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Soldier-like Intercastes in the Rotten-wood Termite *Hodotermopsis sjostedti* (Isoptera: Termopsidae)

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ABSTRACT—All termite species possess a distinct sterile-soldier caste in their colonies, although reproductive soldiers, with soldier characteristics and reproductive ability, have been reported from several species of the family Termopsidae. Such intercastes have been considered the primitive-soldier caste, and based on this many researchers have discussed the evolutionary origin of termite soldiers. We investigated whether such soldier-reproductive intercastes also exist in the Japanese rotten-wood termite *Hodotermopsis sjostedti*. Abnormal individuals with soldier-like characteristics were found and designated as soldier-like intercastes, which appeared to have both soldier and reproductive characteristics. Based on our morphometric analyses and histological examinations, we suggest that the developmental origin of this intercaste is a pseudergate, nymph or sixth-instar larva. In addition, the intercaste was found to have relatively well-developed gonads, although mature oocytes and spermatozoa were not found in female ovaries and male seminal vesicles, respectively. We conclude that the soldier-like intercaste of *H. sjostedti* does not have reproductive ability, which is different from all other known soldier-like intercastes in Termopsidae.

Key words: caste differentiation, gonad, morphometry, reproductive soldier, soldier neotenic

INTRODUCTION

In termites, intercaste individuals that have intermediate morphology of two or more castes have been occasionally found in a colony (e.g. Lefeuvre and Thorne, 1984; Roisin and Pasteels, 1986), and are considered the by-product of plastic post-embryonic development. Among others, the 'reproductive soldier' (or 'soldier neotenic'), found in three species of the genus *Zootermopsis*, is a notable example of an intercaste, because it provides us with insights into the evolutionary origin of termite soldiers (Heath, 1928; Myles, 1986; Roisin, 2000; Thorne *et al.*, 2003). In *Archotermopsis wroughtoni* and *Stolotermes brunneicornis*, soldiers with functional gonads have been reported (Imms, 1919; Gay and Calaby, 1970). Myles (1986) pointed out that 'reproductive soldiers' or 'soldiers with functional gonads' are found in socially-primitive termites, and that soldiers might have originally evolved by individual-level selection under intracolony conflict among replacement reproductives. Thorne (1997) mentioned that soldiers in the primitive termites might have retained reproductive capability, although it was

unclear whether the primitive soldier functioned as a defensive caste or a replacement reproductive. Roisin (1994, 1999) criticized Myles's hypothesis, based on two reasons: there was no evidence for an actual role of soldier weapons in intracolony fight; and it was improbable for soldiers to become sterile if soldiers were originally reproductives. Recently, Thorne *et al.* (2003) revealed that reproductive soldiers are adaptive for intercolony conflicts among incipient colonies, which is conceptually similar to Myles's (1986) hypothesis of intracolony conflict.

In Termopsidae, *Hodotermopsis* is the only genus where the presence of reproductive soldiers remains to be determined (Myles, 1986). Based on the normal caste developmental pathways of *Hodotermopsis sjostedti* (Miura *et al.*, 2000, 2004), we here investigate whether reproductive soldiers exist in this species. Soldier-like intercastes were found from several colonies; therefore, we examined their morphology and fecundity. Based on these results, the developmental and evolutionary origin, and functions of the soldier-reproductive intercastes, are discussed.

MATERIALS AND METHODS

Termite sampling

Recently, seven described species of the genus *Hodotermopsis* were revised into the single species, *H. sjostedti* Holmgren (Takematsu, 1996; Huang *et al.*, 2000). The species is distributed

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throughout northern Vietnam (Holmgren, 1911), southern China (Huang *et al.*, 2000), Taiwan and the Satsunan Islands in southern Japan (Matsumoto and Hirono, 1985; Maekawa *et al.*, 1998). Colonies of *H. sjostedti* were sampled from rotten wood in evergreen forests, on Yakushima Island, Kagoshima Prefecture, Japan, during each May of 2000 to 2002. Soldier-like intercastes found in the field were fixed immediately in FAA fixative (formalin: acetic acid: ethanol=6:1:16). Other colony individuals were kept in the laboratory as stock at approximately 25°C under constant darkness. Soldier-like intercastes differentiated in the laboratory were also fixed in FAA fixative. After fixation, all samples were transferred to 70% ethanol for preservation.

Morphometric study

To evaluate the allometric features of the soldier-like intercaste, we carried out morphometric analysis based on the measurements of body parts. We used the following 17 measurements: 1) head length; 2) maximum head width; 3) head width at base of mandibles; 4) labrum width; 5) postmentum length; 6) postmentum width; 7) left mandible length; 8) maximum head height; 9) head height at base of mandible; 10) eye diameter; 11) pronotum length; 12) pronotum width; 13) mesonotum width; 14) metanotum width; 15) femur width; 16) femur length; and 17) tibia length. The measurements were performed using an image analysis system with a CCD camera, HIM-1N (HOGA, Osaka, Japan). The data set of other castes, according to Koshikawa *et al.* (2002), was also used for comparison with intercastes, and the measurements of eye diameter in other castes were added for the present study. The measurements of neotenics (supplementary reproductives) were also included in this analysis.

We performed principal component analysis (PCA) on the above parameters, using SYSTAT (SPSS, Chicago, IL, USA), to evaluate the integrated morphological information, as used in some previous studies on termite-caste development (Roisin and Pasteels, 1986; Lelis and Everaerts, 1993). A correlation matrix was used for extraction, which is equivalent to using standardized data.

Observations on reproductive organs

We observed the reproductive organs of soldier-like intercaste individuals to compare their fertility with neotenics and soldiers. For paraffin sections, abdomens were dehydrated by transfer into increasing concentrations of ethanol, and then transferred into xylene and finally embedded in paraffin. Successive sections (7 µm in thickness) were stained using the standard hematoxylin-eosin staining method, and enclosed with Canada balsam. We observed the sections using a BX-51 microscope (Olympus, Tokyo, Japan), and captured the images with a DP-50 CCD camera (Olympus) and a Viewfinder Lite program (Eridas, Atlanta, GA, USA). We focused on spermatogenesis in the testes and sperm storage in the seminal vesicles for males, and oogenesis in the ovaries for females.

RESULTS

Soldier-like intercastes in *H. sjostedti*

Of the 28 examined colonies, a total of 20 intercaste individuals (10 males and 10 females), which had soldier-like characteristics, were found from nine colonies (Table 1). Among them, a male intercaste was found from an experi-

Table 1. Summary of intercastes and their sampling conditions.

Colony No.	Approx No. of Nest Mates	Castes in Colony	Date of Colony Sampling	Intercaste No.	Sex of Intercaste	Collection Place	Date of Finding Intercaste
I	1000 – 10000	Pe, L	23. May. 2000	1	M	Field	23. May. 2000
				2	M	Field	23. May. 2000
II	20000	K, Q, Nym, Pe, L	23. May. 2000	3	M	Lab.	8. Apr. 2001
				4	M	Lab.	9. Apr. 2001
III	1000 – 10000	K, Neo, Nym, Pe, L	23. May. 2000	5	F	Field	23. May. 2000
				6	F	Lab.	26. Sep. 2000
IV	1000 – 10000	Pe, L	23. May. 2000	7	F	Field	23. May. 2000
				8	F	Field	23. May. 2000
V	1000 – 10000	Nym, Pe, L	12. May. 2001	9	F	Field	12. May. 2001
VI	10000 – 100000	Neo, Nym, Pe, L	16. May. 2002	10	F	Field	16. May. 2002
				11	M	Field	16. May. 2002
				12	M	Field	16. May. 2002
				13	M	Field	16. May. 2002
VII	10000 – 100000	Neo, Nym, Pe, L	17. May. 2002	14	F	Field	17. May. 2002
				15*	M	Field	17. May. 2002
VIII	1000 – 10000	Neo, Pe, L	18. May. 2002	16	M	Field	18. May. 2002
				17	F	Field	18. May. 2002
IX	1000 – 10000	Neo, Pe, L	18. May. 2002	18	F	Field	18. May. 2002
				19	F	Field	18. May. 2002
II'	50 from colony II	Nym	23. May. 2000	20	M	Lab.	15. Oct. 2000

M: Male, F: Female, K: Primary king, Q: Primary queen, Neo: Neotenic, Nym: Nymph, Pe: Pseudergate, L: Larva. Colony II' was experimentally made in the laboratory of nymphs from Colony II. The individual marked with an asterisk (*) was a presoldier-like intercaste, while others were soldier-like intercastes.

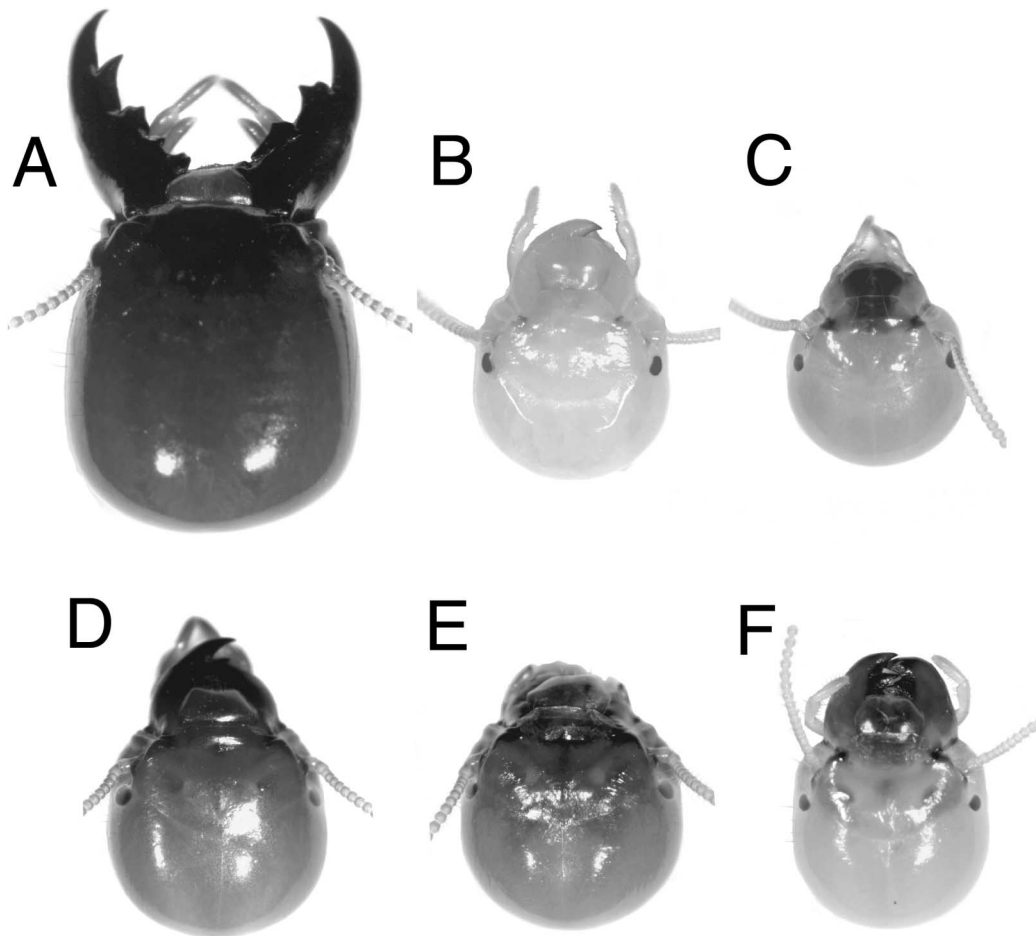


Fig. 1. The head morphology of various castes. A: soldier, B: presoldier, C: pseudergate, D–F: soldier-like intercastes (Nos. 6, 7, 15 in Table 1, respectively). Most of the intercastes have incomplete soldier-like head morphology (D and E). One intercaste individual showed presoldier-like morphology, although the mandibles and an anterior part of the head were pigmented abnormally (F). Bar indicates 2 mm.

mental colony composed only of nymphs for the examination of regressive molts (Koshikawa *et al.*, 2001). There seemed to be no correlation between the appearance of intercastes and colony size or the presence/absence of reproductives. As for head morphology, the intercastes superficially showed the intermediate characters between a pseudergate and soldier (Fig. 1). Although the intercaste mandibles were apparently longer than those of a pseudergate, only the anterior parts of the head were sclerotized, suggesting that they were different from normal soldiers (Fig. 1D and E). Most of the soldier-like intercastes had such morphological characteristics, although there was one exceptional male (Table 1, No.15, and Fig. 1F), with a morphology intermediate to that of a pseudergate and presoldier, with bag-like anterior tips on its mandibles. Most intercastes showed aggressive behavior, suggesting that the intercastes are engaged in colony defense, at least to some extent.

Morphometric study

As shown in our previous study (Koshikawa *et al.*, 2002), the PCA well explained the allometric changes during soldier differentiation (Fig. 2A). The first principal component

accounted for 87.1% of the total variance (eigenvalue: 14.81), and positively correlated with all measurements, showing the general size of insects. The second principal component, which accounted for 7.0% of the total variance (eigenvalue: 1.19), showed allometric differences. The second principal component positively correlated with 1) head length, 2) maximum head width, 3) head width at base of mandibles, 5) postmentum length, 6) postmentum width, 7) left mandible length, 8) maximum head height, 9) head height at base of mandible, 12) pronotum width and 15) femur width, but negatively correlated with 4) labrum width, 10) eye diameter, 11) pronotum length, 13) mesonotum width, 14) metanotum width, 16) femur length and 17) tibia length. Altogether, the first and second principal components expressed 94.1% of the variation among the allometric data. As seen in Fig. 2A, the soldier-like intercaste data grouped separately, indicating that intercastes have apparently different allometry from the other castes.

A scattergram of head width against left-mandible length indicates distinct morphological changes during soldier differentiation, in addition to the morphological differences of intercastes (Fig. 2B). The graph shows that these

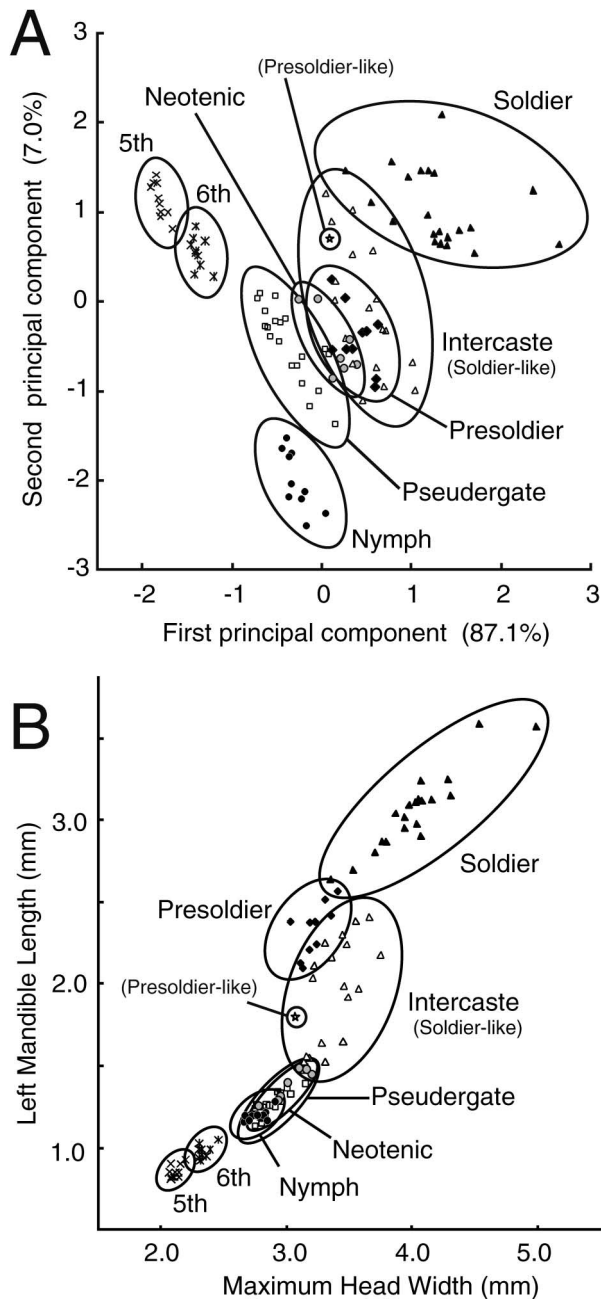


Fig. 2. Results of morphometric analysis. A: Plots from principal component analysis, explained by the first and second principal component axes. B: Plots of left mandible length against maximum head width. Each graph separated the castes distinctly. Inter castes have allometry that is apparently different from any other caste.

characters of the intercastes are intermediate to those of the pseudergates and presoldiers.

Observation of reproductive organs

Among the observed castes (soldier, neotenic and intercaste), the female neotenic (supplementary reproductive) had the largest ovaries with developing oocytes (Fig. 3A). The ovaries of soldier-like intercastes and soldiers were not well developed (Fig. 3D and G), and similar to other non-

reproductive castes, such as pseudergates and presoldiers (data not shown). Therefore, we concluded that the female intercastes did not have reproductive capability.

In the male neotenic, testes were well developed and produced spermatozoa (Fig. 3B), and the seminal vesicles also contained spermatozoa (Fig. 3C). However, in most soldiers, testes were degenerated and spermatozoa were not observed (Fig. 3H), and the seminal vesicles also lacked spermatozoa (Fig. 3I). In the male soldier-like intercastes, testes were less developed than those of neotenic, but greater than those of soldiers and other non-reproductive castes, and all the observed intercastes produced spermatozoa (Fig. 3E). However, no spermatozoa were observed in the seminal vesicles (Fig. 3F), suggesting that the male intercastes did not have ability to inseminate, even though they had more developed reproductive traits than the soldiers.

DISCUSSION

Morphological observations and morphometric analysis are the standard methods to infer developmental pathways in termites (e.g. Roisin and Pasteels, 1986). Therefore, we thought these methods might be useful for the characterization of the soldier-like intercaste in *H. sjostedti*. The morphometrics of the intercastes were intermediate to those of the pseudergates and presoldiers (Fig. 2B). There was one direct observation in which an intercaste was derived from an experimental colony exclusively composed of nymphs, although it remains unknown whether the intercaste was derived through one or two molts from the nymphal stage. We consider that the differentiation through two molts seems more probable, because the meso- and metanotum shapes were not trapezoidal, which is the typical evidence for an individual directly derived from a nymph through a single molt (Koshikawa *et al.*, 2001). This species has only a single nymphal stage (brachypterous nymph) (Miura *et al.*, 2004), and normal soldiers also differentiate from nymphs (Miura *et al.*, 2000; Koshikawa *et al.*, 2001). From these findings, together with the morphometric data, it seems reasonable that the intercastes differentiated from pseudergates, nymphs or sixth-instar larvae. The intercaste differentiation from neotenic, presoldiers and soldiers is thought to be improbable, because neotenic and soldiers are at the developmental end point in the differentiation pathways (Miura *et al.*, 2000), and the regressive molt from presoldier has never been reported.

The fact that the proportion of soldier-like intercastes in a colony was considerably lower (Table 1), suggests that the intercastes are not adaptive in termite colonies, but just developmental abnormalities. We speculate that the semi-developed gonads and incomplete soldier traits in the intercastes were caused by some unusual physiological conditions. Actually, high juvenile hormone (JH) titer causes intercaste differentiations in many termites, including artificial applications of juvenile hormone analogue (JHA) (e.g. Howard and Haverty, 1979; Miura *et al.*, 2003). In some ter-

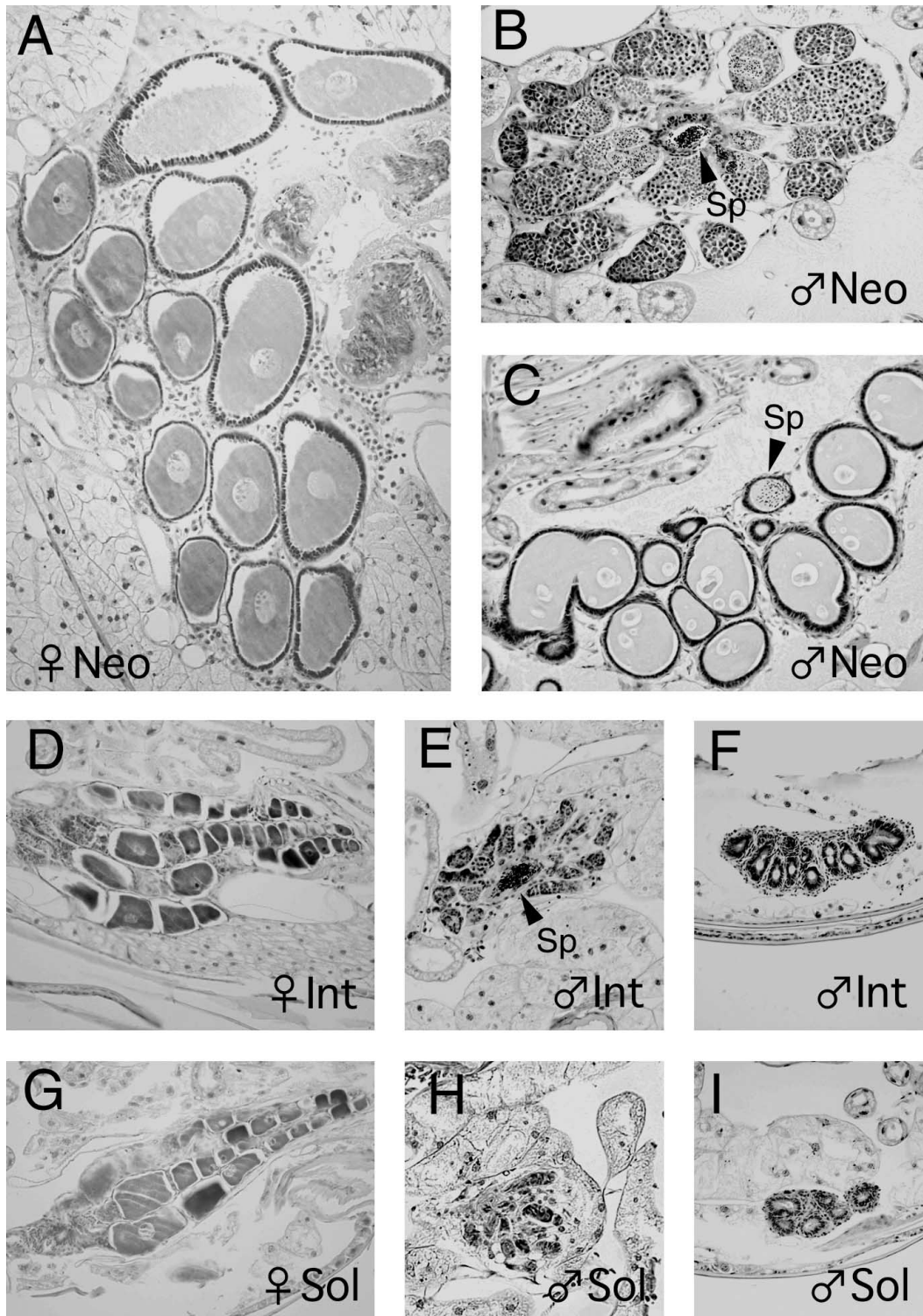


Fig. 3. Paraffin sections of reproductive organs. A–C: neotenics, D–F: intercastes, G–I: soldiers. A, D, G: female ovaries, B, E, H: male testes, C, F, I: male seminal vesicles. Sp: spermatozoa. In the female intercaste, the ovary was not fully developed (D). In the male intercaste, the undeveloped seminal vesicle did not contain spermatozoa (F), although the testis produced them (E). The ovoid structures in the seminal vesicles of the male neotenic (C) were considered as vacant space of seminal-vesicle secretion, which appeared at fixation. Bar indicates 200 μm .

mite species, intercaste differentiation is caused by endoparasitic dipteran larvae parasitizing the head capsule (Grassé, 1986). In *H. sjostedti*, however, intercaste differentiation does not seem to be the result of such parasitism,

because we did not find any larvae in the head capsule of intercastes.

The intercastes in *H. sjostedti* resembled the reproductive soldiers of *Zootermopsis* in head morphology (e.g.

Myles 1986), although the fertility of soldier-like intercastes was not observed in the present study. In *Archotermopsis wroughtoni* and *Stolotermes brunneicornis*, normal soldiers also possess well-developed gonads (Imms, 1919; Gay and Calaby, 1970). However, the situation in *H. sjostedti* is different from those of *Zootermopsis*, *Archotermopsis* and *Stolotermes*; soldiers have degenerated gonads and there is a soldier-like intercaste that does not have reproductive ability. Thus, this intercaste cannot be considered a reproductive-soldier caste, although we cannot entirely exclude the possibility that both intercastes and reproductive soldiers exist, as our limited sampling methods mean that we might not have found them.

Recently, Thorne *et al.* (2003) pointed out that the reproductive soldiers in *Zootermopsis* play an important role in intercolonial conflicts among incipient colonies. However, the adaptive function of the soldier-like intercaste in *H. sjostedti* is obscure. The low proportion of intercastes in a colony suggests that they are not important, at least in a large colony. Although intercastes have not been found from incipient colonies so far, further detailed investigations are required to conclude their adaptive significance. Considering that *Hodotermopsis* and *Zootermopsis* are thought to be closely related (Krishna, 1970), reproductive soldiers in *Zootermopsis* might be derived from such abnormal intercastes through adaptation for defense in incipient colonies.

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