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Social stimuli affect juvenile hormone during breeding in biparental burying beetles (Silphidae: *Nicrophorus*)

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Abstract

Extended biparental care is rare in insects but provides an excellent opportunity to investigate the interaction between the endocrine system and the physical and social environment in the regulation of this behavior. Burying beetles (*Nicrophorus* spp.) have facultative biparental care and depend on locating a small vertebrate carcass that they bury and prepare as food for their young. Commonly, both male and female *Nicrophorus orbicollis* remain in the burial chamber after eggs hatch to feed and guard the larvae. In both sexes, juvenile hormone (JH) rises rapidly in response to the discovery and assessment of the carcass; it returns to near baseline in 24 h; then in females it reaches very high titers at the onset of maternal care. In this paper, we investigate some social (presence of a mate, mating history, larval age) and environmental (carcass size) factors that may affect this endocrine profile. For females, neither the presence of a mate nor mating status (i.e., virginity) affected the initial rise of JH. However, the absence of a mate significantly depressed the JH rise in males. Eighty-seven percent of the single males buried the carcass like paired males but 87% also released pheromones to attract a mate. JH hemolymph titers in females whose broods were replaced every 24 h with newly hatch larvae were significantly higher than those of females rearing aging broods. Lastly, even though larger carcasses took longer to bury and prepare and oviposition was delayed, neither JH titers nor speed of ovarian development was affected by carcass size.

Keywords: Parental care; Juvenile hormone; Social stimuli; Burying beetle; *Nicrophorus*

The interaction between hormones and reproductive behavior has been explored extensively in vertebrate taxa. For example, the endocrine correlates of the stages of parental investment in birds (Buntin, 1996; Wingfield et al., 1990) and mammals (Reburn and Wynne-Edwards, 1999; Rosenblatt, 1992; Wynne-Edwards and Reburn, 2000) are well documented as are the effects on the endocrine system from social stimuli from mates and young (e.g., Lehrman, 1965; Lévy et al., 1996). By comparison, our knowledge of the physiological underpinnings of parental care in insects is much more limited (Trumbo, 1996, 2002). Although maternal care in insects is not that rare (Tallamy and Wood, 1986; Wilson, 1971), only a few taxa have been investigated for hormonal mechanisms (e.g., cockroaches, Engelmann, 1959; Rankin and Stay, 1985 and earwigs; Rankin et al., 1995, 1997; Vancassel et al., 1984, 1987), and very little is known about environmental and social effects on the endocrine system. The honeybee serves as the model for understanding the possible effects of social stimuli on behavior (in this case brood care versus foraging) that is mediated by changes in hormone titers (Robinson, 1987; Robinson et al., 1989). Extended biparental care is rare in insects but offers the best opportunity to investigate the full intricacy of interaction among environmental and social stimuli from mates and offspring, and hormones, as has been done, for example, with birds. Complex behavioral patterns require behavioral plasticity and studies of this plasticity should include information on stimuli (internal and external) that elicit behavioral changes.

Burying beetles (*Nicrophorus* spp.) have facultative biparental care of young and are dependent on locating a small vertebrate carcass that they bury and prepare as food for their young. The unpredictability of this resource has promoted a vertebrate-like plasticity in the timing of reproductive events and parental behaviors as well as a suite of behavioral and physiological adaptations to use

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the resource quickly and to deter competitors. A male and female cooperate to bury a carcass within a few hours of its discovery; they remove fur or feathers and roll it into a ball; they spread anal secretions to retard its degradation (Eggert and Müller, 1997; Pukowski, 1933; Scott, 1998). Mating takes place any time, on or off a carcass (Eggert, 1992), and eggs are oviposited in the soil nearby as soon as 12 h after the discovery of the carcass. In *Nicrophorus orbicollis*, eggs hatch 4 days later and larvae make their way to the carcass where a feeding hole has been opened by the parents. Larvae actively beg from their parents (Rauter and Moore, 1999) and they are fed regurgitant by both parents. The typical pattern in this species is for males to remain until larvae are 3 days old and females to remain until larval development is complete—when they are 6–8 days old (Robertson, 1993; Scott and Traniello, 1990; Trumbo, 1991). Most carcasses are fully utilized, and for this reason, the size of the carcass determines the number of young that can be reared. Once eggs are laid, either parent can rear a brood on their own but if a male arrives on a carcass and no female is present, he typically emits a pheromone to advertise for a mate (Eggert, 1992; Pukowski, 1933).

Like other insects with multiple reproductive cycles, burying beetles have hormonal changes that correlate with reproductive events such as the discovery of a carcass, oviposition, and the onset of parental care. Trumbo et al. (1995) have demonstrated that in females there is a dramatic surge in juvenile hormone (JH) triggered by the behavioral cues associated with the discovery and assessment of the carcass (Fig. 1). The initial rise in JH in paired males is as fast and reaches the same peak as that in females (Panaitof et al., in preparation; this paper). By 24 h, JH in females returns briefly to prebreeding levels and then rises to reach very high hemolymph titers when larvae first appear on the carcass. By 3 days after the appearance of larvae, JH falls to prebreeding levels (Panaitof et al., in preparation; Trumbo, 1996, 1997).

In this paper, we investigate the plasticity of this JH profile and its relationship to the variable and unpredictable social and environmental conditions to which the beetles must respond. Initially, they must respond quickly both behaviorally and physiologically to the size and quality of the resource, the presence or absence of other beetles (mates and competitors), and later to the size of the brood and even to the different stages of development of the young. In light of the need for plastic responses in both reproductive physiology and behavior, we hypothesized that some stimuli might have differential effects on JH titers that in turn may mediate physiology or behavior. In particular, we investigate some social (presence of a mate, mating history, larval age) and environmental (carcass size) factors that may affect the endocrine profile. These factors were chosen because we thought, based on our knowledge of the beetles' natural history, they were the most likely to elicit variable endocrine responses. Furthermore, the effects of some of these factors (e.g., mating history) have been investigated in other insect taxa and have not shown a consistent result (Engelmann, 1959; Schal and Chaing, 1995; Vancassel et al., 1984). We use our results to discuss the question of the principal role of JH in the reproductive cycle of burying beetles: as a gonadotropin or as a regulator of behavior. We predicted that the physiological responses of paired and single females might be the same, but those of paired and single males (who cannot breed without a mate) would differ, that the discovery of a larger carcass, which takes longer to prepare, might slow the initial rise in JH in females, and that high JH titers might be maintained by stimuli from very young larvae.

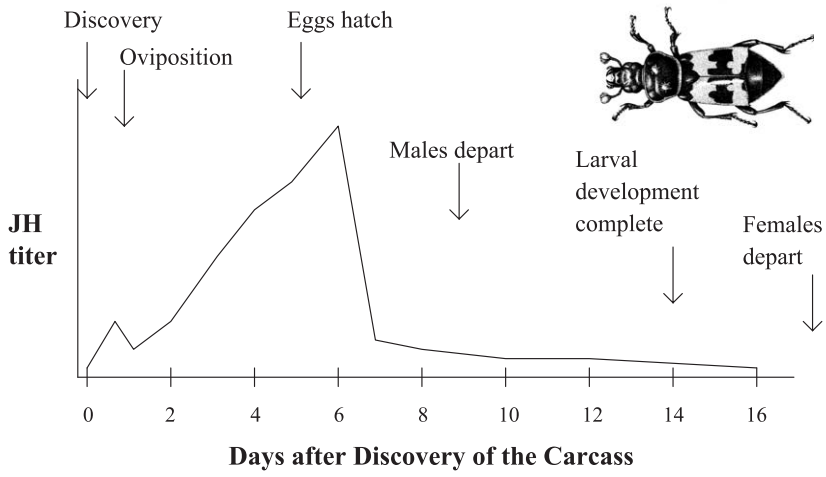

Fig. 1. Schematic diagram of juvenile hormone hemolymph titers in female burying beetles (*Nicrophorus orbicollis*) during a breeding bout and the corresponding physiological and behavioral events.

Methods

Animals and breeding manipulations

All beetles were laboratory-reared from a colony derived from *N. orbicollis* captured in Durham, NH. Beetles were maintained in boxes containing damp paper towels with up to six like-sex individuals, fed mealworms and kidney, and maintained at 20°C and 14:10 L–D. Females are sexually mature by 3 weeks posteclosion (Trumbo et al., 1995; Wilson and Knollenberg, 1984), and males and females were reproductively inexperienced at about 4 weeks old when they were bred.

To breed, a male and female ($n = 67$), a single male ($n = 24$), or a single female ($n = 35$) were placed in a plastic box (19 × 14 × 10 cm) of autoclaved soil with a previously frozen mouse (laboratory culls from the Dana Farber Cancer Institute and the Forsyth Institute). Carcasses were either 15–19 g (small) or 32–35 g (large). These sizes are, respectively, a little larger than the smallest carcass used in the field and a little smaller than the largest carcass that *N. orbicollis* can fully utilize (Scott and Traniello, 1990; Trumbo, 1992).

Social environment during burial

The effect of mating status on JH and visa versa is inconsistent across other insect taxa, and this relationship has never been investigated in males. We hypothesized that the absence of a mate would have no endocrine effect on a female but would alter the endocrine response of a male. We investigated the responses of JH titers and the ovaries to the discovery of a carcass in paired males and females, single mated and unmated females, and single males. Single females were either previously mated by placing them in a small box with a male for 24 h before the experiment or were virgins, having been housed alone for 24 h in similar conditions. (The sperm storage organ, the spermatheca, was dissected after hemolymph was taken to confirm the presence of live sperm.) For this experiment pairs, previously mated and virgin females and single males were given small carcasses.

To ensure that the timing of events was accurate, beetles were placed in the box of soil about 3–4 h before lights out. Starting at lights out, boxes were observed under red light and the carcass was added so that the pair or single beetle could discover it and begin work at a known time. Females were removed and hemolymph was taken 2 or 12 h later. At the same time, both ovaries were removed and the wet weight taken. (After emergence, ovaries develop only to the resting stage until the discovery of a carcass when their weight rapidly increases as a result of vitellogenin uptake by the oocytes (Trumbo et al., 1995; Wilson and Knollenberg, 1984).) Single males were observed for 8 h after they discovered the carcass. Scan samples (Martin and Bateson, 1986) were taken every half hour and the following behaviors were noted: carcass preparation, absent from carcass, and advertising for a mate. Males were removed at 8 h and hemolymph was taken. A control group of paired, breeding males ($n = 10$) was also removed and bled at 8 h. Nonbreeding control males and females ($n = 12$ and $n = 11$, respectively) of the same age were also bled and the wet weight of ovaries taken.

Effect of carcass size

Large carcasses often take 24–48 h longer to bury and prepare than small ones. Because oviposition is delayed, we hypothesized there would be a similar delay in the rise in JH. To measure the effect of carcass size on the initial JH rise and the time to oviposition, hemolymph samples were taken at either 2 or 12 h after discovery from females of pairs burying small or large carcasses ($n = 19$ and $n = 18$, respectively). They were allowed to continue until several days after eggs were observed in the soil on the bottom or sides of the box. Beetles were removed and all the eggs were located and moved to a petri dish with damp towel. Eggs were observed every 2 h (every 4 h between 11 PM and 7 AM) until they hatched. We had previously established that the time from first appearance of eggs to their hatch was 84.8 ± 2.9 h, $N = 15$. Because the timing of oviposition from our first observation of eggs was not always reliable, the time to oviposit was back-calculated from the time of hatching.

Stimulation from young larvae

Eggs hatch and larvae appear on the carcass on about the 5th day. About 24 h later, they molt into 2nd instars and 24 h later molt to the 3rd and final instar. First instar *N. orbicollis* must be fed to survive (Trumbo, 1992), and the frequency of parental regurgitations is highest on the second day (Fetherston et al., 1990) as are JH titers. As larvae age, JH drops quickly and we hypothesized that stimulation from very young larvae might promote high JH. A male and female ($n = 10$) were given a small carcass and allowed to oviposit. On the 4th day, the female was moved with the prepared carcass to a box of clean soil; her eggs were removed and observed. When they hatched, 10 larvae were given to her. Twenty-four hours later, these larvae were removed and counted and she was given 10 new unfed first instars. This was repeated 24 h later so that she reared 1st instar larvae for 3 days, at the end of which, hemolymph samples were taken. Replacements were not continued after 3 days because we intended to compare JH titers to those of control females whose JH returns to baseline by that time (Trumbo, 1997). There were two groups of control females: one group of females ($n = 11$) was given 10 larvae that were replaced after 24 h with 10 different 2-day-old larvae and after another 24 h replaced with 3-

day-old larvae, the other (n = 13) reared undisturbed the original 10 larvae for 3 days. A fourth group of females received no larvae after their eggs hatched (n = 8). Hemolymph was taken from all females 3 days after their eggs hatched.

JH extraction and radioimmunoassay

Hemolymph (4–12 μl, usually 10 μl) was taken from a severed 3rd leg with a calibrated microcapillary tube and placed in 0.5 ml chilled acetonitrile. Extraction and RIA followed the methods of Trumbo et al. (1995) on the same species using the same chiral-specific antiserum to 10R-JH III (Hunnicutt et al., 1989), the naturally occurring enantiomer. Trumbo et al. (1995) and our laboratory have verified that JH titer determinations with this RIA are not affected by the presence of hemolymph lipids. JH was extracted by adding 1 ml 0.9% NaCl and 1 ml hexane. Samples were vortexed, chilled on ice for 10 min, and centrifuged at 2000 RPM at 4 °C for 5 min. The hexane supernatant layer containing the JH was removed, and the extraction was repeated and pooled. It was dried under nitrogen and 100–500 μl MeOH was added. Samples were stored at −20 °C. Two or three tubes with about 1000 DPM radiolabeled JH were extracted at the same time and the extraction efficiency was 91.4 ± 1.6% (mean ± SE).

JH III is the only JH produced by these beetles (Scott et al., 2001; Trumbo et al., 1995). RIAs were performed in duplicate by adding 2 μl extracted sample in MeOH to antibody (1:16,000) and about 3500 DPM radiolabeled JH (NEN 11 Ci/mmol) in 200 μl PBS (0.05 M phosphate buffer, pH 7.3; 0.9% NaCl) with 0.1% gelatin. This was incubated at room temperature for 2 h then cooled on ice for 5 min. Unbound labeled JH was separated from the bound JH by adding 0.7 ml dextran-coated charcoal for 2.5 min and centrifuging at 2000 RPM at 4 °C for 5 min. The supernatant containing the bound, labeled JH was quantified in a liquid scintillation counter (Wallace LKB 1214 Rackbeta). A standard curve based on 3, 10, 30, 100, 300, and 1000 pg racemic JH III (Sigma), maximum binding, and nonspecific binding was included in each assay. A biquadratic equation for the standard curve and the estimates for JH concentration in the samples were calculated using the solver function in Excel ($R^2 \geq 0.995$). Intra-assay variation was 2.6%. The range of antibody binding was 21–33%. The sensitivity of the assay is high (<10 pg JH III/tube, Borst, personal communication). Two standards were run in each assay as internal positive controls. JH concentrations in the hemolymph were calculated from the volume of hemolymph taken, the extraction efficiency, its concentration in MeOH, and multiplied by 0.5 because the standards were of racemic JH and the antibody is chiral specific. Data were log transformed for statistical analysis. Unless otherwise indicated, statistical tests were single-factor ANOVA.

Results

Social environment during burial

For females, the presence of a mate or even virginity made no difference to the rise of JH hemolymph titers upon the discovery of a carcass. There were no significant differences among paired females, previously mated females, and virgins in JH titers after either 2 or 12 h (Fig. 2a). There was a significant increase in JH from prebreeding titers when treatments were combined ($F_{(2,61)}$ = 42.22, P < 0.0001), but there was no significant difference in JH between 2 and 12 h. Similarly, there were no significant differences among paired females, previously mated females, and virgins in ovarian weight after either 2 or 12 h (Fig. 2b). There was a significant increase in ovarian weight from prebreeding, 2 h and 12 h when when treatments were combined ($F_{(2,61)}$ = 23.44, P < 0.0001), and Tukey–Kramer tests for unplanned comparisons with α = 0.01 showed that ovaries after 12 h were significantly heavier than at 2 h or prebreeding.

In contrast, when discovering and assessing a carcass in the absence of a mate, the JH titers of males were significantly depressed ($F_{(2,43)}$ = 7.62, P = 0.001). JH titers of single males were not significantly higher than prebreeding levels and were significantly lower than those of paired

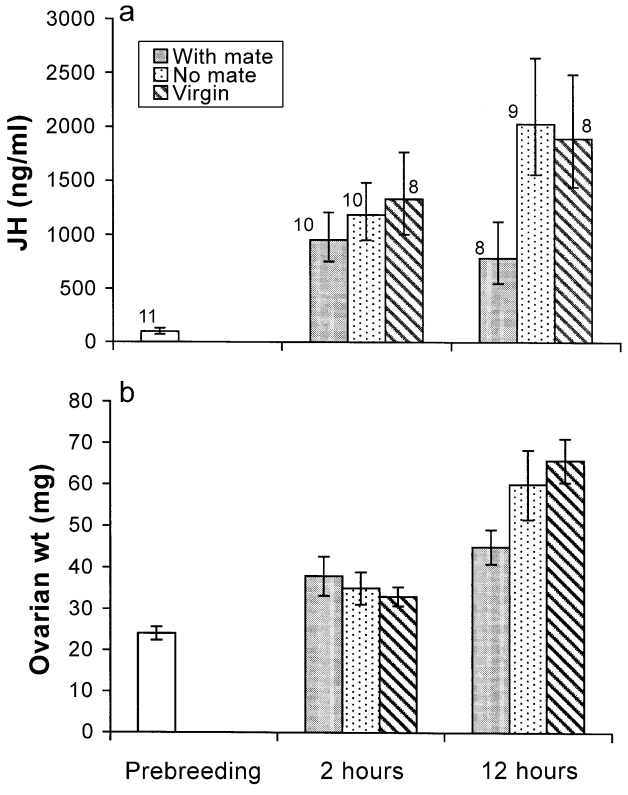

Fig. 2. Juvenile hormone hemolymph titers (a) and ovarian weights (b) for females before breeding, females with a mate, mated females with no male, and virgin females 2 and 12 h after discovery of a carcass. Mean ± SE (of log-transformed data, back transformed, for JH) and sample sizes are shown.

Fig. 3. Juvenile hormone hemolymph titers for males before breeding, and for paired and single males 8 h after discovery of a carcass. The back-transformed mean ± SE of log-transformed data and sample sizes are shown.

males (Tukey–Kramer tests for unplanned comparisons with $\alpha = 0.01$) (Fig. 3). Inspection of individual JH titers suggested that many single males were physiologically unaffected by the presence of a breeding resource. Eleven of 24 (46%) males had titers lower than the mean of prebreeding males; only 1 of 10 (10%) of paired males had such low titers. A moderate proportion of single males had titers within the range of paired males, but only two were above the mean. Twenty-one of 24 males were seen advertising for a mate in at least one of the 15 observations (mean ± SE, 4.5 ± 0.7 observations, range: 0–12). JH titers were neither correlated with the number of observations in which single males were seen to be advertising nor were JH titers correlated with the latency to advertise. There was little variation in the number of observations males were seen to be preparing and burying the carcass (2.9 ± 0.3, mean ± SE, range: 1–5 of 15 observations). In spite of the absence of a mate, most males (87%) buried the carcass at least superficially within 8 h. JH titers were not different between single males that buried the carcass completely within 8 h and those that did not.

Effect of carcass size

Large carcasses required longer to bury and prepare, and oviposition was significantly delayed (Mann–Whitney $U = 270$, $P = 0.001$) (Fig. 4). However, there were no significant differences in JH titers in females burying large or small carcasses after either 2 or 12 h (Fig. 5a). Similarly, there were no significant differences in ovarian weight in females burying large or small carcasses after either 2 or 12 h (Fig. 5b). There was also no difference in the number of eggs oviposited on small and large carcasses.

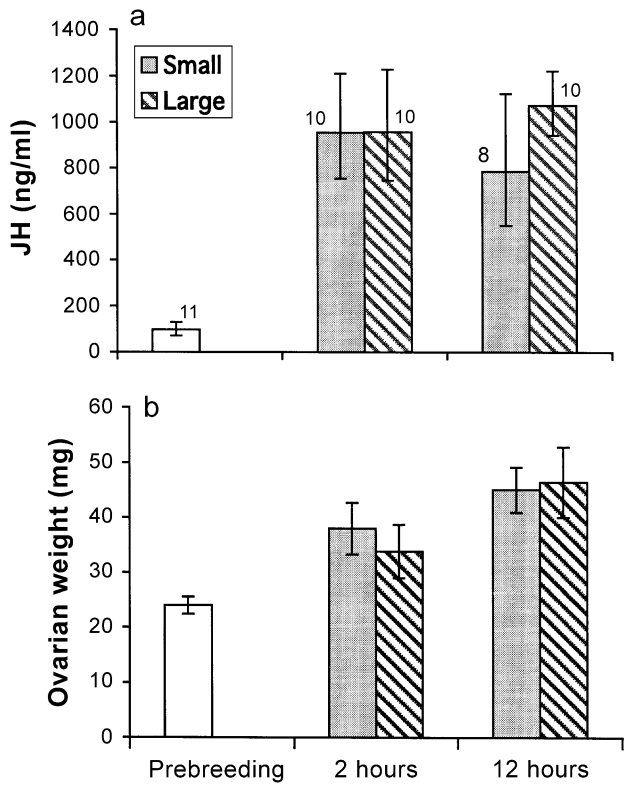

Fig. 5. Juvenile hormone hemolymph titers (a) and ovarian weight (b) for females before breeding, 2 and 12 h after discovery of the carcass for females burying small and large carcasses. Mean ± SE (of log-transformed data, back transformed, for JH) and sample sizes are shown.

Stimulation from young larvae

The replacement of growing larvae with newly hatched larvae for 3 days caused females to maintain very high JH

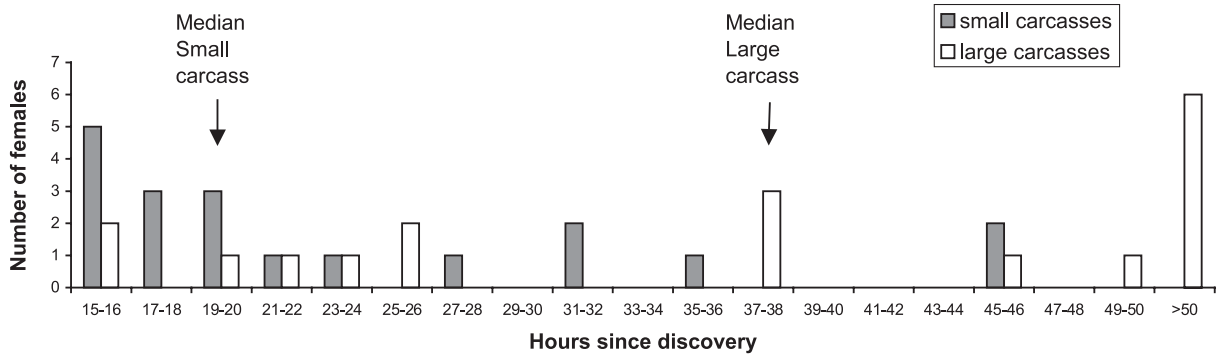

Fig. 4. Distribution of the time required before oviposition for females after they discover a small or a large carcass. Median numbers of hours from discovery to oviposition are indicated for both small and large carcasses.

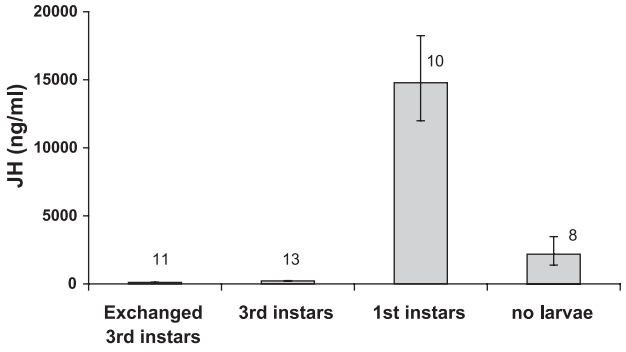

Fig. 6. Juvenile hormone hemolymph titers on the third day after eggs hatched for females whose larvae were replaced with same-age larvae every 24 h for 3 days; females that cared for consistent broods that were third instar by the third day; females whose larvae were replaced with unfed first instars every 24 h for 3 days; females that received no larvae for 3 days. The back-transformed mean ± SE of log-transformed data and sample sizes are shown.

titers (Fig. 6). Each day when larvae were replaced with new first instars, most of the original larvae survived (8.5 ± 0.4, 8.5 ± 0.5, 8.0 ± 0.5, mean ± SE, days 1–3, respectively), had been fed, and had grown normally. Survival of control larvae, those replaced daily with aging larvae, was high (9.3 ± 0.3, 9.5 ± 0.3, 9.9 ± 0.1, days 1–3, respectively). Withholding larvae for 3 days after eggs hatched maintained JH at somewhat higher levels than that of females caring for a consistent brood. JH titers of females after 3 days with either a consistently aging brood of either the same or replaced young (controls), replacement broods of new first instars, or no larvae were significantly different ($F_{(3,38)}$ = 58.93, $P < 0.0001$). Unplanned comparisons of each treatment using the Tukey–Kramer method with $\alpha = 0.01$ indicated that means of females given new first instars each day or no larvae were both significantly different from the controls and from each other.

Discussion

Extended biparental care is rare in insects, burying beetles, dung beetles, the wood roach *Cryptocercus*, and termites being the outstanding examples. Burying beetles are the first for which endocrine studies have been initiated (Trumbo et al., 1995). Although their behavior is well known (Eggert and Müller, 1997; Pukowski, 1933; Scott, 1998) and the JH pattern during a breeding bout has been described (Trumbo, 1996, 1997; Trumbo et al., 1995), studies to date have been correlational. Burying beetles demonstrate considerable behavioral plasticity in response to carcass size (Trumbo, 1991; Trumbo and Fernandez, 1995) and clutch size (Müller, 1987; Rauter and Moore, 1999), and males compensate behaviorally for the loss of a mate (Fetherston et al., 1994). However, nothing is known of the hormonal correlates of this plasticity. Here we have begun to explore the response of JH to some environmental and social stimuli. We find that stimuli from young larvae maintain very high JH and that the hormonal response to the discovery of a carcass of males but not females depends on the presence or absence of a mate.

An artificially maintained brood of young larvae maintained very high JH hemolymph titers in females. When larvae were replaced, JH titers were higher than the peak during a normal breeding cycle (Panaitof et al., in preparation). The stimuli of very young larvae seem to have caused JH to continue to rise in females, rather than fall to prebreeding levels as they normally do after 72 h of brood care. Rates of larval begging and parental regurgitation are high on the first and second day of parental care. Although we cannot say for sure that the cue maintaining high JH is larval begging, it is less likely that adult feeding and regurgitation are the causes because regurgitation rates are highest for second, not first, instar larvae (Fetherston et al., 1994). We also cannot say what the behavioral consequences of this high JH might be. The substitution of younger offspring lengthens the duration of parental care in burrower bugs (Kight, 1997), earwigs (Caussanel, 1970), and dung beetles (Klemperer, 1983), but the hormonal response to these substitutions is unknown. In burying beetles, the duration of maternal care appears to be determined by larval development, and because JH returns to baseline 5–8 days before care is terminated, it is unlikely that JH regulates care directly. However, a possible physiological consequence of high JH is an increase in metabolism which may help beetles to meet the demands of parental care as JH seems to do to coordinate foraging in honey bees (Sullivan et al., 2003).

On the other hand, when larvae were withheld from female burying beetles, their JH titers were significantly higher 72 h later than those of females caring for their third instars. This suggests that the presence of developing larvae hastens the return of JH to baseline and supports the hypothesis that JH may play a role in either the onset or intensity of parental care. This role may be indirect if JH serves to help meet the metabolic demands of parental care (Trumbo, 2002). Whatever the role of JH, the consequences from both manipulations, substitution of young larvae and withholding larvae, indicate that social stimuli can have a strong effect on hormones during care-giving.

The JH response to the discovery of a carcass differed in males and females depending on the presence of a mate. In females, the absence of a mate, or even her unmated status, did not alter her JH response to the discovery of a breeding resource. The relationship between JH and mating history is not consistent in other insect taxa with maternal care. In the cockroach *Diploptera punctata*, the normal cycle of JH titers requires mating (Engelmann, 1959; Stay and Tobe, 1977), but the opposite relationship is the case for *Blatella germanica*; JH is necessary for mating to occur (Schal and Chaing, 1995). In some dung beetles (*Phanaeus*), ovarian maturation, presumably regulated by JH, is dependent on mating (Halffter and López, 1977). However, in earwigs, JH

is not dependent on mating; the application of JH has no effect on mating (Rankin et al., 1995; Vancassel et al., 1984) and mating is not a prerequisite for oviposition or ovipositional behavior (Rankin et al., 1997) but is required for parental behavior (Vancassel, 1977).

In females, the behaviors associated with the assessment of a carcass cause JH to rise significantly within 10 min (Trumbo et al., 1995) and increase 10-fold within a few hours. It seems to bring about a similar rise in paired males. However, the absence of a mate during burial depressed JH titers in males. There was not very much behavioral variation in male responses to finding a carcass without a female; all manipulated the carcass and made forays in the soil nearby; all buried it at least partially; and most males advertised for a female by taking the pheromone-emitting posture. However, there was considerable hormonal intragroup variation with JH rising in a few males to titers similar to those with a mate. There were no consistent behavioral differences to indicate why the endocrine states varied, for example, males that called more were no more likely to have low JH than males that called less. Males that began calling later did not have higher JH, which might be the case if JH rose in response to the discovery of the carcass but was differentially depressed if it took some males longer to realize there was no female. It appears that in males, both the stimuli of the carcass and the cues from a female are necessary for JH to increase.

These differences in hormonal response between male and female and between paired and single males occur because the presence or absence of a mate upon the discovery of a breeding resource has different consequences for a male and female. Females can store sperm and often bury carcasses and rear broods on their own. Males, of course, must find a mate. The difference in endocrine response of males and females to the absence of a mate reflects this requirement. Males must perform the correct behaviors of burial and preparation but the greater need is to be synchronized with his mate. The timing of the necessary behavioral changes during a breeding bout is more difficult for a male. During a reproductive bout, the behavior of male and female must change in concert at appropriate junctures. When males and females first discover a carcass they are aggressive only to consexuals; after oviposition, they are aggressive to all other burying beetles of both sexes (Pukowski, 1933; Scott, 1990, 1994, Trumbo, 1990); both males and females are infanticidal toward larvae until a few hours before their own brood hatches when their behavior changes dramatically to parental (Müller and Eggert, 1990; Scott, unpublished data). To accurately time these events, a male must be synchronized with his mate's oviposition. He might do this by monitoring the actual appearance of eggs or the chemical cues from his mate either at carcass discovery or at oviposition or both. Scott et al. (2001) found evidence that JH biosynthesis in males was tracking that of their mate; in each pair, male rates rose more slowly and dropped later than that of their mate. Thus, males may use the endocrine status of their mates to coordinate their changes in hormones and behavior. Because JH titers did not rise in most single males as it does in paired males, this suggests that the initial steep rise in JH serves to mark the functional beginning of a reproductive bout and behavioral changes can be appropriately synchronized.

We hypothesized that because larger carcasses take longer to bury and prepare than smaller ones, the rise in JH titers and ovarian development might be delayed. Although oviposition was significantly delayed on larger carcasses, the initial JH rise was not slower and there were no differences after either 2 or 12 h in the increase in ovarian weight of females burying large or small carcasses. Thus, the cue for oviposition must depend on the status of carcass preparation.

These results are consistent with the hypothesis that JH plays a major role in coordinating the reproductive behaviors of burying beetles. Typically, JH serves as a gonadotropin (Hardie, 1995; Nijhout, 1994), but in burying beetles, application of JH III or its analogue, methoprene, in the absence of a carcass does not result in vitellogenin uptake by oocytes (Scott et al., 2001). In this study, JH did not appear to regulate oviposition either; hormone rise and ovarian development were equally fast on large carcasses even though oviposition was delayed. In most other insects studied, high titers of JH appear to be incompatible with parental care (reviewed in Trumbo, 2002). For example, in earwigs, a topical application of JH shortens the duration of maternal care, but only in intact females; ovariectomized females continue to provide care after JH application, suggesting that any effect of JH may depend on a responsive ovary. JH application also decreases the proportion of eggs hatching suggesting diminished maternal care (Rankin et al., 1995, 1997). Trumbo (2002) also speculated, in the absence of JH data, that high JH may not be incompatible with parental care in the dung beetle, *Cephalodesmius armiger*, which continues to provision some brood balls while ovipositing in new ones (Monteith and Storey, 1981). However, in this case, brood care is the same behavior as preparation for oviposition. In burying beetles, JH titers are highest during the period of parental care. This raises the question of the role of JH (Scott et al., 2001; Trumbo, 1996, 2002). We have proposed that JH coordinates the shifts in behavior and that JH serves as a behavioral pacemaker (Robinson and Vargo, 1997). In this paper, we have investigated the possible environmental and social effects on JH. The effect of larval stimulation on JH suggests that JH may play a role in the regulation of feeding and other parental behaviors.

In mammals and birds with parental care, the endocrine role is more complex in that more hormones appear to be involved. Among birds, ovarian hormones or androgens in males combined with prolactin are the hormonal basis of incubation and parental care. Often high testosterone is incompatible with paternal care (Wingfield et al., 1990). In addition to these hormones, oxytocin plays a role in some

species of mammal in the onset of maternal behavior whereas the maintenance of parental care appears to be based on stimulation from young (Rosenblatt, 1992). It remains to be seen how many hormones help to regulate parental behavior in burying beetles. Other candidates are the insect ovarian hormone, ecdysone, although it has never been implicated in behavioral regulation, and the biogenic amines, octopamine and serotonin, which have both be shown to be affected by social (aggressive) interactions (Adamo et al., 1995; Kravitz, 1988) in other invertebrates.

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