Brain development in a facultatively social allodapine bee aligns with caste, but not group living

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Introduction: The 'social brain hypothesis' proposes that brain development (particularly primates) is driven by social complexity, more than group size. Yet, small insects with minute brains are capable of the most complex social organization in animals - which warrants further attention. Research has focused on highly eusocial hymenopterans with extreme caste specialization and very large colony sizes that have passed social evolutionary points of no return. However, facultatively social insects that form small colonies (< 20 individuals) are likely to provide greater insight on brain selection at the origin-point of social group living.

Methods: We undertake the first neurobiological investigation of the facultatively social allodapine bees (Apidae: Xylocopinae: Allodapini), an exploratory study comparing single- and multi-female colonies of *Exoneura angophorae*. Using volume as a proxy for neural investment, we measured mushroom body calyces, optic lobes, antennal lobes and whole brains of queens, workers, and single-females to test three theories associating brain development with behavior: social brain hypothesis; distributed cognition hypothesis; sensory environment hypothesis.

Results: Mushroom bodies were reduced in subordinate workers, but did not differ between queens and single-females. Workers had larger optic lobes than queens, but did not differ from single-females. There were no differences in antennal lobes or whole brain volume.

Discussion: Social caste, rather than multi-female versus single-female nesting, influenced mushroom body volume in this allodapine bee – counter to both social brain and distributed cognition theories and in alignment with halictine and ceratinine bees that also form small facultatively social colonies. Optic lobe enhancement is likely a response to dietary niche requirements for extra-nidal foraging behavior – which may be a highly plastic trait capable of rapid transition among allodapine and ceratinine bees that conforms with ecological intelligence hypotheses. These broad volumetric trends require further investigations on the functional neural circuitry involved in the aforementioned environmental contexts.

1 Introduction

The diversity of social organization structures exhibited by insects provide excellent opportunities for studying how social behavior influences neural plasticity and the evolution of brain morphology (*reviewed by* Fahrbach et al. 1998; Fahrbach, 2006; Lihoreau et al., 2012; Farris, 2016; O'Donnell and Bulova, 2017; Godfrey and Gronenberg, 2019). Previous studies on wasps and bees have shown the development of intraspecific differences between individuals of social and solitary nests of facultatively social species (Smith et al., 2010; Rehan et al., 2015; Jaumann et al., 2019). These facultatively social taxa form relatively small colonies (< 20 nestmates), wherein enlarged brain regions (volume as a proxy for neural investment) typically correlate with social caste (egg-laying queens *cf.* non-reproductive workers), social group formation (social queens *cf.* reproductive but solitary foundresses) or dominance status pertaining to social aggression among workers. In a similar vein, the evolution of interspecific differences in brain regions between obligately social species and closely related solitary species has also been demonstrated (Molina and O'Donnell, 2008; O'Donnell et al., 2007, 2015, 2017; Pahlke et al., 2019, 2021).

From an evolutionary perspective, brains are energetically expensive to maintain, so investment in neural tissue should reflect a selective need (Niven and Laughlin, 2008). Originally devised for a range of mammals (particularly primates), the 'social brain hypothesis' posits that the complexities of social interactions (more so than absolute group size or an increased frequency of interactions) should select for increased neural investment in cognition (Dunbar, 1992; Dunbar and Shultz 2007 - but see DeCasien et al., 2017; Kverková et al., 2018). For insects, this should be reflected by larger brains in social species compared to their solitary comparators (Gronenberg and Riveros, 2009; O'Donnell et al., 2015; Pahlke et al., 2021).

The area of the brain that has received the most focus in social insect studies is the mushroom bodies (MB), which are paired neuropils involved with learning, memory, and sensory integration (Fahrbach, 2006); under the correlative assumption that that bigger MBs are associated with more intelligent insects. One study in sweat bees (Halictidae) supports this prediction (Pahlke et al., 2021), but another in wasps (O'Donnell et al., 2015) shows the opposite trend: solitary potter wasps (Eumininae) have larger MBs than their social relatives, the paper wasps (Vespidae). O'Donnell et al. (2015) proposed the 'distributed cognition hypothesis', in which social species can afford to invest less in cognition (and thus have smaller MBs) than their solitary relatives because cognitive effort is distributed across the group.

Differences in brain investment can also arise as a result of plasticity in response to social experience – 'sensory environment hypothesis'. For example, *Drosophila* reared in a group had larger MBs than those reared alone (Heisenberg et al., 1995), typically solitary sweat bees expanded their MBs when caged with a conspecific (Hagadorn et al., 2021) and both ants and bees of social species have reduced MB volume when reared in isolation (Seid and Junge, 2016; Maleszka et al., 2009 - but see Jernigan et al., 2021; Wang et al., 2022; Goolsby et al., 2024). However, recent comparative evidence from vertebrates (primates and birds) provides support for the 'ecological intelligence hypothesis' (Rosati, 2017), which argues that foraging cognition (spatial memory, value-based decision-making, flexible control of behavior) in response to dietary niche can similarly account for the same brain development outcomes that the social brain hypothesis accounts for.

Facultatively social insect species can either form social groups with a reproductive division of labor between an egg-laying queen and non-reproductive worker(s) or nest solitarily - this provides the opportunity to experimentally compare social versus solitary behavior within the same species. In two facultatively social bee species studied to date, the sweat bee *Megalopta genalis* and the small carpenter bee *Ceratina australensis*, there were no consistent social-solitary differences in MB volume, but instead differences between queens and workers within the social nests (Smith et al., 2010; Rehan et al., 2015; Jaumann et al., 2019). This is compatible with equivalent studies in paper wasps, where reproductive dominance status correlated with MB volume (O'Donnell et al., 2007; Molina and O'Donnell, 2007, 2008); and may be a result of cognitive demands associated with maintaining dominance such as keeping track of nestmates and social status (e.g. Tibbetts et al., 2018). It might also result from physiological changes associated with egg laying (ovarian enlargement) and associated ovarian suppression in subordinates (e.g. Smith et al., 2013; Hamilton et al., 2017).

Differential developmental and/or opportunistic adult access to nutrition may allow reproductive dominants to invest more in MBs prior to the establishment of social groups – especially under eusocial colony organization where workers are the offspring of the queen, and thus at least one generation younger (Pahlke et al., 2019). Brain differences associated with social castes may also arise from the requirements of radically different sensory environments, for instance with visually-orienting foragers showing greater development of the optic lobes (Molina et al., 2009; O'Donnell et al., 2014; Valadares et al., 2022); or chemosensory requirements relating to nestmate recognition which might influence development of antennal lobes (Wang et al., 2022; Goolsby et al., 2024).

Here we use the facultatively social allodapine bee *Exoneura angophorae* to test for brain differences associated with social behavior or lack thereof. Tribe Allodapini (Apidae: Xylocopinae) are a radiation of facultatively social bees which represent an independent origin of eusociality in the family Apidae, separate from the corbiculate bees (Schwarz and Tierney, 2020). While recent neurological studies have demonstrated an influence of social caste in sweat bees (Halictidae: Augochlorini), small carpenter bees (Ceratina, Apidae: Xylocopinae), and paper wasps (Vespidae: Polistinae); brain differences between social castes in allodapine bees have yet be investigated, despite their role as a model system for the evolution of eusociality (Michener, 1974; Schwarz et al., 2007; Tierney et al., 2008c).

Nesting biology of *E. angophorae* along the eastern Australian seaboard has been previously reported (Schwarz et al., 1996; Cronin and Schwarz, 1997, 1999, 2001; Bernauer et al., 2021).
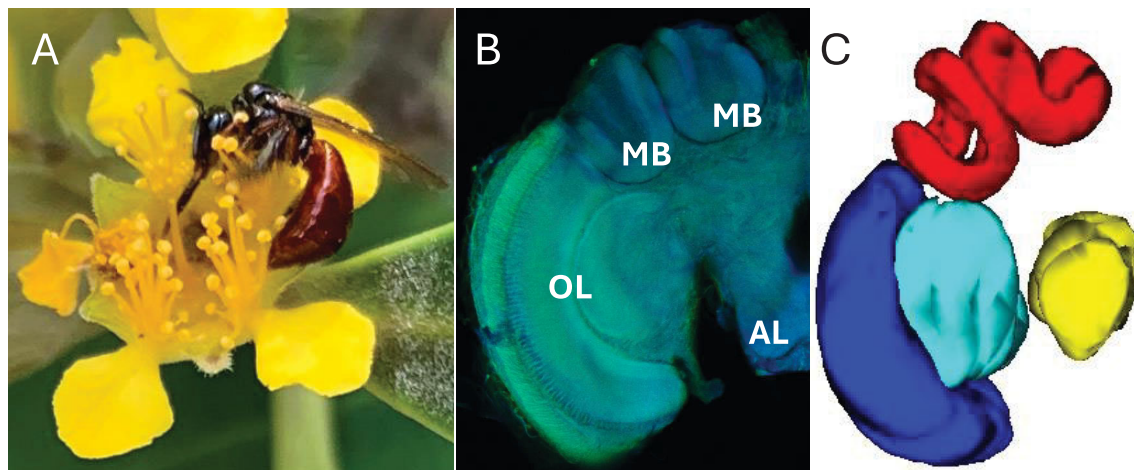
FIGURE 1
Focal allodapine bee and brain morphology. **(A)** Female *Exoneura* bee visiting a *Tristaniopsis* water gum flower in Helensburgh NSW Australia (photo credit: Olivia Bernauer). **(B)** Frontal section confocal micrograph showing the left hemisphere of the brain, including the optic lobe (OL), mushroom body calyces (MB), and antennal lobe (AL); note that most of the antennal lobe is posterior to this section and thus not pictured). **(C)** Three-dimensional reconstruction of the OL lobula (light blue), OL medulla (dark blue), MB calyces (red) and AL (yellow).

Overwintering of eggs provides evidence for the persistence of nests across multiple years, although the peak egg laying period is in spring (Bernauer et al., 2021) and some populations of *E. angophorae* are known to co-found nests (Schwarz et al., 1996; Schwarz, 1988b; Cronin and Schwarz, 1997). Single foundress-female nests become subsocial once brood are present - due to the progressive rearing and defense of immatures in a linear tunnel with no brood cell divisions (Michener, 1969; Bernauer et al., 2021). In some solitary-founded nests, daughters remain as non-reproductive workers for their mother, now a social queen. Should the queen die, semi-social or quasisocial assemblages of sisters can persist or may represent the initial social construct among co-founded nests of closely related individuals (Schwarz, 1988a). If all mature offspring disperse from a solitary-founded nests, the foundress female remains as a solitary reproductive in the absence of brood or a subsocial reproductive in the presence of immature brood – hence from hereon we refer to the latter two scenarios as a 'single-female'.

In this study we explore the evolution and intraspecific development of brain volumes in a montane population of *E. angophorae* (Figure 1). We measured volumes of whole brains and the relative volumetric ratios of MB calyxes, optic lobes (OL) and antennal lobes (AL) of single-females and queens and workers from multi-female colonies. This data was used to test three hypotheses that associate social behavior with neural investment: (i) social brain, (ii) distributed cognition and (iii) sensory environment. The social brain hypothesis predicts that females inhabiting multi-female nests (queens & workers) will exhibit enhanced development of brain regions *cf.* single-females, owing to the increased complexity of cognitive interactions occurring in semisocial and eusocial social colonies. The distributed cognition hypothesis predicts the reverse, that single-females will exhibit larger brain regions *cf.* queens and workers in multifemale nests because the latter can afford to collectively share cognitive duties.

Both of these theories arose from an organismal evolutionary perspective (interspecific comparisons of obligate-solitary versus obligate-social species), but given the facultative nature of allodapine social organization and female totipotency, we also have the opportunity of exploring social brain theorical concepts from an intraspecific developmental perspective within multi-female nests. Hence, we also assess whether queen castes exhibit enhanced brain region development in order to maintain a reproductive dominance hierarchy over worker castes with reduced ovaries (to the exclusion of single-females). The sensory environment hypothesis predicts that the differential sensory experience of individuals nesting in groups *cf.* independent nesting (multi-female nests *cf.* single-female nests) will result in enlarged sensory regions of the brain (to the exclusion of MBs) in multi-female bee nests more so than single-female bees. Hence, we predict that the ALs would be relatively larger in multifemale bee nests (regardless of caste) than single-female nests - because multifemale bees are exposed to the chemical stimulation of adult nestmates and *Exoneura* have been experimentally shown to pheromonally inhibit the ovarian development of subordinate female workers (Schwarz et al., 1987; O'Keefe and Schwarz, 1990). We also expect that females performing foraging tasks outside the nest, will have larger optic lobes than queens, who remain in the nest once their daughters reach adulthood, although these queens are likely to have previously foraged as single-female foundresses.

2 Materials and methods

2.1 Nest collection and dissection

Nests in fronds of the tree fern *Alsophila australis* were collected when bees were not foraging (< 15°C) on 11 October 2018 from

Mount Wilson, New South Wales, Australia (33° 31′ S 150° 22′ E; 906 m elevation). For a comprehensive assessment of life history for this population of *E. angophorae* see Bernauer et al. (2021). We dissected brains from six single-female nests, and six social nests - we were unable to determine whether social nests were solitary or co-founded. Within social nests, queens were determined by ovarian development - measured as the summed length of the three largest oocytes. Reproductive egg-laying queens exhibit enlarged well-developed ovarioles while workers do not. If a nest had more than one worker, we chose the worker with the most worn wings for analysis, as wing wear correlates with bee age and foraging activity (Mueller and Wolf-Mueller, 1993; Tierney et al., 2008a, b; Tierney and Schwarz, 2009). The oldest workers were chosen under the assumption they would be more likely to have experienced social divisions of labor. In total, brain development data was collected for 18 individuals. Social nests contained 3–7 females, including the queen (average = 4.17 ± 1.47 SD). None contained adult males. We measured body size using two metrics: head width from the outer edge of one compound eye to the outer edge of the other, across the antennal insertion points and forewing length from the distal apex of the marginal cell to the axillary sclerites, mounted flat on a microscope slide. All linear measurements were recorded under a stereomicroscope using an ocular micrometer.

2.2 Brain measurements

We preserved bee heads in 4% paraformaldehyde in phosphate buffered saline (PBS) at collection and stored them at 4°C until dissection. We dissected head capsules in PBS to remove the brain which was immediately placed in glutaraldehyde (2%) for 48 hours, bleached in a formamide solution, and dehydrated in a series of ethanol washes of increasing concentration following McKenzie et al. (2016). Prior to imaging, brains were mounted in methyl salicylate. Brains were imaged using an Olympus Fluoview FV1000 confocal microscope using autofluorescence at 10X magnification and a step size of 10 μm. We calculated volumes of the brain and different neuropils (MB calyces, AL, and OL, including both the lobula and medulla) through tracing and serial reconstruction using the software program Reconstruct (Fiala, 2005). Volumes for specific brain areas were analyzed as ratios for each neuropil: whole brain to control for differences in body size. Only one brain hemisphere per individual was used for analyses.

2.3 Statistical analysis

Analyses were conducted in SPSS using non-parametric statistics owning to small sample sizes. We used Kruskal-Wallis tests to compare across the three groups (single-female reproductives, queens, and workers), followed by Bonferroni-corrected pairwise Mann-Whitney U-test pairwise comparisons when the Kruskal-Wallis test showed a significant effect. We used Mann-Whitney U tests to compare social (queens and workers

combined) bees to single-female bees. For queen-worker comparisons we used Wilcoxon sign-rank tests to account for the non-independence of developmental history caused by sharing a nest. We used Spearman's rank correlations to compare continuous variables.

3 Results

3.1 Whole brains, body size and ovarian enlargement

There was no significant difference in head size between queens, workers, and single-females ($H_2 = 2.218$, p = 0.330), nor between queens and workers (Z = -0.524, p = 0.600; Figure 2A). There was no difference in whole brain size between groups ($H_2 = 0.924$, p = 0.630), nor between queens and workers (Z = -0.943, p = 0.345; Figure 2B). There were differences in wing length between groups ($H_2 = 6.34$, p = 0.042; Figure 2C). Single-females had longer wings than workers (Bonferroni corrected *post-hoc* p = 0.037), but other pairwise comparisons were not significant (queens *cf.* workers: p = 0.886; queens *cf.* single-females p = 0.433). Ovarian development differed between groups ($H_2 = 8.257$, p = 0.016; Figure 2D). There was no significant difference between single-female and worker ovaries (Bonferroni corrected *post-hoc* p = 1.00). Queens did not have significantly larger ovaries than single females (Bonferroni corrected *post-hoc* p = 0.098), but they did have significantly larger ovaries than workers (Z = -2.201, p = 0.028). Neither ovary size nor whole brain volume correlated significantly with head size or wing length. Wing length and head width (the two measures of body size) significantly correlated with each other (N = 18, rho = 0.672, p = 0.002).

3.2 Mushroom bodies

There were no differences in absolute MB calyx volume across groups ($H_2 = 3.19$, p = 0.20), or when measured as a ratio of whole brain size across groups ($H_2 = 4.257$, p = 0.119; Figure 3A). Nor was there a difference between social (queens + workers) and single-female bees ($U_{16} = 34.000$, p = 0.892). Within the social nests, though, queens had larger MB calyx ratios than workers (Z = -1.992, p = 0.046; Figure 4A). There was a marginally significant correlation between ovary size and MB calyx volume (N = 18, rho = 0.469, p = 0.050).

3.3 Optic lobes

There were no differences in absolute OL volume across groups ($H_2 = 0.25$, p = 0.88), or when measured as a ratio of whole brain size across groups ($H_2 = 1.836$, p = 0.399; Figure 3B). Nor was there a difference between social (queens + workers) and single-female bees ($U_{16} = 35.000$, p = 0.925) or single-female and worker bees ($U_{11} = 16.0$, *p* = 0.75). Within the social nests, though, workers had
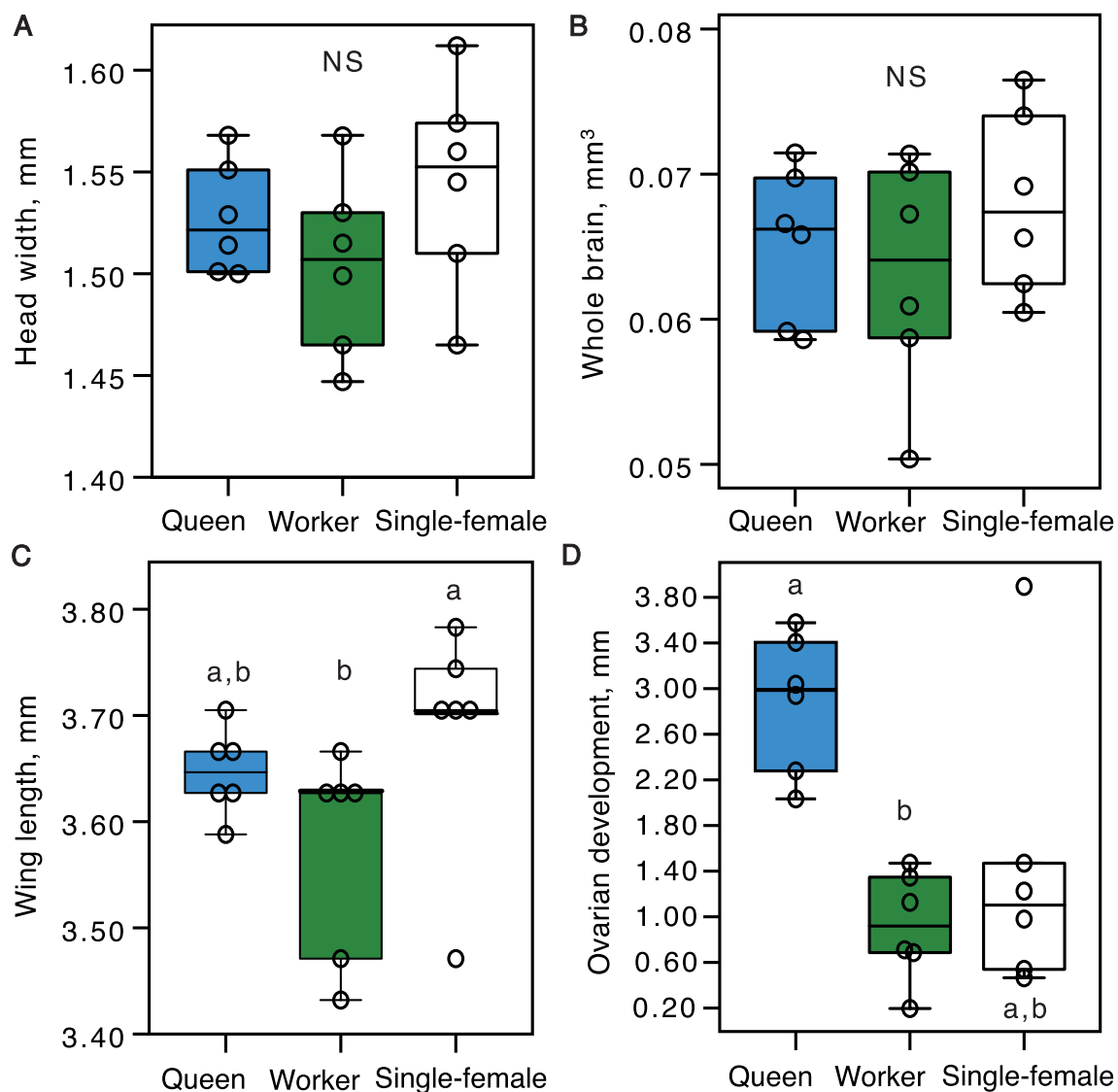
FIGURE 2
Social morphometrics and brain development. Body size and reproductive status are often indicative of social castes in insect colonies, which were assessed relative to brains of Queens (blue), Workers (green) and Single-females (white): head width **(A)**, whole brain volume **(B)**, wing length **(C)** and ovarian development **(D)**. Box-plots indicate median (bold horizontal bar) upper and lower quartiles (box) and 95% confidence intervals (whiskers). Plots assigned with different letters are significantly different from each other - there were no significant differences (NS) in head width or whole brain volume.

larger OL ratios than queens (Z = 2.201, p= 0.028; Figure 4B). There was no correlation between ovary size and OL volume.

3.4 Antennal lobes

There were no differences in absolute AL volume across groups (H_2 = 2.85, p = 0.24), or when measured as a ratio of whole brain size, across groups (H_2 = 1.719, p = 0.423; Figure 3B). Nor was there a difference between social (queens + workers) and single-female bees (U_{16} = 35.000, p = 0.964) or single-female and worker bees (U_{11} = 14,0, p = 0.52). Similarly, there were no differences between castes in the social nests (Z = -1.363, p= 0.173; Figure 4C). There was no correlation between ovary size and AL volume.

4 Discussion

Single-female and multi-female colonies of the facultatively social allodapine bee *E. angophorae* were assessed for differential neural investment in brain regions (MB, OL, AL) to test theories associating intra-specific brain development with behavior. MB calyces were reduced in subordinate workers, but did not differ between queens and single-females. Workers and single-females had larger OLs than queens. There were no differences in ALs or whole brain volumes between groups. Our results concur with previous studies of facultatively social species, whereby differences in social caste (queen *cf*. worker) rather than group or independent nesting, most strongly influence MB volume – which is discussed in relation to social complexity and hypotheses of brain evolution
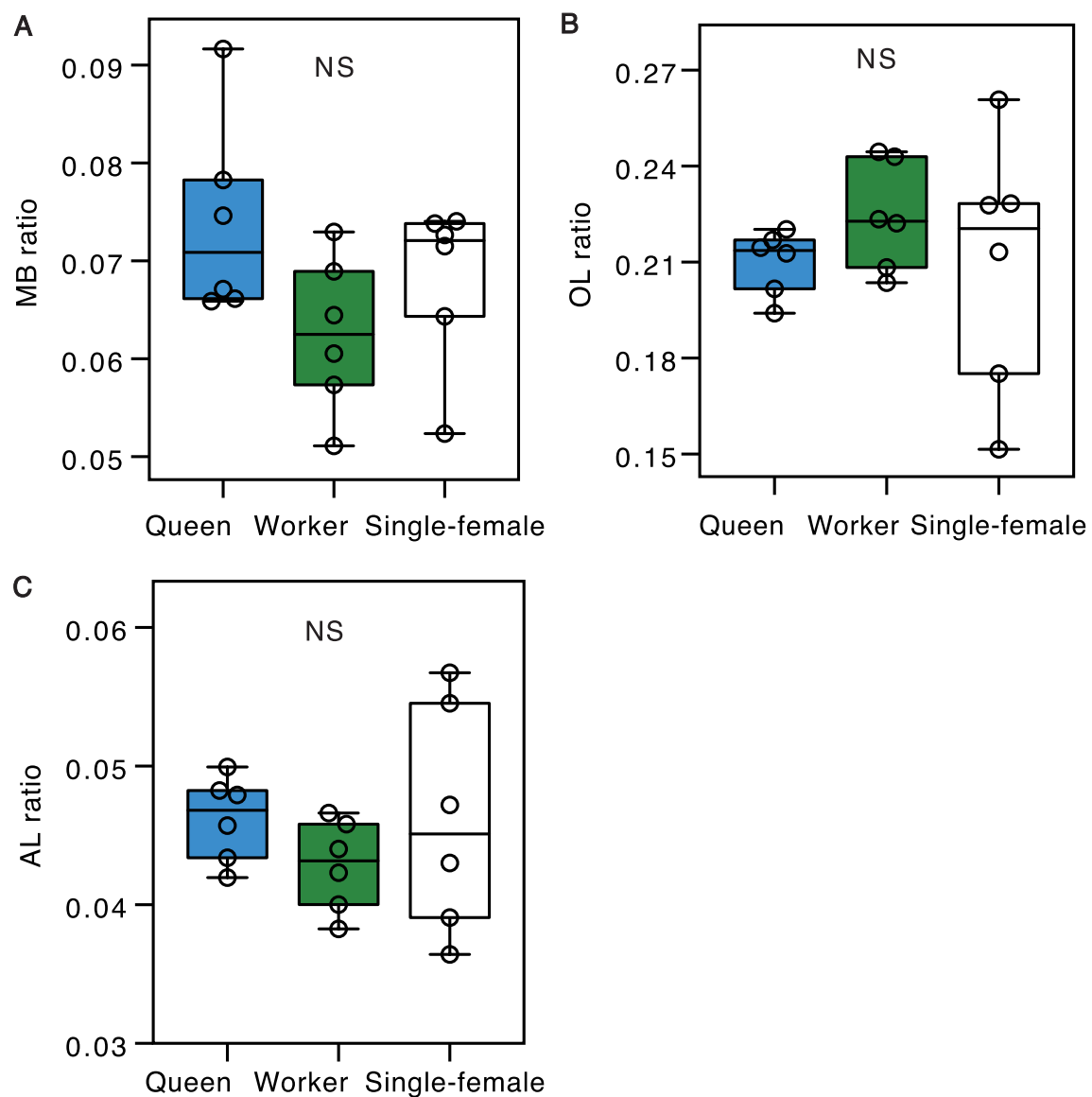
FIGURE 3
Relative development of brain regions. Ratio of mushroom body (MB) volume **(A)**, optic lobe (OL) volume **(B)**, and antennal lobe (AL) volume **(C)** relative to whole brain volume of Queens (blue), Workers (green) and Single-females (white). Box-plots indicate median (bold horizontal bar) upper and lower quartiles (box) and 95% confidence intervals (whiskers). There were no significant differences (NS) among groups, but see pairwise comparisons (Figure 4) for significant pairwise differences between Queens and Workers.

below. OL enhancement in workers and single-females is also consistent with findings in the sister tribe Ceratinini and aligns with ecological intelligence theory more so than social intelligence theory.

4.1 Mushroom body reduction in workers

In our study, *E. angophorae* queens had larger MB calyces than workers. This is consistent with other studies of facultatively social bees (Smith et al., 2010; Rehan et al., 2015; Jaumann et al., 2019) as well as studies of small-colony obligately social bees and wasps (O'Donnell et al., 2007; Molina and O'Donnell, 2007, 2008; Pahlke

et al., 2019). One potential explanation for this is age: queens are usually older than workers (because workers are the queens' daughters unless nests are semisocial), and plasticity based on accumulated experience can lead to MB volume increases (e.g. Withers et al., 1993, 2008; Gronenberg et al., 1996; Farris et al., 2001; Rehan et al., 2015). If accumulated experience due to increased age is responsible for the observed MB calyx volume differences, we would expect single-female reproductives to also have larger MB calyces than workers. The data here are ambiguous: while single-female reproductives have a similar median volume to queens, they are not significantly larger than workers (Figure 3A), although our small sample size limits statistical power. Furthermore, we do not know the history of these single-female
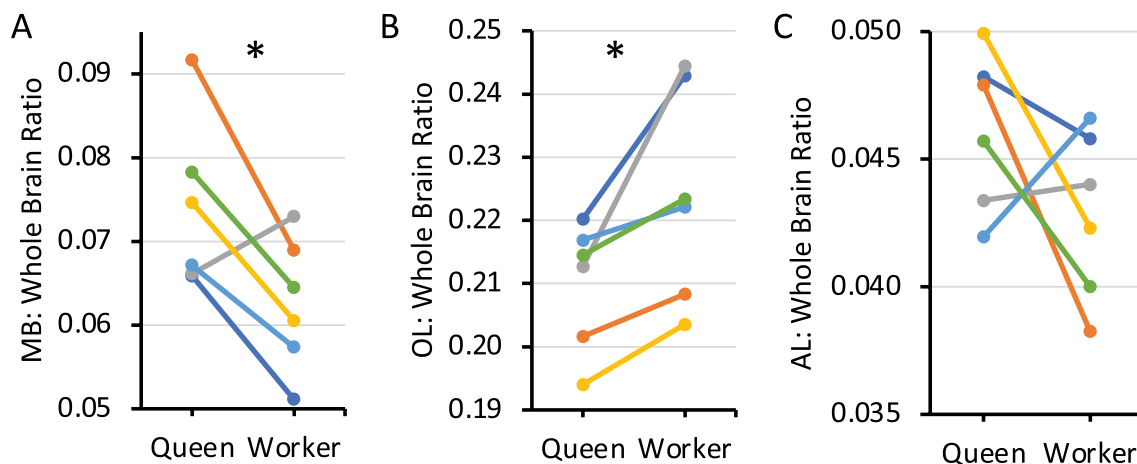
FIGURE 4
Intracolony comparisons of brain development. Pairwise comparisons of different brain regions of queen and worker from the same nest (connected by a line): mushroom body (MB: whole brain ratio) **(A)**, optic lobe (OL: whole brain ratio) **(B)**, and antennal lobe (AL: whole brain ratio) **(C)**. Asterisks (*) indicate a significant difference between groups in paired Wilcoxon sign-rank tests. Each nest is represented by the same color in all three panels.

nests but they likely represent novel spring colonies of dispersing foundresses. We do not have data on the phenology of nest founding or the proportion of co-founding for this population.

However, in two studies controlling for age, *Ceratina* and *Megalopta* queens still had larger MB calyces than workers (Rehan et al., 2015; Jaumann et al., 2019), suggesting that dominance interactions may be responsible for the observed differences in MB volume. In the *C. australensis* bees studied by Rehan et al. (2015), queens had larger MB calyces, and workers smaller ones, than solitary reproductives. In the *M. genalis* bees studied by Jaumann et al. (2019), queen MBs were similar to other treatment groups, but workers' were smaller. This suggests that for subordinate bees, the aggressive interactions typically involved in establishing queen-worker dominance (e.g. Michener and Brothers, 1974; Kapheim et al., 2016) may suppress brain development just as they suppress reproductive development and other physiological processes, including juvenile hormone and brain amine titers (Smith et al., 2013; Hamilton et al., 2017). Brain differences between queens and workers may also result from differential developmental nutrition (Pahlke et al., 2019).

There have been no reports of aggressive behavior in *E. angophorae*, however, in the sister species *E. robusta* reproductively dominant female guards are more likely to physically exclude absentee nestmates from re-entering the nest when they have been experimentally placed in contact with foreign males, more than associates of foreign females or control bees (Bull et al., 1998). Other explicit reports of allodapine agonistic behaviors relate to interactions between inquiline social parasites and their *Braunsapis* hosts, particularly following denial of trophallactic exchange by the host (Batra et al., 1993).

Mass provisioning halictine bees (a) require extra nutrition for diapause, and there is evidence of (b) parental manipulation of brood provisions, which may influence brain development between

castes (Pahlke et al., 2019). Temperate zone allodapine species do exhibit developmental diapause of immatures (eggs and early instar larvae) which overwinter with long-lived adults that also survive this period. Adult ovarian development of both inseminated and uninseminated females of this same population of *E. angophorae* are greatest during winter (Bernauer et al., 2021), which may represent trophic eggs as a nutritional source during winter when foraging is more restricted; as queens and workers from two genera of temperate South African allodapines (*Allodape* and *Braunsapis*) have been observed engaging in oophagy (Skaife, 1953; Mason, 1988). Maternal manipulation of larval nutrients in an open linear nest with no brood cells (in contrast to the individual cells of halictine bees), is feasible but likely difficult to control - especially for species of *Exoneura* where eggs are commonly clumped at the bottom of the nest (not glued to the nest wall as in other genera), rendering maternal discrimination of individual brood during feeding stage larval instars unlikely (Schwarz, 1988a). Also, allodapines are known to exhibit frequent exchange of nutrients between individuals (adults and larvae), which in some instances is representative of a trophallactic 'network' with all adults acting as donors and receivers (Mason, 1988). Thus the caste-based MB differences here may not be a result of maternal manipulation of nutrition.

4.2 Plasticity of sensory brain regions

Our data on OLs, but not ALs, fits with predictions based on the bees' sensory environments. Subordinate worker females forage, which entails visual stimulation and requires navigation based on recognition of visual cues, so it is not surprising that they have larger optic lobes, although queens of the facultatively social Halictid bee *Megalopta genalis* had larger optic lobes than their

workers (Jaumann et al., 2019); but these are dim-light foraging bees (Wcislo and Tierney, 2009) that have made a considerable evolutionary transition in photic environment (Tierney et al., 2012, 2017). Also, *Exoneura* queens used to forage, before their worker daughters were born, which suggests that OLs volume may decrease after the queens become nest-bound. This pattern is seen in post-reproductive females of the socially flexible small carpenter bee *Ceratina calcarata* (Jaumann et al., 2022) and some species of harvester ants once the queen ceases foraging and becomes nest-bound (Gronenberg and Liebig, 1999; Julian and Gronenberg, 2002; Penick et al., 2021). Single-female reproductives also forage, so we would expect that their OLs are similar sized to foragers'. The median OL volume of *E. angophorae* single-females and workers is similar (Figure 3B), but the wide range of variation within the single-female reproductives combined with low sample size makes it difficult to draw conclusions from this group.

Our *a priori* expectation was that the ALs of bees from multi-female nests (queens and workers together) would be larger than that of single-female nest bees because of the chemical stimulation of social nestmate interactions and recognition (Wang et al., 2022; Goolsby et al., 2024). However, this was not the case and is perhaps not unexpected for allodapine bees, given that single-female nests containing brood are sub-social and are progressively feeding these brood during larval feeding stages – hence there is continuous nestmate interaction. Future studies may need to focus on single-females in the complete absence of brood, as only one of our single-female nests lacked brood entirely (Supplementary Table S1), although at the population level single-female nests only lack brood in early-mid autumn (Bernauer et al., 2021).

4.3 Reproductive caste differentiation

Social colonies in this population of *E. angophorae* tend to have one reproductively dominant female (Bernauer et al., 2021), queens that exhibit significantly greater ovarian development compared to all other nestmates. Only spring colonies exhibit per capita benefits to brood production and only summer colonies are known to exhibit sized-based reproductive dominance hierarchies, based on thorough sampling throughout the life cycle: N = 591 nests collected; n = 668 adult females dissected from 121 single-female nests 215 multi-female nests (Bernauer et al., 2021). In alignment with these broader results, our study of spring colonies did not find significant caste-based differences in body size in either wing length or head width, although queens did have larger medians (but not significantly so) for both measures (Figure 2). However, single-females were significantly larger than workers. There may be selection for maximal body size among dispersing foundresses that produce smaller daughters that eclose in summer – these subordinate daughters may overwinter and subsequently inherit reproductive dominance within their natal nest the following spring should the foundress senesce.

4.4 Social brains

Whether complexity of social living is causative of enhanced cognitive abilities (social brain hypothesis), in comparison to solitary living, among insects more broadly remains equivocal. It has been argued that enlarged brain regions may simply be aligned with foraging cognition (ecological intelligence hypothesis), mate competition and defensive behaviors that can be disassociated from sociality (*reviewed by* Poissonnier et al., 2023). The results from our exploratory study represent the first neurobiological data for any species in the bee tribe Allodapini, and suggest that further neurobiological studies would be fruitful. However, our conclusions need to be somewhat tempered given that only six brains per group (18 total) were dissected and analyzed. This is at the lower end of the range of sample sizes for similar studies, most of which used 5–15 individuals per group, with totals of 20 or more individuals, depending on the number of experimental groups (e.g. Withers et al., 1993, 2008; O'Donnell et al., 2007; Molina and O'Donnell, 2007, 2008; Smith et al., 2010; Rehan et al., 2015; Jaumann et al., 2019, 2022; Pahlke et al., 2019, 2021; Valadares et al., 2022).

The differences in MB calyx volume that we found between queens and workers are consistent with dominance-based differences in other social or facultatively social bees and wasps previously studied, namely: queens have larger MBs than workers (Molina and O'Donnell, 2007, 2008; Molina et al., 2009; Rehan et al., 2015; Smith et al., 2010; Jaumann et al., 2019; Pahlke et al., 2021). Our OL data is suggestive of either additional investment to meet sensory needs in foraging workers, or reduction of OL tissue in nest-bound queens. However, more experimental work on the comparative functional neural circuitry in OL's of solitary (allodapine single-female) foragers versus foraging workers in social colonies are needed to confirm this. Lastly, our data suggest that AL volume is apparently not affected by social environment in this allodapine bee.

5 Conclusion

Our study represents the first data on brain volume and social caste in allodapine bees, and suggests that as in other groups of facultatively social or small-colony bees and wasps, social status does correspond with differential development in certain regions of the brain. In this species of *Exoneura* MBs are reduced in workers. However, this does not support social intelligence hypotheses from a comparative developmental standpoint when independently nesting females are taken into account; particularly single-female allodapine nests that contain brood and are considered subsocial. It appears that OL enhancement/reduction may be highly plastic and capable of transitions over relatively short periods of time in allodapine and ceratinine bees, which is in agreement with theoretical concepts of ecological intelligence (foraging cognition) and worthy of further investigation.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because research was undertaken on invertebrates which do not require ethics approval.

Author contributions

ST: Visualization, Conceptualization, Methodology, Formal Analysis, Writing – original draft, Data curation, Validation, Writing – review & editing, Resources, Investigation. SJ: Visualization, Writing – review & editing, Data curation, Methodology, Investigation. OH: Writing – review & editing, Methodology, Data curation, Visualization, Investigation. AS: Methodology, Resources, Funding acquisition, Investigation, Visualization, Formal Analysis, Writing – review & editing, Validation, Data curation, Writing – original draft, Project administration, Supervision, Conceptualization.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2025.1603824/full#supplementary-material

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