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Sexual difference in juvenile-hormone titer in workers leads to sex-biased soldier differentiation in termites

Kouhei Toga [a,b], Shutaro Hanmoto [a], Ryutaro Suzuki [a], Dai Watanabe [c], Toru Miura [c], Kiyoto Maekawa [a,*]

[a] Graduate School of Science and Engineering, University of Toyama, Toyama, Japan
[b] Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan
[c] Graduate School of Environmental Science, Hokkaido University, Sapporo, Japan

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ABSTRACT

In termites, the soldier caste, with its specialized defensive morphology, is one of the most important characteristics for sociality. Most of the basal termite species have both male and female soldiers, and the soldier sex ratio is almost equal or only slightly biased. However, in the apical lineages (especially family Termitidae), there are many species that have soldiers with strongly biased sex ratio. Generally in termites, since high juvenile hormone (JH) titer is required for soldier differentiation from a worker via a presoldier stage, it was hypothesized that the biased soldier-sex ratio was caused by differences in JH sensitivity and/or JH titer between male and female workers. Therefore, we focused on the presoldier differentiation and the worker JH titer in species with only male soldiers (*Nasutitermes takasagoensis*) and with both male and female soldiers (*Reticulitermes speratus*) in natural conditions. In the former species, there are four types of workers; male minor, male medium, female medium and female major workers, and presoldiers differentiate from male minor workers. First, we tried to artificially induce presoldiers from male and female workers. In *N. takasagoensis*, the presoldier differentiation rate and mortality was significantly higher in male minor workers. Morphological analyses showed that both male and female induced presoldiers possessed normal soldier-specific morphologies. It was suggested that female workers, from which soldiers do not differentiate under natural conditions, also maintained the physiological and developmental potential for soldier differentiation. In *R. speratus*, however, no differences were observed in solder differentiation rate and mortality between male and female workers. Second, the JH titers of each sex/type of workers were quantified by high performance liquid chromatography–mass spectrometry in two different seasons (April and December). The results showed that, in *N. takasagoensis*, JH titer in male minor workers was consistently higher than those in other worker types. In *R. speratus*, in contrast, there were no significant differences in JH titers between male and female workers. These results suggested that, in *N. takasagoensis*, male minor workers maintain JH titers at a high level throughout a year, and this may cause the male-biased presoldier differentiation.

1. Introduction

The sex ratio of altruistic castes varies depending on each social animal. For example, social hymenopteran insects (ants and bees) possess only female workers, whereas sex ratios of soldiers are either equal or biased in termites (Roisin, 2000; Bourguignon et al., 2012). The termite soldier is a distinctive caste in terms of its morphology and social function (Deligne et al., 1981). Generally in termites, soldiers differentiate from workers via an intermediate state, the presoldier (Roisin, 2000). Soldiers are sterile and have species-specific defensive morphologies (Weesner, 1969). In the basal lineages (Hodotermitidae, Archotermopsidae, Rhinotermitidae and Serritermitidae), the soldier sex ratio is either equal or only slightly biased (Bourguignon et al., 2012). In the apical lineages (Termitidae), however, there are many species that have soldiers with strongly biased sex ratios (Bourguignon et al., 2012). For example, soldiers are all males in most of the examined species in the Nasutitermitinae, whereas they are females in most species in the Termitinae and Macrotermitinae. Therefore, sexual factors may be associated with the diversity of the soldier-differentiation pathway in the apical species of termites.

In the previous studies, the ultimate mechanism for soldiers with biased sex ratio was discussed in relation to the sexual size dimorphism in rhinotermitid termites *Reticulitermes* spp. (Matsuura, 2006, also refer to Bourguignon et al., 2012). However,

* Corresponding author.
E-mail address: kmaekawa@sci.u-toyama.ac.jp (K. Maekawa).

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the physiological and developmental mechanisms leading to a biased sex ratio in a caste is unknown. In some termitid species (family Termitidae) with a strongly biased sex ratio among soldiers (e.g. *Acanthotermes acanthothorax*, *Microcerotermes parvus*, *Nasutitermes arborum*), the presoldier differentiation from the opposite sex was observed artificially under abnormal caste compositions (Noirot, 1955). This suggests that the soldier differentiation is not completely determined by sex-linked genetic factors (e.g. Lo et al., 2009), although no studies have yet attempted to resolve this issue.

Generally in termites, high juvenile hormone (JH) titer is required for soldier differentiation (reviewed in Watanabe et al., 2014). Therefore, the biased sex ratio in soldiers might be caused by the differences in JH titers, or by the differences in the threshold for JH titers, between male and female workers. To confirm whether sex-specific differences related to JH titers are crucial factors, topical application of JH can be performed. For example, in the ant *Harpegnathos saltator*, artificial induction rates of queen development by JH analogue (JHA) treatment were different depending on the larval instars to which JHA was applied (Penick et al., 2012).

To clarify these hypotheses, we focused on two species, a nasute termite *Nasutitermes takasagoensis* and a rhinotermitid termite *Reticulitermes speratus*. Phylogenetically these genera are not closely related to each other; *N. takasagoensis* is an apical species while *R. speratus* is a basal one (Inward et al., 2007; Legendre et al., 2008, 2013), and the soldier ratios in each colony are probably slightly different (e.g. *Nasutitermes* spp.: 2.8–29.8%, *Reticulitermes* spp.: 0.3–10.8%; Haverty, 1977), although the soldier sex ratio is completely different, being strongly male-biased in *N. takasagoensis* but almost equal in *R. speratus*. In the former species, there are four worker types (male minor, male/female medium, and female major workers), and male minor workers molt into presoldiers and then to soldiers in natural conditions (Fig. 1A). In the latter species, there are male and female presoldiers/soldiers (Fig. 1B). In this study, we tried to artificially induce presoldiers from male or female workers by JH(A) treatment, and compared the presoldier differentiation rate and the worker mortality. Outer and inner morphologies of the induced presoldiers from female workers in *N. takasagoensis* were examined whether they had the normal characteristics of presoldiers. Then, JH titers of male and female workers were quantified by high performance liquid chromatography–mass spectrometry (HPLC–MS). Based on the obtained results, we discuss the role of JH in sex-biased soldier differentiation in termites.

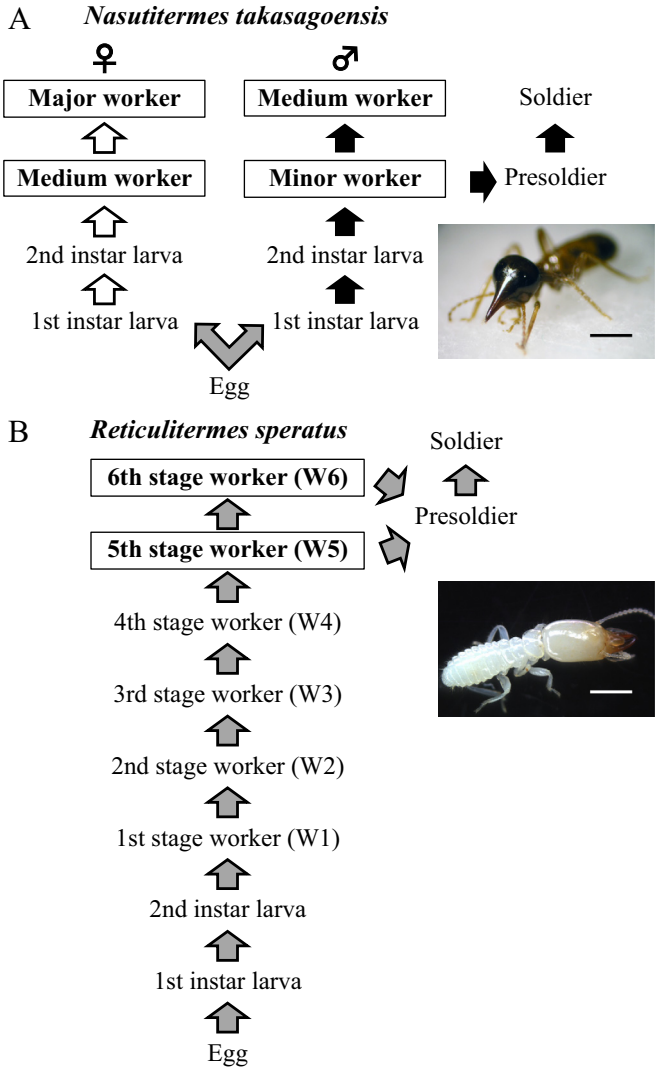

Fig. 1. Developmental pathway of sterile and apterous castes in (A) *Nasutitermes takasagoensis* and (B) *Reticulitermes speratus*. Insets are soldiers of both species. Scale bars indicate 1 mm. In *N. takasagoensis*, presoldiers and soldiers differentiate from only male minor workers (Hojo et al., 2004; Toga et al., 2009). Soldiers have caste-specific head morphologies (horn-like projection called a 'nasus' and an inner 'frontal gland' to produce defensive chemicals), and these structures form during the presoldier molt (Toga et al., 2009; Toga and Maekawa, 2013). In *R. speratus*, both sexes of workers differentiate into presoldiers and soldiers (Takematsu, 1992; Maekawa et al., 2008). Each arrow indicates the molt, and colors show female (white), male (black) and both sexes (gray). Workers used in this study are shown in squares.

2. Materials and methods

2.1. Termites

Six nests of *N. takasagoensis* collected from Iriomote Island (colonies N1 and N2 collected in April 2013, colonies N3 and N4 collected in October 2013) and Ishigaki Island (colonies N5 and N6 collected in October 2014), Okinawa Pref., Japan, were used in this study. All these nests were maintained in separate plastic boxes with well-moistened cotton wool in constant darkness at room temperature (20–25 °C) before use. The relative humidity of the plastic boxes was maintained at more than 80%. Vigorous individuals were used for the following analyses. Each caste (worker, presoldier and soldier) was identified by size, head pigmentation, and abdominal color, according to the criteria of Lenz and Westcott (1985), Miura and Matsumoto (2000), and Hojo et al. (2004). Levels of JH titer may vary during the presoldier molt, and those in workers just before the presoldier molt are constantly high (e.g. Cornette et al., 2008). In this study, we used only workers with non-yellowish white abdomens (non-gut-purged workers; Toga et al., 2009; Masuoka et al., 2013), indicating that they were not just before the molt. The sex ratio in workers and larvae in *N. takasagoensis* are slightly female-biased (Hojo et al., 2004).

Three nests of *R. speratus* collected in September 2014 (colonies R1 and R2) and October 2015 (colony R3) from Toyama shi, Toyama Pref., Japan, were used in this study. These nests were maintained in the same conditions. Sexes of W5 or W6 workers (5th or 6th stage workers; Fig. 1B) were identified by means of the morphological characteristics of the 7th and 8th abdominal sternites (Zimet and Stuart, 1982; Hayashi et al., 2003).

2.2. Induction of presoldier differentiation of N. takasagoensis

Because the JH titers of workers vary depending on the season (Liu et al., 2005b), we performed JHA application experiments in different seasons. The first experimental procedure (1st trial) was