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Authors: Miura, Toru, Roisin, Yves, and Matsumoto, Tadao

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# Developmental Pathways and Polyethism of Neuter Castes in the Processional Nasute Termite *Hospitalitermes medioflavus* (Isoptera: Termitidae)

Toru Miura[1]*, Yves Roisin[2] and Tadao Matsumoto[1]

[1] *Department of Biology, University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan*
[2] *Laboratoire de Biologie Animale et Cellulaire, CP160/12, Université Libre de Bruxelles, Av. F.D. Roosevelt 50, B-1050 Brussels, Belgium*

**ABSTRACT**—The developmental pathways of neuter castes in the processional nasute termite *Hospitalitermes medioflavus*, which has monomorphic soldiers and trimorphic workers, was examined. *Hospitalitermes* is one of the genera of Nasutitermitinae which make processional foraging columns. The castes engaged in foraging are soldiers, and minor, medium and major workers. Sex determination by dissection and histological staining showed that soldiers and minor workers were males, while medium and major workers were females. In larval head width, three well-defined size peaks in the frequency distribution were found. The smallest peak consisted of both males and females of the first instar. The intermediate and largest peaks consisted respectively of males only and females only, of the second instar. Molting individuals and observations of mandibles confirmed the developmental relationships among castes. We concluded that the developmental pathway of the neuter castes in *H. medioflavus* is parallel to that found in *Nasutitermes*. Males change tasks from gnawing food (as minor workers) to defense (as soldiers), whilst females specialize in foraging (gnawing and carrying food). Division of labor during foraging depends primarily on sex and the developmental stage of neuter castes. This species thus displays both temporal and sexual polyethism.

## INTRODUCTION

In eusocial insects, there are helper castes which give up their own reproduction to support a limited number of reproductive individuals. These helpers in Isoptera (termites) are workers and soldiers, often collectively called neuter castes. The caste developmental systems found in termites are very different from those found in the social Hymenoptera because termites have the following distinctive features: (1) hemimetaboly, (2) bisexuality, and (3) diploidy (Noirot, 1989). Several studies covering many species of termites have focused on the developmental pathways of neuter castes (Noirot, 1955, 1969, 1985; Okot-Kotber, 1985; Roisin, 1992, 1996). Such studies have highlighted the diversity of caste developmental pathways among different termite families, genera and species.

The developmental pathway of the sexuals (nymphs and alates) in Termitidae is remarkably constant throughout the whole family (Noirot, 1969). The alates go through one undifferentiated larva followed by five immature nymphal stages. In contrast to the sexuals, the neuter castes show very diverse, often genus-specific, developmental patterns.

In most genera of the subfamily Nasutitermitinae, the soldiers possess a frontal tube, the nasus, through which a defensive secretion is emitted. The subfamily is conveniently divided in two groups: "mandibulate nasutes", whose soldiers possess functional mandibles (e.g. *Syntermes*, *Cornitermes*, *Rhynchotermes*), and "full nasutes", whose soldiers have regressed and non-functional mandibles (e.g. *Nasutitermes*, *Subulitermes*).

The full nasute genus *Hospitalitermes* is widely distributed throughout Southeast Asia (Tho, 1992). These termites forage for lichens or blue-green algae adhering to the surface of tree trunks. Soldiers and workers participate in the foraging parties which start in the evening and continue through to the next morning (Collins, 1979; Jones and Gathorne-Hardy, 1995; Miura and Matsumoto, 1995, 1997, 1998). Soldiers are monomorphic and play the roles of scouting and defending, protecting workers against predators with the help of terpenic substances emitted through their nasus (Chuah *et al.*, 1983; Prestwich, 1983). In *H. medioflavus* there are distinct major, medium and minor workers, among which there is a division of labor. Minor workers gnaw food material while major workers repeatedly receive small pieces of food with their mandibles from the gnawing workers and carry the food balls to the nest. Medium workers are engaged in both tasks. This trimorphism in worker castes was suspected to depend on

* Corresponding author: Tel. +81-3-5454-6652;
FAX. +81-3-5454-4322.

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their developmental stage (Miura and Matsumoto, 1995), but the developmental pathways of these castes had yet to be elucidated.

Therefore, we determined the sex of each caste by histological techniques, and examined molting individuals. The purpose of this study was to gain insights into the relationship between caste systems and task allocation within colonies of this highly derived species.

## MATERIALS AND METHODS

### Study site and specimen collection

Specimens of *H. medioflavus* were collected in Bukit Soeharto Protection Forest, East Kalimantan, Indonesia. We found about 40 nest sites of the termite genus *Hospitalitermes* (two species exist in the study site; 31 of them were *H. medioflavus*) by following the foraging columns or by checking tree stumps and fallen trees. Five nests were opened and a number of individuals constituting the colony were collected randomly. Specimens were fixed in FAA solution for about 24 hr in preparation for sex determination. After fixation, samples were preserved in 70% ethanol. For scanning electron micrographs, individuals were preserved directly in 70% ethanol solution.

### Scanning electron micrographs

Scanning electron microscopy was used to search for morphological differences between sexes in the abdominal sternites and antennal structure in each caste. Alcohol preserved samples were dehydrated by transfer into increasing concentrations of ethanol (up to 100%) and finally into acetone. After being air-dried, samples were metallized with gold by using an ion coater (Eiko IB-3). Scanning electron micrographs were taken with a Hitachi S-405.

### Sex determination

Sex determinations were carried out as described in Noirot (1955). Abdomens were cut in the frontal plane. The dorsal half of the abdomen and the gut were removed. Specimens were stained with alcoholic haematoxylin for 45–60 min, then destained in hydrochloric alcohol (0.5% HCl in 80% ethanol) until the genital apparatus was distinguishable (usually after a few minutes). Females are characterized by rudiments of: (1) oviducts at the posterior margin of sternite 7, (2) spermatheca on sternite 8 and (3) colleterial glands on sternite 9. Males possess rudiments of seminal vesicles at the end of sternite 9. Photographs of the ventral view of abdomen were taken under a binocular microscope (OLYMPUS).

### Measurements of head width

In order to examine the caste composition in each sex, we measured the head width of individuals in a single colony. Using a binocular microscope (OLYMPUS) and micrometer, head width was measured at the widest part of the head capsule, which is just behind the antenna socket in larvae and workers, and in the rear part of the head in soldiers. To detect the caste composition, data was shown in histograms of both sexes from a single colony.

### Observation of mandibles

Molting workers appear pale and fatty, although their cuticle remains pigmented. Mandibles of such workers were mounted on microscope slides. Photographs were taken with a transmitted-light microscope (NIKON OPTIPHOT).

## RESULTS

### Sex discrimination

We initially tried to distinguish males and females among soldiers and workers by scanning electron microscopy. In some lower termites the seventh sternite is longer and the eighth notched or divided in females (Weesner, 1969; Zimet and Stuart, 1982; Henderson and Rao, 1993). However, such differences were not observed among workers and soldiers of *Hospitalitermes*, let alone among larvae. The situation is similar in *Nasutitermes arborum* (Noirot, 1955). Therefore, we applied Noirot's (1955) method, which enabled males and fe-

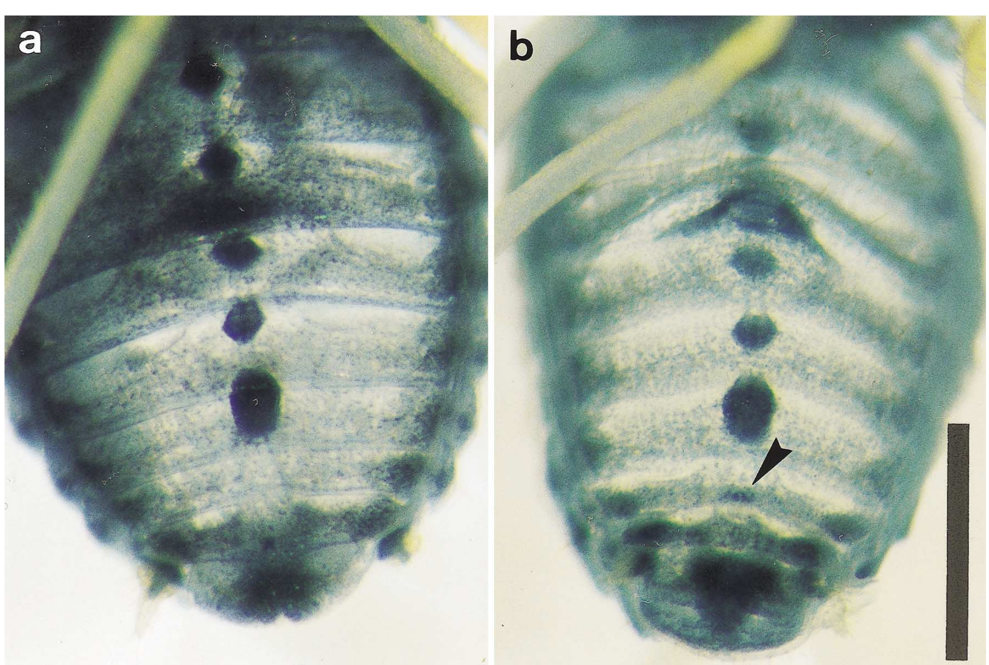


**Fig. 1.** Ventral abdomens of the neuter instars stained with haematoxylin solution for sex discrimination: (**a**) male and (**b**) female. Arrowhead shows the rudiment of spermatheca only found in the eighth sternite of females. Bar indicates 0.5 mm.

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males to be distinguished. In females, rudiments of oviducts are visible near the rear margin of sternite 7, rudiments of the spermatheca on sternite 8 and vestigial colleterial glands on sternite 9. In males, the spermiducts reach a vestigial structure corresponding to the seminal vesicles and ejaculatory duct, on the posterior margin of sternite 9 (Fig. 1).

**Size distribution**

Without sex discrimination three distinct peaks in the distribution of head width are visible in unpigmented larvae from a single colony. After distinguishing males from females, it appears that the first peak (narrow-headed larvae) comprises larvae of either sex. The middle peak comprises only male larvae, the last peak (broad-headed larvae) only females. This suggests that the first peak corresponds to monomorphic first instar larvae, whereas the other peaks correspond to sexually dimorphic second instar larvae (Fig. 2; white columns).

Pigmented and well-sclerotized workers are known to consist of three different size classes (Miura and Matsumoto, 1995). From the sex discrimination, it becomes clear that the minor (smallest) workers are all male and the medium and major (largest) workers all female (Fig. 2; black columns). Monomorphic soldiers (and presoldiers) were all found to be males.

**Number of antennal segments**

Antennal segment number is one of the key indicators to establish developmental pathways accurately (Noirot, 1955). Ten individuals of each caste or instar were checked. The number of antennal segments was constant within each caste (or instar). Figure 3 shows scanning electron micrographs of antennae in all neuter castes. In first instar larvae there were 12 segments in males and females (Fig. 3a, f). The second instar larvae had 14 segments in either sex (Fig. 3b, g). All types of workers (male minor workers, female medium and major workers) had 15 segments (Fig. 3c, h, i). In presoldiers and soldiers (male), the antennae consisted of 14 segments (Fig. 3d, e). The antennal segments of soldiers are more elongated than those of larvae and workers, especially in the basal part.

**Direct evidence from molting workers**

The developmental relationships between larvae were easy to define, so that there were two larval instars in either sex (i.e. two size peaks; Fig. 2). While, the stages of development of the trimorphic workers and soldiers were unclear from size-distribution data. However, we could make it clear by observation of molting individuals although those individuals were scarce. We found medium workers whose thorax was split dorsally in the act of exuviation (n = 9). This indicates that medium workers develop into further instars (i.e. major

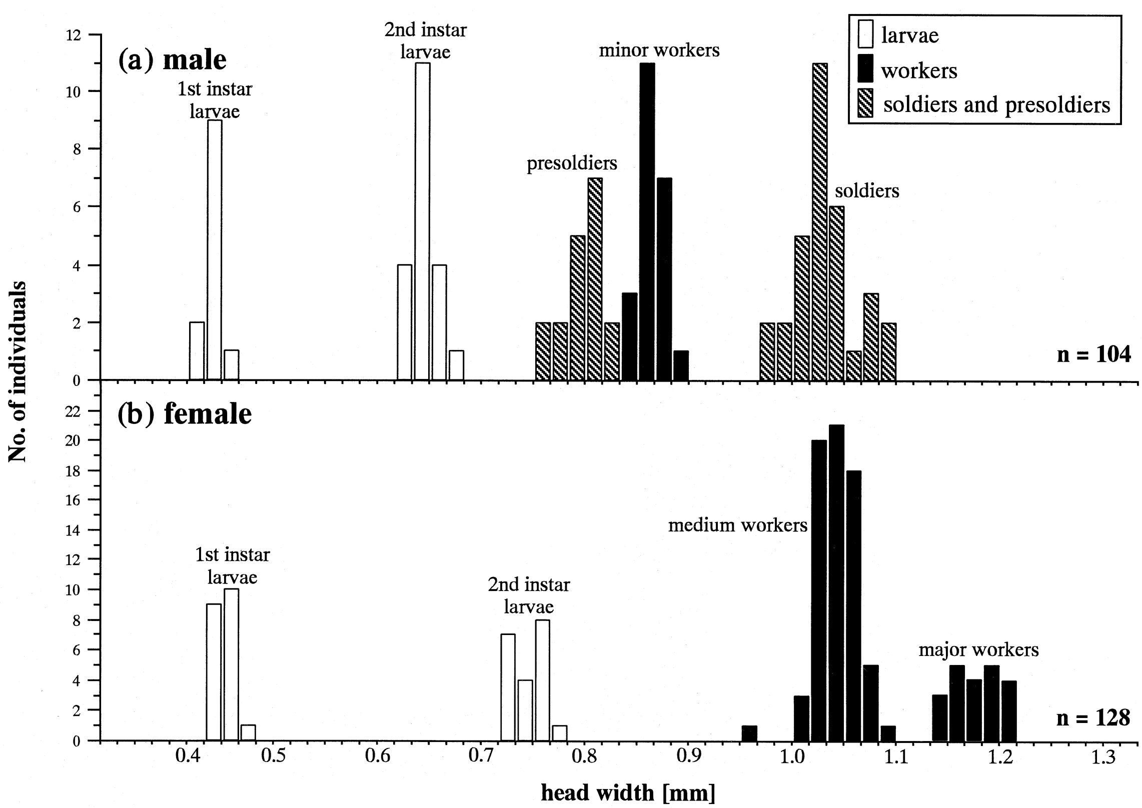


**Fig. 2.** Frequency distributions of the head width in neuters of either sex from a single colony of *Hospitalitermes medioflavus*: (**a**) male, (**b**) female. Open columns, larvae; black columns, workers; shadowed columns, soldiers.

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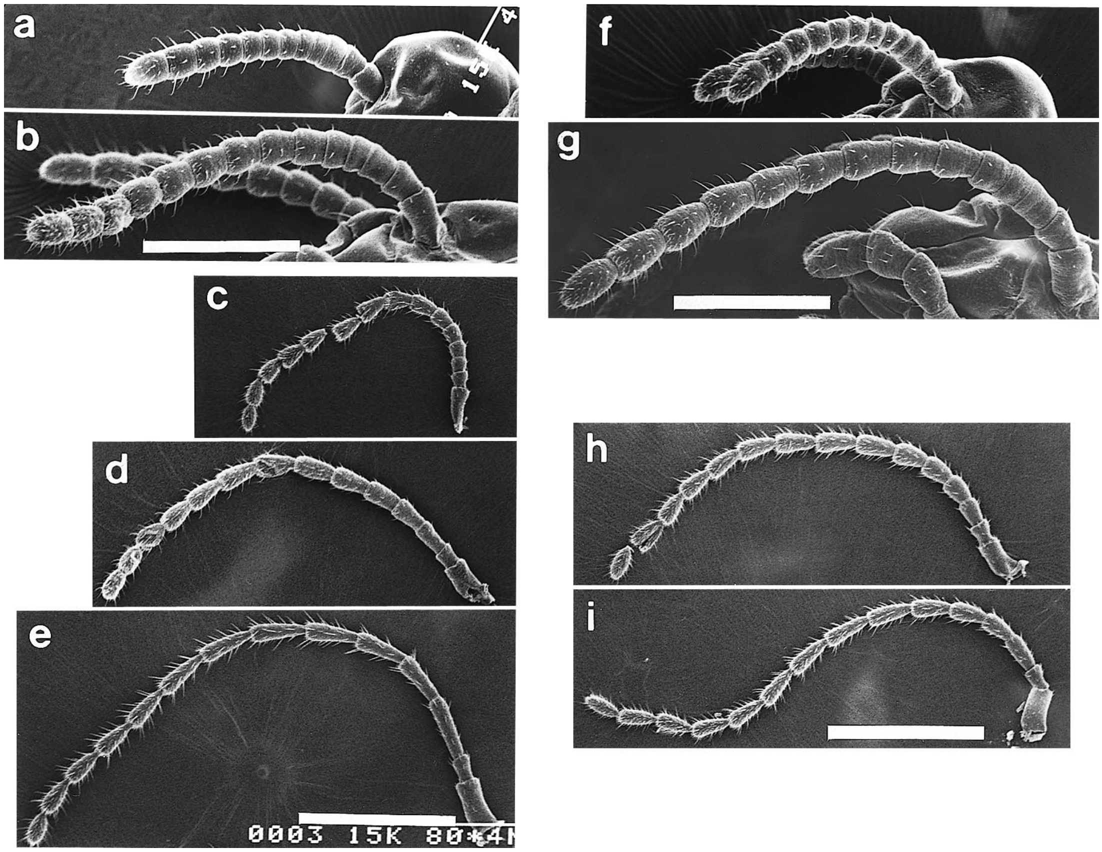


**Fig. 3.** Scanning electron micrographs of the antennae in all neuter instars (males, **a**-**e**; females, **f**-**i**). First and second instar larvae of both sexes possess 12 and 14 antennal segments respectively (first instar, **a**, **f**; second instar, **b**, **g**). In all types of workers (minor, medium and major) there are 15 segments (**c**, **h**, **i**). Presoldiers and soldiers have 14 segments (**d**, **e**). Bars, 0.4 mm (**a**, **b**, **f**, **g**) and 0.8 mm (**c**, **d**, **e**, **h**, **i**).

workers). The finding of newly molted unpigmented major workers with a cast-off pigmented exoskeleton supports this relationship between major and medium workers. Furthermore, molting minor workers (male) were also found, whose thorax was just split dorsally (n = 8). We could not identify the next instar from most of these individuals. However, one of them was clearly molting to a presoldier. Almost the whole body was bared and the frontal projection characteristic of soldiers appeared out of the cast-off head of the worker. We measured the body parts of this individual, confirming the cast-off cuticle was undoubtedly the cuticle of minor worker. In addition, we found 8 minor workers and 5 medium workers approaching a molt: the observation of mandibles revealed that all the small ones showed signs of presoldier differentiation (Fig. 4b), whereas the medium ones were molting to another worker instar (Fig. 4a).

## DISCUSSION

In this species it is easy to distinguish workers from larvae because sclerotization and pigmentation are well-developed in workers. In larvae there are two successive instars both in males and in females. The worker caste is divided into three types; minor, medium and major workers (Miura and Matsumoto, 1995). Our results reveal that the minor workers are male while medium and major workers are female. Furthermore, observation of molting individuals strongly suggests that male minor workers molt into presoldiers. Female medium workers probably molt into major workers. From these facts, we propose the postembryonic developmental schema of neuter castes shown in Fig. 5. In males, number of antennal segments is decreased at the molting from minor worker to presoldier. In this species, soldiers behave as navigators in foraging and their antennation is thought to be important. The decrease of the segment number, and the elongation of each antennal segment may suggest the important function of their antennae.

The caste system of *H. medioflavus* is basically the same as that of *Nasutitermes* spp. especially on three fundamental characteristics; (1) active workers are preceded by two inactive larval instars, (2) a sexual dimorphism appears at the second larval instar; males are smaller than females, (3) presoldiers derive from small (male) workers. The phylogenetic position of *Hospitalitermes* in the clade of full nasute genera can be considered from the comparison of caste systems among those genera. The caste systems of *Trinervitermes* and *Velocitermes* appear to possess the same bases, although male larvae display more complex developmental pathways leading to polymorphic soldiers (Noirot, 1955; Roisin, 1996). By contrast, the humivorous genera *Subulitermes* and

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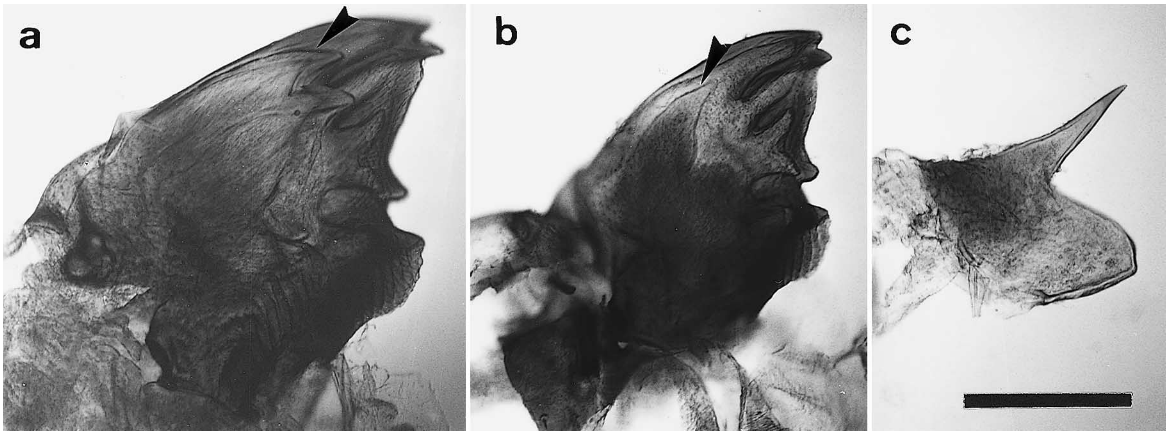


**Fig. 4.** Left mandibles observed under transmitted-light microscope; (**a**) medium worker, (**b**) minor worker and (**c**) soldier. Under the cuticle of the mandible of medium worker, the newly developed mandible of next instar worker (i.e. major worker), with two distinct teeth, is visible (**a**: arrow head). The developing soldier mandible, with a single point, is seen through the mandible of minor worker (**b**: arrow head).

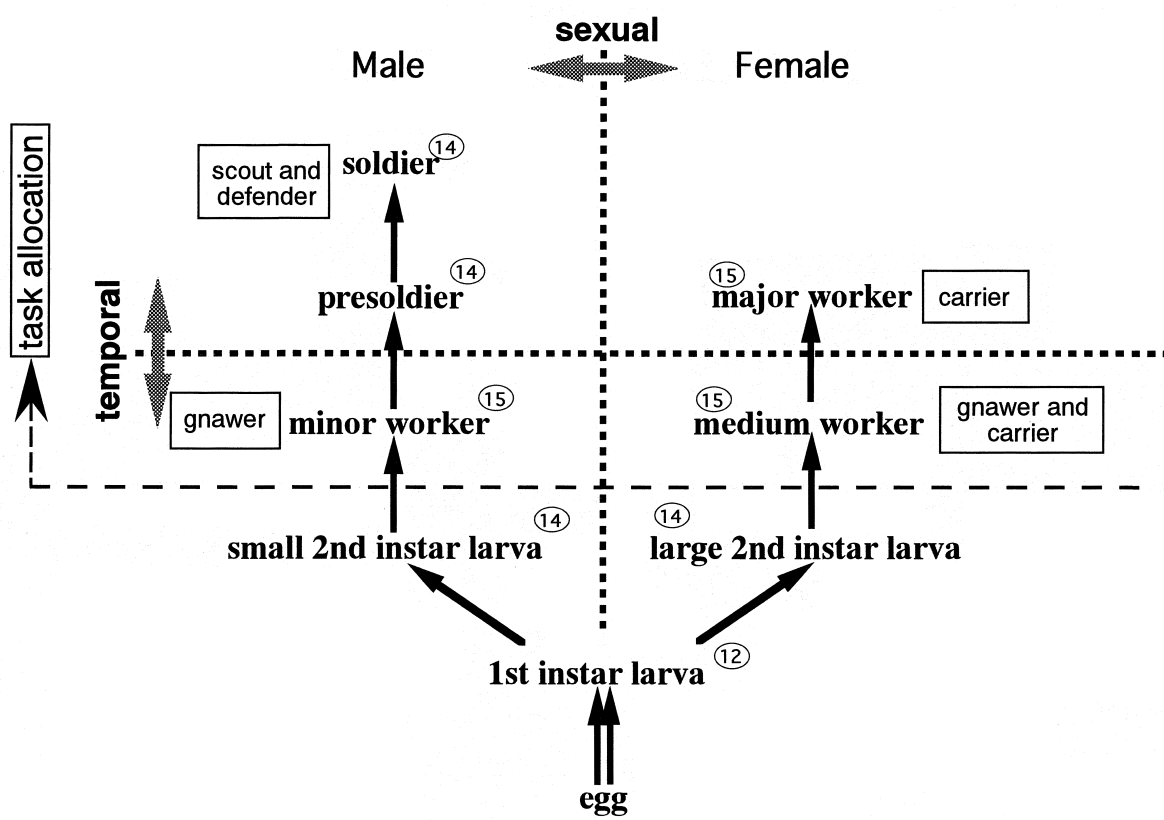


**Fig. 5.** Constructed schema of neuter caste developmental pathways in *Hospitalitermes medioflavus*. Numbers in circles indicate the number of antennal segments. In this species, the division of labor during foraging is realized through sexual and temporal polyethism among the neuter castes. Words in boxes by the castes indicate the tasks when foraging.

*Coatitermes* possess a simpler development, without sexual dimorphism or specialization of either sex in the production of soldiers. These facts may suggest the existence of a clade comprising *Nasutitermes*, *Trinervitermes*, *Velocitermes*, and probably other full nasute genera with similar caste systems, to which some neotropical genera such as *Subulitermes* and *Coatitermes* would not pertain (Roisin, 1996). Our present results support the inclusion of *Hospitalitermes* in this *Nasutitermes*-*Velocitermes* clade. Further studies could reveal whether caste systems of other open-air foraging nasutes, such as *Grallatotermes* or *Longipeditermes* (the latter possessing dimorphic soldiers), would also favor their inclusion in the same clade.

The polyethism pattern of *Hospitalitermes* possesses distinctive features which can be the ecology of this adapted to species, i.e., open-air foraging. It differs from that of

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*Nasutitermes* by the following points. In *Nasutitermes* species, first-instar workers (minor workers) tend to be of light pigmentation, older, more pigmented instars (medium and major workers) perform tasks in exposed conditions, such as initiation of foraging trails (Pasteels, 1965), nest repair (McMahan, 1970, 1977) or defense (Roisin *et al*., 1990). In *Hospitalitermes*, first-instar workers are fully pigmented and do participate in open-air foraging expeditions, where they perform most of gnawing activity (Miura and Matsumoto, 1995).

Based on this study on caste developmental pathways in *Hospitalitermes*, we can consider the relationship between development of the neuters and their tasks allocated in the colony. Although the division of labor in this species has been studied only in terms of foraging behavior, the task allocation among soldiers and the three types of workers is clearly visible (Miura and Matsumoto, 1995, 1998). Male neuters molt from second instar larvae to minor workers and then play the role of gnawers during food collection. Minor workers molt to soldiers (via the presoldier stage), thus dynamically changing their role to defense. On the other hand, female second instar larvae become medium workers. The medium worker, which works as both a gnawer and a food carrier, is part of a vast group (> 50% of all foragers; Miura and Matsumoto, 1998), which enables a very efficient foraging system for the colony. Medium workers can molt to major workers, which exclusively work as food-ball carriers. Task change during development is thus also seen in females, although not as deep as the change observed in males.

In conclusion, task allocation in *H. medioflavus* is realized in a two-dimensional way; firstly on the basis of sex, secondly on the basis of instar (Fig. 5). This species can thus be said to display sexual and temporal polyethism. In termites, several studies of polyethism have distinguished size classes (known to be sex-based) and age classes (Noirot, 1955; Pasteels, 1965; McMahan, 1979). In this study, the social organization of foraging and the caste developmental system were combined to obtain a complete, dual picture of polyethism in *H. medioflavus*.

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