The neuroplasticity of division of labor: worker polymorphism, compound eye structure and brain organization in the leafcutter ant *Atta cephalotes*

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Dedicated to the memory of Eldridge Adams, brilliant behavioral ecologist, colleague, and friend.

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Abstract

Our understanding of how the design of peripheral sensory structures is coupled with neural processing capacity to adaptively support division of labor is limited. Workers of the remarkably polymorphic fungus-growing ant *Atta cephalotes* are behaviorally specialized by size: the smallest workers (minims) tend fungi in dark subterranean chambers while larger workers perform tasks mainly outside the nest. These strong differences in worksite light conditions are predicted to influence sensory and processing requirements for vision. We found that eye structure and visual neuropils have been be selected to maximize task performance according to light availability. Minim eyes had few ommatidia, large interommatidial angles and eye parameter values, suggesting selection for visual sensitivity over acuity. Large workers had larger eyes with disproportionally more and larger ommatidia, and smaller interommatidial angles and eye parameter values, reflecting peripheral sensory adaptation to ambient rainforest light. Additionally, optic lobe and mushroom body collar volumes were disproportionately small in minims, and within the optic lobe, lamina and lobula relative volumes increased with worker size whereas the medulla decreased. Visual system phenotypes thus correspond to task specializations in dark or light environments and reflect a functional neuroplasticity underpinning division of labor in this socially complex agricultural ant.

Keywords (5): task performance, optic lobe, compound eye, ommatidia, social brain

Introduction

Morphology, behavior, and nervous system structure appear to be integrated (Corral-López et al. 2017; Gordon et al. 2017; Iglesias et al. 2018). For example, body size correlates with optical sensitivity and resolution in insect vision (Spaethe and Chittka 2003; Rutowski et al. 2009; Palavalli-Nettimi and Narendra 2018; Taylor et al. 2019). Ants are an ideal model system to examine relationships among behavior, body size, and neuroanatomy because workers have evolved as task specialists in several clades (Hölldobler and Wilson 1990). Scaling patterns of brain size and brain compartment substructure among polymorphic workers, moreover, appear to correspond to foraging ecology and the sensory and cognitive demands of task performance (Gronenberg 2008; Muscedere and Traniello 2012; Gordon et al. 2017). Although olfactory inputs are principal information sources in ants (Hölldobler and Wilson 1990; Czaczkes et al.

2015), vision can be significant in foraging ecology and navigation (Knaden and Graham 2016; Narendra et al. 2017). To home, foragers may use a celestial compass (Wehner 2003; Muller and Wehner 2006), optic flow (Ronacher and Wehner 1995), visual cues and landmark panoramas (Graham and Cheng 2009; Müller and Wehner 2010; Schwarz et al. 2011; Huber and Knaden 2015; Freas et al. 2018), polarized light (Zeil et al. 2014), and canopy patterns (Hölldobler 1980; Beugnon et al. 2005; Rodrigues and Oliveira 2014). Addtionally, visual navigation has been associated with peripheral receptor structure, and primary and higher-order processing brain centers (Gronenberg and Hölldobler 1999; Wehner 2003; Ehmer and Gronenberg 2004; Muller and Wehner 2006; Knaden and Graham 2016), and worker behavioral development may be associated with light-exposure and cued neuroanatomical reorganization in the visual system (Stieb et al. 2010, 2012; Yilmaz et al. 2016).

Ant ommatidia are photoreceptive units that may change in number and structure according to visual needs (Moser et al. 2004; Narendra et al. 2016a). Ommatidia structure affects visual capacity: larger ommatidia enhance light sensitivity, ommatidia number determine image resolution, and lower interommatidial angle improves acuity (Land 1997). Reproductive and worker division of labor in social insects may have selected for differences in compound eye structure (Schwarz et al. 2011; Streinzer et al. 2013). In some ant species, ommatidia number and size scale with worker body size (Menzel and Wehner 1970; Bernstein and Finn 1971; Klotz et al. 1992; Baker and Ma 2006; Schwarz et al. 2011), vary in males and females (Narendra et al. 2016b), and scale differently among polymorphic workers within individual compound eyes and between colonies (Perl and Niven 2016a, b). In bull ants (Greiner et al. 2007; Narendra et al. 2011) and bees (Jander and Jander 2002; Greiner et al. 2004) photoreceptor diameter and eye area increase in nocturnal species in comparison to diurnal species, increasing visual sensitivity. Ommatidia facet diameter is generally smaller in diurnal than nocturnal ants (Narendra et al. 2017), but eye size patterns vary (e.g.(Menzi 1987)).

Visual input from the compound eyes travels to the optic lobes (OL) for primary processing (Gronenberg and Hölldobler 1999). OL investment reflects visual ecology in social insects: in subterranean species, workers are eyeless and OLs are absent whereas diurnal solitary foragers have enormous eyes and their OLs occupy 33% of their brains (Gronenberg and Hölldobler 1999). In paper wasps, queens remain inside the nest and have smaller OLs than foraging workers (O'Donnell et al. 2014), and in the weaver ant *Oecophylla smaragdina*, minor

workers nurse brood, rarely leave the nest, and have disproportionally smaller OLs than majors, which forage and defend territory (Kamhi et al. 2017).

The OL is comprised of three regions: lamina (contrast detection), medulla (color vision processing and small field motion), and lobula (color vision processing, wide field motion detection, and shape and panorama construction) (Strausfeld 1989; Gronenberg 2008; Dyer et al. 2011). OL interneurons project to the collar of the mushroom body (MB) calyx for higher-order processing (Gronenberg 2001; Farris 2016). In ants, males, queens and worker brains show differential investment in the medulla, lobula and MB collar (Ehmer and Gronenberg 2004), reflecting different visual ecologies. Peripheral sensory structure should correlate with higher-order processing ability in task-specialized workers, but this linkage is not well understood.

To investigate visual phenotypes within the worker caste, we investigated variation in the structure of the compound eyes, OL, and MB collar in morphologically and behaviorally differentiated workers of the fungus-growing ant *Atta cephalotes*. Worker head widths (HW) range from 0.6 to 4.5mm; this striking polymorphism is associated with the frequency (Wilson 1980) and efficiency (Wetterer 1991; van Breda and Stradling 1994) of leaf harvesting, fungal comb maintenance, brood care, hygienic behaviors, and colony defense. The smallest workers (minims, HW<1.2mm) primarily tend brood and the fungal comb in dark underground chambers (Wilson 1980) whereas media workers (HW=1.2-3.0mm) harvest plant material, traveling along foraging trails beneath rainforest canopy, and the largest workers (majors, HW>3.0mm), are responsible for defense (Powell and Clark 2004; Hölldobler and Wilson 2010). Medias use vision during orientation along trails (Vilela et al. 1987; Vick 2005). Size-variable workers thus have different social roles and experience environments strongly differing in ambient light intensity and visual complexity. It is unlikely that a single eye structure and sensory processing ability has evolved in all workers. We hypothesized that *A. cephalotes* visual system organization is associated with the visual ecology of size-related division of labor and has resulted from selection for adaptive plasticity in ommatidia structure, OL organization and MB collar investment. Specifically, we predicted that workers engaging in within-nest or outside-nest activities (in darkness or light, respectively) would vary in compound eye structure and relative investment in the OL and its constituent parts, and in the MB collar to support the requirements of vision associated to task performance.

Methods

Laboratory cultures

Queenright *A. cephalotes* colonies were collected in Trinidad (July 2014) and maintained in a Harris environmental chamber (25°C, 50% relative humidity, 12:12h photoperiod). Artificial nests were constructed from multiple plastic boxes (11cm×18cm×13cm each) connected by plastic tubing (ID=2.5cm). Boxes housing fungal combs had dental stone floors with embedded pebbles to provide air circulation for the fungus. Colonies were fed locally collected leaves free of chemicals and organic produce on alternate days, supplemented with rolled oats, apple, and orange mesocarp.

Worker size variation and tissue sampling

We sampled polymorphic workers from three colonies (Ac09, Ac20, and Ac21). *A. cephalotes* appears to exhibit triphasic allometry, with three worker size classes (subcastes): minims (HW across the eyes <1.2mm), medias (HW 1.2-3.0mm) and major workers (HW>3.0mm). Each worker was anesthetized on ice and brains were dissected in ice-cold HEPES buffered saline. Compound eyes were removed and stored in 70% ethanol for processing. Because dissection is delicate, we were not always able to preserve the brain and eyes of the same individual.

Compound eye imaging and structural measurements

Ninety-two intact compound eyes were imaged to create 3D stacks (Fig. 1G) to measure ommatidia number (ON), average ommatidial diameter (D), and interommatidial angle ($\Delta\phi$). Eyes were stored in 70% ethanol, washed in 100% ethanol (3×10 min) before mounting. We measured one eye per worker. Extraneous cuticle was removed to allow eyes to lie flat and were then mounted in methyl salicylate between coverslips and imaged using a Fluoview 1 confocal microscope (λ=488nm, step size=3.1µm) with a 20x air objective (NA=0.5, CA=2). Cuticle has natural fluorescence. Eye data were recorded blind to subcaste by randomly assigning identification numbers to eyes. To quantify ommatidia number, image stacks were flattened in ImageJ (Abràmoff et al. 2004) and facets were counted using the Cell Counter plugin. Volume renderings were viewed in Amira 6.0 to verify counts.

Mean ommatidial diameter was calculated from the average diameter of 5 or 10 randomly selected ommatidia from each eye. Eye surface area was calculated from the mean ommatidial diameter (surface area=$ON\times\pi\times[0.5xD]^2$), and ommatidial density (number of ommatidia per

surface area unit) was calculated by dividing the number of ommatidia by eye surface area (Yilmaz et al. 2014). To quantify interommatidial angle ($\Delta\phi$), image stacks were re-sectioned in the yz plane to obtain a virtual cross section of the eye. ImageJ was used to estimate local eye radius *R* (Schwarz et al. 2011), which with the mean ommatidial diameter (D, in μm) for that eye, interommatidial angle (in radians) could be estimated as $\Delta\phi = D/R$ (Schwarz et al. 2011). Eye parameter (P), which indicates the extent of trade-offs between sensitivity and resolution, was calculated as $\Delta\phi \times D$ (Snyder 1977; Rutowski et al. 2009); lower values of P indicate enhanced acuity, while compromising sensitivity.

Immunohistochemistry and confocal microscopy

After dissection, brains were placed in 16% Zn-formaldehyde (Ott 2008), fixed overnight at approximately 18ºC on a shaker, washed in HBS (6×10 min) and then fixed in Dent's Fixative (80% methanol, 20% DMSO) for minimally 1h. Brains were next washed in 100% methanol and either stored at -17°C or immediately processed. Brains were washed in 0.1M Tris buffer (pH=7.4) and blocked in PBSTN (5% neutral goat serum, 0.005% sodium azide in 0.2% PBST) at 18ºC for 1 hour before incubation for 3 days at room temperature in primary antibody (1:30 SYNORF 1 in PBSTN; monoclonal antibody 3C11obtained from DSHB, University of Iowa, IA, USA). They were washed (6×10 min) in 0.2% PBST and incubated in secondary antibody (1:100 AlexaFluor 488 goat anti-mouse in PBSTN) for 4 days at room temperature. Brains were then washed a final time (6×10 min in 0.2% PBST) and dehydrated in an ethanol series (10min/step, 30/50/70/95/100/100% ethanol in 1x PBS), cleared with methyl salicylate, and mounted on stainless steel slides for imaging.

Sixty-three brains were imaged using an Olympus Fluoview 1 confocal microscope (λ=488nm, step size=3.1μm) with either a 10x air objective (NA=0.3, CA=1), or a 20x air objective (NA=0.5, CA=2). Voxel depth was multiplied by a factor of 1.59 to correct for axial shortening due to mounting in methyl salicylate (Bucher et al. 2000). Brain image stacks were manually segmented using Amira 6.0 and Amira 2019.2 software to quantify neuropil volumes. Given their bilateral symmetry, we segmented one hemisphere per brain, chosen randomly. Our study goal required that only the lamina, medulla and lobula of the OL and MB calyces (separating lip and collar) were segmented separately (Fig. 2A), the rest of the central brain regions and the suboesophageal ganglion were segmented as a whole. Brain data collection was blind to worker HW, although extreme size differences were obvious. Nevertheless, due the

randomized coding of brains, subcaste could not be determined with certainty by the annotator. We calculated the volume of the brain hemisphere, the absolute and relative volume of OL (relative to total brain volume), the absolute and relative volume of MB collar (relative to total brain volume), and the relative volumes of OL subregions (relative to total OL volume).

Statistical evaluation

Statistical evaluations were performed in R (version 3.3.0, Team 2016) using the 'segmented' package to analyze eye and brain metric scaling (V. R. M. Muggeo 2008). To assess allometries in eye structure and brain volumes in relation to worker size, least-square means regression was used on log10-transformed values to estimate a and b in the scaling equation $y=aM^b$, as $log10(y)=log(a)+b\times log(M)$. To test the null hypothesis (H_0) of isometry, a separate linear model was calculated and tested against different slope values depending on the metric. The slope for H_0 were b=0.0 (linear vs. constant values), b=1.0 (linear vs. linear), b=2.0 (linear vs. surface area) and b=3.0 (linear vs. volume) (Kaspari and Weiser 1999).

The Davies test was used to determine if there is a statistically significant change in slope or a 'breakpoint' in a linear relationship (Davies 2002). We observed that the significance of some changes in slope depended on a single data point; therefore, we accepted the change in slope only if its significance was always below 0.05 when removing any point from the dataset. The 'segmented' package was further used to estimate the location of the breakpoint. If the Davies' test revealed two piecewise linear relationships in a scaling relationship, least-square means regression was calculated and tested against isometry independently.

To further explore whether increased investment in primary visual neuropil might have an impact in higher-order visual processing neuropil, we assessed allometry in the ratio of volumes of the optic lobes and MB collar according to HW. We also calculated a least-square means regression on log10-transformed values and tested against isometry (b=0.0).

Results

Eye Structure

The eyes of media and major workers had significantly more ommatidia than minims (Fig. 1A) and showed a significant change in the scaling of ommatidia number and worker size (Davies test, $p<0.001$) at a HW of 1.38mm (95% CI: 1.20 to 1.58mm). Piecewise linear models calculated for both slopes were significant ($p<0.001$, Multiple $R^2=0.989$) with a slope shift from

2.03 (95% CI: 1.91 to 2.13) to 1.35 (95% CI: 1.25 to 1.45). Piecewise linear models were also significantly different from isometry (b=0; p<0.001). Media and major workers also had larger ommatidial diameter (Fig. 1B). The relationship between ommatidial diameter and worker size showed no significant breakpoint (Davies test, p>0.5), and these variables were significantly correlated ($F_{(1,90)}$=1217, p<0.001, R^2=0.93). The slope of the regression line was 0.25 (95% CI: 0.24 to 0.27), which was significantly different from isometry (b=1.0; $F_{(1,90)}$= 1217, p<0.001).

Compound eye size (total eye surface area) was correlated with HW (Fig. 1C). Davies' test showed a significant change in the scaling of total eye size and worker size (p<0.001) at a HW of 1.44mm (95% CI: 1.20 to 1.72mm). Piecewise linear models calculated for both slopes were found to be significant (p<0.001, Multiple R^2=0.988) with a slope shift from 2.52 (95% CI: 2.36 to 2.68) to 1.84 (95% CI: 1.70 to 1.97). Piecewise linear models were significantly different from isometry (b=2.0; p<0.001), although the effect size was small.

The density of ommatidia decreased with HW (Fig. 1D). The relationship between the density of ommatidia and worker size showed no significant breakpoint (Davies test, p>0.5 and these variables showed a significant correlation ($F_{(1,90)}$=1217, p<0.001, R^2=0.93). The slope was -0.50 (95% CI: -0.53 to -0.48), also significantly different from isometry (b=0.0; $F_{(1,90)}$= 1217, p<0.001).

Interommatidial angle decreased as worker size increased (Fig. 1E). Davies' test for a change in slope showed a significant change in the scaling relationship between interommatidial angle and worker size (p<0.001) at a HW of 1.25mm (95% CI: 1.03 to 1.51mm). Piecewise linear models were calculated for both slopes and found to be significant (p<0.001, Multiple R^2=0.84), with a slope shift from -0.98 (95% CI: -1.21 to -0.76) to -0.33 (95% CI: -0.43 to -0.23). Piecewise linear models were also significantly different from isometry (b=0; p<0.001).

Eye parameter decreased with worker size in minims (Fig. 1F). Davies' test showed a significant change in the scaling relationship between eye parameter and worker size (p<0.001) at a HW of 1.26 mm (95% CI: 1.01 to 1.57 mm). Piecewise linear models were calculated for both slopes and found to be significant (p<0.001, Multiple R^2=0.588) with a slope shift from -0.73 (95% CI: -0.95 to -0.51) to -0.07 (95% CI: -0.20 to 0.05). The first segment of the piecewise linear models was found significantly different from isometry (b=0; p<0.001), but the second segment was not (b=0; p=0.205).

Brain Structure

Larger workers had significantly larger brains (Fig. 2B). The relationship between brain volume and worker size showed no significant breakpoint (Davies test, $p>0.05$) and these variables showed a significant positive correlation ($F_{(1,61)}=13.91$, $p<0.001$, $R^2=0.18$) with a slope of 0.37 (95% CI: 0.17 to 0.56) significantly different from isometry ($b=3.0$; $F_{(1,61)}=13.91$, $p<0.001$). We found greater OL investment in media and major workers (Fig. 2C). Relative OL volume and worker size showed a significant positive correlation ($F_{(1,61)}=271.2$, $p<0.001$, $R^2=0.81$) with no significant breakpoint (Davies test, $p=1$). The slope of the regression line was 0.76 (95% CI: 0.67 to 0.85) and significantly different from isometry ($b=0.0$; $F_{(1,61)}=271.2$, $p<0.001$).

Within the OL, relative investment in lamina increased with worker size (Fig. 2C1). Relative lamina volume and HW showed a significant positive correlation ($F_{(1,61)}=37.77$, $p<0.001$, $R^2=0.37$) with no significant breakpoint (Davies test, $p>0.05$). The slope of the regression line was 0.64 (95% CI: 0.43 to 0.85) and significantly different from isometry ($b=0.0$; $F_{(1,61)}=37.77$, $p<0.001$). Relative investment in the medulla, in contrast, decreased with worker size (Fig. 2C2). Relative medulla volume and HW showed a significant negative correlation ($F_{(1,61)}=51.1$, $p<0.001$, R^2 of 0.45) with no significant breakpoint (Davies test, $p>0.05$) and a slope of -0.17 (95% CI: -0.21 to -0.12), significantly different from isometry ($b=0.0$; $F_{(1,61)}=51.1$, $p<0.001$). Finally, as for the lamina, relative investment in the lobula increased with worker size (Fig. 2C3). Relative lobula volume and HW showed a significant positive correlation ($F_{(1,61)}=13.43$, $p<0.001$, $R^2=0.17$) and no significant breakpoint (Davies test, $p=0.362$). The slope of the regression line was 0.21 (95% CI: 0.10 to 0.32) and significantly different from isometry ($b=0.0$; $F_{(1,61)}=13.43$, $p<0.001$).

Media and major workers also invested relatively more in the MB collar (Fig. 2D). Relative collar volume and worker size showed a significant positive correlation ($F_{(1,61)}=13.03$, $p<0.001$, $R^2=0.16$) and no significant breakpoint (Davies test, $p=0.42$). The slope of the regression line was 0.19 (95% CI: 0.08 to 0.29), which was also significantly different from isometry ($b=0.0$; $F_{(1,61)}=13.03$, $p<0.001$). Despite investing more in the MB collar, larger workers had a lower collar:OL volume ratio (Fig. 2E). The relationship between this ratio and worker size showed a significant negative correlation ($F_{(1,61)}=67.17$, $p<0.001$, $R^2=0.52$), no significant breakpoint (Davies test, $p=0.69$), and a slope of -0.57 (95% CI: -0.71 to -0.43), significantly different from isometry ($b=0.0$; $F_{(1,61)}=67.17$, $p<0.001$).

Discussion

Social insect compound eyes and visual information processing neuropils enable adaptive behavioral performance according to cognitive challenges of navigation and ambient light levels (Jander and Jander 2002; Mares et al. 2005; Kapustjanskij et al. 2007; Warrant 2008; Narendra et al. 2011, 2016a; Streinzer et al. 2013; Yilmaz et al. 2014; Bulova et al. 2016). Eye size and ommatidia number correlate with worker size in ants, including polymorphic species, and may be associated with task performance (Menzel and Wehner 1970; Klotz and Reid 1992; Schwarz et al. 2011). In polymorphic *A. cephalotes* workers, in which task performance is strongly correlated with body size-related division of labor, differences in worksite sensory ecology appear to select for visual system polyphenism.

Division of labor and eye structure in A. cephalotes

A. cephalotes workers perform tasks in the complete darkness of fungal comb chambers and in the filtered light epigaeic environment beneath rainforest canopy. Media workers forage day and night (Cherrett 1968), and use trail pheromones as well. Although olfaction appears to be the dominant sensory modality for foraging in many ants, visual information facilitates trail-following in *Atta laevigata* [69], and other ant species (Beugnon and Fourcassié 1988) alter their use of chemicals or vision depending on light conditions. In *A. cephalotes,* improved forager visual ability may enable flexibility in the use of orientation cues and social signals as ambient light levels change.

Minims tend fungi deep underground, medias harvest leaves from their habitat and labor inside the nest, and majors appear to exclusively perform defense and trail maintenance outside. We hypothesized that eye structure variation among subcastes would reflect adaptation to worksite light availability and visual demands for task performance. We expected the eyes of minims to structurally enable light sensitivity over resolution, whereas larger worker eyes were predicted to favor spatial resolution over sensitivity. It is unclear how minims make use of visual information and what level of spatial resolution and sensitivity is needed to work effectively on the fungal comb. Minims, however, also perform some tasks outside the nest, “hitchhiking” on transported leaves during day and night to defend against fly parasites (Linksvayer et al. 2016). We found that the number and size of ommatidia and eye surface area were significantly smaller in minims (Fig. 1A-C), suggesting less capacity to capture light and less reliance on vision to

perform their tasks. The larger ommatidia of media and major workers (Fig. 1B) indicate greater sensitive to light. However, ommatidia size increased hypometrically with body size: although the ommatidia of minims were the smallest, relative ommatidia size was greater in minims than in medias and majors. This may enable minim worker eyes to collect more light than expected from their size, suggesting adaptation to darkness and light (Greiner 2006; Yilmaz et al. 2014). Alternatively, the small size of minim worker eyes and ommatidia may be due to a body size constraint: eye size is as large as developmentally possible to ensure at least a marginal ability to capture light, which may be needed for parasitic fly defenses. Minims also showed a higher density of ommatidia than media and majors (Fig. 1D). Interommatidial angle decreased with worker size, indicating higher visual acuity in larger workers (Fig. 1E). Eye parameter values (Fig. 1F) were significantly higher for minims, but for larger workers, values were lower and not correlated with size. This suggests minim worker eyes are adapted to enhance sensitivity rather than acuity, whereas larger worker eyes structure have been selected for sensitivity and acuity. Higher acuity is adaptive outside the nest, as it allows resolving more distal objects. The significant breakpoints (HW 1.0-1.8mm) found in the linear regressions for ommatidia number, eye surface area, interommatidial angle and eye parameter (Fig. 1A,C,E,F) suggest structural changes to accommodate the body size-associated transition between inside and outside nest division of labor in *A. cephalotes*. Comparisons of eye structure between diurnal, cathemeral, and nocturnal ant (Greiner et al. 2007; Narendra et al. 2013; Yilmaz et al. 2014; Ogawa et al. 2019) and bee species (Greiner et al. 2004) are generally consistent with our predictions.

Although eye structure determines light sensitivity and visual acuity, other anatomical, physiological, and behavioral adaptations modify visual abilities: variations in the size of rhabdomers (Greiner et al. 2004; Gonzalez-Bellido et al. 2011; Narendra et al. 2017), microsaccadic rhabdomere contractions and microvilli refractory time (Juusola et al. 2016), or pupillary systems mediated by pigment ommatidial cells (Narendra et al. 2013, 2016a) Such visual adaptations in *A. cephalotes* polymorphic workers remain to be studied.

Division of labor and visual neuropil size and structure

In ant species characterized by morphological differentiated subcastes, workers are predicted to vary neurobiologically to support the sensory demands of specialized tasks (Muscedere and Traniello 2012; Kamhi et al. 2015; Gordon et al. 2019). If metabolic costs associated with the production and maintenance of brains is high, then selection should favor the

342 reduction of neuropil size (e.g.(Aiello and Wheeler 1995; Niven and Laughlin 2008)). We found
343 that brain volume increased with worker size, but larger workers had brains smaller than
344 expected from their body size (Fig. 2A). We expected *A. cephalotes* workers would invest
345 differentially in brain compartments due to their body size-related task repertoires, and found
346 that larger workers had larger eyes and an allometric increase in OL volume (Fig. 2B).
347 Conversely, some diurnal moths have smaller eyes but larger optic lobes than nocturnal species
348 (Stöckl et al. 2016), a pattern also found in analogous brain region in teleost fishes (Iglesias et al.
349 2018). *A. cephalotes* OL were disproportionally larger in larger workers, and consistent with our
350 prediction, minims showed disproportionately less OL investment. This suggests a task-related
351 increasing need for primary visual information processing in larger workers.
352 Our analysis revealed that lamina, medulla and lobula increased with worker size (Fig.
353 S1), maybe due to higher exposure to light in larger workers active outside the nest (as in
354 (Yilmaz et al. 2016)). Within the OLs, larger workers possessed disproportionally larger lamina
355 and lobula, but a disproportionally smaller medulla (Fig. 2C1-C3). These OL subregion
356 allometries suggest that minims might be better at detecting small-field motion whereas larger
357 workers might be better at processing contrast, wide-field motion, shape, and panorama
358 information. This neuroplasticity seems to adaptively support *A. cephalotes* task specialization
359 inside and outside the nest. We also found a disproportional investment in the MB collar in
360 larger workers (Fig. 2D). Enlarged MBs in social hymenopterans might be the result of ancestral
361 neuroanatomical adaptations to process novel visual information [60]. This evolutionary scenario
362 across phylogenetically diverse ant species appears to be reflected in *A. cephalotes* subcastes that
363 vary in visual ecologies. Our results suggest that the increased need for visual cognition in larger
364 workers is greater for primary processing than for higher-order processing. In *Myrmecia* species,
365 nocturnal workers invested relatively less in OL but relatively more in the MB, including the
366 collar, than diurnal workers (Sheehan et al. 2019). Our results showed that minims had the
367 highest collar:optic lobe ratio (Fig. 2E), apparently as an adaptation to performing tasks in
368 darkness. Collaterally, studies of gene expression differences in whole brains of *A. cephalotes*
369 subcastes revealed a significant worker size-related increase in the level of a gene associated
370 with rod cell development, mirroring the higher demand for visual acuity and larger eye
371 structures in larger workers (Muratore et al., unpublished data). This trend was also true for a

gene associated with growth factor activity, potentially contributing to the allometric OL enlargement and other brain regions.

Conclusions

We found optical and neural plasticity are associated with the complex agrarian division of labor of *A. cephalotes* workers. Previous studies describe differences in eye structure (Menzel and Wehner 1970; Bernstein and Finn 1971; Klotz et al. 1992; Baker and Ma 2006; Schwarz et al. 2011) or visual neuropil investment (O'Donnell et al. 2018). Our results advance our understanding of ant visual system functionality by demonstrating caste-related compound eye and brain plasticity that has evolved in response to worksite light levels. Worker polymorphism has been shown to be correlated with patriline in the several leafcutting ant species (Hughes et al. 2003; Evison and Hughes 2011), suggesting a potential link between genetic variation and the neuroanatomical patterns described here. Division of labor underpinning the fungicultural habits of *A. cephalotes* appears to have played an important selective role in worker visual system evolution. Worker behavior in this species, however, depends on visual and olfactory information that likely varies with the cognitive requirements of tasks. The influence of these factors on the spatial resolving power and sensitivity of eyes and macroscopic and cellular structure of *A. cephalotes* brains requires further study.

Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest

Author's contributions

APH and ESR prepared brains for imaging and registered images; APH, ESR and SA analyzed neuropil volumes; ESR initiated and designed the eye metric imaging protocol, registered eye images, and measured eye structure; APH and SA performed statistical analysis; APH collected ant colonies; IBM analyzed confocal images and contributed to manuscript content; ESR and APH prepared the first draft of the manuscript; JFAT, APH, ESR, and SA conceptualized and designed the study. JFAT secured funding for the study; JFAT and SA wrote the manuscript. All authors edited and approved the final content of the manuscript.

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References

Abràmoff MD, Hospitals I, Magalhães PJ, Abràmoff M (2004) Image Processing with ImageJ. Biophotonics Int 11:36–42

Aiello L, Wheeler P (1995) The expensive-tissue hypothesis: the brain and the digestive system in human and primate evolution. Curr Anthropol 36:199–221

Baker GT, Ma PWK (2006) Morphology and number of Ommatidia in the compound eyes of Solenopsis invicta, Solenopsis richteri, and their hybrid (Hymenoptera: Formicidae). Zool Anz 245:121–125. https://doi.org/10.1016/j.jcz.2006.06.001

Bernstein S, Finn C (1971) Ant compound eye: Size-related ommatidium differences within a single wood ant nest. Experientia 27:708–710. https://doi.org/10.1007/BF02136977

Beugnon G, Fourcassié V (1988) How do red wood ants orient during diurnal and nocturnal foraging in a three dimensional system? II. Field experiments. Insectes Soc 35:106–124

Beugnon G, Lachaud JP, Chagné P (2005) Use of long-term stored vector information in the neotropical ant *Gigantiops destructor*. J Insect Behav 18:415–432. https://doi.org/10.1007/s10905-005-3700-8

Bucher D, Scholz M, Stetter M, et al (2000) Correction methods for three-dimensional reconstructions from confocal images: I. Tissue shrinking and axial scaling. J Neurosci Methods 100:135–143. https://doi.org/10.1016/S0165-0270(00)00245-4

Bulova SJ, Purce K, Khodak P, et al (2016) Into the black and back: the ecology of brain investment in Neotropical army ants (Formicidae: Dorylinae). Sci Nat 103:31. https://doi.org/DOI 10.1007/s00114-016-1353-4

Cherrett JM (1968) The foraging behaviour of *Atta cephalotes* L. (Hymenoptera, Formicidae). J Anim Ecol 37:387. https://doi.org/10.2307/2955

Corral-López A, Garate-Olaizola M, Buechel SD, et al (2017) On the role of body size, brain size, and eye size in visual acuity. Behav Ecol Sociobiol 71:179. https://doi.org/10.1007/s00265-017-2408-z

Czaczkes TJ, Grüter C, Ratnieks FLW (2015) Trail Pheromones, An integrative view of their role in social insect colony organization. Annu Rev Entomol 60:581–599. https://doi.org/10.1146/annurev-ento-010814-020627

443 Davies R (2002) Hypothesis testing when a nuisance parameter is present only under the
444 alternative: Linear Model Case. Biometrika 89:484–489
445 Dyer AG, Paulk AC, Reser DH (2011) Colour processing in complex environments: Insights
446 from the visual system of bees. Proc R Soc B Biol Sci 278:952–959.
447 https://doi.org/10.1098/rspb.2010.2412
448 Ehmer B, Gronenberg W (2004) Mushroom body volumes and visual interneurons in ants:
449 comparison between sexes and castes. J Comp Neurol 469:198–213.
450 https://doi.org/10.1002/cne.11014
451 Evison SEF, Hughes WOH (2011) Genetic caste polymorphism and the evolution of polyandry
452 in *Atta* leaf-cutting ants. Naturwissenschaften 98:643–649. https://doi.org/10.1007/s00114-
453 011-0810-3
454 Farris SM (2016) Insect societies and the social brain. Curr Opin Insect Sci 1–8.
455 https://doi.org/10.1016/j.cois.2016.01.010
456 Freas CA, Wystrach A, Narendra A, Cheng K (2018) The view from the trees: Nocturnal bull
457 ants, *Myrmecia midas*, use the surrounding panorama while descending from trees. Front
458 Psychol 9:1–15. https://doi.org/10.3389/fpsyg.2018.00016
459 Gonzalez-Bellido PT, Wardill TJ, Juusola M (2011) Compound eyes and retinal information
460 processing in miniature dipteran species match their specific ecological demands. Proc Natl
461 Acad Sci U S A 108:4224–4229. https://doi.org/10.1073/pnas.1014438108
462 Gordon DG, Ilieş I, Traniello JFA (2017) Behavior, brain, and morphology in a complex insect
463 society: trait integration and social evolution in the exceptionally polymorphic ant *Pheidole*
464 *rhea* . Behav Ecol Sociobiol 71:166. https://doi.org/https://doi.org/10.1007/s00265-017-
465 2396-z
466 Gordon DG, Zelaya A, Arganda-Carreras I, et al (2019) Division of labor and brain evolution in
467 insect societies: Neurobiology of extreme specialization in the turtle ant *Cephalotes varians*.
468 PLoS One 14:1–16. https://doi.org/10.1371/journal.pone.0213618
469 Graham P, Cheng K (2009) Ants use the panoramic skyline as a visual cue during navigation.
470 Curr Biol 19:R935–R937. https://doi.org/10.1016/j.cub.2009.08.015
471 Greiner B (2006) Adaptations for nocturnal vision in insect apposition eyes. Int Rev Cytol
472 250:1–46. https://doi.org/10.1016/S0074-7696(06)50001-4
473 Greiner B, Narendra A, Reid SF, et al (2007) Eye structure correlates with distinct foraging-bout
474 timing in primitive ants. Curr Biol 17:879–880. https://doi.org/10.1016/j.cub.2007.08.015
475 Greiner B, Ribi WA, Warrant EJ (2004) Retinal and optical adaptations for nocturnal vision in
476 the halictid bee *Megalopta genalis*. Cell Tissue Res 316:377–390.
477 https://doi.org/10.1007/s00441-004-0883-9
478 Gronenberg W (2008) Structure and function of ant (Hymenoptera: Formicidae) brains: strength
479 in numbers. Myrmecol News 11:25–36
480 Gronenberg W (2001) Subdivisions of hymenopteran mushroom body calyces by their afferent
481 supply. J Comp Neurol 435:474–489. https://doi.org/10.1002/cne.1045
482 Gronenberg W, Hölldobler B (1999) Morphologic representation of visual and antennal
483 information in the ant brain. J Comp Neurol 412:229–240.
484 https://doi.org/10.1002/(SICI)1096-9861(19990920)412:2<229::AID-CNE4>3.0.CO;2-E
485 Hölldobler B (1980) Canopy orientation : a new kind of orientation in ants. Science (80-)
486 210:86–88
487 Hölldobler B, Wilson EO (2010) The leafcutter ants: civilization by instinct. WW Norton &
488 Company

Hölldobler B, Wilson EO (1990) The Ants. Harvard University Press, Cambridge, MA

Huber R, Knaden M (2015) Egocentric and geocentric navigation during extremely long foraging paths of desert ants. J Comp Physiol A Neuroethol Sensory, Neural, Behav Physiol 201:609–616. https://doi.org/10.1007/s00359-015-0998-3

Hughes WOH, Sumner S, Van Borm S, Boomsma JJ (2003) Worker caste polymorphism has a genetic basis in *Acromyrmex* leaf-cutting ants. Proc Natl Acad Sci U S A 100:9394–9397. https://doi.org/10.1073/pnas.1633701100

Iglesias TL, Dornburg A, Warren DL, et al (2018) Eyes Wide Shut: the impact of dim-light vision on neural investment in marine teleosts. J Evol Biol 31:1082–1092. https://doi.org/10.1111/jeb.13299

Jander U, Jander R (2002) Allometry and resolution of bee eyes (Apoidea). Arthropod Struct Dev 30:179–193. https://doi.org/10.1016/S1467-8039(01)00035-4

Juusola M, Dau A, Song Z, et al (2016) Microsaccadic information sampling provides *Drosophila* hyperacute vision. Elife 6:e26117. https://doi.org/10.7554/eLife.26117

Kamhi JF, Nunn K, Robson SKA, Traniello JFA (2015) Polymorphism and division of labour in a socially complex ant: neuromodulation of aggression in the Australian weaver ant, *Oecophylla smaragdina*. Proc Biol Sci 282:20150704-. https://doi.org/10.1098/rspb.2015.0704

Kamhi JF, Sandridge-Gresko A, Walker C, et al (2017) Worker brain development and colony organization in ants: Does division of labor influence neuroplasticity? Dev Neurobiol 77:1072–1085. https://doi.org/10.1002/dneu.22496

Kapustjanskij A, Streinzer M, Paulus HF, Spaethe J (2007) Bigger is better: Implications of body size for flight ability under different light conditions and the evolution of alloethism in bumblebees. Funct Ecol 21:1130–1136. https://doi.org/10.1111/j.1365-2435.2007.01329.x

Kaspari M, Weiser MD (1999) The size–grain hipothesis and interespecific scaling in ants. Fuctional Ecol 13:530–538. https://doi.org/10.1046/j.1365-2435.1999.00343.x

Klotz JH, Reid BL (1992) The use of spatial cues for structural guideline orientation in Tapinoma sessile and Camponotus pennsylvanicus (Hymenoptera: Formicidae). J Insect Behav 5:71–82. https://doi.org/10.1007/BF01049159

Klotz JH, Reid BL, Gordon WC (1992) Variation of ommatidia number as a function of worker size in *Camponotus pennsylvanicus* (DeGeer) (Hymenoptera: Formicidae). Insectes Soc 39:233–236. https://doi.org/10.1007/BF01249297

Knaden M, Graham P (2016) The sensory ecology of ant navigation: from natural environments to neural mechanisms. Annu Rev Entomol 61:63–76. https://doi.org/10.1146/annurev-ento-010715-023703

Land MF (1997) Visual Acuity in Insects. Annu Rev Entomol 42:147–177. https://doi.org/10.1146/annurev.ento.42.1.147

Linksvayer TA, Mccall AC, Jensen RM, et al (2016) The function of hitchhiking behavior in the leaf-cutting ant Atta cephalotes. Biotropica 34:93–100. https://doi.org/10.1111/j.1744-7429.2002.tb00245.x

Mares S, Ash L, Gronenberg W (2005) Brain allometry in bumblebee and honey bee workers. Brain Behav Evol 66:50–61. https://doi.org/10.1159/000085047

Menzel R, Wehner R (1970) Augenstrukturen bei verschieden großen Arbeiterinnen von *Cataglyphis bicolor* Fabr. (Formicidae, Hymenoptera). Z Vgl Physiol 68:446–449. https://doi.org/10.1007/BF00297741

Menzi U (1987) Visual adaptation in nocturnal and diurnal ants. J Comp Physiol A 160:11–21.

https://doi.org/10.1007/BF00613437
Moser JC, Reeve JD, Bento JMS, et al (2004) Eye size and behaviour of day- and night-flying leafcutting ant alates. J Zool 264:69–75. https://doi.org/10.1017/S0952836904005527
Muller M, Wehner R (2006) The significance of direct sunlight and polarized skylight in the ant's celestial system of navigation. Proc Natl Acad Sci 103:12575. https://doi.org/10.1073/pnas.0604430103
Müller M, Wehner R (2010) Path integration provides a scaffold for landmark learning in desert ants. Curr Biol 20:1368–1371. https://doi.org/10.1016/j.cub.2010.06.035
Muscedere ML, Traniello JFA (2012) Division of labor in the hyperdiverse ant genus *Pheidole* is associated with distinct subcaste-and age-related patterns of worker brain organization. PLoS One 7:e31618. https://doi.org/10.1371/journal.pone.0031618
Narendra A, Alkaladi A, Raderschall CA, et al (2013) Compound eye adaptations for diurnal and nocturnal lifestyle in the intertidal ant, *Polyrhachis sokolova*. PLoS One 8:e76015. https://doi.org/10.1371/journal.pone.0076015
Narendra A, Greiner B, Ribi WA, Zeil J (2016a) Light and dark adaptation mechanisms in the compound eyes of *Myrmecia* ants that occupy discrete temporal niches. J Exp Biol 219:2435–2442. https://doi.org/10.1242/jeb.142018
Narendra A, Kamhi JF, Ogawa Y (2017) Moving in dim light: behavioral and visual adaptations in nocturnal ants. Integr Comp Biol 57:1104–1116. https://doi.org/10.1093/icb/icx096
Narendra A, Ramirez-Esquivel F, Ribi WA (2016b) Compound eye and ocellar structure for walking and flying modes of locomotion in the Australian ant, *Camponotus consobrinus*. Sci Rep 6:1–10. https://doi.org/10.1038/srep22331
Narendra A, Reid SF, Greiner B, et al (2011) Caste-specific visual adaptations to distinct daily activity schedules in Australian *Myrmecia* ants. Proc R Soc B Biol Sci 278:1141–1149. https://doi.org/10.1098/rspb.2010.1378
Niven JE, Laughlin SB (2008) Energy limitation as a selective pressure on the evolution of sensory systems. J Exp Biol 211:1792–804. https://doi.org/10.1242/jeb.017574
O'Donnell S, Bulova S, Barrett M, Von Beeren C (2018) Brain investment under colony-level selection: Soldier specialization in *Eciton* army ants (Formicidae: Dorylinae). BMC Zool 3:1–6. https://doi.org/10.1186/s40850-018-0028-3
O'Donnell S, Clifford MR, Bulova SJ, et al (2014) A test of neuroecological predictions using paperwasp caste differences in brain structure (Hymenoptera: Vespidae). Behav Ecol Sociobiol 68:529–536. https://doi.org/10.1007/s00265-013-1667-6
Ogawa Y, Ryan LA, Palavalli-Nettimi R, et al (2019) Spatial resolving power and contrast sensitivity are adapted for ambient light conditions in Australian *Myrmecia* ants. Front Ecol Evol 7:1–10. https://doi.org/10.3389/fevo.2019.00018
Ott SR (2008) Confocal microscopy in large insect brains: Zinc-formaldehyde fixation improves synapsin immunostaining and preservation of morphology in whole-mounts. J Neurosci Methods 172:220–230. https://doi.org/10.1016/j.jneumeth.2008.04.031
Palavalli-Nettimi R, Narendra A (2018) Miniaturisation decreases visual navigational competence in ants. J Exp Biol 221:jeb177238. https://doi.org/10.1242/jeb.177238
Perl CD, Niven JE (2016a) Colony-level differences in the scaling rules governing wood ant compound eye structure. Sci Rep 6:1–8. https://doi.org/10.1038/srep24204
Perl CD, Niven JE (2016b) Differential scaling within an insect compound eye. Biol Lett 12:0–3. https://doi.org/10.1098/rsbl.2016.0042
Powell S, Clark E (2004) Combat between large derived societies: A subterranean army ant

established as a predator of mature leaf-cutting ant colonies. Insectes Soc 51:342–351. https://doi.org/10.1007/s00040-004-0752-2

Rodrigues PAP, Oliveira PS (2014) Visual navigation in the Neotropical ant *Odontomachus hastatus* (Formicidae, Ponerinae), a predominantly nocturnal, canopy-dwelling predator of the Atlantic rainforest. Behav Processes 109:48–57. https://doi.org/10.1016/j.beproc.2014.06.007

Ronacher B, Wehner R (1995) Desert ants *Cataglyphis fortis* use self-induced optic flow to measure distances travelled. J Comp Physiol A 177:21–27. https://doi.org/10.1007/BF00243395

Rutowski RL, Gislén L, Warrant EJ (2009) Visual acuity and sensitivity increase allometrically with body size in butterflies. Arthropod Struct Dev 38:91–100. https://doi.org/10.1016/j.asd.2008.08.003

Schwarz S, Narendra A, Zeil J (2011) The properties of the visual system in the Australian desert ant *Melophorus bagoti*. Arthropod Struct Dev 40:128–134. https://doi.org/10.1016/j.asd.2010.10.003

Sheehan ZBV, Kamhi JF, Seid MA, Narendra A (2019) Differential investment in brain regions for a diurnal and nocturnal lifestyle in Australian *Myrmecia ants*. J Comp Neurol 527:1261–1277. https://doi.org/10.1002/cne.24617

Snyder AW (1977) Acuity of compound eyes: Physical limitations and design. J Comp Physiol A 116:161–182. https://doi.org/10.1007/BF00605401

Spaethe J, Chittka L (2003) Interindividual variation of eye optics and single object resolution in bumblebees. J Exp Biol 206:3447–3453. https://doi.org/10.1242/jeb.00570

Stieb SM, Hellwig A, Wehner R, Rössler W (2012) Visual experience affects both behavioral and neuronal aspects in the individual life history of the desert ant *Cataglyphis fortis*. Dev Neurobiol 72:729–742. https://doi.org/10.1002/dneu.20982

Stieb SM, Muenz TS, Wehner R, Rössler W (2010) Visual experience and age affect synaptic organization in the mushroom bodies of the desert ant *Cataglyphis fortis*. Dev Neurobiol 70:408–423. https://doi.org/10.1002/dneu.20785

Stöckl A, Heinze S, Charalabidis A, et al (2016) Differential investment in visual and olfactory brain areas reflects behavioural choices in hawk moths. Sci Rep 6:1–10. https://doi.org/10.1038/srep26041

Strausfeld NJ (1989) Beneath the Compound Eye: Neuroanatomical Analysis and Physiological Correlates in the Study of Insect Vision. In: Facets of Vision. Springer, Berlin, Heidelberg, pp 317–359

Streinzer M, Brockmann A, Nagaraja N, Spaethe J (2013) Sex and caste-specific variation in compound eye morphology of five honeybee species. PLoS One 8:1–9. https://doi.org/10.1371/journal.pone.0057702

Taylor GJ, Tichit P, Schmidt MD, et al (2019) Bumblebee visual allometry results in locally improved resolution and globally improved sensitivity. Elife 8:1–32. https://doi.org/10.7554/eLife.40613

Team RC (2016) R: A Language and Environment for Statistical Computing. R Found. Stat. Comput.

V. R. M. Muggeo (2008) segmented: An R Package to Fit Regression Models with Broken-Line Relationships. R News 3:343–4. https://doi.org/10.1159/000323281

van Breda JM, Stradling DJ (1994) Mechanisms affecting load size determination in *Atta cephalotes* L. (Hymenoptera, Formicidae). Insectes Soc 41:423–435.

https://doi.org/10.1007/BF01240645

Vick K (2005) Use of multiple cues for navigation by the leaf-cutter ant *Atta cephalotes*. University of Maryland, College Park, MD

Vilela EF, Jaffé K, Howse PE (1987) Orientation in leaf-cutting ants (Formicidae: Attini). Anim Behav 35:1443–1453. https://doi.org/10.1016/S0003-3472(87)80017-9

Warrant EJ (2008) Seeing in the dark: Vision and visual behaviour in nocturnal bees and wasps. J Exp Biol 211:1737–1746. https://doi.org/10.1242/jeb.015396

Wehner R (2003) Desert ant navigation: How miniature brains solve complex tasks. J Comp Physiol A Neuroethol Sensory, Neural, Behav Physiol 189:579–588. https://doi.org/10.1007/s00359-003-0431-1

Wetterer JK (1991) Allometry and the geometry of leaf-cutting in *Atta cephalotes*. Behav Ecol Sociobiol 29:347–351. https://doi.org/10.1007/BF00165959

Wilson EO (1980) Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*): II. The ergonomic optimization of leaf cutting. Behav Ecol Sociobiol 7:57–165

Yilmaz A, Aksoy V, Camlitepe Y, Giurfa M (2014) Eye structure, activity rhythms, and visually-driven behavior are tuned to visual niche in ants. Front Behav Neurosci 8:1–9. https://doi.org/10.3389/fnbeh.2014.00205

Yilmaz A, Lindenberg A, Albert S, et al (2016) Age-related and light-induced plasticity in opsin gene expression and in primary and secondary visual centers of the nectar-feeding ant *Camponotus rufipes*. Dev Neurobiol 76:1041–1057. https://doi.org/10.1002/dneu.22374

Zeil J, Ribi WA, Narendra A (2014) Polarisation vision in ants, bees and wasps. In: Polarized Light and Polarization Vision in Animal Sciences, Second Edition

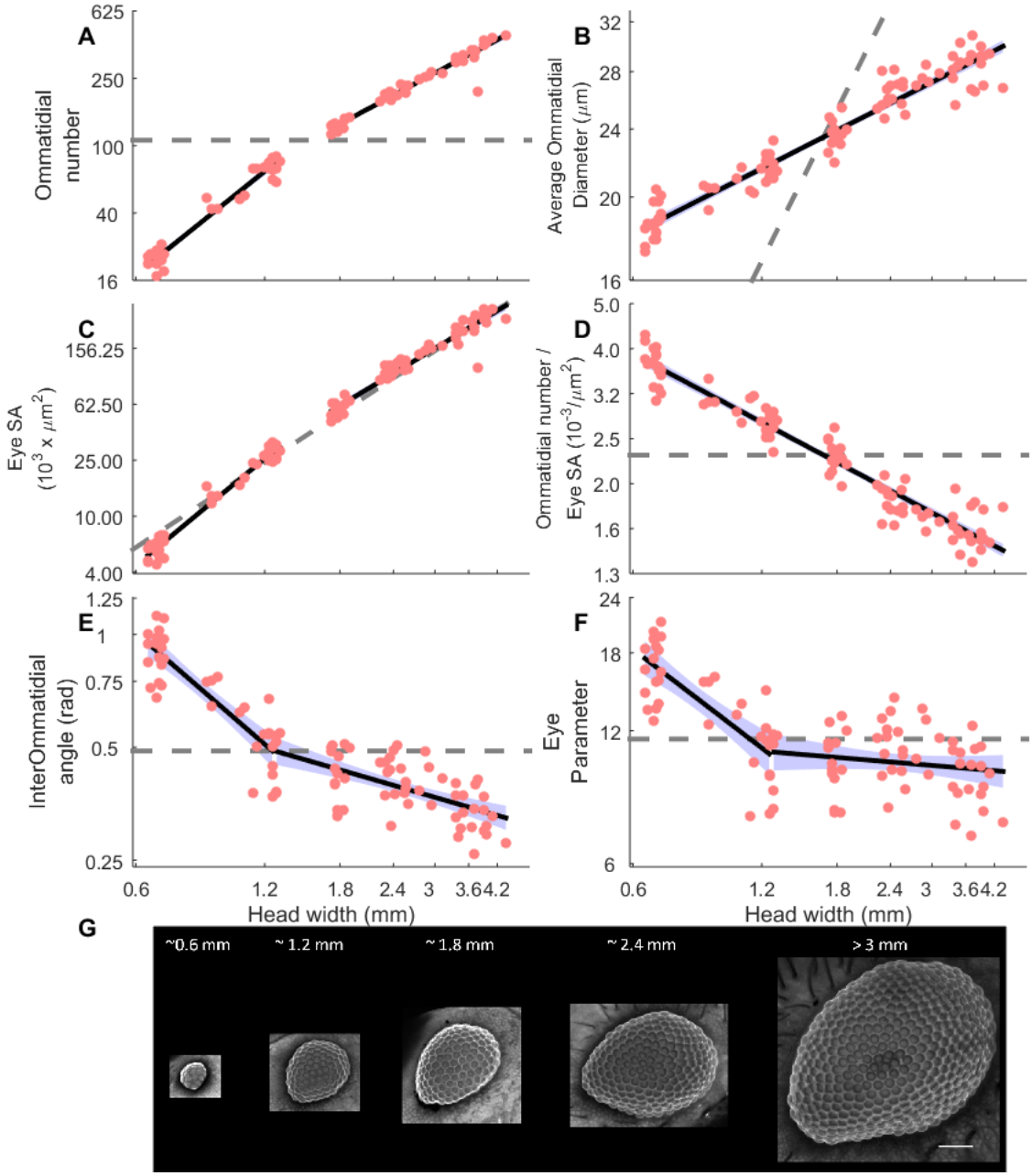
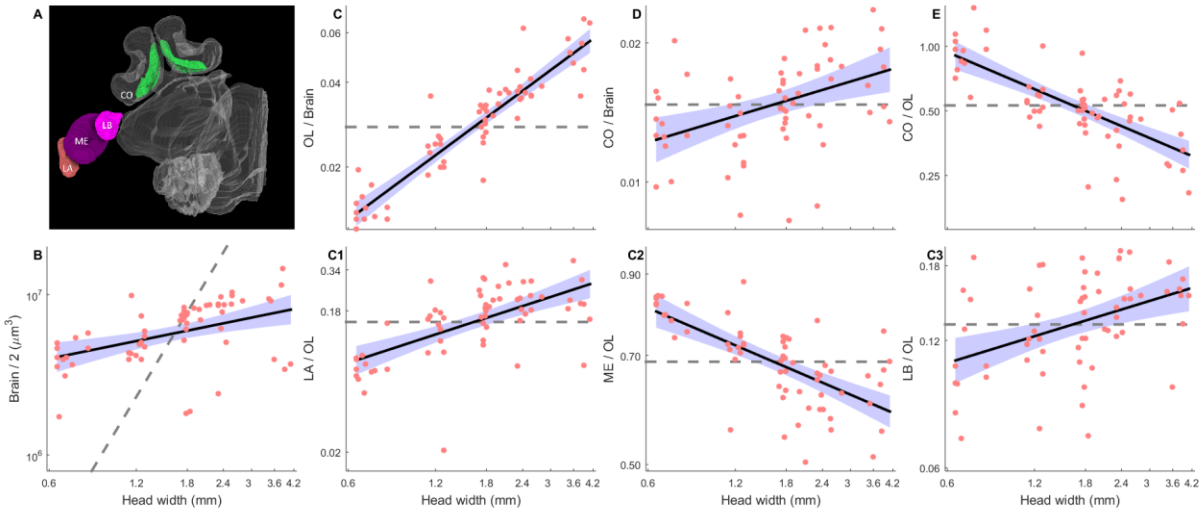


Figure 1: Compound eye structure in polymorphic *A. cephalotes* workers. A. Log-log plot of the ommatidia number as a function of worker HW showing a significant change of slope at 1.38mm. **B.** Log-log plot of average ommatidial diameter as a function of worker HW. **C.** Log-log plot of the eye surface area (SA) as a function of worker HW showing a significant change of slope at 1.44mm. **D.** Log-log plot of ommatidia density (ommatidial number/eye SA) as a function of worker HW. **E.** Log-log plot of the interommatidial angle (rad) as a function of worker HW showing a significant change of slope at 1.25mm. **F** Log-log plot of the eye parameter as a function of worker HW (significant change of slope at 1.26mm). **G.** Z-projections

659 of confocal images of eyes from workers with variable HW (scale bar=100µm). A-F: Each pink
660 point represents a single eye. Solid (significantly different from isometry) or dashed (not
661 significantly different from isometry) black lines show linear regression or piecewise linear
662 regressions as appropriate. Purple patches represent 95% confidence intervals of regression lines.
663 Dashed grey lines are the best-fitting isometric regression models.
664



665

666 **Figure 2: Volumes of polymorphic *A. cephalotes* worker brains and brain compartments.**
667 **A.** 3D reconstruction of the brain hemisphere of an *A. cephalotes* worker (HW ~4mm). **B.** Log-
668 log plot of hemisphere brain volume as a function of worker HW. **C.** Log-log plot of relative OL
669 volume as a function of worker HW. **C1.** Log-log plot of relative volume of OL lamina as a
670 function of worker HW. **C2.** Log-log plot of relative volume of OL medulla as a function of
671 worker HW.**C3.** Log-log plot of relative volume of OL lobula as a function of worker HW.**D.**
672 Log-log plot of relative MB collar volume as a function of worker HW. **E.** Log-log plot of MB
673 collar: OL volume ratio as a function of worker HW. B-E: Legend as in figure 1.
674