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Juvenile hormone titers and caste differentiation in the damp-wood termite *Hodotermopsis sjostedti* (Isoptera, Termopsidae)

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ABSTRACT

Termites are social insects, presenting morphologically distinct castes, performing specific tasks in the colony. The developmental processes underlying caste differentiation are mainly controlled by juvenile hormone (JH). Although many fragmentary data support this fact, there was no comparative work on JH titers during the caste differentiation processes. In this study, JH titer variation was investigated using a liquid chromatography-mass spectrometry (LC-MS) quantification method in all castes of the Japanese damp-wood termite *Hodotermopsis sjostedti*, especially focusing on the soldier caste differentiation pathway, which was induced by treatment with a JH analog. Hemolymph JH titers fluctuated between 20 and 720 pg/μl. A peak of JH was observed during molting events for the pseudergate stationary molt and presoldier differentiation, but this peak was absent prior to the imaginal molt. Soldier caste differentiation was generally associated with high JH titers and nymph to alate differentiation with low JH titers. However, JH titer rose in females during alate maturation, probably in relation to vitellogenesis. In comparison, JH titer was surprisingly low in neotenics. On the basis of these results in both natural and artificial conditions, the current model for JH action on termite caste differentiation is discussed and re-appraised.

1. Introduction

Insect societies are complex structures emerging from the cooperation of a large number of individuals. This phenomenon of cooperation constitutes an important factor in the evident ecological success of social insects. A key for efficient cooperation is the division of labor, with conspicuous castes that accomplish different behavioral tasks (Wilson, 1971). Division of labor is often accompanied by morphological specializations in relation to specific tasks. This distinct polymorphism of social insect castes is subject to environmental factors and results from differential gene expression, it can consequently be considered as a case of polyphenism (Evans and Wheeler, 1999; Miura et al., 1999; Miura, 2001, 2004; Scharf et al., 2003). All members of the society present a similar genetic background, but in response to environmental factors or pheromones, the hormonal status of each individual will be modified and induce gene expression, leading to a specific developmental pathway (Noirot, 1991; Nijhout, 1999; Evans and Wheeler, 2001). In this scenario, hormonal regulations play a central role for caste differentiation, and juvenile hormone (JH) was actually shown to control caste ontogeny in several social insects (Hartfelder, 2000; Hartfelder and Emlen, 2005). For the implications of JH control in social insect polyphenism, the case of the highly dimorphic ant genus *Pheidole* is exemplified. In these ants, high JH levels during embryonic development induce the differentiation of queen caste, whereas a JH sensitive period exists during post-embryonic development, which will determine the differentiation into workers or alternatively into huge soldiers with a heavily armored head (Nijhout, 1994; Sameshima et al., 2004).

Among social insects, termites present one of the most complex and conspicuous caste systems, probably because termites are hemimetabolous insects and most of the castes are derived from immature stages (Thorne, 1996; Roisin, 2000). Many termite species also exhibit a very flexible caste differentiation system, as it is the case for the Japanese damp-wood termite *Hodotermopsis sjostedti*, which was used in the present study. In this species, caste differentiation pathway is linear. The seventh larval instar assume the main part of the work in the society, and such individuals were consequently considered as false workers or pseudergates (Grassé and Noirot, 1947). Pseudergates are totipotent and can differentiate into soldiers through the transient stage presoldier, into alate imagoes through a single nymphal stage, or into neotenics (reproductive castes not derived from winged forms) as well (Miura et al., 2000, 2004). Stationary molts of

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pseudergates into pseudergates and regressive molts from differentiated nymphs to undifferentiated pseudergates have also been reported (Koshikawa et al., 2001).

As supported by the various previous studies, JH plays a central role also in termite caste determination. Early experiments showed that implantation of active corpora allata (the JH-producing endocrine glands) from adult cockroaches or sexually functional termites into pseudergates of *Kalotermes flavicollis* could induce soldier differentiation, whereas corpora allata from pseudergates or nymph did not produce this effect (Lüscher, 1958; Lebrun, 1967). On the basis of Lüscher's (1958) experiments, Nijhout and Wheeler (1982) proposed a model for termite caste differentiation, in which three JH-sensitive periods were defined during the intermolt of the pseudergate instar for successively sexual characters, non-sexual adult characters and soldier characters (Fig. 1). According to this model, a high JH titer through these three sensitive periods would induce soldier differentiation, whereas alate production would appear in a context of continuously low JH titer. Alternatively, a high JH titer at first and then lower during the last period would induce the pseudergate molt, and the opposite pattern the differentiation of neotenics (Fig. 1).

Other supporting evidences for this model are the experimental data using various juvenile hormone analogs (JHA) to induce soldier caste differentiation after the treatment on pseudergates or workers (Hrdý and Křeček, 1972; Howard and Haverty, 1979; Lelis and Everaerts, 1993). Such JHA treatment has also been shown to induce soldier differentiation in *H. sjostedti* (Ogino et al., 1993; Koshikawa et al., 2005; Cornette et al., 2006). Interestingly, the JHA treatment of nymphs at various timings during the nymphal intermolt period induced intercastes with different degrees of soldier characters mixed with adult characters (Miura et al., 2003).

Until recently, only sparse information was available about JH titer in termites and for a long time JH production has been just inferred from data on the size of corpora allata (Noirot, 1969; Noirot and Bordereau, 1991). However, with the development of suitable techniques, the actual JH production in termites has been investigated with various methods, such as radiochemical assay and GC-MS (Greenberg and Tobe, 1985; Park and Raina, 2004; Brent et al., 2005). The general features emerging from all these investigation are low JH levels in workers or pseudergates and soldiers, and high JH levels in presoldiers and in the maturing queens. Various reports indicate that JH levels rise during the soldier differentiation process (Park and Raina, 2004; Liu et al., 2005; Mao et al., 2005). These data, associated to induction experiments with JHA, consequently support the need for high JH levels in the soldier differentiation component of the Nijhout and Wheeler (1982) model. However, no direct data on JH levels during the differentiation process of pseudergates and reproductives is available to validate the model.

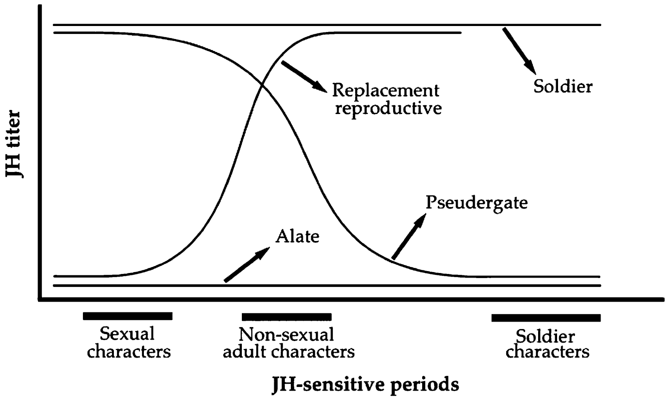

Fig. 1. Model of Nijhout and Wheeler for the JH-mediated control of caste determination in termites. Three JH-sensitive periods were postulated for sexual characters, non-sexual adult characters and soldier characters, respectively (black bars). Each curve represents a hypothetical profile of JH titer changes during the course of the pseudergate instar, leading to differentiation of the indicated caste. (Redrawn from Nijhout and Wheeler, 1982).

In the present study, the adaptation of a direct and rapid quantification method using liquid chromatography-mass spectrometry (LC-MS) allowed the investigation of hemolymph JH titer in every caste of *H. sjostedti*. Induction of the presoldier molt by JHA treatment has also allowed us to monitor the intrinsic JH titer during the course of the developmental transition to presoldier, and these data were compared to natural changes in JH titer during different developmental transitions. Our results fit the model of Nijhout and Wheeler (1982) for soldier and alate caste differentiation, but we found an inverse pattern for JH titer during pseudergate differentiation.

2. Materials and methods

2.1. Insects

Colonies of *H. sjostedti* were sampled from decayed fallen trees in the evergreen forests of Yakushima Island, Kagoshima prefecture, Japan, in May 2005 and May 2006. They were kept in the laboratory as stock colonies in plastic containers at approximately 25 °C under constant darkness. The rotten wood containing the colonies was supplemented with damp pine tree woodcuts, thus allowing colonies to be kept in the laboratory for several years. Summer individuals (pseudergates) were collected within 2 months after colony sampling, until the swarming period. Winter individuals were collected between 7 and 10 months or between 18 and 22 months after colony sampling.

2.2. Hormonal treatment and induction of presoldier differentiation

Pseudergates were isolated in groups of 10 individuals in Petri dishes and fed with filter paper. They were treated with a topical application of 5 μg pyriproxyfen (juvenile hormone analog; Sumitomo chemical, Osaka, Japan) diluted in 5 μl acetone per individual. Presoldier molting was normally induced 2 weeks after treatment (Ogino et al., 1993; Cornette et al., 2006). Some pseudergates were also treated with a topical application of 30 μg JH III (Sigma, St Louis, MO, USA) diluted in 5 μl acetone per individual. However, even at this concentration, JH III did not induce the differentiation to presoldiers.

2.3. Hemolymph collection and JH extraction

Individuals from each natural caste, premolting nymphs with swollen wing buds (just before imaginal molt) and white presoldiers (taken within 1 day after molting) were collected from stock colonies. Premolting pseudergates engaged in stationary molting (white with flattened abdomen) and pseudergates taken just after the stationary molt (with still unsclerotized cuticle) were collected from old stock colonies (more than 6 months after sampling in the field), which produced no more new soldiers. We confirmed the fate of such individuals randomly sampled from those stock colonies and all of them underwent a stationary molt ($n = 20$). JHA-treated pseudergates were taken at 24 h, 3, 7, 10, and 14 days after the treatment and JHA-induced presoldiers, taken on the day of ecdysis or 7 days later, were also sampled. Finally, JH III-treated pseudergates were collected at 24 h and 7 days after