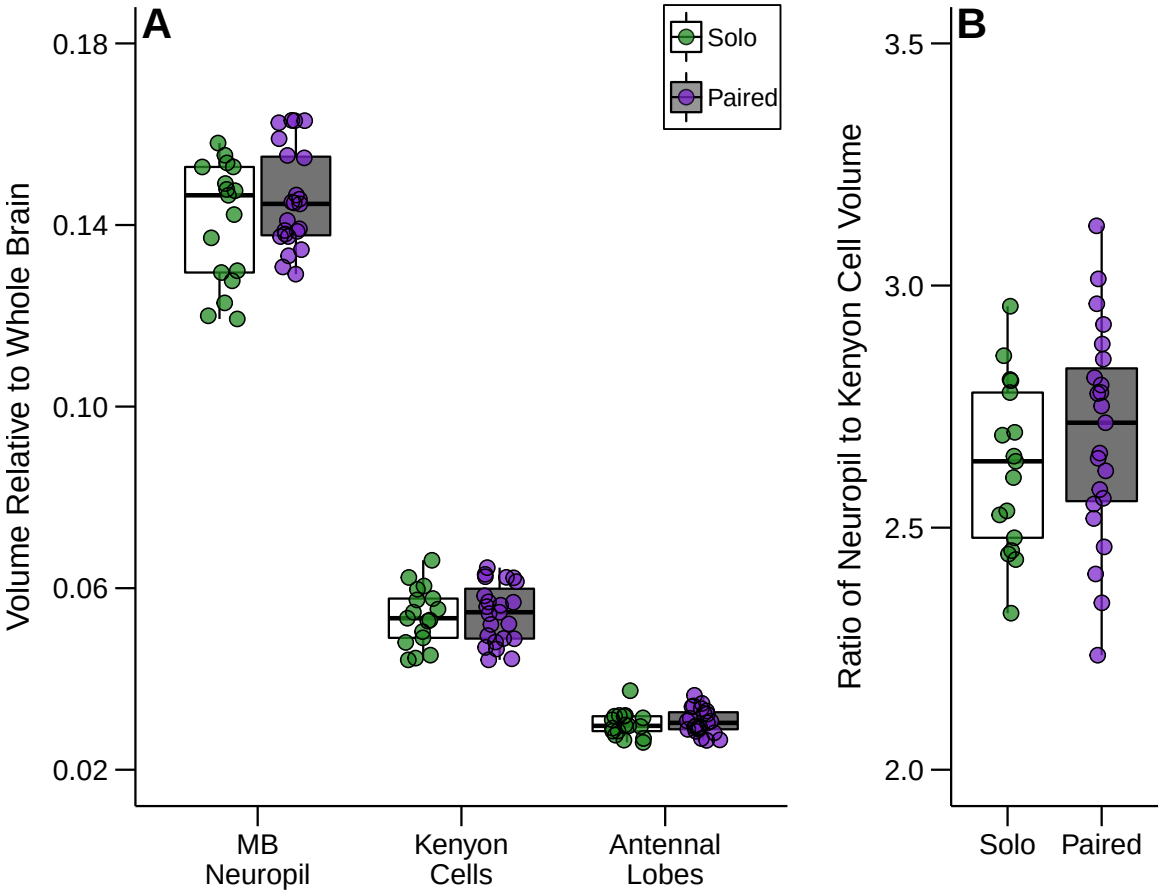
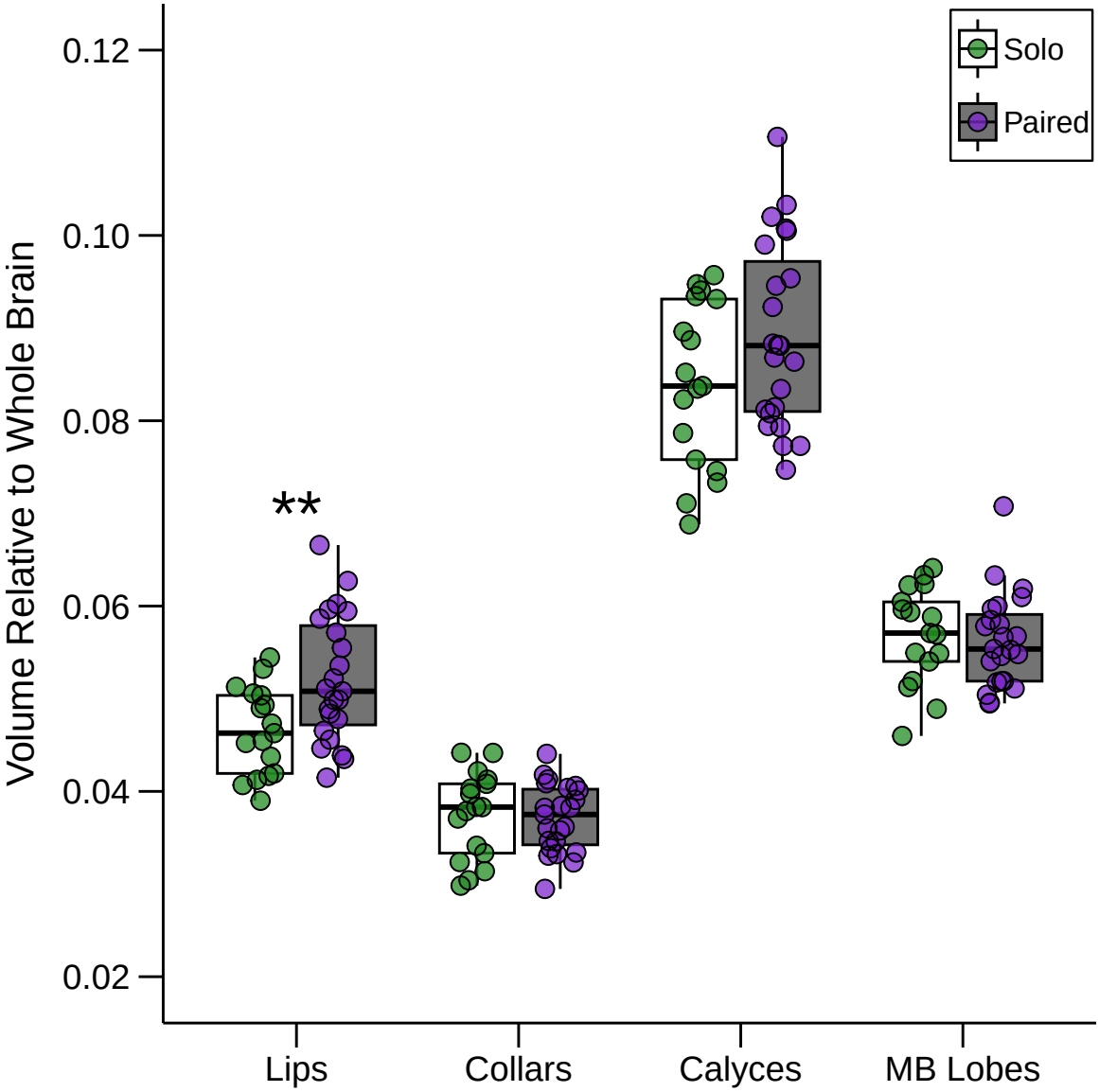


Fig. 2.5: **Effects of social environment on alkali bee mushroom body subregions**. Relative lip volume was significantly larger (t=-2.90, d.f.=38, P=0.01) in females reared with a cage-mate (paired; gray boxes, purple circles; N=23) than those kept alone (solo; white boxes, green circles; N=17). Collar (t=0.22, d.f.=38, P=0.82), calyx (lip+collar; t=-1.73, d.f.=38, P=0.09) and mushroom body lobe (basal ring+peduncle+ventral lobe+medial lobe; t=0.33, d.f.=38, P=0.74) volumes did not significantly differ between the two groups. Volumes are reported as proportion of the whole brain. Boxes represent the interquartile range, with the lines as medians. Whiskers extend to 1.5 times the interquartile range. Filled circles are individual data points. $^{**}P$<0.01.



CHAPTER 3

AGE-RELATED MUSHROOM BODY EXPANSION IN MALE SWEAT BEES AND BUMBLE BEES [2]

Abstract

A well-documented phenomenon among social insects is that brain changes occur prior to or at the onset of certain experiences, potentially serving to prime the brain for specific tasks. This insight comes almost exclusively from studies considering developmental maturation in females. As a result, it is unclear whether age-related brain plasticity is consistent across sexes, and to what extent developmental patterns differ. Using confocal microscopy and volumetric analyses, we investigated age-related brain changes coinciding with sexual maturation in the males of the facultatively eusocial sweat bee, *Megalopta genalis*, and the obligately eusocial bumble bee, *Bombus impatiens*. We compared volumetric measurements between newly eclosed and reproductively mature males kept isolated in the lab. We found expansion of the mushroom bodies—brain regions associated with learning and memory—with maturation, which were consistent across both species. This age-related plasticity may, therefore, play a functionally-relevant role in preparing male bees for mating, and suggests that developmentally-driven neural restructuring can occur in males, even in species where it is absent in females.

Introduction

Some structural and functional brain changes (i.e., neuroplasticity) occur independent of experience, as a natural part of development (Kolb and Gibb, 2008). This age-related, 'experience-expectant' neuroplasticity likely primes neural systems to anticipate predictable

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life events (Frankenhuis and Nettle, 2020; Greenough et al., 1987). Age-related expansion of key brain regions have been well documented in many highly social Hymenoptera, including ants (Gronenberg et al., 1996; Seid et al., 2005), bees (Durst et al., 1994; Fahrbach et al., 1997, 1998; Farris et al., 2001; Jones et al., 2013; Tomé et al., 2014; Withers et al., 1995, 1993) and wasps (Molina and O'Donnell, 2008; O'Donnell et al., 2004). These studies, however, have focused almost exclusively on females (Beani et al., 2014; Gronenberg and Riveros, 2009), and it is unclear to what extent age-related neuroplasticity occurs in males. The function and drivers of age-related brain plasticity are likely different for males and females, and a female-biased focus could thus limit our understanding of how and why neuroplasticity evolves.

We redressed this bias by characterizing age-related neuroplasticity in the males of two bee species within the timespan of reproductive maturation. Cognitive demands prior to nest departure may be minimal in males, which could be accompanied by delayed neural development early in life. However, activities associated with mating and reproductive success likely require enhanced cognitive abilities (Beani et al., 2014). For example, learning odors to avoid in-breeding with close kin, identifying individual females, remembering previously non-receptive mates, orienting spatially and navigating between aerial leks, as well as specializing on certain reproductive tactics may all require adaptive neural reorganization with male maturation (Barrett et al., 2021; Barrows et al., 1975; Fletcher and Michener, 1987; Sovrano et al., 2013, 2012; Wcislo, 1987; Woodgate et al., 2021).

We investigated the effects of age on neuroplasticity in males of two bee species. In the facultatively eusocial sweat bee *Megalopta genalis* (Halictidae), females nest either solitarily or in small groups (Wcislo et al., 2004). These social strategies tend to differ in patterns of sex ratio investment (Smith et al., 2019), but all females are potential mates for males. In the obligately eusocial bumble bee *Bombus impatiens* (Apidae), males mate only with reproductive gynes and not the abundant workers present among large colonies (Goulson, 2010). In both species, males remain in the nest for at least a few days following eclosion, and eventually leave or are ejected, presumably as they become reproductively mature (Goulson,

2010; Kapheim et al., 2013). This period may also be accompanied by neural maturation, but this has never been investigated. To test this hypothesis, we compared mushroom body volumes of aged, mature males relative to young, newly eclosed males. Mushroom bodies are neuropil in the insect brain associated with sensory integration, learning, and memory (Fahrbach, 2006). If neuroplasticity coincides with reproductive maturation, then mushroom bodies should expand with age, independent of experience.

Results

Males of both *M. genalis* and *B. impatiens* exhibit age-related expansion of the mushroom bodies. In *M. genalis*, the relative volumes of calyces were 16.6% higher in mature males than in newly eclosed males (Fig. 3.1a; t = -2.23, df = 12, p = 0.046, Hedges' g = 1.20). Total mushroom body neuropil was 14.7% larger in mature males than in newly eclosed males (Fig. 3.1a; t = -2.30, df = 12, p = 0.040, Hedges' g = 1.24). Although, after adjusting for multiple comparisons, these results are not statistically significant. The ratio of Neuropil to Kenyon cell (N:K) was also higher in mature males than in newly eclosed males (Fig. 3.2a; t = -4.32, df = 12, p = 0.001, Hedges' g = 2.34). In *B. impatiens*, mature males had 24.5% larger calyces (Fig. 3.1b; t = -4.34, df = 16, p = 0.0005, Hedges' g = 2.08), and 19.5% larger mushroom body neuropil (Fig. 3.1b; t = -3.84, df = 16, p = 0.001, Hedges' g = 1.85) than newly eclosed males. *Bombus impatiens* N:K ratios were significantly higher in mature males than in newly eclosed males (Fig. 3.2b; t = -5.12, df = 16, p = 0.0001, Hedges' g = 2.48). Kenyon cells were not significantly different in relative volume between mature and newly eclosed males of either species (Fig. 3.1a,b; *M. genalis*: t = 1.20, df = 12, p = 0.255, Hedges' g = -0.65; *B. impatiens*: t = 1.25, df = 16, p = 0.228, Hedges' g = -0.75). Likewise, mushroom body lobes relative volume were not significantly different between mature and newly eclosed males of either species (Fig. 3.1a,b; M. *genalis*: t = -1.41, df = 12, p = 0.185, Hedges' g = 0.76 and *B. impatiens*: t = -1.42, df = 16, p = 0.175, Hedges' g = 0.69). Results for calyx volume were similar when normalized to Kenyon cell volume instead of whole brain, such that the calyces were larger in mature males in both species, and mushroom body lobes were significantly enlarged in

mature *B. impatiens* males when normalized to Kenyon cell volume (see Appendix C).

Discussion

We found strikingly similar patterns of age-related neuroplasticity in *M. genalis* and *B. impatiens* males. In both species, expansion of mushroom body structures, including calyx and neuropil, occurred with age under experimental conditions void of ecologically-relevant experience. Our work adds to the sparse literature on volumetric neuroplasticity in Hymenopteran males while improving our general understanding of potential functions of experience-expectant neuroplasticity in insects.

Our results provide the first definitive evidence that large-scale volumetric neuroplasticity is driven by age, independent of relevant experience (e.g., social, flight, etc.), in male Hymenoptera. Previous research has documented mushroom body expansion associated with the combination of maturation and experience in paper wasp(Molina and O'Donnell, 2008) and honey bee (Fahrbach et al., 1997) males. Similar to our results, paper wasp and honey bee calyx and neuropil volumes increase, respectively, as males mature. Developmental maturation was observed with flight initiation, social interaction, and mating behavior in these species (Fahrbach et al., 1997; Molina and O'Donnell, 2008). But, since the individual effects of age and experience were not experimentally controlled for in these previous studies, they could not be evaluated independently. Mature males in our study were experimentally deprived of flight, social cues, and mating experience. We aimed to isolate experience-independent from experience-dependent brain changes during adult development. However, social deprivation can adversely affect eusocial insects, leading to impaired brain development, learning, and behaviors (Cabirol et al., 2017; Maleszka et al., 2009; Seid and Junge, 2016). Therefore, we cannot eliminate the possibility that the volumetric plasticity observed may include effects associated with unnatural rearing conditions, inadvertent stress, or unidentified experiences. Nevertheless, our results show that, across multiple species with different rearing conditions, male brains change relatively consistently with age, independent of ecologically-relevant experience, suggesting that age-related neuroplasticity may be common in male Hymenoptera.

Age-related mushroom body expansion coincides with reproductive maturation in male bees, and may represent a common developmental change associated with dispersal from the nest prior to the onset of mating. A primary function of male Hymenoptera is to inseminate a female(s) (Beani et al., 2014; Boomsma et al., 2005; Heinze and Schrempf, 2008; Hrassnigg and Crailsheim, 2005; Michener and Michener, 1974; Wilson, 1971), and experience-expectant neuroplasticity may potentially facilitate this behavior. Our approach cannot identify precisely when age-related brain changes occurred in either species. However, bumble bee (*B. vosnesenskii*) males reach reproductive maturity by 8–10 days post-eclosion (Herndon, 2022), and we observed significant mushroom body expansion in *B. impatiens* males after 10 d of aging. Scent-marking and patrolling is the most common pre-mating strategy in Bombus (Goulson, 2010), whereby males pheromonally mark points along a flight route (Alford, 1975; Goulson, 2010; Valterová et al., 2019). Patrolling is similar to "trap-line" foraging (Goulson, 2010), which is associated with the phylogenetic expansion of mushroom bodies in *Heliconius* butterfly species that also exhibit age-related brain plasticity (Montgomery et al., 2016). Female bumble bees utilize learned aspects of their environment for spatial orientation (Sovrano et al., 2013, 2012), and males have learning capabilities equivalent to females (Muth et al., 2021); therefore, while speculative, the brain changes observed with *Bombus* male maturation may be an important preparation for the potential cognitive challenges related to mate finding behaviors. Similarly, we found that mature (6-d old) sweat bee males had enlarged mushroom bodies relative to newly eclosed males. The mating behavior of *M. genalis* is unknown. However, males typically stay in their natal nest for up to 4 d past emergence (Kapheim et al., 2013), during which time they are fed via trophallaxis by their mothers and sisters (Kapheim et al., 2016). It is presumably during this time that they are becoming reproductively mature. The males of some Halictine species exhibit mate patrolling (Barrows, 1976), but it is unknown whether *M. genalis* conduct these behaviors. In honey bees, neuropil expansion coincides with the time that males reach sexual maturity (6–12 d) (Fahrbach et al., 1997; Harbo, 1986; Snodgrass, 1956). Age-related neuroplasticity observed in male paper wasps (*M.*

mastigophorus) may also coincide with reproductive maturity (Molina and O'Donnell, 2008). Males of this species are atypical of other social insects in that they remain on their natal nests long after eclosion, departing only temporarily to mate (O'Donnell, 1999; O'Donnell et al., 2021). The age at first nest departure (median = 5 d (Molina and O'Donnell, 2009)), however, is still comparable with those of the bees studied here. Our study was not designed to identify the functional relevance of age-related brain development in males, but instead provides new insight for subsequent work. Thus, while the functional roles of age-related plasticity remain unclear, our study and previous studies suggest that the age-related neuroplasticity observed within males across species may be associated with departing the nest in search of mating opportunities—a predictably timed, common event driving the male life-cycle. This pattern of expansion is similar to the 'experience-expectant' neuroplasticity observed in the females of some, but not all, social insects.

Our results also suggest that intraspecific sex differences in age-related neuroplasticity patterns can occur among some social insect species. Neuroanatomical changes in the female workers of highly social bees accompany shifts in colony needs (Durst et al., 1994; Fahrbach et al., 1998; Farris et al., 2001; Tomé et al., 2014; Withers et al., 1995, 1993) coinciding with age-related behavioral transitions from working inside the hive to foraging (Farris et al., 2001; Ismail et al., 2006; Withers et al., 1993). Yet, this age-related task specialization is not universal across social species. In bumble bees, where division of labor is size-based instead of age-related, females exhibit mushroom body expansion within the first few days of life (Jones et al., 2013; Kraft et al., 2019; Riveros and Gronenberg, 2010), which accompanies their capacity for behavioral maturation soon after emergence (Heinrich, 2004). These changes are similar to those observed in our mature *B. impatiens* males. However, experience-expectant neuroplasticity is absent in *M. genalis* females (Jaumann et al., 2019) (though see (Smith et al., 2010)), which also lack age-related task specialization (Smith et al., 2019; Wcislo et al., 2004). Our finding that mushroom body expansion occurs with maturation in male *M. genalis* suggests that experience-expectant

neuroplasticity can occur in males, even when it is absent in females. Future work comparing sex-specific patterns of brain development in additional bee species is needed to determine the pervasiveness of intersexual differences in neuroplasticity. Investigating socioecological drivers of neuroplasticity in both sexes, particularly in solitary species where females lack age-related plasticity (Hagadorn et al., 2021c; Withers et al., 2008), will provide a more robust understanding of the relationship between neuroplasticity and social evolution.

Methods

Field collections and laboratory rearing

We conducted the experiment for *Megalopta genalis* from March to May 2015 on Barro Colorado Island (BCI), Republic of Panama. Twice daily—once in the morning and evening—we collected newly eclosed males from their natal nests, which consisted of dead sticks or branches (Wcislo et al., 2004). We randomly assigned newly eclosed males to either 'newly eclosed' or 'mature' treatment groups. Bees designated as 'newly eclosed' ($N = 8$) were sacrificed within minutes, whereas 'mature' ($N = 6$) males were housed individually in food storage containers for 6 d in an incubator (27°C, 70%, 0:24 L:D) and provided food (36% sugar, 7% protein w/v) *ad libitum*. Food was mixed by dissolving six Nature's Blend Protein tablets (National Vitamin Company, Casa Grande, AZ) in 50 ml distilled water and changed twice daily.

During August to December 2018, we produced *B. impatiens* males from queenless microcolonies ($N = 11$) generated using three commercial colonies from Koppert Biological Systems (Howell, MI, USA). Microcolonies consisted of five *B. impatiens* workers from the same source colony that were housed in custom rearing cages: 173 × 130 × 91 mm food storage containers that included aluminum mesh bottoms and hinged plexiglass tops. We supplied microcolonies with 50% sugar water (cane sugar dissolved in distilled water) supplemented with potassium sorbate, citric acid, Honey B Healthy Essential Oil, and Honey B Healthy Amino Boost, as well as pollen dough *ad libitum*. Our pollen dough

consisted of honey bee collected pollen (Betterbee, Greenwich, NY, USA) mixed with the aforementioned sugar water until it reached a consistency similar to moist, slightly tacky fine-grained sand. We stored microcolonies in an incubator maintained at 27°C and ∼ 60–70% relative humidity on a 16:8 h light:dark cycle. Male brood require ∼ 24 d to develop before eclosion (Cnaani et al., 2002). We checked brood development and for newly eclosed males daily. After eclosion, we randomly assigned new males to one of two treatment groups: 'newly eclosed' ($N = 11$) males were sacrificed immediately, whereas 'mature' ($N = 7$) males were maintained individually for 10 d in the rearing cages and conditions described above.

Where applicable, we followed the recommended guidelines for animal care and use (Percie du Sert et al., 2020).

Preservation and dissection

For each species, we terminated males via decapitation after immobilizing them on ice for ∼ 5 min. We removed eye capsules and mouthparts to facilitate preservation. Head capsules were preserved in 4% zinc paraformaldehyde (PFA) and 4% PFA for *M. genalis* and *B. impatiens*, respectively, and then stored at 4°C until dissection. Prior to dissection, we rinsed head capsules in 1X phosphate-buffered saline (PBS; 3 × 10 min), followed by brain dissections in 1 × PBS. Dissected brains were post-fixed in 2% glutaraldehyde at room temperature for 2 d. After 48 h, brains were rinsed (1X PBS; 3 × 10 min), formamide bleached for ∼ 30–45 min to remove residual pigment (1 × PBS, 3% formamide, 1% triton-X, and 20% hydrogen peroxide) (modified protocol from (Zukor et al., 2010)), rinsed again (1X PBS; 3 × 10 min), and then serially dehydrated via a series of ascending ethanol concentrations (30%, 50%, 70%, 90%, 95%, and 3 × 100%, 10 min each). Lastly, we cleared and stored brains in methyl salicylate at-20°C until imaging.

Confocal microscopy and structure tracing

We imaged brains using autofluorescence on a laser confocal microscope (Zeiss LMS 710). Whole brains were mounted in methyl salicylate and scanned as z-stack series ranging

from 760 to 925 μm thick. We imaged brains as 3 $\times$ 2 tile scans (2867 $\times$ 1946 pixels) with optical slices captured in 5 μm intervals. For both species, brains were imaged simultaneously using two lasers, though the wavelengths, laser power, and gains varied by species. We imaged *M. genalis* at 410–484 nm and 495–538 nm wavelengths, 3.5 and 3.0 power, and 504–535 and 495–517 gains for laser 1 and 2, respectively. For *B. impatiens*, the first laser had a wavelength between 410 and 485 nm, a laser power of 4.0, and a gain range between 527 and 567. The second laser had a wavelength, power, and gain range of 495–538 nm, 3.5, and 518–558. Whole brain image stacks were saved as individual jpegs.

Throughout confocal stacks, we traced individual structures on every other optical slice (10 μm intervals) and estimated volumetric measurements via serial reconstruction using Reconstruct software (Fiala 2005; version 1.1.0.0; available at http://synapses.clm.utexas.edu). Due to occasional tissue damage, we traced each structure unilaterally to maximize sample inclusion. For undamaged brains, we randomly selected either the right or left side to trace, whereas undamaged sides were always traced for brains with tissue damage. The number of right and left side brain traces were distributed similarly across treatment groups (*M. genalis*: Yates corrected χ^2 (1, N = 14) = 0.29, p = 0.589 and *B. impatiens*: Yates corrected χ^2 (1, N = 16) = 0.02, p = 0.896). Whole brain traces were also conducted unilaterally, corresponding with the side used for structure tracing, and always excluded the lamina and retina (Fig. 3.3). We conducted all confocal imaging, tracing, and 3D reconstruction without knowledge of the experimental treatment group to which each sample belonged.

The structures examined included the calyces (lip, collar, and basal ring as one structure), mushroom body lobes (peduncle and lobes as one structure), total neuropil (calyces and mushroom body lobes), and Kenyon cells (Fig. 3.3). Neuropil to Kenyon cell volumetric increases can occur with age-related plasticity (Fahrbach, 2006; Withers et al., 1995, 2008, 1993); therefore, we also assessed neuropil:Kenyon cell ratios (N:K). For each sample, we standardized volumes using two methods (see Appendix C): 1) structure volumes to whole brain, referred to as 'relative volumes', and 2) structure volumes to

Kenyon cells (results reported Supplemental Materials).

Statistical analyses

All statistical analyses were conducted in *R* version 4.0.4 (https://www.r-project.org/). We assessed the relative volumes (structure:wholebrain), structure:KC, and N:K ratios using Student's *t*-tests (*stats*, version 4.0.4) to compare 'newly eclosed' and 'mature' bees independently for each species. We used visual inspection of qq-plots (*car*, version 3.0–10; Fox and Weisberg 2019) and Anderson–Darling normality tests (*Nortest*, version 1.0–4; https://CRAN.R-project.org/package=nortest) to verify normality assumptions. One variable—relative calyx volume for *B. impatiens*—violated normality assumptions, so we applied a Box-Cox transformation (*MASS*, version 7.3–53; Venables and Ripley 2002) using λ = -1.455. We assessed homogeneity of variance using *R* package *car* (version 3.0–10; Fox and Weisberg 2019). Relative Kenyon cell volume violated variance assumptions, therefore we conducted a second Box-Cox transformation using λ = -0.970. We determine effect size between groups by calculating Hedges' *g* (*effsize*, version 0.8.1, Torchiano 2016). To account for multiple comparisons, we applied a Bonferroni correction and adjusted statistical significance to $\alpha = 0.01$.

Data availability

The data are available on Dryad (Hagadorn et al., 2021a) and the code is stored in GitHub: https://github.com/kapheimlab/male_neuroplasticity.

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Author information

Matthew Del Grosso is deceased.

Contributions

K.M.K., M.D.G., K.E., and M.A.H. designed experiments. K.M.K., M.A.H., and W.T.W. contributed resources. M.D.G. and K.E. conducted experiments. M.A.H., M.D.G., K.E., and X.H. contributed to data acquisition. M.A.H. did data analysis and prepared figures. M.A.H. and K.M.K. wrote the main manuscript text. M.A.H. prepared supplementary text. K.E., X.H., and W.T.W. edited the manuscript. M.A.H., K.E., X.H., W.T.W., and K.M.K. reviewed the manuscript.

Competing interests

The authors declare no competing interests.

REFERENCES

Alford, D. V. (1975). *Bumblebees*. Davis-Poynter.

Barrett, M., Schneider, S., Sachdeva, P., Gomez, A., Buchmann, S., and O'Donnell, S. (2021). Neuroanatomical differentiation associated with alternative reproductive tactics in male arid land bees, *Centris pallida* and *Amegilla dawsoni*. *Journal of Comparative Physiology A*, 207:497–504.

Barrows, E. M. (1976). Mating behavior in halictine bees (hymenoptera: Halictidae): I, patrolling and age-specific behavior in males. *Journal of the Kansas Entomological Society*, pages 105–119.

Barrows, E. M., Bell, W. J., and Michener, C. D. (1975). Individual odor differences and their social functions in insects. *Proceedings of the National Academy of Sciences*, 72(7):2824–2828.

Beani, L., Dessì-Fulgheri, F., Cappa, F., and Toth, A. (2014). The trap of sex in social insects: from the female to the male perspective. *Neuroscience & Biobehavioral Reviews*, 46:519–533.

Boomsma, J. J., Baer, B., and Heinze, J. (2005). The evolution of male traits in social insects. *Annu. Rev. Entomol.*, 50:395–420.

Cabirol, A., Brooks, R., Groh, C., Barron, A. B., and Devaud, J.-M. (2017). Experience during early adulthood shapes the learning capacities and the number of synaptic boutons in the mushroom bodies of honey bees (*Apis mellifera*). *Learning & Memory*, 24(10):557–562.

Cnaani, J., Schmid-Hempel, R., and Schmidt, J. (2002). Colony development, larval development and worker reproduction in bombus impatiens cresson. *Insectes Sociaux*, 49:164–170.

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(3):259–263.

Fahrbach, S. E. (2006). Structure of the mushroom bodies of the insect brain. *Annu. Rev. Entomol.*, 51:209–232.

Fahrbach, S. E., Giray, T., Farris, S. M., and Robinson, G. E. (1997). Expansion of the neuropil of the mushroom bodies in male honey bees is coincident with initiation of flight. *Neuroscience letters*, 236(3):135–138.

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(1):115–123.

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(16):6395–6404.

Fiala, J. C. (2005). Reconstruct: a free editor for serial section microscopy. *Journal of microscopy*, 218(1):52–61.

Fletcher, D. J. and Michener, C. D. (1987). *Kin recognition in animals.* John Wiley & Sons, Chichester, UK.

Fox, J. and Weisberg, S. (2019). *An R Companion to Applied Regression.* Sage, Thousand Oaks CA, third edition.

Frankenhuis, W. E. and Nettle, D. (2020). Integration of plasticity research across disciplines. *Current Opinion in Behavioral Sciences*, 36:157–162.

Goulson, D. (2010). *Bumblebees: behaviour, ecology, and conservation.* Oxford University Press on Demand.

Greenough, W. T., Black, J. E., and Wallace, C. S. (1987). Experience and brain development. *Child Development*, 58:539–559.

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(9):2011–2019.

Gronenberg, W. and Riveros, A. (2009). Social brains and behavior, past and present. In Jürgen Gadau, J. F., editor, *Organization of Insect Societies: From Genome to Sociocomplexity*. Harvard University Press, Cambridge, MA.

Hagadorn, M., Eck, K., Del Grosso, M., Haemmerle, X., Wcislo, W., and Kapheim, K. (2021a). Data from: Age-related mushroom body expansion in male sweat bees and bumble bees.

Hagadorn, M. A., Eck, K., Del Grosso, M., Haemmerle, X., Wcislo, W. T., and Kapheim, K. M. (2021b). Age-related mushroom body expansion in male sweat bees and bumble bees. *Scientific Reports*, 11(1):17039.

Hagadorn, M. A., Johnson, M. M., Smith, A. R., Seid, M. A., and Kapheim, K. M. (2021c). Experience, but not age, is associated with volumetric mushroom body expansion in solitary alkali bees. *Journal of Experimental Biology*, 224(6).

Harbo, J. (1986). Propagation and instrumental insemination. *TE Rinderer ed*, pages 361–389.

Heinrich, B. (2004). *Bumblebee economics*. Harvard University Press.

Heinze, J. and Schrempf, A. (2008). Aging and reproduction in social insects–a mini-review. *Gerontology*, 54(3):160–167.

Herndon, J. D. (2022). *Investigating nest box utilization by bumble bees and reproductive development of male bumble bees*. Masters thesis, Utah State University.

Hrassnigg, N. and Crailsheim, K. (2005). Differences in drone and worker physiology in honeybees (*Apis mellifera*). *Apidologie*, 36(2):255–277.

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*, 103(1):207–211.

Jaumann, S., Seid, M. A., Simons, M., and Smith, A. R. (2019). Queen dominance may reduce worker mushroom body size in a social bee. *Developmental neurobiology*, 79(6):596–607.

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(4):250–261.

Kapheim, K. M., Chan, T.-Y., 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., 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.

Kolb, B. and Gibb, R. (2008). Principles of neuroplasticity and behavior. In Stuss, D., Winocur, G., and Robertson, I., editors, *Cognitive neurorehabilitation: Evidence and application*, pages 6–21. Cambridge University Press, NY, USA, Cambridge.

Kraft, N., Spaethe, J., Rössler, W., and Groh, C. (2019). Neuronal plasticity in the mushroom-body calyx of bumble bee workers during early adult development. *Developmental Neurobiology*, 79(4):287–302.

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(8):733–740.

Michener, C. D. and Michener, C. D. (1974). *The Social Behavior of the Bees: a comparative study*. Harvard University Press, Cambridge, MA.

Molina, Y. and O'Donnell, S. (2008). Age, sex, and dominance-related mushroom body plasticity in the paperwasp *Mischocyttarus mastigophorus*. *Developmental Neurobiology*, 68(7):950–959.

Molina, Y. and O'Donnell, S. (2009). Males exhibit novel relationships of dominance with nest departure in the social paper wasp *Mischocyttarus mastigophorus* (hymenoptera: Vespidae). *Ethology*, 115(8):738–746.

Montgomery, S. H., Merrill, R. M., and Ott, S. R. (2016). Brain composition in *Heliconius* butterflies, posteclosion growth and experience-dependent neuropil plasticity. *Journal of Comparative Neurology*, 524(9):1747–1769.

Muth, F., Tripodi, A. D., Bonilla, R., Strange, J. P., and Leonard, A. S. (2021). No sex differences in learning in wild bumblebees. *Behavioral Ecology*, 32(4):638–645.

O'Donnell, S., Donlan, N. A., and Jones, T. A. (2004). Mushroom body structural change is associated with division of labor in eusocial wasp workers (*Polybia aequatorialis*, hymenoptera: Vespidae). *Neuroscience letters*, 356(3):159–162.

O'Donnell, S. (1999). The function of male dominance in the eusocial wasp, *Mischocyttarus mastigophorus* (hymenoptera: Vespidae). *Ethology*, 105(3):273–282.

O'Donnell, S., Fiocca, K., and Congdon, R. (2021). Social network analysis of male dominance in the paper wasp *Mischocyttarus mastigophorus* (hymenoptera: Vespidae). *Journal of Insect Behavior*, 34(3):106–113.

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., Clark, A., Cuthill, I. C., Dirnagl, U., et al. (2020). The arrive guidelines 2.0: Updated guidelines for reporting animal research. *Journal of Cerebral Blood Flow & Metabolism*, 40(9):1769–1777.

Riveros, A. J. and Gronenberg, W. (2010). Brain allometry and neural plasticity in the bumblebee bombus occidentalis. *Brain, behavior and evolution*, 75(2):138–148.

Seid, M. A., Harris, K. M., and Traniello, J. F. (2005). Age-related changes in the number and structure of synapses in the lip region of the mushroom bodies in the ant *Pheidole dentata*. *Journal of Comparative Neurology*, 488(3):269–277.

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

Smith, A. R., Kapheim, K. M., Kingwell, C. J., and Wcislo, W. T. (2019). A split sex ratio in solitary and social nests of a facultatively social bee. *Biology Letters*, 15(4):20180740.

Smith, A. R., Seid, M. A., Jiménez, 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(1691):2157–2163.

Snodgrass, R. E. (1956). *Anatomy of the honey bee*. Comstock Publishing Associates.

Sovrano, V. A., Potrich, D., and Vallortigara, G. (2013). Learning of geometry and features in bumblebees (*Bombus terrestris*). *Journal of Comparative Psychology*, 127(3):312.

Sovrano, V. A., Rigosi, E., and Vallortigara, G. (2012). Spatial reorientation by geometry in bumblebees. *PloS one*, 7(5):e37449.

Tomé, H. V. V., Rosi-Denadai, C. A., Pimenta, J. F. N., Guedes, R. N. C., and Martins, G. F. (2014). Age-mediated and environmentally mediated brain and behavior plasticity in the stingless bee *Melipona quadrifasciata anthidioides*. *Apidologie*, 45(5):557–567.

Torchiano, M. (2016). Effsize - a package for efficient effect size computation.

Valterová, I., Martinet, B., Michez, D., Rasmont, P., and Brasero, N. (2019). Sexual attraction: a review of bumblebee male pheromones. *Zeitschrift für Naturforschung C*, 74(9-10):233–250.

Venables, W. N. and Ripley, B. D. (2002). *Modern Applied Statistics with S*. Springer, New York, fourth edition.

Wcislo, W. T. (1987). The role of learning in the mating biology of a sweat bee *Lasioglossum zephyrum* (hymenoptera: Halictidae). *Behavioral Ecology and Sociobiology*, 20:179–185.

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(3):377–387.

Wilson, E. O. (1971). *The insect societies*. Belknap, Cambridge, MA.

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(1):130–144.

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* (megachilidae). *Developmental neurobiology*, 68(1):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(6434):238–240.

Woodgate, J. L., Makinson, J. C., Rossi, N., Lim, K. S., Reynolds, A. M., Rawlings, C. J., and Chittka, L. (2021). Harmonic radar tracking reveals that honeybee drones navigate between multiple aerial leks. *Iscience*, 24(6):102499.

Zukor, K. A., Kent, D. T., and Odelberg, S. J. (2010). Fluorescent whole-mount method for visualizing three-dimensional relationships in intact and regenerating adult newt spinal cords. *Developmental dynamics*, 239(11):3048–3057.

Figures

Fig. 3.1: Mushroom body (MB) expansion occurs with maturation in male bees. Relative volumes of the mushroom body structures as whole brain proportions for (a) *M. genalis* and (b) *B. impatiens*. In both species, mature males had larger calyces (lip + collar + basal ring; *M. genalis*: t = -2.23, df = 12, p = 0.046; *B. impatiens*: t = -4.34, df = 16, p = 0.0005) and mushroom body neuropil (peduncles + lobes + calyces; *M. genalis*: t = -2.30, df =12, p = 0.04; *B. impatiens*: t = -3.84, df = 16, p = 0.001) relative to newly eclosed bees. Dots represent individual data points for newly eclosed (NE; white boxes; *M. genalis* green dots, N = 8; *B. impatiens* orange dots, N = 11) and mature (gray boxes; *M. genalis* purple dots, N = 6; *B. impatiens* blue dots, N = 7) males. Boxes indicate interquartile range, lines are medians, and whiskers extend to 1.5 the interquartile range. "*" = unadjusted $p < 0.05$ and "**" = unadjusted $p < 0.001$.

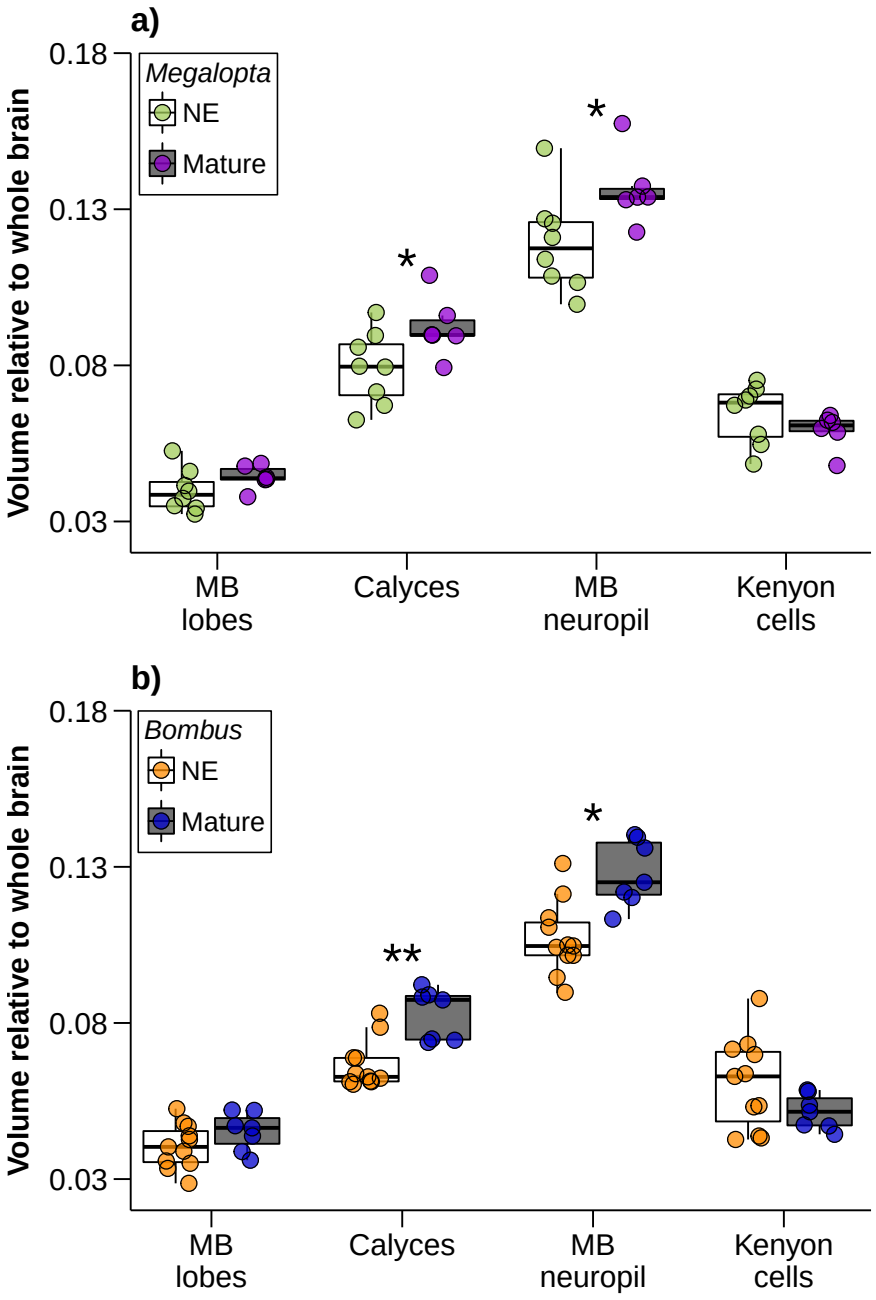


Fig. 3.2: Age-related neuroplasticity in male bees. Neuropil:Kenyon cell ratios are significantly higher in mature, relative to newly eclosed, (a) *M. genalis* (t = -4.32, df = 12, p = 0.001) and (b) *B. impatiens* (t = -5.12, df = 16, p = 0.0001) bees. Dots represent individual data points for newly eclosed (NE; white boxes; *M. genalis* green dots, $N = 8$; *B. impatiens* orange dots, $N = 11$) and mature (gray boxes; *M. genalis* purple dots, $N = 6$; *B. impatiens* blue dots, $N = 7$) males. Boxes indicate interquartile range, lines are medians, and whiskers extend to 1.5 the interquartile range. "*" = unadjusted $p < 0.05$ and "**" = unadjusted $p < 0.001$.

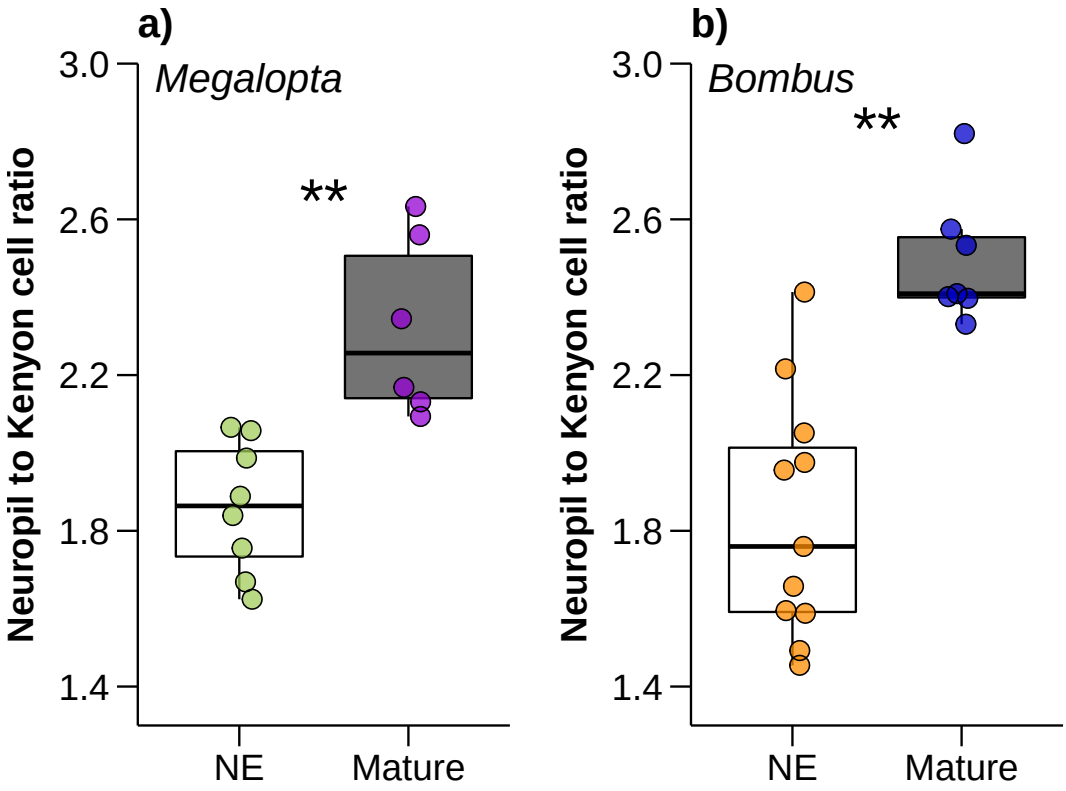
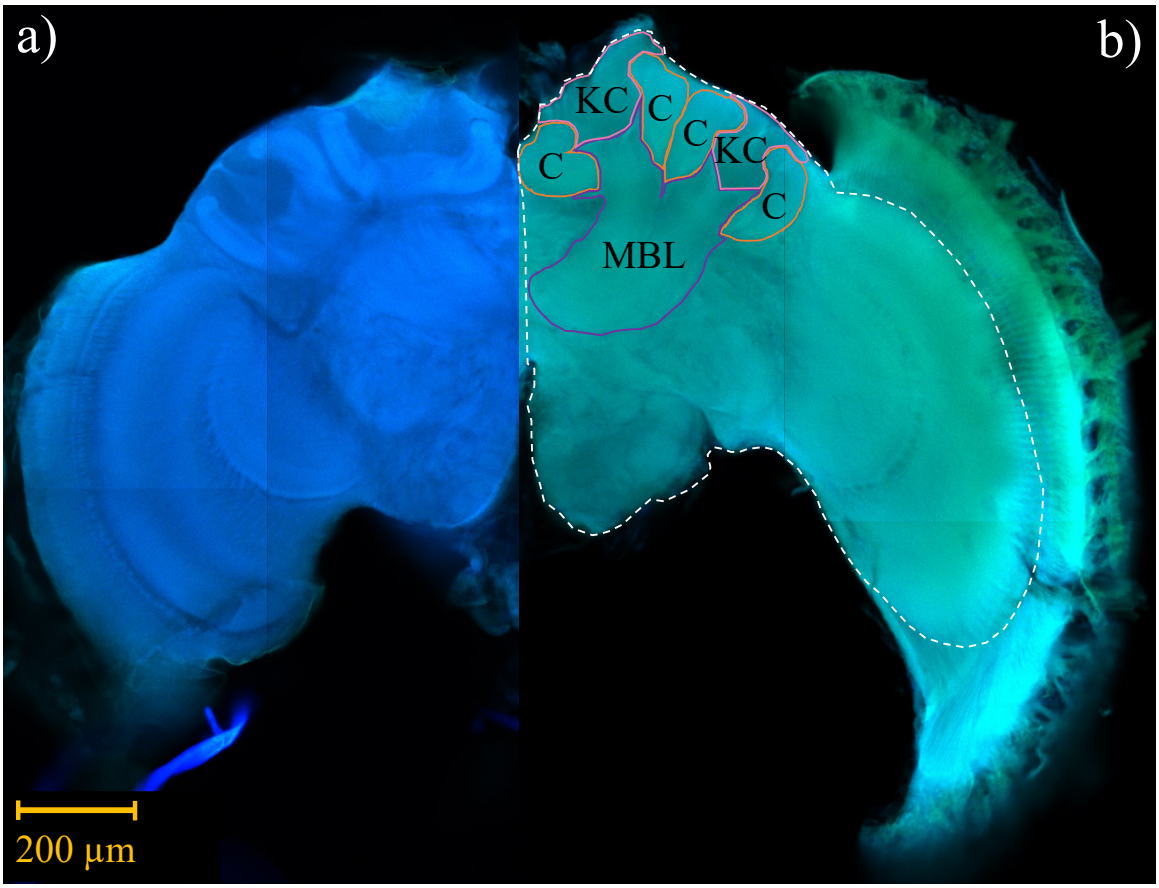


Fig. 3.3: Confocal microscope image of a (a) *Bombus impatiens* and (b) *Megalopta genalis* male brain. Image captures are individual slices taken from raw image stacks. Volumetric measurements were assessed for mushroom body calyces (C; lip, collar, and basal ring as one structure), Kenyon cells (KC), and mushroom body lobes (MBL; peduncle and lobes as one structure). Solid contour lines include structure-specifc boundaries (pink (KC), orange (C), and purple (MBL)), whereas the dotted white line indicates the boundary for a whole brain trace. Scale bar 200 μm.



Supplemental Materials

These supplementary materials accompany Hagadorn *et al.*(2021), and are published online.

Alternative Scaling Methods

Whole brain volumes for *M. genalis*, were not significantly different between groups (Supplementary Fig. S1); t = 1.14, df = 12, p = 0.28). However, mature *B. impatiens* males had significantly larger (23.8%) whole brain volumes compared to newly-eclosed individuals (Supplementary Fig. S1); t = -3.08, df = 16, p = 0.007). We investigated potential methodical factors that may have affected whole brain volumes using Spearman's rho correlation analysis. These results suggested no relationship among whole brain volume and the duration (in days) of storage in PFA before dissection (r_s = -0.34, p = 0.172), number of days between dissection and imaging (r_s = 0.08, p = 0.753), and when brains were traced (r_s =-0.11, p = 0. 654). Thus, in both species, we also normalized the absolute calyx and mushroom body lobe volumes to the Kenyon cells (calyx:KCs and mblobes:KCs; sensu) to verify results. We applied a Bonferroni correction for multiple comparisons and adjusted the significance threshold to α = 0.025. Calyx:Kenyon cells volume of mature males was significantly larger than newly-eclosed males in both *M. genalis* (Supplementary Fig. S2); t = -4.83, df = 12, p = 0.0004, Hedges' g = 2.61) and B. impatiens (Supplementary Fig. S2); t = -4.91, df = 16, p = 0.0002, Hedges' g = 2.37). Mature *B. impatiens* males also had enlarged (26.6%) MB lobe:Kenyon cell volume relative to newly-eclosed bees (Supplementary Fig. S2); t = -3.11, df = 16, p = 0.01, Hedges' g = 1.50). A 20.5% increase in MB lobe:Kenyon cell volume was also observed in mature *M. genalis* males, but the difference is not significant (Supplementary Fig. S2); t = -2.13, df = 12, p = 0.055, Hedges' g = 1.15).

Supplemental Figures

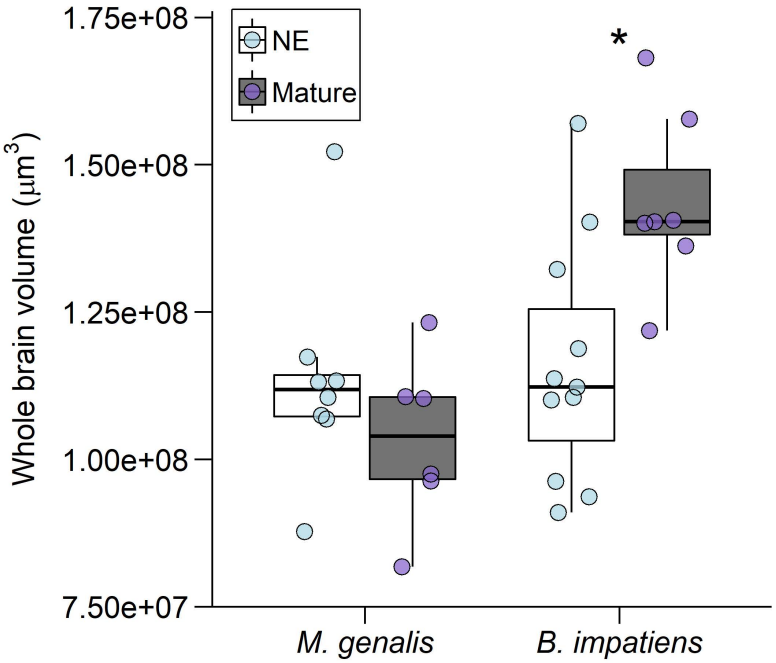


Fig. 3.4: S1. Whole brain volumes by species. Dots represent individual data points for newly-eclosed (NE; white boxes; light blue dots) and mature (gray boxes; light purple dots) males. "*" = unadjusted $p < 0.05$. Boxes represent the interquartile range, with the lines as medians. Whiskers extend to 1.5 the interquartile range.

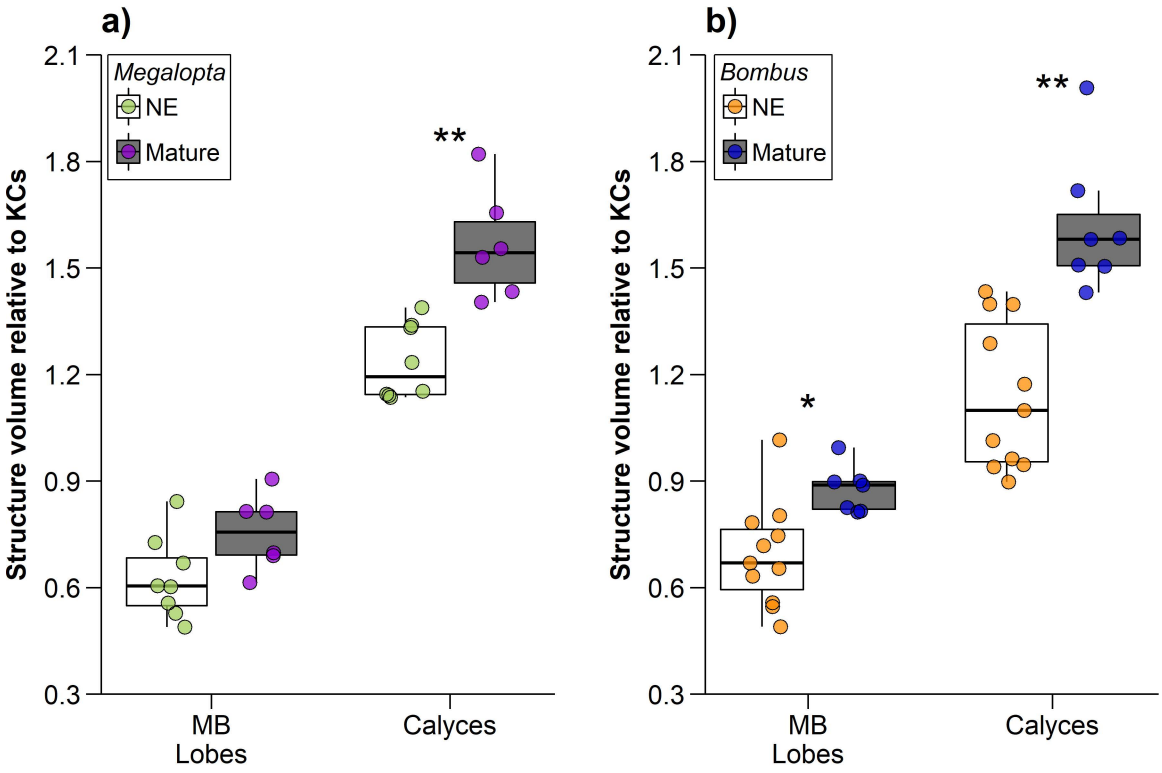


Fig. 3.5: S2. Age-related neuroplasticity for mushroom body structures standardized to Kenyon cell (KC) volumes. Mushroom body lobes and calyx proportions for a) *Megalopta genalis* and b) *Bombus impatiens*. Dots represent individual data points for newly-eclosed (NE; white boxes; *M. genalis* = green dots; *B. impatiens* = yellow dots) and mature (gray boxes; *M. genalis* = purple dots; *B. impatiens* = blue dots) males. "*" = unadjusted $p < 0.05$ and "**" = unadjusted $p < 0.001$. Boxes indicate interquartile range, lines are medians, and whiskers extend to 1.5 the interquartile range.

CHAPTER 4

A QUEEN-LIKE BRAIN: QUEEN AND WORKER BUMBLE BEE BRAINS RESPOND DIFFERENTLY TO EGG-LAYING IN THE ABSENCE OF BROOD CARE[3]

Abstract

In eusociality, cooperative brood rearing often leads to reproductive dominance by a few, while the majority of colony individuals forego their own direct fitness benefits to care for siblings. Sibling care behaviors are thought to be developmentally homologous to, and evolutionarily derived from, maternal care behaviors. One hypothesis for the origin of these alternative female castes is the heterochronic decoupling of reproduction and maternal care over evolutionary time. This decoupling may have facilitated worker engagement in brood care behaviors prior to the onset of egg-laying. A prediction of this hypothesis is that similar neurodevelopmental and neuromolecular mechanisms should regulate maternal and sibling care. These behaviors, therefore, may also be accompanied by similar brain architecture changes (i.e., a maternal brain). Yet, little is known about how maternal and sibling-care behavior influences social insect brains, limiting our understanding of how advanced cooperation evolves. Using behavioral manipulations, confocal microscopy, and volumetric analyses, we address this knowledge gap by exploring neuroplasticity in response to egg-laying and caregiving in bumble bees (*Bombus impatiens*). We compared brain structure volume in queens and workers to measure the effects of reproduction and brood care. We found that neither reproduction nor brood care significantly impact the volume of individual structures within a caste. Interestingly, however, the brains of queens and workers seem to respond differently to egg-laying in the absence of brood care, a hallmark of queen-like behavior and reproductive dominance. This work yields novel insight regarding drivers

[3]The following co-authors contributed to this work: Makenna M. Johnson Bird, Karlee Eck, Anna C. Figgins, Thuy-Tien T. Lindsay, Erica J. Brus, James P. Strange, and Karen M. Kapheim.

of cooperation in bumble bees while furthering our understanding of factors influencing sociality.

Introduction

Maternal care is a widespread phenomenon observed throughout nature. And, while the form and depth may vary taxonomically (Royle et al., 2016), the underlying function is the same—increase offspring, and thereby parental, fitness (Clutton-Brock, 1991). However, the transition to a maternal state is complex. Females must quickly and accurately adapt to the new demands of caring for offspring (Kinsley et al., 2008; Pereira and Ferreira, 2016). As such, this process frequently involves extensive behavioral and physiological changes coordinated by the brain (Kinsley et al., 2008; Kinsley and Lambert, 2006; Pereira and Ferreira, 2016), as well as structural and functional brain plasticity (Kinsley et al., 2008; Kinsley and Lambert, 2006; Leuner et al., 2010; Navarro-Moreno et al., 2022; Pereira, 2016; Pereira and Ferreira, 2016). This maternal-associated neuroplasticity, the development of a maternal brain, is predicted to prepare females for caring for and responding to the changing needs of their offspring (Pereira and Ferreira, 2016). Yet, how this phenomenon translates to non-maternal caregivers is still relatively understudied. Social insects offer a unique opportunity for exploring this question because siblings, not mothers, are the primary caregivers (Wilson et al., 1971). Using social bees, we aim to start closing this gap by exploring brain plasticity associated with maternal and sibling care behaviors.

Cooperative offspring care is a defining feature of eusociality (Batra, 1966). In social insects, this cooperation leads to a reproductive division of labor and, thereby, alternative female castes. More specifically, reproduction is dominated by a few females (i.e., the queens) while the majority forego direct fitness benefits to care for their siblings (i.e., the workers) (Michener, 1969; Michener and Michener, 1974; Wilson et al., 1971). These caregiving behaviors vary, ranging from provisioning resources to thermoregulation and, in some species, actively feeding offspring. Interestingly, sibling care behaviors are also thought to be developmentally homologous to and evolutionarily derived from maternal care behaviors (Evans and Eberhard, 1970; West-Eberhard, 1987, 1996). The developmental

decoupling of reproductive and maternal care over evolutionary time—i.e., the disassociation of egg-laying and caregiving behaviors from one another—has been posited as a mechanism for caste formation in social insects (Evans and Eberhard, 1970). With the origin of castes, instead of these behaviors being exhibited cyclically by a single female, maternal care is now expressed almost exclusively by workers while queens dominate reproduction (Evans and Eberhard, 1970). Therefore, this decoupling may have facilitated pre-reproductive maternal care expression in the worker caste (Evans and Eberhard, 1970; Linksvayer and Wade, 2005).

One critical prediction underlying the heterochrony hypothesis above is that maternal and sibling care behaviors should be regulated by similar neuromolecular and neurodevelopmental mechanisms. Prior studies investigating this focused exclusively on neurogenomic state changes using brain gene expression (Rehan et al., 2014; Toth et al., 2010, 2007; Woodard et al., 2014). Some support for a maternal brain across castes has been observed in paper wasps (Toth et al., 2010, 2007) and carpenter bees (Rehan et al., 2014), but not in bumble bees (Woodard et al., 2014). Paper wasp workers and foundresses performing caregiving behaviors as sisters and mothers, respectively, have more similar whole-brain gene expression profiles relative to those individuals in the colony that do not care for offspring (Toth et al., 2007). This implies the genes underlying brood care are similar between mothers and siblings. Alternatively, the expression profiles in bumble bee foundresses and queens are more similar to each other than to workers (Woodard et al., 2014), suggesting that caste is a better predictor of brain gene expression than is being a caregiver. Exploring both the genetic and developmental basis of sibling care behaviors (Linksvayer and Wade, 2005), however, is required to fully understand how maternal care, and its elaborations, develop in the brain. Thus, while these studies greatly inform our understanding of brain changes occurring over relatively short timescales, i.e., minutes to hours, we still lack foundational knowledge regarding maternal care-associated patterns of neurodevelopmental plasticity, which may occur more slowly. Integrating insight about transitions in maternal care at multiple timescales will yield a more well-rounded view of the relationship between behavioral heterochrony, maternal brains, and caste evolution.

Hitherto, the maternal brain has been best studied in vertebrates, where several elaborate forms of neural plasticity are known to accompany the transition to parenting. Some of these brain changes occur at smaller scales, e.g., altered patterns of neurogenesis (Eid et al., 2019; Rolls et al., 2008), cell proliferation, and dendrite morphology (Hillerer et al., 2014a; Kinsley and Lambert, 2006), while others are more widespread, large-scale rearrangements. For instance, the maternal circuity, an interconnected network of brain structures implicated in the regulation of maternal care and enhanced offspring recognition (Bridges, 2015; Lee et al., 1999; Numan, 1974, 2012), undergoes massive reorganization (Pereira, 2016). Additionally, significant hormonally-induced structural and functional plasticity occurs at the onset of parenting. These changes are predicted to prime the maternal circuitry for responding to offspring at birth and behavioral plasticity postpartum (Kim et al., 2016; Oatridge et al., 2002; Pereira, 2016; Pereira and Ferreira, 2016, and references therein). Especially interesting, developing a maternal brain may also incorporate large-scale volumetric plasticity. In humans, overall brain size decreases with pregnancy, whereas the volume of some subregions increases (Oatridge et al., 2002). Moreover, the relative brain weight and hippocampus volume of lactating female rats are significantly smaller than those of nulliparous females (Hillerer et al., 2014b). Together, these studies characterize response patterns of the vertebrate brain to changes in parental state. However, what remains unclear is the applicability of these patterns to other non-vertebrate taxa.

We aim to begin bridging this gap by investigating the impacts of egg-laying (i.e., reproduction) and brood care behaviors (i.e., maternal care) on neurodevelopment in bumble bee, *Bombus impatiens*. Bumble bees are well-suited for investigating the maternal brains in insects, as well as the neural basis of pre-reproductive maternal care expression in workers. First, they are obligately simple eusocial (Michener, 1969), with annual colonies that generally consist of a single reproductive queen who founded the nest, her non-reproductive daughters (workers; though see Li et al., 2023), and future reproductives (gynes and males) that ultimately leave the nest (Alford et al., 1975; Goulson, 2010). Second, given the annual cycle, both female castes will naturally exhibit brood care, but at different life cycle

stages (Goulson, 2010). Queens rear the first worker generation. After worker emergence, queens cease maternal care and focus almost exclusively on egg-laying while the new workers conduct brood care (Goulson, 2010). Lastly, as with queens, egg-laying in workers can occur (Free, 1955; Goulson, 2010; Li et al., 2023). Specifically, when workers are isolated from the queen their ovaries can develop, stimulating the laying of unfertilized eggs (Free, 1955). These life history traits allow for the simultaneous evaluation of maternal-like brain states across castes. We hypothesized that common neurodevelopmental signatures associated with maternal care behaviors are present across castes. We predicted that both egg-laying (prediction 1) and brood care experience (prediction 2) would significantly impact the volume of substructures in queen and worker bumble bee brains. Additionally, given that they conduct brood care in the absence of reproduction, we predicted that workers would have brains "primed" for conducting brood care behaviors (prediction 3). Our results expand our understanding of the role that neuroplasticity may have played in facilitating cooperative brood care, a defining feature of eusocial life. Further, we are among the first to explore maternal brains in insects; thus, our results also yield foundational insight regarding how maternal behaviors get translated to the brain in a new, non-vertebrate taxon.

Materials and Methods

Study Organism and Queen Preparation

To evaluate the neurodevelopment of the maternal brains in *Bombus impatiens*, we generated samples from eight queenright (i.e., containing a queen) commercial colonies (Koppert Biological Systems, Howell, MI, USA) purchased in 2018. Callow (< 24-h old) gynes were removed from four late-stage colonies (i.e., reproductive pupae present) while callow workers were removed from the four remaining colonies.

Within 24 hours of emergence, gynes were removed from source colonies and housed in rearing containers (150 mm x 150 mm x 100 mm) with age-matched sisters that included pollen dough and enhanced sugar water ad libitum. The sugar water contained 50% sucrose (cane sugar dissolved in distilled water) supplemented with citric acid, potassium sorbate,

Honey B Healthy Essential Oil, and Honey B Healthy Amino Boost (see Rowe et al. (2023) for a detailed description). Our pollen dough was a mixture of finely-ground honey bee-collected pollen (Betterbee, Greenwich, NY, USA) and enhanced sugar water with a consistency similar to moist, slightly tacky fine-grained sand. We maintained gynes in these conditions for 10 d post-emergence, giving them time to sclerotize and mature. After 10 d, we attempted to mate gynes by placing individuals into new cages with on average 6 males (ranging from 13 to 2) of varying age (> 10 d old) and source colonies different than their own for ∼24 h. On day 12, gynes were placed into an artificial cold store (2°C) for 14 days to mimic a short overwintering period. Once removed from cold storage, we considered these individuals' proxies for "queens", described as such from here on out, and they were randomly assigned to treatment groups.

Cage set-up and storage conditions

We used custom-rearing cages to house our bees during experimentation. These consisted of modified food storage containers (173 × 130 × 91 mm) with an aluminum mesh bottom and plexiglass top. Below each cage, we placed a lidded 4 oz portion cup containing enhanced sugar water and gave bees ad libitum access via a wicking system. In each cage, we also placed a weigh boat (44.5 x 44.5 x 9.5 mm) with a small clay nub (Sculpey Original Polymer Sculpting Clay, Stockbridge, GA, USA; prepared as per manufacture instructions) in the center to house pollen and promote egg-laying (Rowe et al., 2023), respectively. The amount of pollen we placed in each tray varied by treatment group (detailed description below). However, the bees were allowed to access the weigh boat and pollen at any time throughout the experiment.

Bees were stored in a humidity and temperature control rearing environment throughout the duration of the experimental trials. We maintained the room at 28°C and ∼60% relative humidity while under complete darkness for ∼24 hr per day. We worked under red light while in the rearing room, which minimized bee exposure to white light. However, some instances, while brief, occurred where white light usage was unavoidable.

Treatment groups and behavioral manipulations

To evaluate maternal brains in *B. impatiens* we designed an experiment where egg-laying and brood care behaviors were highly regulated and manipulated in both castes. We successfully established 3 treatment groups that targeted various combinations of egg-laying and brood care across queens and workers (3 treatments x 2 castes = 6 total treatments) (Fig. 4.1A). We established these treatment groups to mirror various generalized stages that a queen would experience during a typical cycle of colony development (Fig. 4.1B). Throughout the duration of the experimental trials, all samples were housed in cages individually.

We generated foundress-like queens and workers by inhibiting egg-laying, and consequently brood care. We prevented egg-laying by limiting pollen resources to 25 mg (+/- 1 mg) of pollen dough, which was removed and replaced every other day. With this limited pollen and rotation regiment, neither caste laid eggs; therefore, they lacked both egg-laying and brood care experience (Fig. 4.1A). Alternatively, we provided maternal-like individuals with 225 mg (+/- 1 mg) of pollen dough without replacement, which facilitated egg-laying and stimulated care giving behaviors as the brood develop (Fig. 4.1A). For the queen-like treatment groups, we also gave individuals 225mg of pollen dough. In this case, each time a specimen laid an egg, the dough was removed and replaced with 225mg of fresh pollen within 24 h. As such, these individuals were allowed to lay their own eggs, but inhibited from caring for them (Fig. 4.1A). Lastly, for our worker-like group we aimed to isolate brood care behaviors by supplementing pollen dough with brood-patches. We used 225 mg (+/- 1 mg) pollen patches containing worker-laid eggs and larvae to promote fostering, i.e., caring for another's brood, but to discourage egg-laying of their own (Fig. 4.1A).

Queens were placed into treatment groups after being removed from the artificial overwintering period. Alternatively, callow workers were placed into treatment at emergence. As such, queens are ~26 d older than experimental workers. After being placed into treatments, all bees were exposed to 72 h of natural sunlight; aiming to mimic

conditions wild queens may experience shortly after emerging from diapause. Afterward, maternal-like and queen-like samples were kept undisturbed, outside of daily egg checks, until we detected the first laid egg. Following the initiation of egg-laying, bees were maintained for 7 days; maternal-like treatments were left unmanipulated while we removed eggs with each instance of egg-laying in the queen-like group. All individuals were checked daily for eggs. The number of days until egg-lay commenced was variable across and within castes. As such, the duration of the foundress-like treatments was based on the average number of days it took for maternal-like and queen-like specimens to lay eggs in queens (29 d) and workers (22 d).

Behavioral Observations

To verify the occurrence of brood care behaviors in our experiment, we utilized a scan sampling approach. We only collected behavioral data for our maternal-like treatment groups, since these were the only groups that maintained contact with brood. Specifically, each scan was conducted over a 60-minute period with point observations at 10-minute intervals (7 total per bee per scan). After moving specimens to the observation location, we allowed them to habituate for at least an hour prior to initiating scan sampling. An ethogram for behaviors assessed is included in supplementary materials (Supplementary Table S1).

We conducted individual scans three separate times per bee. This included one survey between day 0-1, day 2-4, and day 5-7 for most bees, though the specific number of times during each interval did vary (i.e., some bees were sampled multiple times during one interval and none during another interval). We collected behavioral data for a total of 11 and 9 'maternal-like' queens and workers, respectively. We have three observations per bee ($N_{queens} = 32$ and $N_{workers} = 26$) for all but two of these samples (one queen (K18C120) and one worker (K18J419)), which were observed only twice. All 'maternal-like' queens ($N = 8$) and workers ($N = 8$) used to collect volumetric brain data are among those used for behavioral sampling.

Sample preparation and dissection

Using wet ice, we chilled queens and workers at 4°C for at least 8 and 5 min, respectively. Prior to decapitation, we removed the eye capsules and mouthparts to facilitate permeation. We placed head capsules in a 4% paraformaldehyde (PFA) (Alfa Aesar, Ward Hill, MA, USA) in 1× phosphate-buffered saline (PBS) (Ambion, Austin, TX, USA) preservative solution at 4°C until brains could be dissected. We used dissection and post-processing protocols identical to that of (Hagadorn et al., 2021a,b), but summarized here briefly. Brains were dissected from head capsules in 1X PBS, post-fixed for 48 h in 2% glutaraldehyde, formamide bleached for 20 to 55 min, serial dehydrated using ethanol, and then cleared and stored in methyl salicylate at -20°C until imaging

Confocal microscopy and brain tracing

We used autofluorescence and laser scanning confocal microscopy (Zeiss LMS 710, Jena, Germany) to generate optical slices of each bumble bee's brain. While mounted in methyl salicylate, brains were imaged as 3x2 tile scans (2867 x 1946 pixels; 1.56 sec per tile) in 8 μm intervals ultimately combining to generate approximately 800 to 1200 μm thick image stacks. Due to time and funding constraints, tile scans included most, but not all, of the brain per optical slice. Specifically, we consistently excluded the distal most regions of the optic lobes, though the amount varied per brain. Scanning was bidirectional, including two simultaneous lasers. The first utilized a wavelength of 410-483 nm, a laser power of 4.5, and gain ranges between 537 and 596, whereas the second had a wavelength, power, and a range of gains as 495-570 nm, 4.0, and between 520-581, respectively. Throughout all scans, we maintained the pinhole size at 6.00 to 6.63 airy units.

We hand-traced individual structures throughout image stacks on every other optical slice (16 μm intervals) without knowledge of the experimental treatment group assignment. Using those traces and serial reconstruction (Fiala (2005), Reconstruct version 1.1.0.0, http://synapses.clm.utexas.edu) we estimated volumetric measurements for various subregions of the mushroom bodies (the lip, collar, Kenyon cells, and the mushroom body lobes (multi-structure)) and the antennal lobes (Fig. 4.2A,B). Here, owing to

constraints with image quality, the basal ring, peduncle, and lobes were grouped as one structure, i.e., the mushroom body lobes, to promote consistency across samples. We also wanted to investigate the summation of various grouped structure volumes, including calyx (lip + collar) and total mushroom body neuropil (lip + collar + mushroom body lobes), for a total of seven response variables. We traced one final structure, designated as the "central brain", which includes the entire brain except for the optic lobes and the ocelli pigment (Fig. 4.2A,B). For each sample, we standardized structure volumes to the central brain by calculating structure:central brain ratios, subsequently referred to as "scaled volumes".

Statistical Analyses

For behavioral analyses, we initially compared 4 different binomial analysis of deviance models to determine the best fit. The first was a mixed effects model, including 'source colony' as a random effect with 'caste' and 'grouping day' as fixed effects. 'source colony' had no or minimal effects on behavioral response, therefore we removed that factor. For the second and third models, we included 'caste' and 'grouping day' and fixed effects, but compared the presence or absence of an interaction term, whereas we further simplified the final model by removing 'grouping day'. Across all observed behaviors, AIC scores were lowest for the simplest model (response caste) than that of either model containing 'grouping day'. Therefore, for our behavioral analyses, we used the proportion of occurrence data per scan sample to compare observed behaviors between queens and workers (generalized linear model; family= 'binomial').

For brain volumetric analyses, we assessed changes in relative volume (structure:central brain) across treatment groups. Across the 5 structures (lip, collar, mushroom body lobes, Kenyon cells, and antennal lobes) and the summed groupings (calyx and total mushroom body neuropil), we made comparisons using a one-way ANOVA followed by Tukey post hoc tests (multcomp, version 1.4-10; Hothorn et al. (2008)) where applicable. Normality assumptions were verified using visual inspection of qqplots (car, version 3.0-13; Fox and Weisberg (2018)) and Anderson-Darling normality tests (Nortest, version 1.0-4; https://CRAN.R-project.org/package=nortest), as well as homogeneity of variance (car,

version 3.0-13; Fox and Weisberg (2018)).

We conducted all statistical analyses using R version 4.1.2 (https://www.r-project.org/) and assessed significance at α=0.05.

Results

Brood Care Behaviors

To evaluate similarities and differences in brood care across castes, we compared behaviors between maternal-like queens and maternal-like workers. We found no occurrences of fanning, buzzing, or inactivity, and few instances of seven other behaviors, including manipulating wax, egg-laying, larval-feeding, perching, patrolling, pollen feeding, and drinking sugar water (Supplemental Fig. S10). Additionally, behaviors classified as "other" were also relatively infrequent. Inspecting and incubation behaviors were overwhelmingly the most frequently identified during scan sampling (Supplemental Fig. S10). Behaviors were quite consistent between queens and workers. Therefore, there was no effect of caste on the proportion of each behavior being conducted (Supplemental Table S2).

Volumetric Data: within a caste

We found no evidence suggesting that maternal behaviors (i.e., egg-laying and brood care) significantly impact large-scale neuroplasticity of individual structures within a caste. For mushroom body substructures, relative volume remained consistent across foundress-like, maternal-like, and queen-like queens for the lips (Tukey Post-hoc: Supplemental Table S3; overall model: $F_{5,41} = 2.98$, p = 0.02; Fig. 4.3A), collar (Tukey Post-hoc: Supplemental Table S4; overall model: $F_{5,41} = 9.96$, p = 2.63 x 10^{-6}; Fig. 4.3B), calyces (Tukey Post-hoc: Supplemental Table S5; overall model: $F_{5,41} = 7.42$, p = 4.93 x 10^{-5}; Fig. 4.3C), and mushroom body lobes (overall model: $F_{5,41} = 0.88$, p = 0.50; Fig. 4.3D). Similar patterns were observed for these structures among workers (lips: Fig. 4.3A, Supplemental Table S3; collar: Fig. 4.3B, Supplemental Table S4; calyces: Fig. 4.3C, Supplemental Table S5;

mushroom body lobes: $F_{5,41} = 0.88$, p = 0.50; Fig. 4.3D). We also found no significant differences within castes for Kenyon cells (Tukey Post-hoc: Supplemental Table S6; overall model: $F_{5,41} = 3.24$, p = 0.01; Fig. 4.4A), total mushroom body neuropil (Tukey Post-hoc: Supplemental Table S7; overall model: $F_{5,41} = 8.43$, p = 1.50 x 10^{-5}; Fig. 4.4B), or antennal lobes (Tukey Post-hoc: Supplemental Table S8; overall model: $F_{5,41} = 4.25$, p = 0.003; Fig. 4.4D).

We found only one significant within caste difference across all response variables. Queen-like queens had neuropil:Kenyon cell ratios that were 9.52% larger than foundress-like queens (Tukey Post-hoc: t = 3.14, p = 0.03, Supplemental Table S9; overall model $F_{5,41}$ = 12.37, p = 2.42 x 10^{-7}; Fig. 4.4C). The remaining within caste pairwise comparisons for N:K were not significantly different (Supplemental Table S9).

Volumetric Data: across castes

Across queens and workers, we found that queen-like behaviors (i.e., laying, but not caring for eggs) consistently influenced neuroplasticity. Post-hoc pairwise comparisons show that, relative to queen-like workers, queen-like queens had lip (t = 3.70, p = 0.008, Supplemental Table S3, Fig. 4.3A) and collar (t = 4.86, p < 0.001, Supplemental Table S4, Fig. 4.3B) volumes that were 16.8% and 18.1% larger, respectively. Similarly, total mushroom body neuropil (t = 5.13, p < 0.001, Supplemental Table S7, Fig. 4.4B) was 11% larger in queen-like queens compared to queen-like workers, and N:K ratios that were on average 22.6% higher (t = 6.66, p < 0.001, Supplemental Table S9, Fig. 4.4C). Further, the calyces and antennal lobes of queen-like queens were significantly larger than foundress-like (calyces: t = 4.86, p < 0.001; antennal lobes: t = 3.50, p = 0.01), maternal-like (calyces: t = 3.92, p = 0.004; antennal lobes: t = 3.90, p = 0.004), and queen-like (calyces: t = 4.99, p < 0.001; antennal lobes: t = 3.07, p = 0.04) workers (Supplemental Table S5 and S8, Fig. 4.3C, Fig. 4.4D). The Kenyon cell region in queen-like workers was larger than in both maternal-like (t = -3.16, p = 0.033) and queen-like (t = -3.42, p = 0.02) queens (Supplemental Table S6, Fig. 4.3A). Additionally, we found that both collar volume and N:K ratios, were greater in foundress-like queens compared to queen-like workers (collar: t

= 3.10, p = 0.04; NK: t = 3.64, p = 0.009) while, relative to foundress-like workers, these metrics were larger in maternal-like queens (collar: t = 3.86, p = 0.005; NK: t = 5.05, p < 0.001) (Supplemental Table S4 and S9, Fig. 4.3B, Fig. 4.4C).

Discussion

The cooperative rearing exhibited by siblings in social insects is thought to have evolved from maternal care behaviors, but how the brain regulates this fundamental process is still understudied. Contrary to our expectations (predictions 1 and 2), we found no evidence suggesting that maternal care behaviors impact the volumetric plasticity of individual brain structures within bumble bee queens or workers (i.e., within female castes). This included a lack of neuroplasticity with both egg-laying and brood care in the mushroom bodies, areas in the insect brain that exhibit plasticity in response to other experiences. The lack of plasticity in worker bees, i.e., no additional neural investment with maternal care behaviors, indicates that workers may actually be primed for conducting brood care behaviors (prediction 3). Yet, this same pattern was present in queens, countering our rationale for the basis of this prediction. We did, however, find a relatively consistent pattern when exploring plastic responses across castes. For all but two brain regions, queens that laid eggs, but were inhibited from caring for those eggs, had structures significantly larger than workers under the same conditions. These volumetric changes included both sub-regions of the mushroom bodies, total mushroom body neuropil, and the antennal lobes. Alternatively, no significant differences were observed between queens and workers when both egg-laying and brood care behaviors occurred. Our results identify an interesting transition that may occur in the brain with a queen-like state and suggest this transition may differ between castes. This work is among the first to shed light on the neurodevelopmental nature of maternal behaviors in bees while building our understanding of the role the brain may have played in the evolution of cooperative behaviors.

The insect brain is incredibly plastic. Over the past few decades researchers have documented large-scale changes in brain architecture that are driven by experiences, including foraging (Durst et al., 1994; Farris et al., 2001; Gronenberg et al., 1996; Hagadorn

et al., 2021b; Ismail et al., 2006; Maleszka et al., 2009; Rehan et al., 2015; Riveros and Gronenberg, 2010; Withers et al., 1995, 2008, 1993), oviposition (Van Dijk et al., 2017), and even social interactions (Hagadorn et al., 2021b; Heisenberg et al., 1995; Jaumann et al., 2019; Jernigan et al., 2021; Molina and O'Donnell, 2008; Molina and O'Donnell, 2007; O'Donnell et al., 2007; O'Donnell et al., 2017; Rehan et al., 2015; Smith et al., 2010). Mushroom bodies are paired cognitive structures in the insect brain that are associated with learning, memory, and sensory integration (Gronenberg, 2001; Strausfeld et al., 2009). Among social insects, experience-dependent changes are well-documented within these structures. Therefore, our study predominantly focused on exploring mushroom body plasticity that accompanies maternal care experience. Surprisingly, we found that neither the combination of egg-laying and brood care, nor egg-laying alone, significantly impacted mushroom body size within the queen or worker caste. When comparing volumes of foundress-like to maternal- and queen-like individuals of the same caste we saw no differences in either the lip or the collar, both subregions associated with olfactory and visual input (Fahrbach, 2006; Gronenberg, 2001), respectively. Nor did we observe differences in the calyces (lip + collar) as a whole, mushroom body lobes, or the total mushroom body neuropil. These results indicate that, unlike in vertebrates, bumble bees may lack a maternal brain, or, at least, lack an important feature that characterizes the vertebrate maternal brain (i.e., size changes). Overall, this may suggest that the decoupling of maternal traits that accompany cooperation in social insects may foster brain specialization on caste-dependent tasks without requiring increased neural investment.

The lack of a detectable effect is particularly intriguing when considering the maternal-like treatment groups. We predicted that both egg-laying and brood care behaviors (prediction 1 and 2) would lead to changes in volumetric plasticity. However, individual instances of egg-laying in bumble bees are relatively discrete behaviors (Fisher et al., 2022). Thus, it is possible those distinct, repetitive behavioral instances either do not require or impact brain plasticity or that the changes that occurred were more subtle, i.e., not resulting in large-scale structural rearrangement detectable using our methods.

Differentiating between the two will require using methodological tools that yield more fine-scale neural resolution. Relative to egg-laying, brood care incorporates multiple behaviors that occur continuously (Woodard et al 2013), including multiple forms of thermoregulation and progressive feeding. Interacting with the brood is known to impact both worker reproductive behaviors (Starkey et al., 2019) and brain gene expression patterns (Orlova et al., 2020). Nevertheless, our results indicate that caring for brood does not significantly influence structure volume, including the mushroom bodies, in bumble bee brains. Overall, our results suggest that, unlike other potentially more cognitively demanding experiences (e.g., foraging and social interactions), maternal behavior does not lead to detectable, large-scale volumetric plasticity in bumble bee brains.

Alternatively, it is also possible that the brains of social bees are prepared for providing offspring care early in life, before maternal experiences occur. Bumble bee brains naturally exhibit plasticity within the first few days of life in the absence of experience (Hagadorn et al., 2021a; Jones et al., 2013; Kraft et al., 2019; Riveros and Gronenberg, 2010). In highly eusocial species, such as honey bees, this age-related neuroplasticity occurs at the onset of shifts in behavior, preceding the initiation of foraging (Gronenberg et al., 1996; Seid and Wehner, 2009; Tomé et al., 2014; Withers et al., 1995, 2008, 1993). This suggests that developmentally regulated neural reorganization may act as a priming mechanism facilitating new colony-related tasks (Withers et al., 1995, 1993). Our foundress-like queens and workers, i.e., those lacking maternal experiences, were 29 and 22 days old, respectively, well beyond the period when age-related plasticity occurs in *Bombus impatiens* (Jones et al., 2013). Therefore, perhaps in bumble bees, this early period of neural reorganization may also include preparing females for conducting maternal behaviors. There is some indirect evidence supporting the plausibility of this idea. In honey bees, temporally-associated mushroom body expansion coincides with transitioning to conducting out-of-hive tasks (3 weeks old) (Withers et al., 1995, 1993). Nursing behaviors, on the other hand, occur in advance of this shift, within the first few days of life (Free, 1964). Significant age-related changes, independent of experience, also occur in the honey bee mushroom body within the

first week of life (Fahrbach et al., 1998). This experience-expectant brain plasticity, which is initiated well before the transition to foraging, could suggest that the early expansion of mushroom bodies may facilitate other behaviors as well, including those done by young bees, such as brood care.

Alternatively, care-giving behaviors may not require additional large-scale neural rearrangement. Volumetric analyses similar to our methods have been used repeatedly to explore intraspecific neuroplasticity (Godfrey and Gronenberg, 2019) because they yield unbiased estimates of detectable change (Withers et al., 1993). But, this approach has pitfalls, including the potential for crude extrapolations from too few brain sections or samples and large variation across individuals (Godfrey and Gronenberg, 2019). We included 7-8 samples for all treatment groups and assessed volumes for 47 individual brains. For each brain, a single observer traced distinct structures on sections 16 μm apart while unaware of the treatment group assignment. Given the size of image stacks (see methods for details), we can estimate that each brain had between 50 to 75 traceable sections. Together, these factors help mitigate sample size and tracing limitations that could hamper volumetric work, suggesting that our results are both relatively robust and biologically relevant. That said, interactions between the social environment and neural systems are complex. Thus, studies testing similar hypotheses at different biological levels will be beneficial (Jernigan and Uy, 2023). Specifically, future work investigating the impacts of maternal behavior within and across castes with finer resolution could make things more conclusive. Furthermore, mapping maternal circuitry while exploring more subtle neurodevelopmental changes in synaptic plasticity may be particularly informative when coupled with our results.

An emergent pattern in our data is that queens that lay eggs—but do not care for them—consistently invest more in brain structures than their worker counterparts. These results complement some of the neurogenomic work that has explored gene expression differences associated with the decoupling of egg-laying and brood care behaviors. Work in bumble bee *Bombus terrestris* suggests that reproductive state is a stronger force

acting on brain gene expression patterns than caregiving behaviors (Woodard et al., 2014). Ultimately, this highlights that sibling care behaviors may have originated *de novo* rather than evolving from ancestral maternal care behaviors (Woodard et al., 2014). Our neurodevelopmental results indicate a similar pattern within our queen-like groups. Queen-like queens and workers were conducting the same task (i.e., egg-laying), yet this is the only group within which we observed consistent significant differences across brain structures. Outside of the mushroom body lobes and Kenyon cells, where the pattern was absent (Fig 3d) and reversed (Fig 4a), queen-like queens always had significantly larger structures relative to queen-like workers. This suggests that even while doing the same behavior, the investment in brain structures differs depending on caste. Subsequent studies exploring this derived queen-like state across castes will shed light on how social life has influenced brain evolution.

Conclusion

Understanding the evolutionary origins of alternative female castes requires investigating both the neurodevelopmental and neuromolecular properties of caregiving behavior. However, the neurodevelopmental aspect of the insect maternal brain is still woefully understudied (Godfrey and Gronenberg, 2019). We aim to begin bridging this gap. The novelty of our approach is two-fold: we explored how the same behaviors impact neuroplasticity within and across castes, instead of just the latter, and we assessed these impacts at the neurodevelopmental level. We found that within a caste, egg-laying and brood care behaviors do not significantly impact investment in individual brain structures. This is contrary to what we predicted, but suggests that conducting these behaviors may not require increased mushroom body investment, at least with regard to large-scale volumetric plasticity. Interestingly, we did find that queens invest more in brain tissue when doing queen-like behavior, i.e., laying eggs, but not caring for them, relative to workers exhibiting the same behaviors. This pattern was consistent across the majority of structures assessed, indicating the potential selection for a derived queen-like brain in lieu of a maternal brain in social insects. This work adds to our understanding of caste

development, as well as the impacts of maternal behaviors on brain plasticity.

Conflict of Interest Statement

The authors declare no conflicts of interest.

Author Contributions

Conceptualization: MAH and KMK; Data acquisition: MAH, MMJ, KE, ACF, T-TTL, and EJB; Data curation: MAH; Formal analysis: MAH; Funding acquisition: MAH; Investigation: MAH; Methodology: MAH, T-TTL, JPS, and KMK; Project administration: MAH; Resources: MAH, JPS, and KMK; Supervision: KMK; Visualization: MAH; Writing—original draft: MAH and KMK.

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REFERENCES

Alford, D. V. et al. (1975). *Bumblebees*. Davis-Poynter.

Batra, S. W. (1966). Social behavior and nests of some nomiine bees in india (hymenoptera, halictidae). *Insectes Sociaux*, 13(3):145–153.

Bridges, R. S. (2015). Neuroendocrine regulation of maternal behavior. *Frontiers in neuroendocrinology*, 36:178–196.

Clutton-Brock, T. H. (1991). *The Evolution of Parental Care Princeton University*. Princeton University Press.

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(3):259–263.

Eid, R. S., Chaiton, J. A., Lieblich, S. E., Bodnar, T. S., Weinberg, J., and Galea, L. A. (2019). Early and late effects of maternal experience on hippocampal neurogenesis, microglia, and the circulating cytokine milieu. *Neurobiology of aging*, 78:1–17.

Evans, H. E. and Eberhard, M. J. W. (1970). *The Wasps*. University of Michigan Press.

Fahrbach, S. E. (2006). Structure of the mushroom bodies of the insect brain. *Annu. Rev. Entomol.*, 51:209–232.

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(1):115–123.

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(16):6395–6404.

Fiala, J. C. (2005). Reconstruct: a free editor for serial section microscopy. *Journal of microscopy*, 218(1):52–61.

Fisher, K., Sarro, E., Miranda, C. K., Guillen, B. M., and Woodard, S. H. (2022). Worker task organization in incipient bumble bee nests. *Animal Behaviour*, 185:143–161.

Fox, J. and Weisberg, S. (2018). *An R companion to applied regression*. Sage publications.

Free, J. (1955). The behaviour of egg-laying workers of bumblebee colonies. *The British Journal of Animal Behaviour*, 3(4):147–153.

Free, J. (1964). The allocation of duties among worker honeybees. *Animal behaviour*, 12(2-3):389–390.

Godfrey, R. K. and Gronenberg, W. (2019). Brain evolution in social insects: advocating for the comparative approach. *Journal of Comparative Physiology A*, 205(1):13–32.

Goulson, D. (2010). *Bumblebees: behaviour, ecology, and conservation*. Oxford University Press on Demand.

Gronenberg, W. (2001). Subdivisions of hymenopteran mushroom body calyces by their afferent supply. *Journal of Comparative Neurology*, 435(4):474–489.

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. *The Journal of experimental biology*, 199(9):2011–2019.

Hagadorn, M. A., Eck, K., Del Grosso, M., Haemmerle, X., Wcislo, W. T., and Kapheim, K. M. (2021a). Age-related mushroom body expansion in male sweat bees and bumble bees. *Scientific reports*, 11(1):1–8.

Hagadorn, M. A., Johnson, M. M., Smith, A. R., Seid, M. A., and Kapheim, K. M. (2021b). Experience, but not age, is associated with volumetric mushroom body expansion in solitary alkali bees. *Journal of Experimental Biology*, 224(6):jeb238899.

Heisenberg, M., Heusipp, M., and Wanke, C. (1995). Structural plasticity in the *Drosophila* brain. *Journal of Neuroscience*, 15(3):1951–1960.

Hillerer, K. M., Jacobs, V. R., Fischer, T., and Aigner, L. (2014a). The maternal brain: an organ with peripartal plasticity. *Neural plasticity*.

Hillerer, K. M., Neumann, I. D., Couillard-Despres, S., Aigner, L., and Slattery, D. A. (2014b). Lactation-induced reduction in hippocampal neurogenesis is reversed by repeated stress exposure. *Hippocampus*, 24(6):673–683.

Hothorn, T., Bretz, F., and Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal: Journal of Mathematical Methods in Biosciences*, 50(3):346–363.

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*, 103(1):207–211.

Jaumann, S., Seid, M. A., Simons, M., and Smith, A. R. (2019). Queen dominance may reduce worker mushroom body size in a social bee. *Developmental neurobiology*, 79(6):596–607.

Jernigan, C. M. and Uy, F. M. (2023). Impact of the social environment in insect sensory systems. *Current Opinion in Insect Science*, page 101083.

Jernigan, C. M., Zaba, N. C., and Sheehan, M. J. (2021). Age and social experience induced plasticity across brain regions of the paper wasp *Polistes fuscatus*. *Biology Letters*, 17(4):20210073.

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(4):250–261.

Kim, P., Strathearn, L., and Swain, J. E. (2016). The maternal brain and its plasticity in humans. *Hormones and behavior*, 77:113–123.

Kinsley, C. H., Bardi, M., Karelina, K., Rima, B., Christon, L., Friedenberg, J., and Griffin, G. (2008). Motherhood induces and maintains behavioral and neural plasticity across the lifespan in the rat. *Archives of sexual behavior*, 37(1):43–56.

Kinsley, C. H. and Lambert, K. G. (2006). The maternal brain. *Scientific American*, 294(1):72–79.

Kraft, N., Spaethe, J., Rössler, W., and Groh, C. (2019). Neuronal plasticity in the mushroom-body calyx of bumble bee workers during early adult development. *Developmental Neurobiology*, 79(4):287–302.

Lee, A., Li, M., Watchus, J., and Fleming, A. S. (1999). Neuroanatomical basis of maternal memory in postpartum rats: selective role for the nucleus accumbens. *Behavioral neuroscience*, 113(3):523.

Leuner, B., Glasper, E. R., and Gould, E. (2010). Parenting and plasticity. *Trends in neurosciences*, 33(10):465–473.

Li, J., Zhuang, M., Colgan, T., Guo, Y., Zhang, Z., Liu, F., Xia, Z., Dai, X., Zhang, Z., Li, Y., Wang, L., Xu, J., Guo, Y., Qu, Y., Yao, J., Yang, H., Yang, F., Guo, J., and Brown, M. (2023). Unexpected worker mating and colony-founding in a superorganism. This is a preprint article available on Research Square.

Linksvayer, T. A. and Wade, M. J. (2005). The evolutionary origin and elaboration of sociality in the aculeate hymenoptera: maternal effects, sib-social effects, and heterochrony. *The Quarterly Review of Biology*, 80(3):317–336.

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(8):733–740.

Michener, C. D. (1969). Comparative social behavior of bees. *Annual review of entomology*, 14(1):299–342.

Michener, C. D. and Michener, C. D. (1974). *The social behavior of the bees: a comparative study*. Harvard University Press.

Molina, Y. and O'Donnell, S. (2008). Age, sex, and dominance-related mushroom body plasticity in the paperwasp *Mischocyttarus mastigophorus*. *Developmental Neurobiology*, 68(7):950–959.

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(2):137–144.

Navarro-Moreno, C., Barneo-Muñoz, M., Ibáñez-Gual, M. V., Lanuza, E., Agustín-Pavón, C., Sánchez-Catalán, M. J., and Martínez-García, F. (2022). Becoming a mother shifts the activity of the social and motivation brain networks in mice. *iScience*, page 104525.

Numan, M. (1974). Medial preoptic area and maternal behavior in the female rat. *Journal of comparative and physiological psychology*, 87(4):746.

Numan, M. (2012). Maternal behavior: neural circuits, stimulus valence, and motivational processes. *Parenting, Science and Practice*, 12(2-3):105–114.

Oatridge, A., Holdcroft, A., Saeed, N., Hajnal, J. V., Puri, B. K., Fusi, L., and Bydder, G. M. (2002). Change in brain size during and after pregnancy: study in healthy women and women with preeclampsia. *American Journal of Neuroradiology*, 23(1):19–26.

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(1):39–46.

Orlova, M., Starkey, J., and Amsalem, E. (2020). A small family business: synergistic and additive effects of the queen and the brood on worker reproduction in a primitively eusocial bee. *Journal of Experimental Biology*, 223(3). jeb217547.

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(8):1–9.

Pereira, M. (2016). Structural and functional plasticity in the maternal brain circuitry. In *Maternal brain plasticity: preclinical and human research and implications for intervention*. Jossey-Bass/Wiley.

Pereira, M. and Ferreira, A. (2016). Neuroanatomical and neurochemical basis of parenting: dynamic coordination of motivational, affective and cognitive processes. *Hormones and behavior*, 77:72–85.

Rehan, S. M., Berens, A. J., and Toth, A. L. (2014). At the brink of eusociality: transcriptomic correlates of worker behaviour in a small carpenter bee. *BMC Evolutionary Biology*, 14(1):1–11.

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(2):117–124.

Riveros, A. J. and Gronenberg, W. (2010). Brain allometry and neural plasticity in the bumblebee bombus occidentalis. *Brain, behavior and evolution*, 75(2):138–148.

Rolls, A., Schori, H., London, A., and Schwartz, M. (2008). Decrease in hippocampal neurogenesis during pregnancy: a link to immunity. *Molecular psychiatry*, 13(5):468–469.

Rowe, G., Hagadorn, M. A., Lindsay, T.-T. T., Malfi, R., Williams, N. M., and Strange, J. P. (2023). Production of bumblebees (hymenoptera: Apidae) for pollination and research. In *Mass Production of Beneficial Organisms*, pages 559–579. Elsevier.

Royle, N. J., Alonzo, S. H., and Moore, A. J. (2016). Co-evolution, conflict and complexity: what have we learned about the evolution of parental care behaviours? *Current opinion in behavioral sciences*, 12:30–36.

Seid, M. A. and Wehner, R. (2009). Delayed axonal pruning in the ant brain: a study of developmental trajectories. *Developmental Neurobiology*, 69(6):350–364.

Smith, A. R., Seid, M. A., Jiménez, 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(1691):2157–2163.

Starkey, J., Brown, A., and Amsalem, E. (2019). The road to sociality: brood regulation of worker reproduction in the simple eusocial bee bombus impatiens. *Animal Behaviour*, 154:57–65.

Strausfeld, N. J., Sinakevitch, I., Brown, S. M., and Farris, S. M. (2009). Ground plan of the insect mushroom body: Functional and evolutionary implications. *Journal of Comparative Neurology*, 513(3):265–291.

Tomé, H. V. V., Rosi-Denadai, C. A., Pimenta, J. F. N., Guedes, R. N. C., and Martins, G. F. (2014). Age-mediated and environmentally mediated brain and behavior plasticity in the stingless bee *Melipona quadrifasciata anthidioides*. *Apidologie*, 45(5):557–567.

Toth, A. L., Varala, K., Henshaw, M. T., Rodriguez-Zas, S. L., Hudson, M. E., and Robinson, G. E. (2010). Brain transcriptomic analysis in paper wasps identifies genes associated with behaviour across social insect lineages. *Proceedings of the Royal Society B: Biological Sciences*, 277(1691):2139–2148.

Toth, A. L., Varala, K., Newman, T. C., Miguez, F. E., Hutchison, S. K., Willoughby, D. A., Simons, J. F., Egholm, M., Hunt, J. H., Hudson, M. E., et al. (2007). Wasp gene expression supports an evolutionary link between maternal behavior and eusociality. *Science*, 318(5849):441–444.

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(1866):20171594.

West-Eberhard, M. J. (1987). Flexible strategy and social evolution. In Ito Y., Brown J.L., K. J., editor, *Animal societies: Theories and facts*. Japan Scientific Societies Press.

West-Eberhard, M. J. (1996). Wasp societies as microcosms for the study of development and evolution. In Turillazzi S., W.-E. M., editor, *Natural history and evolution of paper wasps*. Oxford University Press.

Wilson, E. O. et al. (1971). *The insect societies.* Harvard University Press.

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(1):130–144.

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* (megachilidae). *Developmental neurobiology*, 68(1):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(6434):238–240.

Woodard, S. H., Bloch, G. M., Band, M. R., and Robinson, G. E. (2014). Molecular heterochrony and the evolution of sociality in bumblebees (*Bombus terrestris*). *Proceedings of the Royal Society B: Biological Sciences*, 281(1780):20132419.

Figures

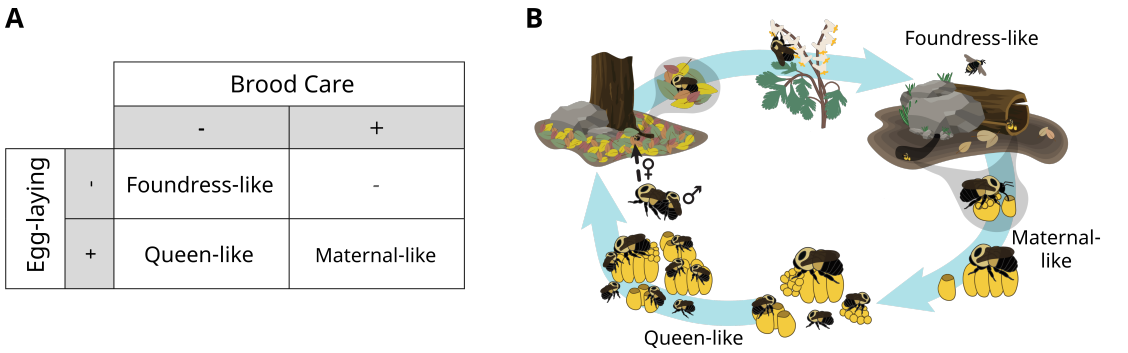

Fig. 4.1: Experimental setup and treatment group rationale. **A)** We applied 3 treatment groups to queens and workers (6 total) with egg-laying and brood care behaviors being present (+) or absent (-) in each. **B)** These treatment groups were designed to mimic various behavioral stages exhibited by queens in a typical colony cycle. Queens emerge in the spring and begin founding a nest. At this time, she is not laying eggs or caring for brood (*foundress-like* state). During the early summer, queens are still in the solitary phase, where she is responsible for caring for the eggs that she lays (*maternal-like* state). After enough workers emerge, queens shift towards predominantly egg-laying while her daughters take over caring for her brood (*queen-like* state). *Graphic by Jeremy Hemberger, which MAH modified by adding study-specific text.*

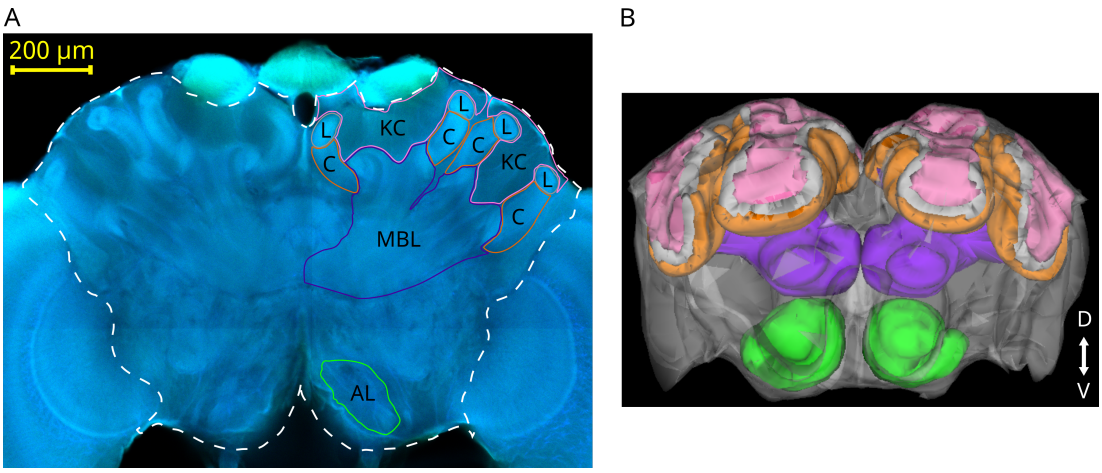

Fig. 4.2: Image of a *Bombus impatiens* brain. **A)** A slice from an image stack generated using a confocal microscope and autofluorescence. Single images were taken every 8 µm. Per slice, individual structures were hand traced; including the lips (L, gray), collar (C, orange), Kenyon cells (KC, pink), mushroom body lobes (MBL (basal ring + pedunculus + lobes), purple), and antennal lobes (AL, green). The white dotted line indicates what we designated as the central brain on this particular slice. Scale bar: 200 μm. **B)** Three-dimensional reconstruction showing the combination of traces across individual structures and the central brain (white). D, dorsal; V, ventral.

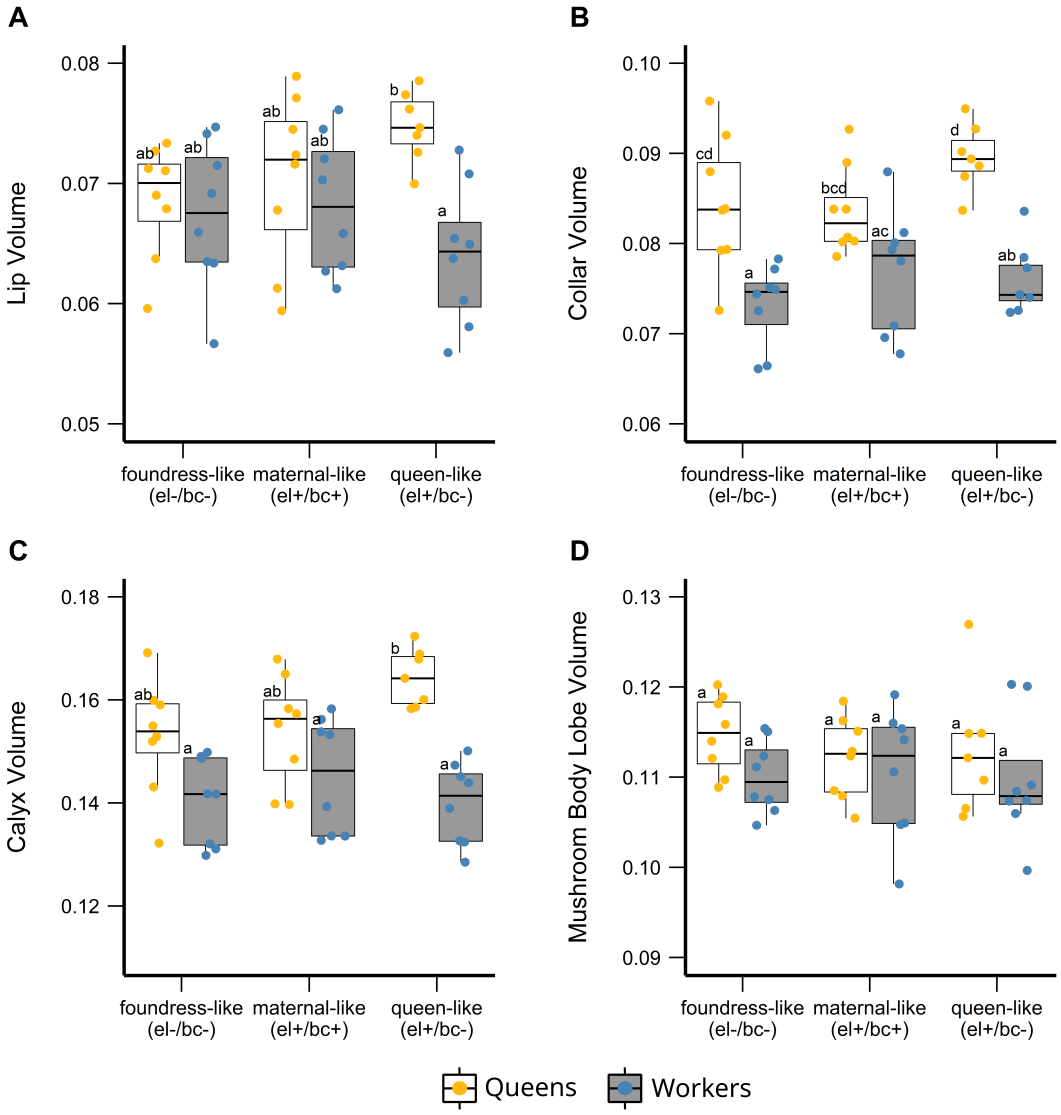

Fig. 4.3: Differences across castes in mushroom body neuroplasticity associated with exhibiting queen-like behaviors. Volumes are scaled proportions of the central brain. Mushroom body substructures include the A) lips ($F_{5,41} = 2.98$, $p = 0.02$), B) collar ($F_{5,41} = 9.96$, $p = 2.63 \text{ x } 10^{-6}$), C) calyces (lip + collar; $F_{5,41} = 7.42$, $p = 4.93 \text{ x } 10^{-5}$), and D) mushroom body lobes ($F_{5,41} = 0.88$, $p = 0.50$). Comparisons are made among foundress-like (no egg-laying (el-) or brood care (bc-)), maternal-like (egg-laying (el+) and brood care (bc+)), and queen-like (egg-laying (el+) but no brood care (bc+)) queens (white boxes, yellow dots, N=8, 8, and 7, respectively) and workers (gray boxes, blue dots, N=8 per group). Dots represent individual data points. Boxes indicate the interquartile range with medians as bolded lines and whiskers that extend to 1.5 the interquartile range. Tukey post-hoc pairwise comparisons are shown using letters, where non-overlapping letters show significant effects.

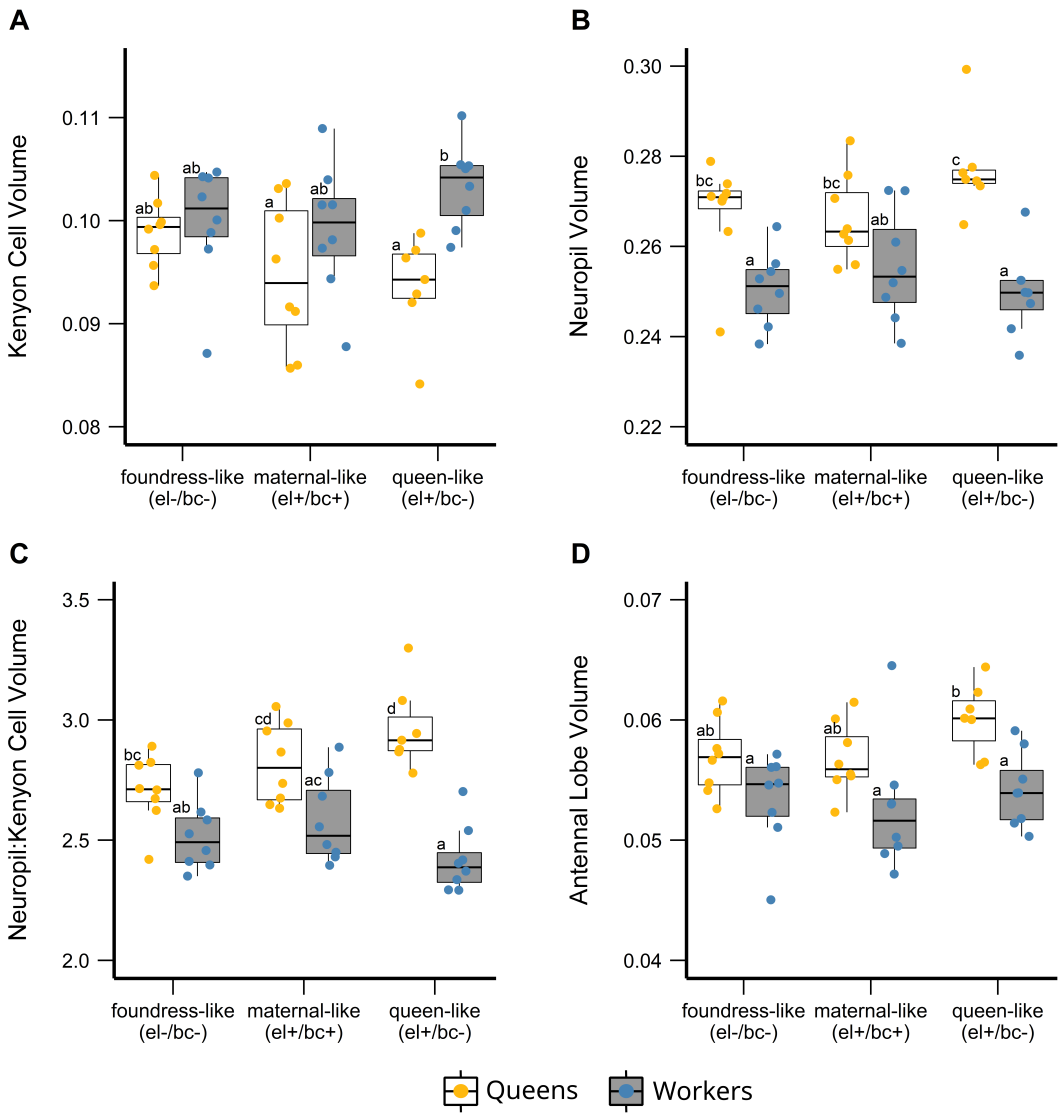

Fig. 4.4: Differences across castes in neuroplasticity associated with exhibiting queen-like behaviors. Volumes are scaled proportions of the central brain. Structures include A) the Kenyon cells ($F_{5,41} = 3.24$, $p = 0.01$), B) total neuropil volume ($F_{5,41} = 8.43$, $p = 1.50$ x 10^{-5}), C) N:K ratios ($F_{5,41} = 12.37$, $p = 2.42$ x 10^{-7}), and D) the antennal lobes ($F_{5,41} = 4.25$, $p = 0.003$). Comparisons are made among foundress-like (no egg-laying (el-) or brood care (bc-)), maternal-like (egg-laying (el+) and brood care (bc+)), and queen-like (egg-laying (el+) but no brood care (bc+)) queens (white boxes, yellow dots, N=8, 8, and 7, respectively) and workers (gray boxes, blue dots, N=8 per group). Dots represent individual data points. Boxes indicate the interquartile range with medians as bolded lines and whiskers that extend to 1.5 the interquartile range. Tukey post-hoc pairwise comparisons are shown using letters, where non-overlapping letters show significant effects.

Supplemental Materials

Supplemental Tables

Table S4.1: S1. Ethogram used for defining behaviors.

Behavior	**Definition**
Brood-associated Behaviors:	
Inspecting	When a bee walks over and antennates a brood clump.
Manipulating Wax	Bees use their mandibles to shape or reshape a portion of wax. This includes both instances that are associated with the brood patch (i.e., shaping the wax to create a brood cup) and those that are not (i.e., constructing or reshaping a honey pot).
Egg-laying	When an bee deposits an egg into a brood cell.
Incubation	When a bee wraps her thorax and abdomen around a brood patch, and a clearly observable abdominal pumping persists throughout.
Fanning	When a stationary bee is standing on a brood clump consistenly beating her wings without attempting to fly.
Larval Feeding	When a bee manipulates the wax covering larval clumps and regurgitates into the larval cell
Perching	Bee standing in a raised posture near the periphery of a brood clump with their heads down and antennae raised above the head.
Patrolling	Here described as when an individual rapidly and repeatedly walks over a brood clump. See Cameron (1989) for more further description.
Behaviors not associated with brood:	
Pollen Feeding	When a bee appears to be ingesting pollen from the pollen patty.
Drinking Sugar Water	Bees accessed sugar water ad libitum using an elevated feeder wick system, making this behavior very distinct. Individuals were observed on the wick while actively drinking (i.e., the proboscis was touching the wick).
Buzzing	The bee generates a conspicuous noise in response to external disturbance.
Inactivity	Individuals are motionless during the scan.
Other	All other behaviors done by bees were deemed not directly relevant to the study.

Table S4.2: S2. Analyses comparing behaviors exhibited by maternal-like queens and maternal-like workers. Abbreviations: AIC = Akaike information criterion, SE = Standard error, z = z-score, p = p-value

Behavior	**AIC**	**Estimate**	**SE**	**z**	***p***
Inspecting	35.37	0.672	0.698	0.962	0.34
Manipulating Wax	5.41	-1.191	3.000	-0.397	0.69
Egg-laying	4.28	-18.139	15583.782	-0.001	1.00
Incubation	68.67	-0.690	0.554	-1.246	0.21
Larval Feeding	8.63	-0.033	1.364	-0.024	0.98
Perching	4.58	18.080	8519.950	0.002	1.00
Patrolling	5.44	18.990	8519.950	0.002	1.00
Pollen Feeding	6.86	-0.665	1.861	-0.357	0.72
Drinking Sugar Water	8.05	0.498	1.456	0.342	0.73
Other	15.39	0.821	1.213	0.677	0.50

Table S4.3: S3. *Post-hoc* results for the lip region of the mushroom bodies volumetric analysis.

Treatment Groups		**Estimate**	**SE**	**t**	***p***
Within a caste:					
Queens					
maternal-like	foundress-like	0.00180	0.00281	0.639	0.987
queen-like	foundress-like	0.00618	0.00291	2.123	0.296
queen-like	maternal-like	0.00438	0.00291	1.506	0.663
Workers					
maternal-like	foundress-like	0.00087	0.00281	0.309	1.000
queen-like	foundress-like	-0.00337	0.00281	-1.199	0.834
queen-like	maternal-like	-0.00424	0.00281	-1.508	0.661
Across castes:					
Queens	Workers				
foundress-like	foundress-like	0.00120	0.00281	0.428	0.998
foundress-like	maternal-like	0.00034	0.00281	0.119	1.000
foundress-like	queen-like	0.00458	0.00281	1.628	0.586
maternal-like	foundress-like	0.00300	0.00281	1.067	0.891
maternal-like	maternal-like	0.00213	0.00281	0.758	0.973
maternal-like	queen-like	0.00638	0.00281	2.266	0.231
queen-like	foundress-like	0.00739	0.00291	2.536	0.137
queen-like	maternal-like	0.00652	0.00291	2.238	0.243
queen-like	queen-like	0.01076	0.00291	3.695	0.008

Table S4.4: S4. *Post-hoc* results for the collar region of the mushroom bodies volumetric analysis.

Treatment Groups		**Estimate**	**SE**	**t**	***p***
Within a caste:					
Queens					
maternal-like	foundress-like	-0.0007	0.00272	-0.252	1.000
queen-like	foundress-like	0.0053	0.00282	1.865	0.437
queen-like	maternal-like	0.0059	0.00282	2.108	0.303
Workers					
maternal-like	foundress-like	0.0037	0.00272	1.370	0.744
queen-like	foundress-like	0.0027	0.00272	1.008	0.913
queen-like	maternal-like	-0.0010	0.00272	-0.362	0.999
Across castes:					
Queens	Workers				
foundress-like	foundress-like	0.0112	0.00272	4.109	0.002
foundress-like	maternal-like	0.0075	0.00272	2.739	0.089
foundress-like	queen-like	0.0084	0.00272	3.101	0.038
maternal-like	foundress-like	0.0105	0.00272	3.857	0.005
maternal-like	maternal-like	0.0068	0.00272	2.487	0.152
maternal-like	queen-like	0.0078	0.00272	2.849	0.069
queen-like	foundress-like	0.0164	0.00282	5.834	< 0.001
queen-like	maternal-like	0.0127	0.00282	4.510	< 0.001
queen-like	queen-like	0.0137	0.00282	4.860	< 0.001

Table S4.5: S5. *Post-hoc* results for the calyx (includes the lip and collar) region of the mushroom bodies volumetric analysis.

Treatment Groups		**Estimate**	**SE**	**t**	***p***
Within a caste:					
Queens					
maternal-like	foundress-like	0.00111	0.00474	0.235	1.000
queen-like	foundress-like	0.01144	0.00491	2.332	0.205
queen-like	maternal-like	0.01033	0.00491	2.105	0.304
Workers					
maternal-like	foundress-like	0.00460	0.00474	0.971	0.924
queen-like	foundress-like	-0.00063	0.00474	-0.133	1.000
queen-like	maternal-like	-0.00523	0.00474	-1.104	0.877
Across castes:					
Queens	Workers				
foundress-like	foundress-like	0.01240	0.00474	2.616	0.116
foundress-like	maternal-like	0.00779	0.00474	1.645	0.575
foundress-like	queen-like	0.01302	0.00474	2.748	0.087
maternal-like	foundress-like	0.01351	0.00474	2.850	0.069
maternal-like	maternal-like	0.00891	0.00474	1.879	0.429
maternal-like	queen-like	0.01414	0.00474	2.983	0.051
queen-like	foundress-like	0.02383	0.00491	4.859	< 0.001
queen-like	maternal-like	0.01923	0.00491	3.921	0.004
queen-like	queen-like	0.02446	0.00491	4.987	< 0.001

Table S4.6: S6. *Post-hoc* results for the Kenyon cells region of the mushroom bodies volumetric analysis.

Treatment Groups		**Estimate**	**SE**	**t**	***p***
Within a caste:					
Queens					
maternal-like	foundress-like	-0.00420	0.00273	-1.536	0.644
queen-like	foundress-like	-0.00524	0.00283	-1.854	0.444
queen-like	maternal-like	-0.00105	0.00283	-0.370	0.999
Workers					
maternal-like	foundress-like	-0.00064	0.00273	-0.233	1.000
queen-like	foundress-like	0.00351	0.00273	1.284	0.792
queen-like	maternal-like	0.00415	0.00273	1.517	0.655
Across castes:					
Queens	Workers				
foundress-like	foundress-like	-0.00092	0.00273	-0.338	0.999
foundress-like	maternal-like	-0.00029	0.00273	-0.105	1.000
foundress-like	queen-like	-0.00443	0.00273	-1.622	0.589
maternal-like	foundress-like	-0.00512	0.00273	-1.874	0.432
maternal-like	maternal-like	-0.00448	0.00273	-1.641	0.577
maternal-like	queen-like	-0.00863	0.00273	-3.158	0.033
queen-like	foundress-like	-0.00617	0.00283	-2.180	0.269
queen-like	maternal-like	-0.00553	0.00283	-1.955	0.385
queen-like	queen-like	-0.00967	0.00283	-3.420	0.017

Table S4.7: S7. *Post-hoc* results for the total mushroom body neuropil volumetric analysis.

Treatment Groups		**Estimate**	**SE**	**t**	***p***
Within a caste:					
Queens					
maternal-like	foundress-like	-0.00150	0.00520	-0.289	1.000
queen-like	foundress-like	0.00966	0.00539	1.794	0.481
queen-like	maternal-like	0.01117	0.00539	2.073	0.321
Workers					
maternal-like	foundress-like	0.00496	0.00520	0.953	0.930
queen-like	foundress-like	-0.00087	0.00520	-0.168	1.000
queen-like	maternal-like	-0.00583	0.00520	-1.120	0.870
Across castes:					
Queens	Workers				
foundress-like	foundress-like	0.01709	0.00520	3.284	0.024
foundress-like	maternal-like	0.01213	0.00520	2.332	0.205
foundress-like	queen-like	0.01797	0.00520	3.452	0.015
maternal-like	foundress-like	0.01559	0.00520	2.995	0.049
maternal-like	maternal-like	0.01063	0.00520	2.042	0.337
maternal-like	queen-like	0.01646	0.00520	3.163	0.033
queen-like	foundress-like	0.02676	0.00539	4.967	< 0.001
queen-like	maternal-like	0.02180	0.00539	4.046	0.003
queen-like	queen-like	0.02763	0.00539	5.129	< 0.001

Table S4.8: S8. *Post-hoc* results for antennal lobe volumetric analysis.

Treatment Groups		**Estimate**	**SE**	**t**	***p***
Within a caste:					
Queens					
maternal-like	foundress-like	-0.00012	0.00185	-0.067	1.000
queen-like	foundress-like	0.00318	0.00191	1.661	0.565
queen-like	maternal-like	0.00330	0.00191	1.725	0.524
Workers					
maternal-like	foundress-like	-0.00076	0.00185	-0.413	0.998
queen-like	foundress-like	0.00081	0.00185	0.440	0.998
queen-like	maternal-like	0.00158	0.00185	0.852	0.955
Across castes:					
Queens	Workers				
foundress-like	foundress-like	0.00352	0.00185	1.901	0.416
foundress-like	maternal-like	0.00428	0.00185	2.313	0.212
foundress-like	queen-like	0.00270	0.00185	1.461	0.690
maternal-like	foundress-like	0.00339	0.00185	1.834	0.456
maternal-like	maternal-like	0.00416	0.00185	2.247	0.239
maternal-like	queen-like	0.00258	0.00185	1.394	0.730
queen-like	foundress-like	0.00670	0.00191	3.497	0.014
queen-like	maternal-like	0.00746	0.00191	3.896	0.004
queen-like	queen-like	0.00588	0.00191	3.072	0.041

Table S4.9: S9. *Post-hoc* results for Neuropil:Kenyon cell volumetric analysis.

Treatment Groups		**Estimate**	**SE**	**t**	***p***
Within a caste:					
Queens					
maternal-like	foundress-like	0.06750	0.07928	0.851	0.956
queen-like	foundress-like	-0.09615	0.07928	-1.213	0.828
queen-like	maternal-like	-0.16365	0.07928	-2.064	0.326
Workers					
maternal-like	foundress-like	-0.00076	0.00185	-0.413	0.998
queen-like	foundress-like	0.00081	0.00185	0.440	0.998
queen-like	maternal-like	0.00158	0.00185	0.852	0.955
Across castes:					
Queens	Workers				
foundress-like	foundress-like	0.19268	0.07928	2.430	0.170
foundress-like	maternal-like	0.12518	0.07928	1.579	0.616
foundress-like	queen-like	0.28884	0.07928	3.643	0.009
maternal-like	foundress-like	0.30386	0.07928	3.833	0.005
maternal-like	maternal-like	0.23636	0.07928	2.981	0.051
maternal-like	queen-like	0.40002	0.07928	5.046	< 0.001
queen-like	foundress-like	0.45057	0.08206	5.491	< 0.001
queen-like	maternal-like	0.38307	0.08206	4.668	< 0.001
queen-like	queen-like	0.54672	0.08206	6.662	< 0.001

Supplemental Figures

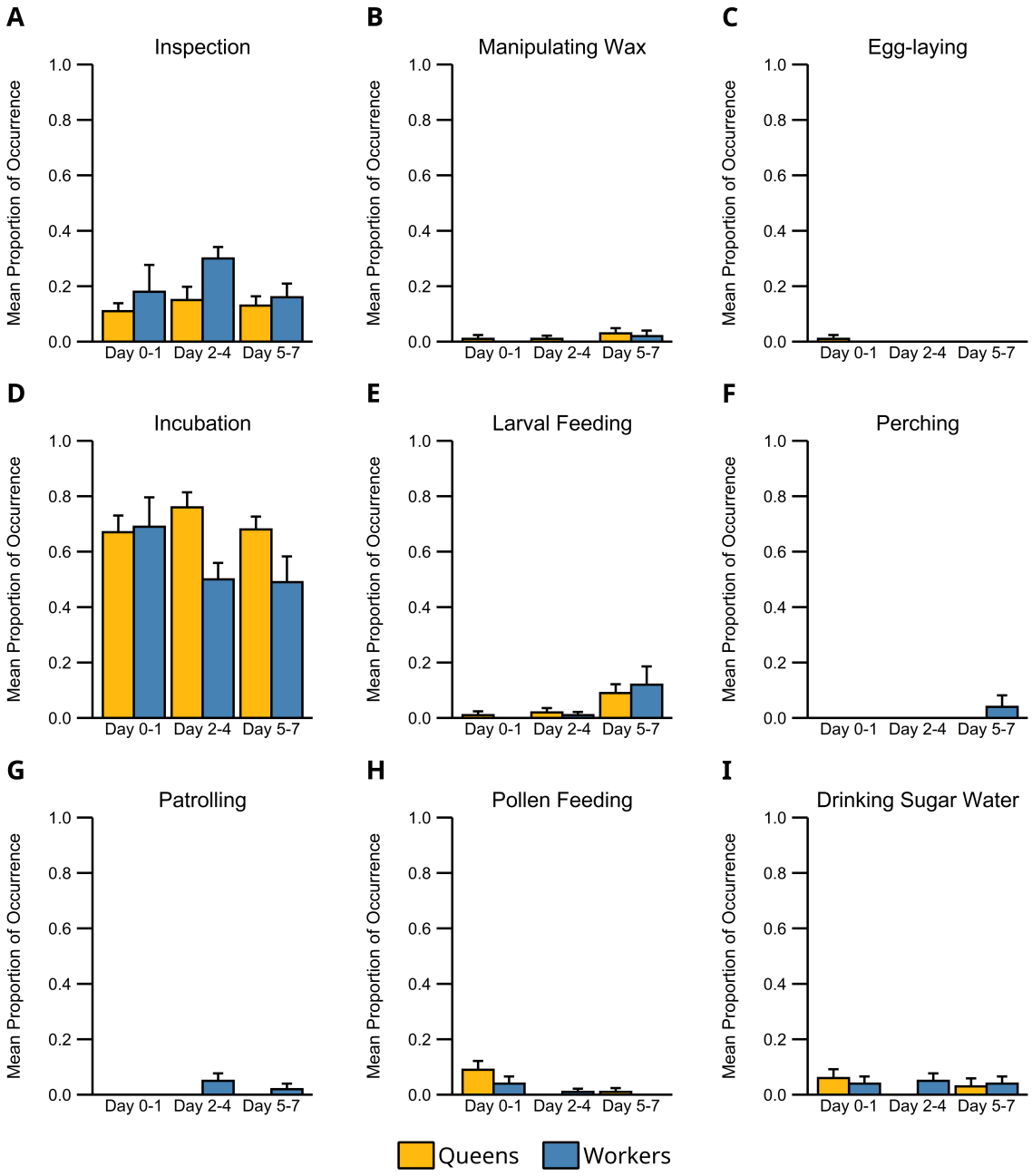

Fig. 4.5: S10. The proportion of individual behaviors exhibited by maternal-like queens (gold) and workers (blue). Behaviors are assayed 0-1 d, 2-4 d, and 5-7 d after the first instance of egg-laying. Bees were culled 7 days after the first instance of egg-laying.

CHAPTER 5

SUMMARY AND CONCLUSIONS

The transition to living eusocially incorporates numerous behavioral changes, including division of labor among and within castes and cooperatively rearing offspring. Interestingly, in social insect females, these shifts in behaviors are accompanied by elaborate structural and functional changes in the brain; neuroplasticity in response to colony life. This brain plasticity has been demonstrated with maturation (i.e., age-related changes) and specific experiences (i.e., experience-dependent). However, how sociality influences neuroplasticity and drives patterns in brain investment is still unclear. Moreover, prior work is sex, species, and behavior-biased, limiting our understanding of the evolutionary relationship between neuroplasticity and sociality and its prevalence within and across taxa. Exploring relationships between the brain and social behaviors in both solitary and social insects will yield insight into neuroplasticity's role in facilitating the evolution of eusociality. In this dissertation, I used brain plasticity data from three different bee species to begin addressing the current limitations. My results showed that the males of social species have brains that exhibit age-related plasticity in the absence of experience, similar to social females. Alternatively, the females of solitary species appear to lack brains with this capability. Together, this suggests that the developmentally-associated neuroplasticity that occurs in anticipation of shifts in colony need and maturation appears to have evolved with sociality, but likely not before. Further, how social experiences impact the brain seems to vary. Specifically, solitary bees may be predisposed to responding to social cues, whereas the decoupling of reproductive and caregiving behaviors—a hallmark of eusocial life—might be characterized by differing patterns of brain plasticity across castes. I summarize the results from my chapters below.

In Chapter Two, I asked whether the combination of age-related and experience-dependent neuroplasticity is a pre-adaptation to eusociality or an adaptive response

to social life. To distinguish between these two competing alternatives, I investigated neuroplasticity in a solitary species that is closely related to social taxa. Alkali bees, *Nomia melanderi*, have preadaptations to eusociality but are ancestrally solitary, making them an ideal species for exploring this question. I used experimental manipulation, fluorescent confocal microscopy, and volumetric plasticity to assess brain changes associated with age, nesting experience, and the social environment. First, we compared the volume of individual brain regions among naive newly-emerged females, laboratory-reared females deprived of reproductive and nesting experiences, and free-flying, nesting females. I found that experienced females invest significantly more in brain regions associated with learning and memory relative to similarly-aged, inexperienced females. However, unlike in highly eusocial species, age-related plasticity appears to be absent in solitary alkali bees. This suggested that developmentally-driven neuroplasticity may be unique to social species, whereas plasticity with experience seems relatively common. Next, I investigated how the brains of solitary bees respond to a social environment. I paired newly-emerged, naive females with a conspecific partner and compared their brain volumes to age-matched females housed in isolation. I found that alkali bees invest more in mushroom body substructures associated with olfactory processing when paired with a partner, suggesting that the brains of solitary bees may be pre-adapted to respond to social stimuli. Together, these findings provide some of the most compelling insights to date regarding the role of neuroplasticity in social evolution, as well as novel insight into brain investment patterns in solitary bees.

In Chapter Three, I continued exploring the relationship between developmentally-driven brain changes and sociality by asking if similar age-related neuroplasticity patterns occur in males. In highly social females, age-related plasticity generally coincides with a systematic, age-based behavioral maturation. However, this phenomenon was virtually unexplored in males. Unlike females, the behavior of male bees is expected to be hyper-focused on mating; therefore, the drivers of age-related plasticity likely differ between sexes. Thus, a lack of male data biased our understanding of age-related plasticity and its role in

social evolution. Using sweat bees and bumble bees, two species with varying degrees of sociality, I characterized how male bee brains are modified with sexual maturation. Using fluorescent confocal microscopy and volumetric measurements, I compared the brains of newly-emerged and older, reproductively mature males, both lacking experience, for each species independently. In both species, reproductively mature males had significantly larger mushroom bodies. Further, strikingly similar patterns in mushroom body expansion were observed across sweat bee and bumble bee males. Overall, this suggested that while males exhibit similar age-related changes to highly social females, the evolutionary drivers of that phenomenon may differ between the sexes.

In Chapter Four, I asked if there were common neural signatures underlying the behaviors that typify alternative female castes in social insects. In highly social species, females (generally) take on one of two roles: reproduction or brood care. Here, reproductive dominance is exhibited by a queen(s) while workers forego their own reproductive potential to care for their siblings. To understand the relationship between neuroplasticity and caste-specific behaviors, I compared the brains of queen and worker bumble bees conducting reproductive and brood care behaviors to age-matched counterparts lacking these behaviors. Using behavioral manipulations, fluorescent confocal microscopy, and volumetric measurements, I compared the brains of foundress-like, maternal-like, and queen-like queens and workers. Foundress-like individuals lacked egg-laying (i.e., reproductive) and brood care behaviors, whereas maternal-like individuals had experience with both. Queen-like queens and workers were allowed to lay eggs but did not care for them, exemplifying typical queen-like behaviors in a colony. My results indicated no significant differences in brain volume within either caste across the three treatment groups. Specifically, brains did not seem to change with reproductive or brood care behaviors. However, relative to workers, queens invested more in mushroom body neuropil when laying eggs they do not care for. This suggested that caste-specific differences in the brain could reinforce a queen-like state. These results add to our understanding of factors that may be facilitating alternative female castes while identifying experiences that do not induce detectable,

large-scale architecture changes.

Conclusion

Eusociality is a major evolutionary and behavioral innovation. Yet, how the brain impacts, and is impacted by, social life remains inconclusive. This dissertation aimed to redress this issue by asking fundamental questions about the relationship between volumetric neuroplasticity and social behavior across multiple solitary and social bee species. My work suggests that brain evolution likely coincided with the transition to sociality, potentially as adaptive responses to the demands of a social lifestyle. This includes an additional form of neuroplasticity, e.g., experience-expectant plasticity, that appears to be unique to social taxa and differences in caste-specific responses to alternative behavioral roles inside a colony. Additionally, this research continues to highlight the need for incorporating males into our understanding of eusocial evolution, without which a female bias will persist. The results of my dissertation have generated novel insight regarding multiple facets underlying the interconnectedness of neurodevelopment and social evolution. As we move forward, testing similar hypotheses across various timescales and organisms will be critical for elucidating a fine-tuned role of how sociality influences neuroplasticity and drives patterns in brain investment.

APPENDICES

APPENDIX A

Coauthor Permission Letters

I hereby give permission to Mallory A. Hagadorn to include the following published material in their dissertation, of which I am a co-author.

1. Hagadorn, Mallory A., Makenna M. Johnson, Adam R. Smith, Marc A. Seid, and Karen M. Kapheim. 2021. Experience, but not age, is associated with volumetric mushroom body expansion in solitary alkali bees. *Journal of Experimental Biology*, 224(6). doi:10.1242/jeb.238899.

2. Hagadorn, Mallory A., Karlee Eck, Matthew Del Grosso, Xavier Haemmerle, William T. Wcislo, and Karen M. Kapheim. 2021. Age-related mushroom body expansion in male sweat bees and bumble bees. *Scientific Reports*, 11(17039). doi: 10.1038/s41598-021-96268-w.

Karen M. Kapheim

Date: 8/9/2023

I hereby give permission to Mallory A. Hagadorn to include the following published material in their dissertation, of which I am a co-author.

Hagadorn, Mallory A., Makenna M. Johnson, Adam R. Smith, Marc A. Seid, and Karen M. Kapheim. 2021. Experience, but not age, is associated with volumetric mushroom body expansion in solitary alkali bees. *Journal of Experimental Biology*, 224(6). doi:10.1242/jeb.238899.

Makenna Mary Johnson Bird

Makenna M. Johnson

Date: 4/15/23

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Adam R. Smith

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Hagadorn, Mallory A., Makenna M. Johnson, Adam R. Smith, Marc A. Seid, and Karen M. Kapheim. 2021. Experience, but not age, is associated with volumetric mushroom body expansion in solitary alkali bees. *Journal of Experimental Biology*, 224(6). doi:10.1242/jeb.238899.

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Ph.D. Candidate in Biology, *Expected graduation: Fall 2023*

Dissertation: "The impacts of maturation and experience on volumetric neuroplasticity in solitary and social bees."

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Undergraduate Research: "Quick guide to the Scarabaeoidea of Maryland"

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the ground and in the canopy. *Submitted to the Journal of the Lepidopterists' Society September 2023.*

2. Rowe, G., **M.A. Hagadorn**, T-T.T. Lindsay, R.L. Malfi, N.M. Williams, and J.P Strange. **2023**. Production of bumblebees (Hymenoptera: Apidae) for pollination and research. In *Mass Production of Beneficial Organisms: Invertebrates and Entomopathogens*, 2nd Edition, pp. 559-579, Academic Press. doi:10.1016/B978-0-12-822106-8.00004-X

3. **Hagadorn, M.A.**, F.K. Hunter, M.M. Johnson, T. DeLory, T.L. Pitts-Singer, K.M. Kapheim. **2023**. Maternal body condition and season influence RNA deposition in the oocytes of alfalfa leafcutting bees (*Megachile rotundata*). *Frontiers in Genetics*, 13: 1064332. doi:10.3389/fgene.2022.1064332

4. **Hagadorn, M.A.**, K. Eck*, M. Del Grosso, X. Haemmerle*, W.T. Wcislo, and K.M. Kapheim. **2021**. Age-related mushroom body expansion in male sweat bees and bumble bees. *Scientific Reports*, 11: 17039. doi:10.1038/s41598-021-96268-w

5. **Hagadorn, M.A.**, M.M Johnson, A.R. Smith, M.A. Seid, and K.M. Kapheim. **2021**. Experience, but not age, is associated with volumetric mushroom body expansion in solitary alkali bees. *Journal of Experimental Biology*, 224. doi:10.1242/jeb.238899

6. **Hagadorn, M.A.**, K. Mitchell*, J. Restein*, and D.L. Price. **2021**. Relationship of Dung Beetle (Coleoptera: Scarabaeidae and Geotrupidae) Abundance and Parasite Control in Cattle on Pastures throughout Maryland. *Coleopterists Bulletin*, 75(2): 382-402. doi:10.1649/0010-065X-75.2.382

7. Smith, A.R., T. DeLory, M.M. Johnson, A.C. Figgins*, **M.A. Hagadorn**, K.M. Kapheim. **2019**. Small body size is associated with increased aggression in the solitary sweat bee *Nomia melanderi* (Hymenoptera, Halictidae). *Journal of Insect Behavior*, 1-9. doi:10.1007/s10905-019-09736-7

8. Simons, P.*, M. Molina*, **M.A. Hagadorn**, and D.L. Price. **2018**. Natural History and Succession of Dung Beetles (Scarabaeidae and Geotripidae) in Seven Coastal Plain Forests of Maryland. *North Eastern Naturalist.* doi:10.1656/045.025.0108

MANUSCRIPT IN PREPARATION (*Undergraduate Researcher)

1. **Hagadorn, M.A.**, M.M. Johnson, K. Eck*, A.C. Figgins*, T-T.T. Lindsay, J.P Strange, K.M. Kapheim. **In Prep**. A Queen-like Brain: queen and worker bumble bee brains respond differently to egg-laying in the absense of brood care. *Target Journal: Journal of Insect Behavior.*

2. **Hagadorn, M.A.**, K. Mitchell*, A. Estes, P.D. Anderson, J.C Dunning Hotopp, and D.L. Price. In Prep. Core microbiome harbored in dung beetle *Onthophagus taurus* (Schreber) (Coleoptra: Scarabaeidae: Scarabaeinae) taken from Maryland cattle pastures. *Target Journal: Environmental Microbiology.*

OTHER PUBLICATIONS

1. **Hagadorn, M.A**. and D.L. Price. **2012**. Quick Guide for the Identification of Maryland Scarabaeoidea. Salisbury University. Pp. 54.

RESEARCH GRANTS ($163,354 acquired to date)

2022 International Union for the Study of Social Insects, North American Section Travel Award ($1570)

2021 Animal Behavior Society Student Research Grant ($2,000)

2021 American Genetic Association Evolutionary, Ecological, Conservation Genomics Research ($6000)

2021 Utah State University, Graduate Enhancement Award ($4000)

2020 Utah State University, Graduate Research and Creative Opportunities Award ($1000)

2020 Utah State University, College of Science Claude E. ZoBell Scholarship ($1000)

2020 Utah State University, James A. and Patricia A. MacMahon Endowed Ecology Graduate Student Scholarship ($2000)

2018 Sigma Xi Grants in Aid of Research Award ($1000)

2017 Utah State University, Department of Biology Joseph E. Greaves Endowed Scholarship ($6000)

2017 Utah State University, College of Science Piette Graduate Scholarship ($600)

2017 Utah State University, Research and Graduate Studies Travel Award ($200)

2014 Salisbury University, Student Academic Research Award ($500)

2013 Salisbury University, Graduate Research and Presentation Grant ($500)

2013 National Science Foundation Graduate Research Fellowship Program Award ($132,000)

2012 Salisbury University, Student Academic Research Award ($172)

2012 Salisbury University, Henson Undergraduate Research Award ($175)

2012 Salisbury University, Guerrieri Undergraduate Research Summer Program ($4500)

2012 Salisbury University, Henson Undergraduate Research Award ($137)

HONORS AND AWARDS

2023 Utah State University College of Science PhD Researcher of the Year

2022 Explore College Teaching Certificate–Empowered Teaching Excellence Program, Utah State University.

2021 Animal Behavior Society George W. Barlow Award–for the top-ranked student research proposal

2021 Second place, LGBTQ+ STEMinar Flash Talk and Poster Competition, Virtual Conference

2017 First place, SysEB: Hymenoptera Graduate Student Poster Competition, National Entomological Society of America Meeting, Denver, CO

2015 Second place, Graduate Student Poster Competition, Eastern Branch Entomological Society of America Meeting, Elizabethtown, PA

2014 Second place, Masters Oral Competition, Eastern Branch Entomological Society of America Meeting, Williamsburg, VA

2013 First place, Graduate Student Poster Competition, Mid-Atlantic Ecological Society of America Meeting, Delaware State University

2013 Salisbury University Biology Department Faculty Award

2012 Cum Laude from Salisbury University

2012 Dhimitra S. Davenport-Hopkins Scholarship

2012 Salisbury University Scholar Holler Recipient (November)

2012 Inducted into Beta Beta Beta Biological Honor Society

DIVERSITY, EQUITY, AND INCLUSION, AND JUSTICE (* **while at Utah State University)**

2022: Diversity Mentorship and Inclusive Teaching Learning Circle (Lead Facilitator)*

Reading Material: Modeling Mentoring Across Race/Ethnicity and Gender: Practices to Cultivate the Next Generation of Diverse Faculty

As the lead facilitator, I selected the reading material, organized meetings, documented progress, and directed the discussion. My goals for this reading group were: 1) to draw awareness of disparities in the mentorship approach and the ability to be mentored across systemic barriers in academia and 2) to foster discussion about implementing current best practices while developing the next steps.

2021: Department of Biology Diversity, Equity, and Inclusion Committee Member*

I was a Diversity, Equity, and Inclusion Committee member for the USU Biology Department. The primary goal of this committee is to increase diversity, equity, and inclusion within our department and help support underrepresented students. To date, I have assisted with two DEI-associated events: 1) a "Lunch and Learn" Panel Discussion on inequity in STEM and 2) a Panel Discussion on Non-Academic Career Paths aimed at post-docs and graduate students.

Spring 2021: Assisted the Moderator for Picture a Scientist "Lunch and Learn" Panel Discussion *

My responsibilities included collecting questions in advance and helping to relay those questions (anonymously) to the panel.

2019: Initiated the scheduling for department-wide inclusivity training*

My contribution included working to coordinate and initiate implicit bias and up-stander training in the Biology Department. These trainings aimed to promote a healthy department culture while continuously encouraging "us" to better our personal and professional selves.

<u>TEACHING EXPERIENCE</u> (*while at Utah State University)

Animal Behavior, *Graduate Teaching Asst.* *Fall 2022, 2023**

Explore College Teaching Certificate *Summer 2022**

Empowered Teaching Excellence Program

Biology and the Citizen, *Primary Instructor of Record* *Spring 2020**

This is an introductory biology course for non-majors designed to give students a broad overview of biological concepts and facilitate their understanding of how these topics relate to real-world issues. As the instructor, I created a syllabus and assessments, designed and presented lectures, and promoted class discussions and critical thinking opportunities. I also implemented two exam-alternative assignments to assess the understanding of introductory content, scientific enterprise, and communication skills. This course started in a face-to-face format but moved to online instruction due to the COVID-19 outbreak.

Evolutionary Biology, *Graduate Teaching Asst.* *Spring 2018*, 2019**

Human Physiology, *Graduate Teaching Asst.* *Fa. 2018*; Sp. 2013, 2022*, 2023**

Human Anatomy, *Graduate Teaching Asst.* *Fall 2015; Spring 2016*

Guest Lectures

Animal Behavior (Utah State University) *Fall 2021*

Genetic Analysis (Salisbury University) *Spring 2014, 2015*

TRiO ACHiEVE Tutor *2012*

Molecular Genetics

Cell Biology

Introductory Biology

PRESENTATIONS (*Undergraduate Researcher, **High School)

1. **Hagadorn, M.A.**, M.M. Johnson, K. Eck*, A.C. Figgins*, T-T.T. Lindsay, J.P. Strange, K.M. Kapheim. Do bees have a mom brain?: investigating how egg-laying and brood care impact neurodevelopmental plasticity in bumble bees. Hansen Life Sciences Retreat. Utah State University, Logan, UT. October 2023. (*Invited Talk*)

2. **Hagadorn, M.A.**, M.M. Johnson, K. Eck*, A.C. Figgins*, T-T.T. Lindsay, J.P. Strange, K.M. Kapheim. Mom Brain: investigating how egg-laying and brood care impact neurodevelopmental plasticity in bumble bees. Department of Biology Graduate Student Seminar. Utah State University, Logan, UT. February 2023. (*Invited Talk*)

3. **Hagadorn, M.A.**, M.M. Johnson, K. Eck*, A.C. Figgins*, T-T.T. Lindsay, J.P Strange, K.M. Kapheim. Maternal brains: exploring neuroplasticity associated with egg-laying and brood care in bumble bees. International Union for the Study of Social Insects (IUSSI) International Meeting. Themed Symposium: *What can we learn from simple insect societies?* San Diego, USA. July 2022. (*Talk*)

4. **Hagadorn, M.A.**, M.M. Johnson, A.R. Smith, M.A. Seid, K. Eck*, M. Del Grosso, X. Haemmerle*, W.T. Wcislo, and K.M. Kapheim. Experience and maturation as factors influencing brain plasticity in solitary and social bees. Biology Department Seminar Series. Salisbury University, Salisbury, MD. April 2021. (*Invited Talk*)

5. *Hagadorn, M.A.*, M.M. Johnson, A.R. Smith, M.A. Seid and K.M. Kapheim. Social and nesting experience induces neuroplasticity in solitary bees. LGBTQ+ STEMinar. Remote Attendance. The University of Oxford. Oxford, UK. January 2021. (*Poster*)

6. **Hagadorn, M.A.**, M.M. Johnson, A.R. Smith, M.A. Seid, K. Eck*, M. Del Grosso, X. Haemmerle*, W.T. Wcislo, and K.M. Kapheim. Experience and maturation as factors influencing volumetric mushroom body plasticity in solitary and social bees. Department of Biology Seminar Series. Utah State University, Logan, UT. December 2020. (*Invited Talk*)

7. Pearse, W.D., M.G. Branstetter, J. Bravo, S.B. Franklin, **M.A. Hagadorn**, S. Kinosian, M. Licht, S. Hudson, R.J.R. McCleary, E.H. Mooney, E.G. Simpson, K. Hafen, K.B. Weedop, H. Wilson, M.R. Helmus. Augmenting Research Grounded On NEON (ARGON): Using new data compilation techniques to contextualize NEON

site diversity. Ecological Society of America Annual Meeting. New Orleans, LA. August 2018. (*Talk*)

8. **Hagadorn, M.A.**, M.M. Johnson, A.R. Smith, M.A. Seid and K.M. Kapheim. Neuroplasticity associated with juvenile hormone and social cues in solitary alkali bees Nomia melanderi. Entomological Society of America National Meeting. Denver, CO. November 2017. (*Poster*)[First place winner in graduate poster competition]

9. **Hagadorn, M.A.**, A.M. Estes, J.C. Dunning Hotopp, P.D. Anderson, K. Mitchell*, and D.L. Price. Analysis of 16S rRNA community and core microbial taxa present in dung beetle species *Onthophagus taurus* Schreber taken from Maryland cattle pastures. Ecological Society of America Annual Meeting. Portland, OR. August 2017. (*Talk*)

10. **Hagadorn, M.A.**, A.M. Estes, J.C. Dunning Hotopp, P.D. Anderson, K. Mitchell*, and D.L. Price. 16s rRNA community analysis of the gut microbiome in Maryland populations of the dung beetle species Onthophagus taurus Schreber Mid-Atlantic Ecological Society of America, Kutztown University, Kutztown, PA. April 2016. (*Talk*)

11. **Hagadorn, M.A.** Dung beetles, their endosymbionts, and the power of Next-Generation Sequencing. The American Society for Biochemistry and Molecular Biology, Salisbury University Chapter Meeting. October 2015. (*Talk*)

12. **Hagadorn, M.A.**, K. Mitchell*, A.M. Estes, J.C. Dunning Hotopp, and D.L. Price. Gut microbial community in Maryland populations of Onthophagus taurus Schreber. Mid-Atlantic Ecological Society of America, Elizabethtown College, Elizabethtown, PA. April 2015. (*Poster*) [Second place winner in graduate poster competition]

13. Mitchell, K.*, **M.A. Hagadorn**, J. Restein, and D.L. Price. Dung Beetles on Organic and Conventionally Managed Cattle Pastures. Mid-Atlantic Ecological Society of America, Elizabethtown College, Elizabethtown, PA. April 2015. (*Poster*)

14. Estes, A.M., K. King**, E. Snell-Rood, **M.A. Hagadorn**. D.L. Price, B. Doube, A. Moczek, J.C. Dunning Hotopp. The Dung Beetle Microbiome: An Essential, Beneficial Microbiome Assisting in Dung Degradation and Harboring Antibiotic Resistance. 5th American Society for the Microbiology Conference on Beneficial Microbes, Washington D.C. September 2014. (*Talk*)

15. Mitchell. K.*, **M.A. Hagadorn**, A.M. Estes, D.L. Price, J.C. Dunning Hotopp. The Gut Microbiome of *Onthophagus taurus* on cattle farms. Guerrieri Undergraduate Research Symposium 2014. Salisbury University, Salisbury, MD. August 2014. (*Poster*)

16. Mitchell, K.*, **M.A. Hagadorn**, J.A. Restein*. The Effects of Diverse Farming Practices on Dung Beetle Populations Across Maryland. Salisbury University Student Research Conference, Salisbury University, Salisbury, MD. April 2014. (*Talk*)

17. Hagadorn, M.A., J.A. Restein*, K. Mitchell*, D.L. Price. Not all dung is created equal: Dung beetles on organic and conventionally managed cattle pastures. Entomological Society of America Eastern Branch Meeting. Entomology: Key Science. Williamsburg, VA. March 2014. (*Poster*) [Second place winner in master's student competition]

18. Estes, A.M., E. Snell-Rood, **M.A. Hagadorn**, D.L. Price, B. Doube, B. Ma, D. Fadrosh, J. Ravel, A. Moczek, J.C. Dunning Hotopp. A global core dung beetle microbiome with varying taxonomic abundance across life stage. Mechanisms and Consequences of Invertebrate-Microbe Interactions, Keystone Symposia, Tahoe City, CA. January 2014. (*Poster*)

19. **Hagadorn, M.A.**, J.A. Restein*, K. Mitchell*, D.L. Price. Not all dung is created equal: Dung beetles on organic and conventionally managed cattle pastures. Entomological Society of America National Meeting. Entomology 2013: Science

Impacting a Connected World, SOLA Symposium. Austin, TX. November 2013. (*Invited Talk*)

20. Mitchell, K.*, J.A. Restein*, **M.A. Hagadorn**, and D.L. Price. 2013. Dung Beetles on Organic and Conventionally Managed Cattle Farms. Guerrieri Undergraduate Research Symposium 2013, Salisbury University, Salisbury MD. August 2013. (*Poster*)

21. **Hagadorn, M.A.**, J.A. Restein*, and D.L. Price. Dung Beetles and their Gut Endosymbionts. Mid-Atlantic Ecological Society of America, Delaware State University, Dover, DE. April 2013. (*Talk*) [First place winner in graduate poster competition]

22. Restein, J.A.*, **M.A. Hagadorn**, and D.L. Price. 2013. Dung Beetles on Organic and Conventionally Managed Dairy Cattle Pastures. Salisbury University Student Research Conference, Salisbury University, Salisbury MD. April 2013. (*Talk*)

23. **Hagadorn, M.A.***, A.M. Estes, and D.L. Price. Not All Dung is Created Equal. Biodiversity: From Evolutionary Origins to Ecosystem Function, Drexel University, Philadelphia, PA. October 2012. (*Poster*)

24. **Hagadorn, M.A.***, A.M. Estes, and D.L. Price. Not All Dung is Created Equal. Guerrieri Undergraduate Research Symposium 2012, Salisbury University, Salisbury MD. August 2012. (*Poster*)

25. **Hagadorn, M.A.*** and D.L. Price. 2012. Quick Guide to the Scarabaeoidea of Maryland. Salisbury Univerisity Student Research Conference, Salisbury University, Salisbury MD. April 2012. (*Talk*)

OUTREACH AND SERVICE(* while at Utah State University)

2023: BUGfest at the Natural History Museum of Utah, Rio Tinto Center, University of Utah*

2022-present: Empowered Teaching Excellence Program: Explore Track Development Subcommittee Member*

2021–2022: International Union for the Study of Social Insects (IUSSI) 2022 Meeting Graduate Student and Post-doctoral Committee Member*

2021–2022: Department of Biology Diversity, Equity, and Inclusion Committee Member*

2019: Biology Graduate Student Association Chair Member*

2017–2020: Native American Summer Mentorship Program (NASMP)*

2017–2018: Department of Biology Graduate Program Committee (NASMP)*

2017–2018: Nerd Herd Coding Club–Arduino Projects*

2017–2018: Entomology Club Zoo Keeper–Science Unwrapped Events*

2014: Genetics Instructor–Wicomico County Thinking and Doing program

2013–2015: SU Graduate Student Liaison: Biology Living, Learning, Community

2012–2015: Co-Instructor: Introductory Biology, Assateague Island Field Trip

2012–2014: Instructors Assistant: Insect Night at SU for Brownies Troop 1140

2012–2015: Instructors Assistant: Bug Day at Bundles of Joy Daycare

SOCIETY MEMBERSHIPS

International Union for the Study of Social Insects—North America Section (IUSSI-NAS)

International Society for Neuroethology

Society for the Study of Evolution

Animal Behavior Society

American Genetic Association

SOFTWARE PACKAGES

MADtraits–Make A Database of Traits (R Package).**(2018)**. Pearse, W.D., K. Hafen,

M.A. Hagadorn, M. Haupt, S.B. Hudson, S. Kinosian, R. McClearly, A. Rego, and K.M. Weglarz. GitHub repository: https://github.com/willpearse/MADtraits

UNDERGRADUATES MENTORED

Utah State University: 12 students; 1 undergraduate honors theses

Salisbury University: 4 students; 1 undergraduate honors thesis

PROFESSIONAL REVIEW

Journal of Applied Entomology: 1 manuscript

Empowered Teaching Excellence–open-book series: 2 book chapters