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Central projections of the sensory hairs on the gemma of the ant *Diacamma*: substrate for behavioural modulation?

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Abstract. In the ant genus *Diacamma*, all workers eclose from their cocoons with little clublike thoracic appendages, called gemmae. Whether these gemmae are mutilated determines individual behaviour, and ultimately reproductive role, in two of the three species examined. The gemmae are covered with sensory hairs, which probably serve a mechanoreceptive function. The sensory afferents arising from these hairs were stained and traced into the central nervous system (CNS). They feature widely distributed collaterals invading all three thoracic ganglia as well as the suboesophageal and the second abdominal ganglia. The multisegmental arborization pattern of the gemma afferents is very similar to that of wing-hair afferents of other ants (queens and males) or other insects in general. This implies that gemmae and wings are homologous structures. We discuss the morphology of the gemma afferents with respect to their possible involvement in the behavioural changes associated with mutilation. The neuronal processing may be modulated by (1) the decrease of sensory input onto interneurons (suggested by the afferents' extensive arborizations); or (2) by the effect of neuromodulatory substances (suggested by the finding that terminals occur within the cell body rind of the ganglion).

Key words: Insect hair sensilla – Mechanoreception – Thoracic ganglia – Wings – Neuromodulation – *Diacamma australe*, *D. rugosum*, *D. vagans* (Insecta)

Introduction

The ants (together with the termites, some bees and some wasps) are unique among animals because of the occurrence of two morphological classes of female adults, queens and workers, that differentiate during larval development. The morphological differences between queens and workers are associated with division of labor within the colonies. Ant queens are specialized to establish new colonies and to reproduce; in most species they have wings that they shed after being mated. Ant workers are usually sterile and they always are wingless. In a small number of species belonging to the phylogenetically primitive ant subfamily Ponerinae, queens have disappeared and some of the workers mate and reproduce instead (Peeters 1991). Thus in the tropical genus *Diacamma* (comprising 20–30 species), queens never exist. A unique characteristic of the workers in *Diacamma* is the occurrence of a pair of tiny clublike mesothoracic appendages (Tulloch 1934; Bitsch and Peeters 1991). These structures, which have not been found in any other ant genus, have been termed "gemmae" (Peeters and Billen 1991).

All workers of *Diacamma* have gemmae when they eclose from their cocoons, and in *D. vagans* these are retained throughout adult life (Peeters et al. 1992). In contrast, in *D. rugosum* (the only species occurring in Japan; currently under taxonomic revision) and in *D. australe*, the gemmae are bitten off soon after emergence. Only one worker retains them in each colony, and this individual is inseminated and monopolizes egg laying (Fukumoto et al. 1989; Peeters and Higashi 1989). She aggressively removes the gemmae in all newly eclosed workers. Mutilation of the gemmae is associated with changes in individual behaviour. Unmutilated workers behave aggressively towards nestmates, but if their gemmae are surgically removed, they become timid and lose their dominant position in the social hierarchy (Peeters and Higashi 1989). The retention of the gemmae also appears to be essential for mating: mutilated workers are always virgin, and during laboratory experiments males copulated only with workers having gemmae. While it is clear that the gemmae play a crucial role in reproductive control in *D. australe* and *D. rugosum*, the physiological basis for the behavioural changes triggered by mutilation remain poorly understood.

In *D. australe*, the gemmae are completely filled with about 500 unicellular glands, which are individually connected via minute ducts to pores on the outer surface of the gemma (Billen and Peeters 1991; Peeters and Bil-

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len 1991). These glandular cells presumably release pheromones to the outside, which have been speculated to function as male attractants. However it has not yet been possible to analyze the chemical composition of these pheromones, and the functional significance of the putative gemma pheromones awaits further investigations.

Although the internal and external morphology of the gemma has been described in some detail, no mention has been made of one fact that is obvious from the various published scanning electron micrographs: the gemmae are covered with hairs. As in other insects, these might be sensory hairs with chemoreceptive or mechanoreceptive functions. If they are indeed sensory structures, then we expect that the sensory cells associated with them send their axons (sensory afferents) into the central nervous system (CNS). Mutilation of the gemmae would then change the ongoing sensory input to the CNS of an individual. Furthermore, mutilation would doubtlessly cause degeneration of the sensory axons since their cell bodies are located inside the gemma. Thus mutilation of the gemma is associated with two distinct effects: an altered sensory input to the CNS, and a halt in the release of a pheromone. Both effects may result in behavioural changes.

Here we demonstrate that (1) the hairs on the gemmae are indeed innervated, (2) the axons of these putative mechanosensory cells exhibit a characteristic pattern of arborization within the CNS that is reminiscent of the afferent projections from sensory hairs on the wings of other ants, and (3) some collaterals of the gemma afferents project into the cell body layer surrounding the neuropil of the CNS.

Materials and methods

Experimental animals

Specimens of *D. australe* originated from a colony excavated near Townsville, north Queensland, Australia. *D. rugosum* was collected in Nakijin castle, Okinawa Island, Japan; *D. vagans* was collected in Masunigodi, Tamil Nadu, south India. To produce unmutilated workers, cocoons of *D. australe* and *D. rugosum* were placed together with small groups of mutilated workers, thus avoiding mutilation by the dominant reproductive worker. In addition, males and queens of *Ectatomma ruidum* (collected at Barro Colorado, Costa Rica) and *Camponotus ligniperda* (collected in forests around Würzburg, Germany) were examined to compare afferents of wing sensilla with the gemma afferents.

Counting of sensilla

Sensilla on the gemmae were counted from scanning electron micrographs (*D. australe*, *D. vagans*, *D. rugosum*) or directly under the microscope employing the green autofluorescence of the cuticular structures under blue epifluorescence excitation (*D. vagans*, *D. rugosum*). For scanning electron microscopy (SEM), ants were immobilized by cooling, fixed in phosphate-buffered (pH 6.8) 2.5% glutaraldehyde, dehydrated in a graded series of acetones, and critical-point dried (acetone/carbon dioxide). Some specimens that had been kept in 70% ethanol for more than a year were airdried at 30° C. All specimens were gold-coated and observed using a Zeiss DSM 962 SEM.

Staining procedures

Workers of *D. rugosum* and *D. vagans* were immobilized by cooling and mounted sideways with a wax/resin mixture so as to allow access to the gemma. Some of the hairlike structures on the gemma were carefully scraped off with a sharpened insect pin or with a broken glass microelectrode, avoiding any other damage to the fragile appendage. Next, the gemma and surrounding cuticle were covered with vaseline except for a small area on the gemma, thus enclosing 4–15 bases of the cut sensilla in a vaseline well. This well was then filled with an aqueous solution of either cobalt-chloride (5%) or 2% -5% Biocytin (Fluka) or Neurobiotin tracer (Vector). A positive current was routinely applied to the tracer well for 1–3 h using a thin silver wire connected to a constant current source (90 nA) or to a 9V battery (negative electrode on the ant's gaster). Alternatively, the dye solutions were applied using glass capillary micropipettes. The overall diffusion times ranged from 2–12 h, typically 5 h. The longer diffusion periods sometimes produced good staining event without the use of current. Subsequently, the ants were processed according to the tracer used (cobalt or Biocytin).

Male *Diacamma* and males and queens of *Ectatomma* were mounted and treated as above in order to stain the hairlike sensilla on the wings.

Cobalt histology. The thoraces of ants that had been treated with cobalt-chloride were cut open and immersed for 3 min in 70% ethanol containing ammonium sulphide (about 0.5%) to precipitate cobalt as sulphide. Afterwards the thoraces were fixed in Bouin's fixative or Carnoy's fixative, the ganglia were dissected out and silver-intensified (Strausfeld and Obermayer 1976; Bacon and Altman 1977). The ganglia were then dehydrated in graded ethanols, embedded in Durcupan (Fluka) and sectioned at 10–25 µm on a sliding microtome.

Biocytin histology. The histological procedure to visualize the afferents filled with Biocytin (or Neurobiotin tracer) was based on Horikawa and Armstrong's (1988) original method and on a protocol for marine crustacea (Schmidt and Ache 1990). The present procedure was developed to work on intact insect CNS (Gronenberg and Strausfeld 1992) and has been adapted for backfilling sensory afferents from their sensilla.

After the tracer diffusion period the thorax was opened and immersed in 4% formaldehyde in 0.2 M Sorensen's phosphate buffer or in 0.16 M Karnovsky's cacodylate buffer (buffer osmolarity adjusted with 3% sucrose; pH 7). After 2–4 h fixation at room temperature (or overnight at 4° C) the ganglia were removed and washed in buffer for at least 4 h. Then the tissue was incubated for 8–24 h in buffer containing the avidin-conjugated horseradish peroxidase (HRP; Vectastain ABC-kit; Vector Labs.) and 0.5% Triton-X-100. Instead of using a high concentration of Triton X-100, in some cases the ganglia were dehydrated in ascending ethanols, transferred into xylene and then rehydrated in descending ethanols before incubation in the avidin-conjugate.

After this incubation the tissue was rinsed thoroughly in buffer for 4 12 h before it was presoaked in the DAB solution (0.05% diaminobenzidine and 0.5% Triton X-100 in 0.2 M phosphate buffer or 0.16 M cacodylate buffer, pH 7). After presoaking for 3 h in the dark at 4° C the tissue was transferred to the same DAB solution containing 0.01% hydrogen peroxide in which it was processed in the dark for 3 h at 4° C and then for an additional 1 h at 20° C. Subsequently the tissue was rinsed in buffer overnight and then incubated in the dark in 1% osmium tetroxide in 0.16 M cacodylate buffer for 1 h at 4° C and for an additional hour at 20° C. Tissue was afterwards rinsed in buffer, then distilled water, dehydrated in graded ethanols, transferred into Araldite (Fluka Durcupan) and after polymerization blocks were serially sectioned at 10–20 µm on a sliding microtome. Reconstructions of material containing cobalt or DAB reaction product were made from serial sections using a camera lucida attachment to the microscope at an initial magnification of 560- 1400 ×.

Alternatively, the fixed tissue was incubated in a solution of avidin conjugated to either Lucifer yellow or Cascade blue (Molecular Probes). The conjugates were reconstituted in 0.1 M bicarbonate buffer with 2 mM sodium azide added to give 'stock solutions' of 0.1%. When frozen, these stock solutions worked well for more than a year. For use, they were diluted in phosphate- or cacodylate buffer containing 0.5% Triton X-100 to give a final concentration of 0.0025% conjugate. Ganglia were incubated in this solution for 8–20 h, repeatedly washed in buffer, dehydrated in ethanols, embedded in Spurr's (1969) embedding medium, polymerized and sectioned at 10 µm–15 µm. Neurons labelled with Lucifer yellow or Cascade blue were viewed under epifluorescence illumination using the appropriate excitation and barrier filters (Zeiss Axiophot), photographed on Kodak Ektachrome 400 and graphically reconstructed from the slides.

Results

External morphology

The gemmae are clublike appendages of the dorsolateral mesothorax (Fig. 1a–f) that are reminiscent of dipteran or strepsipteran halteres. They fit smoothly into a thoracic cavity ("gemmarium," Billen and Peeters 1991; Fig. 1c, f). The length of the main body of the gemma ranges from 290 µm (*D. rugosum*) to 360 µm (*D. australe*), the width from 190 µm to 230 µm, and the stalk length from 125 µm to 140 µm.

In all three *Diacamma* species examined, the gemma's surface facing the gemmarium has soft and membranous cuticle whereas the cuticle on the stalk and upper surface of the gemma is stiff and much thicker. In *D. rugosum* and *D. australe* the inner surface of the gemma is ridged (Fig. 1e) while in *D. vagans* this membrane looks rather smooth (arrow in Fig. 1f). In the region of the mesonotum/mesopleurum transition, the stalk is connected to the lateral mesothorax by flexible cuticle so that it can (passively) be bent outwards by about 60° (Fig. 1d) without breaking (it has been bent too far in Fig. 1e, hence the stalk is partly broken). In the pupae the gemmae protrude from the body wall (Peeters and Billen 1991), but in adults they are always recessed in their gemmarium. No muscles insert at the gemmae, hence they cannot be moved voluntarily.

The ventral edge of the gemmarium carries thin, hairlike structures in the vicinity of the stalk (arrows in Fig. 1c). These structures do not exhibit any specialization at their bases, and we infer that they are trichomes, i.e. noninnervated cuticular structures. Similar trichomes were found inside the gemmarium: only one trichome in *D. rugosum*, about 10–15 trichomes in *D. vagans*, and 20–30 in *D. australe*. Attempts to backfill these structures in the same way as sensory hairs (see Materials and methods) did not reveal any afferent sensory fibres originating from those structures, thus confirming that they are trichomes.

Hairlike sensilla

Unlike the gemmarium and the gemma surface facing it, the outward surface of the gemma carries hairlike structures, on which we focus in this paper. The length of these structures ranges from 40 µm to 150 µm, most being about 60 µm long. Since their bases are cuplike (arrows in Fig. 1h–j), they seem to be mechanoreceptive sensilla. They are bent towards the gemma and run parallel to its surface (Fig. 1f–h). The sensilla on the antennae and on other regions of the body surface have similar directional characteristics, which is normal for mechanoreceptive sensilla in other insects.

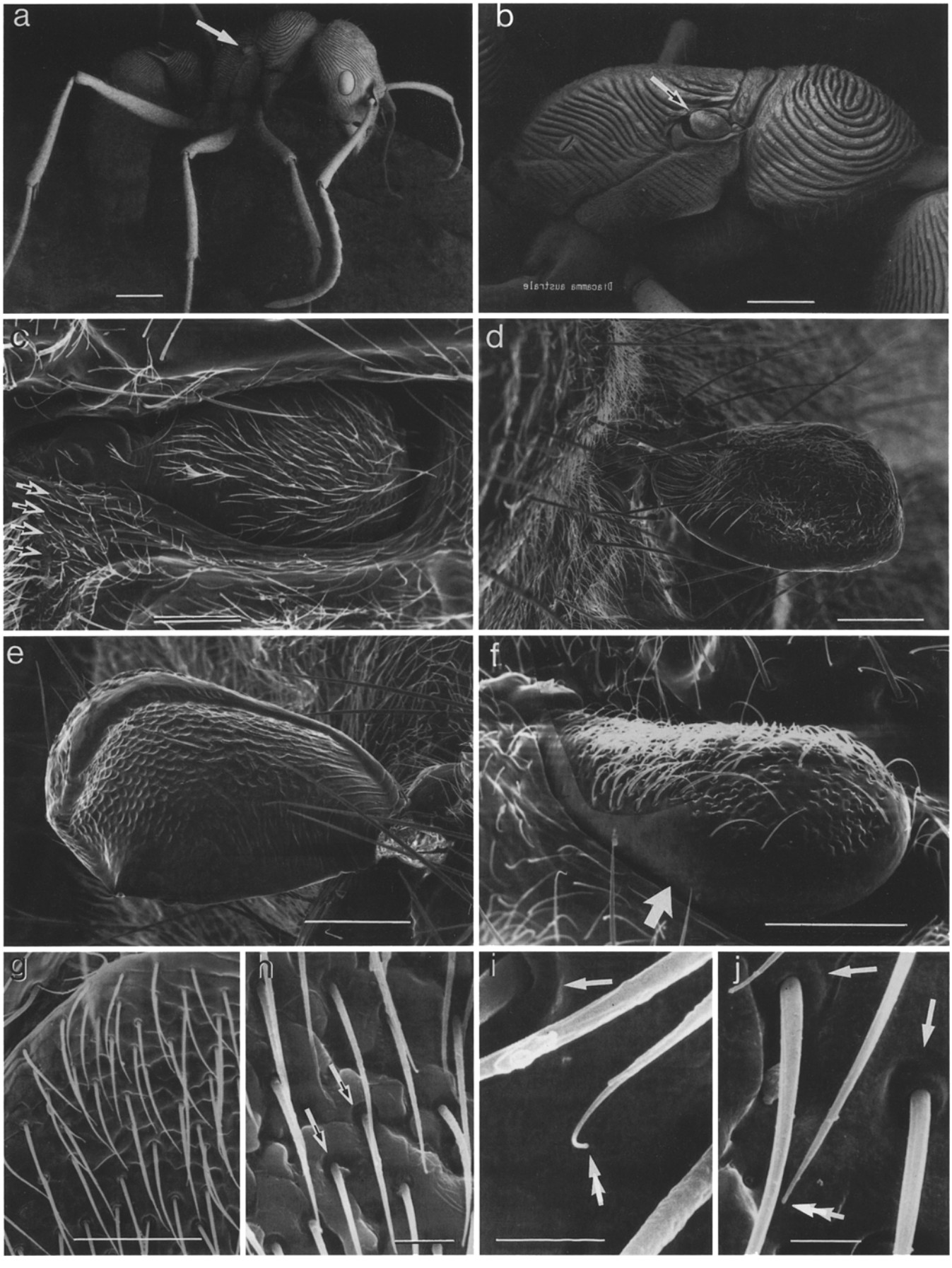
All sensilla in *D. rugosum* and some sensilla in *D. australe* feature recurved endings pointing towards the gemma (Fig. 1i). The tips of all sensilla on the gemmae in the three species end in a little swelling (Fig. 1i, j). Neither on the rounded ends of the sensilla nor on their shafts did we find any pores that would suggest a chemosensory function. The cuticle in the vicinity of the gemma also carries hairlike sensilla that look similar to those on the gemma. Some of these, however, are much longer (200–350 µm; Fig. 1c, d). *Diacamma* carries similar long sensilla all over her body, as can be seen in Fig. 1b.

In the three species examined, the stalk of the gemma carries 6–16 sensilla. In the present context, the numbers of sensilla on the main body of the gemma are of particular interest because in *D. rugosum* and in *D. australe* this part is lost during mutilation (in some workers the stalk of the gemma remains on the thorax). We counted 77 ± 13 sensilla (mean $\pm$ standard deviation) in *D. rugosum* ($n=7$), 73 ± 13 in *D. australe* ($n=5$), and 97 ± 19 in *D. vagans* ($n=4$).

Sensory projections

In both *D. rugosum* and *D. vagans*, backfills with cobalt chloride, Lucifer yellow, and Biocytin or Neurobiotin, reveal that all the sensilla on the gemmae give rise to afferent axons, thus confirming their sensory nature. However, we could not unambiguously detect the position of most cell bodies or show the path of the sensory axons within the main part of the gemma for two reasons: (1) sectioning of the gemma was difficult because of an often insufficient impregnation of this tiny, cuticle-enclosed structure by the embedding medium; and (2) the gemma's surface was often contaminated with tracer, and in most cases internal structures could not be seen through the cuticle. Some of the successful sections revealed the same unicellular glands found by Billen and Peeters (1991) in *D. australe*, as well as sensory axons inside the stalk as demonstrated in Fig. 2b. Fig. 3f in Billen and Peeters (1991) shows a cross section through the nerve in the stalk region of the gemma of *D. australe*. From this transmission electron micrograph kindly provided by Johan Billen, we were able to count about 70 axons in this region, which is consistent with the numbers of sensilla mentioned above.

The bundle of afferent axons from the gemma projects ventroposteriorly towards the thoracic ganglia (which are unfused in ants). Together with other axon bundles that supply dorsolateral cuticle regions and probably also mesothoracic muscles, the gemma afferents contribute to a nerve that enters the pro-mesotho-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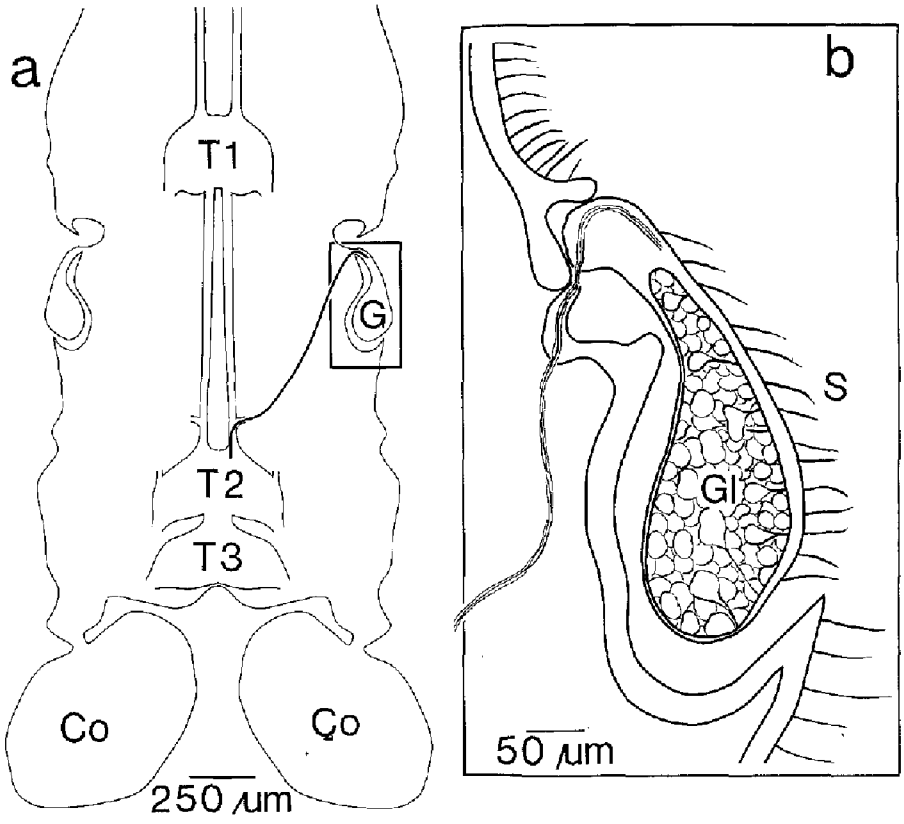


Fig. 2. Schematized horizontal cross section of the thorax (**a**) of a *D. rugosum* worker showing relative size and position of the gemma (*G*) and of the thoracic ganglia *T1* (prothoracic ganglion), *T2* (mesothoracic ganglion), and *T3* (metathoracic ganglion). Nerve connecting gemma and CNS indicated by *bold line*; *box* in **a** represents area depicted in **b**. *Co* Coxae of the hindlegs; *Gl* glandular cells inside the gemma; *S* hairlike sensilla

racic connectives about 100 µm anteriorly with respect to the mesothoracic ganglion (Fig. 2a). This nerve, which we will here refer to as the gemma nerve, is homologous to the wing nerve in male *Diacamma* (see below).

Afferent arborizations in the thoracic ganglia

After entering the pro-mesothoracic connectives the afferent axons project into the mesothoracic ganglion where they branch profusely (Figs. 3, 4a, b), giving rise to a plexus of densely interwoven collaterals (Figs. 3, 4d) with bleblike swellings. These tangles of fibres (Fig. 4d, e, g) are so dense that from the preparations (each comprising at least four or five afferents), single fibres cannot be resolved. The fibre plexus reaches up to the dorsoanterior margin of the mesothoracic neuropil. From this dense plexus arise varicose collaterals, some of which extend ipsilaterally to the lateral margins of the ganglion (Fig. 3), others project to the contralateral side of the neuropil. The collaterals that extend to the metathoracic ganglion also originate from the plexus but generally they are not a simple continuation of the afferent axons that enter the mesothoracic ganglion. The plexus also gives rise to collaterals that project up to the prothoracic ganglion (Figs. 3, 5, 7b; arrow in Fig. 4d).

The path of a single gemma afferent could be traced through the mesothoracic ganglion (Fig. 7c). This particular axon was easily recognized and thus resolved from accompanying afferents by its larger diameter and beadlike varicosities. Unlike its companion axons (Fig. 7b) it remained in the ipsilateral hemiganglion and, in contrast to the majority of afferents described so far, one collateral of this thick axon continued directly towards the metathoracic ganglion. Similar direct meso-metathoracic axonal projections are shown in the color photomontage (Fig. 6); here axons emerge from the mesothoracic plexus, which is only partly shown.

In the metathoracic ganglion the axon collaterals are generally more restricted to the central neuropil and do not extend laterally as far as they do in the mesothoracic ganglion. In particular, contralateral branches are shorter or are missing altogether (Fig. 3, 5, 6). In ants, the metathoracic ganglion is fused with the first abdominal neuromere (Markl 1966). As can be seen in Figs. 3 and 6, beaded collaterals project posteriorly into the neuropil

Fig. 1a–j. Scanning electron micrographs of gemmae and sensilla of *Diacamma*. Side view of a *D. australe* worker (**a**) and of its thorax (**b**), *arrows* point at the gemma. Gemma of *D. rugosum* inside its gemmarium with *arrows* pointing at a region covered by trichomes (**c**); gemmae of *D. australe*, bent out of the gemmarium and showing the surface facing outwards (**d**) and the structured surface facing the gemmarium (**e**) of the gemma; side view of the gemma of *D. vagans* with the hair-covered outer cuticle of the gemma and its smooth cuticle facing the gemmarium (*arrow*) (**f**); sensilla on the gemmae of *D. vagans* (**g**, **j**) and of *D. rugosum* (**h**, **i**) at different magnifications; *arrows* in **h**–**j** point at cup-shaped hair bases, *double-headed arrows* point at the tips of the sensilla. *Calibration bars*: 1 mm in **a**; 500 µm in **b**; 100 µm in **c**–**f**; 50 µm in **g**; 10 µm in **h**; 5 µm in **i** and **j**

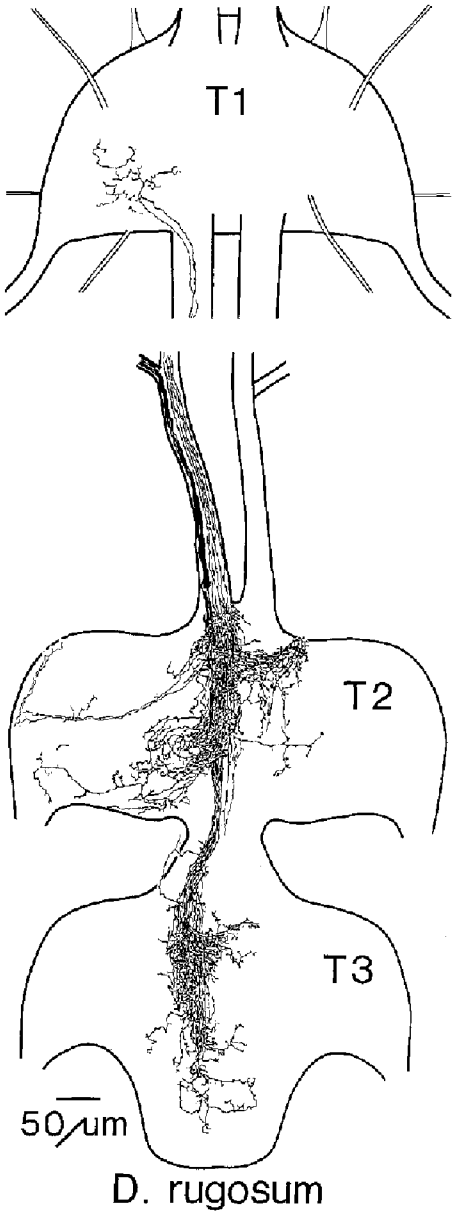


Fig. 3. Horizontal reconstruction of 5–7 afferents arising from sensilla on the gemma of *D. rugosum* and stained with cobalt chloride. Dorsal view; abbreviations as in Fig. 2

pertaining to the first abdominal neuromere. In the prothoracic ganglion we found a similar arborization pattern as in the metathoracic ganglion (Figs. 3, 5, 8): axon collaterals arise from the mesothoracic plexus and project through the pro-mesothoracic connectives, passing the entry point of the gemma nerve and proceeding upwards to the prothoracic ganglion. Again, those ascending collaterals are not simple extensions of the incoming primary afferent fibres or of the branches descending to the metathoracic ganglia. The ascending collaterals mainly branch within the ipsilateral prothoracic hemiganglion (Figs. 3, 5, 8). Generally, we found fewer collaterals in the prothoracic ganglion than in the mesothoracic or in the metathoracic one. This is most probably due to the fact that the pro-mesothoracic connectives are substantially longer than those between the meso- and metathoracic ganglia (Fig. 2a); hence the dyes have to be transported over much longer distances.

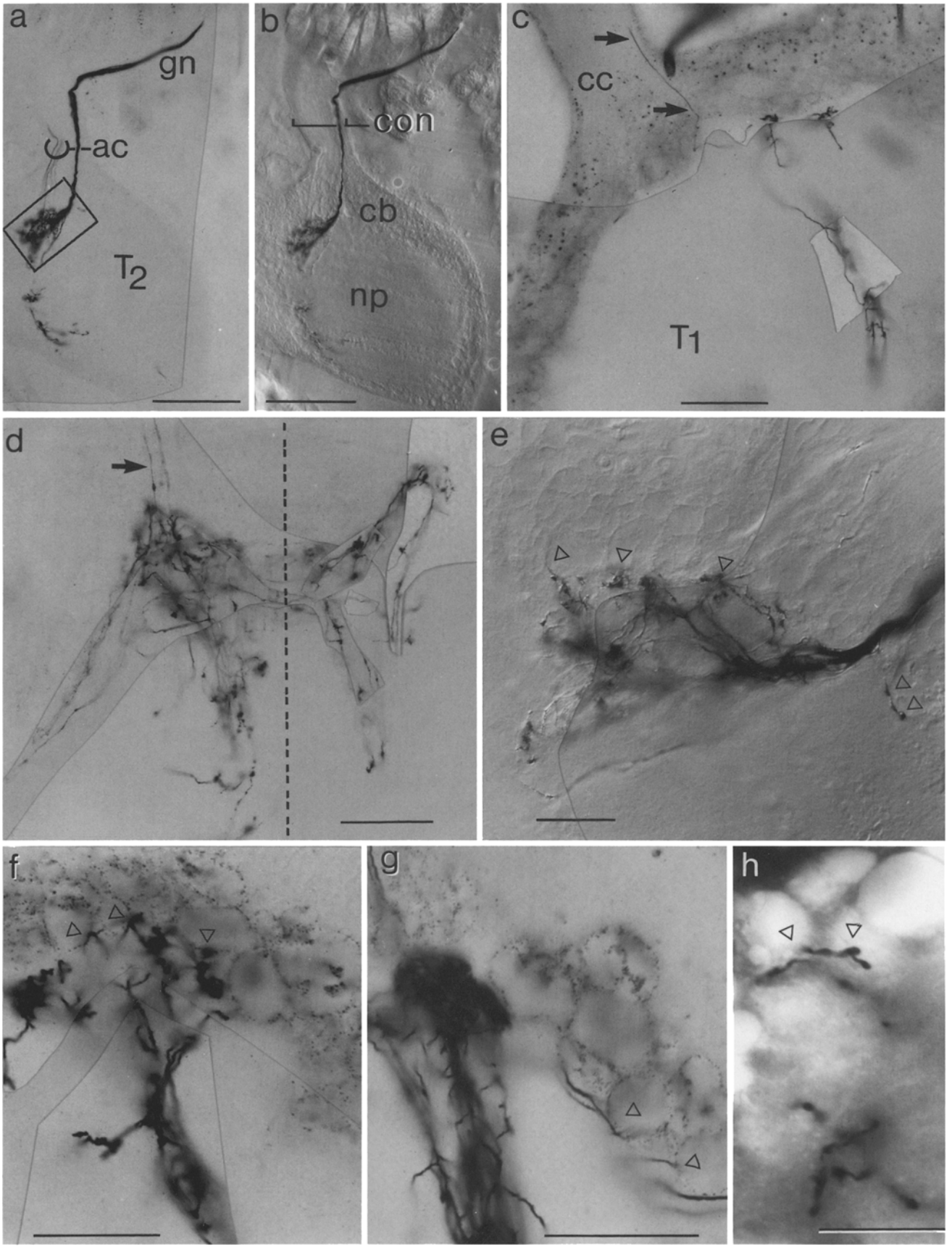
Though there is no direct evidence, we assume that the majority, if not all, of gemma afferents project to all three thoracic ganglia. In the prothoracic as well as in the metathoracic ganglion we found densely interwoven tangles like those described for the mesothoracic ganglion (compare Fig. 4c, prothoracic, and Fig. 4g, metathoracic, with Fig. 4d, e, mesothoracic). As in the mesothoracic ganglion (Fig. 9), in the first and third thoracic ganglia varicose axon collaterals are widely distributed in the dorsoventral plane and reach up to the dorsal margins of the neuropil (see below).

In some of the preparations we found prothoracic axon collaterals ascending through the cervical connectives (Figs. 4c, 8a) or metathoracic collaterals descending through the connective to the petiolus ganglion (the second abdominal ganglion; Fig. 8b), indicating an extension of gemma afferents within the ganglia of at least six body segments (suboesophageal to second abdominal ganglion). For the technical reasons stated above, we assume that many more collaterals extend farther than actually revealed by this staining technique.

Collaterals in the cell body rind

The afferent branches and terminals are not only confined to the neuropil of the thoracic ganglia but they reach the proximal somata of the cell body rind of the ganglia. Fig. 9 illustrates the dorsoventral extent of mesothoracic neuropil contacted by the gemma afferents. The arrowheads in Fig. 9b also indicate regions of putative contact among afferent collaterals and cell bodies in the ventral and lateral regions of the ganglion. The same is illustrated for the prothoracic (Fig. 10a, b) and the metathoracic ganglion (Fig. 4h, 10d). In some cases, the visualization of extraneuropilar collaterals was facilitated by the fact that strong silver intensification

Fig. 4. Photomicrographs (**b, g, h**) and photomontages (**a, c–f**) of horizontal sections (15–20 µm) featuring gemma afferents stained with cobalt chloride (**a–g**; *D. rugosum*) or Biocytin (**h**; *D. vagans*; HRP-conjugated avidin). **a** Path of the afferent axons through the gemma nerve (*gn*) and terminating in the mesothoracic ganglion (*T2*). Collaterals ascending to the prothoracic ganglion (*ac*); *box* outlines area depicted in **e**. **b** Same section as in **a** viewed with Nomarski optics and showing the neuropil (*np*) and cell body (*cb*) regions of the ganglion and afferent axons entering the pro-mesothoracic connective (*con*). **c** Arborizations in the prothoracic ganglion (*T1*) and a single collateral *arrows* ascending through the cervical connective (*cc*). **d** Plexus of bilateral mesothoracic collaterals in dorsomedial neuropil (midline indicated by *broken line*); *arrow* marks collaterals ascending to prothoracic ganglion. **e** Higher magnification (Nomarski optics) of the mesothoracic area *boxed* in **a**; details of varicose axon collaterals close to the cell body rind in the mesothoracic **f** and in the metathoracic ganglion **g**, **h**. *Open triangles* in **e–h** mark some of the cell bodies and point at regions of close proximity (and putative contact) among sensory afferents and cell bodies. *Calibration bars*: **a, b** 100 µm; **c** 30 µm; **d** 40 µm; **e** 30 µm; **f, g** 20 µm; **h** 15 µm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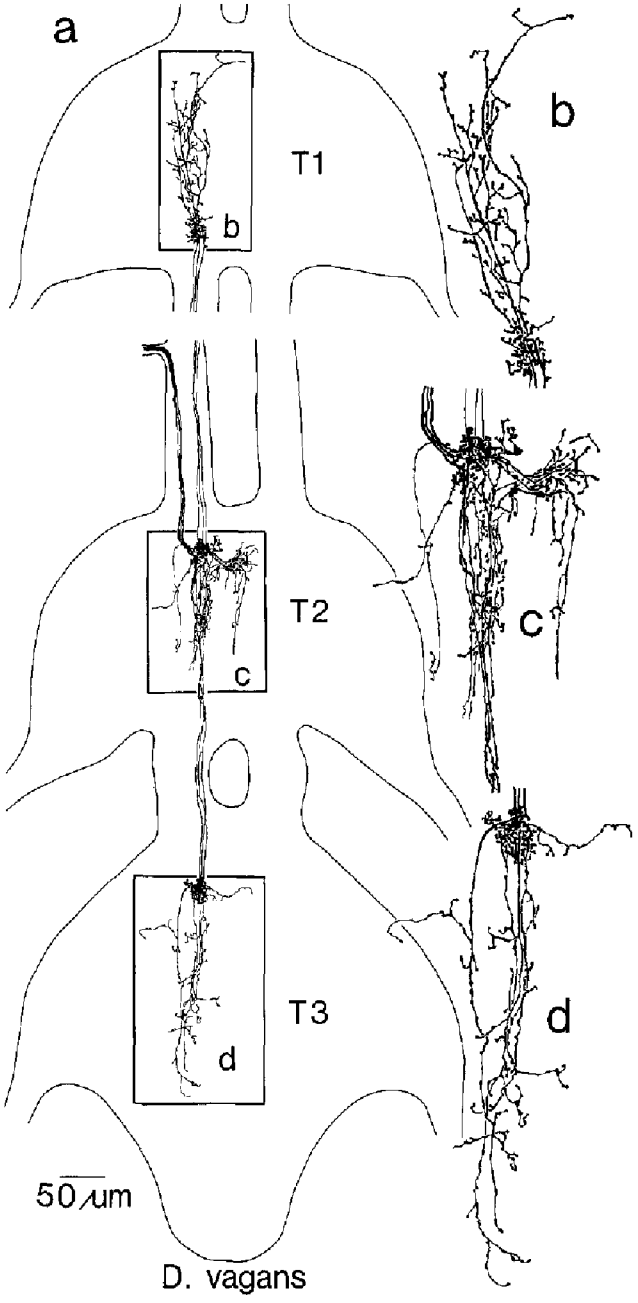


Fig. 5. a Horizontal reconstruction of 3–5 afferents arising from sensilla on the gemma of *D. vagans* and stained with Biocytin (Lucifer-yellow conjugated avidin); **b–d** details of the areas *boxed* in **a**. Abbreviations as in Fig. 3

led to an unspecific silver deposit around many cell bodies, thus simultaneously revealing cell bodies and axon collaterals using normal bright-field illumination (Figs. 4f, g; 10a, b). Fig. 10c gives an example of the depth of cell body layer penetration by the afferent axons. While collaterals do not reach as far into the cell body rind as has been shown for some interneurons in crustaceans (Sandeman et al. 1990), it is clear from the drawings and photomicrographs (Fig. 4e–h) that afferent branches were not restricted to the neuropil proper but travel in the intercellular space between neuronal somata (Fig. 4g) and occasionally seem to make contacts with cell bodies as indicated by the bleblike terminals on (or near) the cell body membrane (Fig. 4e, h).

Interspecific similarities

So far we have not discriminated between the two species examined neuroanatomically. In spite of their behavioural differences (mutilation occurs in *D. rugosum*, but not in *D. vagans*) we did not find any significant anatomical variation. The size of the gemma, the number and shape of the sensilla, the path of the gemma nerve, and the overall arborization pattern of the gemma afferents are all quite similar. Also, we did not observe any notable differences between preparations stained with cobalt or with Biocytin. Although some of the mesothoracic collaterals of *D. rugosum* gemma afferents depicted in Fig. 3 seem to reach more laterally than those shown for *D. vagans* in Figs. 5, 6, and 7, this does not indicate significant dissimilarities. We have other preparations of *D. rugosum* featuring collaterals of more restricted lateral extent. Regarding the pattern of axonal arborization, there was more variation within each species than between species. Because we did not stain all the sensillum afferents in their entirety in any preparation, we ascribe the differences among preparations to variation among the different sensillar projections. Generally, the similarities were much more striking than the dissimilarities. Compare, for instance, the mesothoracic plexus in Fig. 3 (*D. rugosum*) and in Fig. 7 (*D. vagans*). The same is true for the amount and nature of putative contacts between gemma afferents and neuronal cell bodies found in *D. rugosum* as well as in *D. vagans* (compare Fig. 10b and 10c).

Similarities with wing afferents

The 'gemma nerve' of unmutilated *Diacamma* workers was also present in mutilated workers. In the latter the unstained nerve could be recognized by the distinctive way in which it joins the pro-mesothoracic connectives rather than the mesothoracic ganglion proper. The 'gemma nerve' receives other tributary nerves in the periphery, which may explain why it is still present in mutilated *Diacamma* workers in which the gemma afferents presumably are degenerated. It suggests that the nerve carries many more fibres than those of the approximately 70–100 sensilla on the gemma. The term 'gemma nerve' would thus strictly apply only for the distalmost part of the nerve as it emerges from the gemma and before it joins any other tributary nerve.

In addition, we found a comparable nerve in male *Diacamma*, as well as in the two other species examined (*Camponotus ligniperda* and *Ectatomma ruidum*). This nerve is particularly prominent in alate ants (males and queens) and appears to be the wing nerve. Markl (1966) shows a similar nerve in *Formica polyctena*, which he tentatively homologizes with the wing nerve of *Apis mellifica* (nerve II N1). In male *Diacamma* and other alate ants this nerve extends more anteriorly than it does in

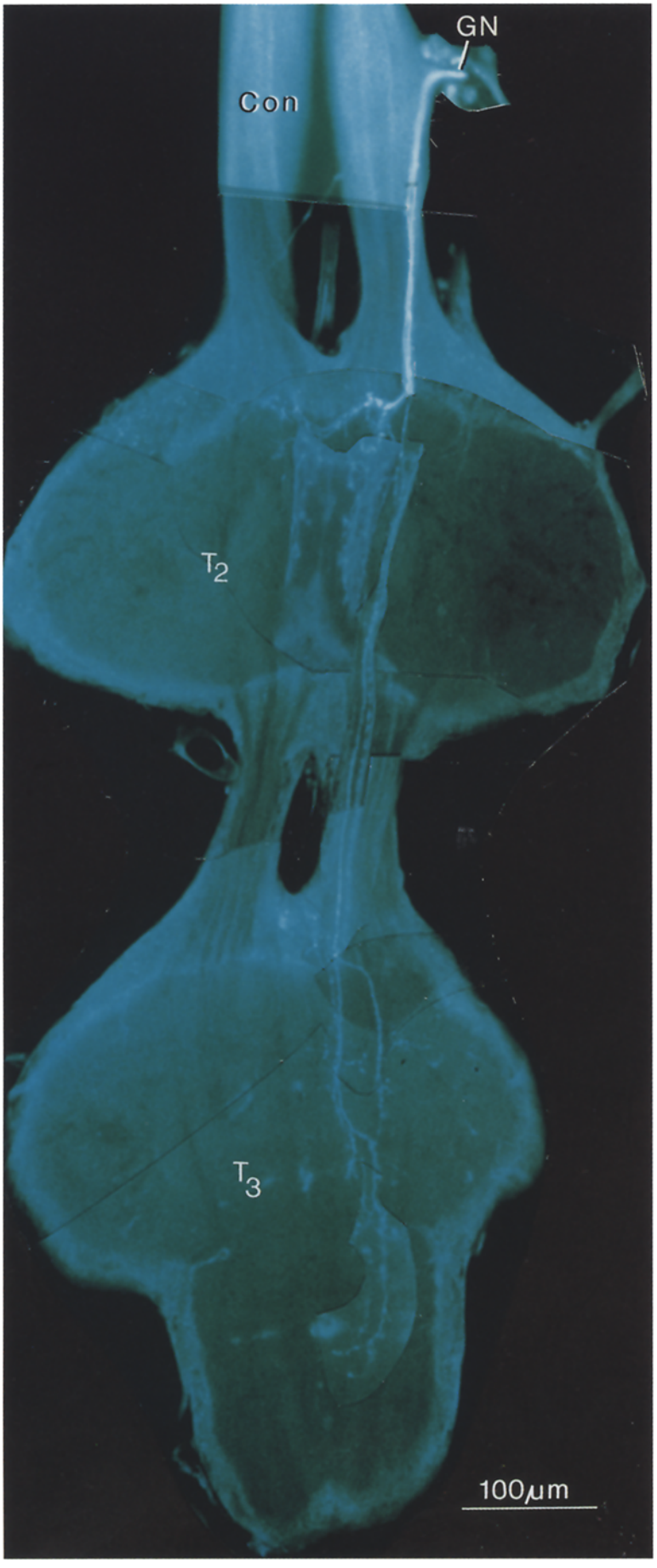


Fig. 6. Montage of photomicrographs (dorsal view) showing meso- and metathoracic collaterals of 4 sensory afferents arising from the gemma of *D. vagans* and simultaneously stained with Biocytin (Cascade-blue conjugated avidin). *Con* Pro-mesothoracic connective; *GN* gemma nerve; *T2*, *T3* meso- and metathoracic ganglion, respectively

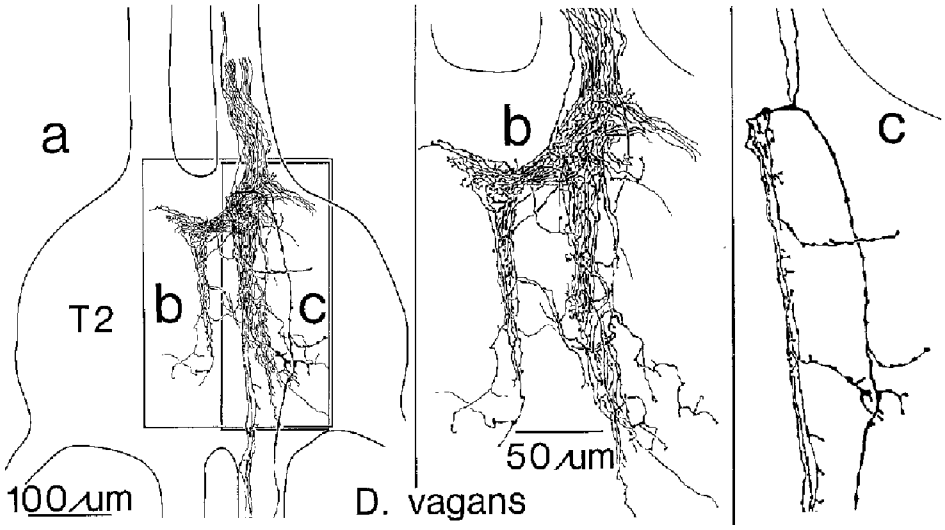


Fig. 7 a–c. Mesothoracic (*T2*) arborizations of sensory afferents from the gemma of *D. vagans*. Reconstructed (dorsal view) from Biocytin avidin – Lucifer-yellow conjugated material. **a** Combination of the many fine collaterals situated centrally and ventrally in the neuropil and drawn separately in **b** and of the dorsalmost thick branches drawn in **c**

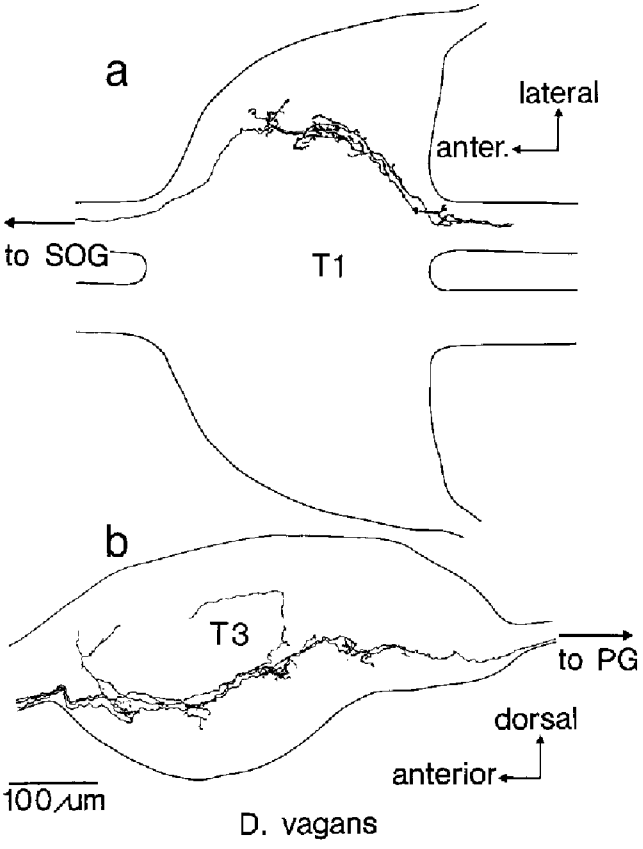


Fig. 8. Afferents from sensilla on the gemma of *D. vagans* in the prothoracic (*T1*) and metathoracic (*T3*) ganglia showing collaterals ascending to the suboesophageal ganglion (*SOG*) in **a** (dorsal view) or descending to the ganglion of the petiole (*PG*) in **b** (sagittal view). Directions *dorsal*, *lateral* and *anterior* indicated by *arrows*

the worker because it projects around the big dorsoventral flight muscles (Fig. 11 a). There can be no doubt, however, that it is the same as the 'gemma nerve.' This nerve is substantially larger in diameter in the winged forms because it carries the sensory axons of the many wing sensilla as well as at least part of the wing muscle motor neurons that are presumably absent in ant workers. No obvious size differences of this particular nerve have been found between *Diacamma* workers and workers of *Camponotus*.

The two examples of afferents originating from hair-like sensila on the wings of male *Diacamma* or an *Ectatomma* queen (depicted in Fig. 11 b and c, respectively) demonstrate that the wing afferents share their basic pattern of arborization within the thoracic ganglia with gemma afferents. This has been confirmed for all the sexes and species studied. While the wing hair afferents do not invade the neuropil as extensively as do the gemma afferents and, particularly, do not project contralaterally within the mesothoracic ganglion (Fig. 11 b, c), their regions of arborization are comparable to those of gemma afferents. Moreover, they also send collaterals to the prothoracic ganglion that originate from a densely interwoven fibre plexus in the dorsoanterior region of the mesothoracic ganglion, as has been established earlier for the gemma afferents. In the prothoracic ganglion, too, wing afferents arborize more sparsely than do gemma afferents (compare prothoracic arborizations of two fibres in Fig. 3 or of three fibres in Fig. 5 with the few collaterals of the many wing afferents in Fig. 12). As demonstrated for gemma afferents in Fig. 8 a, afferents supplying hair sensilla on the wings may also project through the cervical connectives and probably up to the suboesophageal ganglion (Fig. 12), again revealing the similarity between the two groups of sensory afferents. In addition, the axon terminals of afferents originating from hairs on the wings often terminated in the vicinity of cell bodies. So far, we could not, however, unambiguously establish the actual penetration of the cell body rind by these wing afferents of male and female ants.

Discussion

Similarity of gemma and wing afferents

In general, most insect mechanosensory afferents send their axonal projections into the neuromere corresponding to the body segment on which they are located. This has comprehensively been documented for various types

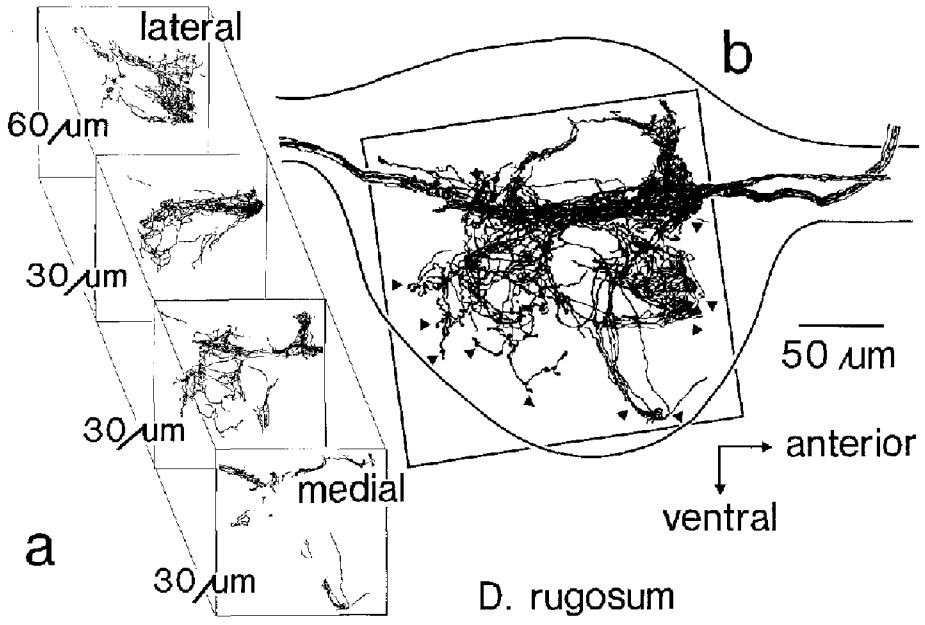
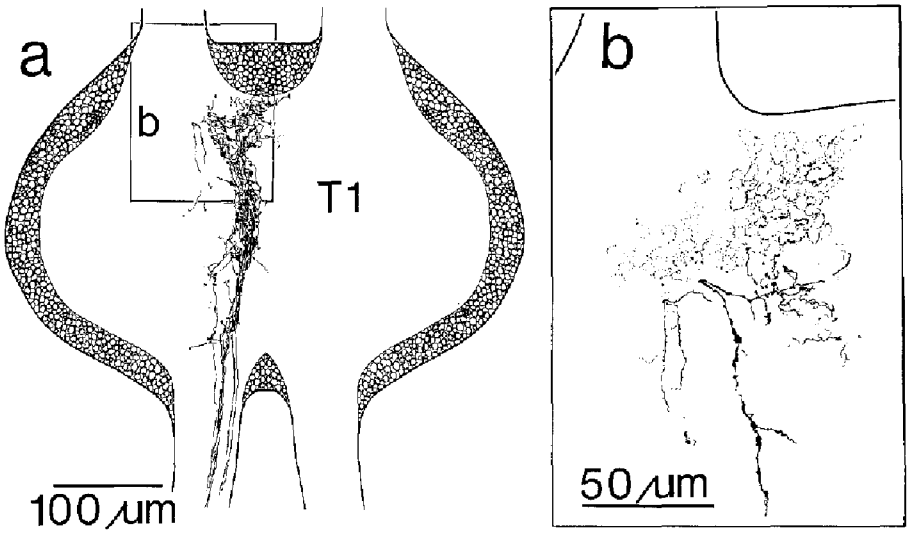


Fig. 9a, b. Mesothoracic arborizations of afferents from sensilla on the gemma of *D. rugosum*, sagittal view. **a** Reconstruction in four different sagittal planes and progressing from *lateral* to *medial*, the first one incorporating the lateralmost four sections (15 µm each, *60 µm* together), the following ones combining two sections each (hence *30 µm*). **b** Combined reconstruction; *box* indicates area depicted in **a**; *triangles* point at regions of putative contacts between sensory afferents and cell bodies



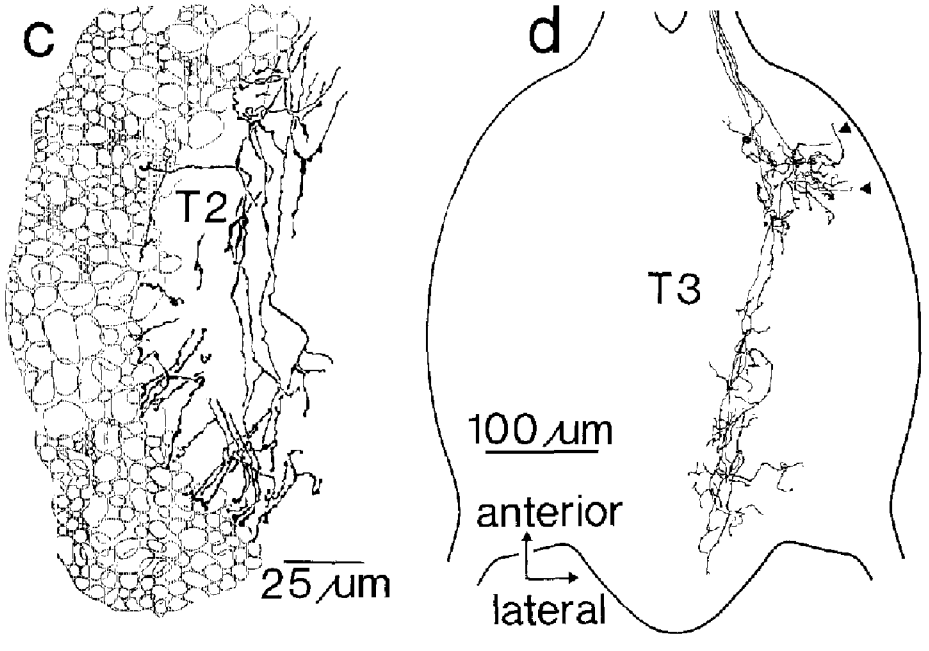


Fig. 10. Regions of putative contact between cell bodies and sensory afferents of the gemma in the prothoracic (**a, b**), mesothoracic (**c**), and metathoracic ganglia (**d**); dorsal views. Cell body rind in **a** schematized, in **b** and **c** reconstructed (cell bodies *hatched* in **c**). Dotted appearance of somata in **b** (unspecific silver precipitation) resulted from strong silver intensification. Area depicted in **b** *boxed* in **a**. *Triangles* in **d** point towards putative contact regions. **a, b,** and **d** *D. rugosum*, reconstructed from cobalt-fills; **c** *D. vagans*, reconstructed from Biocytin/HRP fill

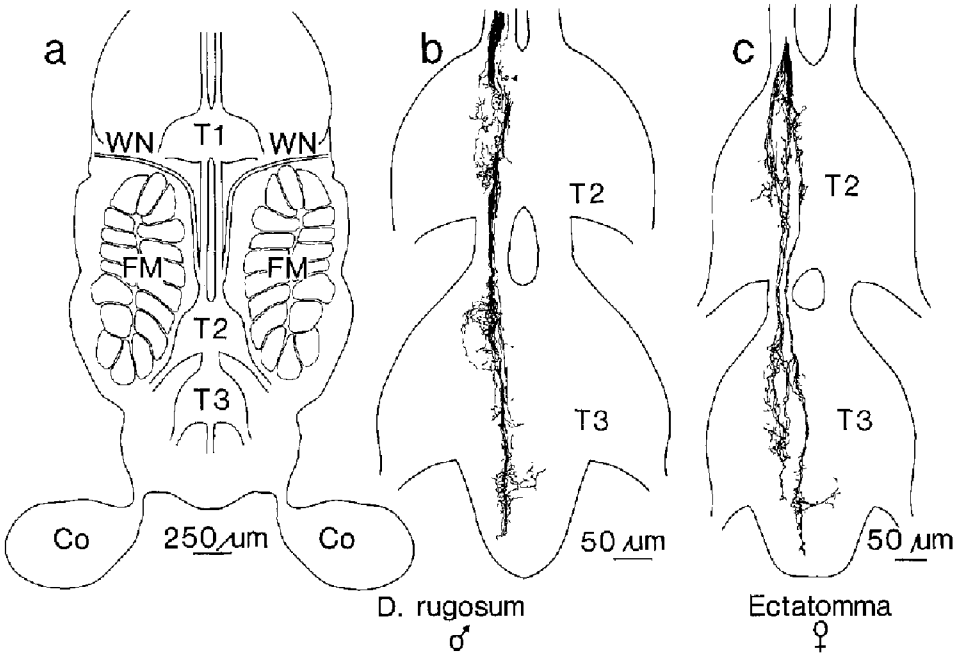


Fig. 11. Basic layout (horizontal views) of the thoracic nervous system of a male *Diacamma* (**a**) and of the afferents of hair sensilla on the left front wing of a male *D. rugosum* (**b**) and of an *Ectatomma* queen (**c**). *FM* Dorsoventral flight muscle; *WN* wing nerve; other abbreviations as in Fig. 2

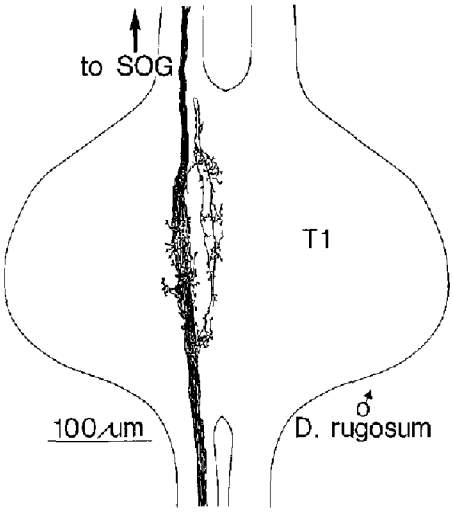


Fig. 12. Reconstruction of about 10 afferent projections originating from hairlike sensilla on the left front wing of a male *D. rugosum* and stained with cobalt chloride; dorsal view of the prothoracic ganglion *T1*. The axons ascend through the cervical connective to the suboesophageal ganglion *SOG*

of sensilla on the legs of different insect species, e.g., locusts (Pflüger et al. 1981, 1988; Newland 1991), crickets (Eibl and Huber 1979; Hustert 1985), tettigoniids (Oldfield 1983), or *Tenebrio* (Breidbach 1990). Like afferents originating from the cerci (Murphey 1981), the projections of sensilla on different leg segments are somatotopically organized within the corresponding ganglion, proximal leg segments (coxae) projecting more centrally than distal ones (tarsi; Pflüger et al. 1981; Johnson and Murphey 1985; Newland 1991). Most afferent arborizations originating from sensilla on the legs reside in ipsilateral neuropil of the corresponding neuromere. Only some chordotonal organs (proprioreceptors) feature multisegmental and bilateral arborizations (Hustert 1978; Bräunig et al. 1981, 1983; Pflüger et al. 1988). Many tactile or wind-sensitive hairs on the thorax also feature axonal projections that are restricted to a single neuromere (Pflüger 1980; Pflüger and Tautz 1982). The same is true for *Diacamma* as revealed by a few (fortuitous) stainings of mesothoracic hair sensilla situated close to the gemma (not shown in the figures). They exhibited the unilaterally restricted projection pattern characteristic for tactile hairs in other insects.

Sensory structures on wings (as well as on wing-homologous halteres) differ in this respect. While some bristles on the edge of dipteran wings show a simple uniganglionic and unilateral arborization pattern (Palka and Ghysen 1982), all other sensory structures related to wings (and examined so far) do not. In *Drosophila*, afferents from campaniform sensilla on the wing surface or close to the wing joint send collaterals into all three thoracic neuromeres and up to the brain (Palka and Ghysen 1982). Sensilla on the (metathoracic) dipteran halteres show similar multisegmental projections that reach up to the brain and also feature contralateral thoracic collaterals (Sandeman and Markl 1980; Palka and Ghysen 1982). Breidbach (1990) documented projections of hindwing sensilla afferents (*Tenebrio*; revealed from wing nerve backfills) that invade mesothoracic neuropil although they are most prominent in the (corresponding) metathoracic ganglion. In locusts, sensory afferents originating from hair plates at the wing base (tegula; Bräunig et al. 1983), from the wing hinge stretch receptors (Altman and Tyrer 1977), or from the wing itself (Bräunig et al. 1983), also feature multisegmental axon collaterals. So the multisegmental nature of wing afferents seems to be a general feature in hemimetabolous as well as in holometabolous insects. As demonstrated in this study, ants are no exception. There are only minor differences between the examples given here (Figs. 11, 12)

and those published for other insects. While in most other insect families examined, the sensory wing nerve enters the CNS at the meso- or metathoracic ganglion, in ants the wing nerve merges with the pro-mesothoracic connective, which leads to a different overall pattern of arborizations in the mesothoracic ganglion. Upon closer inspection, however, the branching points of secondary axon collaterals as well as the dorsoventral extent within each ganglion is similar to those exhibited in other insects.

The general resemblance between gemma afferents and wing afferents of ants has already been pointed out in the Results section. When gemma afferents are compared with locust sensory wing afferents such as those shown in photomicrographs of whole-mount preparations from wing nerve backfills (Bräunig et al. 1983), the similarity is striking. In ants, there is no general description of the internal organization of the thoracic CNS available, as exists for some other insects (e.g., locusts: Tyrer and Gregory 1982; Pflüger et al. 1988; stick insect: Kittmann et al. 1991; the sphinx moth *Manduca sexta*: Suder et al. 1992). Thus the different regions in the thoracic ganglia of *Diacamma* where axon terminals of gemma afferents have been found cannot be related to any known tracts or neuropil regions. Hence it is not possible to match known sensory wing projections of other insects to the gemma afferents on a point-to-point basis. However, from their distribution within the ganglia, it is obvious that gemma afferents are very similar to afferents from wing sensilla not only in ants but in other insect species. This is certainly a strong criterion for homologizing the hairlike sensilla on the two structures and, accordingly, homologizing the entire appendages, front wings and gemmae.

How could the loss of sensory input from the gemmae affect behaviour?

Regardless of the functional significance of the sensory hairs on the gemma, mutilation will have several consequences on the information processing in the CNS: electrical activity in the gemma afferents (probably caused by touch stimuli) causes transmitter release at terminals that are presynaptic to central neurons (e.g. spiking and nonspiking interneurons and motor neurons). Once the gemmae are removed, any input from the missing sensilla would stop immediately. Moreover, it is most likely that the axonal arborizations of the gemma would degenerate after mutilation because the sensory neurons have their cell bodies within the gemma. Hence all the axon collaterals and their synapses described in this paper would disappear from the CNS within hours or days.

These physiological and morphological changes in the CNS might underlie the transition from aggressive to timid behaviour. These behavioural changes are complex (they undoubtedly are composed of many different motor programs), they are relatively unspecific (as compared to simple reflexes), and they are long-lasting. Here we want to speculate upon two neuronal mechanisms that can account for the observed behavioural transformation.

In other animals, complex long-term behavioural changes may involve the action of so-called neuromodulatory substances. Unlike "classical" neurotransmitters, such neuromodulators may act on many target cells simultaneously. In the lobster it has been demonstrated that antagonistic biogenic amines bring about the postural changes associated with dominance or submission of competitors after a single or a few fights (reviewed by Kravitz 1988). These behavioural changes seem comparable to those seen in *Diacamma*.

Neuromodulatory substances may bring about long-term changes in cellular chemistry and physiology, which might explain the profound behavioural modifications that occur in mutilated *Diacamma* workers. The only evidence yet to support this idea is the fact that afferent gemma axons contact the cell body rind of the ganglia. Similar, though more pronounced, arborization characteristics have been described for interneurons, but not for sensory afferents, in some other arthropods. In the bee brain, Schürmann et al. (1989), Schäfer and Rehder (1989), and Schürmann and Erber (1990) characterized fibres with synapses (boutons) terminating among cell bodies of different protocerebral soma clusters. In crustaceans, giant interneurons with terminals among olfactory cell bodies have also been shown (Sandeman et al. 1990; Johansson 1991). All these neurons have been revealed by immunocytochemistry that indicated that they contain either biogenic amines (dopamine: bee; serotonin: crustaceans) or neuropeptides (FMRFamide: bee; substance P: crustaceans), respectively. While neuropeptides as well as biogenic amines can have neuromodulatory effects in different animals, in *Diacamma* immunocytochemical evidence suggesting the involvement of neuromodulators remains to be demonstrated.

There is an alternative explanation of the behavioural changes that follow mutilation that is not based on the action of neuromodulatory substances. Immediately after mutilation the interneurons and motor neurons that are postsynaptic to the gemma afferents would receive less excitatory input. Hence the equilibrium of these neurons (e.g. the resting potential, input resistance, or threshold for activation) would be altered. In locusts, small shifts in the membrane potential of spiking and nonspiking interneurons resulting from mechanosensory input (Laurent and Burrows 1988) can dramatically change the output characteristics of sensory-motor pathways (Laurent 1990). Thus it is reasonable to assume that in *Diacamma* the decrease in sensory input onto interneurons may result in a shift to a different motor program.

Nevertheless, we need to consider the thousands of other sensory inputs simultaneously impinging onto the thoracic interneurons at any time. Is it possible that the missing input from those 70–100 sensory afferents (per gemma) onto their postsynaptic elements could directly account for the behavioural changes triggered by mutilation? Even though relatively few afferent neurons are involved, their impact onto postsynaptic neurons may be substantial because of the afferents' extensive axonal

arborizations and synapses in all thoracic ganglia. These characteristics would be expected to strongly amplify the sensory input, the cessation of which could certainly alter the neuronal processing responsible for the behavioural output.

The homology of gemmae and wings is interesting in this context. When ant queens have mated they shed their wings and their behaviour then changes conspicuously. While virgin, their behaviour is focussed on the nuptial flight and on mating, whereas after the wings are shed, queens of many ant species burrow a hole and start living underground. Their motor output is modified; they metabolize the flight muscles and start to lay eggs. If in *D. rugosum* and *D. australe* the presence or absence of gemma afferents controls the behavioural state, is it possible that the degeneration of wing sensillum afferents in queens causes analogous behavioural changes? We propose that the halt in sensory input and the physical disruption of sensory cell axons associated with dealation in queens may underlie the profound behavioural changes observed. In *Diacamma*, the loss of the gemmae certainly causes the behavioural changes observed (we even have circumstantial evidence from a few laboratory observations in which the loss of only one gemma leads to an intermediate level of aggressiveness; these individuals dominated mutilated workers but were submissive when confronted with unmutilated workers). It remains to be demonstrated, however, that in queen ants the loss of wings is the actual cause for the behavioural changes described above.

Adaptive significance of the gemmae

The gemmae are unknown in all other ant genera. Gemmae are homologous with the wings in males and queens of other species, but ant workers never have wings. Thus the existence of gemmae seems to represent the novel expression of various genes normally associated with the development of queens. Although there are some morphological similarities between wings and gemmae, including (1) site of articulation on the thorax, (2) sensory hairs, and (3) central projections of sensory neurons, there are also differences. For example, there is only one pair of gemmae, and they contain exocrine glands and are not movable. What may have been the initial adaptive significance of these new structures?

More details are needed on the behavioural significance of the gemma hairs (e.g. ablation experiments). We postulate that sensory hairs are present on the outside of a gemma as a consequence of the homology with wings (i.e., a proportion of the "wing genes" are expressed during gemma development). These hairs, which serve to detect local air currents when present on wings, may instead have a general tactile function on the gemmae (similar to that of other mechanoreceptors elsewhere on the thorax). However, what is of evolutionary interest is that, because of the extensive central projections of their sensory afferents, the existence of gemmae represents a preadaptation for the modulation of behaviour. They are not involved in *D. vagans*, because all adults retain them. In *D. australe* and *D. rugosum* however, mutilation of the gemmae has become an essential component of the mechanism of reproductive control. Future research must determine whether the situation in *D. vagans* represents the ancestral condition, or is derived.

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