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Gene expression changes in the tyrosine metabolic pathway regulate caste-specific cuticular pigmentation of termites

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ABSTRACT

In social insects, all castes have characteristic phenotypes suitable for their own tasks and to engage in social behavior. The acquisition of caste-specific phenotypes was a key event in the course of social insect evolution. However, understanding of the genetic basis and the developmental mechanisms that produce these phenotypes is still very limited. In particular, termites normally possess more than two castes with specific phenotypes (i.e. workers, soldiers, and reproductives), but proximate developmental mechanisms are far from being fully understood. In this study, we focused on the pigmentation of the cuticle as a model trait for caste-specific phenotypes, during the molts of each caste; workers, soldiers, presoldiers (intermediate stage of soldiers), and alates (primary reproductives) in *Zootermopsis nevadensis*. Expression patterns of cuticular tanning genes (members of the tyrosine metabolic pathway) were different among each molt, and high expression levels of several "key genes" were observed during each caste differentiation. For the differentiation of castes with well-tanned cuticles (i.e. soldiers and alates), all focal genes except *DDC* in the former were highly expressed. On the other hand, high expression levels of *yellow* and *aaNAT* were observed during worker and presoldier molts, respectively, but most other genes in the pathway were expressed at low levels. RNA interference (RNAi) of these key genes affected caste-specific cuticular pigmentation, leading to soldiers with yellowish-white heads and pigmented mandibular tips, presoldiers with partly pigmented head cuticles, and alates with the yellow head capsules. These results suggest that the pigmentation of caste-specific cuticles is achieved by the regulation of gene expression in the tyrosine metabolic pathway.

1. Introduction

Castes of social insects show multiple characteristic phenotypes specialized for each task in their colony. All individuals have the same genetic information, and thus the regulation of gene expression is crucial for the formation of caste-specific phenotypes. For instance, highly developed ovaries or testes are observed in reproductives, and special weapons are possessed by soldiers. By understanding the developmental bases of these caste-specific traits, important insights into the intrinsic mechanisms of caste differentiation can be obtained (Miura and Scharf, 2011).

Termite castes have distinctive morphological phenotypes compared with other social insects (Watanabe et al., 2014). However, understanding of the developmental mechanisms of caste-specific phenotypes is still limited. According to previous studies, juvenile hormone (JH) is the central factor for caste differentiation, and some genes (for example, hexamerins, vitellogenins and cytochrome P450 genes) may work for the caste regulation (reviewed in Watanabe et al., 2014; Korb, 2015; Scharf, 2015). Because all castes are formed through the molting process, molting hormone (20-hydroxyecdysone: 20E) may be also crucial for caste differentiation. Therefore, to analyze the caste-specific phenotypes, it is important to focus on the traits which are affected by hormonal (JH and 20E) control.

Moreover, to understand the development of caste-specific phenotypes, comparative developmental analysis during the molting processes of all castes (reproductives, workers, and soldiers) in the same species is an important and useful experimental method. However, there are no reports in the literature comparing detailed developmental processes of caste differentiation during alate (imago, winged adult) formation, because to identify and monitor individuals during caste differentiation is relatively difficult. Previous developmental studies focused on presoldier (a

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precursor stage of soldier) differentiation (Koshikawa et al., 2003; Toga et al., 2012), because it could be induced from workers by artificial JH or JH analog (JHA) treatments (Scharf et al., 2003; Cornette et al., 2006; Tsuchiya et al., 2008). In incipient colonies of the damp wood termite *Zootermopsis nevadensis*, the molting schedule of worker–worker, worker–presoldier, and presoldier–soldier differentiation has been identified (Maekawa et al., 2012; Masuoka et al., 2015; see Fig. 1). If the molting schedule of alates in *Z. nevadensis* could be identified under natural conditions, developmental processes of all castes could then be compared. Because the phenotypes of soldiers and workers may be the result of developmental arrest or a change in the process of post-embryonic imaginal formation (Maekawa et al., 2008), comparative studies including alates will provide important information to understand the origin of castes.

Because each caste possesses a unique cuticular nature, and the pigmentation process is well conserved in insects and affected by the hormonal (especially 20E) control (Arakane et al., 2009; Zhan et al., 2010; Futahashi et al., 2010; Elias-Neto et al., 2010), cuticular pigmentation is a useful phenomenon for the study of caste determination. The cuticles of workers show a low tanning level, but the edge of the mandibles is composed of highly tanned cuticle in order to facilitate feeding on hard wood. Presoldiers have soft and colorless cuticles, and they immediately molt into soldiers. Soldiers have an extraordinarily tanned cuticle, especially in parts of the head (e.g. mandibles and head capsule). Alates have an encrusted black cuticle over the whole body, and they fly out from the natal nest to swarm.

Generally in insects, a tanning process through the tyrosine metabolic (TM) pathway is involved in cuticle pigmentation. In the TM pathway, two catecholamines (dopa and dopamine) are synthesized by tyrosine hydroxylase (*TH*) and dopamine decarboxylase (*DDC*), and melanins required for pigmentation are synthesized from these catecholamines by phenoloxidase (Wittkopp and Beladade, 2009; Arakane et al., 2009; see Fig. 2). Furthermore, two other catecholamines (N-acetyldopamine [NADA], N-β-alanyldopamine [NBAD]) are synthesized from dopamine by arylalkylamine N-acetyltransferase (*aaNAT*) and NBAD synthase (*ebony*), respectively, and quinones are also produced from catecholamines. These quinones function in cuticular hardening and pigmentation through the crosslinking of cuticular proteins (Arakane et al., 2009; Andersen, 2010). For termite soldier differentiation (presoldier–soldier molt), high gene expression levels of *laccase2* (*Lac2*), which works as the phenoloxidase in the TM pathway (Fig. 2), were observed in the rhinotermitid termite *Reticulitermes speratus*, and it might be involved in the cuticular tanning of soldiers (Masuoka et al., 2013). However, the functions of the TM pathway genes (including *Lac2*), or how they control caste-specific cuticular pigmentation are not fully known in termites.

In this study, to understand the cuticular pigmentation mechanisms during caste differentiation, we performed expression and functional analyses of the TM pathway genes during the molting processes of castes (worker–worker, worker–presoldier, presoldier–soldier, and nymph–alate molts) in *Z. nevadensis*. Based on the results obtained, we discuss the role of hormone and signaling activity for the formation of caste-specific phenotypes including cuticles.

2. Materials & methods

2.1. Insects

Z. nevadensis colonies were collected from laurel forests in Hyogo Prefecture, Japan, in May 2014 and 2015. Colonies were kept in plastic cases at approximately 25 °C in constant darkness until the emergence of a gut-purged nymph or newly molted alates. In accordance with previous studies (Itano and Maekawa, 2008; Maekawa et al., 2012), incipient colonies were founded by artificial mating of male and female alates in petri dishes with wood chips, and kept at approximately 25 °C in constant darkness. In an

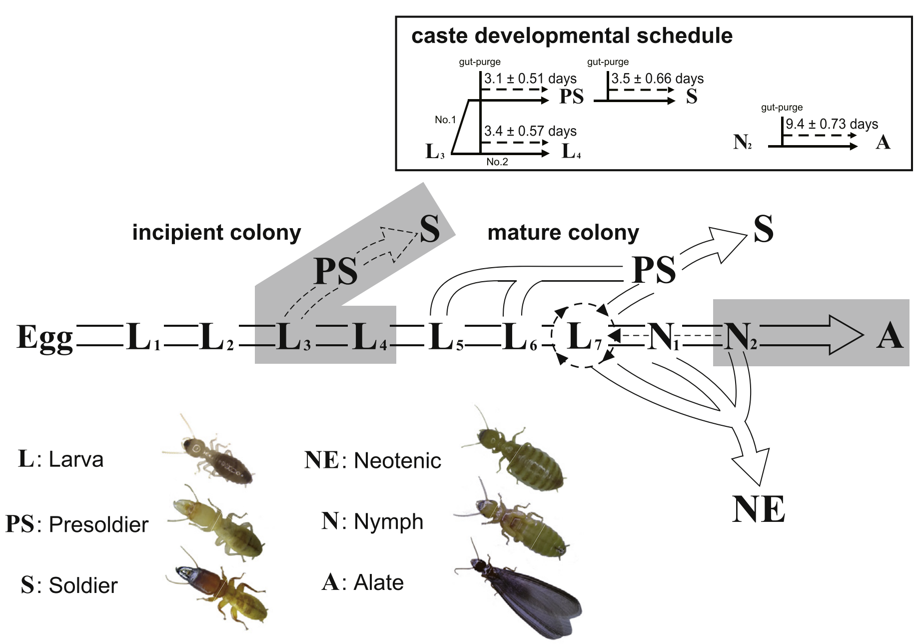

Fig. 1. Caste developmental pathway of *Z. nevadensis* (modified from Heath, 1927; Castle, 1936). This is called the "linear developmental pathway" and individuals can develop into all castes including alates (primary reproductives). Although the soldier caste is developed from the older larvae (5–7th instar larvae) in the mature colony, the first emerged 3rd instar larva (called "No. 1 larva") can develop into a soldier in the incipient colony (Maekawa et al., 2012; Masuoka et al., 2015). Each caste possesses distinctive cuticular features, especially in the head. Gray boxes indicate the developmental stages focused on in this study. The inset shows the molting periods during each caste differentiation. See the text for further details.