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Sexual Dimorphism in the Antennal Lobe of the Ant *Camponotus japonicus*

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The carpenter ant, a social hymenopteran, has a highly elaborated antennal chemosensory system that is used for chemical communication in social life. The glomeruli in the antennal lobe are the first relay stations where sensory neurons synapse onto interneurons. The system is functionally and structurally similar to the olfactory bulbs of vertebrates. Using three-dimensional reconstruction of glomeruli and subsequent morphometric analyses, we found sexual dimorphism of the antennal lobe glomeruli in carpenter ants, *Camponotus japonicus*. Female workers and unmated queens had about 430 glomeruli, the highest number reported so far in ants. Males had a sexually dimorphic macroglomerulus and about 215 ordinary glomeruli. This appeared to result from a greatly reduced number of glomeruli in the postero-medial region of the antennal lobe compared with that in females. On the other hand, sexually isomorphic glomeruli were identifiable in the dorsal region of the antennal lobe. For example, large, uniquely shaped glomeruli located at the dorso-central margin of the antennal lobe were detected in all society members. The great sexual dimorphism seen in the ordinary glomeruli of the antennal lobe may reflect gender-specific tasks in chemical communications rather than different reproductive roles.

Key words: social insects, Hymenoptera, insect brain, glomeruli, macroglomerulus, isomorphism

INTRODUCTION

Ants have an elaborate chemosensory system in the brain, since they communicate with nestmates largely by chemical signals, so-called pheromones (Hölldobler and Wilson, 1990; Gronenberg and Hölldobler, 1999; Ehmer and Gronenberg, 2004; Ozaki et al., 2005). As in other insect species, antennae of ants have various kinds of olfactory sensilla housing several sensory neurons (Renthal et al., 2003). Axons of antennal olfactory neurons synapse onto dendrites of a variety of interneurons in the glomeruli of the first-order olfactory center, the antennal lobe.

In insects, individual glomeruli receive convergence from olfactory afferents expressing the same receptors, thus acting as a functional module (Gao et al., 2000). Furthermore, glomeruli receiving afferents expressing similar receptors tend to be located close to each other (Takahashi et al., 2004; Couto et al., 2005). For example, in moths a large, male-specific glomerular complex is specialized for process-

ing female sex pheromone (Boeckh and Tolbert, 1993; Hansson, 1999). In the ant *Camponotus obscuripes*, it has recently been reported that glomeruli responsive to alarm pheromone are clustered in the dorsal region of the antennal lobe (Yamagata et al., 2006).

Antennal lobe glomeruli differ greatly in number across different insect species (Hansson, 1999), even in phylogenetically close insects (e.g., orthopteran insects (Ignell et al., 2001)). Ants of different castes and genders in a colony vary considerably in body and head sizes, and their roles in chemical communication are also different (Hölldobler and Wilson, 1990). It is therefore not surprising that antennal lobe structures show gender- or caste-dependent specialization.

There have been recent attempts to correlate morphological characteristics of the nervous system with the behavior of ants (Gronenberg and Hölldobler, 1999; Ehmer and Gronenberg, 2004). Morphometric analyses of the brains of *Camponotus* ants have revealed that relative volumes of major neuropils such as the mushroom body, central complex, optic lobe, and antennal lobe vary in society members, including workers, unmated queens and males (Ehmer and Gronenberg, 2004; Kubota et al., 2006). In the leaf-cutting

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ants *Atta vollenweider* and *Atta sexdens*, a macroglomerulus assumed to process potentially trail pheromone has been identified in the antennal lobe of large workers but not in that of small workers (Kleineidam et al., 2005). However, there has been no comprehensive analysis of the glomerular organization across castes and genders in ants.

In this study, we created almost complete three-dimensional glomerular maps for all society members of *Camponotus japonicus*, including workers, unmated queens, and males. The results showed that there is great sexual dimorphism in the number of ordinary glomeruli. This difference was not detected between workers and unmated queens.

MATERIALS AND METHODS

Animals

Ants (*Camponotus japonicus*) were caught around a nest on the campus of Fukuoka University during the mating season in spring. Workers (Fig. 1A), unmated queens (Fig. 1B), and males (Fig. 1C), collected from the same colony, were used for histological experiments.

Neuroanatomical procedures

To visualize the brain neuropils and the antennal lobe glomeruli, we used tissue autofluorescence enhanced by glutaraldehyde fixation. Ants that had been anaesthetized by cooling on ice were decapitated, and their brains were dissected in cooled ant saline (4.8 mM TES, pH 7.4, containing 127 mM NaCl, 6.7 mM KCl, 2 mM CaCl₂, and 3.5 mM sucrose). The brains were fixed with 4% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.0), kept overnight at 4°C, dehydrated in an ethanol series, and cleared in methyl salicylate.

Observation using confocal microscopy and image processing

Whole-mount preparations of ant brains were viewed frontally using a confocal imaging system (LSM-510; Carl Zeiss, Jena, Germany). The brain neuropils and glomeruli were visualized with 458-nm excitation and a long-pass emission filter (>475 nm). Serial optical sections were acquired at 2-μm intervals throughout the entire depth of the brain. All images were taken at a resolution of 1024×1024 pixels and were saved as TIFF files for later analysis.

Three-dimensional reconstructions and volumetric analysis of glomeruli

TIFF images were processed using image processing software (Amira; TGC, Berlin, Germany) and analyzed. The brain neuropils and glomeruli were manually outlined for each optical slice and were three-dimensionally reconstructed. The volumes of brain neuropils and glomeruli were measured and calculated from reconstructed 3D-images using the image processing software (Amira).

The number of specimens used for morphometric analysis is shown in parenthesis in the Results section. The t-test was used to determine significant differences in volumes of brain neuropils and numbers of glomeruli. The orientation of the brain is referred to with respect to the neuraxis, which is tilted against the head-body axis by about 90°.

RESULTS

Appearances of society members of *Camponotus japonicus*

Fig. 1 shows a worker, an unmated queen, and a male of the carpenter ant, which are about 10 mm, 20 mm and 10 mm in body length, respectively. Flightless workers (Fig. 1A) have no wings or ocelli, while unmated queens (Fig. 1B)

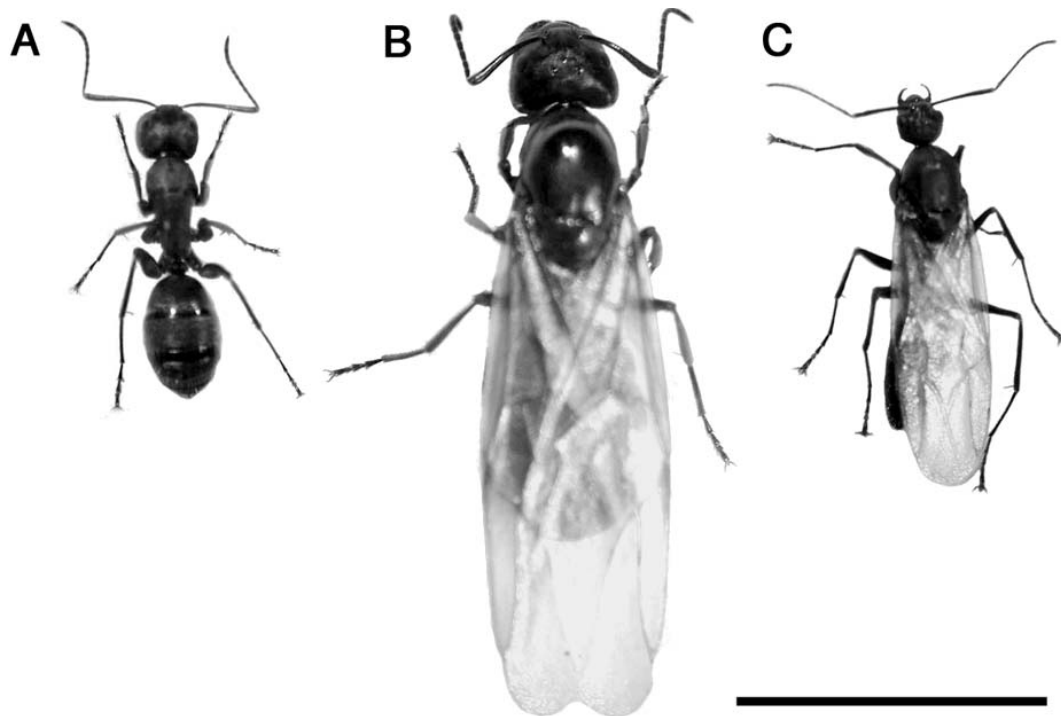


Fig. 1. Carpenter ants: (A) a worker, (B) an unmated queen, and (C) a male. Body lengths are about 10 mm, 20 mm, and 10 mm, respectively. Scale bar=10 mm.

have ocelli and wings during the mating season, as do males (Fig. 1C).

Gross organization of brain neuropils

The basic brain organization was similar in all society members. Fig. 2 shows a worker brain that was visualized by autofluorescence and viewed ventrally (frontally). Fig. 2A, B and C are confocal images of 2- μ m optical sections at depths of 80 μ m, 128 μ m, and 176 μ m from the ventral surface of the brain, respectively.

The brain is comprised of the protocerebrum and deutocerebrum (Fig. 2). The subesophageal ganglion is situated just dorso-posteriorly to the brain (not shown in Fig. 2).

The mushroom body is located at the center in each hemisphere of the protocerebrum and comprises intrinsic neurons, the Kenyon cells (Fig. 2). The cell bodies of Kenyon cells are located in the peripheral region of the calyx (Fig. 2). The mushroom body comprises five compartments: lateral and medial calyces, peduncles, vertical lobe, and medial lobe (Fig. 2A, B). The central complex is located in the midline of the protocerebrum, just anteriorly to the medial lobe of the mushroom body. The central complex consists of four compartments: a fan-shaped upper division and a lower division of the central complex (Fig. 2B), noduli (not shown), and protocerebral bridge (Fig. 2C).

The optic lobe protrudes laterally from the protocerebrum, and it consists of three neuropils: lamina, medulla, and lobula (Fig. 2C).

The deutocerebrum consists of the glomerular neuropil, the antennal lobe (Fig. 2A, B), and the non-glomerular neuropil, the dorsal lobe (Fig. 2C).

Volumetric analysis of major brain neuropils

The brain/subesophageal ganglion (SEG) complex was reconstructed three-dimensionally in the worker (Fig. 3A), unmated queen (Fig. 3B), and male (Fig. 3C) for volumetric comparison. Absolute volumes of the brain/SEG complex (Br+SEG), mushroom body (MB), and antennal lobe (AL) were largest in unmated queens and smallest in males, while absolute volumes of the optic lobe (OL) and central complex (CC) were largest in unmated queens and smallest in workers (Fig. 4A). Except for the central complex, there was a significant difference among absolute volumes in the three members ($p < 0.05$, t-test, $n = 5$) (a-c; Fig. 4A). The mean value of absolute volumes of antennal lobes in workers was about 60% that in unmated queens (Fig. 4A). However, there was no significant difference in relative volumes of antennal lobes to the brain/SEG complex between workers and unmated queens ($p < 0.05$, t-test, $n = 5$) (Fig. 4B). Relative volumes of the mushroom bodies were significantly larger in workers than in unmated queens (Fig. 4B). Relative volumes of optic lobes were extremely large in males and small in workers (Fig. 4B). Considering this large difference between relative volumes of optic lobes, other relative values, i.e., relative volumes of mushroom bodies or antennal lobes to the brain/SEG complex excluding optic lobes, were calculated. Relative values of mushroom bodies or antennal lobes in males or unmated queens were slightly larger than those in Fig. 4B, but the overall results were almost the same as the relative values in Fig. 4B.

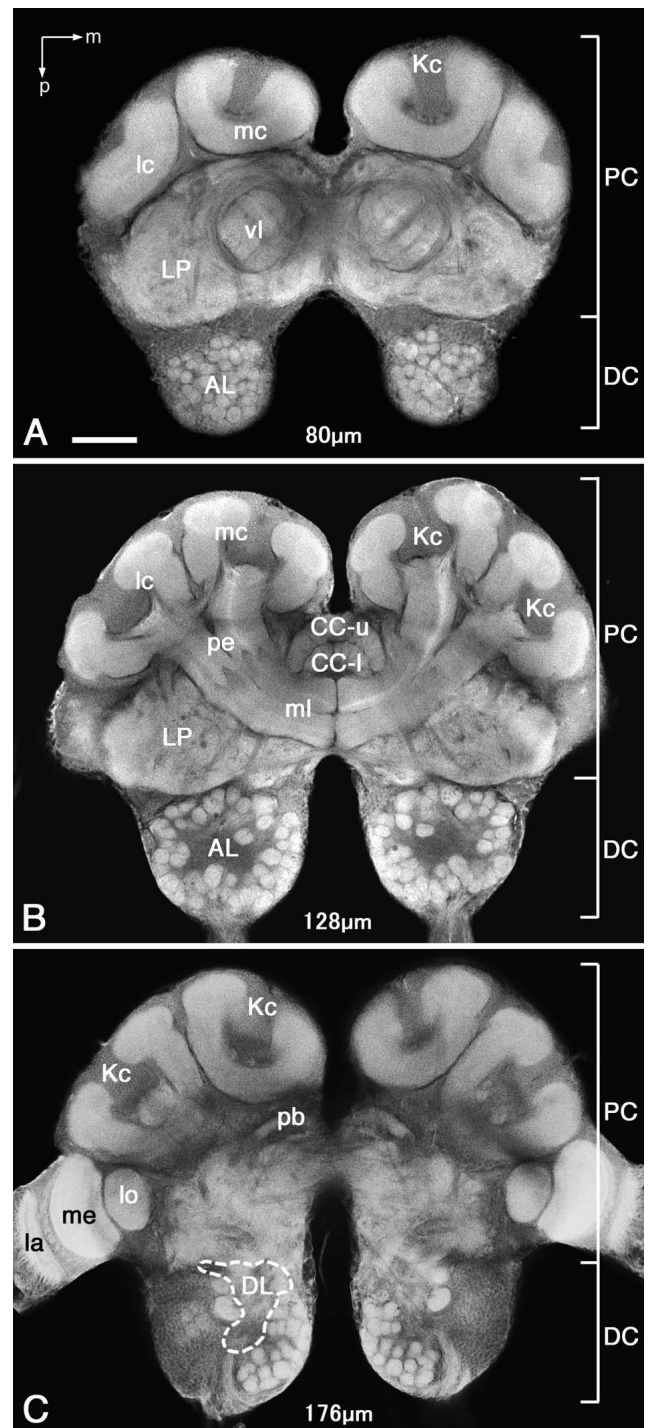


Fig. 2. Major neuropils in the brain of the worker ant. **A**, **B**, and **C** show ventral side views of LSM optical sections at depths of 80 μ m, 128 μ m, and 176 μ m from the brain surface, respectively. AL, antennal lobe; CC-l, lower division of the central complex; CC-u, upper division of the central complex; DC, deutocerebrum; DL, dorsal lobe (broken line); Kc, Kenyon cells; lc, lateral calyx; la, lamina of the optic lobe; lo, lobula of the optic lobe; LP, lateral protocerebrum; mc, medial calyx; me, medulla of the optic lobe; ml, medial lobe of the mushroom body; PC, protocerebrum; pe, peduncle of the mushroom body; vl, vertical lobe of the mushroom body. Directions are indicated in the coordinate planes: m, medial; p, posterior. Scale bar=100 μ m.

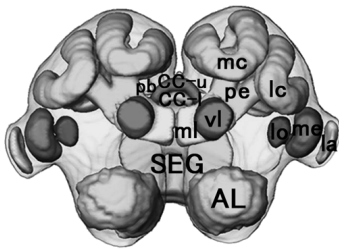
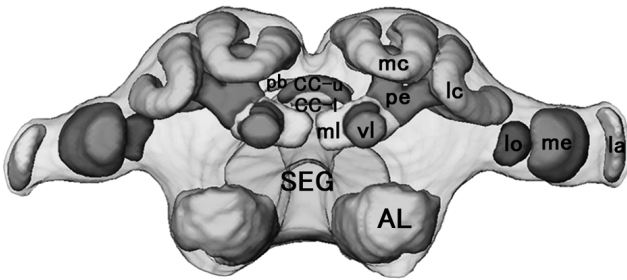
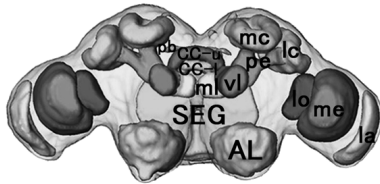
A Worker**B Unmated queen****C Male**

Fig. 3. Three-dimensionally reconstructed major neuropils in brains of (A) a worker, (B) an unmated queen, and (C) a male. Lateral (lc) and medial (mc) calyces, medial (ml) and vertical (vl) lobes, and peduncles (pe) are components of the mushroom body. Upper (CC-u) and lower (CC-l) divisions, noduli (not shown), and protocerebral bridge (pb) are components of the central complex. The lamina (la), medulla (me), and lobula (lo) are components of the optic lobe. AL, antennal lobe; AN, antennal nerve; SEG, subesophageal ganglion. Scale bar=100 μm .

Glomerular organization of the antennal lobe

Fig. 5 shows the left antennal lobe glomeruli of a worker (A–C), an unmated queen (D–F), and a male (G–I) in optical sections at 2- μm intervals. The images in the first row (Fig. 5A, D, G) show the ventral region of the antennal lobe at depths of 34, 34, and 32 μm from the surface of the antennal lobe in a worker, an unmated queen, and a male, respectively. Since brain sizes of members are different, these optical sections were taken not from the same depth but from similar levels based on the characteristic glomerular structure. The macroglomerulus (MG; Fig. 5G) is located at the entrance of the antennal nerve in males but not in workers (Fig. 5A) or unmated queens (Fig. 5D). The diameter of the macroglomerulus is about 50 μm , while the diameters of ordinary glomeruli in the ventral region of the antennal lobe are about 20 μm .

The images in the second row (Fig. 5B, E, H) are the middle regions of antennal lobes at depths of 60, 72, and 52

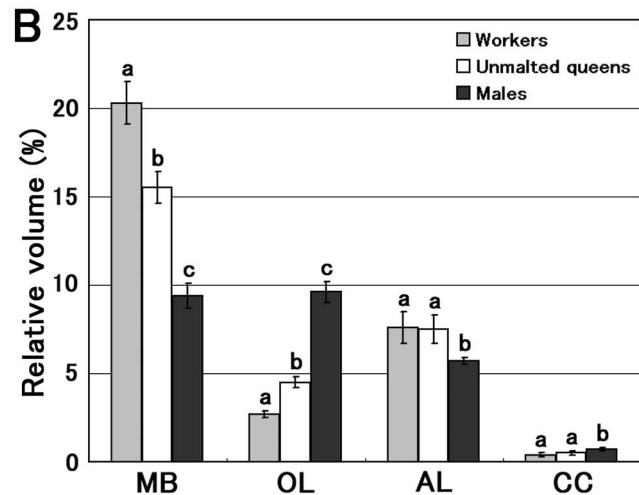
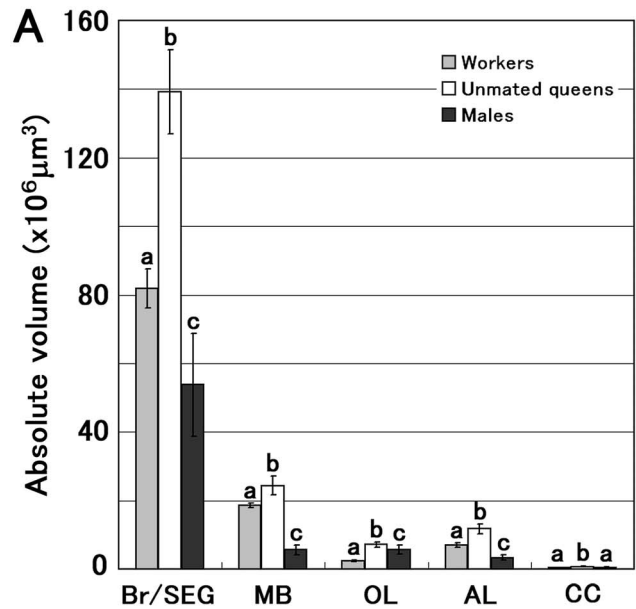


Fig. 4. (A) Mean values of absolute volumes of brain neuropils in workers ($n=5$), unmated queens ($n=5$) and males ($n=5$). The mean volumes of the central complex (CC) of workers, unmated queens and males are 0.40 ± 0.06 , 0.74 ± 0.03 and $0.42 \pm 0.13 \times 10^6 \mu\text{m}^3$ (mean \pm SD), respectively. (B) Mean values of relative volumes of neuropils to brain/SEG complex. Different letters (a, b, c) denote significant differences at least at the 0.05 level (t-test, $n=5$) among workers, unmated queens, or males. AL, antennal lobe; Br+SEG, brain/subesophageal ganglion complex; CC, central complex; MB, mushroom body; OL, optic lobe.

μm from the surface of the antennal lobe in a worker, an unmated queen, and a male, respectively. In the middle regions, the ordinary glomeruli are about 30 μm in diameter, larger than those in the ventral region, and are distributed peripherally in antennal lobes. In the center of the antennal lobe, there are many neuronal tracts and fibers devoid of autofluorescence (white asterisks; Fig. 5B, E, H).

The images in the third row (Fig. 5C, F, I) are the dorsal regions of antennal lobes in a worker, an unmated queen, and a male at depths of 108, 140, and 104 μm from the surface of the antennal lobe, respectively. Glomeruli in the dor-

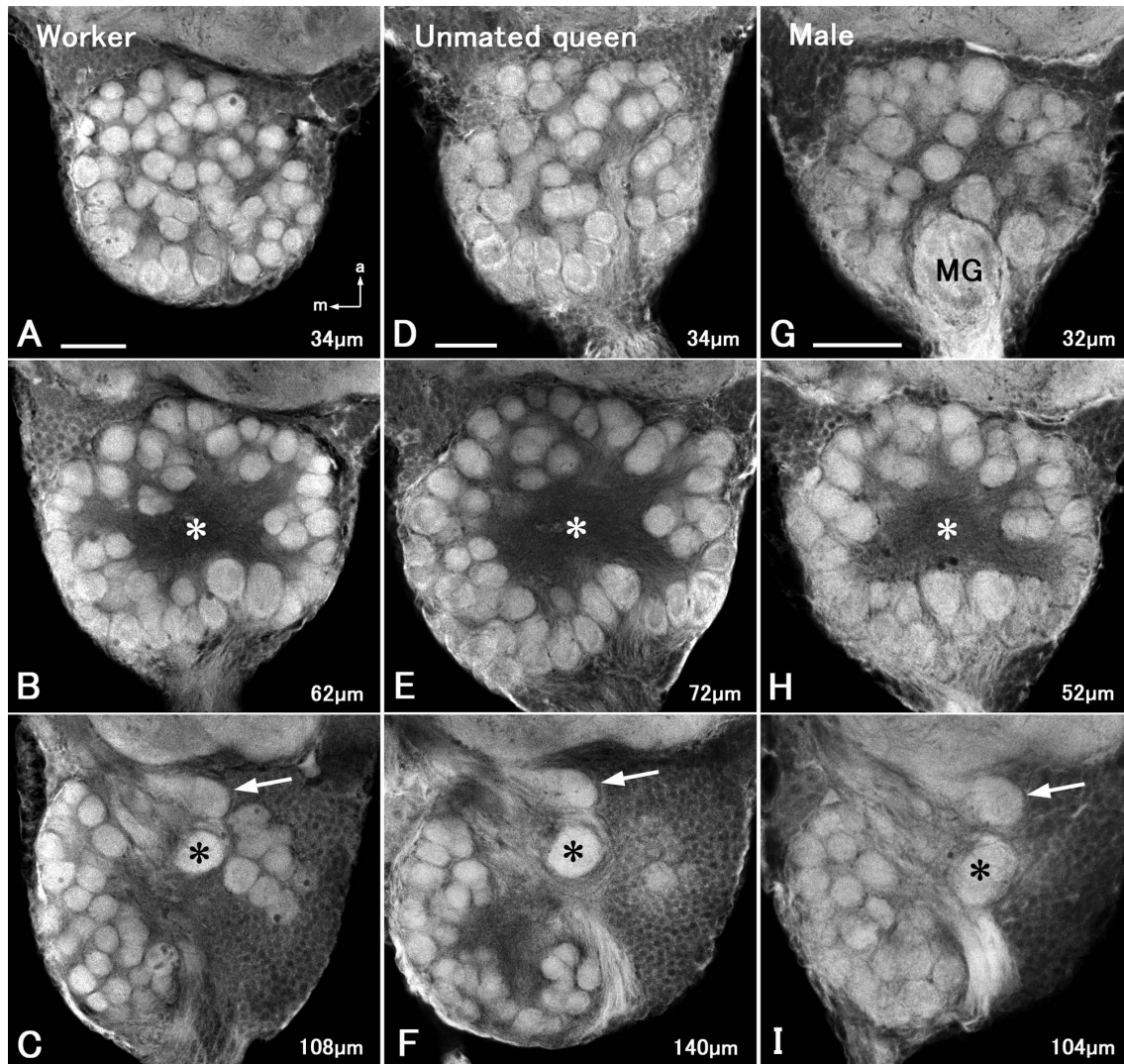


Fig. 5. Optical sections of the left antennal lobe glomeruli in (A–C) a worker, (D–F) an unmated queen, and (G–I) a male. Value in micrometers on each image denotes the depth of the optical section from the brain surface. Images in the same horizontal row show glomerular regions of almost similar levels of the antennal lobes. A macroglomerulus (MG) is evident at the entrance of the antennal nerve only in the male antennal lobe (G). In the middle regions, glomeruli are distributed peripherally in the antennal lobes, and there is no glomerulus in the centers (white asterisks; B, E, H). Landmark glomeruli are located in the dorsal regions and include relatively large glomeruli (black asterisks; C, F, I) at the bifurcations of the tracts and elongate, curved glomeruli (white arrows; C, F, I) at the anterior edge next to the large glomeruli. The images of worker, unmated queen, and male are at the same magnification. Directions are indicated in the coordinate planes: a, anterior; m, medial. Scale bar=50 µm.

sal region are over 30 µm in diameter and are much larger than those in the ventral or medial region. However, the glomeruli in the postero-medial region are as small as those in the ventral region.

Several glomeruli located in the dorsal margin of the antennal lobe were reliably identifiable between different individuals based on their unique location and shape. For example, an elongate, curved glomerulus (arrows; Fig. 5C, F, I) is located in the antero-dorsal region of the antennal lobe just ventrally to the DL in all members. A relatively large spherical glomerulus (black asterisks; Fig. 5C, F, I) was distinguished between the bifurcations of the main tract in the dorso-central region of the antennal lobe in all members as well.

Three-dimensional representation of antennal lobe glomeruli

Entire antennal lobe glomeruli were reconstructed three-dimensionally in the worker (Fig. 6A–C), unmated queen (Fig. 6D–F), and male (Fig. 6G–I). The first to third rows in Fig. 6 show ventral (A, D, G), dorsal (B, E, H), and lateral (C, F, I) side views of the left antennal lobe, respectively. The macroglomerulus (yellow; Fig. 6C) is situated at the entrance of the antennal nerve in the male antennal lobe, viewed ventrally (Fig. 6C) and laterally (Fig. 6I).

In dorsal side views of antennal lobes, the elongate, curved glomerulus (red; Fig. 6B, E, H) and the large spherical glomerulus (green; Fig. 6B, E, H) are located at the dorsalmost region of the antennal lobe in all members.

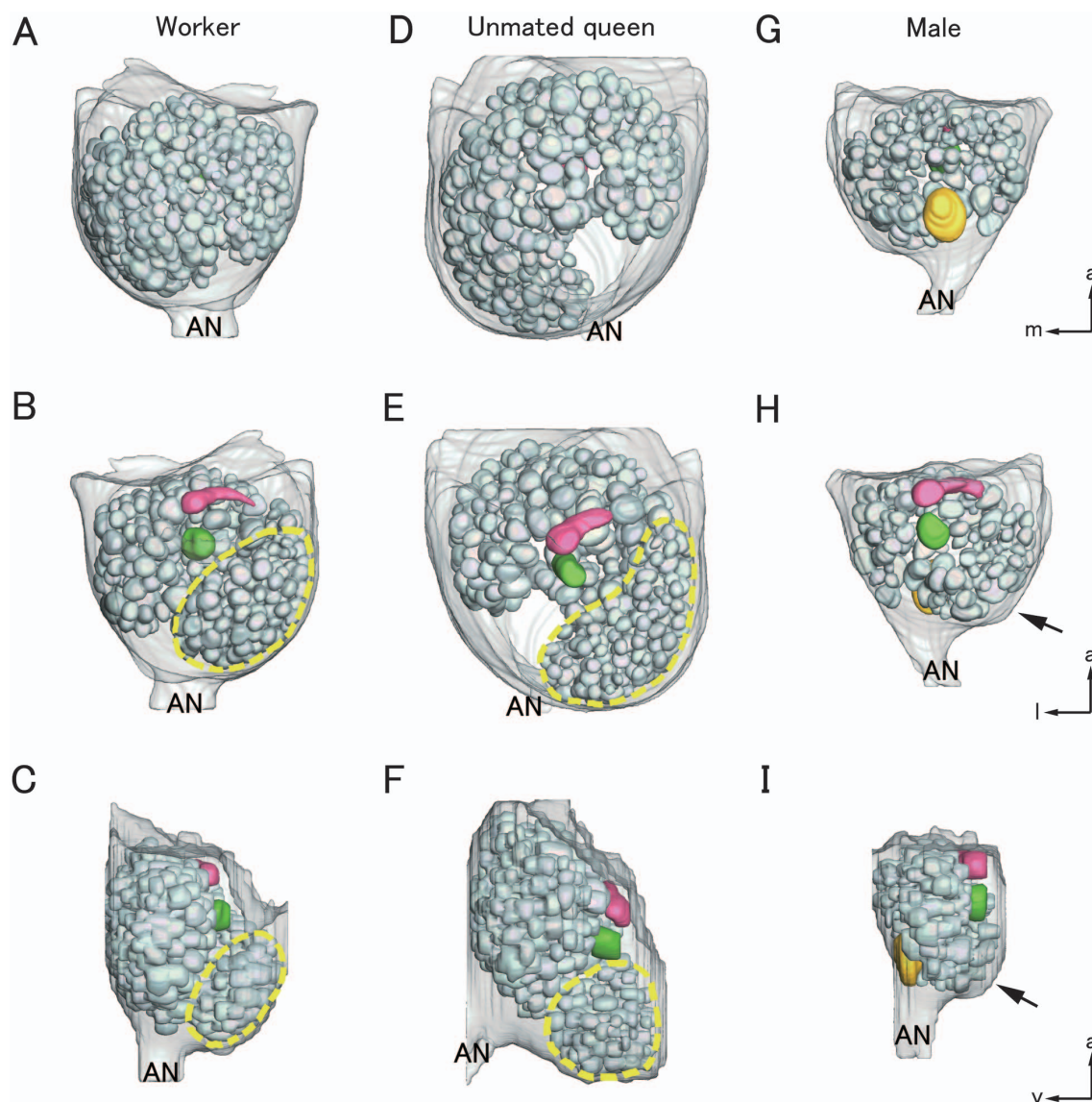


Fig. 6. Three-dimensionally reconstructed glomeruli of the left antennal lobes in (A–C) the worker, (D–F) unmated queen, and (G–I) male. The first row (A, D, G) shows ventral side views, the second row (B, E, H) shows dorsal side views, and the third row (C, F, I) shows lateral side views. Elongate, curved (red) and large dorsalmost (green) glomeruli are common to all members as a landmark. A macroglomerulus (yellow) is located at the entrance of the antennal nerve (AN) in the male (G, H, I). Yellow broken lines enclose the glomeruli located in the postero-medial region of the antennal lobe in the worker (B, C) and unmated queen (E, F) but not in the male (arrows; H, I). Directions are indicated in the coordinate planes: a, anterior; l, lateral; m, medial; v, ventral. Scale bar=100 μ m.

In dorsal and lateral side views of antennal lobes, a considerable number of glomeruli seemed to form a cluster in the dorso-postero-medial region of the antennal lobe of a worker (broken line; Fig. 6B, C) and an unmated queen (broken line; Fig. 6E, F). These glomeruli were relatively small and seemed to be clustered outside the arrays of other glomerular regions (Fig. 6B, C, E, F). In males, the number of postero-medially located glomeruli seemed to be much smaller (arrow; Fig. 6H, I).

Number of glomeruli

Based on the three-dimensional reconstructions of antennal lobe glomeruli, the total number of glomeruli in a

hemisphere was counted in workers, unmated queens, and males. Averages and standard deviations are shown in Fig. 7. The mean numbers of glomeruli were 438 ± 15 (mean \pm SD) in workers ($n=6$), 435 ± 12 in unmated queens ($n=7$), and 215 ± 7 in males ($n=6$) (Fig. 7). The numbers of glomeruli were not significantly different between workers and unmated queens (a-a; Fig. 7), but the numbers in workers and unmated queens were significantly different from the number in males ($p < 0.05$, t-test) (a-b; Fig. 7).

Volumetric analysis of glomeruli

Fig. 8 shows histograms of mean values of absolute glomerular volumes in workers ($n=6$) (Fig. 8A), unmated

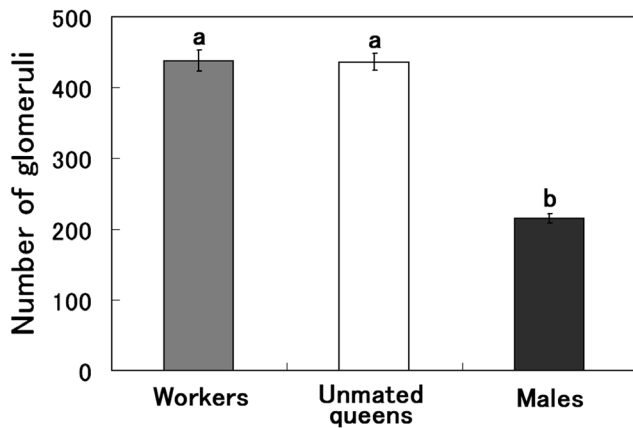


Fig. 7. Numbers of glomeruli in workers, unmated queens, and males. The mean numbers of glomeruli were 438 ± 15 (mean \pm SD) in workers ($n=6$), 435 ± 12 in unmated queens ($n=7$), and 215 ± 7 in males ($n=6$). There was no significant difference in number of glomeruli between workers and unmated queens (a-a), but the numbers of glomeruli in workers and unmated queens were significantly different from the number in males (a-b) ($p < 0.05$, t-test).

queens ($n=6$) (Fig. 8B), and males ($n=6$) (Fig. 8C). In all members, over 80% of all glomeruli in the antennal lobe ranged from 1×10^3 to $10 \times 10^3 \mu\text{m}^3$ in volume. The sizes of most glomeruli were $4\text{--}5 \times 10^3 \mu\text{m}^3$ in workers, $5\text{--}6 \times 10^3 \mu\text{m}^3$ in unmated queens, and $2\text{--}3 \times 10^3 \mu\text{m}^3$ in males (Fig. 8).

Glomerular distributions seemed to be roughly divided into two or three classes of sizes in all members (Fig. 8). Major classes consist of ordinary glomeruli: $1\text{--}20 \times 10^3 \mu\text{m}^3$ in workers (Fig. 8A), $1\text{--}23 \times 10^3 \mu\text{m}^3$ in unmated queens (Fig. 8B) and $1\text{--}18 \times 10^3 \mu\text{m}^3$ in males (Fig. 8C). Minor classes include three kinds of large glomeruli: 1) the elongate, curved glomerulus (Figs. 5, 6) in all society members, 2) the large spherical glomerulus between the bifurcations of the main tract (Figs. 5, 6) in all society members, and 3) a large glomerulus located in the postero-medial region in males and in the postero-lateral region in workers and unmated queens (not shown). Sizes of glomeruli in minor classes are $25\text{--}34 \times 10^3 \mu\text{m}^3$ in workers (asterisk; Fig. 8A), $25\text{--}39 \times 10^3 \mu\text{m}^3$ in unmated queens (asterisk; Fig. 8B), and $22\text{--}32 \times 10^3 \mu\text{m}^3$ in males (asterisk; Fig. 8C). These large glomeruli are 5–10 times larger than most ordinary glomeruli in all members. A third class in males is macroglomeruli $41\text{--}45 \times 10^3 \mu\text{m}^3$ in size (MG; Fig. 8C), which are about 20 times larger than most ordinary glomeruli in males.

Although the numbers of glomeruli in the antennal lobes of workers and unmated queens were very similar, the sizes of glomeruli differed. The number of glomeruli smaller than $5 \times 10^3 \mu\text{m}^3$ was much larger in workers (53% of total number of glomeruli) than in unmated queens (31%), while the number of glomeruli larger than $5 \times 10^3 \mu\text{m}^3$ was much larger in unmated queens (69%) than in workers (47%) (Fig. 8A, B).

Relative sizes of individual glomeruli to the respective antennal lobe volumes in all members are shown in Fig. 9. The number of glomeruli with relative sizes in the range of $1.5\text{--}2.5 \times 10^{-3}$ was significantly larger in workers than in unmated queens, while the number of glomeruli with relative sizes in the range of $0.5\text{--}1.5 \times 10^{-3}$ was significantly smaller

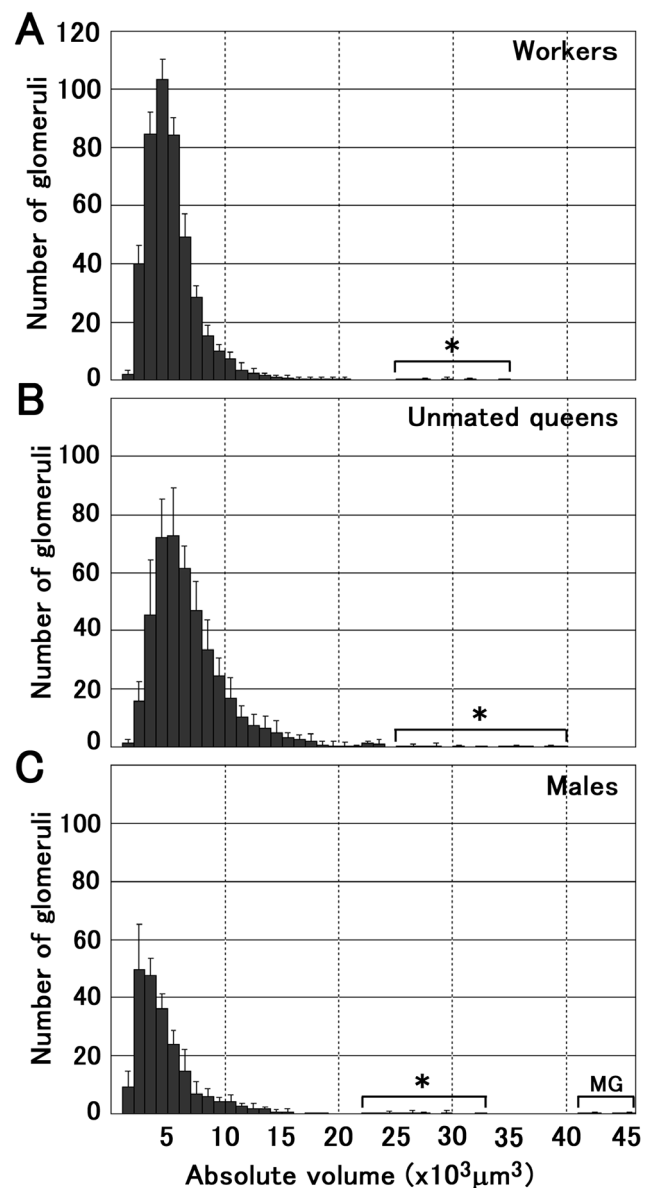


Fig. 8. Histograms of mean values of absolute glomerular sizes in (A) workers ($n=6$), (B) unmated queens ($n=6$), and (C) males ($n=6$). Ranges of most glomerular sizes were $4\text{--}5 \times 10^3 \mu\text{m}^3$ in workers (A), $5\text{--}6 \times 10^3 \mu\text{m}^3$ in unmated queens (B), and $2\text{--}3 \times 10^3 \mu\text{m}^3$ in males (C). An asterisk (A, B, C) shows a minor cluster of large glomeruli including an elongate, curved glomerulus, a dorsalmost large glomerulus, and large glomeruli near the entrance of the antennal nerve. MG, cluster of macroglomeruli in males.

in workers than in unmated queens (Fig. 9A, B). Absolute volumes of glomeruli in workers were smaller than those in unmated queens, but relative sizes of glomeruli in workers were larger than those in unmated queens (Figs. 8, 9).

The total volume of glomeruli in unmated queens was about 1.38 times larger than that in workers, while the inter-glomerular space was about 2.01 times larger in unmated queens than in workers. Furthermore, relative values of total glomerular volumes to whole antennal lobe volumes were larger in workers (0.51) than in unmated queens (0.43),

while relative values of interglomerular spaces to whole antennal lobe volumes were smaller in workers (0.37) than in unmated queens (0.46) (Table 1). These measurements suggest that individual glomeruli are more tightly packed in

the antennal lobe of workers than in the antennal lobe of unmated queens.

DISCUSSION

General features of the antennal lobe structure in the ant *Camponotus japonicus*

Volumetric analyses of major brain neuropils in *Camponotus japonicus* demonstrated that the volume ratio of each neuropil in society members (Figs. 3, 4) (Kubota et al., 2006) was fundamentally similar to those in *Camponotus ocreatus* (Ehmer and Gronenberg, 2004). In this study, we obtained further insight into the difference between antennal lobe structures in society members.

In the ant *Camponotus japonicus*, glomeruli in the ventral region were relatively small, while those in the dorsal region were large (Figs. 5, 6). Generally, glomeruli tend to be progressively larger from the ventral to dorsal region of the antennal lobe in most insects (Ignell et al., 2001). The absolute volumes of most glomeruli were $4\text{--}5\times10^3\text{ }\mu\text{m}^3$ in workers, $5\text{--}6\times10^3\text{ }\mu\text{m}^3$ in unmated queens and $2\text{--}3\times10^3\text{ }\mu\text{m}^3$ in males (Fig. 8). The absolute volume of the antennal lobe was smallest in males and largest in unmated queens (Figs. 3, 4) (Ehmer and Gronenberg, 2004; Kubota et al., 2006). Although the absolute volume of the antennal lobe in unmated queens was about 1.6 times larger than that in workers (Fig. 4, Table 1), the numbers of glomeruli in workers and unmated queens were almost the same (Fig. 7). Furthermore, relative glomerular sizes to the antennal lobe volume in workers were larger than those in unmated queens (Fig. 9). To solve the problem of space limitation, two strategies were seen in the antennal lobe structure of the worker. First, absolute volumes of individual glomeruli of workers were generally smaller than those of unmated queens: the number of glomeruli over $5\times10^3\text{ }\mu\text{m}^3$ in volume was much smaller in workers than in unmated queens (Fig. 8A, B). Second, individual glomeruli were packed tightly in the antennal lobe: the relative glomerular size against the antennal lobe volume was larger in workers than in unmated queens (Fig. 9), and the interglomerular space was smaller in workers than in unmated queens (Table 1).

The large elongate, curved glomerulus and the large spherical glomerulus were located in the dorsalmost region of the antennal lobe close to the dorsal lobe in all members (arrows; Fig. 5). In the honeybee, a deformed glomerulus has been detected in the dorsalmost region of the antennal lobe (Kirschner et al., 2006). In the cockroach, two types of glomeruli with an elongate, curved shape in the dorsalmost region of the antennal lobe receive specifically thermosensory or hygrosensory afferents (Nishikawa et al., 1995;

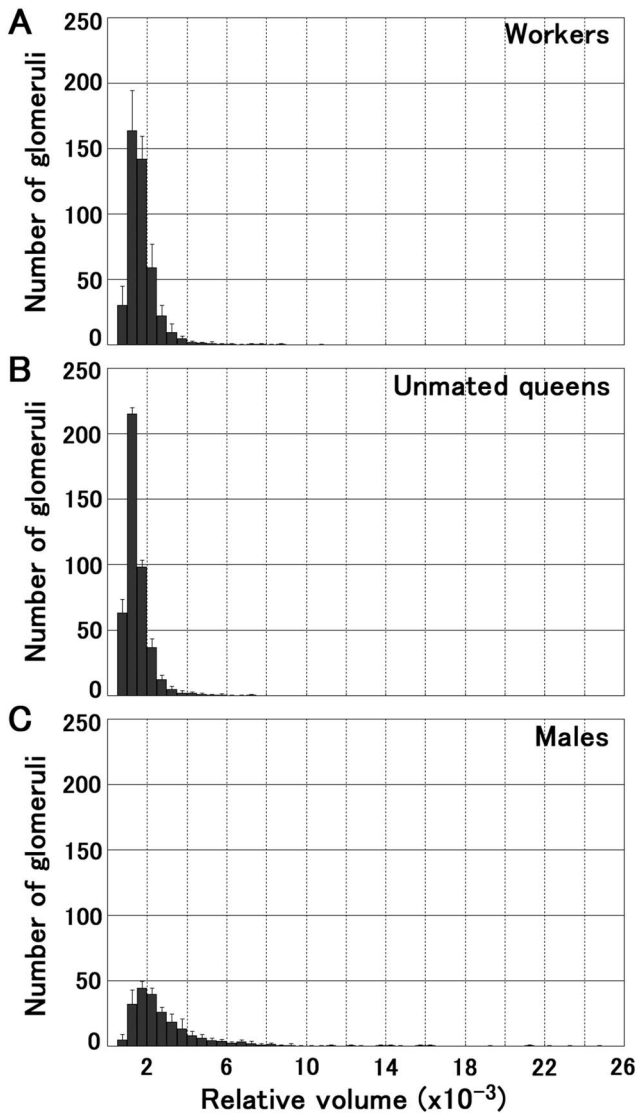


Fig. 9. Histograms of mean values of relative sizes of glomeruli to antennal lobes in (A) workers (n=6), (B) unmated queens (n=6), and (C) males (n=6). Ranges of relative sizes of most glomeruli were $1\text{--}1.5\times10^{-3}\text{ }\mu\text{m}^3$ in workers (A) and unmated queens (B) and $1.5\text{--}2\times10^{-3}\text{ }\mu\text{m}^3$ in males (C).

Table 1. Measurements of four characters in workers and unmated queens of *Camponotus japonicus*. Absolute volumes ((mean \pm SD) $\times10^6\text{ }\mu\text{m}^3$, n=6) are whole antennal lobes, cores of antennal lobes, total glomeruli, and interglomerular spaces. Relative values are total glomeruli (gl) or interglomerular spaces (space) to whole antennal lobes (AL). Ratios are the respective values of unmated queens (U) to those of workers (W).

	Absolute volume (mean \pm SD) (n=6)				Relative value	
	Whole antennal lobe	Core of antennal lobe	Total glomeruli	Inter-glomerular space	gl/AL	space/AL
Workers	3.79 \pm 0.37	0.47 \pm 0.03	1.93 \pm 0.11	1.39 \pm 0.37	0.51	0.37
Unmated queens	6.12 \pm 0.88	0.67 \pm 0.10	2.66 \pm 0.34	2.79 \pm 0.48	0.43	0.46
Ratio of U/W	1.61	1.43	1.38	2.01	0.85	1.24

Nishino et al., 2003). Although physiological identification of these glomeruli is clearly needed in the ant or honeybee, such sensory modalities may be well conserved across genders and insect species and represented in the dorsal margin of the antennal lobe.

A macroglomerulus has been reported at the entrance of the antennal nerve in the antennal lobe in the large worker of the leaf-cutting ants *A. vollenweideri* and *A. sexdens* (Kleineidam et al., 2005). We observed a relatively large glomerulus near the antennal nerve entrance in all members of the carpenter ant. However, further investigation is needed to confirm whether these glomeruli in carpenter ants are identical to those in leaf-cutting ants.

Comparison of the number of glomeruli in ants and other insect species

The present study revealed that both workers and unmated queens of *Camponotus japonicus* had over 430 glomeruli in the antennal lobe (Fig. 7). Among insects, the largest number of antennal lobe glomeruli has been reported in the locust *Schistocerca gregaria*, reaching about 1000 microglomeruli (Hansson, 1999). In contrast to the single olfactory receptor axons that project to single glomeruli seen in most insects (Hansson, 1999), including the ant (Nishikawa, unpublished observation), those in *Schistocerca* project to up to six glomeruli (Ignell et al., 2001). Thus, the actual number of glomeruli in *Schistocerca* is estimated to be less than 200. These results show that the number of glomeruli in the ant *Camponotus japonicus* appears to be the largest in insects so far. Such a large number of glomeruli in the ant may reflect the broad spectrum of chemicals received by chemosensory neurons on the antennae and the complexity of sensory processing in the primary olfactory centers.

In comparison with other ant species, the number of glomeruli of *Camponotus* is much larger than the numbers in large workers of leaf-cutting ants: 243 ± 19 in *A. vollenweideri* and 189 ± 16 in *A. sexdens* (Kleineidam et al., 2005). *Atta* leaf-cutting ants are food specialists, whereas most *Camponotus* species are food generalists. The large number of glomeruli seen in *Camponotus* may reflect the requirement to discriminate many food odors compared to leaf-cutting ants.

Sexual dimorphism in glomerular number

In the male antennal lobe of the ant, a macroglomerulus was found to be located at the entrance of the antennal nerve (Figs. 5, 6). This macroglomerulus was not observed at the entrance of the antennal nerve in workers or unmated queens. Although the function of the macroglomerulus has not yet been determined, it is probably related to the processing of sex pheromone, as indicated in other insect species (Arnold et al., 1985; Boeckh and Tolbert, 1993; Hansson, 1999; Kondoh et al., 2003; Kurtovic et al., 2007).

In honeybees, the worker has 156–176 glomeruli and the drone has 103 glomeruli (Arnold et al., 1985; Flanagan and Mercer, 1989; Galizia et al., 1999). Male ants had about 215 glomeruli, about a half the number of those in workers and unmated queens (Fig. 7). Such a large difference in ordinary glomeruli between castes or genders has not been reported before in insects. Worker ants and unmated queen

ants are genetically females (Hölldobler and Wilson, 1990) and have almost the same numbers of glomeruli (Fig. 7). Therefore, we conclude that there is sexual dimorphism in the society members of *Camponotus japonicus*.

In the calyx of the mushroom body, the relative volume of lip region that receives olfactory input is reduced in males of *Camponotus ocureatus*; it is more than 50% smaller than that in females (Ehmer and Gronenberg, 2004). This decrease in volume of the lip region is in proportion to the decrease in glomerular number seen in this study.

Since we did not stain sensory tracts, the clustering manner of glomeruli remains obscure. Nevertheless, it is very likely that the number of glomeruli located in the postero-medial region of the antennal lobe is greatly decreased in males (Fig. 6). Behaviorally, males do not perform any of the colony's tasks (Hölldobler and Wilson, 1990). It would therefore be interesting if the postero-medially located glomeruli seen in workers and unmated queens are related to the processing of chemicals used for the colony's tasks.

Functional and morphological identification of glomeruli in ants should be the focus of future studies.

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