Journal of Insect Physiology 61 (2014) 25–33

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Endocrine control of cuticular hydrocarbon profiles during worker-to-soldier differentiation in the termite *Reticulitermes flavipes*

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ARTICLE INFO

Article history:
Received 29 August 2013
Received in revised form 12 December 2013
Accepted 18 December 2013
Available online 27 December 2013

Keywords:
Juvenile hormone
Chemical signature
Caste differentiation
Soldier

ABSTRACT

The social organization of termites, unlike that of other social insects, is characterized by a highly plastic caste system. With the exception of the alates, all other individuals in a colony remain at an immature stage of development. Workers in particular remain developmentally flexible; they can switch castes to become soldiers or neotenics. Juvenile hormone (JH) is known to play a key role in turning workers into soldiers. In this study, we analyzed differences in cuticular hydrocarbon (CHC) profiles among castes, paying particular attention to the transition of workers to soldiers, in the subterranean termite species *Reticulitermes flavipes*. CHCs have a fundamental function in social insects as they serve as cues in inter- and intraspecific recognition. We showed that (1) the CHC profiles of the different castes (workers, soldiers, nymphs and neotenics) are different and (2) when workers were experimentally exposed to a JH analog and thus induced to become soldiers, their CHC profiles were modified before and after the worker-presoldier molt and before and after the presoldier-soldier molt.

1. Introduction

Unlike in other social insects like bees, wasps or ants, social organization in termites is highly plastic. An important feature of termite social organization is the caste system. Termites are hemimetabolous insects and, except for the primary reproductives (alates), all individuals remain at an immature stage of development. They are organized into castes (workers, soldiers, nymphs and reproductives) that result from larval polyphenism (Korb and Katrantzis, 2004). Workers' development is plastic; they can switch to another caste, becoming soldiers or secondary reproductives (i.e. apterous neotenics) or even develop regressively into a former instar, which is a unique developmental pattern (Korb and Katrantzis, 2004). As a result, individuals from the same colony with the same genetic background can develop different phenotypes (or into different castes) depending on intrinsic and/or extrinsic factors (Scharf et al., 2007). One such intrinsic factor is endocrinal: exposure to juvenile hormone (JH) (Mao et al., 2005; Park and Raina, 2005; Scharf et al., 2003). This hormone is produced by the corpora allata, a pair of neurosecretory glands, and has a broad range of developmental and physiological effects (Gilbert et al., 2000). JH plays several roles: it controls larval/nymphal development and metamorphosis, diapause, migratory behavior, wing length, reproduction and caste determination (Hartfelder, 2000). JH is also involved in termite caste polyphenism (Mao et al., 2005; Park and Raina, 2004), especially when individuals differentiate into soldiers (Hartfelder and Emlen, 2005; Miura, 2001; Scharf et al., 2003) and probably when they differentiate into neotenics (Elliott and Stay, 2007, 2008; Leniaud et al., 2011). Different forms of JH have been found in insects (Darrouzet et al., 1998; Darrouzet and Desneux, 2013); however, JH-III is the only JH moiety released by the termite corpora allata (Brent et al., 2005) and detected in hemolymph (Brent et al., 2005; Cornette et al., 2008; Park and Raina, 2004). In termites, it is now well known that JH induces the differentiation of workers into presoldiers and then into soldiers (Brent, 2009; Morel and Blum, 1988; Park and Raina, 2004). Several studies have shown that ectopic exposure of workers to JH-III readily induces their differentiation into soldiers (Scharf et al., 2003, 2005, 2007; Tarver et al., 2009, 2010; Zhou et al., 2006a,b, 2007). In 1972, Hrdy and Krecek similarly induced differentiation by ectopically applying a JH analog (JHA). Their results demonstrate that JH can act via exogenous exposure. Moreover, other studies have demonstrated that, under natural conditions, an increase in JH hemolymph titers in workers can cause them to differentiate into soldiers (Mao et al., 2005; Park and Raina, 2004). A brief peak in JH production is sufficient to induce such differentiation (Cornette et al., 2008).

The insect cuticle is covered by a thin lipid layer largely consisting of cuticular hydrocarbons (CHCs). Because of their chemical properties, CHCs primarily act to limit insect water loss by creating a surface barrier (Blomquist and Dillwith, 1985; Gibbs and Pomonis, 1995; Hadley, 1985). However, they also serve as recognition cues. Inter- and intraspecific recognition among insects involve CHCs, which constitute the chemical signatures of individuals (reviewed by Blomquist and Bagnères, 2010; Howard

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0022-1910/$ - see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jinsphys.2013.12.006

and Blomquist, 2005). Several studies have demonstrated a strong link between the social behavior of insects and their cuticular profiles (Bagnères et al., 1991; Breed et al., 1995; Howard and Blomquist, 2005; Leconte and Hefetz, 2008). Indeed, CHCs have an important function in eusocial insects: these compounds compose a mosaic of hierarchical information that is involved in nestmate recognition (Howard, 1993; Howard and Blomquist, 2005). Moreover, within a given colony, individuals of different castes have different CHC signatures (Bagnères et al., 1998; Haverty et al., 1996; Howard and Blomquist, 1982; Kaib et al., 2000; Smith and Taylor, 1990). Therefore, chemical signatures can vary, and the degree of variation depends on a wide range of endogenous factors, such as the endocrine system or enzymes (Fan et al., 2004; Lengyel et al., 2007; Schal et al., 2003), and exogenous factors, such as climate and season (Bagnères et al., 1990), diet (Liang and Silverman, 2000), parasitism (Lebreton et al., 2010), or time (Bagnères et al., 2011; Cuvillier-Hot et al., 2001; Lebreton et al., 2009). The relative importance of these factors varies among species (VanderMeer and Morel, 1998). Moreover, CHC profiles are modified not only by the social or biotic environment but are also under genetic control in both social and solitary insects (Bonavita-Cougourdan et al., 1996; Dronnet et al., 2006; Etges et al., 2009; Thomas and Simmons, 2008; Vauchot et al., 1996).

Several studies have shown that a relationship exists between individual hemolymph JH titers and the division of labor in *Apis mellifera*. For instance, brood-tenders have low JH titers, while foragers have high JH titers (Huang et al., 1994; Huang and Robinson, 1995). Because there is also a relationship between a worker's caste and its CHC profile, it may be that JH is involved in the production of CHCs and the composition of CHC profiles, as has been observed in the ant *Myrmicaria eumenoides* (Lengyel et al., 2007).

In this study, we analyzed intercaste differences in CHC profiles in the subterranean termite species *Reticulitermes flavipes*; we also experimentally explored how profiles change as workers become soldiers. First, we analyzed the CHC profiles of the different castes: workers, soldiers, nymphs and neotenics. Second, we exposed workers to a JH analog to induce their differentiation into soldiers and characterized their chemical profiles at different time points, i.e. before and after the worker-presoldier molt and before and after the presoldier-soldier molt.

2. Materials and methods

2.1. Rearing conditions

Colonies of *R. flavipes* were collected from the St. Trojan forest on the Ile d'Oléron (Charente-Maritime, France) in October 2005, 2006 and 2007. This forest is largely composed of pine trees (*Pinus pinaster*), which cover more than 80% of the area. Termite species were identified based on worker post-clypeus morphology, which was examined for each individual sampled on the day of collection (Clément et al., 2001). Species identity was then confirmed using CHC profiles (Bagnères et al., 1990, 1991). Termite samples were collected from wood fragments or pine tree stumps. Colonies were kept in these pieces of wood, stocked with soil in the laboratory, and regularly watered. Colonies were kept in permanent darkness at 26 °C.

2.2. Determination of caste-specific chemical profiles

Individuals were sampled from eight colonies. The different castes and stages were distinguished as described in Büchli (1958) (Fig. 1). All individuals were placed in a plastic box with Fontainebleau sand and a piece of pine wood.

A total of 86 chemical analyses were performed: 19 analyses of L1–L3 workers, 27 analyses of L4–L8 workers, 26 analyses of nymphs, 7 analyses of neotenics and 7 analyses of soldiers. To characterize caste-specific CHC profiles, pools of 5 termites were formed and analyzed as described below.

2.3. Chemical analysis

To extract their CHCs, 5 individuals were placed in 150 μL of pentane for 5 min (including 1 min of gentle vortexing). A total of 100 μL of the extract was then transferred to a new clean tube and stored at −20 °C until analyses could take place. Immediately prior to analysis, 5 μL of *n*-C18 (*n*-octadecane: $C_{18}H_{38}$) was added to each tube as an internal standard. Samples of 2 μL were then analyzed using gas chromatography (GC); we employed a CPG AGILENT Technologies 6850 Network GC System equipped with a

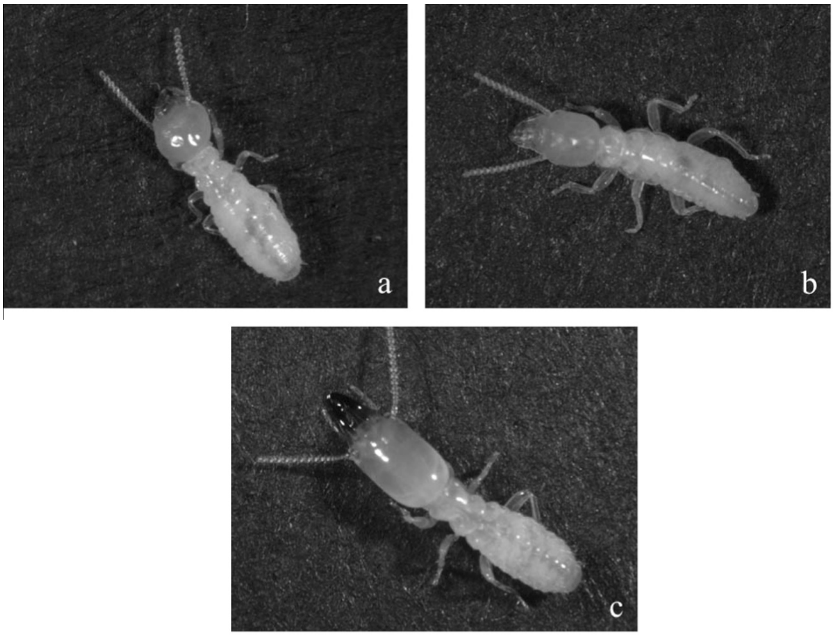

Fig. 1. Morphology of a *R. flavipes* (a) worker, (b) pre-soldier and (c) soldier.