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ScienceDirect
General and Comparative Endocrinology 152 (2007) 102–110
GENERAL AND COMPARATIVE ENDOCRINOLOGY
www.elsevier.com/locate/ygcen

# Juvenile hormone synthesis as related to egg development in neotenic reproductives of the termite *Reticulitermes flavipes*, with observations on urates in the fat body

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Received 15 November 2006; revised 2 March 2007; accepted 5 March 2007

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## Abstract

The relationship between juvenile hormone (JH) synthesis and egg development, which is well documented in cockroaches, is much less studied in their close relatives, the termites. In this study of neotenic reproductives of the subterranean termite *Reticulitermes flavipes*, in vitro rates of juvenile hormone (JH) synthesis by corpora allata (CA) are related to vitellogenic egg development and the size of CA. The first study compared brachypterous and apterous neotenics in their first cycle of egg development and a second study compared physogastric and non-physogastric brachypterous and apterous neotenics. In both studies, rates of JH synthesis correlated with the size of CA as indicated by their length. Unlike the cockroach in which all basal oocytes are in the same stage of development, those in termites are in various stages. In brachypterous and apterous in the first cycle of egg development, CA with high rates of JH synthesis were from females with early vitellogenic basal oocytes, whereas CA with low rates of JH synthesis were from females with either pre-vitellogenic or mature basal oocytes. This pattern of JH synthesis is similar to the cycle of JH synthesis correlated with oocyte development in several cockroach species. In later oocyte maturations, CA from physogastric apterous females with ovaries containing mature, as well as growing oocytes, showed a wide range of JH production; the CA with the highest rates of JH synthesis were from females with the highest proportion of early vitellogenic oocytes suggesting that both mature and early vitellogenic oocytes interact to regulate JH synthesis. Rates of JH synthesis were related to the number of vitellogenic ovarioles. Physogastric brachypterous neotenics, compared to the other classes of neotenic females, had CA with 2- to 4-fold higher rates of JH synthesis and ovaries with 2.5- to 8-fold greater number of vitellogenic ovarioles. However, both physogastric brachypterous and apterous neotenics had more vitellogenic basal oocytes and less urate in their fat bodies than the respective non-physogastric neotenics. These results demonstrate the similarities and differences between the classes of neotenic termites and between reproductive females in cockroaches and termites.

*Keywords:* *Reticulitermes flavipes*; Termite; Juvenile hormone; Vitellogenesis; Neotenic reproductives; Urate

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## 1. Introduction

The relationship between juvenile hormone (JH) synthesis and egg development in cockroaches has been well investigated. Much that is known about this relationship in cockroaches comes from radiochemical assays for rates of JH synthesis by corpora allata (CA) in vitro (Tobe and Stay, 1985). In contrast, only a few studies have investigated this relationship in termites. The first measurements of in vitro rates of JH synthesis were carried out on *Zootermopsis angusticollis* and showed that JH production increased after neotenic reproductives emerged from larval instars (Greenberg and Tobe, 1985). Later, it was shown in this species that the increase in rates of JH synthesis as alates matured into queens correlated with an increase in the number of vitellogenic oocytes (Brent et al., 2005). Many studies have proposed that increase in CA volume is accompanied by an increase in JH synthesis and this has been found to be true in reproductive cycles of

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doi:10.1016/j.ygcen.2007.03.003

cockroaches, but not necessarily in all insects (Szibbo and Tobe, 1981; Chiang and Schal, 1994; Tobe and Stay, 1985). In several species of termites, the observed enlargement of CA of neotenic reproductives was presumed to be accompanied by increased JH synthesis (Lüscher, 1965; Noirot, 1969; Okot-Kotber, 1980; Miles and Chang, 1984; Noirot and Bordereau, 1990). JH regulation of ovarian development in termites was demonstrated by application of JH analogue to nymphs that resulted in the maturation of ovaries in the soldier-alate intercastes of *Zootermopsis nevadensis* (Miura et al., 2003). Also, application of JH II to workers of *Reticulitermes flavipes* resulted in ovarian development in pre-soldiers and soldiers (Scharf et al., 2003). JH III is the product of CA of *R. flavipes* (Yagi et al., 2005). Application of this JH to workers of *R. flavipes* resulted in an increase in expression of two vitellogenin genes (Scharf et al., 2005b) and it was found that the highest expression of two vitellogenin genes occurred in alates and neotenics (Scharf et al., 2005a).

The present study investigates the relationship between JH synthesis and egg development in neotenic reproductives of the eastern subterranean termite *R. flavipes*. Caste development in this species, as in other lower termites, is flexible so that environmental conditions can direct the developmental pathway (Watson and Sewell, 1981). Newly hatched individuals (larvae) develop into workers or nymphs, distinguished by the absence or presence of wing pads, respectively. In the absence of the primary reproductives, the king and queen, some of the workers and nymphs develop into secondary reproductives to continue colony growth. The workers become apterous neotenics and the nymphs, brachypterous neotenics (Miller, 1969; Thorne, 1999). The reproductive potential of these neotenics is far less than that of the queen, but as they age, under favorable conditions, they increase in egg production and become physogastric with abdomens enlarged by fat bodies and ovaries (Thorne, 1999). Increased egg production increases the demand for fat body production of vitellogenin. Urate stored in the fat body could potentially be utilized for synthesis of vitellogenin because gut bacteria have been shown to recycle uric acid nitrogen in *R. flavipes* (Potrikus and Breznak, 1981).

We have investigated the relationships between in vitro rates of JH synthesis and the number of vitellogenic ovarioles in the first cycle of egg development in brachypterous and apterous neotenics and, in a second study, compared the differences in these parameters between non-physogastric and physogastric brachypterous and apterous neotenics. To confirm, as proposed by others, that in neotenic reproductives, enlargement of CA is related to increase in hormone production, we have measured the length of the CA as well as their rates of JH synthesis. In addition, we have investigated JH synthesis as related to the length of the longest basal oocyte in first cycle brachypterous and apterous neotenics as well as physogastric apterous neotenics. Finally, the presence or absence of conspicuous urate deposits in the fat body of physogastric and non-physogastric neotenics was related to oocyte development.

## 2. Methods

### 2.1. Animals

Stock laboratory cultures of *R. flavipes* were established by collecting rolls of corrugated cardboard placed in the ground at a site near the University of Iowa campus in Coralville, IA between May and October 2005, and April and June 2006. Rolls were placed in 15 × 20 × 10 in. plastic containers with covers and kept at 24 °C, 12 h:12 h light:dark, and 85% relative humidity. Petri dish cultures with 50–100 nymphs and late-instar workers or late-instar workers alone were established between August and November 2005 and in February 2006 (after 0 days to 4 months in the laboratory). The 100 × 15 mm plastic Petri dishes were lined with moist filter paper, supplied with two-folded pieces for harborage (Whatman #2), and stored in tightly sealed plastic containers at 24 °C. These were examined weekly for development of neotenics.

Brachypterous neotenics ($n = 41$) emerged more often from Petri dishes containing 10 or more nymphs than from those with fewer than 10 nymphs regardless of the number of workers. However, apterous neotenics ($n = 39$) generally developed in dishes containing at least 30 workers regardless of the presence or absence of nymphs. Both types of neotenics emerged in Petri dishes between December 2005 and August 2006 (after 2–8 months of isolation from the stock cultures) and were assayed within the week of emergence. These neotenics were in the first cycle of egg development.

Physogastric ($n = 22$) and non-physogastric ($n = 18$) brachypterous neotenics used for the second set of assays were taken directly from stock laboratory cultures in June and July 2006 and therefore their history is unknown. Physogastric apterous neotenics used for the second set of assays ($n = 24$) were taken from stock laboratory cultures ($n = 9$) in June and July 2006 (history unknown), as well as from Petri dish cultures ($n = 15$) between February 2006 and May 2006 (after 4–8 months of isolation from stock cultures). Non-physogastric apterous neotenics used for the second set of assays ($n = 33$) were taken from Petri dish cultures between February and May 2006 (after 4–8 months of isolation from stock cultures).

### 2.2. Characterization of neotenic females

All neotenic females were weighed before assay. Brachypterous neotenics weighed between 6 and 14 mg and apterous neotenics weighed between 4 and 9 mg. Mean wet weights in milligrams for the females in the first study were: first cycle brachypterous neotenics, $8.9 \pm 0.2$ ($n = 41$) and first cycle apterous neotenics, $6.5 \pm 0.1$ ($n = 39$). Mean wet weights for the females in the second study were: physogastric brachypterous neotenics, $11.8 \pm 0.3$ ($n = 22$); non-physogastric brachypterous neotenics, $9.5 \pm 0.1$ ($n = 18$); physogastric apterous neotenics, $7.5 \pm 0.1$ ($n = 24$); and non-physogastric apterous neotenics, $6.1 \pm 0.1$ ($n = 33$).

Brachypterous neotenics were identified by the presence of wing buds and a darker cuticular color than that of nymphs. Those designated as physogastric had large abdomens and weights of 10 mg or more. Those with thin abdomens and weights less than 10 mg were designated as non-physogastric.

Apterous neotenics were identified by the lack of wing buds and longer, wider abdomens and darker color than that of workers. Apterous neotenics were designated as physogastric if they had enlarged abdomens and typical weights of 7 mg or more. Those with long, thin opaque abdomens and typical weights of 7 mg or less were designated as non-physogastric.

### 2.3. Juvenile hormone synthesis assay

Termites were chilled on ice for 5–10 min. Pairs of CA (attached to a small piece of esophagus to facilitate handling) were dissected in saline (1.093% NaCl, 0.157% KCl, 0.085% $CaCl_2$, 0.017% $MgCl_2$, 0.006%