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Rapid Elevation of Juvenile Hormone Titer During Behavioral Assessment of the Breeding Resource by the Burying Beetle, *Nicrophorus orbicollis*

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Female burying beetles (*Nicrophorus orbicollis*) rapidly complete ovarian maturation upon discovering a suitable carrion resource for breeding. In this study, we examined changes in hemolymph titers of juvenile hormone (JH) over the first 30 days of adult female life, and in response to the discovery of a mouse carcass. Levels of JH were found to increase gradually over the first 20 days, and then increased abruptly within 24 h of discovery of a carcass. Changes in JH titer were correlated with increases in ovarian mass and length of terminal oocytes. To more precisely determine the timing of the endocrine response to a carcass, hemolymph titers of JH were measured 2, 10, 20 and 60 min after carcass discovery. Titers of JH were significantly elevated (112% over controls) in just 10 min. To confirm this resource discovery-related increase in JH, hemolymph samples were taken from the same individuals both 2 days before, and 10 min after, discovery of a carcass. Again, JH titers rose significantly (170%) in beetles 10 min after carcass discovery. Prominent behaviors observed during the 10-min period following discovery included palpating, lifting, walking around the carcass and making forays into the surrounding soil. Feeding did not occur. These results suggest that the rapid JH surge in female burying beetles is triggered by information obtained during behavioral assessment of a breeding resource, and not by mating or feeding cues. The association between ovarian development and JH titer further suggests a role for JH in co-ordinating reproduction in a temporally and spatially unpredictable environment.

Juvenile hormone Ovarian development Burying beetle *Nicrophorus* Behavior Reproduction

INTRODUCTION

In most insects, females must encounter appropriate environmental conditions to induce the hormonal changes that initiate ovarian maturation and reproductive behavior. The role of the environment in cuing reproduction can be viewed in two ways. In insects that typically breed shortly after adult emergence, the important endocrine events may be dependent on cues such as photoperiod, which indicate whether the individual should attempt to reproduce or enter reproductive diapause (Engelmann, 1970). In species that rely on an unpredictable event, such as acquisition of a critical resource, individuals often attain reproductive competence and then remain in an arrested state until the appropriate stimuli are experienced. When a blood meal is required for reproduction, for instance, ovarian maturation is stimulated after the ingestion of a blood meal. In the mosquitoes *Culex* spp (Edman and Lynn, 1975; Mitchell and Millian, 1981) and *Aedes aegypti* (Klowden and Lea, 1979), and the sandfly *Phebotomus papatasi* (Schlein and Warburg, 1985), the proximate stimulus appears to be endogenous (extension of the gut) rather than a direct assessment of the environment. Similarly, when the timing of mating is unpredictable, endogenous cues resulting from copulation may stimulate oviposition and terminate sexual receptivity (reviewed in Davey, 1985). Important mating-related cues can be products of the male reproductive system (Loher and Edson, 1973; Freidel and Gillot, 1976), or mechanical stimulation (Sugawara, 1979).

We might expect that the completion of reproductive maturation in response to favorable conditions would

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be especially rapid when the critical resource is both temporally and spatially unpredictable, as well as highly valued by competitors. Burying beetles (*Nicrophorus* spp) must outcompete congeners, carrion-breeding flies and scavengers to exploit small vertebrate carcasses as a breeding resource. Many of these competitors have evolved behavioral and physiological adaptations to exploit carrion quickly, such as larviposition (Denno and Cothran, 1976). Burying beetles also respond rapidly to a suitable resource. After emerging as adults, burying beetles feed on insects and carrion until reproductive competence is attained (12–20 days). Females become sexually receptive shortly after emerging, and will store sperm obtained during matings at feeding aggregations or away from carcasses (Eggert, 1992). In competent females, ovarian development plateaus until the discovery of a suitable resource triggers a 2–3 fold increase in ovarian mass, followed by oviposition within 24–36 h (Wilson *et al.*, 1984; Scott and Traniello, 1987).

Scott and Traniello (1987) found that handling a carcass, rather than nutritional benefits or mating, was the cue that triggered ovarian maturation in burying beetles. Discovery of a carcass initiates assessment of the resource. Following discovery, beetles begin lifting and burying the carcass, preparing the nest and depositing antifungal anal secretions (Fabre, 1919; Pukowski, 1933; Milne and Milne, 1944; Halffter *et al.*, 1983). A male and female will co-operate to prepare a carcass and care for the young, although a lone female will complete the entire nesting cycle on her own if a male fails to discover the resource (Trumbo, 1994). Scott and Traniello (1987) thus argued that assessment, preparation and burying of the carcass were the critical cues. If this is the case, then rapid reproductive maturation in burying beetles is regulated by a complex interaction of environmental, behavioral and endocrine factors in a manner analogous to many vertebrates (Lehrman, 1965; Crews, 1975). However, support for this intriguing hypothesis was limited to measurement of increase of ovarian mass; the behavioral and endocrinological regulation of reproduction in burying beetles has not been studied.

In this study, we report findings from the first hormonal analysis of a burying beetle (*Nicrophorus orbicollis*). We present profiles of juvenile hormone (JH) titers during the first 30 days of adult life for starved and fed females. In addition, we demonstrate an extremely rapid increase in JH titer (<10 min) in response to the discovery of a breeding resource, and show that this response cannot be explained by feeding or mating. Four behaviors that occur shortly after the location of a carcass are described, and we suggest that information obtained during assessment of the resource triggers the JH response.

MATERIALS AND METHODS

Experimental animals

Experimental animals were taken from a laboratory population of *N. orbicollis* that is descended from a wild population collected at The University of Michigan Biological Station. To ensure an outbred and genetically diverse laboratory population, beetles are trapped in the field each summer and introduced into the population. Information concerning habitat associations, phenology and diurnal activity patterns of the wild population can be found in Wilson and Fudge (1984) and Trumbo (1991). Beetles were housed in mixed-sex groups at 23°C and 15L:9D, fed one meal of chicken liver after emerging as adults and thereafter maintained on mealworms. Females were isolated 2 days before experimental trials in plastic containers ($8 \times 15 \times 30$ cm) three-quarters filled with soil. Mouse carcasses were obtained from local biomedical facilities, stored frozen and thawed overnight prior to experimental trials.

Measurement of JH titer

Titers of JH were measured using a recently developed chiral-specific RIA (Hunnicutt *et al.*, 1989). Hemolymph samples were obtained (1.2–10.8 μl) by severing one or more legs near the base and applying a calibrated micro-capillary tube (DrummondTM) to the wound. Samples were placed into 500 μl acetonitrile (OmniSolv, HPLC grade) in 13×100 mm glass culture tubes with Teflon-lined screw caps, and stored at -70°C. JH was extracted by adding 1 ml 0.9% NaCl and 1.5 ml hexane (Fisher, HPLC grade) to the acetonitrile–hemolymph mixture. After vortexing, samples were cooled on ice for 10 min, vortexed again and centrifuged at 3000 ***g*** for 8 min (4°C). The supernatant hexane phase, containing the JH, was removed. The extraction was repeated and the pooled supernatants were dried in a vacuum centrifuge (Savant SC110). All glassware was baked prior to use.

To perform the RIA, 50 μl methanol was added to each extract and a 2.5 μl aliquot was transferred to a tube (10×75 mm) containing 200 μl premixed antiserum (1:34, 750) and 11,400 dpm of [10-^{3}H(N)]-JH (NEN, 629 GBq/mmol). The mixture was incubated for 2 h at room temperature and then cooled in an ice–water bath for 10 min. Unbound radiolabeled JH was separated from bound JH by adding dextran-coated charcoal (for 2.5 min) and centrifuging for 3 min at 2000 ***g*** (4°C). Radioactivity in the supernatant (containing radiolabeled JH bound to antiserum) was quantified by liquid scintillation spectrometry (Beckman LS6000IC). A standard curve based on analyses of 1, 3, 10, 30, 100, 300, 1000, 3000 and 10,000 pg 50% racemic JH III (Sigma) was constructed for each assay. Parameters of the standard curve were estimated by non-linear regression (Wilkinson, 1989) according to the following formula:

$$\log(\text{amount of JH}) = \frac{\log_e\left(\frac{\text{maximum binding}}{\text{dpm bound}} - 1\right) + A}{B}$$

where "maximum binding" is the value obtained (in dpm) when no competing JH is present, and "dpm bound" is the value obtained from sample tubes containing

unknown amounts of JH (Huang *et al.*, 1994). The formula fits the standard curve data very well ($R^2 = 0.994 \pm 0.001$, $N = 10$ curves), enabling accurate estimates of JH titer in biological samples. JH equivalents were multiplied by 0.5 because the racemic JH III used to generate the standard curve contains 50% of each enantiomer and the antibody only recognizes the biologically active enantiomer (Hunnicutt *et al.*, 1989; Huang *et al.*, 1994).

Qualitative analyses of JH

The specificity of the RIA was investigated by fractionating the hexane extracts of hemolymph samples ($N = 4$) with normal-phase high performance liquid chromatography (HPLC) (5 μ, 4.6 mm x 25 cm silica column; 5 or 10% diethyl ether in hexane; 1 ml/min). One-minute fractions were collected, dried, resuspended in 25 μl ethanol and analyzed for immunoactivity by RIA as described above.

The RIA used in this study involves minimal purification of hemolymph samples and is sensitive enough to be used on single individuals. It is therefore attractive for behavioral studies involving large numbers of individuals. To determine whether analyses with this assay are affected by hemolymph lipids, hemolymph samples ($N = 16$) were divided into two, and half of each sample was subject to a purification procedure that removed lipids. A reverse-phase C_{18} Sep-Pak cartridge (Waters) was washed with 1 ml 100% methanol and 3 ml 60% methanol. JH extracts (re-dissolved in 1 ml 60% methanol) were passed through the cartridge, followed by two washes of the sample tubes with 1 ml 60% methanol. The JH fraction was eluted with 3 ml 85% methanol, dried to 1 ml with a vacuum centrifuge and the JH re-extracted with hexane. To monitor JH recovery, radiolabeled JH (approx. 5000 dpm) was added to each sample as an internal standard prior to splitting. JH titers from lipid-purified and non-purified halves were determined by RIA as described above.

To verify that lipids were removed with this procedure, purified and non-purified samples were analyzed by thin layer chromatography (TLC) according to Goodman *et al.* (1990). The TLC plate was prepared by washing with 100% methanol, and then with a 7:7:1 hexane:chloroform:ethyl acetate mixture. A pooled hemolymph sample ($N = 20$ individuals) was divided into two halves, one part of which was subjected to Sep-Pak purification. Purified and non-purified sample halves were dried down, suspended in 100 μl ethanol and applied to the TLC plate along with a JH III standard. In addition, crushed beetle abdomens ($N = 5$) were extracted with hexane, and the extract spiked with JH III and divided into two sample halves. Half of the samples were subjected to the Sep-Pak purification procedure, and purified and non-purified sample halves were applied to the TLC plate. An iodine vapor staining method for staining lipids (Goodman *et al.*, 1990) was used to compare bands from purified hemolymph, non-purified hemolymph, purified crushed abdomen extracts, non-purified crushed abdomen extracts and from the JH III standard.

Lifetime patterns of JH titer and reproductive development

Hemolymph samples were taken from females maintained on mealworms 3, 6, 9, 12, 20 or 30 days after adult emergence, and from starved females on days 3, 6 and 9 ($N = 8$–12 per treatment). An additional group of females was kept on mealworms for 29 days, and on day 30 was given a 15–25 g mouse carcass for 24 h. Body mass of females was measured before and after presentation of a carcass, and hemolymph samples were taken at the end of the 24 h period. Abdomens from all females were frozen immediately at −20°C for later dissection. After thawing, the right ovary was removed and placed into saline. Three of the 12 ovarioles were selected randomly and their terminal oocytes were measured at 30 × using an ocular micrometer (Olympus SZH dissecting microscope). The left ovary was removed and weighed.

Hormonal and behavioral responses to a carcass in the first 60 min

To determine whether there is a measurable change in JH titer in the first hour subsequent to discovery of a carcass, reproductively competent females (25–35 days) were isolated as before in soil-filled containers. After the onset of scotophase (the active period of *N. orbicollis*), a mouse carcass was placed in the path of a walking beetle to cause an encounter. Beetles were monitored under red light. Hemolymph samples were taken at 2, 10, 20 or 60 min after the "discovery" of the resource ($N = 10$–16 females per treatment). Control beetles (no carcass) were sampled either prior to the onset of scotophase (C1) or during the first 2 h of scotophase (C2) along with those individuals exposed to a carcass.

To confirm the rapid increase in JH upon encountering a carcass, additional individuals were sampled twice, once before and once after carcass discovery. Eleven females had a hemolymph sample taken (2.1–5.2 μl) from one severed leg 2 days prior to trials during the first 3 h of scotophase [(beetles receive similar leg injuries during contests for resources, and recover and reproduce successfully shortly thereafter (Pukowski, 1933; Trumbo, 1990)]. Immediately prior to trials, females were taken one at a time, weighed to the nearest 0.01 g and introduced into experimental containers over the first 3 h of scotophase. Ten minutes later, the beetle was weighed again, and a second hemolymph sample was taken. The carcass was then inspected to determine whether the skin had been punctured, which would indicate that feeding had occurred. Eight additional females (controls) were maintained on mealworms and had two hemolymph samples taken, 48 h apart as described above, during the first 3 h of scotophase.

To observe the behavioral response to a carcass, observations were made of these same females under red

light during the 10 min trial. The following behaviors were recorded:

—*palpate*: the beetle moves its mouthparts back and forth over the surface of the carcass;
—*lift*: the beetle moves under the carcass and pushes it off the soil surface;
—*circumambulate*: the beetle walks completely around the mid-section of the carcass, or walks the length of the carcass from head to tail;
—*foray*: the beetle leaves the carcass, uses its head to plow through soil and returns to the carcass.

These behaviors have been well described and can be categorized unambiguously (Fabre, 1919; Pukowski, 1933; Milne and Milne, 1944; Halffter *et al.*, 1983).

RESULTS

Qualitative analyses of JH

For each hemolymph sample ($N = 4$) the majority of the immunoactivity eluting from the HPLC column was detected as a single peak with a retention time identical to the JH III standard (Fig. 1). No other hemolymph fraction had immunoactivity higher than the background levels (≈ 8 pg). This result suggests that JH III is the only detectable form of JH in burying beetles, as has been found in other beetles (de Kort *et al.*, 1982; Khan *et al.*, 1982; Schooley *et al.*, 1984). The TLC analysis of a pooled hemolymph sample demonstrated that the Sep-Pak purification procedure effectively eliminated lipids from beetle hemolymph without removing JH (Fig. 2). This procedure likewise eliminated lipids from crushed abdomen samples (plate not shown). Removal of lipids, however, was not necessary for accurate determination of JH titer in hemolymph of burying beetles. Estimates of JH titers in split samples were significantly correlated ($R^2 = 0.93$, $P < 0.001$; Fig. 3). Further, the slope (0.96 ± 0.07) of the regression line was not significantly different from unity ($P > 0.05$, t-test), and the intercept (-43.8 ± 31.2) was not different from zero ($P > 0.05$, t-test). These results indicate that JH titer determinations with this RIA are not affected by the presence of hemolymph lipids in this species, as also has been demonstrated for honey bees (Huang *et al.*, 1994).

Lifetime patterns of JH and reproductive development

Ovarian mass and length of terminal oocytes increased until day 20 in adult females that were fed mealworms [Fig. 4(A) and (B)]. There was no increase in either measure of reproductive development in fed females between days 20 and 30 ($P > 0.20$, Mann–Whitney U-tests). Starved females had significantly lighter ovaries on days 6 and 9 than did comparably aged fed females. Oocyte length could not be measured in starved females because of a lack of definition in ovariole structure. The presentation of a carcass to 30-day-old females triggered significant increases in both mean ovarian mass (+160%) and mean length of the terminal oocyte (+59%) within 24 h [Fig. 4(A) and (B)]. At 24 h, these two measures of reproductive development are near their maximum (Trumbo, unpublished results). Mean body mass also increased ($19 \pm 4\%$, SE) during the 24 h on the carcass. Each female on a carcass gained mass (range 8–28%, $P < 0.01$, Sign test).

Titers of JH approximately paralleled measures of reproductive development in fed beetles, increasing nearly 4-fold from day 3 to day 30 [Fig. 4(C)]. Starved females, on the other hand, showed no increase in JH titer over the first 9 days of adult life. When 30-day-old fed females were presented a carcass, JH titers increased significantly (+195%) within 24 h of discovery, paralleling increases in ovarian mass and length of terminal oocytes.

Hormonal and behavioral responses to a carcass in the first 60 min

Females exhibited extremely rapid hormonal and behavioral responses to the discovery of a resource. JH titers were significantly higher in females that had been on a carcass for 10 min as compared to control females without a carcass that were sampled either prior to scotophase or during the first 2 h of scotophase (Fig. 5). There was an additional significant rise in JH titer

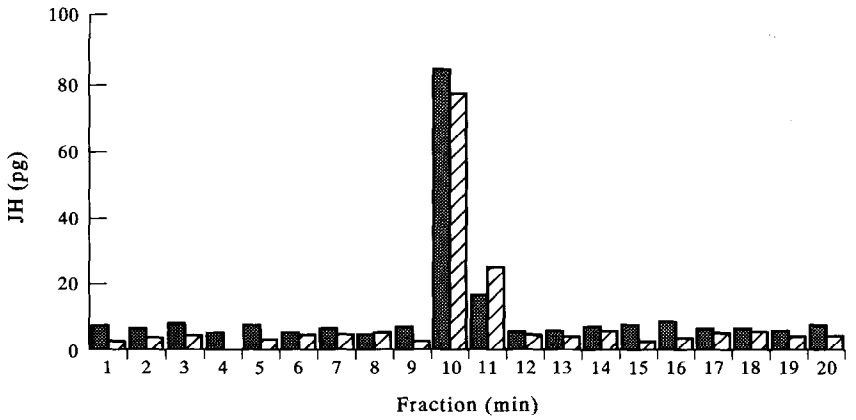

FIGURE 1. Qualitative analysis of JH in hemolymph of adult female burying beetles: immunoactivity of the different fractions of a hemolymph extract (hatched bars), and of a ^{3}H-labeled JH III standard (solid bars) following HPLC separation.

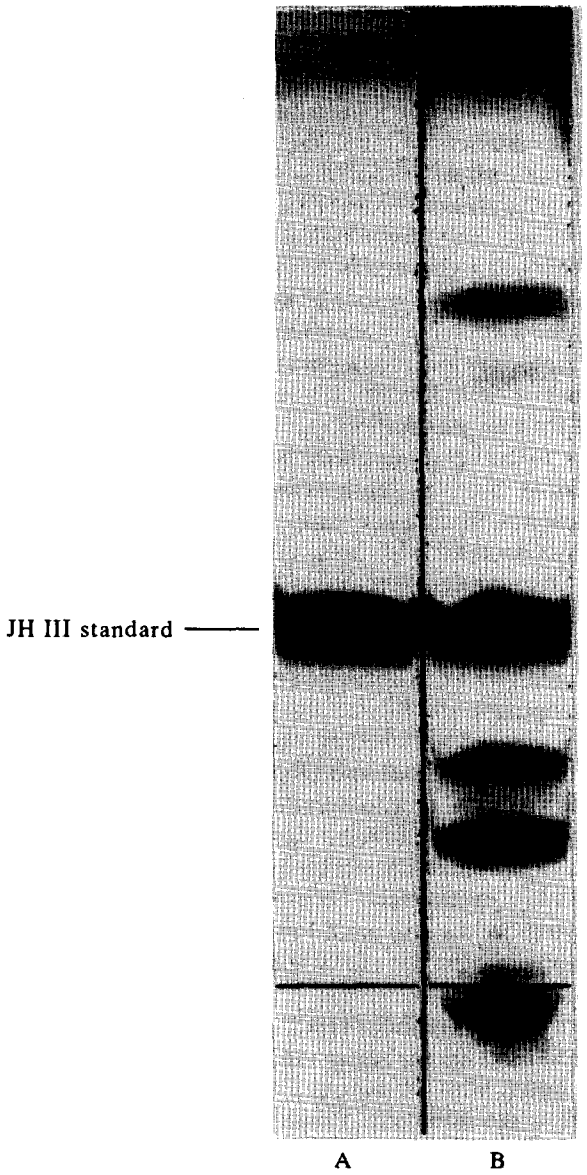

FIGURE 2. Effect of Sep-Pak purification on hemolymph lipids. Computer scans of a TLC plate showing bands produced by JH III (based on standard not shown) and lipids in hemolymph samples. Lane A, purified with Sep-Pak; lane B, not purified with Sep-Pak.

between 10 and 60 min. To confirm the extremely rapid increase in JH titer in response to carcass discovery, a second experiment was performed in which hemolymph was sampled twice from the same individuals. Ten of 11 females had higher JH titers after handling a carcass for 10 min than they did 2 days earlier during the same part of the activity period (mean increase of 170%; Wilcoxon's matched pairs signed ranks test, $P < 0.001$; Fig. 6). In contrast, there was no significant change in JH titer between the first and second hemolymph samples in control females that were maintained on mealworms.

Several behaviors were exhibited during the 10 min following the discovery of a carcass. These behaviors were organized temporally. Upon encountering the resource, each female ($N = 11$) first stroked their mouthparts over the carcass, but was not observed to feed. Subsequently, each female lifted and circumambulated the carcass, and made at least two forays into the surrounding soil prior to the end of the trial. All females either lifted or circumambulated the carcass before making the first foray ($P < 0.01$, Sign test). Mean ($\pm$SE) time to the first lift, circumambulation and foray were 39.1 $\pm$ 9.7, 92.3 $\pm$ 19.5 and 180.0 $\pm$ 40.9 s, respectively. By the end of the observation period females made an average of 3.6 ($\pm$0.4) forays. Four of 11 had begun to move the carcass (minimum of 5 cm displacement of the head).

Feeding did not occur in the 10 min subsequent to the discovery of the carcass. None of the carcasses had marks which indicated puncturing of the skin. No beetle had an increase in body mass of greater than the inter-measurement error (0.01 g). Finally, the mean body mass of females before and after exposure to the carcass was identical (0.48 g).

DISCUSSION

In most insects, JH plays an important role in regulating ovarian development (Ferenz, 1981; Koeppe *et al.*, 1985; Okuda and Chinzei, 1988). In burying beetles, there was a clear association of measures of ovarian condition (mass and length of terminal oocytes) with levels of JH. This association was maintained from emergence as an adult until the attainment of reproductive competence, and through the 24 h period following discovery of a resource. This suggests that JH is a primary regulator of ovarian maturation in this species. This conclusion will be tested in the future by direct application of JH analogs to competent females. Ecdysteroids also play a role in regulation of ovarian development, sometimes in concert with JH (Ma *et al.*, 1988; Wennauer *et al.*, 1989). The role of ecdysteroids in adult burying beetles is not known at present.

The peak titers of JH in burying beetles (which occur between 1 and 4 h subsequent to carcass discovery,

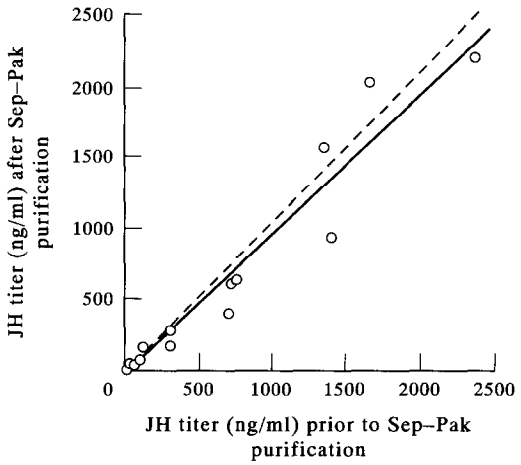

FIGURE 3. Effect of hemolymph lipids on RIA performance. Sixteen samples each were divided into two, half of each was passed through a C_{18} Sep-Pak cartridge to remove lipids prior to analysis of JH titer by RIA. The dotted line (slope of 1.0 and an intercept of 0) is the expected relation if lipids do not interefere with JH titer determinations.

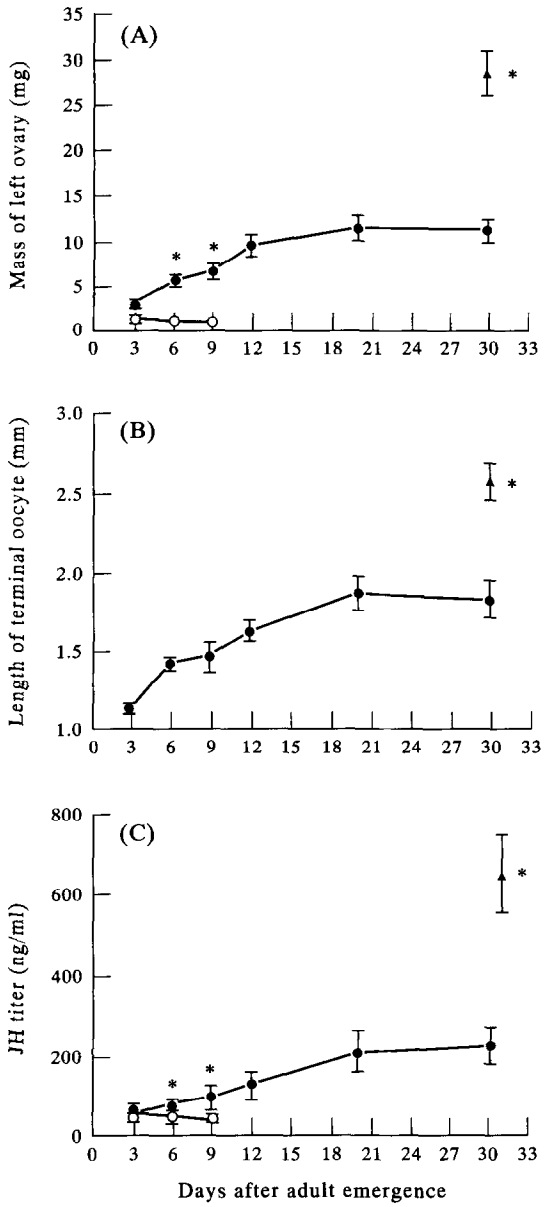

FIGURE 4. Reproductive development and JH titer in adult females. Mean (±SE) ovarian mass (A), length of terminal oocyte (B) and hemolymph JH titer (C) in fed (●) and starved (○) females. In each graph, a single point (△) also is shown for 30-day-old beetles that have been on a carcass for 24 h (*$P < 0.01$; $N = 8$–12 per data point). Mann–Whitney U-tests compare starved and fed females (days 3, 6 and 9), and 30-day-old females with and without a carcass.

unpublished results) are quite high relative to titer determinations in other insects. Peak JH titers are an order of magnitude greater than the highest recorded levels in the Colorado potato beetle as measured by gas chromatography–mass spectroscopy (Khan *et al.*, 1983; de Kort *et al.*, 1985), and five times greater than peak titers in worker honey bees as measured by the Borst RIA (Huang *et al.*, 1994). Since the present study is the first endocrinolgical analysis within the superfamily Staphylinoidea, it is not certain how to account for these differences. Qualitative JH analyses and the results of the lipid removal experiment indicate that the high titers observed in this study are real. Since JH levels appear to be higher than necessary to trigger vitellogenesis (Davey, 1993), perhaps the high titers are related in some way to the multiple roles of JH in regulating burying beetle reproduction and behavior.

The latency of the JH increase (< 10 min) was extremely short in females that discovered a suitable carcass, and is the fastest change in JH titer of which we are aware. The physiological mechanisms that regulate JH titer in burying beetles have not yet been explored. The rapidity of the JH response suggests that burying beetles may be a valuable model for exploring the dynamics of synthesis, release and degradation during modulation of hormone titer. Ecologically, a rapid hormonal response and ovarian maturation can best be understood by the need to compete successfully for a large, protein-rich resource that is both spatially unpredictable and ephemeral. In a similar manner in vertebrates, androgens often associated with long-term effects on behavior and reproduction can increase rapidly in titer (< 10 min) when an organism is confronted with a critical environmental challenge (Wingfield *et al.*, 1987).

In many insects, hormonal events that regulate ovarian development are elicited by endogenous cues resulting from mating or feeding, or the perception of an appropriate photoperiod (Engelmann, 1970; Klowden, 1990). Among female vertebrates, on the other hand, reproductive development may not occur unless critical behaviors such as inspecting a nest or assessment of male courtship takes place (Lehrman, 1965; Hinde, 1965). Burying beetles also seem to require the performance of assessment behaviors for triggering important endocrine events.

Scott and Traniello (1987) suggested that behaviors such as assessment, burial and preparation of the carcass, but not feeding or mating, were the cues that caused ovarian development. Results from the present study suggest that the rapid elevation in JH titer in response to carcass discovery may be triggered by a minimal period of assessment alone, since preparation and burying did not begin in the first 10 min. The additional events that must occur for ovarian maturation and oviposition are not completely understood. Results from other studies suggest that repeated contact, but not extensive handling of the carcass, is necessary. When a carcass is presented and then removed several hours later, females fail to oviposit (Trumbo, unpublished results). This suggests that contact with the resource must be ongoing to complete ovarian maturation and initiate oviposition. Observations made when two females discover a carcass suggest that intermittent contact with the carcass is sufficient for at least some oviposition. When two females discover a carcass, the smaller subordinate female is normally excluded by the larger, dominant individual (Bartlett and Ashworth, 1988; Otronen, 1988). In *N. vespilloides*, subordinates were able to spend only 5.3% of their time on a carcass during the 24 h period following discovery, while dominants were on the resource 78.2% of the time (Müller *et al.*, 1990). Even so, subordinates often laid a few eggs (Müller *et al.*, 1990), suggesting

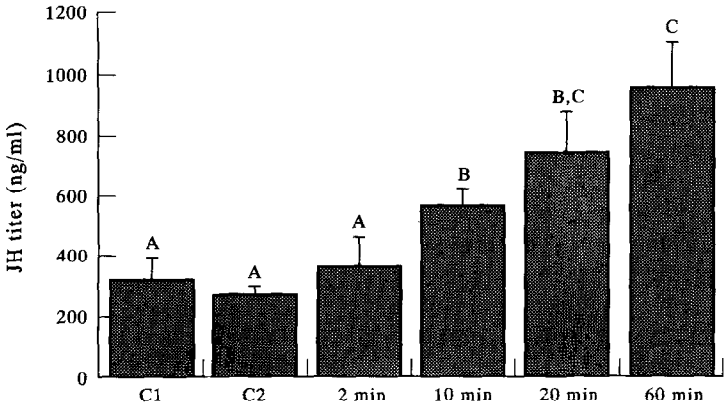

FIGURE 5. Hormonal response to discovery of a carcass. JH titer (mean ±SE) in females after 2, 10, 20 or 60 min on a carcass. Control 1 (C1) females were sampled prior to the onset of scotophase, control 2 (C2) females were sampled during the first 2 h of scotophase. Different letters above the bars indicate statistically significant differences [$P < 0.05$, Tukey's test, Wilkinson (1989), $N = 10$–16 per treatment except C2 ($N = 24$)].

that some oviposition can occur following intermittent contact and assessment of the resource. It would be interesting to know whether the extent to which a subordinate contacts and handles a carcass affects either the magnitude of the ovarian increase or the number of eggs oviposited.

Mating was not found to be important for the initial JH response, and there is little evidence that it affects ovarian maturation or oviposition. Female burying beetles readily mate shortly after emerging as adults and will mate away from carcasses throughout life (Eggert, 1992). Previously-mated *N. orbicollis* females readily accept carcasses and will complete an entire reproductive cycle in the absence of a mate (Scott, 1989; Trumbo, 1991). Scott and Traniello (1987) noted that the increase in ovarian mass within 24 h of carcass discovery was equivalent in females with and without a mate. Even virgin females of reproductive age (>20 days) will bury carcasses and oviposit infertile eggs [*N. orbicollis* (Robertson, 1995); *N. vespilloides* (Eggert, personal communication)]. Mating, therefore, is not necessary for the initial JH increase, ovarian maturation or oviposition.

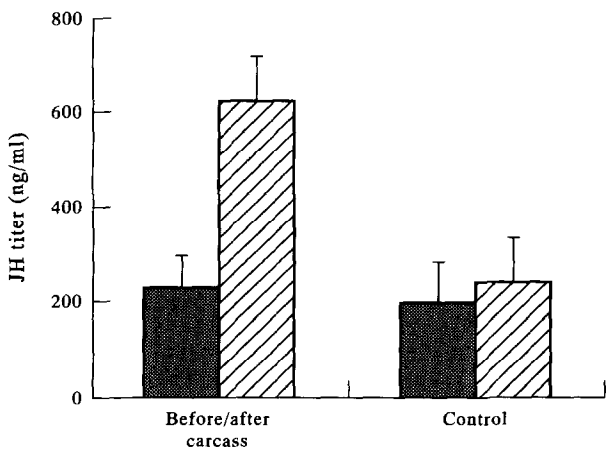

FIGURE 6. JH titer before and after discovery of a carcass. Mean (±SE) JH titer in females 2 days prior to (solid bars) and 10 min following (hatched bars) the discovery of a carcass. Control females were not provided a carcass. Results of statistical analyses in text.

The relationship between feeding following the discovery of a carcass and reproduction is not fully understood. It is clear that feeding is not necessary for the initial surge in JH titer since feeding did not occur in our study in the 10 min following carcass discovery. Similarly, Scott and Traniello (1987) demonstrated that nutritional benefits from the carcass are not sufficient to induce ovarian maturation. This conclusion was based on the lack of complete ovarian development in females that are fed to satiation on small pieces of mouse, but were not given a carcass suitable for reproduction. In the present study, all females fed on carcasses during the 24 h following the discovery of a resource and the weight gain (mean of 19%) during that time was substantial. Similarly, Müller *et al.* (1990) reported that *N. vespilloides* gain weight during the 24 h following discovery of a carcass. Thus, while feeding in the absence of a carcass is not sufficient to trigger ovarian maturation (Scott and Traniello, 1987), it is not yet known whether feeding on a carcass has quantitative effects on the latency to oviposition, or the total number of eggs that are matured.

Cues obtained during the assessment of the resource appear sufficient to trigger the increase in JH titer. Prominent behaviors immediately following discovery of a potential resource include palpating the carcass, lifting, circumambulating and making forays on the surrounding soil. Although these behaviors were intermixed, there was a degree of temporal organization. The initial palpation preceded the initial lifting or circumambulating, which preceded the initial foray. Subsequent behaviors were less ordered. A female that forayed, for example, often returned to lifting and crawling around the carcass. Although functions of these behaviors have not been determined, we hypothesize that assessment occurs in three stages: determining whether the resource is carrion (palpating); determining whether the resource is of proper size and in a suitable state of decomposition (lifting and circumambulating); and determining whether the substrate is suitable for burial and oviposition (forays).

Burying beetles have a rich behavioral repertoire which includes nest-building, contests for resources, parental care (during which time ovarian development is suppressed), facultative quasisociality, emission of sex attractants and subordinate reproductive strategies (Pukowski, 1933; Trumbo, 1990; Müller *et al.*, 1990; Eggert and Müller, 1992; Scott and Williams, 1993; Trumbo and Wilson, 1993; Trumbo and Eggert, 1995). The ability to obtain multiple hemolymph samples from the same individuals, and employ a sensitive assay for determining titers of JH, will assist in understanding the complex integration of these behaviors.

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