

Research article

Comparative studies on alate wing formation in two related species of rotten-wood termites: *Hodotermopsis sjostedti* and *Zootermopsis nevadensis* (Isoptera, Termopsidae)

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Summary. Termite colonies are comprised of several types of castes that differentiate throughout postembryonic development. In termopsid termites (family Termopsidae), alates are normally differentiated from apterous instars by two nymphal instar stages and three moulting events. Here, we report that of the rotten-wood termite *Hodotermopsis sjostedti*. There is only a single nymphal instar between the pseudergate and alate stages. During the annual alate production season in June/July, we observed some nymphs with small wing buds which were dorsally swollen. Those nymphal individuals subsequently moulted into alates through only a single moult. We examined their histology and internal morphology and observed that the folding pattern of the newly formed wings was very different from that seen in second stage nymphs of the closely related species *Zootermopsis nevadensis*. The newly formed wings of *H. sjostedti* are formed inside the relatively smaller wing buds and therefore must be folded in a complicated manner. Our observations revealed that the tips of the folded wings were elongated and bent, such that they overlapped in the median plane. We suggest that heterochronic evolutionary change accounts for the compression of nymphal development into a single instar. We also suggest that this probably occurred at either the individual or colony level in this species.

Key words: Alate differentiation, caste differentiation, hemimetaboly, nymphal instar, wing formation.

Introduction

Caste differentiation is deeply related to the postembryonic development of insects. However, Hymenoptera and Isoptera have different caste systems, based on different developmental modes known as holometaboly and hemimetaboly, respectively (Nijhout and Wheeler, 1982). Morphologically

different types of individuals, or castes, are produced by means of ontogenetic regulation, which is affected by extrinsic factors such as interactions among colony members (Noirot, 1991; Miura, 2001).

The worker caste of the seven termite families, diverges from the alate developmental line at the first or second moult in the Mastotermitidae, Hodotermitidae, Termitidae and in some Rhinotermitidae. This developmental pattern is called the bifurcated pathway (Watson and Sewell, 1985). In the alate developmental line of these termite groups, alates develop through several moulting events, with the gradual growth of alate features such as wing buds (Miller, 1969; Noirot, 1969; Roisin, 2000). In contrast, the developmental lines to alates and to neuters in the Kalotermitidae, Termopsidae, and the remaining of Rhinotermitidae are not separated until the older instars. This is called the linear pathway (Watson and Sewell, 1985). Nymphs derive from these older apterous instars, which play a worker role and are therefore called 'pseudergates' (Grassé and Noirot, 1947). Most species possess at least two nymphal instars with wing buds before the imaginal moult to alates. There are, however, some exceptions. In the Rhinotermitidae, two species are reported to have a single nymphal instar: *Prorhinotermes inopinatus* (Roisin, 1988) and *Termitogetonr. planus* (Parmentier and Roisin, 2003). Until the present report, all known Kalotermitidae and Termopsidae were reported to have at least two nymphal instars (Noirot, 1985).

In this paper, we report the process of wing development in the rotten-wood termites *Hodotermopsis sjostedti* (previously *H. japonica*) and *Zootermopsis nevadensis* (Isoptera: Termopsidae). The Termopsidae is thought to be positioned in a relatively basal part of the termite order Isoptera (Kambhampati et al., 1996; Kambhampati and Eggleton, 2000; Thompson et al., 2000). Species of this family reveal that older instar individuals, the so-called pseudergates, play the role of workers. Some researchers have even defined the pseudergate more strictly as a worker-like individual derived

through regressive or stationary moults (Grassé and Noirot, 1947; Thorne, 1996). We actually documented that *H. japonica* individuals derived from regressive moults, also work as pseudergates (Koshikawa et al., 2001). The older instars of *H. japonica* are totipotent and can differentiate into any other caste, e. g., winged alates, soldiers, and supplementary reproductives (Miura et al., 2000). In contrast to the developmental pathways of castes in *Zootermopsis* spp. (Thompson, 1922; Heath, 1927; Thorne, 1997), those of *H. sjostedti* are less well studied. In a previous study, however, we did notice that the number of nymphal instars was different in *H. sjostedti* than in other species of Termopsidae (Miura et al., 2000). This paper presents evidence of the loss of a nymphal instar in this species by comparing the developmental stages of *H. sjostedti* with those of *Z. nevadensis*. In addition, it suggests that this evolutionary event has occurred in relation to the social interactions of this species.

Materials and methods

Insects

The rotten-wood termite *Hodotermopsis sjostedti* is distributed throughout East Asia, from the Satsunan Islands in Japan to northern Vietnam (Matsumoto et al., 1990). The Japanese population of this species was formerly described as *H. japonica* (Holmgren, 1912; Esaki, 1956); however all *Hodotermopsis* species were recently integrated as *H. sjostedti* (Takematsu, 1996; Huang et al., 2000). We collected colonies of *H. sjostedti* on Yaku-shima Island in May or June of every year from 1997 to 2001. This species rarely produces winged alates, although we sometimes found nymphs with tiny wing buds. In June 2001, however, some of the laboratory colonies produced nymphs with swollen wing buds, which later became winged alates.

We also collected colonies of the American rotten-wood termite *Zootermopsis nevadensis* from Kawanishi in Hyogo Prefecture, Japan, in October 2000. This species, which was recently introduced into Japan (Morimoto, 2000), possesses nymphs with apparent wing buds throughout the year and produces alates annually. The sampled colonies of both species were kept in the laboratory in plastic boxes at $25 \pm 1^\circ\text{C}$ and 70% humidity, under constant darkness.

We collected various stages of nymphs and alates and fixed them in FAA (formalin: ethanol: acetic acid = 6:16:1) for about 24 hours. They were then transferred to 70% ethanol and preserved for histological examination and scanning electron microscopy.

Histological examination

To observe the wing formation histologically, paraffin sections were made and stained with hematoxylin and eosin. The thoracic part of the termites was dehydrated in increasing concentrations of ethanol transferred to xylene, and finally embedded in paraffin. Successive sections (5 μm in thickness) were mounted on glass slides coated with egg-white glycerin. Paraffin was removed from the sections with xylene. The sections were then rehydrated and stained with hematoxylin solution (0.1% hematoxylin, 0.02% NaIO_3 , 5% $\text{AlK}(\text{SO}_4)_2$, 0.1% citric acid, 5% trichloroacetaldehyde) and 0.5% eosin solution. After staining, the slides were washed, dehydrated, and sealed with Canada balsam and cover glasses.

Scanning electron microscopy

Scanning electron microscopy (SEM) was used to investigate the formation of the wing structure in the nymphal stage. Fixed samples were

dehydrated by transfer through increasing concentrations of ethanol (up to 100%), acetone, and finally hexamethyldisilazane. After air-drying, the samples were coated with gold using an ion coater (Eiko IB-3). Scanning electron micrographs were taken in a Hitachi S-405 electron microscope.

Results

Alate production

There were several nymphal individuals with small wing buds in the sampled colonies of *H. sjostedti*. These individuals were designated as being in the first nymphal stage. Under the laboratory conditions used, we found that alates were produced in some of the colonies in June 2001. Similar occurrences had been recorded in previous years during our continuous investigations on the species. We were able to identify a number of nymphal individuals which had swollen thoracic segments with small wing buds in the colonies producing alates. These wing buds were not as elongated as those seen in many other species of termites (Fig. 1a).

All of the *Z. nevadensis* colonies had a number of nymphs with apparent wing buds. These individuals were designated as second instar nymphs. Alates were produced in these colonies a few times during the year, probably because we

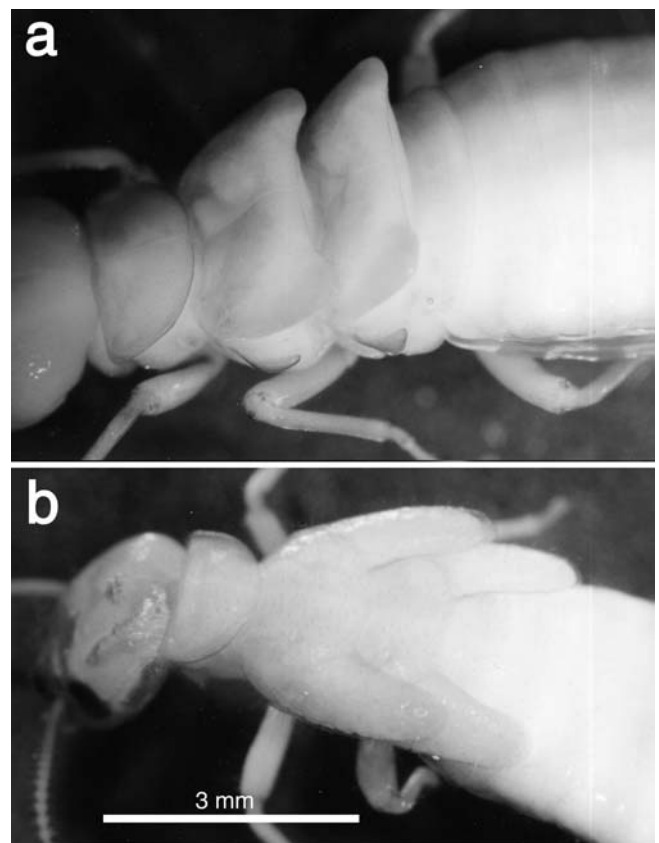


Figure 1. Photographs of final instar nymphs with swollen wing buds, just prior to their moult to alates: (a) *Hodotermopsis sjostedti*, (b) *Zootermopsis nevadensis*

kept the colonies under constant warm conditions ($25 \pm 1^\circ\text{C}$). The second-stage nymphs had swollen wing buds (Fig. 1 b) just prior to their moult into alates.

Histological findings

We made cross sections of the thoracic segments of *H. sjostedti* and *Z. nevadensis* nymphs that possessed swollen wing buds (Figs. 2, 3). Well-developed and folded wings were seen inside the swollen wing buds in *H. sjostedti*, (Fig. 2 a, c). In addition, we could easily recognize well-developed wing muscles inside the trunk of the thorax (Fig. 2 d). Compared with the folding pattern of the wings in *Z. nevadensis*, the thickness of the wing buds in *H. sjostedti* was greater (compare Figs. 2 a and 3 a) and the right and left wings overlapped each other (Fig. 2 c). When the median planes of both species

were examined, we could see folded wings in *H. sjostedti* (Fig. 2 b), which were elongated from the lateral side, while no wing structure could be seen in *Z. nevadensis* (Fig. 3 b).

Scanning electron microscopy

The SEM images of the nymphal instars of both *H. sjostedti* and *Z. nevadensis* (Fig. 4) revealed that the single nymphal stage of *H. sjostedti* (Fig. 4a) was similar in structure to that of the first stage nymph of *Z. nevadensis* (Fig. 4c). There was an obvious difference in wing bud morphology between the first and second stage nymphs of *Z. nevadensis* (Fig. 4c and d). In order to understand the folding pattern of the wing just before the moult into alates, we dissected the cuticle of the swollen wing buds of nymphs of both species. The tips of the wings of the *H. sjostedti* nymphs were turned towards the

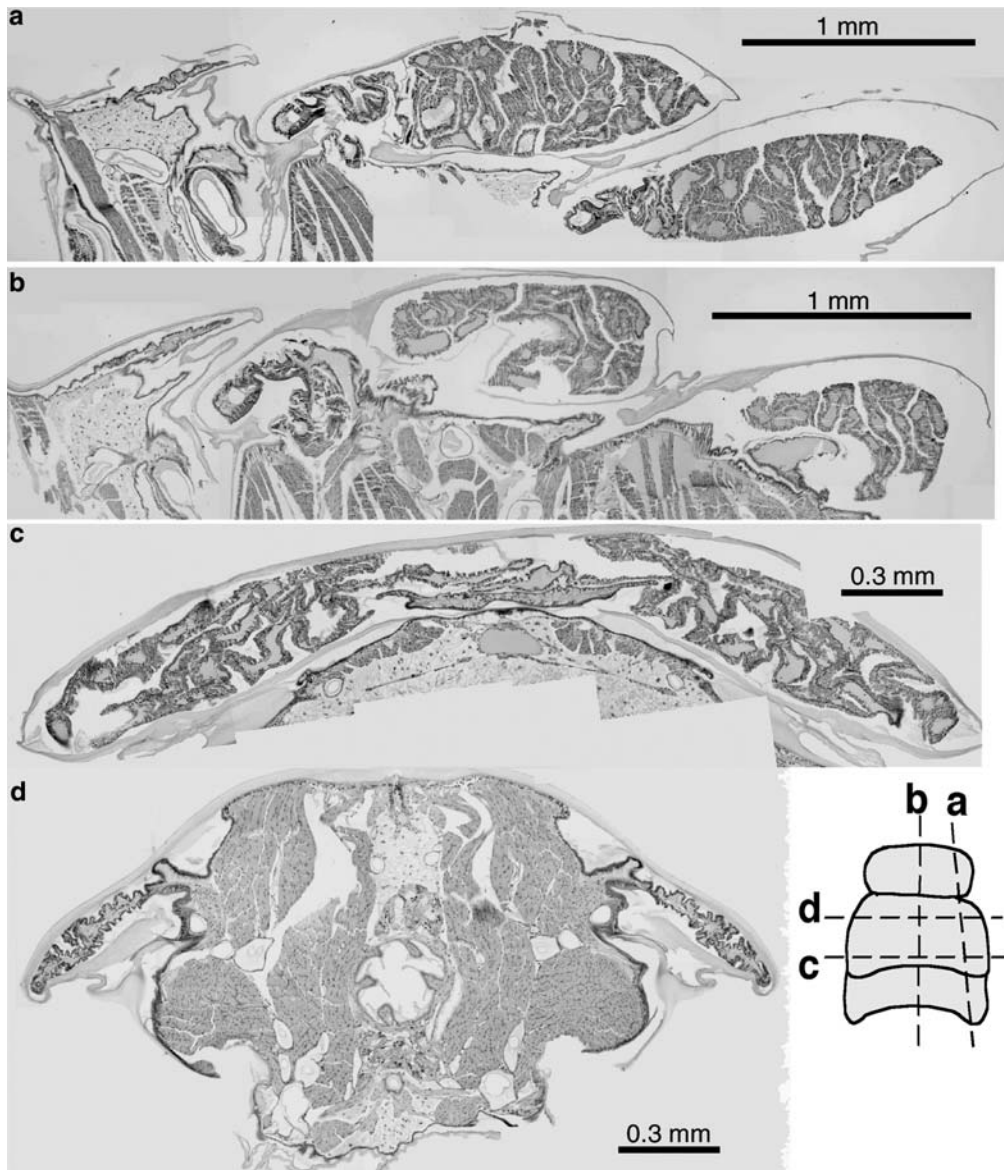


Figure 2. Cross sections of swollen wing buds of nymphs of *H. sjostedti*: (a, b) sagittal sections (lateral and median planes, respectively); transverse sections showing (c) folding wings and (d) flight muscles

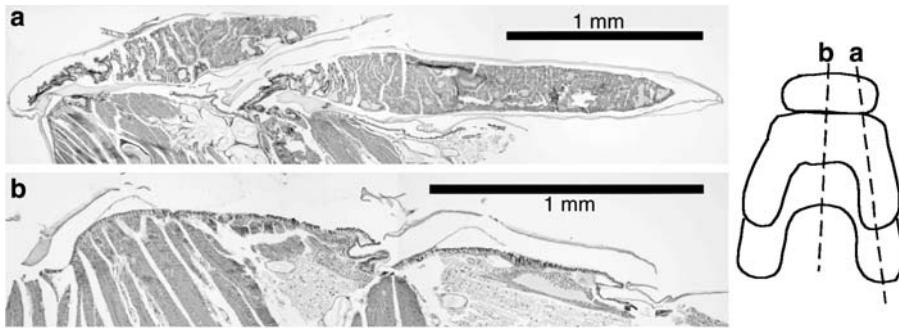


Figure 3. Cross sections (sagittal planes) of swollen wing buds of nymphs of *Z. nevadensis*: (a) lateral plane showing folded wings, (b) median plane showing no wing structure on the thoracic segments

medial region of the body (Fig. 4b), while the wings of *Z. nevadensis* were folded towards the tips along the wing buds and there was no wing structure at the median thorax (Fig. 4e). These results indicate that alate development apparently differs between the two species: Namely, alates derive from a single nymphal stage with specific wing formation in *H. sjostedti*.

Discussion

Our morphological observations on wing buds in final stage nymphs indicate that there are obvious differences in alate development between the two termopsid species, *H. sjostedti* and *Z. nevadensis*. As described in Castle (1934), *Z. nevadensis* has two nymphal instars, which possess short and long wing buds, respectively (Fig. 4c, d). This seems to be the normal developmental pattern in many lower termites (Noirot, 1985). In contrast, *H. sjostedti* has only a single nymphal stage with tiny wing buds, a stage that derives from the seventh instar. In our previous study on caste systems (Miura et al., 2000), we were sceptical about the existence of a second nymphal stage. Based on the results of the present study, we can redraw the caste developmental pathways as shown in Fig. 5. According to this schema, the morphogenesis of alate characters, including wing formation, occurs only during a single nymphal stage with tiny wing buds. Indeed, an accelerated wing preparation was observed under the thoracic cuticle of these nymphs. Both the wing itself and the well-developed wing muscles were observed (Fig. 2d), indicating that these individuals were prepared for the nuptial flight.

During the accelerated wing formation in *H. sjostedti*, which is substantially different from that occurring in multiple nymphal stages, newly formed wings were folded inside the small wing buds and the folded wings were directed towards the median region of the thorax (Figs. 2, 4). As a result, the meso- and metathorax was thicker (Fig. 2). This unusual folding pattern is apparently caused by the constraint of wing development inside the small wing buds, which make it impossible for the wings to spread posteriorly.

Alate development during a single nymphal stage as in *H. sjostedti*, possibly evolved from termites that have two nymphal stages. This is based on the theory that *Hodoter-*

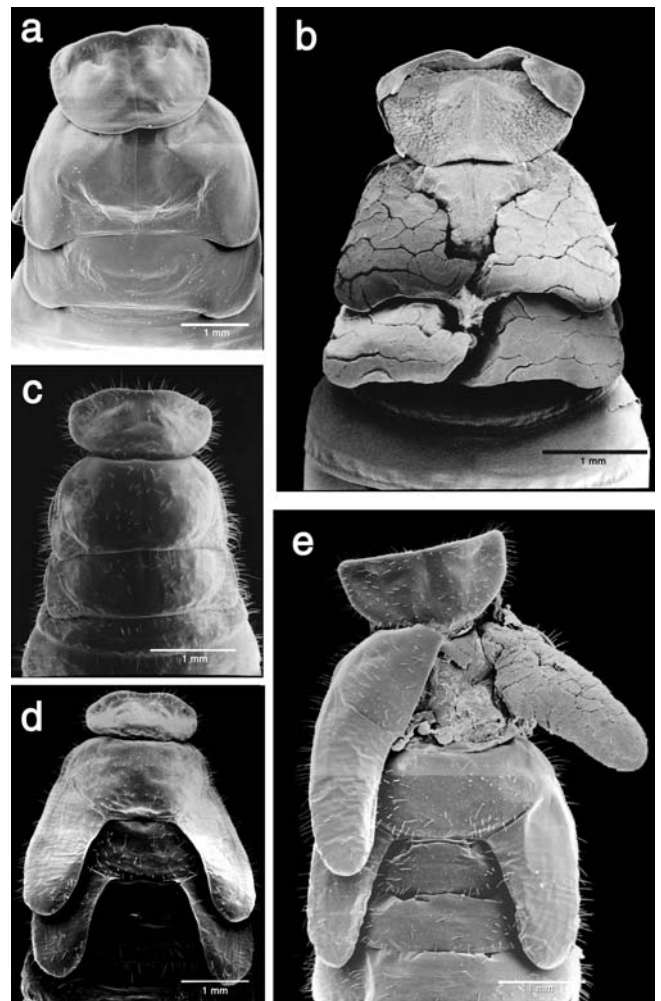


Figure 4. Scanning electron microscopy of the folded wings of nymphs: (a) *H. sjostedti* single instar nymph before wing bud swelling, (b) *H. sjostedti* nymph with swollen wing buds (cuticle of thorax removed), (c) *Z. nevadensis* first stage nymph with small wing buds, (d) *Z. nevadensis* second instar nymph before wing bud swelling, (e) *Z. nevadensis* second instar nymph with swollen wing buds (cuticle of the right forewing was removed)

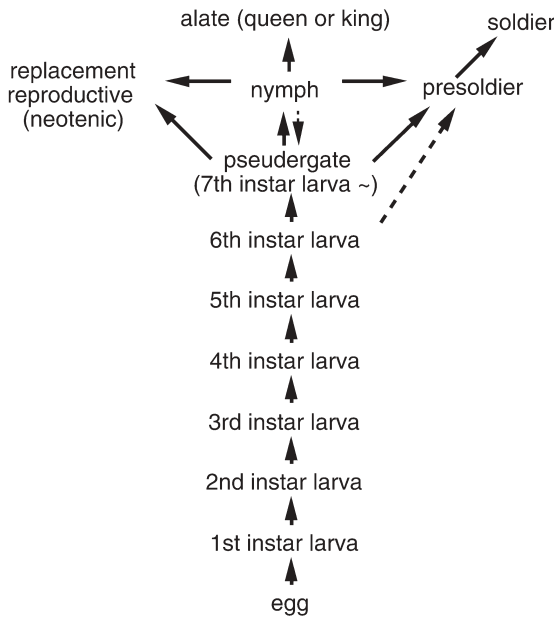


Figure 5. Proposed schema of the caste developmental pathways of *H. sjostedti*. Each arrow indicates a moult. Dotted lines indicate potential moults, which are suggested to occur under natural conditions. Here, we designate as ‘pseudergate’ an individual older than the 7th instar larva, which performs most of the colony tasks although it maintains the potential to become a reproductive

mopsis and *Zootermopsis* are thought to be sister taxa at the apical position in the phylogeny of Termopsidae (Krishna, 1970; Kitade, pers. comm.) and that two nymphal stages with wing buds have been reported in all *Zootermopsis* species (Heath, 1927; Thorne, 1997) and in *Archotermopsis wroughtoni* (Imms, 1919), *Stolotermes ruficeps* (Morgan, 1959), and *S. brunneicornis* (Miura, unpublished data). Even outside the family, few termite species are known to produce alates through a single nymphal instar (Miller, 1969; Noirot, 1969; Noirot and Pasteels, 1987). The only exceptions have been reported in *Prorhinotermes* and *Termitogeton* in the Rhinotermitidae, both of which show unique wing formation (Roisin, 1988; Parmentier and Roisin, 2003). It would seem erroneous to conclude that the second stage has been skipped in *Hodotermopsis*, rather that the alate formation has been accelerated and compacted within the final nymphal instar stage. In either case, a heterochronic change in alate wing formation has apparently occurred in the lineage of *Hodotermopsis*.

The adaptive meaning of nymphal compression in *H. sjostedti* may have an individual or colony level basis. Considering that nymphs also pursue colony tasks like pseudergates (Thorne, 1997), the single-instar nymphs might play a worker role until their final moult into alates. In other words, colonies may be able to rapidly produce alates from working individuals, as a countermeasure against emergencies.

A number of studies have suggested that conflicts exist among the reproductives in termite colonies (Myles, 1986, 1988). In such cases, wing-budded nymphs will fight with each other, which can result in nymphs with mutilated wing

buds. These damaged nymphs cannot develop into alates, but will undergo regressive moulting (Roisin, 1994). We did indeed observe mutilated nymphs in colonies of *H. sjostedti* and *Z. nevadensis*. Wing mutilation has also been reported in other termopsid species (Heath, 1927; Myles, 1988; Lenz et al., 1991). It might be possible, therefore, that nymphs of *H. sjostedti* do not develop long wing buds in order to avoid wing-pad biting as a consequence of intracolony conflicts. Tiny wing buds are certainly more difficult to bite than larger wing buds (such as those in second-stage nymphs). The developmental consequence of intracolony conflict has also been reported as caste mimicry in *Zootermopsis* (Hahn and Myles, 1994). To test this hypothesis, further studies are necessary to compare wing mutilation in *H. sjostedti* with that in other species.

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