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Endocrine changes in maturing primary queens of *Zootermopsis angusticollis*

Colin S. Brent[a,*], Coby Schal[b], Edward L. Vargo[b]

[a]*School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA*
[b]*Department of Entomology and W.M. Keck Center for Behavioral Biology, North Carolina State University, Box 7613, Raleigh, NC 27695-7613, USA*

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Abstract

Termite queens are highly specialized for reproduction, but little is known about the endocrine mechanisms regulating this ability. We studied changes in the endocrinology and ovarian maturation in primary reproductive females of the dampwood termite *Zootermopsis angusticollis* following their release from inhibitory stimuli produced by mature queens. Winged alates were removed from their natal nest, manually dewinged, then paired in an isolated nest with a reproductive male. Development was tracked by monitoring ovarian development, in vitro rates of juvenile hormone (JH) production by corpora allata, and hemolymph titers of JH and ecdysteroids. The production rate and titer of JH were positively correlated with each other but negatively correlated with ecdysteroid titer. Four days after disinhibition, JH release and titer decreased while ecdysteroid titer increased. The new levels persisted until day 30, after which JH increased and ecdysteroids decreased. Fully mature queens had the highest rates of JH production, the lowest ecdysteroid titers, and the greatest number of functional ovarioles. The results support the hypothesis that JH plays a dual role in termite queens depending on their stage of development; an elevated JH titer in immature alates may maintain reproductive inhibition, but an elevated JH titer in mature queens may stimulate ovarian activity. The decline in JH production and the elevation in ecdysteroid titer correspond to a period of physiological reorganization and activation. The specific function of ecdysteroids is unknown but they may help to modulate the activity of the corpora allata.

Keywords: Reproduction; Isoptera; Queen; Juvenile hormone; Ecdysteroids

1. Introduction

The maintenance of a reproductive caste within colonies of social insects is a complex process, the mechanics of which can vary considerably among species. Reproductively dominant individuals maintain their position within the colony through a variety of direct and indirect interactions with nestmates. These can range from overt aggressive behaviors to the production and distribution of pheromones that can act as physiological primers to suppress development in recipients (reviewed in Wilson, 1971). Regardless of the particular form of interaction, the recipient of inhibitory stimuli must undergo a process of sensory and neuroendocrine transduction to cause the release or suppression of hormones that ultimately regulate reproductive behavior, development and gametogenesis. Although developing a means to regulate nestmate reproduction is a cornerstone to the evolution of eusociality, the physiological mechanisms involved are poorly characterized in all but a few well-studied systems such as the honey bee (reviewed in Robinson and Vargo, 1997). In this paper we investigate the endocrine changes occurring in reproductively maturing termites to begin to elucidate the mechanisms by which reproductive castes are maintained within the Isoptera.

*Corresponding author at: Department of Entomology and W.M. Keck Center for Behavioral Biology, North Carolina State University, Box 7613, Raleigh, NC 27695-7613, USA. Tel.: +1 919 515 3784; fax: +1 919 515 7746.
E-mail address: csbrent@ncsu.edu (C.S. Brent).

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Among the termites, *Zootermopsis angusticollis* (Hagen) has one of the best-characterized systems of reproductive regulation. This dampwood termite belongs to a family considered to be basal within the Isoptera (Nalepa, 1994; Kambhampati, 1995), so that many of the regulatory mechanisms are likely to have conserved elements in more derived termite species. In this species a healthy queen is able to prevent female nestmates from becoming reproductively active (Light, 1943; Light and Weesner, 1951; Greenberg and Stuart, 1979, 1982). In particular, alates, winged sexuals which have already undergone a molt into an "adult" reproductive form, have inactive ovaries and exhibit no mating behavior until they disperse away from their natal nest to establish a new colony (Castle, 1934; Weesner, 1969). The reproductive suppression of alates is likely to have been the first social control mechanism to have evolved in the subsocial ancestors of termites, and may be the basis for the derivation of other social controls crucial to termite colony dynamics.

Subsequent to leaving their natal nest, alates go through a period of development during which their ovaries develop additional functional ovarioles, and they begin to produce vitellogenic oocytes (Castle, 1934; Greenberg and Tobe, 1985; Brent and Traniello, 2001a). Thirty days after founding a new nest, maturing queens will copulate with their mate and will lay their first eggs 15–20 days later (Brent and Traniello, 2001a, b). Profound hormonal changes are necessary to elicit these major behavioral and physiological events. In most insects, the hormones responsible for such changes are juvenile hormone (JH) and ecdysteroids (Hardie, 1995; Leather, 1995). In *Z. angusticollis*, JH III is the primary homologue produced by the paired corpora allata (CA) (Meyer et al., 1976; Greenberg and Tobe, 1985), and is involved in the production of the yolk protein precursor vitellogenin (Greenberg and Tobe, 1985). JH has also been shown to regulate the differentiation of immature termites into reproductive forms (Lüscher, 1972, 1974; Okot-Kotber, 1980, 1982, 1983; Nijhout and Wheeler, 1982). Ecdysteroids occur at high concentrations in adult castes of some termite species (Bordereau et al., 1976; Delbecque et al., 1978; Okot-Kotber et al., 1993; Raina et al., 2003), although their site of synthesis, composition and specific function are unknown (Noirot, 1977; Delbecque et al., 1978; Noirot and Bordereau, 1990; Okot-Kotber et al., 1993). However, recent experiments with newly flown alates of the lower termite *Coptotermes formosanus* (Raina et al., 2003), suggest that ecdysteroids may play a significant role in regulating ovarian development and oogenesis in termites.

Although both JH and ecdysteroids may play a role in mediating the social inhibitory effect of functional queens, little is known about the endocrine events following separation from the queen and how they are coordinated with the physiological changes preparatory to egg production. Here, we report changes in the biosynthetic activity of the CA, the hemolymph titer of JH and ecdysteroids, and ovarian maturation of primary females before and during the first 45 days after being released from a queen's inhibitory influence. We present evidence that the physiological events that follow disinhibition are triggered by a rapid decline in JH production and a concurrent surge in ecdysteroid production. We also develop a model for the endocrine control of reproduction in *Z. angusticollis* and for how functional queens can manipulate this system in nestmates.

2. Materials and methods

2.1. Source and maintenance of termites

Termites were selected from stock colonies of *Z. angusticollis* collected from the Del Monte Forest in Pebble Beach, CA in September of both 2003 and 2004. Parent colonies were kept in plastic boxes containing moistened nest wood maintained in an environmental chamber under a 14L:10D light cycle at 23 °C. All termites used in the experiments were randomly selected from multiple colonies over and extended period of time, and were of various ages. Animal health was monitored during data collection. Female alates attempting to disperse upon opening the parent colony were dewinged and randomly paired with a maturing primary male within covered 67 ml plastic cups (Solo Cup Co.) for up to 45 days. The cups contained 2 g (dry weight) of birch sawdust moistened with distilled water and compressed to remove excess water and form a solid mass.

2.2. Measurement of in vitro JH production

The in vitro rate of JH production of paired CA dissected from individual maturing primary females was measured using a rapid partition radiochemical assay (RCA) (Pratt and Tobe, 1974; Tobe and Pratt, 1974; modified by Feyereisen and Tobe, 1981) as previously described (Brent and Vargo, 2003). Females were removed from the inhibitory stimuli within their natal nest, paired with a reproductive male and allowed to mature. They were destructively sampled after each of the first 5 days following release from disinhibition and after 10, 15, 20, 25, 30 and 45 days. At least 20 females were sampled for each day. Additional samples were obtained from 23 virgin queens prior to isolation (day 0), 27 ovipositing mature queens known to have been dealates for at least 180 days, and 33 workers (6–7th instar). The paired CA glands were dissected from immobilized termites, cleaned of attached tissue, and

preincubated for 30 min in a Petri-dish at 26 °C in 100 µl of modified TC199 medium (Specialty Media, Phillipsburg, NJ), with 50 mm Hepes buffer, pH 7.4, without methionine or bicarbonate, and containing 2% Ficoll 400 (Sigma Chemical Co.). Following preincubation, the CA were transferred to a 6 × 50 mm borosilicate culture tube containing 100 µl fresh medium supplemented with 0.2 MBq L-[methyl- ^{3}H]-methionine (specific activity of 2.7–3.0 TBq mmol^{-1}; PerkinElmer Life Sciences, Inc.) for a final concentration of 5 µCi/100 µl. All glands were floated on the surface of the medium to ensure adequate oxygenation (Holbrook et al., 1997). Culture tubes were maintained at 26 °C and were rotated at 90 rpm at a 15° pitch on an orbital shaker. Following incubation, radiolabeled JH was extracted from the medium and CA together with 250 µl ice-cold iso-octane. A 100 µl aliquot from each sample was evaporated under N_2, and then mixed with 3 ml Scintiverse BD (Fisher) scintillation fluid. Radiolabeled methionine incorporation was measured using a scintillation spectrometer (Beckman LS-5801). Because CA was extracted together with the medium we express the results as "JH production" rather than "release". Nonetheless, only a small percentage of the produced JH is retained within the CA, so that rates of production and release are nearly equivalent.

An appropriate incubation time for the RCA was established by measuring the time course of JH production over a 6-h period. For each hour, 11–16 CA were destructively sampled as described above. Each CA pair was taken from a virgin queen that had been reared away from a functional queen for 4 days and was used in only one sample. Based on constant rates of JH production by CA for at least 6 h, a standard incubation time of 5 h was used for the remainder of the experiments.

2.3. Measurement of hemolymph JH titer

JH titer was measured using the GC-MS method developed by Bergot et al. (1981). Using a glass micropipette, hemolymph was collected from the females out of which the CA pair was dissected for determining JH production rate. Samples from 4–8 primary reproductives at the same stage of development were pooled for a final volume of 10 µl. This hemolymph was diluted into 500 µl methanol (90%, HPLC grade), partitioned against hexane three times (Robinson et al., 1991), and the hexane phase was stored at −80 °C until analysis. The methanol phase was saved for the ecdysteroid determinations described later. JH samples were processed according to Bergot et al. (1981) as modified by Shu et al. (1997). Calibration curves were established using known quantities of standards containing d_3-methoxyhydrins of JH I, JH II, JH III and JH III ethyl ester supplied by S. Ramaswamy.

JH d_3-methoxyhydrins were analyzed on a HP6890 GC equipped with a 30m × 0.25 mm Carbowax Econo-Cap column (Alltech) coupled to a HP5973 MSD running in the SIM mode, with helium as the carrier gas at 33.5 cm s^{-1}. To each sample 200 pg JH III ethyl ester, prepared from synthetic JH III (Sigma Chemical Co.) according to Mori et al. (1973), was added as an internal standard. Two ions of each JH d_3-methoxyhydrins at m/z 76 and 225 were monitored to ensure JH specificity.

2.4. Measurement of hemolymph ecdysteroid titer

Ecdysteroid titer was determined using a modified radioimmunoassay developed by Warren et al. (1984) and Zera and Bottsford (2001). Hemolymph pooled from several termites (collected as described for JH Titer) was extracted with 500 µl chilled 90% methanol, lyophilized, resuspended in 1 ml methanol, and stored at −80 °C until analyzed. Duplicate 10 µl aliquots of each sample were incubated overnight with 100 µl of [^{3}H]-ecdysone (5.0 µBq ml^{-1}, NEN) in Borate Buffer, and 100 µl of a polyclonal ecdysteroid antiserum (H-22 antibody, gift from L. Gilbert) at 4 °C on an orbital shaker. The antiserum has been shown to be cross-reactive for ecdysone, ecdysterone, 20-hydroxyecdysone and makisterone A (Warren and Gilbert, 1986). A standard competition curve was generated using 20-hydroxyecdysone (Sigma) in quantities from 15.6–2000 pg. After 18 h, 20 µl of cleaned Protein-A solution (Pansorbin, CalBiochem) was added to each tube to precipitate the complex during another hour of incubation at room temperature. Samples were centrifuged at 5000g and the remaining pellet was washed twice with 100 µl borate buffer. Radioactivity was determined by a scintillation spectrometry and ecdysteroid concentrations were estimated by nonlinear regression.

2.5. Ovarian development

Ovarian development was assessed in maturing primary females for each sample day by dissecting virgin queens in 70% ethanol under a dissecting microscope and counting the total number of functional ovarioles and the number of vitellogenic terminal oocytes, as described in Brent and Traniello (2001a). Ovarioles were considered functional if they were not filamentous and contained oocytes at some stage of development. Oocytes were considered vitellogenic if yolk protein could be observed and the volume equaled or exceeded 0.01 mm^3 (Hewitt et al., 1972; Brent and Traniello, 2001b). Similar measures were taken for alates sampled directly from their natal colony (day 0), and for fully functional queens heading mature colonies.

2.6. Statistical analysis

Statistical tests used are reported with the results.

3. Results

3.1. JH production and hemolymph titer

An appropriate incubation time for the radiochemical assay used to measure the rate of JH production was established by measuring hourly the amount of JH over a 6-h period. Similar to the results of Greenberg and Tobe (1985), JH production was found to be linear (Fig. 1; $r = 0.97, F = 72.03$, $P < 0.001$), indicating a constant rate; therefore a standard incubation period of 5 h was used for all experiments. GC-MS confirmed that only the JH III homologue was found in *Z. angusticollis*; JH I and JH II were never detected.

The rate of JH production was significantly correlated with the concentration of JH in hemolymph (Fig. 2; linear regression, $r = 0.953$, $F = 109.27$, $P < 0.001$), and varied considerably among females at different stages of development (Fig. 3A). This suggests that hemolymph JH is modulated mainly by JH production by the CA and that the latter is an appropriate correlate of JH titer. In reproductively inhibited alates sampled directly from their natal nest (day 0), JH production was moderate (3.63 ± 1.11 pmol h^{-1}), and comparable to that found in similarly aged and functionally sterile workers (4.23 ± 0.68 pmol h^{-1}; Kruskal–Wallis ANOVA, $H = 2.637$, df = 1, $P = 0.104$). After the females had been isolated with a reproductive male, JH production stayed constant through the third day (3.81 ± 0.58 pmol h^{-1}), but by the fourth day CA activity dropped 78% (0.85 ± 0.29 pmol h^{-1}; Kruskal–Wallis

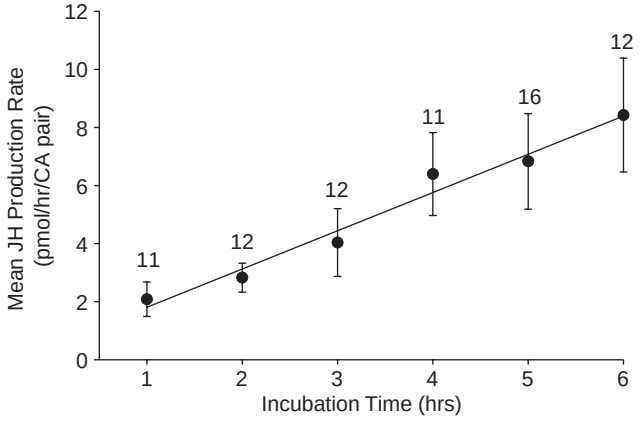

Fig. 1. Mean ($\pm$ s.e.) in vitro JH production rate by corpora allata pairs dissected from primary females sampled independently over 6 h. Production rate is linear (linear regression, $r = 0.97$, $F = 72.03$, $P < 0.001$). Sample sizes are indicated above each mean.

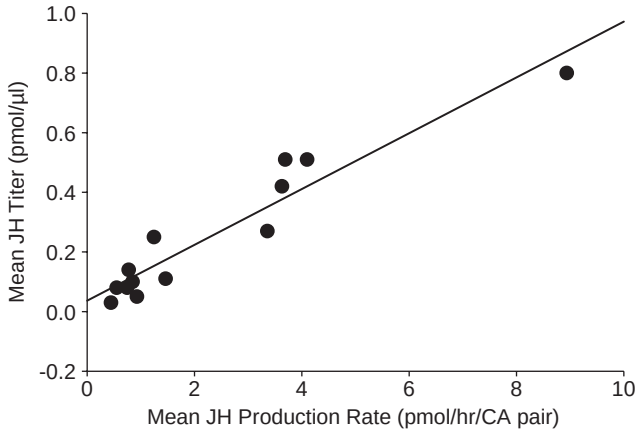

Fig. 2. Correlation between the means for production rate and titer of JH in mature and maturing primary females (linear regression, $r = 0.95$, $F = 109.27$, $P < 0.001$).

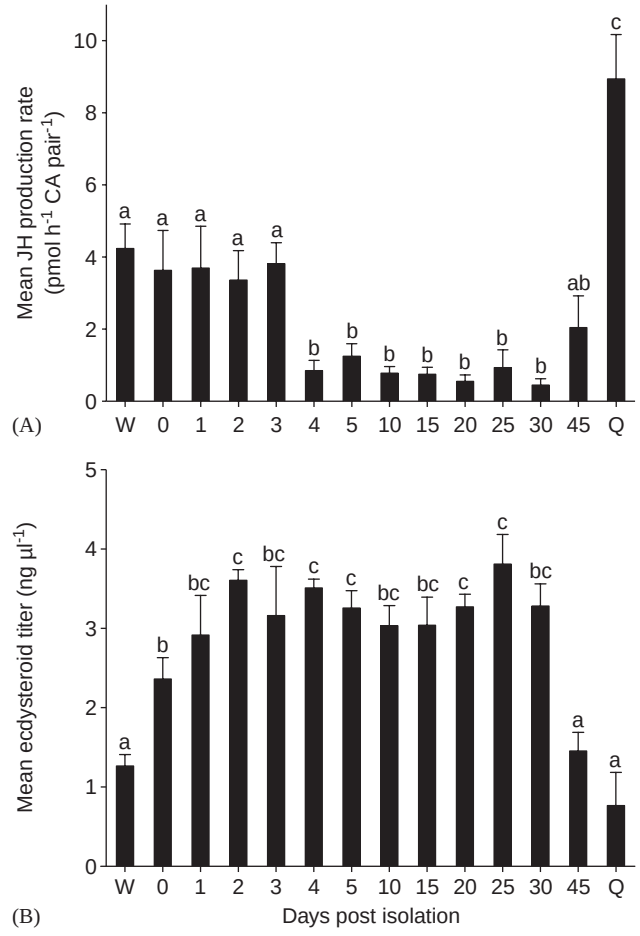

Fig. 3. Mean ($\pm$ s.e.) in vitro JH production rates (A) and ecdysteroid titers (B) in workers (W), mature queens (Q), and maturing primary females during the first 45 days after isolation from the inhibitory influence of their natal nest. Each mean represents 15–33 determinations for JH production and 4–15 determinations for ecdysteroid titers. Bars with the same letter are not significantly different ($P < 0.05$) by one way Kruskal–Wallis ANOVA on ranks followed by Dunn's test.

ANOVA, $H = 15.263$, df $= 1$, $P < 0.001$). JH production remained low through the first month, but between 30 and 45 days, JH production nearly quintupled, from 0.44 ± 0.18 to 2.04 ± 0.88 pmol h^{-1}. Although the difference was not statistically significant (Kruskal–Wallis ANOVA, $H = 1.210$, df $= 1$, $P = 0.271$) due primarily to high variance in the 45 day females, the results do indicate an upswing in CA activity. Such an increase was certainly the case in the fully mature queens (Q), which had the highest rate of JH production (8.94 ± 1.23 pmol h^{-1}), more than double the rate found in alates drawn directly from their natal nest (Kruskal–Wallis ANOVA, $H = 11.670$, df $= 1$, $P < 0.001$). Similar to the changes in JH production rate, JH titer dropped between days 0 and 5, from 0.41 ± 0.02 to 0.11 ± 0.03 pmol µl^{-1} (ANOVA, $F = 75.704$, df $= 1$, $P < 0.001$). The titer stayed low through day 30 (0.08 ± 0.02, 0.05 ± 0.01, and 0.03 ± 0.01 pmol µl^{-1}, respectively, for Days 10, 20 and 30), began to rise in day 45 females (0.18 ± 0.01 pmol µl^{-1}), then more than quadrupled in mature queens (0.82 ± 0.03 pmol µl^{-1}, ANOVA, $F = 322.855$, df $= 1$, $P < 0.001$).

3.2. Measurement of hemolymph ecdysteroid titer

Similar to our observations with JH production and JH titer, we found that the titer of circulating ecdysteroids varied considerably in maturing primary females, but in a pattern inverse to that of JH (Fig. 3B). Reproductively inhibited alates drawn directly from their natal nest had a moderate level of ecdysteroid in their hemolymph (2.36 ± 0.27 ng µl^{-1}), which was significantly higher than that of similarly aged workers (1.26 ± 0.15 ng µl^{-1}; ANOVA, $F = 14.814$, df $= 1$, $P < 0.001$). Just two days after the primary females were isolated from their natal nest and paired with a male, the ecdysteroid concentration surged 53% (3.60 ± 0.14 ng µl^{-1}, ANOVA, $F = 7.289$, df $= 1$, $P = 0.018$) and remained high for at least 30 days. Between days 30 and 45, the ecdysteroid titer dropped significantly (ANOVA, $F = 10.756$, df $= 1$, $P = 0.007$) to a concentration comparable to that in found immature alates on day 0. The titer was at its lowest in mature queens (0.77 ± 0.42 ng µl^{-1}). A comparison of the mean JH production rates and mean ecdysteroid titers over the sampled developmental period indicates a significant negative correlation between the two parameters (linear regression, $r = -0.88$, $F = 28.17$, $P < 0.001$).

3.3. Ovarian development

Few changes occur in the ovaries of maturing primary females for the first 30 days after removal from the natal nest (Fig. 4). They start with a significantly greater number of functional ovarioles per ovary than workers (30.80 ± 0.65 vs. 26.88 ± 0.88, Kruskal–Wallis ANOVA, $H = 8.201$, df $= 1$, $P = 0.004$), but this number does not change significantly until after 45 days of maturation, when ovarioles increase to 35.52 ± 0.88 per ovary (one way ANOVA, $F = 13.999$, df $= 1$, $P < 0.001$). The number of functional ovarioles per ovary was highest in the mature queens (43.60 ± 1.10). The number of vitellogenic oocytes followed the same pattern as ovariole number, increasing 36% between days 30 and 45, from 4.64 ± 0.54 to 6.33 ± 0.57 (Kruskal–Wallis ANOVA, $H = 4.421$, df $= 1$, $P = 0.035$), and increasing another 62% in mature queens to 10.34 ± 1.01.

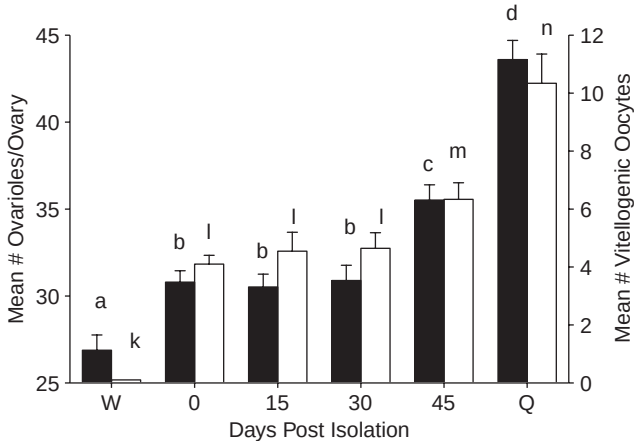

Fig. 4. Mean (± s.e.) number of functional ovarioles (white bars) and vitellogenic oocytes (black bars) found in the ovaries of workers (W), mature queens (Q) and maturing primary females during the first 45 days after isolation from the inhibitory influence of their natal nest. Each mean represents 28–40 determinations for ovariole numbers and 26–39 determinations for vitellogenic oocytes. Bars with the same letter are not significantly different ($P > 0.05$) by one-way Kruskal–Wallis ANOVA on ranks followed by Dunn's test.

4. Discussion

Termite queens maintain their reproductive status within a colony through the production of specific stimuli that result in the inhibition of ovarian activity in nestmates (Noirot, 1990; Noirot and Bordereau, 1990). This suppressive effect extends even to nestmates that have reached what is considered to be an "adult" reproductive form, such as other primary females waiting to disperse away from their natal nest as alates (reviewed in Weesner, 1969). This effect may be achieved via inhibitory primer pheromones produced by a queen (Miller, 1969; Lüscher, 1974, 1976; Bordereau, 1985; but see Stuart, 1979), or by self-regulated inhibition brought about by recognition of a queen within the nest (Spradbury, 1965, 1991; West-Eberhard, 1969, 1978; Ross, 1988; Keller and Nonacs, 1993). Although previous research had indicated that hormones were involved in termite caste differentiation (Lüscher, 1972, 1974; Okot-Kotber, 1980, 1982, 1983; Nijhout and

Wheeler, 1982) and reproduction (Lüscher, 1972; Meyer et al., 1976; Delbecque et al., 1978; Greenberg and Tobe, 1985; Okot-Kotber et al., 1993), little was known about the specific mechanisms involved. We present the first substantive evidence supporting a model of reproductive inhibition maintained through modulation of a female's endocrine system. Such reversible endocrine castration of reproductively competent nestmates has also been observed in ants, bees and wasps (reviewed in Robinson and Vargo, 1997; Brent and Vargo, 2003; Cuvillier-Hot et al., 2004).

Our results indicate that within a few days of a female alate being separated from her natal nest and being paired with a male reproductive, both the rate at which her CA produce JH (Fig. 3A), and the hemolymph ecdysteroid titer (Fig. 3B) change dramatically. It is possible that male-specific stimuli influence the female's endocrine system, but because separation from the natal nest without a male is sufficient to significantly promote ovarian development (Brent and Traniello, 2001b), the observed hormonal changes are most likely due to disinhibition from queen stimuli. Immediately upon separation from the natal nest CA activity is moderate, comparable to that observed in non-reproductive workers. We suggest that the resulting JH titer exceeds a threshold necessary to suppress ovarian development in the alates. This rate of JH production is maintained in alates for 3–5 days after they have been removed from their nest, at which point it declines significantly. The delay prior to the decline in JH may indicate that periodic, but not necessarily constant, contact with a queen's inhibitory stimulus may be sufficient to maintain an alate's maturational stasis. It is also possible that the inhibitory signals are highly persistent and are slowly eliminated after separation. The subsequent decline in JH may facilitate a final period of development towards becoming a functional queen. This idea is consistent with the observation that in many insects, a high JH titer during critical periods of maturation, in particular during an intermolt, can result in the maintenance of a juvenile form after the molt, whereas a lower titer produces an adult molt (Nijhout, 1994). This normal organizational effect of JH that directs development may have acquired an extended functionality in directing reproduction in termites, providing a mechanistic basis for the evolution of social control mechanisms. Similar elaboration on the basic function of JH has been identified in a number of social insect species (reviewed in West-Eberhard, 1996; Robinson and Vargo, 1997).

A queen may control circulating JH concentration in immature alates by one of two means. First, she may directly or indirectly manipulate CA activity, causing an increase in the production and release of JH. Queens of *Bombus terrestris* have been shown to control reproductive development in workers by releasing a pheromone with just such an effect (Röseler et al., 1981; Bloch et al., 1996; but see Bloch et al., 2000). Second, a termite queen may have little or no direct influence over the CA of an alate, but instead she could inhibit the production or activity of JH-esterases resulting in an increased JH titer (Hammock, 1985). However, the strong correlation between JH production and titer (Fig. 2) suggests that esterase activity is relatively constant, despite large fluctuations in JH production during the period sampled. Although the role of esterases cannot be wholly excluded without direct measurements, it is likely that CA activity in large part determines JH titer, as appears to be the case in honey bees (Rachinsky and Hartfelder, 1990; Huang et al., 1991), fire ants (Brent and Vargo, 2003) and cockroaches (Tobe and Stay, 1985).

One means by which the queen may be able to influence CA activity is through manipulating ecdysteroid titer. There was a significant negative correlation between JH release rate (and hence JH titer) and ecdysteroid concentration in hemolymph, which may indicate that the presence of one hormone regulated the production of the other. Following separation from a functional queen, the ecdysteroid titer of female alates increased two days prior to the decrease in JH production. Ecdysteroids have been shown to inhibit CA activity in cockroaches (Stay et al., 1980; Lanzrein et al., 1981; Rankin and Stay, 1987; Chiang et al., 1995), and this control may have been conserved in the closely related Isoptera. Indeed, Raina et al. (2003) have shown that maturing alates of *Coptotermes formosanus* exposed to an ecdysone agonist produce eggs later than controls, and had a reduced CA volume. The ecdysteroids might act either directly on the CA, but given the delay observed before JH production declined, it is more likely to act via a neurohormonal pathway involving the brain and the corpora cardiaca. Ecdysteroids may inhibit CA activity by promoting the release of allatostatins, or by causing variations in the sensitivity of the CA to the presence of the neuropeptides (Feyereisen, 1985; Tobe and Stay, 1985; Stay et al., 1996, 1997). Alternatively, an elevated JH titer may inhibit ecdysteroidogenesis (reviewed in Riddiford, 1994; Hartfelder, 2000). A third possibility is that the CA and ecdysteroidogenic glands are responding independently to changes in the social environment. In some adult insects, the production of one hormone has no obvious effect on the production of the other (Hagedorn, 1985). Considering the strong negative correlation between JH and ecdysone titers, the finding that the increase in ecdysteroid titer preceded the decrease in JH, and the close evolutionary ties, it is more likely that termites follow the cockroach model. Until hormonal manipulation studies are conducted, however, none of these possibilities can be discounted.

Subsequent to the initial decline in JH following separation from the natal nest, the circulating concentration of the hormone stays low for at least 1 month (Fig. 3A). During this time, there is no change in the number of ovarioles or vitellogenic oocytes in these maturating females (Fig. 4). By Day 45, both of these indicators of ovarian activity increase, while at the same time the JH release rate and titer also rise. This trend continues until the female is a fully mature queen. The coordination of these two parameters during this later stage of maturation suggests that the role of JH may be shifting, transitioning from a developmental regulator to a promoter of oogenesis. Similar shifts in functionality are observed in other insects, in which a high JH titer prevents the adult molt, but is necessary to stimulate oocyte production in mature adults (Nijhout, 1994). This shift in JH function may become effective within a few days or weeks following separation of the alates from the inhibitory influences of their natal nest, and may be the result of organizational changes within the brain or the ovaries. The concurrent variation observed in ecdysteroid titer (Fig. 3B) may actually stimulate such changes, since these hormones have been shown to have both organizational and activational effects in other insects (Tsuchida et al., 1987; Hagedorn, 1989; Bitsch and Bitsch, 1988; Stay et al., 1997). Greenberg and Tobe (1985) have already demonstrated that vitellogenin production is correlated with JH titer in secondary reproductives of *Z. angusticollis*, and the very rapid release rate of JH observed in mature queens may reflect their need to maintain a constant, high rate of vitellogenin production to support their increased fecundity.

During the same period that JH production is increasing, ecdysteroid titers are dropping (Fig. 3B). This decline may be the result of a changed capacity to produce the hormone(s). The specific origin of ecdysteroids in adult termites is unknown, but one potential source during the initial development of maturing alates is the prothoracic glands. The prothoracic glands are the primary source of ecdysteroids in immature insects (Hagedorn, 1985), and have been shown to persist in other adult castes of some termite species (reviewed in Noirot, 1969). After the adult molt, these glands tend to degenerate in the majority of insects (Hagedorn, 1985; Nijhout, 1994), although they are known to persist and regulate reproduction in *Thermobia* (Bitsch and Bitsch, 1988). The prothoracic glands of *Z. angusticollis* may remain intact after the alate molt, remaining active until the primary reproductive has undergone the final stages of maturation following its developmental disinhibition. Ecdysteroid production may decline once these glands begin to degenerate. Intact prothoracic glands have not been found in mature termite queens, so it is likely that the ecdysteroids we observed in these older females have a different origin. In other adult insects the ovaries are ecdysteroidogenic (Rees, 1985; Delbecque et al., 1990; Whiting and Dinan, 1990; Weidener et al., 1992; Gillot and Ismail, 1995) and the hormones are responsible for driving oocyte maturation or are incorporated into the eggs to control embryonic development (Hagedorn, 1985, 1989; Hoffmann and Lageux, 1985). Further study will be necessary to define the role and origin of these hormones in termite queens.

In summary, these results indicate that although alates have an adult form, profound internal changes must occur before they can become functional reproductives. Following their imaginal molt, their hormonal physiology and ovarian development more closely resemble those of non-reproductive forms. Release from a queen's inhibitory stimuli results in significant changes in both JH and ecdysteroid production, supporting the hypothesis that queens maintain reproductive dominance by manipulating the endocrinology of nestmates. Once free of this suppressive effect, an alate goes through a major period of neuroendocrine reorganization. The maturational changes affect the way in which the termite perceives and responds to its environment, activates reproductive behaviors, and initiates oogenesis. The hormones that are regulating this transformation appear to undergo their own changes in function. Future research will further elucidate the endocrine mechanisms controlling reproduction, and will identify the specific inhibitory stimuli used by functional queens to control their nestmates.

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