

Brain organization mirrors caste differences, colony founding and nest architecture in paper wasps (Hymenoptera: Vespidae)

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The cognitive challenges that social animals face depend on species differences in social organization and may affect mosaic brain evolution. We asked whether the relative size of functionally distinct brain regions corresponds to species differences in social behaviour among paper wasps (Hymenoptera: Vespidae). We measured the volumes of targeted brain regions in eight species of paper wasps. We found species variation in functionally distinct brain regions, which was especially strong in queens. Queens from species with open-comb nests had larger central processing regions dedicated to vision (mushroom body (MB) calyx collars) than those with enclosed nests. Queens from advanced eusocial species (swarm founders), who rely on pheromones in several contexts, had larger antennal lobes than primitively eusocial independent founders. Queens from species with morphologically distinct castes had augmented central processing regions dedicated to antennal input (MB lips) relative to caste monomorphic species. Intra-specific caste differences also varied with mode of colony founding. Independent-founding queens had larger MB collars than their workers. Conversely, workers in swarm-founding species with decentralized colony regulation had larger MB calyx collars and optic lobes than their queens. Our results suggest that brain organization is affected by evolutionary transitions in social interactions and is related to the environmental stimuli group members face.

Keywords: social behaviour; brain organization; caste differences; tissue allocation

1. INTRODUCTION

Neural tissue is expensive. Both production and maintenance costs of neural tissue are high relative to many other tissues (Niven *et al.* 2003; Nawroth *et al.* 2007; Niven 2007). The anatomical organization of brains often indicates differences in relative investment among functionally distinct brain regions (Hampton & Shettleworth 1996; Clayton 1998; Bingman 2004). Brain structure covaries with behavioural differences, suggesting that brain tissue allocation reflects species-typical cognitive challenges (Barton & Harvey 2000; Gronenberg 2001). Sociality poses a number of substantial cognitive challenges to animals, including spatial learning of nest/home sites, group mate discrimination and assessment of relative rank (Anderson 1998; Capaldi *et al.* 1999; Tibbetts & Lindsay 2008). At the individual level, social roles within groups correspond to differences in brain volume and activity (Withers *et al.* 1993; Øyvind *et al.* 1999; Groh *et al.* 2006; Molina & O'Donnell 2007, 2008).

The social intelligence hypothesis was developed to explain how the evolution of sociality could impact brain evolution (Shultz & Dunbar 2006; Dunbar & Shultz 2007). This hypothesis assumes that sociality imposes novel cognitive challenges (e.g. group mate recognition) to a species in addition to those faced by its

solitary ancestors. Social species often have enlarged brains and/or larger specific brain regions than their non-social relatives (Croney & Newberry 2007; Silk 2007). For example, comparative studies of neuroanatomy in Hymenoptera suggested that some brain neuropils were larger in social species than in closely related solitary species (Howse 1974).

The goal of our study was to extend the comparative analysis of brain structure evolution beyond social–solitary species comparisons. The origin of sociality within a clade is an important transition, but there can be further changes in behaviour as a more complex social structure evolves (Bourke 1999; Anderson & McShea 2001; Jeanne 2003). The modification of behaviour in social lineages can include changes in group size, modes of communication and the degree of division of labour among group members (Jeanne 2003; Hölldobler & Wilson 2009). We propose that evolutionary transitions in group structure and behaviour within social taxa can select for additional changes in brain architecture. We predicted that species variation in cognitive challenges would explain differences in brain architecture (Farris & Roberts 2005; Farris 2008).

We used social paper wasps (Vespidae) as subjects. Paper wasp phylogeny is well characterized at the genus level (Carpenter 1991; Arévalo *et al.* 2004). Paper wasps exhibit two distinct grades of social organization, which are associated with colony initiation behaviour: independent-founding (IF) species and swarm-founding (SF) species (Jeanne 1991, 2003; Smith *et al.* 2002).

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IF and SF differ in colony size: minimum mature colony sizes of SF barely overlap the maximum mature colony sizes of IF (Jeanne 1991, 2003). Division of labour is more complex in SF, with greater worker partitioning of tasks, worker team organization and well-developed temporal polyethism (O'Donnell & Jeanne 1992; Jeanne 2003). Within the IF/SF grades of social structure, there is further variation in nest architecture, colony size and mechanisms of caste determination (Jeanne 1991; O'Donnell 1998a; Noll *et al.* 2004). We extended previous comparisons of IF and SF brain plasticity (O'Donnell *et al.* 2007; Molina & O'Donnell 2007, 2008) by increasing the number of genera sampled and by accounting for the effects of phylogeny in the analysis (Felsenstein 1985; Martins & Hansen 1996, 1997).

We measured the size (volume) of anatomically discrete brain regions as an indicator of investment in brain tissue. We measured both peripheral and central processing brain regions allocated to two major sensory organs: the compound eyes (vision) and the antennae (chemosensation and tactility). The central processing tissues were in the mushroom bodies (MBs), neuropils located within the insect forebrain. Peripheral sensory processing centres of the insect brain—the optic and antennal lobes (ALs)—innervate different MB calyx sub-regions: the lip primarily processes olfactory and tactile input from the ALs, whereas the MB calyx collar receives visual information from the optic lobes (OLs; Farris 2005; Fahrbach 2006).

We assessed whether the volume of targeted brain regions was associated with three behavioural and caste covariates. We list the cognitive challenges individuals face and predicted patterns of brain investment (see also electronic supplementary material).

(a) *Mode of colony founding*

Unlike IF, SF species coordinate movement to new nest sites with pheromone trails (Naumann 1975; Jeanne 1981, 1991). Only SF queens employ pheromones to suppress reproductive development in nest mates (West-Eberhard 1977; Landolt *et al.* 1998; Jeanne 2003). We predicted that SF would have greater investment in olfactory processing brain regions than IF because of an increased dependence on olfactory communication (pheromones).

We expected the brain structure of IF and SF to differ further because of the dramatic changes in colony organization that accompanied the evolution of SF from IF ancestors (Smith *et al.* 2002; Jeanne 2003). Principal among these changes is a shift from centralized, hierarchical control of division of labour in IF to relatively decentralized control in SF (Bourke 1999; Beshers & Fewell 2001). As insect colony organization evolves towards decentralization, the cognitive demands on any given colony member may actually decrease. In centralized societies (e.g. IF), the queen regulates colony activity by interacting directly with nestmates (Reeve 1991). IF queens may therefore be capable of individual recognition and associative memory, and queens may assess colony needs as well as the reproductive abilities of nestmates. IF worker behaviour is linked to their dominance rank and workers are plastic in their task performance (Reeve 1991; Premnath *et al.* 1996; Molina &

O'Donnell 2009). In contrast, SF workers specialize on a set of tasks (O'Donnell & Jeanne 1990; Jeanne 2003). SF wasp workers may use only the information necessary for performing specific tasks (Jeanne 1991; Gordon 1996; Chittka *et al.* 1999), and higher order cognitive processing may be less crucial. IF bumble-bee workers have relatively larger MBs than SF honeybee workers (Howse 1974; Mares *et al.* 2005). We predicted that brain structure would vary with social complexity in paper wasps: MB calyx volume should be smaller in SF species, whose colony organization is more similar to that of honeybees.

(b) *Nest architecture*

In both solitary and social insects, ambient light intensities affect the development of visual processing brain regions (Barth & Heisenberg 1997; Gronenberg & Liebig 1999; Julian & Gronenberg 2002). All IF and some SF species build open-comb nests where the nest surface is minimally sheltered from ambient light. In contrast, most SF species either nest in cavities or cover their brood combs with an envelope, reducing light levels on the brood comb (Jeanne 1975; Wenzel 1991). SF species with open nests may use visual cues more heavily than SF species with enclosed nests (Hunt *et al.* 1995; Nascimento & Tannure-Nascimento 2005; Greiner 2006; Warrant 2008). We predicted that brains from species with open nests would have significantly larger visual processing regions (e.g. MB calyx collar, OL). Because they are largely nestbound (O'Donnell 1998a; Strassmann 2001), queens are especially likely to experience only on-nest light environments. We predicted that queens in closed-nest species would have the most reduced visual processing regions.

(c) *Degree of morphological caste differentiation*

We compared females of both worker and queen castes for each subject species. As noted above, including queens is important because the effects of variation in colony environment, such as nest architecture (e.g. open versus closed nests), may be most strongly reflected in queen brain structure. Furthermore, increased physiological specialization on egg laying by queens leads to a reduction in their behavioural repertoires (Richards 1978; Anderson & McShea 2001; Jeanne 2003). Fewer cognitive demands may permit reductions in queens' neural tissue investment.

In addition to our comparative analysis of caste differences, we measured intraspecific patterns of queen/worker brain structure differences. Paper wasp species differ in the degree of queen/worker caste differences and in the developmental mechanisms of caste determination (Keller 1993; O'Donnell 1998a; Hunt *et al.* 2003; Noll *et al.* 2004; Deshpande *et al.* 2006). Morphologically distinct paper wasp queens are often smaller than their workers in some body regions, particularly head capsule size, but typically have larger abdomens (Jeanne *et al.* 1995; O'Donnell 1998a; Noll *et al.* 2004). We predicted that caste differences in brain structure would be most dramatic for species with morphologically distinct queens and workers.

In SF, workers depart the nest to forage, relying on cues from multiple sensory modalities (Raveret-Richter & Jeanne 1991; Weiss *et al.* 2004; McPheron & Mills

2007; Richter 2007). Nestbound SF queens, like ant and honeybee queens, may rely predominantly on olfactory cues, including brood pheromones and other queens' pheromones (Landolt *et al.* 1998; Jeanne 2003). We expected queens in species with closed nests to have significantly reduced brain regions dedicated to visual processing relative to their workers.

2. METHODS

(a) Study sites and subject collection

We collected subject nests from 20 June to 20 July 2004, 27 June to 24 July 2005 and 27 July to 7 August 2006 in Monteverde (10°18' N, 84°49' W) and near Cañas in the Guanacaste Province, Costa Rica (10°26' N, 85°07' W), and from 6 June to 23 June 2007 at the Tiputini Biodiversity Station and Yasuni National Park in Ecuador (0°38' S, 76°08' W; see electronic supplementary material for more details). Nests were located on the eaves of buildings or in vegetation. Wasps were collected and stored in an aldehyde-based fixative (Prefer, Anatech Ltd).

(b) Ovary dissections and identifying caste

We dissected the ovaries from each female's gaster and photographed them under a dissecting microscope at $\times 10$ magnification using a digital camera. We used the mean area of the two largest oocytes as an index of ovary development (Keeping 2000, 2002). Within each colony, we assigned caste based on ovary development: we labelled females with filamentous oocytes as workers and females with the largest oocytes as queens. We used analyses of morphometric data from Noll *et al.* (2004) to determine whether each species had morphologically distinct queen/worker castes (see electronic supplementary material).

(c) Neuroanatomical measurements

For each nest, we collected neuroanatomical data from one to four adult females per caste (see the electronic supplementary material for further information on histology, tissue preparation and quantification).

We measured the volume of the following brain subregions: the OL (medulla and lobula), the AL (only the glomeruli) and the MB calyx (lip and collar + basal ring; see electronic supplementary material; Gronenberg 2001; O'Donnell *et al.* 2004, 2007; Molina & O'Donnell 2007, 2008). The cell body regions and fibres between the subregions of quantified neuropils were not included in analyses. The MB calyx collar and basal ring (BR) were grouped because boundaries between these subdivisions were ambiguous for some species, whereas boundaries between the lip and collar were always distinct.

(d) Statistical analyses

We tested whether neuroanatomy corresponded to nest architecture (open versus enclosed nests), mode of colony founding (IF/SF) and the presence/absence of morphologically distinct castes (see electronic supplementary material; Wenzel 1991; Jeanne 2003; Noll *et al.* 2004). As in previous studies, we used volume ratios as response variables (Withers *et al.* 1993; O'Donnell *et al.* 2004, 2007; Molina & O'Donnell 2007, 2008). We calculated ratios of each brain region to the sum of all quantified brain regions (total quantified volume was the sum of the OL, AL, the MB calyx and lobes and the central complex; Ehmer & Hoy 2000; Ehmer *et al.* 2001; Ehmer & Gronenberg 2004). We used volume

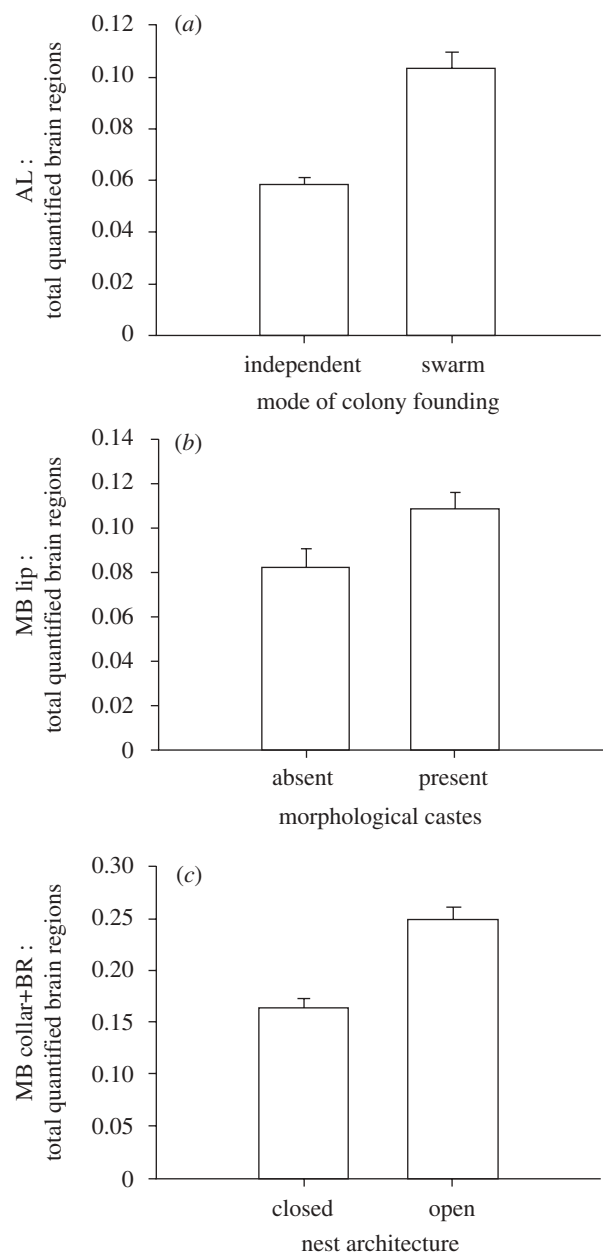


Figure 1. Bar graphs depicting relationships between brain organization and behavioural and caste covariates. (a) Mode of colony founding: bar graph representing means and s.e. of AL volume relative to the total volume of quantified brain regions in IF ($n = 11$: 4Q, 7W) and SF species ($n = 54$: 29Q, 25W). (b) Bar graph representing means and s.e. of the volume of the MB calyx lips relative to total quantified brain volume in species with ($n = 24$: 12Q, 12W) and without ($n = 41$: 21Q, 20W) morphological castes. (c) Bar graph representing means and s.e. of MB calyx collar + BR volume relative to total quantified brain volume for queens in species with open ($n = 10$ queens) and closed ($n = 23$ queens) nests.

ratios because body size may influence absolute volumetric measurements (Wehner *et al.* 2007).

For interspecific comparisons, we used PHYLIP software (<http://evolution.genetics.washington.edu/phytip.html>) to estimate phylogenetic branch lengths from mitochondrial cytochrome oxidase subunit I (COI) sequences (sequence divergence: Arévalo *et al.* 2004). For the intraspecific comparisons of caste differences in brain region volume, we used general linear models/multiple regression methods

(SPSS Inc., SPSS 15.0, 2007). Unless otherwise noted, all significant relationships shown are the results of partial correlation analysis after accounting for colony effects.

3. RESULTS

(a) Overall species differences

When queen and worker castes were analysed together, neuroanatomical brain regions associated with olfactory processing did not covary with nest architecture (see electronic supplementary material). SF species had significantly larger ALs than IF species (phylogenetic GLC multiple regression analysis, $t_7 = 3.00$, $p = 0.02$; figure 1a), and species with morphological castes had larger MB calyx lips than caste monomorphic species (phylogenetic GLC multiple regression analysis, $t_4 = 4.00$, $p = 0.02$; figure 1b). None of the covariates—nest architecture, colony founding, nor caste differences—were significantly related to the size of visual processing regions (the MB calyx collar + BR and the OLs).

(b) Caste-related species differences

Variation among queens appeared to account for species differences in brain structure. SF queens had larger ALs than IF queens (phylogenetic GLC multiple regression analysis, $t_7 = 5.00$, $p = 0.002$). Queens from species with morphologically distinct castes had larger MB calyx lips than queens from monomorphic species (phylogenetic GLC multiple regression analysis, $t_7 = 4.00$, $p = 0.02$). Furthermore, queens in species with open nests had larger MB calyx collar + BR values than queens in species with enclosed nests (GLS multiple regression analysis, $t_4 = -3.50$, $p = 0.02$; figure 1c). When analysed separately, species differences among workers were weaker or absent (see electronic supplementary material).

(c) Intraspecific caste differences

In both of the IF species, which have open nests, queens had greater MB calyx volume dedicated to visual processing than workers (MB calyx collar + BR: *Mischocyttarus mastigophorus*, $F_{1,6} = 6.21$, $p = 0.04$; *Polistes instabilis*, $F_{1,2} = 26.64$, $p = 0.04$). In contrast, SF queens in some species with enclosed nests had decreased brain volume dedicated to visual processing relative to their workers (*Agelaia xanthopus*, OL: $F_{1,6} = 10.12$, $p = 0.02$; *Protopolybia exigua*, MB calyx collar + BR: $F_{1,7} = 8.27$, $p = 0.02$). In both the IF species, queens had more brain volume dedicated to olfaction than their nestmates (*M. mastigophorus*, AL: $F_{1,6} = 7.28$, $p = 0.04$; *P. instabilis*, MB calyx lip: $F_{1,2} = 27.83$, $p = 0.03$).

4. DISCUSSION

We found that species differences in paper wasp brain structure correspond to variation in mode of colony founding, strength of queen/worker caste differences and nest architecture. The patterns further suggest that there is differential investment in modality-specific brain regions.

(a) Mode of colony founding

SF paper wasps had larger peripheral olfactory processing regions (ALs). Olfactory communication becomes increasingly important in social interactions during the

evolution of advanced eusocial species (paper wasps, honeybees and ants; Van der Vecht 1959; Darchen 1976; Richards 1978; Le Conte & Hefetz 2008). Only SF paper wasps are known to use trail pheromones to communicate the location of new nest sites (Jeanne 1991; Smith *et al.* 2002). A greater proportion of SF species may employ alarm pheromones to indicate danger to the nest than IF species (Landolt *et al.* 1998), and at least some swarm founders rely on queen pheromones to regulate interactions with workers (Naumann 1975; West-Eberhard 1977; Forsyth 1978).

Our prediction of reduced central processing capacity in SF workers, as indicated by MB calyx volume, was not supported. We did not find significant differences in central processing regions between IF and SF species.

(b) Nest architecture: open versus closed nests

Greater constant exposure to light (i.e. open comb nests) was positively linked to larger visual processing regions (MB calyx collars + basal rings). Our data suggest that differential exposure to visual stimuli (e.g. light levels) correlates most strongly with differences in queen brain structure. Foraging away from the nest may provide similar exposure to visual stimuli for workers living in open and closed nests, but queens generally remain nest-bound. Furthermore, IF may use visual cues in dominance interactions (Jeanne 1972; Tibbetts 2002; Tibbetts & Dale 2007).

(c) Caste differences

Queens accounted for most of the species differences we documented: brain structure corresponded more strongly with mode of colony founding and nest architecture in queens than in workers. We suggest that queen brain structures are more diverse because queen behavioural roles vary more widely across species than worker roles. IF queens directly police their nestmates' reproduction and control or eliminate worker-laid eggs (Gamboa *et al.* 1990; Reeve 1991; Premnath *et al.* 1996). IF queens often forage for building materials, while SF queens apparently never do so (O'Donnell 1998b,c). SF queens, in contrast, rarely interact physically with nestmates and may rely on broadcast chemical signals (queen pheromones; Naumann 1975; West-Eberhard 1977; Forsyth 1978). The reduction in SF queen behaviour to on-nest activity means markedly less exposure to environmental stimuli, especially for those living in closed nests, and a greater reliance on information within the nest (pheromones). The greater investment in olfactory processing regions (ALs) in SF species parallels this increased reliance on olfactory information on the nest. Smaller visual processing areas (MB collars) in queens living in closed nests also suggest that queen brain structure reflects the information most relevant for a nest-bound lifestyle.

We did not find evidence for the expected pattern of stronger queen/worker differences in species with morphological castes. Both the IF species we examined, but not most of the SF species, showed augmented olfactory processing neural tissue in queens relative to their workers. One possible explanation for the lack of queen/worker differences in SF is that the evolution of increased reliance on pheromonal communication (e.g. recruitment

signals, trail pheromones) has impacted both castes (Landolt *et al.* 1998; Smith *et al.* 2002).

(d) Conclusion

Our species and caste comparisons suggest that paper wasps can provide an excellent venue for further comparative investigation in expanding the social intelligence hypothesis. The diversity of paper wasp social structures allowed us to test the effects of the species-specific cognitive challenges that each species faces. Paper wasp brain structure did not merely change with the evolutionary origin of sociality in Vespidae (Howse 1974). Wasp brain architecture mirrors the complex evolution of caste role specialization and social interactions in the Polistinae. Our data emphasize the importance of interpreting social interactions in terms of the sensory information being utilized and the cognitive demands they impose (Farris 2008). Our data provide a basis for further behavioural studies on these species to elucidate these cognitive demands. Our study generates predictions for relationships between sociality and brain structure in other social insects as well as in social vertebrates. For example, the mode of communication used in social interactions may correlate with greater investment in modality-specific brain regions. The need to discriminate between individual group mates, and to track individual identity across multiple interactions, may select for greater investment in higher-order brain regions. We believe that these cognitive demands, which can include higher-order processes such as sensory integration, learning and memory, underlie the positive relationships between brain size and sociality seen in many taxa (Howse 1974; Shultz & Dunbar 2006; Croney & Newberry 2007; Silk 2007).

More detailed analyses of the neural structure of brain regions, such as quantifying the number of sensory neurons in the antennae or the glomerular organization of ALs, would be useful in elucidating the developmental basis of the brain volume differences we measured. Although brain tissue dedicated to vision differed with nest architecture and light environments, we found evidence for changes in peripheral visual processing (OLs) in some species and central visual processing (MB collar + BR) in others. We cannot assess whether and how these brain regions might interact during development (Molina & O'Donnell 2008). It remains to be determined when central versus peripheral processing capacities will respond evolutionarily to changes in species light environments and social parameters (e.g. communication).

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