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**Differences in mechanosensory hairs among castes of the damp-wood termite**

***Hodotermopsis sjostedti* (Isoptera: Termopsidae)**

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*Running title:* Sensory system differences among termite castes

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## ABSTRACT

Termites are one of the major groups of social insects, which comprise alates, workers (pseudergates), and soldiers within a species. These castes have different roles and behaviors, and undertake division of labor to increase the inclusive fitness of their colony. On the basis of the different behavioral repertoires, caste-specific neural modifications are predicted, such as modification of sensory systems, i.e., inputs into the nervous system. This study evaluated these sensory-system differences based on mechanoreceptive sensilla length among castes of the damp-wood termite *Hodotermopsis sjostedti*. We found that soldiers and alates had longer sensilla than pseudergates, and that this variation among castes differed with body region. Specifically, the differences were particularly conspicuous on head capsules and pronota, while sensilla on mouthparts and legs were of similar lengths among castes. It is proposed that soldiers and alates use these long mechanoreceptive structures to sense faint vibrations, an important capability for their defensive role in detecting enemies and cracks in nest wood.

*Key words:* caste differentiation, defensive behavior, mechanoreceptive sensillum, sensory system, soldier, termite.

## INTRODUCTION

Termites constitute a major group of eusocial insects, which show elaborate polyethism. Colony members differentiate into morphologically different castes such as alates, workers (pseudergates), and soldiers. Different tasks are allocated to each caste to increase the inclusive fitness within colonies. Each caste also shows a distinctive behavior. The job of the soldier termite is to patrol the nest and defend it against invading predators or competitors. Workers (pseudergates) function mainly in nest-maintenance, foraging, and nursing (Stuart 1969). Alates find partners at the time of nuptial flight, found new colonies, and then dedicate their lives to reproduction (Nutting 1969). These caste differences in morphology and behavior derive from polyphenism, a process whereby various phenotypes are produced from similar (even identical) genome information. These behavioral differences would necessitate development of caste-specific neural modifications within the species, triggered by environmental signals such as interactions among colony members (Noirot 1991, Miura 2001).

In the central nervous system (CNS), external information is transmitted in a controlled manner from sensory systems, via integration systems to motor systems (Delcomyn 1998). The CNS could be modified in a caste-specific manner at any of these stages, and we are gradually learning more about such modifications in social hymenopterans. For example, a recent study of sensory systems showed that the glomeruli in antennal lobes of the leaf-cutting ant are enlarged in large-bodied workers (Kleineidam 2006). In addition, the mushroom bodies of integrating systems

in honeybee are modified according to the age and foraging experience of the insect (Farris et al. 2001, O'Donnell 2004). Moreover, the concentration of biogenic amines and expression of their receptors in this species are influenced by social contexts (Sasaki and Nagao 2002, Humphries et al. 2003, Harano et al. 2005).

Termites are hemimetabolous insects that have acquired social characteristics independent of hymenoptera, and little is known about their caste-specific neural modifications (Richard 1969). Recently, we discovered such neural modification in the motor systems of *Hodotermopsis sjostedti*, in which mandibular motor neurons are extremely enlarged in soldier termites (Ishikawa and Miura, in preparation). However, caste differences in sensory and integrating systems are almost unknown (Heath 1927, Deng et al. 2006). Sorting out the neurophysiological mechanisms underlying social behavior in termites will thus require a careful investigation of caste-specific neural modifications. In the present study, we focused on the sensory systems of termites. On destructions of the nest, termite soldiers detect the sign and start defensive behavior instantly. Therefore, some caste differences in sensory systems, involving with the detection ability of invasion, were predicted.

We observed different sensilla dimensions among castes of *H. sjostedti* (Family Termopsidae; Order Isoptera), which have large bodies and ancestral biting-type soldiers. These termites principally comprise three castes: alate, pseudergate, and soldier. The larvae undergo six molts after hatching to become pseudergates, which function as workers. Pseudergates can differentiate to the soldier line or alate line if needed, although many of them remain as pseudergates and repeat the stationary molts. Individuals that enter the alate line first become nymphs, then molt into alates, while

individuals in the soldier line differentiate into presoldiers, then to soldiers (Miura et al. 2000, 2004).

## MATERIALS AND METHODS

### Insects

*Hodotermopsis sjostedti* (Family Termopsidae) is distributed in the Satsunan Islands of Kagoshima Prefecture in Southern Japan (Matsumoto and Hirono 1985, Maekawa et al. 1998). Colonies were sampled in May 2006 from rotten wood in primary evergreen forests on Yakushima Island, Kagoshima Prefecture, and then stored with nest wood at approximately 25°C in the laboratory.

### Measurement of sensillum length

To clarify differences in sensory systems among alates, soldiers, and pseudergates in *H. sjostedti*, we measured the length of hairs on antennae (5th antennal segments and most distal segments), labura, maxilla (most distal segments), labia (most distal segments), head capsules, pronota, pretarsi of fore-, mid- and hindlegs, and cerci. The hair lengths were measured using an image analysis system with a CCD camera (HIM-1, HOGA, Kyoto, Japan). Insects were fixed in FAA fixative (formalin: ethanol: acetic acid = 6: 16: 1), and then preserved in 70% ethanol. We then collected two of the longest hairs from each body part ( $n = 5$ ).

### Scanning electron microscopy (SEM)

Termite samples were fixed in FAA, dehydrated through increasing concentrations of ethanol, and then immersed in acetone. Samples were air-dried, and then coated with gold using an Ion Sputter E-1010 (Hitachi, Tokyo, Japan). Detailed morphological

structures in the thoracic region were observed on a JSM-5510LV scanning electron microscope (JEOL, Tokyo) (Miura et al. 2004).

### **Silver staining**

Sensilla can be categorized based on porosity. Porous sensilla usually have chemosensory functions, and nonporous ones perform mechanosensory functions (Navasero and Elzen 1991). We used silver staining to examine sensilla porosity, using a method modified from Navasero and Elzen (1991). Live insects were washed three times for 10 sec each in 10% acetone, soaked in 0.1 M AgNO<sub>3</sub> for 15 min at room temperature or for 30 min at 60°C, and then washed three times for 15 min each in distilled water. The insects were later soaked in developing solution (Rendol FUJIFILM, Tokyo) for 5 min, rinsed in 3% acetic acid for 1 min, and then dehydrated serially in ethanol. Dehydrated samples were cleared in a xylene/phenol mixture (3:1 ratio + 1% ethanol) and mounted using glycerol. Mounted samples were observed on a BX-51 light microscope (Olympus, Tokyo), and images captured using a DP-50 CCD camera (Olympus) and the Viewfinder Lite program (Eridas, Atlanta, GA).

## RESULTS

### Measurement of sensillum length

Two of the longest sensilla for each body part were measured. The sensilla of alates and soldier were longer on average than those of pseudergates (Fig. 1A) (average lengths: 0.084 mm for workers, 0.179 mm for soldier, 0.179 mm for alates). The degree of differences in sensillum length among castes depended on the body parts sampled. In comparison between soldiers and pseudergates, sensilla on the head capsule and pronotum showed the most remarkable differences (5.5 times longer in soldiers than in pseudergates), followed by antennae and cerci (2.5-3.0 times longer in soldiers than in pseudergates). Sensilla on legs and mouthparts of soldiers were not significantly different, being only 1.5-2.0 times longer than those of pseudergates (Fig. 1B). Sensillum lengths in alates were similar to those of soldiers on head capsule, pronotum, and foreleg while almost identical to those of pseudergates on labrum, maxilla and the tip of antenna. Sensillum lengths on labium, midleg, hindleg, and cercus were only moderately different on average between soldiers and pseudergates (Fig. 1A, B).

### Detailed morphology of sensillum structure

Detailed structural analysis of the sensilla by SEM confirmed the presence of pores noted with silver staining. Antennae possessed bristle sensilla of 50-100  $\mu\text{m}$  in length and basiconic sensilla of 5-10  $\mu\text{m}$  (Fig. 2 A-C). The basiconic sensilla stained with silver (Fig. 2M, white arrowhead), while the bristle sensilla were not stained (Fig. 2M,

black arrowhead). The labrum tip showed approximately 200- $\mu$ m-long bristle sensilla, which also did not stain with silver, indicating a lack of porosity.

The maxillary palp structure showed bristle sensilla (100-180  $\mu$ m in length) and curved-tip bristle sensilla (30-40  $\mu$ m in length) (Fig. 2E, right inset) at the lateral and distal parts, respectively (Fig. 2D-E). Basiconic sensilla were also found among these sensilla (Fig. 2E, left inset); these were entirely stained with silver, while only the tip of the curved bristle sensilla showed silver staining in a punctate pattern. Although long bristle sensilla were not stained after 15-min silver treatment at room temperature, some tips were faintly stained after 30 min incubation at 60°C. The tips positioned at the distal part of the maxillary palp tended to be stained, while those at the base remained unstained. The long and curved bristle sensilla of soldiers and alates were longer than those of pseudergates. The characteristics of sensilla on the labia were similar to those on maxillae.

Extremely long bristle sensilla were found on the head capsules of soldiers and alates (approximately 250  $\mu$ m long in soldiers and approximately 150  $\mu$ m long in alates), while hardly any were found on the same body part of pseudergates (Fig. 2G-I). There were more of these sensilla on the alates compared to the soldier termites. Another bristle sensilla of 20-50  $\mu$ m in length existed in all castes (Fig. 2G, inset), as did coeloconic sensilla, approximately 2-3  $\mu$ m long and not silver stained (Fig. 2I inset).

Similarly to a head capsule, pronotum showed extremely long bristle sensilla in soldiers and alates, but not in workers. On average, these were longer in soldiers compared to alates (~200  $\mu$ m long in soldiers and ~150  $\mu$ m long in alates), but fewer

in number. Other bristle sensilla 20-30  $\mu\text{m}$  long existed in all castes. The long bristle sensilla were not stained, while the sockets of the short bristle sensilla showed positive silver staining.

The distal tarsomere of legs possessed bristle sensilla, which did not stain with silver and were longer on average in soldiers and alates (approximately 100  $\mu\text{m}$ ) than in pseudergates. The alates also had more such sensilla than pseudergates. Almost all sensilla found in cerci were bristle sensilla, 120-150  $\mu\text{m}$  long in soldiers and alates, and 70  $\mu\text{m}$  long in pseudergates (Fig. 2J-L). None of these sensilla showed silver staining (Fig. 2N). Between segments of the cercus, the discriminative filiform sensilla were found. These structures are almost 200  $\mu\text{m}$  in length and have accessory structures consisting of seven or eight domes circularly arranged at the base of the sensilla (Fig. 2J-L, arrowheads and 2L, inset). These sensilla were arranged in rows on the cercus, transverse to the cercus axis. This structure is typical in filiform sensilla that are deflected by faint air currents and low-frequency sound, or medium vibration (Keil 1997). The sockets of these sensilla were stained with silver although the hairs themselves were not stained (Fig. 2N).

## DISCUSSION

The exquisite social behaviors seen in termites rely on modifications to various body parts in the course of caste differentiation (Miura 2004, 2005). Previous studies highlighted morphological specialization of defensive structures such as mandibles and frontal glands (Miura and Matsumoto 2000, Koshikawa et al. 2002, 2003) and the ability to synthesize secretory substances for attack (Hojo et al. 2005, 2007) as modifications of soldier differentiation. These specializations directly relate to physical features of the soldier armory. Successful defense behavior of the soldier caste must also involve modifications to the nervous systems to elicit functions including enemy detection, deciding on a defense behavior, attacking enemies, and head banging.

In spite of reduced eyes, soldiers are able to detect precisely the position of invading enemies and perform defensive behavior aggressively (Weesner 1969, Stuart 1969). We recently found that the mandibular motor neurons, which are the output for the neural cascade controlling defense behavior, were specifically enlarged in soldiers (Ishikawa and Miura, in preparation). This study also revealed elongation of the mechanoreceptive sensilla, which are the input for defense behavior signals, in accordance with the soldier differentiation. These observations indicate that the nervous systems of termites are specifically modified at different levels of neural regulation to enable soldier-specific defense behaviors.

The present study showed that the average length of the sensilla of soldiers and alates was more than twice longer than that of pseudergates. However, the degree of

length variation among castes ranged from 1.5 to 5.5 times, depending on the body part sampled. This unequal elongation suggested that sensillum length is determined by site-specific regulatory mechanisms operating during caste differentiation. It is also likely that sensillum elongation is required for soldiers and alates to perform their distinctive roles, and therefore has adaptive significance. Neotenic, which reproduce in their natal nest, also possess elongated sensilla (data not shown), further suggesting common regulatory cascades triggered by factors that induce caste differentiation such as juvenile hormone.

The present study also showed that sensilla on the head capsule and pronotum were particularly elongated in soldiers and alates. Based on their thickness, length, and other structural properties, these sensilla are probably contact-mechanoreceptive. On the other hand, no significant size differences were detected in the putative chemoreceptive basiconic and coeloconic sensilla, or wind-receptor structures such as filiform sensilla. Our results suggest an adaptive significance for this variation in mechanoreceptive sensilla, although their actual function remains unclear. In the case of amblyopic soldiers, contact stimulus is an invaluable source of information. When colony members detect an enemy invasion, soldiers put their heads toward the exterior of the nest wood and wait for the approach of invaders (Fig. 2O). On first contact, soldiers aggressively poke their heads and simultaneously bite with their mandibles. As with our focal termite species, soldiers of a eusocial aphid species, *Tuberaphis styraci*, inhabit dark and closed environments, and accordingly possess longer sensilla than other individuals (Shibao, H. personal communication). In contrast to ants and bees, which forage over external environments using visual information, soldiers of

termites and aphids defend colonies under darkness, and may therefore facilitate contact rather than visual reception.

The intermediate length of the alate sensilla may also reflect their role in defense, which is to protect the nests of the incipient colonies in the absence of soldiers. This idea is consistent with mandibular motor neurons in alates also being of an intermediate size between those of soldiers and workers (Ishikawa and Miura, in preparation). In addition, alates experience the external world, necessitating dense and long sensilla to aid in gathering surrounding information and preventing infection (Nutting 1969).

In summary, our results together with previous findings indicate that sensilla elongation in termite soldiers and alates might correlate to their caste functions within a colony. Future studies will focus on the mechanisms underlying these social behaviors. We need to analyze neural cascades with respect to behavioral differences among castes using molecular biological and neurophysiological techniques.

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## FIGURE LEGENDS

**Fig. 1.** Sensilla lengths in body parts of alates, soldiers, and pseudergates, in absolute values (**A**) and relative values to pseudergates (**B**) (mean±SD,  $n = 10$  for each caste). The different letters (a-c) on bars denote significant differences between bars (one-way ANOVA followed by the Tukey-Kramer test,  $P < 0.05$ ).

**Fig. 2.** Detailed morphological comparisons of sensilla among castes. Scanning electron microscopy showed the detailed sensillum structures of antennae (**A-C**), maxillae (**D-F**), head capsules (**G-I**), and cerci (**J-L**) in pseudergate (**A, D, G, J**), soldier (**B, E, H, K**), and alate (**C, F, I, L**) termites. Light microscope images of silver staining in pseudergate antenna (**M**) and cercus (**N**). Soldiers utilize elongated sensilla for the defense of their colonies (**O**).





