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ORIGINAL PAPER

Behavioral performance and division of labor influence brain mosaicism in the leafcutter ant *Atta cephalotes*

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Abstract

Brain evolution is hypothesized to be driven by behavioral selection on neuroarchitecture. We developed a novel metric of relative neuroanatomical investments involved in performing tasks varying in sensorimotor and processing demands across polymorphic task-specialized workers of the leafcutter ant *Atta cephalotes* and quantified brain size and structure to examine their correlation with our computational approximations. Investment in multisensory and motor integration for task performance was estimated to be greatest for media workers, whose highly diverse repertoire includes leaf-quality discrimination and leaf-harvesting tasks that likely involve demanding sensory and motor processes. Confocal imaging revealed that absolute brain volume increased with worker size and functionally specialized compartmental scaling differed among workers. The mushroom bodies, centers of sensory integration and learning and memory, and the antennal lobes, olfactory input sites, were larger in medias than in minims (gardeners) and significantly larger than in majors ("soldiers"), both of which had lower scores for involvement of olfactory processing in the performance of their characteristic tasks. Minims had a proportionally larger central complex compared to other workers. These results support the hypothesis that variation in task performance influences selection for mosaic brain structure, the independent evolution of proportions of the brain composed of different neuropils.

Keywords Social brain evolution · Distributed cognition · Task performance · Mushroom body · Sensory processing

Abbreviations

AL	Antennal lobe
CX	Central complex
DMSO	Dimethylsulfoxide
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
MB	Mushroom body
OL	Optic lobe
PBS	Phosphate-buffered saline
PBST	Phosphate-buffered saline with Triton
ROCB	Remainder of central brain
SEZ	Subesophageal zone

Introduction

Identifying the selective forces that contribute to the evolution of brain size and patterns of investment in functionally specialized brain centers is key to understanding the organization of behavior. Social life is predicted to contribute to selection for both increased (Dunbar 1998, 2009; Adolphs 2003; Kamhi et al. 2016) and decreased brain size (Jaffe and Perez 1989; Riveros et al. 2012; Sulger et al. 2014; O'Donnell et al. 2018a; DeSilva et al. 2021; Reséndiz-Benhumea et al. 2021), and/or brain compartment scaling relationships (Muscedere and Traniello 2012; Smaers and Soligo 2013; O'Donnell et al. 2015, 2018b; DeCasien and Higham 2019). Assessments of sensory, motor, and processing demands for behavioral performance should inform predictions about brain size and scaling given the cost of neural tissue, but the nature and extent of such demands are rarely estimated in analyses that link behavior and neuroanatomy.

Insects are important models to examine behavioral and/or cognitive evolution (Boogert et al. 2018; Lihoreau et al. 2019; Godfrey and Gronenberg 2019; Simons and Tibbetts 2019; Muratore and Traniello 2020). Social insect worker

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Dedicated to the memory of Edward O. Wilson in recognition of his inspiring work on leafcutter ants.

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brains are composed of compartments responsible for visual and olfactory processing (optic lobes [OL] and antennal lobes [AL], respectively), higher-order processing, learning, and memory (mushroom bodies [MB]), navigation, orientation, and movement (central complex [CX]; also associated with the MBs) (Le Moël et al. 2019; Green et al. 2019; Sun et al. 2020; Pisokas et al. 2020; Kamhi et al. 2020; Currier et al. 2020), and mandibular control and gustation (the suboesophageal zone [SEZ]). The remainder of the central brain (ROCB; see "Methods" for anatomical details) is composed of several protocerebral regions thought to integrate sensory information (Strausfeld 2012; Green et al. 2019; Currier et al. 2020). Complexity in colony organization may involve selection for either smaller, neurally differentiated worker brains (Riveros Rivera and Gronenberg 2009; Riveros et al. 2012; Lihoreau et al. 2012; Sulger et al. 2014; O'Donnell et al. 2015, 2018b; Feinerman and Traniello 2015), involving reductions in the volume of subsets of described insect brain compartments, or larger brains (Wehner et al. 2007) potentially able to metabolically offset increased production and operation costs (Kamhi et al. 2016).

Division of labor in socially complex fungus-growing ants is characterized by morphologically differentiated workers in derived species (Schultz and Brady 2008; Hölldobler and Wilson 2010; Muratore and Traniello 2020). Colonies of leafcutter ants, *Atta*, have strongly polymorphic workers categorized into size-based groups (subcastes) differing in labor roles and efficiencies of task performance (Fig. 1) (Wilson 1980a, b). Worker size-related specializations include fungal care, nursing immatures, leaf selection, cutting and transport, waste management and hygienic behavior, and colony defense. These tasks differ in the extent to which they involve multimodal sensory integration and motor functions associated with monitoring the growth of fungus and brood development, decoding plant chemistry, leaf harvesting, navigation, recognizing infectious agents, detecting environmental hazards, and combating predators and parasites (Saverschek and Roces 2011; Groh et al. 2014; Arenas and Roces 2016a, b, 2017; Green and Kooij 2018; Goes et al. 2020; Buehlmann et al. 2020; Fleischmann et al. 2020). This pattern of division of labor characterizes *Atta cephalotes*, whose workers range in size from 0.5 to more than 4.5 mm in head width and are divisible into groups according to task repertoires (Table 1b). Brain size and structural variation could reflect differences in behavioral diversity: workers displaying the broadest task repertoire and corresponding behavioral and/or cognitive demands could have experienced selection for a greater volume of neural tissue.

To examine factors contributing to brain evolution in *A. cephalotes*, we evaluated the expected distribution of the relative involvement of different modalities of sensory perception, integration, and sensorimotor functions in task performance across polymorphic workers. Our study explores stable differences among adult worker brains that have evolved with behavioral specialization as a likely selective factor, rather than experience-dependent plasticity occurring over the course of an individual's lifespan. We determined patterns of variation in behavioral demands associated with task performance and work environments to provide a semi-quantitative score reflecting how brain tissue would be scaled to efficaciously perform tasks. This is based on the assumption that neuropil volumes correlate with the degree of involvement of task-associated sensory processes. We acknowledge that this assumption does not encompass all influences on brain region scaling, such as limits on circuitry miniaturization (Seid et al. 2011; Niven and Farris 2012; Groh et al. 2014), and that brain compartment volumes are not necessarily equivalent to

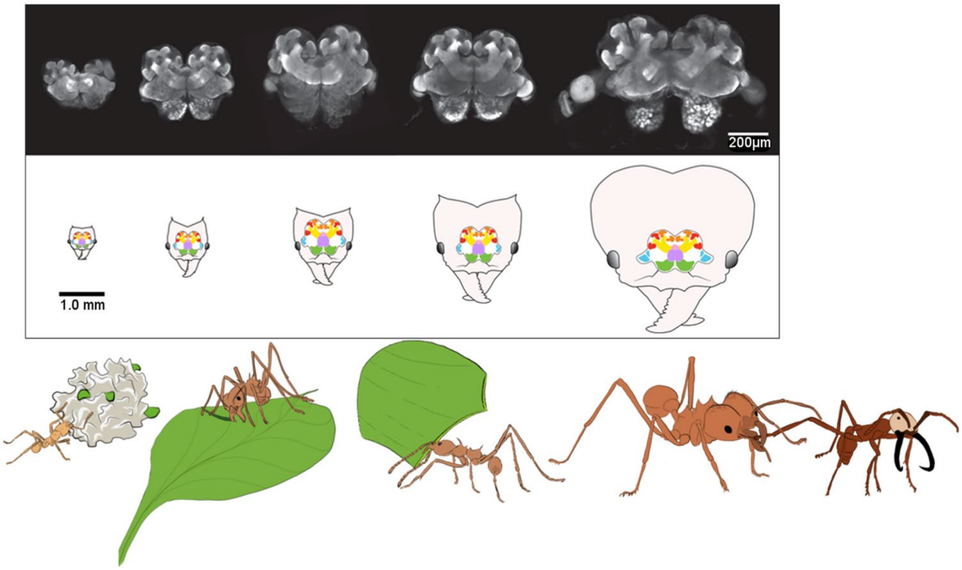


Fig. 1 Confocal images of *A. cephalotes* polymorphic worker brains (top row). Structural diagrams of color-coded neuropils (middle row; compartments not drawn to scale). Blue = OL, green = AL, orange = MB medial calyces, red = MB lateral calyces, yellow = MB peduncle, purple = SEZ, pink = CX. Illustration of task performance by polymorphic workers (bottom row; minim, two images of medias, major attacking an army ant, an important predator)

Table 1 Tasks performed by *A. cephalotes* workers and their relative reliance on sensory modalities, sensory input processing, high-order integration, and motor coordination in relation to division of labor

(a) Sensory/cognitive process

Task	Vision	Olfaction	Gustation; contact chemoreception	Seismic signal perception	Anemotaxis; air current mechanoreception	Magnetoreception	Thermoreception	Hygroreception	Proprioception; somatosensory processing; kinesthesis	Sensory integration	References
Foraging											
Trail pheromone deposition	0	3	1	0	0	0	0	0	2	2	Wilson (1980a), Evison et al. (2008)
Search	3	3	1	0	0	2	0	0	1	3	Travaglini et al. (2015)
Trail navigation and orientation	3	3	0	0	0	2	0	0	1	3	Banks and Srygley (2003)
Liquid food collection	0	2	3	0	0	0	0	0	1	2	Kooij et al. (2016), Rytter and Shik (2016)
Trail clearing	1	2	0	0	0	0	0	0	3	1	Dupuis and Harrison (2017)
Leaf harvesting											
Leaf-quality assessment	0	3	3	0	0	0	0	1	2	3	Hubbell et al. (1984), Howard et al. (1988), Thiele et al. (2014)
Short-range recruitment	0	3	3	3	0	0	0	0	2	2	Markl 1965, Roces et al. (1993)
Leaf cutting	0	2	3	0	0	0	0	0	3	2	Nichols-Orians and Schultz (1989)
Leaf fragment size selection	0	0	0	0	1	0	0	0	3	2	Segre and Taylor (2019)
Leaf fragment transport	1	3	0	0	2	2	0	0	3	3	Segre and Taylor (2019)
Leaf fragment load stabilization	0	0	0	0	3	0	0	0	3	3	Moll et al. (2013)
Leaf fragment transfer	0	1	0	0	1	0	0	0	3	1	Kwaku et al. (2020)
Fungal gardening											
Navigating in fungal comb	0	2	0	0	0	1	0	0	2	2	Farias et al. (2020)
Macerating plant material	0	2	3	0	0	0	0	0	2	1	Hölldobler and Wilson (2010)
Mulching garden	0	2	2	0	0	0	0	0	2	1	Khadempour (2018)
Monitoring fungal growth	0	3	3	0	0	0	0	0	0	2	Green and Kooij (2018), Khadempour et al. (2021), Römer et al. (2017)

Table 1 (continued)

(a) Sensory/cognitive process											
Task	Vision	Olfaction	Gustation; contact chemoreception	Seismic signal perception	Anemotaxis; air current mechanoreception	Magnetoreception	Thermoreception	Hygroreception	Proprioception; somatosensory processing; kinesthesis	Sensory integration	References
Licking mycelium	0	2	3	0	0	0	0	0	0	1	Wilson (1983)
Fertilizing fungus with feces	0	2	3	0	0	0	0	0	0	1	Mueller (2002), Kooij et al. (2016)
Removing fungal pathogens	0	2	2	0	0	0	0	0	2	1	Currie and Stuart (2001), Rodrigues et al. (2008), Goes et al. (2020)
Applying metapleural gland secretion	0	2	2	0	0	0	0	0	1	1	Poulsen et al. (2002)
Applying bacterial antibiotics	0	2	2	0	0	0	0	0	1	1	Poulsen et al. (2002)
Transplanting hyphae	0	2	2	0	0	0	0	0	2	1	Mueller (2002)
Pruning hyphae	0	2	2	0	0	0	0	0	2	1	Bass and Cherrett (1996)
Brood care											
Brood sorting	0	3	3	0	0	0	0	0	2	2	Franks and Sendova-Franks (1992), Schultner and Pulliainen (2020)
Adjusting brood microclimate	0	0	0	0	0	0	3	3	2	2	Roces and Núñez (1995), Bollazzi and Roces (2002), Groh et al. (2004), Ruchty et al. (2009, 2010), Römer et al. (2018)
Grooming brood	0	3	3	0	0	0	0	0	1	1	Tragust et al. (2013)
Covering brood with mycelia	0	2	2	0	0	0	0	0	1	1	Armitage et al. (2016)
Transporting brood	0	2	0	0	0	1	0	0	2	1	Römer and Roces (2014)
Feeding larvae	0	2	2	0	0	0	0	0	1	1	Wilson (1983)
Hygiene											
Removing inactive mycelium	1	3	2	0	0	0	0	0	1	1	Mueller (2002)

Table 1 (continued)

(a) Sensory/cognitive process

Task	Vision	Olfaction	Gustation; contact chemo-reception	Seismic signal perception	Anemotaxis; air current mechanoreception	Magneto-reception	Thermo-reception	Hygro-reception	Proprioception; somatosensory processing; kinesthesis	Sensory integration	References
Necrophoresis	1	3	1	0	0	0	0	0	1	1	Hart and Ratnieks (2001)
Policing waste-worker segregation	0	3	1	0	0	0	0	0	0	1	Hart and Ratnieks (2001)
Colony security											
Nestmate discrimination	0	3	1	0	0	0	0	0	0	2	Lenoir et al. (2001)
Parasitic fly defense	1	1	0	0	0	0	0	0	2	1	Feener and Moss (1990), Orr (1992)
Worker rescue	0	2	0	3	0	0	0	0	3	2	Roces et al. (1993)
Secreting alarm pheromone	0	3	0	0	0	0	0	0	1	2	Norman et al. (2017)
Attacking intruders	3	3	0	0	1	0	0	0	3	2	Powell and Clark (2004b)

(b) Behavioral performance by size (HW, mm)

Task	0.6	1.2	1.8	2.4	≥3	Reference
Foraging						
Trail pheromone deposition	3	3	3	3	0	Wilson (1980a), Evison et al. (2008)
Search	0	1	3	3	0	Travaglini et al. (2015)
Trail navigation and orientation	1	2	3	3	1	Banks and Srygley (2003)
Liquid food collection	0	1	3	3	0	Kooij et al. (2016), Rytter and Shik (2016)
Trail clearing	0	0	1	2	3	Dupuis and Harrison (2017)
Leaf harvesting						
Leaf-quality assessment	0	2	3	3	0	Hubbell et al. (1984), Howard et al. (1988), Thiele et al. (2014)
Short-range recruitment	0	2	3	3	0	Markl (1965), Roces et al. (1993)
Leaf cutting	0	2	3	3	0	Nichols-Orians and Schultz (1989)
Leaf fragment size selection	0	2	3	3	0	Segre and Taylor (2019)
Leaf fragment transport	0	2	3	3	0	Segre and Taylor (2019)
Leaf fragment load stabilization	0	2	3	3	0	Moll et al. (2013)
Leaf fragment transfer	1	3	3	3	0	Kwaku et al. (2020)
Fungal gardening						
Navigating in fungal comb	3	2	1	0	0	Farias et al. (2020)
Macerating plant material	3	2	1	0	0	Hölldobler and Wilson (2010)

Table 1 (continued)

(b) Behavioral performance by size (HW, mm)

Task	0.6	1.2	1.8	2.4	≥ 3	Reference
Mulching garden	3	2	1	0	0	Khadempour (2018)
Monitoring fungal growth	3	2	1	0	0	Green and Kooij (2018), Khadempour et al. (2021), Römer et al. (2017)
Licking mycelium	3	2	1	0	0	Wilson (1983)
Fertilizing fungus with feces	3	2	1	0	0	Mueller (2002), Kooij et al. (2016)
Removing fungal pathogens	3	2	1	0	0	Currie and Stuart (2001), Rodrigues et al. (2008), Goes et al. (2020)
Applying metapleural gland secretion	3	2	1	0	0	Poulsen et al. (2002)
Applying bacterial antibiotics	3	2	1	0	0	Poulsen et al. (2002)
Transplanting hyphae	3	2	1	0	0	Mueller (2002)
Pruning hyphae	3	2	1	0	0	Bass and Cherrett (1996)
Brood care						
Brood sorting	2	3	2	0	0	Franks and Sendova-Franks (1992), Schultner and Pulliainen (2020)
Adjusting brood microclimate	2	3	1	0	0	Roces and Núñez (1995), Bollazzi and Roces (2002), Römer et al. (2018)
Grooming brood	2	3	1	0	0	Tragust et al. (2013)
Covering brood with mycelia	2	3	1	0	0	Armitage et al. (2016)
Transporting brood	2	3	2	0	0	Römer and Roces (2014)
Feeding larvae	2	3	1	0	0	Wilson (1983)
Hygiene						
Removing inactive mycelium	2	3	2	0	0	Mueller (2002)
Necrophoresis	2	3	2	0	0	Hart and Ratnieks (2001)
Policing waste-worker segregation	2	2	2	2	0	Hart and Ratnieks (2001)
Colony security						
Nestmate discrimination	2	2	2	2	2	Lenoir et al. (2001)
Parasitic fly defense	3	2	0	0	0	Feener and Moss (1990), Orr (1992)
Worker rescue	0	2	2	2	0	Roces et al. (1993)
Secreting alarm pheromone	2	3	3	3	2	Norman et al. (2017)
Attacking intruders	0	0	2	3	3	Powell and Clark (2004b)

(a) Ratings: 1 = possible role, 2 = likely role, and 3 = significant role (details in "Methods"). The "proprioception, somatosensory processing and kinesthesis" category refers to involvement above common body and appendage motor control systems across polymorphic workers that reflect greater coordination than standing, walking without load carriage, feeding, mouthpart and appendage movement, self-grooming, and allogrooming. Scores are based on published data. (b) Performance frequency of a given task in the repertoire of different-size workers (rating based on the scale presented above). Supporting references and task categories are repeated in each table metrics such as glomeruli number in the antennal lobes (Kelber et al. 2009b) in understanding the organization of the brain. Nonetheless, brain size and compartmental allometries correlate with variability in worker size and task performance in ants (Muscedere and Traniello 2012; Ilieş et al. 2015; Gordon et al. 2017; Kamhi et al. 2019). Furthermore, the volumes and structural elaboration of visual and olfactory neuropils and the MBs in ants correlate with processing capability and have ethological significance (Gronenberg 2001; Farris 2011).

To identify how brain size, compartmental scaling, and differential task performance demands of division of labor correlate with our estimates, we quantified patterns of sensory, higher-order processing, and motor neuropil investment. This allowed us to correlate variation in task repertoires and their associated sensory challenges among workers to size scaling among functionally differentiated brain compartments. Based on an assessment of worker size-related sensory and motor functions, olfaction, and higher-order processing, we hypothesized that intermediate-size (media, leaf harvesting) workers would have higher MB proportional volume than small (minims, fungus gardening) or large (majors, defensive) workers. We also hypothesized that our scores would accurately describe the pattern of proportional AL volume, with this neuropil predicted to be proportionally largest in medias due to selection for processing diverse olfactory cues. We also determined the fit of our scores to the proportional volumes of the CX (also a neuropil of multisensory integration; Plath and Barron 2015; Le Moël et al. 2019) and the OLs (a primary sensory input neuropil).

Methods

Colony collection and culturing

Unrelated *A. cephalotes* colonies, recently founded by a newly inseminated queen and having relatively few workers and a small fungal comb, were collected in Trinidad in 2016. Colonies (Ac09, Ac16, Ac20, Ac21) were cultured in a Harris environmental chamber under a 12-h light: 12-h dark cycle at 55% humidity and 25 °C at Boston University. All colonies were housed in large plastic bins (30 cm × 46 cm × 28 cm) that provided a foraging arena and area for waste disposal. Plastic boxes (11 cm × 18 cm × 13 cm) interconnected by plastic tubes (1 cm diameter) served as chambers for the fungus. Colonies were provisioned with washed pesticide-free leaves of rhododendron, rose, lilac, andromeda, bramble, oak, sugar maple, willow, and beech (as available), organic baby spinach, romaine, arugula, frisée, and oat flakes.

Behavioral performance demands and estimates of associated neuroanatomical support

We integrated fungus-growing ant brain morphology and behavior (Wilson 1980a, b; Hölldobler and Wilson 2010) with data from the literature (references in Table 1) to inform our estimate of needs for sensory integration and motor control in *A. cephalotes* worker task performance. Based on our results of research on visual system evolution in *A. cephalotes* (Arganda et al. 2020), we assumed greater volume in neuropils such as the MBs, ALs, and CX would process more diverse stimulus arrays and coordinate sensorimotor processes. For example, tasks such as leaf selection, cutting, and transport involve olfactory discrimination, proprioception and mechanosensory and muscular systems to control the mandibles, appendages, head position, and direction of movement while excising plant tissue (Khalife et al. 2018; Green et al. 2019; Currier et al. 2020), whereas other tasks differ significantly in these needs. Scores for task performance frequency were based on results of studies of worker size-related behavior (references listed in Table 1). Levels of involvement of sensory integration and other cognitive processes were based on overlap in known sensory capacities of ants and documented instances of behaviors being disrupted through manipulations of the brain or sensory pathways (see references in Table 1).

Approximations of neuropil investment for sensory integration were calculated as the sum of each worker group task performance process combination from scores in Table 1 according to the following equation:

$$D_w = \sum_{t=1}^{n} x_t y_{wt},$$

where D_w is the score reflecting investment in neuronal substrate to process and integrate sensory and/or sensorimotor inputs for a given worker size group. A higher D_w value predicts greater investment (proportional volume) in a given brain compartment functionally related to the processes in question, x_t is the multisensory integration-task demand score, the estimated degree of sensory integration involved in performing a given task t, y_{wt} is the worker size group task performance score, estimating the tendency of a given worker group w to perform a given task t, n is the total number of tasks.

This equation integrates contributions from the type of neuropil investments likely to be necessary for the performance of individual tasks with the frequency with which polymorphic workers are likely to perform them to generate hypotheses concerning the likelihood of selection acting to prioritize or deprioritize sensory integration in the brains of size-variable workers. x_t was rated on a 0–3 scale according to the role an input or process is considered to underpin a specific task: 0 = not involved, 1 = possible role (little direct empirical evidence but logical justification for hypothesizing involvement, e.g., evidence in other insects), 2 = likely role (evidence of involvement in ants, under certain conditions), and 3 = significant role (compelling evidence of involvement in ants, including *A. cephalotes* or closely related species). y_{wt} was rated on a similar scale according to the likelihood that a given worker size group performed a specific task where: 0 = does not perform task, 1 = possible occasional role in performing task, 2 = likely to contribute to task, and 3 = known to frequently perform task.

We recognize that task performance observations contributing to our scores included studies of lab and field colonies that may vary in the type of behavioral data they generate. For example, majors specialized on colony security may not be exposed in lab cultures to stimuli that induce defensive actions. However, we do not believe that such variation affects the inferences of our computational model.

Immunohistochemistry and confocal microscopy

Mature workers, as identified by complete darkening and hardening of the exoskeleton, collected from colonies Ac09, Ac16, Ac20, and Ac21 were decapitated immediately prior to brain dissection and fixation. We selected mature workers to control for the influences of age and experience as best as possible given the nature of our study. Individuals were sampled from five worker size groups identified by head width (HW): minims (0.6 mm ± 0.1 mm), medias (1.2 mm ± 0.1 mm, 1.8 ± 0.1 mm, or 2.4 mm ± 0.1 mm), and majors (3.0 mm or larger). HW was measured as the widest distance from the outer margin of one eye to the other across the face in front view. Brains (n = 30) from workers sampled from Ac09, Ac20, and Ac21 were dissected in ice-cold HEPES-buffered saline, placed in 16% zinc-formaldehyde (Ott 2008), and fixed overnight at room temperature on a shaker. Whole brains were processed to visualize the presynaptic protein synapsin. Fixed brains were washed in HEPES-buffered saline six times, 10 min per wash, and fixed in Dent's Fixative (80% MeOH, 20% DMSO) for minimally 1 h. Brains were then washed in 100% methanol and either stored at − 17 °C or immediately processed. Brains were washed in 0.1 M Tris buffer (pH 7.4) and blocked in PBSTN (5% normal goat serum, 0.005% sodium azide in 0.2% PBST) at room temperature for 1 h before incubation for 3 days at room temperature in primary antibody (1:30 SYNORF 1 in PBSTN; monoclonal antibody anti-synorf 3C11 obtained from DSHB, University of Iowa, IA, USA; 62). They were washed 6 × 10 min in 0.2% PBST and incubated in the 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 and PBS series (10 min per concentration, 30/50/70/95/100/100% ethanol in 1 × PBS), then cleared with and immersed in methyl salicylate, and mounted on stainless steel glass windowed slides for imaging.

Brains were imaged with a Nikon C2 confocal microscope and images were manually annotated using Amira 6.0 software to quantify neuropil volumes (not including cell bodies). The individual who annotated all brains for the study did not have any expectation of specific outcomes and did not have knowledge of predictions generated by our model. The annotation process involved using paintbrush- or magic wand-style tools to select areas to be included in a given neuropil in a given single scan of a 3D stack. The margins of focal neuropil regions were identified visually (or automatically when using the magic wand tool) based on the presence of synapsin staining. The magic wand-style tool was used primarily to annotate the antennal lobe glomeruli. Every third frame was annotated manually (or every other frame in the case of the antennal lobes) and intervening frames were filled in using the interpolation function of Amira. Interpolated frames were also checked and edited for accuracy. Annotated slices were then used to calculate the 3D volume of each neuropil using Amira and these data were exported for analysis. We recorded the volumes of OL, AL, MB, CX, SEZ, and ROCB. We use the term ROCB for simplicity and to correspond with our ability to associate specific compartments with sensorimotor functions to describe the tissue composed of the superior neuropils, lateral horn, ventrolateral neuropils, inferior neuropils, and ventromedial neuropils, as designated in a fruit fly brain (Ito et al. 2014). For the ALs, only glomerular tissue was included (excluding aglomerular neuropil and all soma layers). For the OLs, we measured only the medulla and lobula neuropils, excluding surrounding cell bodies. Similarly, measurements of the SEZ did not include somata. We also measured and separately examined substructures of the MB: the medial calyces (MB medial calyces), lateral calyces (MB lateral calyces), and peduncle and lobes (MB peduncle). Our peduncle measurements incorporated vertical and medial lobes; these metrics are included in all discussions of the peduncle. The volumes of these components were combined to quantify total MB size (total MB) across worker size groups. For bilateral structures, one hemisphere was measured, and for compartments located along the brain midline (SEZ and CX), the whole structure was measured (Supplementary Table 1; Supplementary Table 2). When calculating total brain volume, we excluded all soma layers and used only neuropil volumes.

Volumetric analysis

Statistical comparisons among worker size groups and brain compartment metrics were performed using R (version 3.6.2). We compared absolute volumes of total measured brain volume, total brain volume scaled to head width, all individual brain compartments, and normalized brain compartments. Normalized volumes were calculated by dividing the volume of the compartment of interest by total brain volume. We invoke the central limit theorem due to our relatively large sample size ($n=50$). To account for any unevenness in the sampling of different worker groups across colonies, we included colony identity as a random effect within a linear mixed effects model (using the *lmer* package in R; Bates 2005) and tested differences using ANOVA. If ANOVA results showed a significant effect of worker size group on the proportional size of a brain compartment, we performed pairwise comparisons with a Bonferroni correction for multiple comparisons using the *contrast* and *eemeans* (Lenth 2018) functions in R to determine the significance of compartment size differences among groups.

Linear regression was used to assess the significance of correlations between values of D_w for each worker size group and either proportional volumes of brain compartments or total brain volume scaled to worker size (i.e., the sum of all measured neuropils/HW).

Principal component analysis (PCA) was performed on log-transformed proportional volumes using the prcomp function from the base stats package in R. Linear discriminant analysis (LDA) was performed using the lda function from the MASS package in R (Liaw and Wiener 2001). Previously collected data of *A. cephalotes* brain volume measurements taken by a different observer were used as a training set (Supplementary Table 3).

Results

Division of labor and sensory involvement in task performance

Scores for sensory integration were highest in medias (1.2 mm $D_w=124$, 1.8 mm = 127, 2.4 mm = 102) and the score for minims (0.6 mm $D_w=82$) exceeded that for majors (3 mm+ $D_w=20$) (Fig. 2). A linear regression of total MB volume on D_w showed a significant correlation ($p=0.002$) between our scores and the pattern of proportional volume in the MBs (Table 2, Fig. 3). Similarly, MB peduncle volume showed moderate but significant correlation to D_w values. MB medial calyx, MB lateral calyx (and medial and lateral calyx summed volume), OL, AL, CB, SEZ, and ROCB volumes were also compared to D_w values, all explaining very low levels of variance with mixed significance (Table 2).

Division of labor and neural phenotypes

Absolute total brain volume of increased with worker size (Fig. 4a; Table 3a; Supplementary Table 5a). Only majors had significantly larger brains. When adjusted for body size, total brain volumes were not significantly different (Fig. 4b; Table 3a; Supplementary Table 4b). The absolute volumes of all compartments except the CX significantly increased with worker size (Fig. 5; Table 3a; Supplementary Table 5). Consistent with the pattern of total brain size, many brain compartments were significantly larger in majors. The proportional volumes of all brain compartments except the MB medial calyces, MB lateral calyces (as well as the proportional volume of the sum of medial and lateral calyces), and the SEZ significantly differed among workers of different-size groups (Table 3b). In contrast to the relatively uniform pattern of increase in absolute volumes, the directions of these trends differed (Fig. 6; Table 3b; Supplementary Table 6).

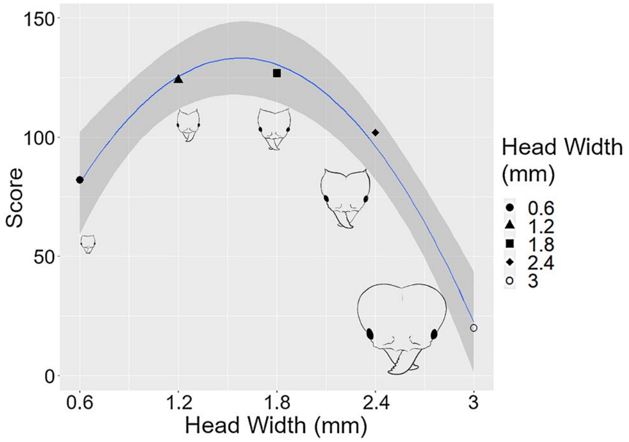


Fig. 2 Calculated scores (D_w) for neuronal substrate investment based on tasks performed by *A. cephalotes* worker size groups. *Y* axis values are the sum of each sensory integration/sensorimotor function task score multiplied by the corresponding worker group-size task performance score (see "Methods"). Local regression curve approximates neuronal substrate investment score as a function of HW plus HW squared (score ~ HW + HW^2; blue line) and 95% confidence interval (grey band). Heads are drawn to scale

Principal component analysis of log-transformed proportional brain volumes explained a significant portion (PC1 = 15.83%, PC = 66.04%) of the observed variance (Fig. 7a). Linear discriminant analysis, using a model that included proportional volumes of all neuropils except for the ROCB (which was colinear with other variables) and trained on a separate data set of *A. cephalotes* brain volumes, classified samples in the main data set with 95.8% accuracy (Fig. 7b). However, this result was found only when 1.2, 1.8, and 2.4 mm worker groups were clustered as medias. LDA using five worker size groups (0.6, 1.2, 1.8, and 3 mm+) and the same testing and training data sets achieved 54.2% accuracy.

Table 2 Fit of D_w scores to observed patterns of proportional brain compartment investment

Brain compartment	Multiple R-squared	Adjusted R-squared	*F* statistic	*p* value
Total neuropil volume/HW	0.012	− 0.008	0.597	0.4434
OL	0.351	0.338	25.990	5.770e−06
AL	0.001	− 0.020	0.062	0.805
Total MB	0.186	0.169	10.960	0.002
MB medial calyces	0.087	0.087	0.068	0.037
MB lateral calyces	0.002	− 0.019	0.071	0.791
MB medial and lateral calyces sum	0.039	0.019	1.952	0.169
MB peduncle	0.155	0.138	8.822	0.005
CX	0.104	0.085	5.559	0.023
SEZ	0.079	0.060	4.129	0.048
ROCB	0.025	0.005	1.250	0.269

Linear regression statistics for the fit of a subset of categories of behavioral performance/sensory/cognitive process as predictors for the brain compartments whose function most closely corresponds to these demands. Degrees of freedom = 1, 48

AL antennal lobe, *CX* central complex, *MB* mushroom body, *OL* optic lobe, *ROCB* remainder of central brain, *SEZ* subesophageal zone

Discussion

We assessed how estimated sensory and motor aspects of task performance involved in the agricultural division of labor correlate with variation in brain size and compartmental allometries (i.e., mosaic structure) among polymorphic workers of *A. cephalotes*. We identified significant differences in absolute size and proportional investment across polymorphic task-differentiated workers, supporting the hypothesis that variation in task performance has been a contributing selective factor in the evolution of worker neural phenotypes. Scaling patterns broadly correlated with sensory, somatosensory, and integrative information-processing demands associated with the specialized repertoires of polymorphic workers, consistent with demands in species characterized by large colony size and task differentiation (Riveros et al. 2012).

Behavioral performance challenges in social insects have been typically inferred from interspecific and intraspecific variation in neuropil scaling patterns and general assessments of sensory environments and socioecological influences (Gronenberg et al. 1996; Gronenberg 1999; Muscedere and Traniello 2012; Amador-Vargas et al. 2015; O'Donnell et al. 2018b), casually correlated with sociobiological characteristics such as colony size, foundation strategy and queen/worker differentiation (for example the reduction in MB volume in solitary compared to social wasps; O'Donnell et al. 2015), and/or diet and life history (Sayol et al. 2020). To the best of our knowledge, our study is the first to employ a model that estimates contributing factors to selection on brain investment in relation to sensorimotor integration and to establish a significant correlation with neuroanatomy.

Modeling investment in sensory integration processes in the brain

We assessed the need for neuropil investment to serve sensory input processing and sensorimotor functions, conservatively evaluated the size-related involvement of polymorphic workers in performing these tasks (Fig. 2) and used this metric to generate predictions concerning brain evolution. Notably, our scores were broadly consistent with total MB and MB peduncle scaling patterns, although the percentage of explained variance was moderate, lending modest support to the concept that selection on brain compartment volume optimizes for sensory involvement in task integration. However, several other factors likely contribute to levels of investment. These may include differences in the

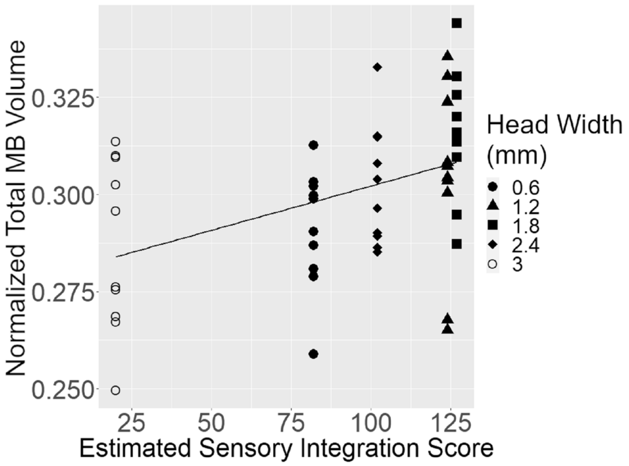


Fig. 3 Normalized total MB volume as a function of sensory integration score (D_w) with regression line. R-squared = 0.169. $p = 0.002$

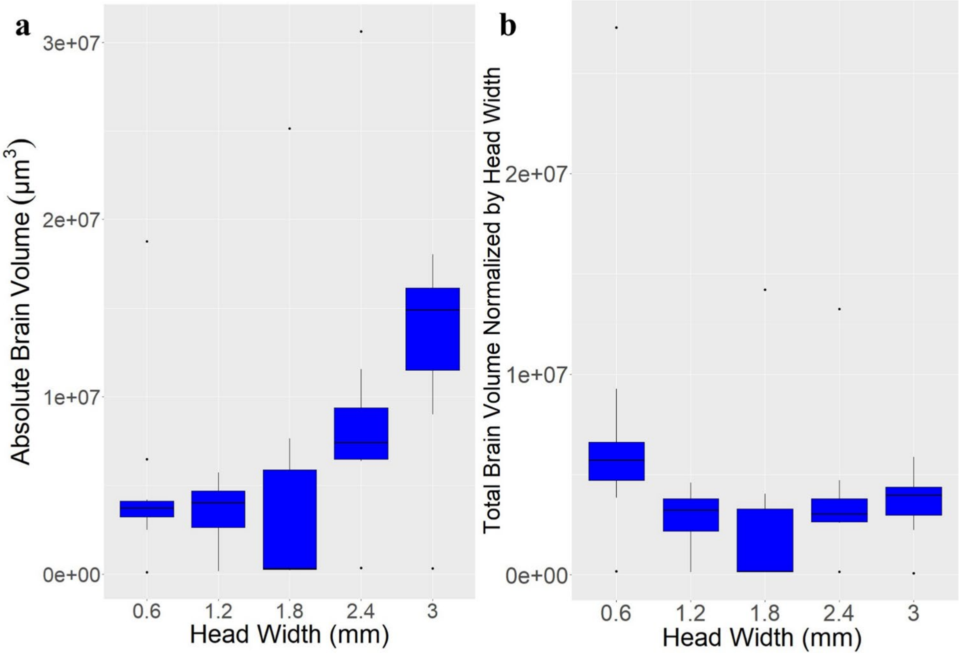


Fig. 4 **a** Absolute total brain volume (sum of the OLs, ALs, total MB, CX, SEZ, and ROCB) across worker size groups ($p=0.002$). **b** Total brain volume scaled to body size (total volume/HW for each sample across worker size groups ($p=0.143$). Y axis values are noted in scientific e notation

Table 3 Statistical analysis of brain volume and brain compartment absolute (a) and proportional (b) volume among worker size groups

Brain compartment	Chi-squared	*p* value
(a) Absolute total brain and compartmental volumes		
Total of neuropils	17.245	0.002
Total neuropil volume/HW	6.872	0.143
OL	55.265	2.859e−11
AL	11.889	0.0182
Total MB	14.275	0.006
MB medial calyces	15.220	0.004
MB lateral calyces	15.646	0.004
MB medial and lateral calyces sum	15.460	0.004
MB peduncle	12.316	0.015
CX	3.127	0.537
SEZ	21.699	2.301e−04
ROCB	15.320	0.004
(b) Proportional brain compartment volumes		
OL	332.680	< 2.200e−16
AL	11.710	0.020
Total MB	14.237	0.007
MB medial calyces	6.454	0.168
MB lateral calyces	4.480	0.345
MB medial and lateral calyces sum	2.277	0.685
MB peduncle	14.961	0.005
CX	29.453	6.324e−06
SEZ	7.683	0.104
ROCB	12.933	0.012

Degrees of freedom = 4

AL antennal lobe, *CX* central complex, *MB* mushroom body, *OL* optic lobe, *ROCB* remainder of central brain, *SEZ* subesophageal zone

Fig. 5 **a** Absolute volume of the OL (*p* = 2.859e−11); **b** AL (**b** p = 0.0182); **c** total MB (*p* = 0.006); **d** MB medial calyces (*p* = 0.004); **e** MB lateral calyces (*p* = 0.004); **f** MB medial and lateral calyces sum (*p* = 0.004); g. MB peduncle (p = 0.015); **h** CX (*p* = 0.537), **i** SEZ (*p* = 2.301e−04); and **j** ROCB (*p* = 0.004) across worker size groups. *Y* axis values are noted in scientific e notation

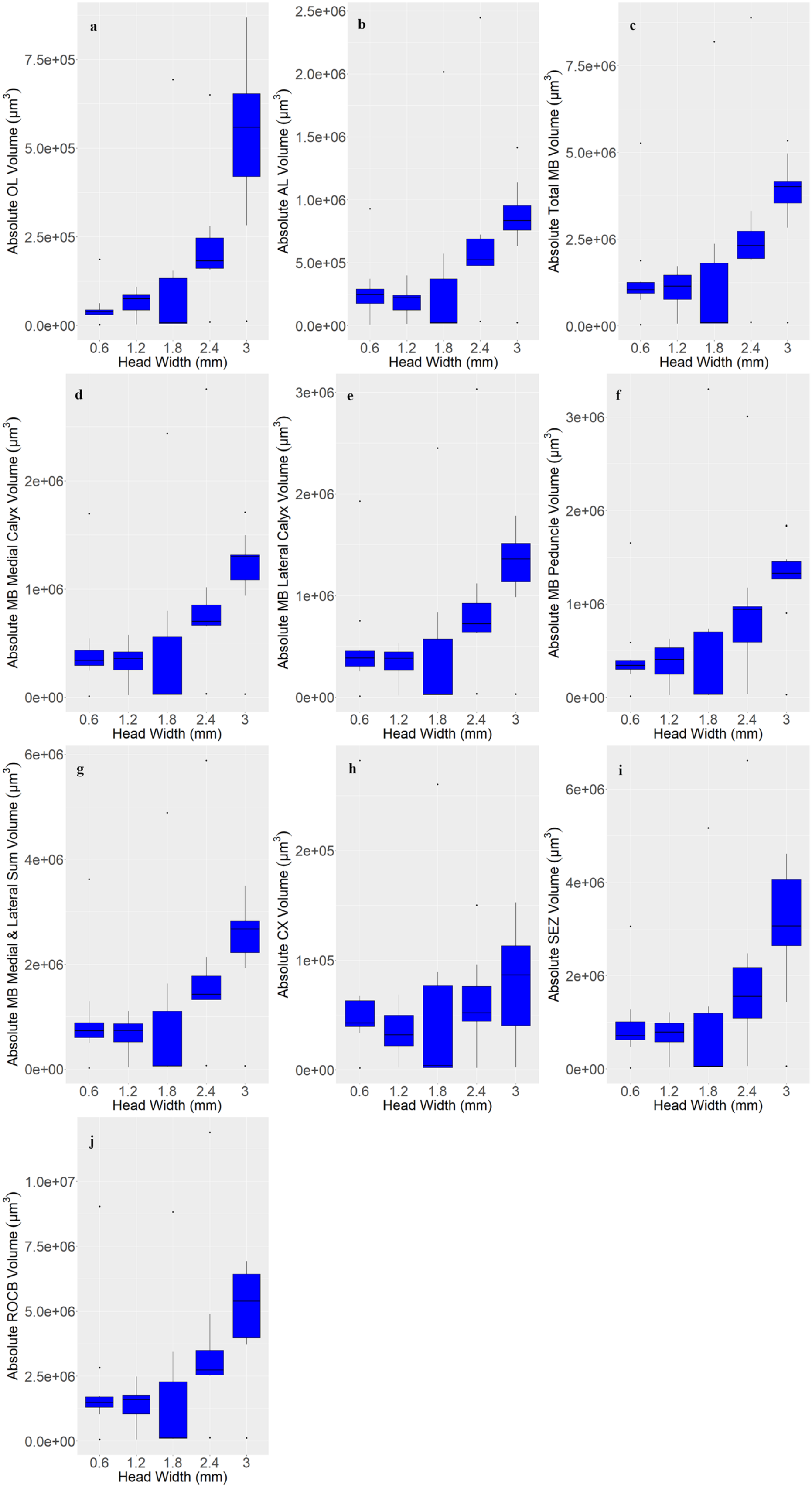
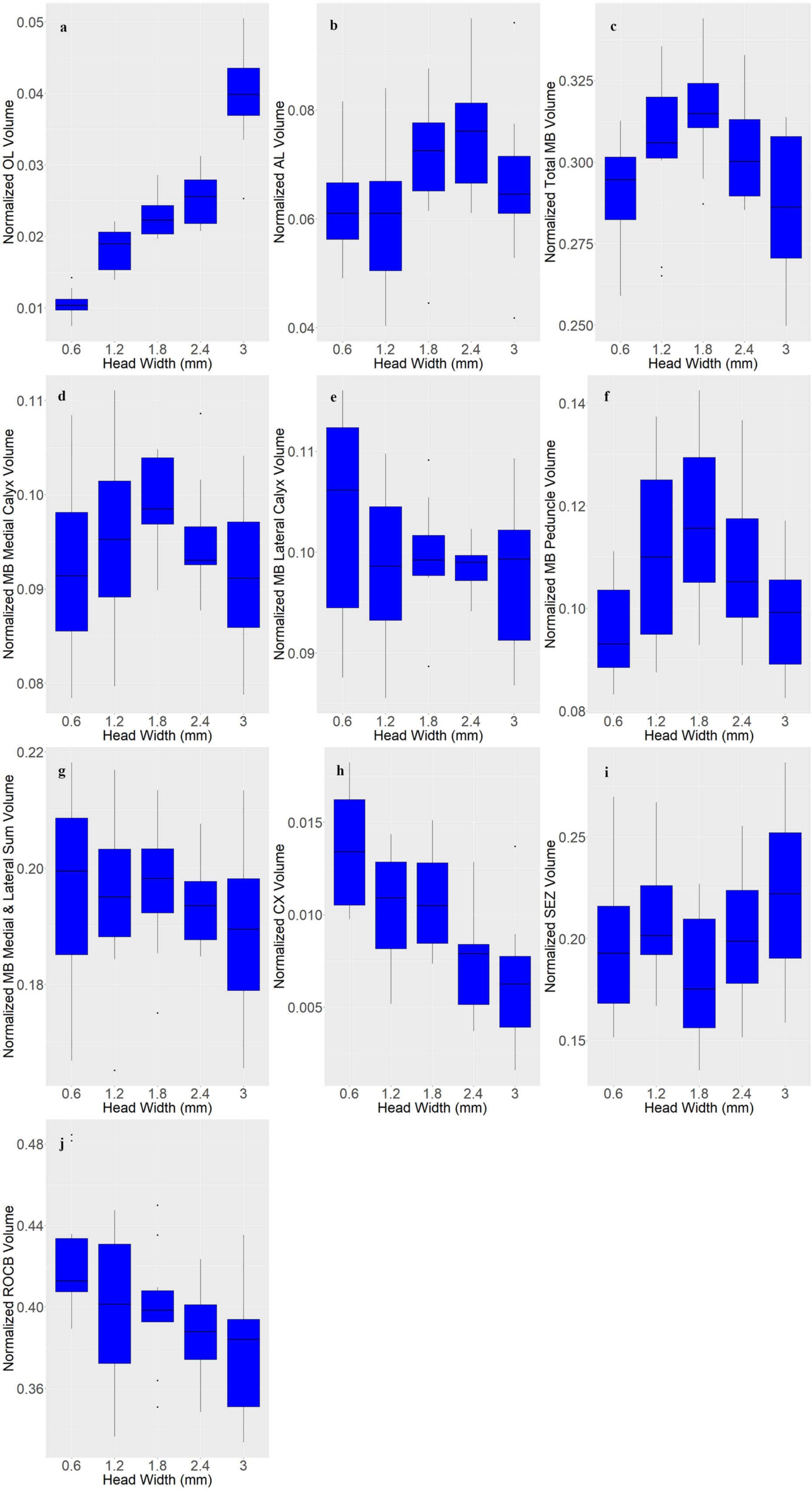


Fig. 6 Relative investment (compartment volume as a percent of total brain volume, indicated on *Y* axis) in the **a** OL ($p \leq 2.200e{-}16$); **b** AL ($p = 0.020$); **c** total MB ($p = 0.007$); **d** MB medial calyces ($p = 0.168$); **e** MB lateral calyces ($p = 0.345$); **f** MB medial and lateral calyces sum; **g** MB peduncle ($p = 0.005$); **h** CX ($p = 6.324e{-}06$); **i** SEZ ($p = 0.104$); and **j** ROCB ($p = 0.012$) across worker size groups



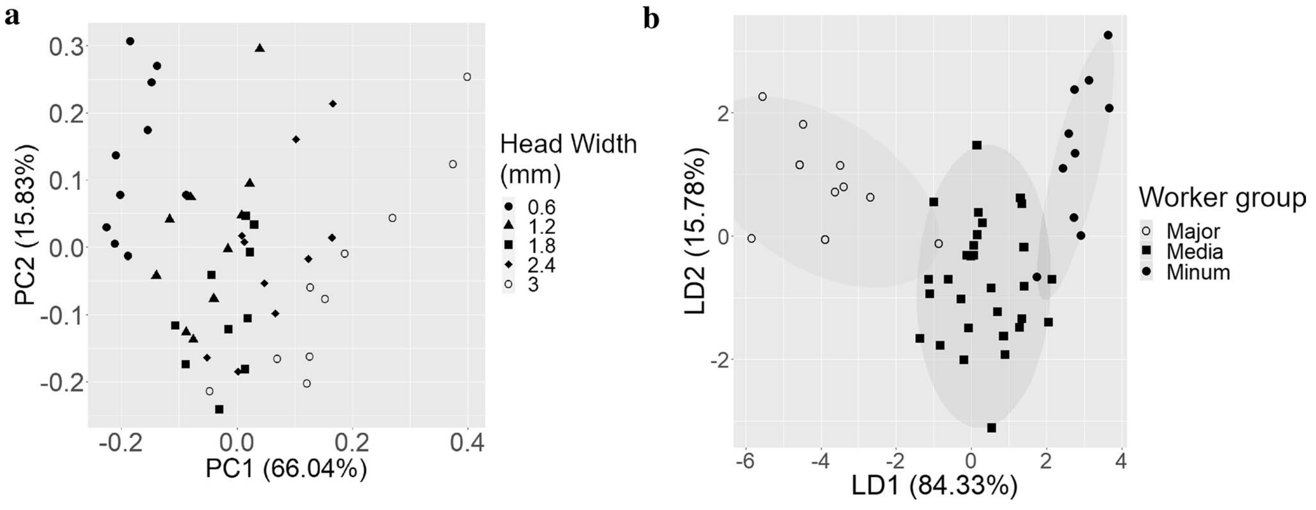


Fig. 7 **a** Principal component analysis plot of all log-transformed compartmental volumes normalized to total brain volume. **b** Linear discriminant analysis of samples according to worker size group (minims: 0.5–0.7 mm, medias: 1.1–2.5 mm, majors: 3 mm+) based on compartmental volumes (excluding the ROCB, which was colinear with other variables) normalized to total brain size. Classification accuracy = 95.8%

amount of tissue involved in maintaining baseline neural functions across workers that vary in body size and metabolic expenses (Kamhi et al. 2016; Packard 2020), or other size-related constraints (Finlay and Darlington 1995; Herculano-Houzel 2012; Herculano-Houzel et al. 2014; O'Donnell et al. 2018b).

Differentiation of worker neural phenotypes

Principal component and linear discriminant analysis distinguished individual *A. cephalotes* brains from different worker groups on the basis of compartment volumes. LDA demonstrated that the degree to which brains can accurately be classified in terms of worker body size is greater when all media size classes (1.2, 1.8, and 2.4 mm) are pooled, but is nevertheless able to distinguish samples belonging to five different groups. These results suggest that medias are readily distinguished in terms of neuroanatomy from the largest and smallest worker specialists in relation to task performance and although differentiation among size groups of medias is less substantial, it is consistent with our scores (Fig. 2). Similarly, we identified relatively few significant differences in the proportional volume of any brain compartment among different media size groups in contrast to the differences identified in medias in comparison to minims and medias in comparison to majors. Such a lack of distinction in the boundaries between media groups could reflect evolutionary origins of worker differentiation in *A. cephalotes*, in terms of how different groups "split off" (Rajakumar et al. 2012) or may reflect constraint from the task-diverse behavioral profile of most media workers (see Bernays and Funk 1999; Farris and Roberts 2005).

Absolute total brain volume

Interestingly, although total brain volume differed between majors in comparison to minims and medias, we found no significant change in total brain size in 0.6 to 2.4 mm workers despite a fourfold increase in body size. Such a pattern could theoretically result from limits on miniaturization of total brain size in smaller workers (Groh et al. 2014), making them undifferentiated with respect to total absolute brain volume from medias. Alternatively, this could result from either differential demands affecting the entire brain that have an inflection point at majors or from broader constraints/influences from specific developmental trajectories governing worker size (Trible and Kronauer 2017; O'Donnell et al. 2018b).

Antennal lobes

Medias were predicted to have greater sensory integration needs than other worker groups (Fig. 2) due to their large task repertoire and collaterally diverse behavioral challenges involved in selecting and harvesting plant material and navigating to and from food sources (Wilson 1980b; Hubbell et al. 1983, 1984; Blanton and Ewel 1985; Howard 1987; Howard et al. 1988; Falibene et al. 2015; Arenas and Roces 2017). Media workers (specifically the 1.8 mm and 2.4 mm size groups) had greater AL proportional volume compared with minims and majors. A significant change in proportional AL size occurred in the transition from 1.2

to 2.4 mm medias. Increased input to the AL from antennal olfactory sensory neurons is associated with increased olfactory sensitivity (Acebes and Ferrús 2001; Kuebler et al. 2010). Consistent with their social roles, media worker ALs are enlarged, potentially to process more diverse olfactory information, similar to the linkage of AL enlargement and olfaction-based tasks in *Atta vollenweideri* (Kleineidam et al. 2005; Kelber et al. 2009a, b; Kuebler et al. 2010). Nonetheless, little of the variance in AL volume could be explained by our model, suggesting multiple factors may influence investment in primary sensory neuropils, even those potentially sensitive to diverse stimuli.

Mushroom bodies

Medias (1.8 mm) had significantly higher relative total MB volume compared to majors and their total MB volume tended to be larger than that of other workers. This trend was consistent for the MB peduncle, which was significantly enlarged in 1.8 mm medias relative to minims, and for the MB medial calyces, although differences in proportional MB medial calyces volume were not significant. The MB lateral calyces, in contrast, showed no proportional increase in either minims, medias, or majors. Further, when analyzed as a sum, the combined absolute volume of the MB medial calyces and MB lateral calyces did not significantly differ among worker size groups. The functions of the MB medial calyces and the MB lateral calyces may differ; differences in the volume of the medial calyx in a bumblebee species correlated to differences in experience (Riveros and Gronenberg 2010). The significant differences we found in total MB volume were thus primarily driven by the volume of the MB peduncle, a fibrous, bi-partite region containing Kenyon cell neurites and neurites from MB extrinsic neurons connecting to other brain compartments, embedded in the protocerebral lobe and innervated by MB extrinsic neurons connecting it to regions in the protocerebrum (Goll 1967; Rybak and Menzel 1993; Ito et al. 2014; Schürmann 2016). Our results suggest that the nature and diversity of the leaf-harvesting task repertoire of 1.8 mm medias contributes to selection for relatively larger MBs, specifically MB peduncle. Since the medial and lateral MB calyces process sensory inputs while the MB peduncle relays outputs from the MBs to other brain regions (Rybak and Menzel 1993; Schürmann 2016), the significant differences we observed in MB peduncle proportional volume, but not in MB calyx proportional volume, may reflect increased selection in medias for efficient higher-order processing in the MBs and signaling to other areas of the brain. Task experience correlates with MB size (Durst et al. 1994; Gronenberg et al. 1996; Fahrbach 2006) and large MBs may be associated with increased behavioral flexibility (Riveros Rivera and Gronenberg 2009; Riveros et al. 2012; O'Donnell et al. 2015). Specifically, MBs play an essential role in olfactory learning in insects (Connolly et al. 1996; Zars 2000; Komischke et al. 2005; Busto et al. 2010) and investing more in this compartment may allow medias to react with specificity to a wide array of learned chemical cues associated with the suitability of plant material for fungal growth. With respect to specialist or generalist beetles (Farris and Roberts 2005), the enlargement of the MBs is consistent with the idea that generalists—in this case, medias that have a larger task repertoire—are selected to elaborate neural tissue to fulfill their diverse tasks. *A. vollenweideri* workers show decreasing proportional volume in the MB calyces with increasing worker size (Groh et al. 2014). Our data indicate greater (non-significant) MB calyx investment in *A. cephalotes* medias, perhaps due to their generalist plant tissue harvesting that contrasts with the specialized grass harvesting of *A. vollenweideri*. However, MB circuitry, rather than volume, may be related to task specialization (Groh et al. 2012).

Additional compartmental allometries

We found that CX volume was proportionally largest in minims, and inversely related to worker size. Greater investment in the CX may represent circuitry to enable multisensory navigation within dark three-dimensional labyrinthal fungal comb chambers. Minims mainly perform fungal-gardening tasks that likely rely on non-visual navigational strategies, perhaps involving CX circuitry (Mamiya et al. 2018; Le Moël et al. 2019; Green et al. 2019; Shiozaki et al. 2020; Sun et al. 2020; Pisokas et al. 2020; Currier et al. 2020).

A. cephalotes worker OL proportional volume increases with worker size and is highest in majors, which have greater visual acuity (Arganda et al. 2020). In addition to enlarged OLs, majors also possess a greater number of ommatidia, the photoreceptive subunit of the compound eye, and have larger ommatidia compared to medias and minims, although ommatidia size relative to body size is largest in minims (Arganda et al. 2020). The number and size of major worker ommatidia relative to other worker groups is consistent with their increased proportional and absolute volume of OL tissue, enabling greater visual acuity. The repertoire of *Atta* majors appears limited to defense (Wilson 1980a; Powell and Clark 2004a), a task mainly taking place outside the nest that likely involves target detection.

Total brain volume

Total brain volume sharply increased in the largest two worker size groups. When scaled to body size, minims had the largest brains, consistent with Haller's rule (Rensch 1956), although these scaled differences were not significant, perhaps due to limits on neural circuit miniaturization

(Beutel et al. 2005; Chittka and Niven 2009; Seid et al. 2011; Niven and Farris 2012; Groh et al. 2014). However, Haller's rule does not apply to all hymenopteran species, including some parasitoid wasps smaller in size than *A. cephalotes* minims (van der Woude et al. 2013; van der Woude and Smid 2016; Groothuis and Smid 2017). *A. cephalotes* fungal gardening and nursing tasks may select for more neural tissue than involved in parasitoid behavior. Size-adjusted total brain volume showed very little correlation to estimates from our model reflecting task performance. Given the more significant correlation found in some brain compartments and coupling of scaling pattern of compartments to sensory processing, total brain volume may be a too general and imprecise metric to reflect behavioral differences (Chittka and Niven 2009; Muscedere and Traniello 2012; Muscedere et al. 2014; Logan et al. 2018) among *A. cephalotes* workers.

Conclusions

Our results provide insight into the associations between task specializations and sensorimotor and higher-order processes in brain evolution. Our estimates of the involvement of processes in task performance and the extent to which different worker groups perform these tasks allowed us to model the influence of behavioral repertoire on brain scaling. Selection on neuroanatomy in *A. cephalotes* polymorphic workers appears to support the social organization of agricultural division of labor. The relationship of our score to total MB and MB peduncle volumes suggests our method effectively characterizes the involvement of neuropils that integrate diverse stimuli and facilitate higher-order processing, but does not adequately explain variances in investment in specialized primary input neuropils. This is likely due to the emphasis our model places on behavioral diversity, and thus the integration and processing of multimodal stimuli, and differences in the frequency with which different worker groups perform tasks.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00359-021-01539-6.

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Author contributions IBM and JFAT designed the study. IBM drafted the manuscript. IBM, EMF, and JFAT developed and edited the manuscript. IBM and EMF prepared and imaged brains. EMF measured neuropils and IBM and EMF statistically analyzed volumetric data. JFAT secured funding.

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Availability of data and material All data used in this study are included as supplementary tables. Additionally, data are available via: https://doi.org/10.5061/dryad.wm37pvmnq.

Code availability Code used is available via: https://github.com/Imuratore-bio/ant-brains.

Declarations

Conflict of interest The authors have no conflicts of interest or competing interests to declare. Colonies were collected in Trinidad in compliance with the laws of Trinidad and Tobago and imported to USA in compliance with the conditions of permit USDA APHIS PPQ526P–12–04067. All ants were housed in conditions promoting colony health with minimal disturbance and varied foraging options. The USDA inspected and approved our containment facility. We collected sufficient samples for study while minimizing impact on colony populations. This study necessitated humanely killing sterile workers by cold anaesthetization to stain and image brains.

References

Acebes A, Ferrús A (2001) Increasing the number of synapses modifies olfactory perception in *Drosophila*. J Neurosci 21:6264–6273. https://doi.org/10.1523/JNEUROSCI.21-16-06264.2001

Adolphs R (2003) Cognitive neuroscience of human social behaviour. Nat Rev Neurosci 4:165–178. https://doi.org/10.1038/nrn1056

Amador-Vargas S, Gronenberg W, Wcislo WT, Mueller U (2015) Specialization and group size: brain and behavioural correlates of colony size in ants lacking morphological castes. Proc Biol Sci 282:20142502. https://doi.org/10.1098/rspb.2014.2502

Arenas A, Roces F (2016a) Gardeners and midden workers in leaf-cutting ants learn to avoid plants unsuitable for the fungus at their worksites. Anim Behav 115:167–174. https://doi.org/10.1016/j.anbehav.2016.03.016

Arenas A, Roces F (2016b) Learning through the waste: olfactory cues from the colony refuse influence plant preferences in foraging leaf-cutting ants. J Exp Biol 219:2490–2496. https://doi.org/10.1242/jeb.139568

Arenas A, Roces F (2017) Avoidance of plants unsuitable for the symbiotic fungus in leaf-cutting ants: Learning can take place entirely at the colony dump. PLoS ONE 12:e0171388. https://doi.org/10.1371/journal.pone.0171388

Arganda S, Hoadley AP, Razdan ES, Muratore IB, Traniello JFA (2020) The neuroplasticity of division of labor: worker polymorphism, compound eye structure and brain organization in the leafcutter ant *Atta cephalotes*. J Comp Physiol A. https://doi.org/10.1101/2020.03.04.975110

Armitage SAO, Fernández-Marín H, Boomsma JJ, Wcislo WT (2016) Slowing them down will make them lose: a role for attine ant crop fungus in defending pupae against infections? J Anim Ecol 85:1210–1221. https://doi.org/10.1111/1365-2656.12543

Banks AN, Srygley RB (2003) Orientation by magnetic field in leaf-cutter ants, *Atta colombica* (Hymenoptera: Formicidae).

Ethology 109:835–846. https://doi.org/10.1046/j.0179-1613.2003.00927.x

Bass M, Cherrett JM (1996) Leaf-cutting ants (Formicidae, Attini) prune their fungus to increase and direct its productivity. Funct Ecol 10:55–61. https://doi.org/10.2307/2390262

Bates D (2005) Fitting linear mixed models in R. R News 5:27–30

Bernays EA, Funk DJ (1999) Specialists make faster decisions than generalists: experiments with aphids. Proc R Soc Lond B 266:151–156. https://doi.org/10.1098/rspb.1999.0615

Beutel RG, Pohl H, Hünefeld F (2005) Strepsipteran brains and effects of miniaturization (Insecta). Arthropod Struct Dev 34:301–313. https://doi.org/10.1016/j.asd.2005.03.001

Blanton CM, Ewel JJ (1985) Leaf-cutting ant herbivory in successional and agricultural tropical ecosystems. Ecology 66:861–869. https://doi.org/10.2307/1940548

Bollazzi M, Roces F (2002) Thermal preference for fungus culturing and brood location by workers of the thatching grass-cutting ant *Acromyrmex heyeri*. Insect Soc 49:153–157. https://doi.org/10.1007/s00040-002-8295-x

Boogert NJ, Madden JR, Morand-Ferron J, Thornton A (2018) Measuring and understanding individual differences in cognition. Philos Trans R Soc b, Biol Sci 373:20170280. https://doi.org/10.1098/rstb.2017.0280

Buehlmann C, Aussel A, Graham P (2020) Dynamic multimodal interactions in navigating wood ants: what do path details tell us about cue integration? J Exp Biol. https://doi.org/10.1242/jeb.221036

Busto GU, Cervantes-Sandoval I, Davis RL (2010) Olfactory learning in *Drosophila*. Physiology 25:338–346. https://doi.org/10.1152/physiol.00026.2010

Chittka L, Niven J (2009) Are bigger brains better? Curr Biol 19:R995–R1008. https://doi.org/10.1016/j.cub.2009.08.023

Connolly JB, Roberts IJH, Armstrong JD, Kaiser K, Forte M, Tully T, O'Kane CJ (1996) Associative learning disrupted by impaired gs signaling in *Drosophila* mushroom bodies. Science 274:2104–2107. https://doi.org/10.1126/science.274.5295.2104

Currie CR, Stuart AE (2001) Weeding and grooming of pathogens in agriculture by ants. Proc Biol Sci 268:1033–1039. https://doi.org/10.1098/rspb.2001.1605

Currier TA, Matheson AM, Nagel KI (2020) Encoding and control of orientation to airflow by a set of *Drosophila* fan-shaped body neurons Calabrese, RL, editor. Elife 9:e61510. https://doi.org/10.7554/eLife.61510

DeCasien AR, Higham JP (2019) Primate mosaic brain evolution reflects selection on sensory and cognitive specialization. Nat Ecol Evol 3:1483–1493. https://doi.org/10.1038/s41559-019-0969-0

DeSilva JM, Traniello JFA, Claxton AG, Fannin LD (2021) When and why did human brains decrease in size? A new change-point analysis and insights from brain evolution in ants. Front Ecol Evol 9:712. https://doi.org/10.3389/fevo.2021.742639

Dunbar RIM (1998) The social brain hypothesis. Evol Anthropol 6:178–190. https://doi.org/10.1002/(sici)1520-6505(1998)6:5%3c178::aid-evan5%3e3.0.co;2-8

Dunbar RIM (2009) The social brain hypothesis and its implications for social evolution. Ann Hum Biol 36:562–572. https://doi.org/10.1080/03014460902960289

Dupuis EC, Harrison JF (2017) Trunk trail maintenance in leafcutter ants: caste involvement and effects of obstacle type and size on path clearing in *Atta cephalotes*. Insect Soc 64:189–196. https://doi.org/10.1007/s00040-016-0530-y

Durst C, Eichmüller S, Menzel R (1994) Development and experience lead to increased volume of subcompartments of the honeybee mushroom body. Behav Neural Biol 62:259–263. https://doi.org/10.1016/s0163-1047(05)80025-1

Evison SEF, Hart AG, Jackson DE (2008) Minor workers have a major role in the maintenance of leafcutter ant pheromone trails. Anim Behav 75:963–969. https://doi.org/10.1016/j.anbehav.2007.07.013

Fahrbach SE (2006) Structure of the mushroom bodies of the insect brain. Annu Rev Entomol 51:209–232. https://doi.org/10.1146/annurev.ento.51.110104.150954

Falibene A, Roces F, Rössler W (2015) Long-term avoidance memory formation is associated with a transient increase in mushroom body synaptic complexes in leaf-cutting ants. Front Behav Neurosci 9:84–84. https://doi.org/10.3389/fnbeh.2015.00084

Farias AP, da Camargo R, S, Andrade Sousa KK, Caldato N, Forti LC, (2020) Nest architecture and colony growth of *Atta bisphaerica* grass-cutting ants. Insects 11:741. https://doi.org/10.3390/insects11110741

Farris SM (2011) Are mushroom bodies cerebellum-like structures? Arthropod Struct Dev 40:368–379. https://doi.org/10.1016/j.asd.2011.02.004

Farris SM, Roberts NS (2005) Coevolution of generalist feeding ecologies and gyrencephalic mushroom bodies in insects. PNAS 102:17394–17399. https://doi.org/10.1073/pnas.0508430102

Feener DH, Moss KAG (1990) Defense against parasites by hitchhikers in leaf-cutting ants: a quantitative assessment. Behav Ecol Sociobiol 26:17–29. https://doi.org/10.1007/bf00174021

Feinerman O, Traniello JFA (2015) Social complexity, diet, and brain evolution: modeling the effects of colony size, worker size, brain size, and foraging behavior on colony fitness in ants. Behav Ecol Sociobiol 70:1063–1074. https://doi.org/10.1007/s00265-015-2035-5

Finlay B, Darlington R (1995) Linked regularities in the development and evolution of mammalian brains. Science 268:1578–1584. https://doi.org/10.1126/science.7777856

Fleischmann PN, Grob R, Rössler W (2020) Magnetoreception in hymenoptera: importance for navigation. Anim Cogn 23:1051–1061. https://doi.org/10.1007/s10071-020-01431-x

Franks NR, Sendova-Franks AB (1992) Brood sorting by ants: distributing the workload over the work-surface. Behav Ecol Sociobiol 30:109–123. https://doi.org/10.1007/BF00173947

Godfrey RK, Gronenberg W (2019) Brain evolution in social insects: advocating for the comparative approach. J Comp Physiol A 205:13–32. https://doi.org/10.1007/s00359-019-01315-7

Goes AC, Barcoto MO, Kooij PW, Bueno OC, Rodrigues A (2020) How do leaf-cutting ants recognize antagonistic microbes in their fungal crops? Front Ecol Evol. https://doi.org/10.3389/fevo.2020.00095

Goll W (1967) Strukturuntersuchungen am Gehirn von Formica. Z Morphol Okol Tiere 59:143–210

Gordon DG, Ilieş I, Traniello JFA (2017) Behavior, brain, and morphology in a complex insect society: trait integration and social evolution in the exceptionally polymorphic ant *Pheidole rhea*. Behav Ecol Sociobiol 71:166. https://doi.org/10.1007/s00265-017-2396-z

Green PWC, Kooij PW (2018) The role of chemical signalling in maintenance of the fungus garden by leaf-cutting ants. Chemoecology 28:101–107. https://doi.org/10.1007/s00049-018-0260-x

Green J, Vijayan V, Mussells Pires P, Adachi A, Maimon G (2019) A neural heading estimate is compared with an internal goal to guide oriented navigation. Nat Neurosci 22:1460–1468. https://doi.org/10.1038/s41593-019-0444-x

Groh C, Tautz J, Rössler W (2004) Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development. PNAS 101:4268–4273. https://doi.org/10.1073/pnas.0400773101

Groh C, Lu Z, Meinertzhagen IA, Rössler W (2012) Age-related plasticity in the synaptic ultrastructure of neurons in the mushroom body calyx of the adult honeybee *Apis mellifera*. J Comp Neurol 520:3509–3527. https://doi.org/10.1002/cne.23102

Groh C, Kelber C, Grübel K, Rössler W (2014) Density of mushroom body synaptic complexes limits intraspecies brain miniaturization in highly polymorphic leaf-cutting ant workers. Proc Biol Sci 281:20140432–20140432. https://doi.org/10.1098/rspb.2014.0432

Gronenberg W (1999) Modality-specific segregation of input to ant mushroom bodies. Brain Behav Evol 54:85–95. https://doi.org/10.1159/000006615

Gronenberg W (2001) Subdivisions of hymenopteran mushroom body calyces by their afferent supply. J Comp Neurol 435:474–489. https://doi.org/10.1002/cne.1045

Gronenberg W, Heeren S, Hölldobler B (1996) Age-dependent and task-related morphological changes in the brain and the mushroom bodies of the ant *Camponotus floridanus*. J Exp Biol 199:2011–2019

Groothuis J, Smid HM (2017) *Nasonia* parasitic wasps escape from Haller's rule by diphasic, partially isometric brain-body size scaling and selective neuropil adaptations. Brain Behav Evol 90:243–254. https://doi.org/10.1159/000480421

Hart AG, Ratnieks FLW (2001) Task partitioning, division of labour and nest compartmentalisation collectively isolate hazardous waste in the leafcutting ant *Atta cephalotes*. Behav Ecol Sociobiol 49:387–392. https://doi.org/10.1007/s002650000312

Herculano-Houzel S (2012) The remarkable, yet not extraordinary, human brain as a scaled-up primate brain and its associated cost. PNAS 109:10661–10668

Herculano-Houzel S, Manger PR, Kaas JH (2014) Brain scaling in mammalian evolution as a consequence of concerted and mosaic changes in numbers of neurons and average neuronal cell size. Front Neuroanat. https://doi.org/10.3389/fnana.2014.00077

Hölldobler B, Wilson EO (2010) The leafcutter ants: civilization by instinct. W. W. Norton & Company

Howard JJ (1987) Leafcutting ant diet selection: the role of nutrients, water, and secondary chemistry. Ecology 68:503–515. https://doi.org/10.2307/1938455

Howard JJ, Cazin J, Wiemer DF (1988) Toxicity of terpenoid deterrents to the leafcutting ant *Atta cephalotes* and its mutualistic fungus. J Chem Ecol 14:59–69. https://doi.org/10.1007/bf01022531

Hubbell SP, Wiemer DF, Adejare A (1983) An antifungal terpenoid defends a neotropical tree (Hymenaea) against attack by fungus-growing ants (*Atta*). Oecologia 60:321–327. https://doi.org/10.1007/bf00376846

Hubbell SP, Howard JJ, Wiemer DF (1984) Chemical leaf repellency to an attine ant: seasonal distribution among potential host plant species. Ecology 65:1067–1076. https://doi.org/10.2307/1938314

Ilieş I, Muscedere ML, Traniello JFA (2015) Neuroanatomical and morphological trait clusters in the ant genus *Pheidole*: evidence for modularity and integration in brain structure. Brain Behav Evol 85:63–76. https://doi.org/10.1159/000370100

Ito K, Shinomiya K, Ito M, Armstrong JD, Boyan G, Hartenstein V, Harzsch S, Heisenberg M, Homberg U, Jenett A, Keshishian H, Restifo LL, Rössler W, Simpson JH, Strausfeld NJ, Strauss R, Vosshall LB (2014) A systematic nomenclature for the insect brain. Neuron 81:755–765. https://doi.org/10.1016/j.neuron.2013.12.017

Jaffe K, Perez E (1989) Comparative study of brain morphology in ants. Brain Behav Evol 33:25–33. https://doi.org/10.1159/000115895

Kamhi JF, Gronenberg W, Robson SKA, Traniello JFA (2016) Social complexity influences brain investment and neural operation costs in ants. Proc Biol Sci 283:20161949. https://doi.org/10.1098/rspb.2016.1949

Kamhi JF, Ilieş I, Traniello JFA (2019) Social complexity and brain evolution: comparative analysis of modularity and integration in ant brain organization. Brain Behav Evol 93:4–18. https://doi.org/10.1159/000497267

Kamhi JF, Barron AB, Narendra A (2020) Vertical lobes of the mushroom bodies are essential for view-based navigation in australian *Myrmecia* ants. Curr Biol 30:3432-3437.e3. https://doi.org/10.1016/j.cub.2020.06.030

Kelber C, Rössler W, Kleineidam CJ (2009a) Phenotypic plasticity in number of glomeruli and sensory innervation of the antennal lobe in leaf-cutting ant workers (*A. vollenweideri*). Dev Neurobiol 70:222–234. https://doi.org/10.1002/dneu.20782

Kelber C, Rössler W, Roces F, Kleineidam CJ (2009b) The antennal lobes of fungus-growing ants (Attini): Neuroanatomical traits and evolutionary trends. BBE 73:273–284. https://doi.org/10.1159/000230672

Khadempour L (2018) Microbial mediation of herbivory in leaf-cutter ant fungus gardens. Ph.D., The University of Wisconsin-Madison: United States—Wisconsin

Khadempour L, Kyle JE, Webb-Robertson B-JM, Nicora CD, Smith FB, Smith RD, Lipton MS, Currie CR, Baker ES, Burnum-Johnson KE (2021) From plants to ants: fungal modification of leaf lipids for nutrition and communication in the leaf-cutter ant fungal garden ecosystem. mSystems. https://doi.org/10.1128/mSystems.01307-20

Khalife A, Keller RA, Billen J, Hita Garcia F, Economo EP, Peeters C (2018) Skeletomuscular adaptations of head and legs of *Melissotarsus* ants for tunnelling through living wood. Front Zool 15:30. https://doi.org/10.1186/s12983-018-0277-6

Kleineidam CJ, Obermayer M, Halbich W, Rössler W (2005) A macroglomerulus in the antennal lobe of leaf-cutting ant workers and its possible functional significance. Chem Senses 30:383–392. https://doi.org/10.1093/chemse/bji033

Komischke B, Sandoz J-C, Malun D, Giurfa M (2005) Partial unilateral lesions of the mushroom bodies affect olfactory learning in honeybees *Apis mellifera L*. Eur J Neurosci 21:477–485. https://doi.org/10.1111/j.1460-9568.2005.03879.x

Kooij PW, Pullens JWM, Boomsma JJ, Schiøtt M (2016) Ant mediated redistribution of a xyloglucanase enzyme in fungus gardens of *Acromyrmex echinatior*. BMC Microbiol 16:81. https://doi.org/10.1186/s12866-016-0697-4

Kuebler LS, Kelber C, Kleineidam CJ (2010) Distinct antennal lobe phenotypes in the leaf-cutting ant (*Atta vollenweideri*). J Comp Neurol 518:352–365. https://doi.org/10.1002/cne.22217

Kwaku KM, Gonick EA, Ostapovich EM, Weinberg IP (2020) The frequency of leaf transfer in *Atta cephalotes* along horizontal and vertical surfaces near the bases of trees. Insect Soc 67:481–486. https://doi.org/10.1007/s00040-020-00784-3

Le Moël F, Stone T, Lihoreau M, Wystrach A, Webb B (2019) The central complex as a potential substrate for vector based navigation. Front Psychol. https://doi.org/10.3389/fpsyg.2019.00690

Lenoir A, D'Ettorre P, Errard C, Hefetz A (2001) Chemical ecology and social parasitism in ants. Annu Rev Entomol 46:573–599. https://doi.org/10.1146/annurev.ento.46.1.573

Lenth R (2018) Package 'lsmeans.' Am Stat 34:216–221

Liaw A, Wiener M (2001) Classification and regression by randomForest. R News 2:18–22

Lihoreau M, Latty T, Chittka L (2012) An exploration of the social brain hypothesis in insects. Front Physiol 3:442–442. https://doi.org/10.3389/fphys.2012.00442

Lihoreau M, Dubois T, Gomez-Moracho T, Kraus S, Monchanin C, Pasquaretta C (2019) Putting the ecology back into insect cognition research. Adv in Insect Phys. https://doi.org/10.1016/bs.aiip.2019.08.002

Logan C, Avin S, Boogert N, Buskell A, Cross FR, Currie A, Jelbert S, Lukas D, Mares R, Navarrete AF, Shigeno S, Montgomery S

(2018) Beyond brain size: uncovering the neural correlates of behavioral and cognitive specialization. Comp Cogn Behav Rev. https://doi.org/10.17863/CAM.25916

Mamiya A, Gurung P, Tuthill JC (2018) Neural coding of leg proprioception in *Drosophila*. Neuron 100:636-650.e6. https://doi.org/10.1016/j.neuron.2018.09.009

Markl H (1965) Stridulation in leaf-cutting ants. Science 149:1392–1393. https://doi.org/10.1126/science.149.3690.1392

Moll K, Roces F, Federle W (2013) How load-carrying ants avoid falling over: mechanical stability during foraging in *Atta vollenweideri* grass-cutting ants. PLoS ONE 8:e52816. https://doi.org/10.1371/journal.pone.0052816

Mueller UG (2002) Ant versus fungus versus mutualism: ant-cultivar conflict and the deconstruction of the attine ant-fungus symbiosis. Am Nat 160(Suppl 4):S67-98. https://doi.org/10.1086/342084

Muratore IB, Traniello JFA (2020) Fungus-growing ants: models for the integrative analysis of cognition and brain evolution. Front Behav Neurosci 14:599234–599234. https://doi.org/10.3389/fnbeh.2020.599234

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–e31618. https://doi.org/10.1371/journal.pone.0031618

Muscedere ML, Gronenberg W, Moreau CS, Traniello JFA (2014) Investment in higher order central processing regions is not constrained by brain size in social insects. Proc Biol Sci 281:20140217. https://doi.org/10.1098/rspb.2014.0217

Nichols-Orians CM, Schultz JC (1989) Leaf toughness affects leaf harvesting by the leaf cutter ant, *Atta cephalotes* (l.) (Hymenoptera: Formicidae). Biotropica 21:80–83. https://doi.org/10.2307/2388446

Niven JE, Farris SM (2012) Miniaturization of nervous systems and neurons. Curr Biol 22:R323–R329. https://doi.org/10.1016/j.cub.2012.04.002

Norman VC, Butterfield T, Drijfhout F, Tasman K, Hughes WOH (2017) Alarm pheromone composition and behavioral activity in fungus-growing ants. J Chem Ecol 43:225–235. https://doi.org/10.1007/s10886-017-0821-4

O'Donnell S, Bulova SJ, DeLeon S, Khodak P, Miller S, Sulger E (2015) Distributed cognition and social brains: reductions in mushroom body investment accompanied the origins of sociality in wasps (Hymenoptera: Vespidae). Proc Biol Sci 282:20150791. https://doi.org/10.1098/rspb.2015.0791

O'Donnell S, Susan B, Barrett M, von Beeren C (2018a) Brain investment under colony-level selection: soldier specialization in *Eciton* army ants (Formicidae: Dorylinae). BMC Zoology 3:3. https://doi.org/10.1186/s40850-018-0028-3

O'Donnell S, Bulova Susan J, Barrett M, Fiocca K (2018b) Size constraints and sensory adaptations affect mosaic brain evolution in paper wasps (Vespidae: Epiponini). Biol J Linn Soc Lond 123:302–310. https://doi.org/10.1093/biolinnean/blx150

Orr MR (1992) Parasitic flies (Diptera: Phoridae) influence foraging rhythms and caste division of labor in the leaf-cutter ant, *Atta cephalotes* (Hymenoptera: Formicidae). Behav Ecol Sociobiol 30:395–402. https://doi.org/10.1007/BF00176174

Packard GC (2020) Rethinking the metabolic allometry of ants. Evol Ecol 34:149–161. https://doi.org/10.1007/s10682-020-10033-5

Pisokas I, Heinze S, Webb B (2020) The head direction circuit of two insect species. Elife 9:e53985. https://doi.org/10.7554/eLife.53985

Plath JA, Barron AB (2015) Current progress in understanding the functions of the insect central complex. Current Opin Insect 12:11–18. https://doi.org/10.1016/j.cois.2015.08.005

Poulsen M, Bot AN, Nielsen MG, Boomsma JJ (2002) Experimental evidence for the costs and hygienic significance of the antibiotic metapleural gland secretion in leaf-cutting ants. Behav Ecol Sociobiol 52:151–157. https://doi.org/10.1007/s00265-002-0489-8

Powell S, Clark E (2004a) Combat between large derived societies: a subterranean army ant established as a predator of mature leaf-cutting ant colonies. Insect Soc 4:342–351. https://doi.org/10.1007/s00040-004-0752-2

Powell S, Clark E (2004b) Combat between large derived societies: a subterranean army ant established as a predator of mature leaf-cutting ant colonies. Insect Soc 51:342–351. https://doi.org/10.1007/s00040-004-0752-2

Rajakumar R, Mauro DS, Dijkstra MB, Huang MH, Wheeler DE, Hiou-Tim F, Khila A, Cournoyea M, Abouheif E (2012) Ancestral developmental potential facilitates parallel evolution in ants. Science 335:79–82. https://doi.org/10.1126/science.1211451

Rensch B (1956) Increase of learning capability with increase of brain-size. Am Nat 90:81–95. https://doi.org/10.1086/281911

Reséndiz-Benhumea GM, Sangati E, Sangati F, Keshmiri S, Froese T (2021) Shrunken social brains? A minimal model of the role of social interaction in neural complexity. Front Neurorobot. https://doi.org/10.3389/fnbot.2021.634085

Riveros AJ, Gronenberg W (2010) Brain allometry and neural plasticity in the bumblebee *Bombus occidentalis*. Brain Behav Evol 75:138–148. https://doi.org/10.1159/000306506

Riveros AJ, Seid MA, Wcislo WT (2012) Evolution of brain size in class-based societies of fungus-growing ants (Attini). Anim Behav 83:1043–1049. https://doi.org/10.1016/j.anbehav.2012.01.032

Riveros Rivera AJ, Gronenberg W (2009) Social brains and behavior: past and present. In: Organization of insect societies. Organization of insect societies: from genome to sociocomplexity Harvard University, Cambridge, pp 377–401. doi: https://doi.org/10.1007/s00265-015-2035-5.

Roces F, Núñez Josué A (1995) Thermal sensitivity during brood care in workers of two *Camponotus* ant species: circadian variation and its ecological correlates. J Insect Physiol 41:659–669. https://doi.org/10.1016/0022-1910(95)00019-Q

Roces F, Tautz J, Hölldobler B (1993) Stridulation in leaf-cutting ants. Naturwissenschaften 80:521–524. https://doi.org/10.1007/BF01140810

Rodrigues A, Bacci M Jr, Mueller UG, Ortiz A, Pagnocca FC (2008) Microfungal "weeds" in the leafcutter ant symbiosis. Microb Ecol 56:604–614. https://doi.org/10.1007/s00248-008-9380-0

Römer D, Roces F (2014) Nest enlargement in leaf-cutting ants: relocated brood and fungus trigger the excavation of new chambers. PLoS ONE 9:e97872. https://doi.org/10.1371/journal.pone.0097872

Römer D, Bollazzi M, Roces F (2017) Carbon dioxide sensing in an obligate insect-fungus symbiosis: CO2 preferences of leaf-cutting ants to rear their mutualistic fungus. PLoS ONE 12:e0174597. https://doi.org/10.1371/journal.pone.0174597

Römer D, Bollazzi M, Roces F (2018) Carbon dioxide sensing in the social context: leaf-cutting ants prefer elevated CO2 levels to tend their brood. J Insect Physiol 108:40–47. https://doi.org/10.1016/j.jinsphys.2018.05.007

Ruchty M, Romani R, Kuebler LS, Ruschioni S, Roces F, Isidoro N, Kleineidam CJ (2009) The thermo-sensitive sensilla coeloconica of leaf-cutting ants (*Atta vollenweideri*). Arthropod Struct Dev 38:195–205. https://doi.org/10.1016/j.asd.2008.11.001

Ruchty M, Helmchen F, Wehner R, Kleineidam C (2010) Representation of thermal information in the antennal lobe of leaf-cutting ants. Front Behav Neurosci 4:174. https://doi.org/10.3389/fnbeh.2010.00174

Rybak J, Menzel R (1993) Anatomy of the mushroom bodies in the honey bee brain: the neuronal connections of the alpha-lobe. J Comp Neurol 334:444–465. https://doi.org/10.1002/cne.903340309

Rytter W, Shik JZ (2016) Liquid foraging behaviour in leafcutting ants: the lunchbox hypothesis. Anim Behav 117:179–186. https://doi.org/10.1016/j.anbehav.2016.04.022

Saverschek N, Roces F (2011) Foraging leafcutter ants: olfactory memory underlies delayed avoidance of plants unsuitable for the symbiotic fungus. Anim Behav 82:453–458. https://doi.org/10.1016/j.anbehav.2011.05.015

Sayol F, Collado MÁ, Garcia-Porta J, Seid MA, Gibbs J, Agorreta A, San Mauro D, Raemakers I, Sol D, Bartomeus I (2020) Feeding specialization and longer generation time are associated with relatively larger brains in bees. Proc Biol Sci 287:20200762–20200762. https://doi.org/10.1098/rspb.2020.0762

Schultner E, Pulliainen U (2020) Brood recognition and discrimination in ants. Insect Soc 67:11–34. https://doi.org/10.1007/s00040-019-00747-3

Schultz TR, Brady SG (2008) Major evolutionary transitions in ant agriculture. PNAS 105:5435–5440. https://doi.org/10.1073/pnas.0711024105

Schürmann F-W (2016) Fine structure of synaptic sites and circuits in mushroom bodies of insect brains. Arthropod Struct Dev 45:399–421. https://doi.org/10.1016/j.asd.2016.08.005

Segre PS, Taylor ED (2019) Large ants do not carry their fair share: maximal load-carrying performance of leaf-cutter ants (*Atta cephalotes*). J Exp Biol. https://doi.org/10.1242/jeb.199240

Seid MA, Castillo A, Wcislo WT (2011) The allometry of brain miniaturization in ants. Brain Behav Evol 77:5–13. https://doi.org/10.1159/000322530

Shiozaki HM, Ohta K, Kazama H (2020) A multi-regional network encoding heading and steering maneuvers in *Drosophila*. Neuron 106:126-141.e5. https://doi.org/10.1016/j.neuron.2020.01.009

Simons M, Tibbetts E (2019) Insects as models for studying the evolution of animal cognition. Curr Opin Insect 34:117–122. https://doi.org/10.1016/j.cois.2019.05.009

Smaers JB, Soligo C (2013) Brain reorganization, not relative brain size, primarily characterizes anthropoid brain evolution. Proc Biol Sci 280:20130269. https://doi.org/10.1098/rspb.2013.0269

Strausfeld NJ (2012) Arthropod brains: evolution, functional elegance, and historical significance. Harvard University Press, Cambridge

Sulger E, McAloon N, Bulova SJ, Sapp J, O'Donnell S (2014) Evidence for adaptive brain tissue reduction in obligate social parasites (*Polyergus mexicanus*) relative to their hosts (*Formica fusca*). Biol J Linn Soc 113:415–422. https://doi.org/10.1111/bij.12375

Sun X, Yue S, Mangan M (2020) A decentralised neural model explaining optimal integration of navigational strategies in insects. Elife. https://doi.org/10.7554/elife.54026.sa2

Thiele T, Kost C, Roces F, Wirth R (2014) Foraging leaf-cutting ants learn to reject *Vitis vinifera ssp. vinifera* plants that emit herbivore-induced volatiles. J Chem Ecol 40:617–620. https://doi.org/10.1007/s10886-014-0460-y

Tragust S, Ugelvig LV, Chapuisat M, Heinze J, Cremer S (2013) Pupal cocoons affect sanitary brood care and limit fungal infections in ant colonies. BMC Evol Biol 13:225. https://doi.org/10.1186/1471-2148-13-225

Travaglini R, Forti L, Camargo R (2015) Foraging behavior of leaf cutting ants: How do workers search for their food? Sociobiology 62:347–350. https://doi.org/10.13102/sociobiology.v62i3.714

Trible W, Kronauer DJC (2017) Caste development and evolution in ants: it's all about size. J Exp Biol 220:53–62. https://doi.org/10.1242/jeb.145292

van der Woude E, Smid HM (2016) How to escape from Haller's rule: Olfactory system complexity in small and large *Trichogramma evanescens* parasitic wasps. J Comp Neurol 524:1876–1891. https://doi.org/10.1002/cne.23927

van der Woude E, Smid HM, Chittka L, Huigens ME (2013) Breaking Haller's rule: brain-body size isometry in a minute parasitic wasp. Brain Behav Evol 81:86–92. https://doi.org/10.1159/000345945

Wehner R, Fukushi T, Isler K (2007) On being small: brain allometry in ants. Brain Behav Evol 69:220–228. https://doi.org/10.1159/000097057

Wilson EO (1980a) Caste and division of labor in leaf-cutter ants (Hymenoptera, Formicidae, *Atta*). 1. The overall pattern in *Atta sexdens*. Behav Ecol Sociobiol 7:143–156. https://doi.org/10.1007/BF00299520

Wilson EO (1980b) Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*) II. The ergonomic optimization of leaf cutting. Behav Ecol Sociobiol 7:157–165. https://doi.org/10.1007/bf00299521

Wilson EO (1983) Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: Atta). Behav Ecol Sociobiol 14:55–60. https://doi.org/10.1007/BF00366656

Zars T (2000) Behavioral functions of the insect mushroom bodies. Curr Opin Neurobiol 10:790–795. https://doi.org/10.1016/S0959-4388(00)00147-1