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Sex-specific and caste-specific brain adaptations related to spatial orientation in *Cataglyphis* ants

Robin Grob [ID] | **Niklas Heinig** | **Kornelia Grübel** | **Wolfgang Rössler** [ID] |
Pauline N. Fleischmann [ID]

Behavioral Physiology and Sociobiology (Zoology II), Biocentre, University of Würzburg, Würzburg, Germany

Correspondence
Robin Grob, Behavioral Physiology and Sociobiology (Zoology II), Biocentre, University of Würzburg, 97074 Würzburg, Germany.
Email: robin.grob@uni-wuerzburg.de

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Abstract

Cataglyphis desert ants are charismatic central place foragers. After long-ranging foraging trips, individual workers navigate back to their nest relying mostly on visual cues. The reproductive caste faces other orientation challenges, i.e. mate finding and colony foundation. Here we compare brain structures involved in spatial orientation of *Cataglyphis nodus* males, gynes, and foragers by quantifying relative neuropil volumes associated with two visual pathways, and numbers and volumes of antennal lobe (AL) olfactory glomeruli. Furthermore, we determined absolute numbers of synaptic complexes in visual and olfactory regions of the mushroom bodies (MB) and a major relay station of the sky-compass pathway to the central complex (CX). Both female castes possess enlarged brain centers for sensory integration, learning, and memory, reflected in voluminous MBs containing about twice the numbers of synaptic complexes compared with males. Overall, male brains are smaller compared with both female castes, but the relative volumes of the optic lobes and CX are enlarged indicating the importance of visual guidance during innate behaviors. Male ALs contain greatly enlarged glomeruli, presumably involved in sex-pheromone detection. Adaptations at both the neuropil and synaptic levels clearly reflect differences in sex-specific and caste-specific demands for sensory processing and behavioral plasticity underlying spatial orientation.

KEYWORDS
antennal lobe, central complex, learning and memory, mushroom bodies, optic lobes, polymorphism, synaptic plasticity

1 | INTRODUCTION

Cataglyphis desert ants are well adapted to the harsh environment of arid habitats where ground temperatures regularly reach up to 70°C. Unlike many other desert dwellers, *Cataglyphis* workers do not avoid the burning heat, but rather forage during the hottest hours of the day. To avoid staying out in the sun for too long, the ants show

impressive navigational capabilities (Wehner, 2020). For that reason, *Cataglyphis* foragers have been well-studied experimental models for the neuroethology of spatial orientation and navigation for several decades (Wehner, 2019). Desert ants use a wide range of navigational strategies to not get lost during their far-ranging foraging runs (Wehner, 2020). Their navigational repertoire includes path integration based on a step integrator for estimating distance (Wittlinger et al., 2006) combined with a celestial (Müller & Wehner, 1988) or magnetic (Fleischmann, Grob, et al., 2018) compass for determining

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direction. In addition, desert ants use visual landmarks (Buehlmann et al., 2012; Fleischmann et al., 2016; Steck et al., 2009), panoramic scenes (Fleischmann, Rössler, & Wehner, 2018) and learned routes (Collett, 2010) for finding their ways back home. Before the ants become foragers, they go through an age-related sequence of different behavioral stages (age-related polyethism). Female *Cataglyphis* workers start out as more or less motionless callows, then take care of the brood, act as food storage units, bring out waste and help to maintain the nest (Schmid-Hempel & Schmid-Hempel, 1984). After about 4 weeks of indoor tasks, workers leave the nest to calibrate their visual guidance systems (Grob et al., 2019). For that, they perform well-structured learning walks around their nest entrance while using the earth's magnetic field as a reference system (Fleischmann et al., 2020b; Zeil & Fleischmann, 2019). After up to 3 days of learning walks, the ants go out and forage for their colony (Schmid-Hempel & Schmid-Hempel, 1984). Consequently, workers have to be equipped with plastic brains that flexibly support visual-learning based behavioral tasks (Grob et al., 2017; Habenstein et al., 2020, 2021; Schmitt et al., 2016; Stieb et al., 2012).

While the worker caste only includes female ants, the reproductive caste comprises both sexes (Wilson & Hölldobler, 2005). In the genus *Cataglyphis*, the social structure and the ways of division of reproduction are diverse. *Cataglyphis* colonies may have single or multiple reproductive queens, which can be either singly or multiply mated. In other cases, workers may also be able to produce offspring (Boulay et al., 2017). *Cataglyphis* ants perform different mating (Leniaud et al., 2011) and dispersal strategies (Peeters & Aron, 2017). This diversity during mating and dispersal allowed *Cataglyphis* to populate a vast distributional range of arid habitats with many different species (Wehner, 2020). In any case, reproductive and worker castes face vastly different behavioral tasks characterized by specific orientation challenges. In contrast to some *Cataglyphis* species, both sexes of the reproductive caste in *C. nodus* are winged and fly out from their nest of origin. This could indicate formations of "mating swarm" (also called "male aggregation") mating behavior, where reproductive animals of several colonies meet at some distance from their natal nest to mate (Peeters & Aron, 2017). Afterward, mated queens found a new colony on their own. In contrast, workers are purely ambulatory and undergo an age-related polyethism from performing interior tasks to outdoor foraging. When males fly out of their natal nest, their primary goal is to find a virgin queen (gyne) and mate before they subsequently die (Wehner, 2020). Their behavioral repertoire comprises largely innate behaviors with little behavioral flexibility, a feature that applies to most ant species (Gronenberg, 2008). Gynes, on the other hand, aim at founding a new colony after mating, which requires advanced orientation abilities to be able to return to the newly founded nest. These task differences outlined above call for specific adaptations in sensory systems and high-order brain centers related to spatial orientation.

Both the interior–exterior transition and the period of learning walks at the beginning of the ants' foraging career are accompanied by plastic synaptic changes in neurocircuits of two visual pathways (Rössler, 2019; Wehner, 2020). The brain of *Cataglyphis* shows typical features or hymenopteran brains. Visual information from the

compound eyes is processed via the optic lobes (OL), and two distinct pathways project to two sensory integration centers — the central complex (CX) and the mushroom bodies (MB) (Grob et al., 2017, 2019; Habenstein et al., 2020; Rössler, 2019; Schmitt et al., 2016). Panoramic visual information is stored in the MBs, centers for learning and memory, while directional information from the sky-compass network is fed into the CX via the bulbs of the lateral complex (LX) (Grob et al., 2019; Rössler, 2019). The ants' antennae house sensory structures for mechanosensory and olfactory information. Olfactory information is relayed to the glomeruli, odor processing units in the antennal lobes (ALs) and then also transferred to the MBs (Habenstein et al., 2020). Mechanosensory information is processed in the antennal mechanosensory and motor center (AMMC) and several integration centers in posterior brain neuropils (Grob, Tritscher, et al., 2021; Habenstein et al., 2020; Stieb et al., 2011).

Brain neuropil volumes can serve as a first proxy to look at adaptations to specific behavioral and orientation needs (Gronenberg, 2008). We therefore compared relative volumes of major neuropils and their substructures in primary and secondary brain centers important for spatial orientation and navigation (AL, OL, CX, and MB) of males, gynes and workers from the Mediterranean desert ant *Cataglyphis nodus*. We extended the analyses to the level of neurocircuits by comparing absolute numbers of synaptic complexes in two visual pathways to the MBs and CX. We hypothesize that the different lifestyles of *Cataglyphis* castes and sexes are reflected in specific neuronal adaptations for spatial orientation — while the female brain needs to be equipped for flexible navigation tasks with large centers for learning and memory, male brains should be more narrowly tuned to innate behaviors for finding a mate.

2 | METHODS

2.1 | Animals

Winged males (♂) (Figure 1(a)) and gynes (♀, virgin queens, Figure 1(b)) of *Cataglyphis nodus* (Brullé, 1832) were collected while leaving the nest entrance of two colonies over the course of 1 day at Kotychi-Strofylia National Park, Lapas, Greece in the summer of 2019. Additionally, worker ants (☿, Figure 1(c)) that brought food back to the colony (foragers) were collected at Schinias National Park, Marathonas, Greece. Ants were kept one night in darkness prior to dissection following the same procedure as established in Grob et al. (2017).

2.2 | Antibody characterization

To determine the volume of neuropils in the *Cataglyphis* brain, a monoclonal antibody to synapsin (SYNORF1, mouse@synapsin; kindly provided by E. Buchner and C. Wegener, University of Würzburg, Germany) was used to visualize synapse-rich neuropils (Table 1). Synapsin is highly conserved among invertebrates and present in presynaptic terminals. The specificity of the antibody has been characterized previously for *Cataglyphis* ants (Grob, Tritscher, et al., 2021; Habenstein et al., 2020; Schmitt et al., 2016, 2017; Stieb et al., 2010, 2012).
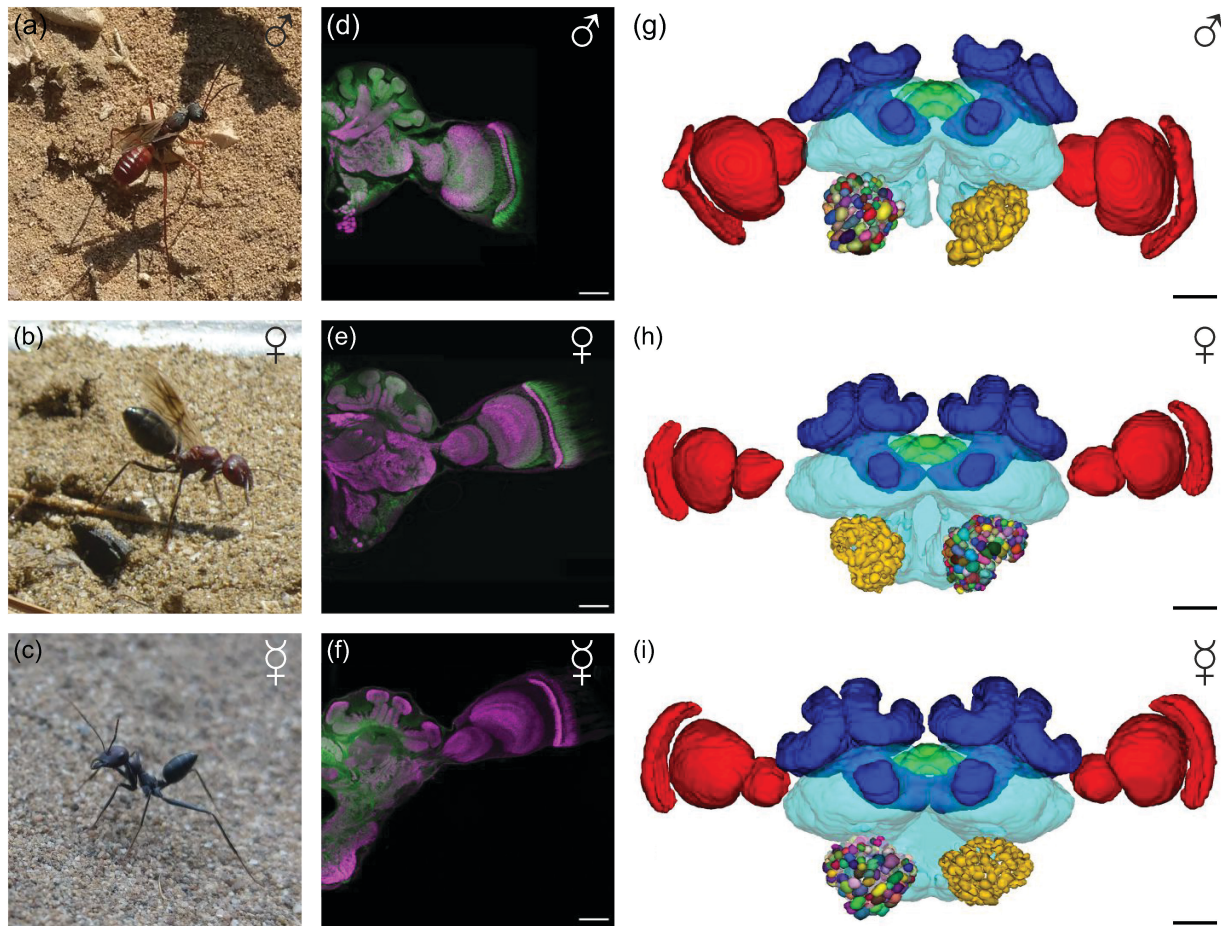
FIGURE 1 Phenotypes of males and the two female castes of *Cataglyphis nodus* and their brains. (a–c) The reproductive castes of *C. nodus* (a) males (♂) and (b) gynes (virgin queens, ♀) are winged and capable of flight, while (c) the infertile female workers do not have wings and walk (☿) [images taken from (Fleischmann et al., 2020a)]. (d–f) To compare the brains of (d) male, (e) gyne, and (f) worker ants, their brains were double labeled with anti-synapsin antibodies (magenta) and f-actin labeling with fluor-phalloidin (green) (only the left brain hemispheres are shown). (g–i) The 3D-reconstruction of the brains revealed significant differences between the brains of (g) male, (h) gyne, and (i) worker ants, specifically in the following neuropils of interest: Mushroom bodies (MB, blue), central complex (CX, green), optic lobes (OL, red), and antennal lobes (AL, yellow). The individual glomeruli of the AL of one side (side chosen pseudo-randomized) are shown multicolored. The protocerebral lobes and gnathal ganglia are shown in turquoise. Scale bars = 100 μm [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Antibody characterization

Antibody	Immunogen	Manufacturer; species; clonality; Cat #; RRID
Synapsin	Drosophila synapsin glutathione-S-transferase fusion protein	E. Buchner, Theodor-Boveri-Institute, University of Würzburg, Germany; mouse; monoclonal; Cat # 3C11 (SYNORF1); RRID: AB_528479

2.3 | Neuroanatomical procedures

Brains were double stained using a primary antibody to synapsin (SYNORF1, E. Buchner, University of Würzburg, Germany) and CF633 Phalloidin (00046, Biotium Inc., Fremont, California) using a previously established protocol (Habenstein et al., 2020).

For brain dissection, all experimental ants were anesthetized on ice. The ants were then decapitated and the brains were immediately dissected under cooled ringer solution (127 mM NaCl, 7 mM KCl, 1.5 mM CaCl₂, 0.8 mM Na₂HPO₄, 0.4 mM KH₂PO₄, 4.8 mM TES, and 3.2 mM trehalose, pH 7.0). The brains were transferred to a 64-well-plate and fixated in 4% formaldehyde phosphate-buffered saline (PBS) at 4°C overnight. Subsequently, the brains were rinsed in PBS, three times for 10 min each. To permeabilize cell membranes for antibody application on the whole-mount brains, the brains were rinsed once with 2% Triton-X 100 for 10 min and twice with 0.2% Triton-X 100 for 10 min. The brains were then incubated for 1 h at room temperature on a shaker in a 0.5% Triton-X 100 solution in PBS with 2% of Normal Goat Serum (NGS, Jackson ImmunoResearch Laboratories), to block unspecific binding sites. Then they were incubated on a shaker using a primary antibody to synapsin from mouse 1:50 in PBS with 0.5% Triton-X 100 and 2% NGS for 5 days in a fridge at 4°C. After incubation, the brains were rinsed three times in PBS for 20 min.

Subsequently, the brains were incubated in a secondary antibody coupled to AlexaFluor 568 (A12380, Molecular Probes, Eugene, Oregon) dye (1:250), and CF633 Phalloidin (2.5 μL Phalloidin from Methanol stock solution in 500 μL PBS) in PBS with 0.5% Triton-X 100 and 1% NGS for 3 days on a shaker at 4°C. The brains were rinsed in PBS, four times for 20 min each, and post-fixated in 4% formaldehyde in PBS overnight at 4°C. Afterward, they were washed four times in PBS for 20 min each and subsequently dehydrated in an ascending series of ethanol in water dilution (30%, 50%,70%, 90%, and 95% ethanol in water for 3–4 min each, and two times in 100% ethanol for 5 min). The brains were finally cleared in methyl salicylate (4529.1, Carl Roth Gmbh & Co. Kg, Karlsruhe, Germany).

2.4 | Data analyses

2.4.1 | Neuroanatomical analyses

A confocal laser-scanning microscope (Leica TCS SP8, Leica Microsystems GmbH, Wetzlar, Germany) was used for scanning the brains as image stacks at a step size of 5 μm. We used an APO 20×/0.7 IMM water immersion objective for overviews with 0.75 digital zoom for whole-brain scans, with 1.6 zoom for closeups of the CX, with 2.0 zoom for the MB calyx, and with 4.0 zoom for detailed scans of the LX. The PL APO 63×/1.2 W objective with 2.0 digital zoom was used for high-resolution scans in the lip (Li) and collar (Co) of the MB calyx.

The major neuropils and their subunits were easily distinguishable in anti-synapsin labeled whole mount brains (Figure 1(d–f)). Volumes of brain neuropils of interest were analyzed using the 3D-reconstruction software TrakEM2 (Cardona et al., 2012) plugin for ImageJ 1.52n (Wayne Rasband, National Institutes of Health, The United States).

To compare numbers of synaptic complexes between sexes and castes, microglomeruli (MG) were quantified with Amira (Amira-Avizo Software 2019.1, Thermo Fisher Scientific Inc., Waltham, Massachusetts) in visual and olfactory subregions of the MB calyx (Co, Li) using the AMIRA tracking tool following the protocol by Groh et al. (2012) [for further details see Rössler et al., 2017]. MG densities were analyzed by averaging multiple sample volumes, in the Li (two) and the Co (three) as numbers of MG per 1000 μm³ following the procedure introduced by Groh et al. (2014). The total numbers of MG per neuropil were estimated by multiplying the densities with the volume of Li, and Co respectively. This represents a good approximation, as in *C. nodus* MG densities are largely homogeneous throughout the Li and Co neuropils (Grob et al., 2017; Stieb et al., 2010).

In the LX bulbs, numbers of synaptic complexes in one brain hemisphere were counted using the TrakEM2 (Cardona et al., 2012) plugin for ImageJ 1.52n (Wayne Rasband, National Institutes of Health, The United States) to trace individual synaptic complexes.

2.4.2 | Statistical analyses

For statistic comparison, we used a non-parametric Kruskal–Wallis-test ($\alpha = 0.05$), and post hoc Mann–Whitney U-test with Bonferroni correction ($\alpha = 0.05$, after Bonferroni correction: $\alpha_{corrected} = 0.0167$). Since the absolute brain volumes differed significantly between the sexes and castes (♂, ♀, and ☿), the proportion of each neuropil (OLs, MBs, CX, and ALs) was used to compare relative neuropil volumes. The volume of each neuropil was divided by the total volume of the brain (method adapted from Nishikawa et al., 2008). In the case of synaptic complexes, we compared absolute numbers in the MB Li, Co, and LX. Absolute numbers of AL glomeruli were also compared statistically. Since the total numbers of glomeruli and AL sizes differed significantly between sexes and castes, relative measurements of glomerular volumes were used to compare AL glomeruli. For each AL, the median of glomerular volumes was calculated. By dividing the glomerular volume by the median, the relative volume of each glomerulus was calculated [method adapted from Stieb et al., 2011]. The relative volumes show how much larger the volume of a glomerulus is compared with the median size of all glomeruli. Outliers were defined as glomeruli with a higher value than 1.5 times the interquartile range of the 25th and 75th percentiles of the ALs. All statistical analyses were done with MATLAB (2015a or 2018a, The MathWorks Inc., Natick, Massachusetts).

2.5 | Nomenclature

We refer to Habenstein et al. (2020) (see also https://www.insectbraindb.org for 3D data of the *Cataglyphis* brain) and Ito et al. (2014) for the nomenclature of neuropils in the ant brain. For terms related to spatial orientation and navigation, we refer to Grob, el Jundi, and Fleischmann (2021).

3 | RESULTS

While the different sexes and castes in *C. nodus* have vastly different tasks and locomotory demands (flying, ambulatory), we found that the overall structure of their brains is remarkably similar (Figure 1(d–i)). The total brain volumes, including OLs, ALs, MBs, CX, protocerebral lobes, and gnathal ganglia, differ markedly between the sexes. Male *C. nodus* (median ± median absolute deviation [MAD]: $(58.0 ± 5.51) × 10^6$ μm³) have a significantly smaller brain volume compared with females, both gynes (median ± MAD: $(85.2 ± 0.91) × 10^6$ μm³) and workers (median ± MAD: $(78.8 ± 6.19) × 10^6$ μm³) (Kruskal–Wallis test: Total Brain Volume: $\chi^2 = 8.97$; $n = 14$; $p = .0113$; Mann–Whitney U-test with Bonferroni correction [$n_♂ = 4$; $n_♀ = 5$; $n_☿ = 5$]: ♂ vs. ♀ $p = .0159$; ♂ vs. ☿ $p = .0159$; ♀ vs. ☿ $p = .222$; Figure 2). The total brain size of workers and males scatter more than the brain volumes of gynes.

3.1 | Relative volumes of neuropils

Since the total brain volume differed significantly between the sexes and there might be size-dependent differences within the groups, subsequent analyses used relative neuropil volumes to compare between

sexes and castes (for absolute volumes of the neuropils and their variation within and across groups, see Table 2). Despite the small sample sizes ($n_\male = 4$; $n_\female = 5$; and $n_\worker = 5$), the results show significant differences between sexes and castes.

The OLs of males are relatively larger compared with those in both female castes (median ± MAD: $\male = (26.78 ± 2.31)\%$; $\female = (16.15 ± 0.70)\%$; $\worker = (14.27 ± 2.07)\%$; Kruskal–Wallis test: relative volume OLs: $\chi^2 = 8.97$; $n = 14$; $p = .0113$; Mann–Whitney U-test with
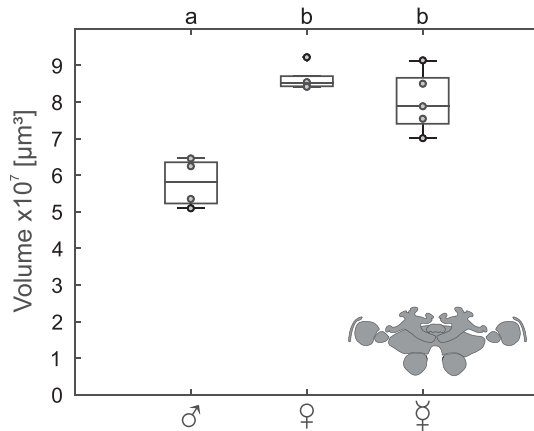

FIGURE 2 Total brain volume of male ($\male$, $n = 4$), gyne ($\female$, $n = 5$), and worker ($\worker$, $n = 5$) *Cataglyphis nodus* ants. The central line of each boxplot depicts the median of the data. The upper and lower limits of the boxes show the 25th and 75th percentiles, while the whiskers extend to the extreme data points without outliers. Additionally, all data points are plotted as gray circles. Significant differences between the groups are marked with lower case letters (Kruskal–Wallis test and post hoc Mann–Whitney U-test with Bonferroni correction (after correction $p < .0167$)). Plotted neuropiles are filled in the brain diagram. For statistical details, see text

Bonferroni correction ($n_\male = 4$; $n_\female = 5$; $n_\worker = 5$): $\male$ vs. $\female$; $p = .0159$; $\male$ vs. $\worker$ $p = .0159$; $\female$ vs. $\worker$ $p = .222$; Figure 3(a)).

The MB volumes are smallest in males and largest in female workers (median ± MAD: $\male = (21.08 ± 0.74)\%$; $\female = (25.46 ± 0.35)\%$; $\worker = (33.64 ± 0.59)\%$; Kruskal–Wallis test: relative volume MBs: $\chi^2 = 11.57$; $n = 14$; $p = .00307$; Mann–Whitney U-test with Bonferroni correction ($n_\male = 4$; $n_\female = 5$; $n_\worker = 5$): $\male$ vs. $\female$ $p = .0159$; $\male$ vs. $\worker$ $p = .0159$; $\female$ vs. $\worker$ $p = .00794$; Figure 3(b)).

While the relative volumes of the CX do not differ between the female castes, the relative CX volumes are significantly larger in males (median ± MAD: $\male = (1.25 ± 0.07)\%$; $\female = (1.06 ± 0.00)\%$; $\worker = (1.09 ± 0.00)\%$; Kruskal–Wallis test: relative volume CX: $\chi^2 = 8.46$; $n = 14$; $p = .0145$; Mann–Whitney U-test with Bonferroni correction ($n_\male = 4$; $n_\female = 5$; $n_\worker = 5$): $\male$ vs. $\female$ $p = .0159$; $\male$ vs. $\worker$ $p = .0159$; $\female$ vs. $\worker$ $p = .421$; Figure 3(c)).

The AL relative volumes were found largest in males, while the smallest relative volumes were measured in workers (median ± MAD: $\male = (5.67 ± 0.71)\%$; $\female = (3.72 ± 0.14)\%$; $\worker = (2.28 ± 0.15)\%$; Kruskal–Wallis test: Relative Volume ALs: $\chi^2 = 11.57$; $n = 14$; $p = .00307$; Mann–Whitney U-test with Bonferroni correction ($n_\male = 4$; $n_\female = 5$; $n_\worker = 5$): $\male$ vs. $\female$ $p = .0159$; $\male$ vs. $\worker$ $p = .0159$; $\female$ vs. $\worker$ $p = .00794$; Figure 3(d)).

3.2 | Organization of antennal lobes

The overall AL structure characterized by its glomerular organization was found similar across sexes and castes (Figure 4), but the number of glomeruli in the AL of males (median ± MAD: 198 ± 2) is significantly lower compared with both female castes (median ± MAD: $\female$: 245 ± 0; $\worker$: 250 ± 1) (Kruskal–Wallis test: number of glomeruli in the AL: $\chi^2 = 9.85$; $n = 14$; $p = .00730$; Mann–Whitney U-test with Bonferroni correction ($n_\male = 4$; $n_\female = 5$; $n_\worker = 5$): $\male$ vs. $\female$ $p = .0159$; $\male$ vs.

TABLE 2 Absolute volumes of the neuropils in μm^3

Absolute volumes in μm^3	Total brain	Optic lobes	Mushroom bodies (MBs)	MB collar	MB lip	Central complex	Antennal lobes
Male 1	64,623,296	16,408,479	10,840,396	1,901,354	5,339,490	793,542	4,039,227
Male 2	62,431,130	19,484,811	13,089,777	2,254,900	6,430,775	789,091	3,178,627
Male 3	50,949,617	12,002,449	11,441,079	1,785,094	5,991,816	726,655	3,916,246
Male 4	53,598,907	15,102,832	11,354,829	2,075,398	5,512,066	607,677	2,588,787
Gyne 1	85,241,652	15,104,644	21,704,108	3,359,623	10,722,992	799,604	3,283,202
Gyne 2	85,349,570	14,541,797	23,604,343	4,019,076	12,297,651	904,033	3,156,365
Gyne 3	84,331,499	13,079,062	21,754,022	3,596,508	11,207,233	901,657	2,778,856
Gyne 4	92,185,437	14,241,097	23,145,185	3,377,755	12,017,572	1,009,589	3,425,313
Gyne 5	84,183,384	13,596,244	19,639,564	3,247,239	9,869,060	895,655	3,395,878
Worker 1	78,834,648	9,620,570	26,518,635	4,879,379	13,583,573	792,951	1,545,798
Worker 2	91,317,985	15,122,095[a]	31,715,744	4,804,828	15,917,356	999,193	2,118,880
Worker 3	85,027,529	12,132,646	27,323,110	4,513,799	13,389,884	925,384	2,065,849
Worker 4	75,324,271	12,401,030	25,714,441	4,352,230	13,398,593	812,843	1,716,782
Worker 5	70,207,013	9,466,550	23,196,240	3,990,656	11,745,636	768,341	1,193,527

[a]One optic lobes was missing in this preserved brain. Thus, the remaining optic lobe was doubled for the total volume.
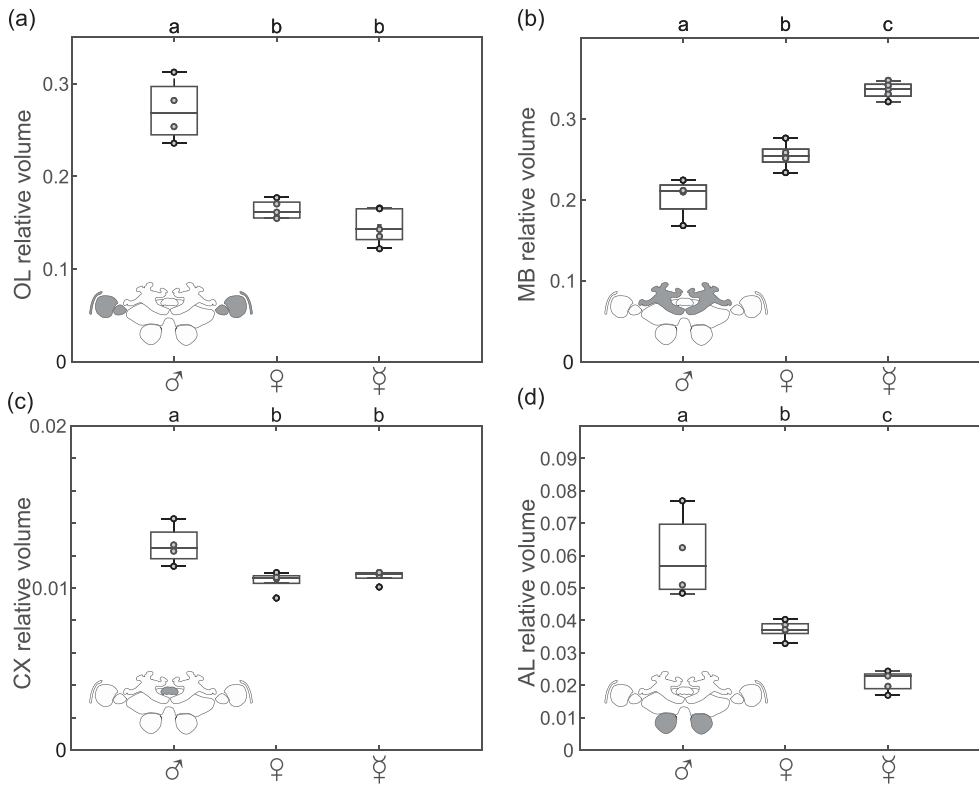
FIGURE 3 Relative volumes of neuropils of interest. (a) Optic lobes (OL), (b) mushroom bodies (MB), (c) central complex (CX), and (d) antennal lobes (AL) in male ($\male$, $n = 4$), gyne ($\female$, $n = 5$), and worker ($\worker$, $n = 5$) ants. Plotted neuropiles are gray in the brain diagram. For figure conventions, see Figure 2, and for statistical details, see text
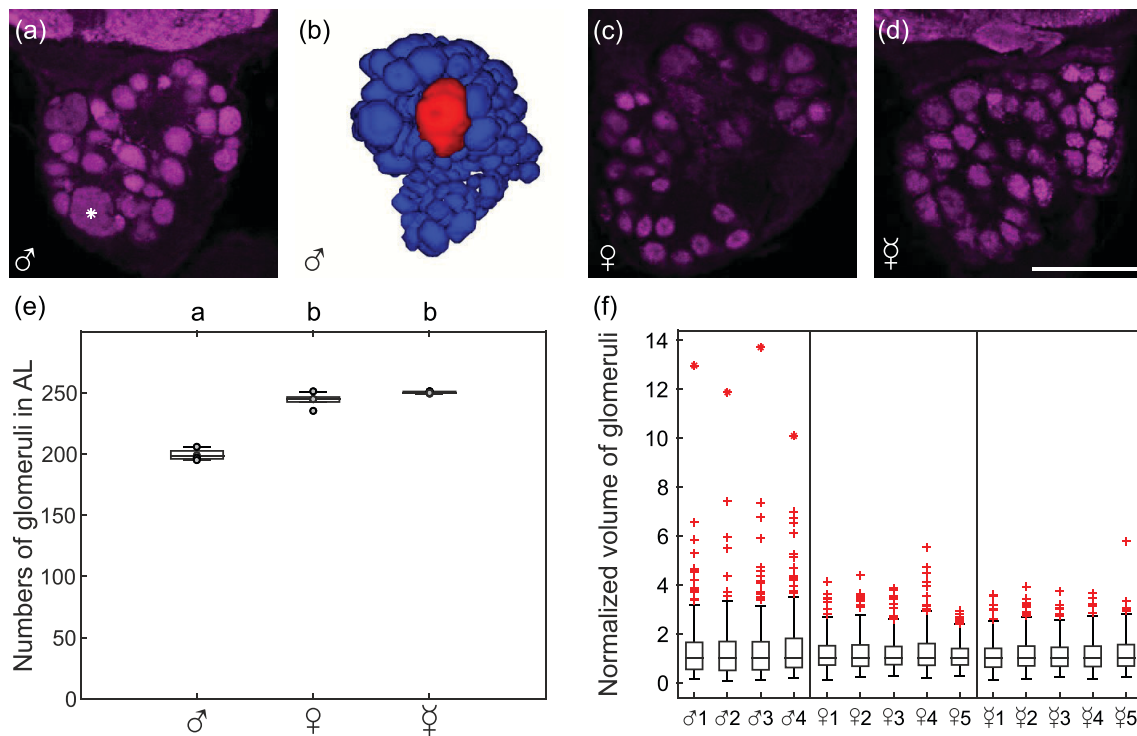

FIGURE 4 Numbers and relative sizes of antennal lobe (AL) glomeruli in male ($\male$, $n = 4$), gyne ($\female$, $n = 5$), and worker ($\worker$, $n = 5$) ants. (a,c,d) Labeling with anti-synapsin antibody (magenta) of the AL glomeruli of (a) males, (c) gynes, and (d) workers. Highly enlarged macroglomerulus is marked with an asterisk (view from frontal). (b) The highly enlarged macroglomerulus is highlighted (red) in the 3D reconstruction of the male AL glomeruli (blue) (view from dorsal). (e) Numbers of enlarged glomeruli in the ALs. (f) Normalized volumes of the glomeruli. Outliers are shown as red crosses, with significant outliers highlighted as an asterisk. For figure conventions, see Figure 2, and for statistical details, see text [Color figure can be viewed at wileyonlinelibrary.com]

☿ $p = .0159$; ♀ vs. ☿ $p = .0952$; Figure 4(e)). Only the ALs of males contain highly enlarged glomeruli (so called macroglomeruli) exceeding more than 10 times the median size of the glomeruli in the ALs (Figure 4(f)).

3.3 | Synaptic complexes in the mushroom bodies and lateral complex

Comparison of the absolute numbers of synaptic complexes (microglomeruli, MG) in the MB calyx revealed significant differences between the sexes and castes (Table 3). The estimated total numbers of MG in the MB Co are significantly lower in males (median ± MAD: 195,542 ± 18,107) than in female *C. nodus* (median ± MAD: ♀: 397,614 ± 23,272; ☿: 394,864 ± 34,211) (Kruskal–Wallis test: Number of MG in the MB Co: $\chi^2 = 8.72$; $n = 17$; $p = 0.0128$; Mann–Whitney U-test with Bonferroni correction ($n_{\male} = 4$; $n_{\female} = 5$; $n_{☿} = 8$): ♂ vs. ♀ $p = .0159$; ♂ vs. ☿ $p = .0159$; ♀ vs. ☿ $p = .833$; Figure 5(a)). The density of Co MG is significantly higher in gynes compared with workers, but we found no statistical difference between males and both female castes (Kruskal–Wallis test: density of MG in the Co: $\chi^2 = 9.63$; $n = 17$; $p = .00812$; Mann–Whitney U-test with Bonferroni correction ($n_{\male} = 4$; $n_{\female} = 5$; $n_{☿} = 8$): ♂ vs. ♀ $p = .0635$; ♂ vs. ☿ $p = .299$; ♀ vs. ☿ $p = .00155$; Figure 5(b)).

In the MB Li the pattern is similar to the one found in the Co. Males have significantly smaller numbers of MG in the Li than both female castes (median ± MAD: ♂: 282,629 ± 15,316; ♀: 751,819 ± 44,536; ☿: 695,431 ± 81,293) (Kruskal–Wallis test: number of MG in the Li: $\chi^2 = 9.29$; $n = 17$; $p = .00960$; Mann–

Whitney U-test with Bonferroni correction ($n_{\male} = 4$; $n_{\female} = 5$; $n_{☿} = 8$): ♂ vs. ♀ $p = .0159$; ♂ vs. ☿ $p = .00404$; ♀ vs. ☿ $p = .354$; Figure 5(c)). MG are packed more densely in the Li of gynes compared with both males and workers (Kruskal–Wallis test: density of MG in the MB Li: $\chi^2 = 10.14$; $n = 17$; $p = .00627$; Mann–Whitney U-test with Bonferroni correction ($n_{\male} = 4$; $n_{\female} = 5$; $n_{☿} = 8$): ♂ vs. ♀ $p = .0159$; ♂ vs. ☿ $p = .651$; ♀ vs. ☿ $p = .00155$; Figure 5(d)).

In the LX, the number of synaptic complexes (giant synapses, GS) was lowest in males (median ± MAD: 88.5 ± 1.5) and slightly higher counts were found in workers (median ± MAD: 95 ± 4) and gynes (median ± MAD: 103 ± 0.5). No statistical difference was found between the sexes and castes after Bonferroni correction (Kruskal–Wallis test: Number of GSs in the LX: $\chi^2 = 10.18$; $n = 14$; $p = .00615$; Mann–Whitney U-test with Bonferroni correction ($n_{\male} = 4$; $n_{\female} = 4$; $n_{☿} = 5$): ♂ vs. ♀ $p = .0286$; ♂ vs. ☿ $p = .0317$; ♀ vs. ☿ $p = .0317$; Figure 5(e)).

4 | DISCUSSION

4.1 | Polymorphisms in olfactory neuropils

Due to their special role in eusocial insect colonies, the behavioral repertoires of males are focused on few specific tasks, namely finding a virgin queen and mating. It has previously been suggested, that in *Cataglyphis fortis* sex pheromone communication plays an important role during courtship and mating behavior (Stieb et al., 2011). The brains of hymenopteran males usually reflect this specialization. In the AL the number of glomeruli in males is largely

TABLE 3 Number of synaptic complexes in mushroom bodies and the lateral complex and number of glomeruli in the antennal lobes

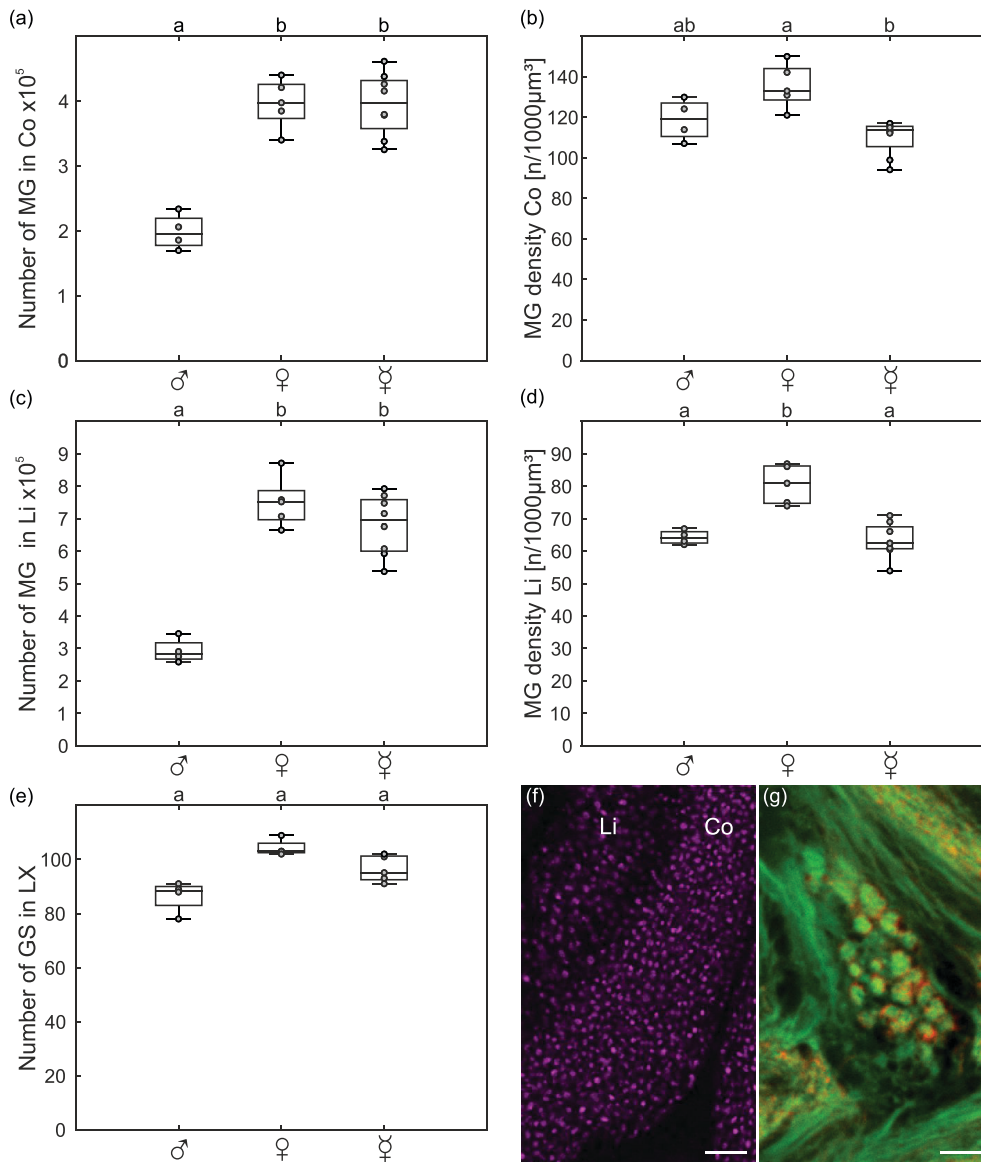|	Microglomeruli in MB collars	Microglomeruli in MB lips	Giant synapses in one lateral complex	Glomeruli in one antennal lobe
Male 1	205,452	275,874	91	199
Male 2	233,006	345,654	89	206
Male 3	185,633	289,383	78	197
Male 4	169,238	258,751	88	195
Gyne 1	420,886	665,719	103	245
Gyne 2	439,866	758,355	103	245
Gyne 3	397,614	751,819	–	245
Gyne 4	339,652	871,274	109	251
Gyne 5	384,257	707,283	102	235
Worker 1	460,830	747,096	101	250
Worker 2	377,713	716,281	102	249
Worker 3	437,588	792,235	–	251
Worker 4	425,551	770,419	93	251
Worker 5	379,112	592,176	95	250
Worker 6	325,275	607,834	91	–
Worker 7	337,239	537,762	–	–
Worker 8	415,606	674,581	–	–

FIGURE 5 Differences in the total numbers and densities of synaptic complexes in the mushroom bodies (MBs) and lateral complex (LX). (a–d) Microglomeruli (MG) in MB calyx subdivisions in males (♂, n = 4), gynes (♀, n = 5), and workers (☿, n = 8). (a) Total number of MG in the visual input region of the MB, the collar (Co). (b) Density of MG in the Co. (c) Total number of MG in the olfactory input region of the MBs, the lip (Li). (d) Density of MG in the Li. (e) Total number of giant synapses (GS) in the LX in males (♂, n = 4), gynes (♀, n = 4), and workers (☿, n = 5). For figure conventions, see Figure 2, and for statistical details, see text. (f,g) Confocal images of synaptic complexes in (f) the MB (☿), and (g) the LX (♀). The synaptic complexes are labeled with an antibody to the presynaptic protein synapsin (magenta). In the LX, double staining with f-actin by fluor-phalloidin (green). Scale bars = 10 μm [Color figure can be viewed at wileyonlinelibrary.com]

reduced compared with females. This is true for desert ants like *C. nodus*, or *C. fortis* (Stieb et al., 2011), but also for other ants like *Atta vollenweideri* (Kuebler et al., 2010), *Camponotus floridanus* (Zube & Rössler, 2008), *Camponotus japonicus* (Nishikawa et al., 2008), and *Harpegnathos saltator* (Hoyer et al., 2005), as well as for the honey bee *Apis mellifera* (Arnold et al., 1985; Brockmann & Robinson, 2007). The number of AL glomeruli between the female castes of *C. nodus* does not differ. This is different in *C. fortis*, where queens have more AL glomeruli than workers (Stieb et al., 2011). In this case, *C. nodus* is more similar to other ant species, where the number of AL glomeruli in queens are comparable to those of workers (Kuebler et al., 2010; Nishikawa et al., 2008). Since many olfactory tasks involved in the maintenance of the colony are solely performed by females (Hölldobler & Wilson, 1990), a higher number of glomeruli is needed, for example for brood and nest related communication. In absolute numbers, the numbers of glomeruli in the ALs of *C. nodus* exceed those found in *C. fortis* (Stieb et al., 2011), but was also found to be smaller than in other ant species less specialized for visual

navigation (Kuebler et al., 2010; Nishikawa et al., 2008; Zube & Rössler, 2008).

While *C. nodus* males have the lowest number of glomeruli in the ALs, they have a larger relative volume of ALs compared with females. This is in line with the results found in *C. japonicus* (Nishikawa et al., 2008). The large size of male ALs is mainly due to massively enlarged individual macroglomeruli. The occurrence of macroglomeruli has been described for males of several ant species, like *A. vollenweideri* (Kuebler et al., 2010), *C. fortis* (Stieb et al., 2011), *C. japonicus* (Nishikawa et al., 2008), and *H. saltator* (Hoyer et al., 2005). In a variety of insects, macroglomeruli are exclusively receiving input from sex-pheromone-sensitive olfactory receptor neurons [e.g., *Manduca sexta* (Hansson et al., 1991), *A. mellifera* (Arnold et al., 1985)]. It has been previously suggested, that pheromone communication plays an important role in the mating behavior of *Cataglyphis* (Stieb et al., 2011). However, thus far, only very little is known about *Cataglyphis*' mating behavior and the use of sex pheromones (Wehner, 2020). Large numbers of olfactory receptor neurons converge and terminate in

macroglomeruli, largely contributing to their increased volume (Berg et al., 1998; Vickers & Baker, 1997). The results in *C. nodus* demonstrate that the structural organization of the ALs expresses both sexual-dimorphism (macroglomeruli are present exclusively in males) and caste-specific polymorphism (difference in the relative volumes of the ALs).

Olfactory information leaves the AL via two olfactory pathways to the Li of the MB and the lateral horn (LH), the medial and lateral antennal lobe tracts (ALTs) (Habenstein et al., 2020). Compared with females, the medial ALT is reduced in males of *C. floridanus* (Zube & Rössler, 2008). In the honeybee, the lateral ALT does not show learning-dependent plasticity (Peele et al., 2006). It has been suggested, that the reduction of the medial ALT in male *Camponotus* ants may reflect the dominance of less plastic olfactory circuits to higher brain centers (Zube & Rössler, 2008). Male *C. nodus* have the relatively smallest MBs and workers the largest, which is reflected in about twice the absolute numbers of MB calyx synaptic complexes in the Li and Co. Between female ants, the total number of synaptic complexes does not differ significantly, although the MB volume is smaller in gynes. Interestingly, gynes make up for their smaller MBs with an increased synaptic density, mainly in the Li. The MG density has previously been shown to be an intraspecies limiting factor in the neuronal architecture of the brain in leaf-cutting ant worker castes (Groh et al., 2014). The high numbers of synaptic complexes in the MB calyx Li and of glomeruli in the ALs emphasize the importance of olfaction-related behavioral tasks in both female castes of *Cataglyphis*. In contrast, the male olfactory neuropils reflect fewer and more specific olfactory tasks like finding a gyne, potentially by olfactory non-compass orientation based on sex pheromone communication.

4.2 | Polymorphisms in visual neuropils

Visual information is transferred via visual pathways from the OLs into high-order integration centers in the ant brain (Grob et al., 2017; Habenstein et al., 2020; Rössler, 2019; Schmitt et al., 2016). In *C. nodus*, we found a sexual dimorphism in the relative size of the OLs. Males possess larger OLs compared with both female castes. Similar results have been found in *A. vollenweideri* (Kuebler et al., 2010) and *C. japonicus* (Nishikawa et al., 2008). Large optic lobes correlate with high amounts of sensory input. Likewise, more ommatidia in the compound eye allow a better spatial resolution. A higher number of ommatidia in male ants compared with their female conspecifics is common among ants (Gronenberg, 2008), indicating that vision is an important cue for males to find mates. In some species, workers may possess even fewer ommatidia than gynes (Narendra et al., 2016). In some insects males even have specialized region that is tuned for small field motion detection, so-called "love spots," in their eyes (Perry & Desplan, 2016). It will be interesting in future work to analyze how differences and similarities in ommatidia numbers and organization are reflected in the numbers and volumes of cartridges and columns in the optic lobes.

In *C. nodus*, one obvious distinction between the non-reproductive female worker caste and the reproductive caste is that

workers are purely ambulatory, while males and gynes are winged and capable of flight. This difference in locomotion might also lead to different neuronal needs for spatial orientation and navigation. Flight might lead to the need for a higher temporal visual resolution. Like the ants' eyes, also other sensory organs are adapted to the different types of locomotion. One example is the mechanosensory Johnston's organ (JO) in the ants' antennae that is involved in several navigational tasks including wind-compass orientation, and flight control. Interestingly, the number of JO sensory neurons is higher in the alates than in *C. nodus* workers (Grob, Tritscher, et al., 2021).

In *Cataglyphis*, visual information in general is an important navigational cue for the ants (Wehner, 2020). Information from polarized skylight is projected via a highly conserved pathway from the OLs via the anterior optic tract and several stages to the LX to finally terminate in the CX (Grob et al., 2017, 2019; Schmitt et al., 2016). Our present study, for the first time in any insect, shows that the number of large synaptic complexes does not differ between males and the two female castes. These giant synapses form an important relay station of the sky-compass pathway and have been proposed to play an important role in the processing of sky-compass relevant cues by allowing for a fast and reliable signal transmission along this specialized visual pathway (Held et al., 2016).

The CX is a highly conserved neuropil (Homberg, 2008) that plays a crucial role in integration of orientation cues and motor output (Honkanen et al., 2019). Our results revealed that while the absolute volume of the CX is smallest in males, the relative size of the CX in males is larger than in females. This suggests the importance of neuronal circuits for high-order integration of sensory stimuli relevant for spatial orientation and locomotion control, even in short-lived males, most likely due to the necessity for promoting the efficacy of male mating behavior. This sex-specific dimorphism in CX volume is consistent with results from *A. vollenweideri* (Kuebler et al., 2010) and *C. japonicus* (Nishikawa et al., 2008). To better address the smaller absolute size of the CX in males, synaptic counts within subunits of the CX would be helpful in the future. However, this was not possible with confocal imaging techniques as anti-synapsin labeled neuropil is very dense in this region. Therefore, we decided to use relative CX volumes as a suitable first proxy to look at neuronal adaptations (Gronenberg, 2008).

Information about the visual scenery (panorama) is transferred from a retinotopical organization in the OLs to the MB Co via the anterior superior optic tract (Grob et al., 2017, 2019; Schmitt et al., 2016). The MBs are essential for learning-based orientation, like visual landmark orientation (Buehlmann et al., 2020; Kamhi et al., 2020). Comparable to conditions in the olfactory compartments (Li), the numbers of synaptic complexes in the Co are significantly smaller in males but show no difference between the female castes. The vast numbers of synaptic complexes and the associated parallel MB microcircuits in *Cataglyphis* workers are well suited for storing visual images, which can be used for orientation based on view memories (Ardin et al., 2016; Grob et al., 2017). Foraging ants need large computational capacities for learning the visual panorama, routes, and landmarks to navigate successfully back to their nest every time they leave it. Additionally, foragers rely on a time-

compensated sun compass during their long foraging runs, for which they have to learn and memorize the course of the sun over the day, potentially in relation to panoramic features, whenever available (Wehner & Lanfranconi, 1981; Wehner & Müller, 1993). In *Cataglyphis* species that show mating swarm behavior, mated queens find their new colony alone, without the help of workers (Boulay et al., 2017; Peeters & Aron, 2017). Thus, young queens face similar navigational challenges as workers do and need to learn the position of their newly founded nest. Males, in contrast, have small MBs. They do not need to learn or remember the location of a nest, since they will never return to a nest after leaving their natal colony (Boulay et al., 2017; Hölldobler & Wilson, 1990; Peeters & Aron, 2017). Male ants of all examined species thus far possess a large CX that is comparable in size to the CX of workers (Gronenberg, 2008). The voluminous OLs together with a large CX and a small synaptic capacity in the MB of *C. nodus* males suggest the requirement for a high degree of specification and precision during visual orientation at the expense of behavioral flexibility, most importantly learning-related changes in behaviors.

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CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTION

Robin Grob: Conceived the study, collected the data, analyzed the data, visualized the data, drafted. **Niklas Heinig:** Collected the data, analyzed the data. **Kornelia Grübel:** Analyzed the data. **Wolfgang Rössler:** Conceived the study, led the study, revised the manuscript. **Pauline N. Fleischmann:** Conceived the study, led the study, analyzed the data, visualized the data, revised the manuscript. All authors approved the final version of the manuscript for submission.

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DATA AVAILABILITY STATEMENT

All data are supplied within the manuscript and supplement. Raw confocal microscopy images and 3D data are available from the authors upon reasonable request.

ORCID

Robin Grob https://orcid.org/0000-0002-0096-4040

Wolfgang Rössler https://orcid.org/0000-0002-5195-8214

Pauline N. Fleischmann https://orcid.org/0000-0002-5051-884X

REFERENCES

Ardin, P., Peng, F., Mangan, M., Lagogiannis, K., & Webb, B. (2016). Using an insect mushroom body circuit to encode route memory in complex natural environments. *PLOS Computational Biology*, *12*, e1004683.

Arnold, G., Masson, C., & Budharugsa, S. (1985). Comparative study of the antennal lobes and their afferent pathway in the worker bee and the drone (*Apis mellifera*). *Cell and Tissue Research*, *242*, 593–605.

Berg, B. G., Almaas, T. J., Bjaalie, J. G., & Mustaparta, H. (1998). The macroglomerular complex of the antennal lobe in the tobacco budworm moth *Heliothis virescens*: Specified subdivision in four compartments according to information about biologically significant compounds. *Journal of Comparative Physiology A. Neuroethology, Sensory, Neural, and Behavioral Physiology*, *183*, 669–682.

Boulay, R., Aron, S., Cerdá, X., Doums, C., Graham, P., Hefetz, A., & Monnin, T. (2017). Social life in arid environments: The case study of cataglyphis ants. *Annual Review of Entomology*, *62*, 305–321.

Brockmann, A., & Robinson, G. E. (2007). Central projections of sensory systems involved in honey bee dance language communication. *Brain, Behavior and Evolution*, *70*, 125–136.

Buehlmann, C., Hansson, B. S., & Knaden, M. (2012). Desert ants learn vibration and magnetic landmarks. *PLoS One*, *7*, e33117.

Buehlmann, C., Wozniak, B., Goulard, R., Webb, B., Graham, P., & Niven, J. E. (2020). Mushroom bodies are required for learned visual navigation, but not for innate visual behavior, in ants. *Current Biology*, *30*, 3438–3443. https://doi.org/10.1016/j.cub.2020.07.013

Cardona, A., Saalfeld, S., Schindelin, J., Arganda-Carreras, I., Preibisch, S., Longair, M., Tomancak, P., Hartenstein, V., & Douglas, R. J. (2012). TrakEM2 software for neural circuit reconstruction. *PLoS One*, *7*(6), e38011-1-8.

Collett, M. (2010). How desert ants use a visual landmark for guidance along a habitual route. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 11638–11643.

Fleischmann, P. N., Christian, M., Müller, V. L., Rössler, W., & Wehner, R. (2016). Ontogeny of learning walks and the acquisition of landmark information in desert ants, *Cataglyphis fortis*. *Journal of Experimental Biology*, *219*, 3137–3145.

Fleischmann, P. N., Grob, R., Müller, V. L., Wehner, R., & Rössler, W. (2018). The geomagnetic field is a compass cue in *Cataglyphis* ant navigation. *Current Biology*, *28*, 1440-1444.

Fleischmann, P. N., Grob, R., & Rössler, W. (2020a). Kompass im Kopf. *Biologie in unserer Zeit*, *50*, 100–109.

Fleischmann, P. N., Grob, R., & Rössler, W. (2020b). Magnetoreception in hymenoptera: Importance for navigation. *Animal Cognition*, *23*(6), 1051–1061.

Fleischmann, P. N., Rössler, W., & Wehner, R. (2018). Early foraging life: Spatial and temporal aspects of landmark learning in the ant *Cataglyphis noda*. *The Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, *204*, 579–592.

Grob, R., el Jundi, B., & Fleischmann, P. N. (2021). Toward a common terminology for arthropod spatial orientation. *Ethology Ecology & Evolution*, *33*(3), 338–358.

Grob, R., Fleischmann, P. N., Grübel, K., Wehner, R., & Rössler, W. (2017). The role of celestial compass information in *Cataglyphis* ants during learning walks and for neuroplasticity in the central complex and mushroom bodies. *Frontiers in Behavioral Neuroscience*, *11*, 1–14.

Grob, R., Fleischmann, P. N., & Rössler, W. (2019). Learning to navigate – How desert ants calibrate their compass systems. *NeuroForum*, *25*, 109–120.

Grob, R., Tritscher, C., Grübel, K., Stigloher, C., Groh, C., Fleischmann, P. N., & Rössler, W. (2021). Johnston's organ and its central projections in *Cataglyphis* desert ants. *Journal of Comparative Neurology*, *529*, 2138–2155.

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. *Proceedings of the Royal Society B: Biological Sciences*, *281*, 20140432.

Groh, C., Lu, Z., Meinertzhagen, I. A., & Rössler, W. (2012). Age-related plasticity in the synaptic ultrastructure of neurons in the mushroom body calyx of the adult honeybee *Apis mellifera*. *Journal of Comparative Neurology*, 520, 3509–3527.

Gronenberg, W. (2008). Structure and function of ant (Hymenoptera: Formicidae) brains: Strength in numbers. *Myrmecological News*, 11, 25–36.

Habenstein, J., Amini, E., Grübel, K., el Jundi, B., & Rössler, W. (2020). The brain of *Cataglyphis* ants: Neuronal organization and visual projections. *Journal of Comparative Neurology*, 528(18), 3479–3506.

Habenstein, J., Thamm, M., & Rössler, W. (2021). Neuropeptides as potential modulators of behavioral transitions in the ant *Cataglyphis nodus*. *Journal of Comparative Neurology*, 529(12), 3155–3170.

Hansson, B. S., Christensen, T. A., & Hildebrand, J. G. (1991). Functionally distinct subdivisions of the macroglomerular complex in the antennal lobe of the male sphinx moth *Manduca sexta*. *The Journal of Comparative Neurology*, 312, 264–278.

Held, M., Berz, A., Hensgen, R., Muenz, T. S., Scholl, C., Rössler, W., Homberg, U., & Pfeiffer, K. (2016). Microglomerular synaptic complexes in the sky-compass network of the honeybee connect parallel pathways from the anterior optic tubercle to the central complex. *Frontiers i Behavioral Neuroscience*, 10, 186.

Hölldobler, B., & Wilson, E. O. (1990). *The ants*. Belknap Press of Harvard University Press.

Homberg, U. (2008). Evolution of the central complex in the arthropod brain with respect to the visual system. *Arthropod Structure & Development*, 37, 347–362.

Honkanen, A., Adden, A., da Silva, F. J., & Heinze, S. (2019). The insect central complex and the neural basis of navigational strategies. *Journal of Experimental Biology*, 222(Suppl_1), 1–15.

Hoyer, S. C., Liebig, J., & Rössler, W. (2005). Biogenic amines in the ponerine ant *Harpegnathos saltator*: Serotonin and dopamine immunoreactivity in the brain. *Arthropod Structure & Development*, 34, 429–440.

Ito, K., Shinomiya, K., Ito, M., Armstrong, J. D., Boyan, G., Hartenstein, V., Harzsch, S., Heisenberg, M., Homberg, U., Jenett, A., Keshishian, H., Restifo, L. L., Rössler, W., Simpson, J. H., Strausfeld, N. J., Strauss, R., & Vosshall, L. B. (2014). A systematic nomenclature for the insect brain. *Neuron*, 81, 755–765.

Kamhi, J. F., Barron, A. B., & Narendra, A. (2020). Vertical lobes of the mushroom bodies are essential for view-based navigation in Australian *Myrmecia* ants. *Current Biology*, 30, 3432–3437. https://doi.org/10.1016/j.cub.2020.06.030

Kuebler, L. S., Kelber, C., & Kleineidam, C. J. (2010). Distinct antennal lobe phenotypes in the leaf-cutting ant (*Atta vollenweideri*). *Journal of Comparative Neurology*, 518, 352–365.

Leniaud, L., Heftez, A., Grumiau, L., & Aron, S. (2011). Multiple mating and supercoloniality in *Cataglyphis* desert ants. *Biological Journal of the Linnean Society*, 104, 866–876.

Müller, M., & Wehner, R. (1988). Path integration in desert ants, *Cataglyphis fortis*. *Proceedings of the National Academy of Sciences of the United States of America*, 85, 5287–5290.

Narendra, A., Ramirez-Esquivel, F., & Ribi, W. A. (2016). Compound eye and ocellar structure for walking and flying modes of locomotion in the Australian ant, *Camponotus consobrinus*. *Scientific Reports*, 6, 1–10.

Nishikawa, M., Nishino, H., Misaka, Y., Kubota, M., Tsuji, E., Satoji, Y., Ozaki, M., & Yokohari, F. (2008). Sexual dimorphism in the antennal lobe of the ant *Camponotus japonicus*. *Zoological Science*, 25, 195–204.

Peele, P., Ditzen, M., Menzel, R., & Galizia, C. G. (2006). Appetitive odor learning does not change olfactory coding in a subpopulation of honeybee antennal lobe neurons. *The Journal of Comparative Physiology A. Neuroethology, Sensory, Neural, and Behavioral Physiology*, 192, 1083–1103.

Peeters, C., & Aron, S. (2017). Evolutionary reduction of female dispersal in *Cataglyphis* desert ants. *Biological Journal of the Linnean Society*, 122, 58–70.

Perry, M. W., & Desplan, C. (2016). Love spots. *Current Biology*, 26, R484–R485. https://doi.org/10.1016/j.cub.2016.02.020

Rössler, W. (2019). Neuroplasticity in desert ants (Hymenoptera: Formicidae): Importance for the ontogeny of navigation. *Myrmecological News*, 29, 1–20.

Rössler, W., Spaethe, J., & Groh, C. (2017). Pitfalls of using confocal-microscopy based automated quantification of synaptic complexes in honeybee mushroom bodies (response to Peng and Yang 2016). *Science Report*, 7, 9786.

Schmid-Hempel, P., & Schmid-Hempel, R. (1984). Life duration and turn-over of foragers in the ant *Cataglyphis bicolor* (Hymenoptera, Formicidae). *Insectes Sociaux*, 31, 345–360.

Schmitt, F., Stieb, S. M., Wehner, R., & Rössler, W. (2016). Experience-related reorganization of giant synapses in the lateral complex: Potential role in plasticity of the sky-compass pathway in the desert ant *Cataglyphis fortis*. *Developmental Neurobiology*, 76, 390–404.

Schmitt, F., Vanselow, J. T., Schlosser, A., Wegener, C., & Rössler, W. (2017). Neuropeptides in the desert ant *Cataglyphis fortis*: Mass spectrometric analysis, localization, and age-related changes. *Journal of Comparative Neurology*, 525, 901–918.

Steck, K., Hansson, B. S., & Knaden, M. (2009). Smells like home: Desert ants, *Cataglyphis fortis*, use olfactory landmarks to pinpoint the nest. *Frontiers in Zoology*, 6, 5.

Stieb, S. M., 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*. *Developmental Neurobiology*, 72, 729–742.

Stieb, S. M., Kelber, C., Wehner, R., & Rössler, W. (2011). Antennal-lobe organization in Desert ants of the genus *Cataglyphis*. *Brain, Behavior and Evolution*, 77, 136–146.

Stieb, S. M., Muenz, T. S., Wehner, R., & Rössler, W. (2010). Visual experience and age affect synaptic organization in the mushroom bodies of the desert ant *Cataglyphis fortis*. *Developmental Neurobiology*, 70, 408–423.

Vickers, N. J., & Baker, T. C. (1997). Chemical communication in heliothine moths. VII. Correlation between diminished responses to point-source plumes and single filaments similarly tainted with a behavioral antagonist. *Journal of Comparative Physiology A. Neuroethology, Sensory, Neural, and Behavioral Physiology*, 180, 523–536.

Wehner, R. (2019). The *Cataglyphis* mahrèsienne: 50 years of Cataglyphis research at Mahrès. *Journal of Comparative Physiology A. Neuroethology, Sensory, Neural, and Behavioral Physiology*, 205, 641–659. https://doi.org/10.1007/s00359-019-01333-5

Wehner, R. (2020). *Desert navigator: The journey of an ant*. The Belknap Press of Havard University Press.

Wehner, R., & Lanfranconi, B. (1981). What do the ants know about the rotation of the sky? *Nature*, 293, 731–733.

Wehner, R., & Müller, M. (1993). How do ants acquire their celestial ephemeris function? *Naturwissenschaften*, 80, 331–333.

Wilson, E. O., & Hölldobler, B. (2005). Eusociality: Origin and consequences. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 13367–13371.

Wittlinger, M., Wehner, R., & Wolf, H. (2006). The ant odometer: Stepping on stilts and stumps. *Science*, 312, 1965–1967.

Zeil, J., & Fleischmann, P. N. (2019). The learning walks of ants (Hymenoptera: Formicidae). *Myrmecological News*, 29, 93–110.

Zube, C., & Rössler, W. (2008). Caste- and sex-specific adaptations within the olfactory pathway in the brain of the ant *Camponotus floridanus*. *Arthropod Structure & Development*, 37, 469–479.