Brain Size and Visual Environment Predict Species Differences in Paper Wasp Sensory Processing Brain Regions (Hymenoptera: Vespidae, Polistinae)

Sean O’Donnell\textsuperscript{a} Marie R. Clifford\textsuperscript{b} Sara DeLeon\textsuperscript{a} Christopher Papa\textsuperscript{a} Nazaneen Zahedi\textsuperscript{a} Susan J. Bulova\textsuperscript{a}

\textsuperscript{a}Department of Biodiversity, Earth and Environmental Science, Drexel University, Philadelphia, Pa., and
\textsuperscript{b}Department of Biology, University of Washington, Seattle, Wash., USA

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Abstract
The mosaic brain evolution hypothesis predicts that the relative volumes of functionally distinct brain regions will vary independently and correlate with species’ ecology. Paper wasp species (Hymenoptera: Vespidae, Polistinae) differ in light exposure: they construct open versus enclosed nests and one genus (Apoica) is nocturnal. We asked whether light environments were related to species differences in the size of antennal and optic processing brain tissues. Paper wasp brains have anatomically distinct peripheral and central regions that process antennal and optic sensory inputs. We measured the volumes of 4 sensory processing brain regions in paper wasp species from 13 Neotropical genera including open and enclosed nesters, and diurnal and nocturnal species. Species differed in sensory region volumes, but there was no evidence for trade-offs among sensory modalities. All sensory region volumes correlated with brain size. However, peripheral optic processing investment increased with brain size at a higher rate than peripheral antennal processing investment. Our data suggest that mosaic and concerted (size-constrained) brain evolution are not exclusive alternatives.

When brain regions increase with brain size at different rates, these distinct allometries can allow for differential investment among sensory modalities. As predicted by mosaic evolution, species ecology was associated with some aspects of brain region investment. Nest architecture variation was not associated with brain investment differences, but the nocturnal genus Apoica had the largest antennal:optic volume ratio in its peripheral sensory lobes. Investment in central processing tissues was not related to nocturnality, a pattern also noted in mammals. The plasticity of neural connections in central regions may accommodate evolutionary shifts in input from the periphery with relatively minor changes in volume.

Introduction

The central nervous system (CNS), particularly the brain, lies at the interface between animal physiology and behavior. The CNS comprises some of the most expensive animal tissues in both production and maintenance costs, and these costs impose strong penalties for excess CNS tissue growth [Laughlin, 2001; Niven and Laughlin, 2008; Navarette et al., 2011]. Functional compartmentalization of brain tissues is a common if not universal feature of...
CNS architecture. Distinct cognitive processes are often performed by anatomically discrete brain regions. The mosaic brain evolution hypothesis links this functional compartmentalization to adaptive brain investment [Barton and Harvey, 2000; Smaers and Soligo, 2013]. Under mosaic brain evolution, the sizes of brain regions evolve independently in response to selective pressures on the cognitive demands they process [Chittka and Niven, 2009; Shultz and Dunbar, 2010]. Investment in brain regions should evolve rapidly and should closely match the particular cognitive demands animal species face. As predicted, comparative studies show that investments in brain regions covary with species’ sensory environments [Cooper et al., 1993; Catania, 2005; Linsey et al., 2007]. For example, evolutionarily independent invasions of cave environments by Astyanax fish were followed by convergent but genetically distinct reductions of eyes and optic processing brain tissues [Borowsky, 2008; Jeffery, 2009]. Quantitative genetic and artificial selection studies demonstrate the feasibility of mosaic brain evolution: there are independent genetic effects on the sizes of different brain regions [Hager et al., 2012; Kolb et al., 2013].

A corollary of the mosaic brain hypothesis is the prediction of trade-offs (negative relationships) between the sizes of different brain regions: if functionally distinct regions are free to evolve independently, and overall CNS investment is limited, then brain regions may compete for limited resources [Niven and Laughlin, 2008]. Evidence of brain region trade-offs is often sought in CNS structures that process distinct sensory modalities, such as vision versus mechanosensation [Cooper et al., 1993; Catania, 2005]. Perception of different sensory modalities is typically accomplished by unique sensory structures, and this functional discretization is often echoed in central processing brain regions [Farris, 2008].

We tested predictions of mosaic brain evolution, including tests for trade-offs, using a comparative analysis of brain structure in Neotropical paper wasps (Vespidae, Polistinae). Paper wasps use powered flight for locomotion, and metabolic constraints on CNS investment may be especially great for flying animals. All paper wasps are eusocial, with obligate group living in nests where offspring are reared cooperatively. Sensory structures and brain anatomy are well characterized for social Hymenoptera, including paper wasps [Ehmer and Hoy, 2000; Gronenberg, 2001; O’Donnell et al., 2011]. All paper wasps have image-forming compound eyes and chemo-sensory/tactile antennae. Anatomically distinct peripheral lobes process optic and chemosensory input [optic and antennal lobes, respectively; Gronenberg, 2001], and these lobes innervate distinct regions of central processing neuropils called mushroom bodies [MB; Strausfeld et al., 1998; Fahrbach, 2006]. Optic lobes innervate the collar region of the MB calyx, and antennal lobes innervate the lip region of the MB calyx [Gronenberg, 1999].

Paper wasp genera differ in exposure to light levels. Some Neotropical genera build open-comb nests, while others construct enclosing nest envelopes or nest in dark cavities [Wenzel, 1991]. Wasps in the genus Apoica are the only nocturnally active Neotropical paper wasps [Greiner, 2006].

We used a phylogeny for the 19 currently recognized genera of paper wasps in the western hemisphere to account for potential effects of evolutionary relationships on brain structure (fig. 1) [Wenzel and Carpenter, 1994; Carpenter et al., 2000; Carpenter, 2004]. We measured the volumes of antennal processing regions (antennal lobes and MB lips) and optic processing regions (optic lobes and MB collars), and we used the total volume of all other brain structures (henceforth brain remainder) to control for variation in total brain size. Brain remainder volume was greater than all structures against which it was compared, making it a robust index of overall brain size. Using brain remainder as a brain size index avoids statistical confounds that result from including the structures being analyzed in a measure of total brain volume.

The evolution of nocturnal behavior is associated with changes in CNS investment across several taxa. Investment in optic processing tissue decreases, while investment in other modalities increases in derived nocturnal taxa [Barton et al., 1995; Catania, 2005]. In bats, the evolution of roosting in dark, sheltered sites is associated with the evolutionary loss of UV vision capacity [Xuan et al., 2012]. Based on these comparative studies of vertebrate brain investment, we expected paper wasp species with darker nest environments and nocturnal species to invest relatively more in chemosensory processing brain regions (antennal lobe and lip region of the MB calyx) and less in optic processing brain regions (optic lobe and collar region of the MB calyx) [Barton et al., 1995].

We first tested whether paper wasp genera differed in the volume of the four sensory processing regions. We then asked whether the volumes of the optic and antennal sensory processing regions were negatively correlated as expected if there was an investment trade-off. We examined evidence for trade-offs in two ways. First, we tested whether the absolute volumes of the optic and antennal processing regions were negatively correlated. Positive correlations between brain region volumes are expected if evolutionary changes in overall brain size affect all brain regions,
and this pattern could mask trade-offs among brain regions [concerted brain evolution: Herculano-Houzel, 2011; Powell and Leal, 2012]. To correct for possible concerted brain size effects, we also tested whether the ratios of volumes of each region to brain remainder were negatively correlated. As an alternative to direct trade-offs, we then tested whether overall brain size explained patterns of optic versus antennal brain investment. Investment in central versus peripheral processing brain regions depends on total brain size in paper wasps [O’Donnell et al., 2011]. Finally, to test whether brain region investment was associated with ecology (light levels), we asked whether nest architecture and nocturnal behavior predicted the size of the four sensory brain structures. We also used the ratios of the antennal to optic processing region volumes (antennal lobe/optic lobe and MB lip/collar) as indexes of relative tissue allocation among sensory modalities.

**Materials and Methods**

**Subject Species**

We analyzed the brain architecture of one species from each of 13 genera of Neotropical eusocial paper wasps (Polistinae). Our subjects spanned the 19 currently recognized Neotropical paper wasp genera, including relatively basal and derived taxa (fig. 1). All wasps were collected from nests in the field except *Brachygaster smithii* (collected from a swarm). Wasps were collected into and stored in buffered aldehyde-based fixative (Prefer fixative; Anatech, Ltd.). Wasp species, collection dates, and locations were:

- *Polistes instabilis*: July 2005, Costa Rica, 10°27.2’N, 85°7.5’W;
- *Mischocyttarus mastigophorus* and *Agelaia xanthopus*: August 2006, Costa Rica, 10°18.1’N, 84°47.9’W;
- *Nectarinella championi*: August 2006, Costa Rica, 10°14.4’N, 84°54.3’W;
- *Apoica pallens*, *Angiopolybia zischkai*, *Chartergellus fulves*, *Leipomeles dorsata*, *Parachartergus smithii*, *Polybia dimidiata*, *Protopolybia exigua*, and *Synoeca septentrionalis*: June 2007, Ecuador, 0°40.3’S, 76°24.0’W, and *B. smithii*: July 2012, Costa Rica, 10°16.3’N, 84°49.4’W. We collected neuroanatomical data on 4–9 female wasps per species. Three subject genera build open-comb nests; the remainder construct enclosing envelopes or nest in cavities [Wenzel, 1991]. One genus (*Apoica*) is nocturnal [Greiner, 2006].

**Histology and Neuroanatomy**

Only mature wasps with fully hardened, deeply colored cuticle were used as subjects. We cut wasps’ head capsules from the thorax at the narrow neck-like juncture behind the gena and removed the antennae and mandibles. We dehydrated head capsules through an ethanol series, acetone, then increasing concentrations of plastic resin. We incubated individual wasp heads in 0.5 ml resin in BEEM capsules at 60 °C for 72 h. We sectioned each head into 12- to 16-μm-thick sections (depending on the species) using a rotary microtome with disposable steel histology blades. We mounted sections on gelatin-coated microscope slides and stained the tissue with toluidine blue. We cleared in an ethanol series and coverslipped under transparent mounting medium.
Results

Species Differences

Species differed significantly in the relative amount of investment (ratio of brain region volume to brain remainder volume) for all four sensory processing brain regions (fig. 2; antennal lobes: F\(_{12,74}\) = 24.87, \(p < 0.001\); optic lobes: F\(_{12,74}\) = 35.45, \(p < 0.001\); MB lip: F\(_{12,74}\) = 9.92, \(p < 0.001\); MB collar: F\(_{12,74}\) = 11.27, \(p < 0.001\)).

Tests for Trade-Offs

We found no evidence for direct trade-offs (negative relationships) between the sizes of paper wasp antennal and optic brain regions. For peripheral processing brain tissues, species mean antennal lobe volumes were significantly positively correlated with species mean optic lobe volumes (r = 0.75, n = 13, \(p < 0.001\); PIC r = 0.73, n = 12, \(p < 0.001\)). For central processing brain tissues, species mean MB lip volumes were significantly positively correlated with species mean MB collar volumes (r = 0.97, n = 13, \(p < 0.001\); PIC r = 0.97, n = 12, \(p < 0.001\)). Similar patterns held when analyzing brain size-corrected volumes (fig. 2; peripheral lobes: r = 0.92, n = 13, \(p < 0.001\), PIC r = 0.93, n = 12, \(p < 0.001\); MB calyx: r = 0.31, n = 13, \(p = 0.31\), PIC r = 0.30, n = 12, \(p = 0.34\) (both not significant but positive)).

Relationships with Brain Size

Species mean total brain volumes ranged from 0.084 mm\(^3\) (\(Leipomeles\)) to 0.51 mm\(^3\) (\(Polistes\)), a 6-fold difference. Brain remainder volume ranged from 0.041 mm\(^3\) (\(Leipomeles\)) to 0.22 mm\(^3\) (\(Polistes\)), a 5.4-fold difference. Both antennal lobe and optic lobe volumes increased with brain remainder volume (fig. 3; F\(_{1,22}\) = 26.49, \(p < 0.001\)). Optic lobes were larger than antennal lobes (test for brain region main effects F\(_{1,22}\) = 27.93, \(p < 0.001\)) and increased in volume with brain remainder faster than antennal lated with the size of the remainder of the brain. The (target region volume × brain remainder volume) interaction term tested whether the slopes of the two region-remainder correlations differed from each other.

For all analyses of species mean data, we conducted parallel analyses on phylogenetically independent contrasts (PIC) using the method of Felsenstein [1985] for two continuously varying characters. Independent contrasts were calculated using web-based software [COMPARE; Martins, 2004]. We calculated independent contrasts using a fully resolved genus level phylogeny for paper wasps [Wenzel and Carpenter, 1994] with all branch lengths set to one [O’Donnell et al., 2011]. In all cases, analyses of the raw data and PIC analyses led to similar conclusions.
lobes (test for differences in slope $F_{1,22} = 13.64, p = 0.001$; PIC ANCOVA $F_{1,20} = 10.10, p = 0.005$). The two largest-brained species had particularly large optic lobes (fig. 3). MB calyx lip and collar volumes both increased with brain remainder (fig. 3; $F_{1,22} = 25.52, p < 0.001$). MB collars were larger than MB lips (test for brain region main effects $F_{1,22} = 12.25, p = 0.002$), but these regions increased with brain remainder at similar rates [test for differences in slope $F_{1,22} = 2.40, p = 0.136$ (n.s.), PIC ANCOVA $F_{1,20} = 0.70, p = 0.41$].

**Associations with Ecology: Light Levels**

Species mean relative investment did not covary with nest architecture (open vs. closed nests) for any brain region (fig. 2, 3; antennal lobes: $F_{1,11} = 0.36, p = 0.55$; optic lobes: $F_{1,11} = 1.42, p = 0.26$; MB lip: $F_{1,11} = 0.45, p = 0.52$; MB collar: $F_{1,11} = 1.98, p = 0.19$). Nest architecture (open vs. closed nests) was not related to the antennal lobe:optic lobe ratio (fig. 3; $F_{1,11} = 1.22, p = 0.29$) or to the MB lip:collar ratio ($F_{1,11} = 2.23, p = 0.16$).

The evolution of nocturnality was associated with differences in sensory modality investment in the peripheral lobes. The nocturnal wasp *A. pallens* had the highest antennal lobe:optic lobe volume ratio among the species we studied (fig. 4; $F_{1,11} = 9.64, p = 0.010$), but this pattern was not reflected in the MB calyx lip:collar ratio ($F_{1,11} = 0.88, p = 0.37$).

**Discussion**

Paper wasp species differed in relative investments in the optic and antennal processing regions we measured, but increases in investment in one modality were not associated with decreases in investment in the other. Our data did not support the existence of direct trade-offs between sensory modalities. As seen in ants (Formicidae), volumes of optic and antennal regions were positively associated, suggesting concerted brain size effects on evolutionary changes in sensory structure volumes [Gronenberg and Hölldobler, 1999; Powell and Leal, 2012]. Evidence for direct evolutionary trade-offs between brain regions is rarely found [Barton and Harvey, 2000; Gatenby et al., 2011; Warren and Iglesias, 2012]. Many cases of apparent brain tissue trade-offs between sensory modalities involve taxa that have shifted to novel sensory environments such as caves or nocturnal activity [Zhao et al., 2009]. These cases may involve simultaneous selection for enhancing investment in one sensory capacity and selection against the other (or loss of function via neutral process such as drift). Recent suggestions that cave fish chemosensory tissue gain and optic tissue loss are genetically associated through negative pleiotropy have been called into question [Yoshizawa et al., 2012; Gunter and Meyer, 2013].
Relationships with Brain Size

Paper wasp species differences in brain architecture were largely explained by brain size effects [also see O’Donnell et al., 2011]. Optic investment changed more rapidly with brain size than antennal investment in the peripheral sensory neuropils. Although a number of studies have attempted to test between concerted and mosaic brain evolution as alternatives [Barton et al., 1995; Herculano-Houzel, 2011; Powell and Leal, 2012; Gunter and Meyer, 2013; Smaers and Soligo, 2013], our findings suggest that this dichotomy is an oversimplification. Our data suggest that brain size is a key factor to be considered in analysis of brain architecture because brain regions that covary with brain size can do so at different rates [Kaskan et al., 2005]. While brain region investment may be constrained by overall brain size (concerted evolution), different rates of change with brain size can allow for evolutionary flexibility in tissue allocation to functionally distinct brain regions (mosaic evolution).

Both visual and optic sensory inputs are processed in hymenopteran brain regions we could not quantify due to limitations of our staining method. In honeybees (Apis mellifera) and other Hymenoptera, olfactory information is processed in the lateral horn region of the protocerebrum. Some axonal tracts that leave the antennal lobes innervate the lateral horn and are distinct from tracts innervating the MB lobes, suggesting parallel processing of sensory input in the lateral horn [Rössler and Zube, 2011]. Similarly, visual input is processed in the anterior optic tubercule as well as in the MB collar region [Pfeiffer and Kinoshita, 2012]. Quantification of the size of these other sensory processing regions could be used to test the generality of our findings.

Relationships with brain size differed between paper wasp antennal and optic peripheral lobes. The two largest-brained species had particularly large optic lobes (fig. 5). These species, i.e. P. instabilis and S. septentrionalis, differ from each other in the size of new and mature colonies and in nest architecture (open stalked combs vs. envelope-covered sessile combs), suggesting that there is a general effect of brain size on optic lobe investment in paper wasps.

Species Ecology: Visual Environments

Contrary to our predictions, paper wasp nest architecture variation was not associated with species differences in brain sensory structure sizes. In addition to lower light levels, the interiors of enclosed nests are likely to have relatively simple visual environments (e.g. less light intensity variation, fewer visual edges, less color variation). However, the relative darkness of wasp nest interiors may vary among species. Enclosed-nest subject species in our study ranged from cavity nesters (A. xanthopus) to species with thin, translucent nest paper (L. dorsata). Furthermore, swarm-founding wasp workers are often active on the exterior nest surface, and workers of all species must depart the nest to forage for food and other materials [O’Donnell and Jeanne, 1992]. These temporary exposures to exterior light environments may affect paper wasp brain investment in optic processing independently of nest visual environments.

Fig. 4. Bar graphs showing the species mean values ± 95% CI (error bars) of the ratios of antennal:optic tissue volumes in the sensory periphery (top) and the central brain (bottom). Shaded bars are the nocturnal genus Apoica; hatched bars are species that build open-comb nests. Bars are ordered from species with the highest to lowest antennal lobe (AL):optic lobe (OL) ratio in both graphs.
The evolution of nocturnality in the genus *Apoica* led to dramatic changes in light levels experienced by active wasps. As predicted, the nocturnal paper wasp *Apoica* differed from other genera in its greater antennal lobe-to-optic lobe volume ratio. The evolution of nocturnality in flying Hymenoptera (bees and wasps) is associated with changes in compound eye structure: eye size (number of facets), light-gathering lens size, and/or the size of light receptive surfaces increase compared to diurnal relatives [Warrant et al., 2004; Greiner, 2006; Warrant, 2008]. However, these changes are not sufficient to maintain visual acuity under the eight-order-of-magnitude-lower light levels experienced during night flight, and neural processing such as summation of light inputs across eye facets may be necessary to permit nocturnal foraging [Greiner, 2006; Warrant, 2008; Kelber et al., 2011]. Our data suggest that, as in nocturnal vertebrates, the relative reliance on olfaction increases even when anatomical adaptations for low-light visual acuity arise. Nocturnal foraging evolved independently in the Paleotropical social hornet *Provespa* (Vespidae, subfamily Vespinae), providing an opportunity for additional comparative tests of the generality of our findings on sensory brain investment in wasps [Warrant, 2008].

The strongest brain architecture differences for *Apoica* were in peripheral sensory processing tissues, and these differences were not reflected in associated sensory processing regions of the MB. Similar evolutionary lability in the sensory periphery combines with central processing conservation in the evolution of novel sensory systems in vertebrates [Wilczynski, 1984; Kaskan et al., 2005]. Changes in patterns of innervation and neural connectivity in central processing regions such as vertebrate cortex and insect MB can accommodate developmental changes in environmental experience, individual differences in sensory structure neuron composition, and even loss of sensory input such as blindness [Kaskan et al., 2005; Jones et al., 2009; Merabet et al., 2010; Groh et al., 2012]. This plasticity may allow central processing tissues to accommodate evolutionary transitions in input from the sensory periphery without requiring proportional changes in the size of the central processing region.

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