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Changes in biosynthesis and degradation of juvenile hormone during breeding by burying beetles: a reproductive or social role?

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Abstract

Burying beetles, *Nicrophorus orbicollis*, depend on the location of an unpredictable resource, a small vertebrate carcass, for reproduction. When they discover a carcass, they undergo a correlated rapid rise in titers of juvenile hormone (JH) in the hemolymph and ovarian development. This study investigates the regulation of the changes in JH during breeding in both male and female burying beetles and the role of JH in ovarian development. JH biosynthesis by the corpora allata (CA), measured in vitro, increased in females within an hour of their discovery of a carcass and increased later in males. After returning to low rates as oviposition began, JH biosynthesis rose again 3 days later in females but not in males. Neither the ovaries nor testes synthesized JH. There was a concomitant fall in JH esterase activity within 12 h of discovery of the carcass in both males and females. Although the rise in JH titers and biosynthesis and the fall in JH esterase is correlated with ovarian development, application of methoprene or JH III in the absence of a carcass did not result in vitellogenin uptake by the oocytes. Therefore, we conclude that, in spite of the rapid rise in JH before oviposition, it is not sufficient to regulate vitellogenin synthesis and/or its uptake by the ovaries. We suggest that its role has been preempted to organize social behavior and coordinate parental behavior between mates.

Keywords: JH biosynthesis; JH esterase; Vitellogenesis; Ovarian development; *Nicrophorus*

1. Introduction

Reproduction has been shown to be dependent on juvenile hormone (JH) in virtually every insect species studied. Its mechanism and exact role is quite variable, but in most cases JH is the main hormone that regulates the synthesis of vitellogenin by the fat bodies and its uptake by the developing oocytes (Englemann, 1984; Koeppe et al., 1985; Nijhout, 1994; Wyatt and Davey, 1996). One of the few exceptions is found in mosquitoes — opportunistic breeders — for which vitellogenesis is initiated by ecdysone in response to a blood meal. However, JH is necessary to stimulate previtellogenic growth of the primary follicles and to act on the fat body to make it competent to synthesize vitellogenin (Hagedorn, 1985). In some highly social insects, JH may play an entirely non-reproductive role. Whereas JH acts as a gonadotropin in primitively eusocial Hymenoptera, in the highly eusocial honeybee, it has been preempted to regulate age-dependent division of labor among workers (Robinson and Vargo, 1997).

JH titer in the hemolymph is the result of a balance between its synthesis by the corpora allata (CA) and its clearance through degradation, modified by JH binding protein (Hammock, 1985; Roe and Venkatesh, 1990; de Kort and Granger, 1996; Gilbert et al., 2000). JH esterase appears to be an important mode of regulation of JH titers in Coleoptera (Kramer and deKort, 1976), Lepidoptera (Venkatesh et al., 1987) and Diptera (Lassiter et al., 1994). Roe et al. (1997) found that a novel germinal diol was a selective, potent inhibitor of hemolymph JH

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esterase from *Trichoplusia ni*. This inhibitor in vivo eliminated JH esterase activity and initiated egg maturation in adult moths.

Burying beetles, *Nicrophorus orbicollis* Say (Coleoptera: Silphidae) are opportunistic breeders that require the location of an unpredictable resource. JH titers have been shown to rise and fall dramatically during a reproductive bout (Trumbo et al., 1995; Trumbo 1996, 1997). Ovarian development is accompanied by a rise in JH hemolymph titers. In this study we establish the patterns of JH biosynthesis and degradation during a reproductive cycle. We show that, in spite of correlated changes of hemolymph titers, rate of biosynthesis and degradation, JH is not sufficient to regulate synthesis and/or the uptake of vitellogenins by the developing oocytes in this species.

After emerging as adults, burying beetles feed on insects and carrion. Females reach reproductive competence within 12–20 days (Wilson and Knollenberg, 1984; Trumbo et al., 1995) when their ovaries cease developing and reach a resting stage. Mating takes place any time, on or off a carcass, and within a week or so of emerging all females have sperm stored in their spermathecae. Burying beetles utilize small vertebrate carcasses, a rich but unpredictable resource, as a food source for their young. They must out compete many competitors including other burying beetles, carrion-breeding flies and scavengers, and as a consequence have evolved several behavioral and physiological adaptations to exploit carrion quickly. The discovery of a carcass triggers a two-fold increase in JH titers within 10 min and within 24 h there is a two- to three-fold increase in ovarian mass and a three- to four-fold increase in JH titers (Scott and Traniello, 1987; Trumbo et al., 1995). Oviposition typically begins 15–36 h after the discovery of the carcass.

Burying beetles have facultative bi-parental care; the common pattern in *N. orbicollis* is for a single male and female to bury and prepare the carcass, for the male to remain until larvae are 3–4 days old (about 9 days after the discovery of the carcass), and for the female to remain until larval development is complete (about 17 days after discovery of the carcass). If the female dies, deserts or is removed experimentally, the male remains with the brood and compensates behaviorally by increased rates of care (Fetherston et al., 1994) and by a longer duration of care (Trumbo, 1991). Females also can rear broods successfully on their own (Scott and Traniello, 1990; Trumbo, 1991). Both males and females are reproductively competent as soon as they leave the brood chamber.

2. Materials and methods

2.1. Rearing and breeding protocols

Laboratory colonies of *N. orbicollis* were established from adults collected in Durham, NH (JH biosynthesis and ovarian development studies) and Orange Co., NC (JH esterase activity, 1-naphthyl acetate esterase, and hemolymph protein studies). Beetles were reared to the adult stage and breeding was conducted at 20–25°C, 14L:10D (NH) or 15L:9D (NC). Adults were kept in small groups in containers without soil (to inhibit reproductive activity) and were fed mealworms or small (<2 g) pieces of chicken liver or beef kidney. At 24–30 days after eclosion, male–female pairs were provided a thawed mouse carcass (20–25 g) in a container (24×12×9 cm or 19×14×10 cm) three-quarters filled with soil. When they were so placed just at "lights out", they usually began burial immediately. Hemolymph samples for all JH esterase assays were taken on a 12- or 24-h schedule whereas some JH biosynthesis assays were timed by the physiological events of oviposition and hatching. The carcass was added for the biosynthesis study so that both members of the pair would discover it simultaneously and the timing of subsequent events could be precise. Eggs were oviposited within 24 h and could usually be seen on the bottom or sides of the boxes which were checked every 2 h. Similarly, when hatching was expected to be imminent, the carcass was carefully uncovered and monitored every 4 h. Beetles were checked for injuries after breeding. To assay females, post parental care, we removed breeding females from rearing containers after larvae dispersed from the nest and returned the females to small containers without soil.

2.2. JH biosynthesis during breeding

The rate of synthesis of JH by the CA was established by removing both the male and female from the brood chamber at a predesignated time. The CA along with the corpus cardiacum (CC), which is contiguous with the CA and not distinct, was dissected in mosquito saline buffer (138 mM NaCl, 8.4 mM KCl, 4 mM $CaCl_2$, 2 mM $MgCl_2$, 12 mM NaH_2PO_4, 12 mM Na_2HPO_4, 42.5 mM sucrose). Each pair of glands was incubated in 5 μl of modified L-15B tick medium (Munderloh and Kurtti, 1989; Holbrook et al., 1997) lacking non-radiolabeled methionine, with a final concentration of 50 μM [^{3}H-methyl]-methionine (70–85 Ci/mmol, New England Nuclear, Wilmington, DE) and 0.5 μM OTFP (3-octylthio-1,1,1-trifluoropropan-2-one), a JH esterase inhibitor. This medium has been validated and used for short- and long-term culture of the CA for diverse arthropods (Holbrook et al., 1997) including beetles (Tillman et al., 1998). OTFP was prepared as described by Hammock et al. (1982, 1984). We also incubated separately one

ovary and one testis from each of two females and two males, respectively, in 5 μl medium. The CAs of two additional males and females were incubated in medium with 40 μM farnesoic acid as well. Farnesoic acid was prepared by the method of Borovsky et al. (1994). Tissues were incubated on a hexane-rinsed, oven-baked glass coverslip contained in a 24-well tissue culture plate in a water-saturated atmosphere. After 3 h at room temperature, the tissues and medium were extracted with 5×20 μl each of dH_2O and hexane.

Standards were purchased or prepared for comparison with the retention times of products from *Nicrophorus* CA. JH III was obtained from Sigma Chemical and JH I and II from Calbiochem (San Diego, CA). Methyl farnesoate (MF) and JH III bisepoxide (JHB_3) were synthesized according to the methods of Yin et al. (1995). JH III diol was prepared enzymatically according to the methods of Linderman et al. (1995) as well as chemically by the method of Rembold et al. (1980).

Detection of radiolabeled products was done with a Beckman HPLC system with model 125 pumps, model 166 UV detector, and a model 171 radioactive-flow detector. Data from HPLC were detected and analyzed using the Beckman System Gold software. Samples were chromatographed on an Econosphere C_{18} reverse-phase HPLC column (4.6 mm×25 cm, 5 mm particle size) using a linear gradient program of 40–100% acetonitrile. The run conditions were as follows: run length 50 min, solvent flow rate 1 ml/min, mobile phase of 40% acetronitrile: 60% 5 mM HEPES pH 7.5 to 100% acetronitrile over 45 min with a 5 min hold, scintillation fluid (Beckman Ready-Flow III) flow of 3.0 ml/min.

2.3. JH esterase activity

To collect hemolymph, we applied micropipettes to the severed hind legs of adults. Slight pressure on the thorax increased the flow. At least 4 μl of hemolymph was collected from each beetle, allowing for assays of individuals. The hemolymph of known volume was transferred to culture tubes containing 200 μl of sodium phosphate buffer (I=0.2 M, pH 7.4, 0.01% phenylthiourea, 10% sucrose). Hemolymph was vortexed vigorously and stored at −80°C until used for analysis. JH esterase activity was assayed by the partition method of Hammock and Roe (1985) using JH III as substrate. 1-Naphthyl acetate esterase activity was assayed according to Sparks et al. (1979). The final substrate concentration was 5×10^{-6} M for the JH esterase and 2.2×10^{-4} M for the 1-naphthyl acetate esterase assay. Incubation times for the assays were 15 min; hydrolysis rates were linear over the first 4 h of incubation and crossed the origin. The protein concentration of hemolymph was determined according to the Bio-Rad protein assay (Bio-Rad Laboratories 1977) using bovine serum albumin (Fraction V, Sigma) as the standard.

2.4. Ovarian development

In order to investigate if JH promotes ovarian development, we treated 21–28 day-old virgin females with 4 μl acetone (controls) or with the JH analogue, racemic methoprene (200, 600 and 1000 μg/g beetle) (Dr David Cerf, Sandoz Corporation, Palo Alto, CA) or with racemic JH III (200 μg/g beetle) (Sigma-Aldrich) applied topically to the ventral cervical membrane in <4 μl acetone. Similarly, 7 week-old females that had been mated 4 weeks previously and had reared a brood were treated with acetone or 1000 μg methoprene/g beetle. The high doses of methoprene and JH III were warranted by the naturally occurring very high JH titers measured. Twenty-two hours later we sacrificed the beetles and took the wet weight of both ovaries. Ovarian weights were compared to those of untreated females 17 h after being given a mate and a carcass. We allowed only 17 h for the ovarian development of untreated females because we wanted to be sure they had not yet begun to oviposit when we sacrificed them.

3. Results

3.1. JH biosynthesis

Typical radiochromatograms of products synthesized from [^{3}H-methyl] methionine by the CA from male and female beetles are shown in Fig. 1. Positive controls for the beetle assays were fourth stadium cockroaches, *Diploptera punctata* (Eschscholtz) or *Gromphadorina portentosa* Schaum (supplied by G.L. Holbrook, North Carolina State University, Raleigh). The major product produced by the CA of the beetles co-chromatographed (in separate runs) exactly with JH III from the cockroaches and with authentic JH III standard. JH III was found to be the only JH in the hemolymph of *N. orbicollis* by Trumbo et al. (1995). JH III diol and methyl farnesoate were present in smaller amounts in some samples. No radio-products were produced that co-chromatographed in separate runs with JH I, JH II or JH III bisepoxide. A few samples, however, had additional peaks that did not correspond to our JH standards or JH metabolites. The CAs of two males and two females, removed just before oviposition and incubated with farnesoic acid and [^{3}H-methyl] methionine, had high rates of synthesis of JH III (males: 4.01 and 6.31 pmol gland-pair^{-1} h^{-1}; females: 15.09 and 17.77 pmol gland-pair^{-1} h^{-1}).

The rates of JH synthesis of males and females at six different times during a breeding cycle are shown in Fig. 2a. JH biosynthesis rapidly increased with the discovery of a carcass and then fell rapidly before oviposition. The rise and the fall in JH biosynthesis in females seemed to precede those in her mate (Wilcoxon's signed-ranks

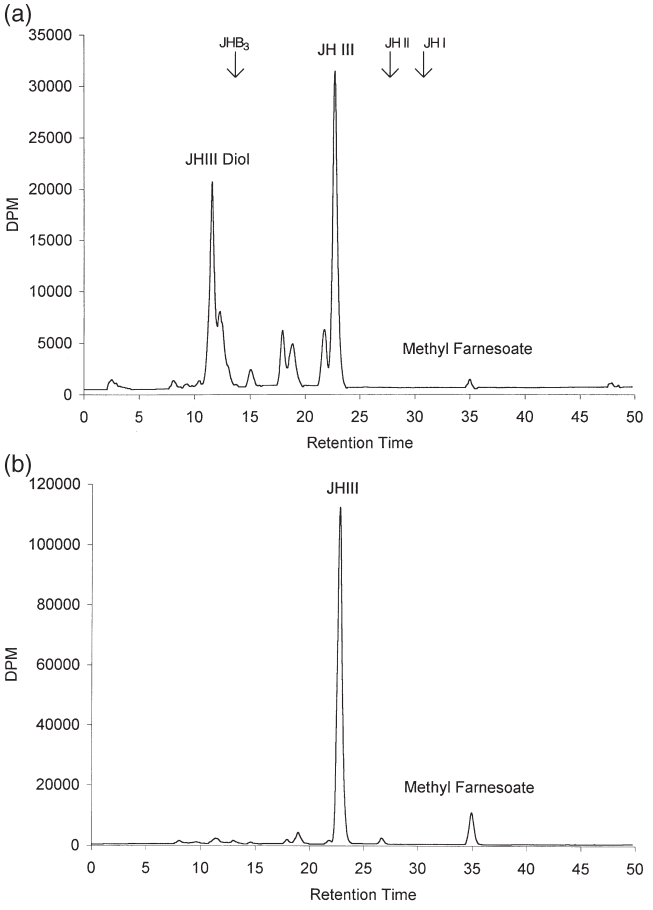

Fig. 1. Chromatographs of products of CA of a male (a) and female (b) *N. orbicollis* with high rates of biosynthesis.

tests at 1 h and just before oviposition: N=5 paired observations, P=0.06; N=8 paired observations, P=0.02, respectively). There was considerable variation and neither profile demonstrated statistically significant differences in synthesis rates (ANOVA for males P=0.12, females P=0.14; the power for α=0.05 of the ANOVAs was estimated to be 0.15 and 0.25, respectively, Zar, 1984). Planned comparisons showed that the increase in synthesis from pre-breeding to just before oviposition was not statistically significant in males (P=0.07) or females (P=0.64); nor was the increase in biosynthesis by females from pre-breeding to the appearance of larvae (P=0.09). However, the apparent increases in JH III biosynthesis (Fig. 2a) were positively correlated with increases in the hemolymph JH III titers (Fig. 2b).

Neither the ovary nor testis (N=2 each) incubated without farnesoic acid synthesized JH (data not shown).

3.2. JH esterase activity

JH esterase activity was reciprocally related to JH biosynthesis and hemolymph titers over the reproductive cycle for both females and males (Fig. 2c). At 12 h after the onset of a reproductive opportunity (presentation of

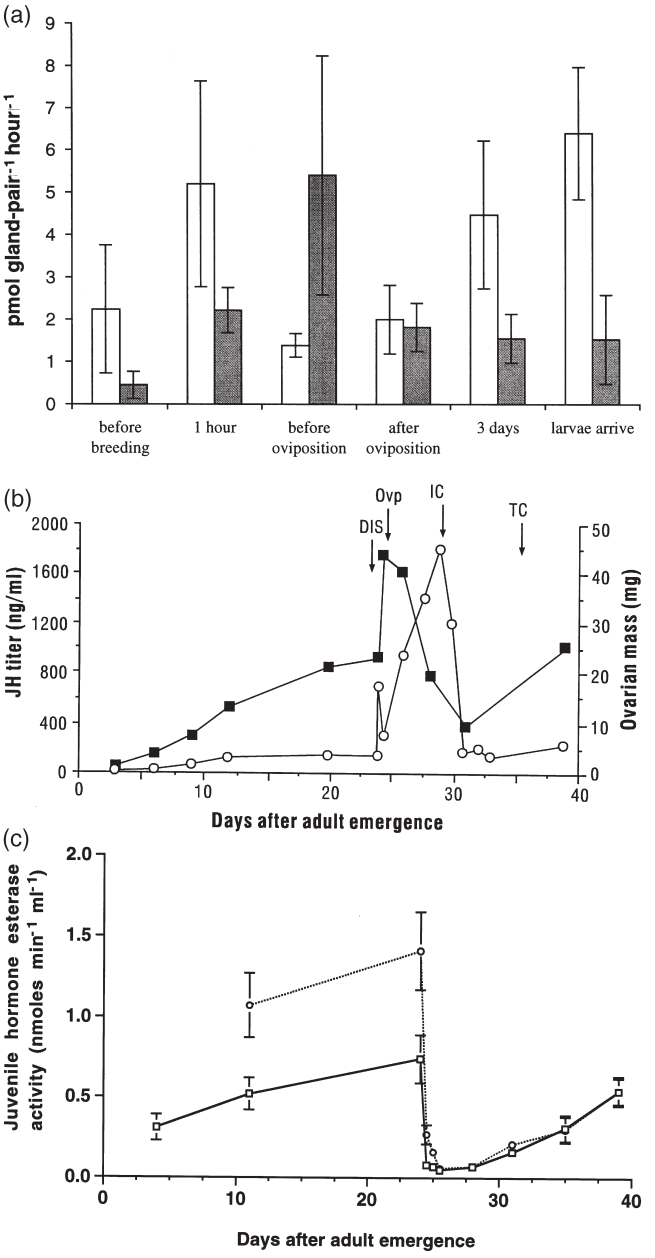

Fig. 2. JH hemolymph titers, biosynthesis rates and degradation in *N. orbicollis*. (a) Mean (±SE) rate of JH III biosynthesis in males (gray) and females (white) at different periods of the breeding cycle: before breeding, 1 h after discovery of the carcass, just before or just after oviposition had begun (15–18 h after discovery), 3 days after discovery of the carcass, and within 2 h of the appearance of larvae on the carcass (about the 6th day after the discovery of the carcass). Sample sizes were five pairs of males and females removed at 1 h, six pairs removed with new larvae and 8–11 beetles for each of the remaining data points. (b) Hemolymph titers of JH III (open circles) and ovarian mass (closed squares) in females during the reproductive cycle. DIS=discovery of the carcass; Ovp=oviposition; IC=initiate care of larvae; TC=terminate care. (modified from Trumbo et al., 1995; Trumbo, 1997.) (c) Mean (±SE) JH esterase activity during the adult life cycle of females (unbroken line) and males (dashed line). Hemolymph was analyzed from 7–10 females or 3–5 males for each time point (non-pooled). The carcass was presented on day 24.

the carcass) JH esterase activity had dropped to less than 1/10th of the pre-reproductive level. This coincides with a rapid increase in JH titer in females and males after carcass discovery and a rapid increase in ovarian mass (Fig. 2b). The change in hemolymph JH metabolic activity appears to result from changes in JH specific esterases, as 1-naphthyl acetate esterase activity follows a different pattern during the reproductive/parental cycle (Fig. 3a). Hemolymph protein levels fell after presentation of a carcass to half their prebreeding level in females and to a fifth of the prebreeding level in males (Fig. 3b). They rose to prebreeding levels gradually during the reproductive bout in both males and females.

3.3. Ovarian development

Since ovarian development is triggered by the behaviors of assessing the carcass (Scott and Traniello, 1987) and the discovery of the carcass is closely followed by a rapid rise in JH hemolymph titers (Trumbo et al., 1995), we originally hypothesized that JH is directly responsible for ovarian development. However, JH alone appears insufficient to support ovarian development. Neither the JH analogue, methoprene, nor JH III itself brought about ovarian development in mature virgin females within 24 h, whereas breeding females with a carcass showed more than a three-fold increase in ovarian weight from prebreeding levels, as represented by acetone-treated females (Fig. 4). Similarly, the ovarian weight of previously mated and bred females treated with 1000 μg methoprene/g beetle (N=6) was not significantly different from those treated with acetone only (N=6) (mean±SE=25.0±1.6 vs 29.9±2.2 mg, respectively, t=0.10, P=0.92) but was significantly less than that of females given a carcass (t=5.20, P=0.001).

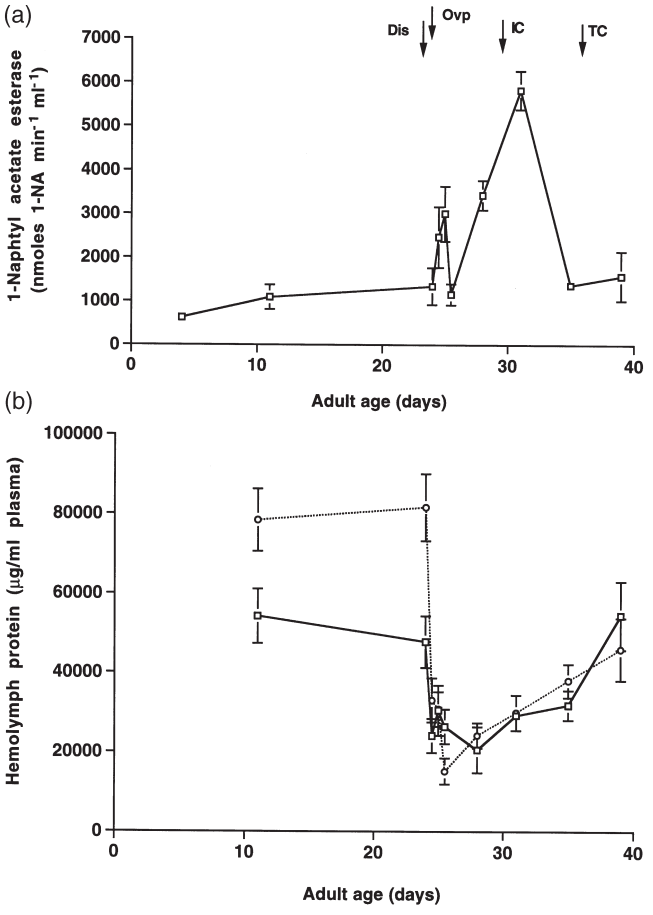

Fig. 3. 1-Naphthyl acetate esterase activity and hemolymph protein. (a) Mean (±SE) 1-naphthyl acetate esterase activity during the adult life cycle of *N. orbicollis* females. Hemolymph was analyzed from 3–4 individuals for each time point (non-pooled). (b) Mean (±SE) hemolymph protein in females (unbroken line) and males (dashed line). Hemolymph was analyzed from 3–5 individuals for each time point (not pooled).

4. Discussion

Evidence that burying beetles are synthesizing JH III is provided by the co-chromatography in separate analyses with racemic JH III (non-radiolabeled), with C10-^{3}H-racemic JH III and with naturally occurring 10R-JH III produced by the two cockroach species. The cockroach JH III produced from radiolabeled methionine provided the most direct proof by co-chromatography that the beetle product, by the same biosynthesis method, was JH III. The supplementation of the medium with farnesoic acid increased beetle JH III radiosynthesis, as we would expect for a product derived from farnesoic acid. We also found the JH III precursor, methyl farnesoate. No JH I, JH II or JH III bisepoxide was produced by the beetle CA. The metabolite, JH III diol, was produced. This was not surprising since no epoxide hydrolase inhibitors were added to the incubation medium. These results, however, indicate the presence of JH epoxide hydrolase in the dissected tissues (the CC–CA complex).

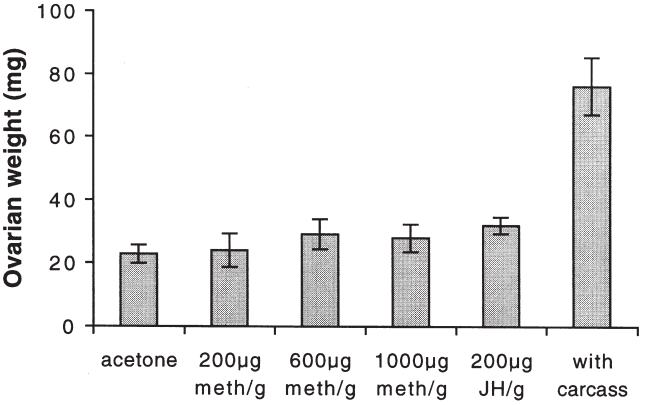

Fig. 4. Mean (±SE) of wet weight of ovaries of female *N. orbicollis*, 22 h after treatment with acetone, methoprene or JH III and of untreated females 17 h after being given a carcass (just before oviposition would begin). Sample sizes are 5–6 beetles per treatment. ANOVA: $P<0.0001$.

The role of this epoxide hydrolase activity in JH regulation is unclear. Although the CA is the principal site of JH biosynthesis, the mosquito ovary makes JH III de novo (Borovsky et al., 1994). However, neither the ovaries nor testes of the examined burying beetles produced JH.

Changes in JH biosynthesis and degradation were correlated with hemolymph JH titers for burying beetles over a reproductive cycle. As females became reproductively competent after emergence and before presentation of a carcass, ovarian weight increased while JH III was being synthesized, hemolymph JH esterase was high and hemolymph JH concentration was low. These results suggest that JH is not important for the maturation of the ovary to resting stage. The rapid increase of hemolymph titers triggered by the discovery of the carcass is regulated in females by an equally rapid increase in JH biosynthesis and decrease in JH esterase activity. The first increase in the rate of biosynthesis appeared to last less than 18 h in females and there was a longer-lasting increase during the 3 days preceding the arrival of larvae on the carcass. The increase in biosynthesis by males that precedes oviposition by his mate is as great as that of females and may be triggered by the discovery of the carcass — as is the case for females — and may also be socially facilitated; both the rise and fall of JH biosynthesis seem to follow those of his mate.

The rate of JH biosynthesis appears to be quite variable, especially for non-breeding females (range from 0–16 pmol gland-pair^{-1} h^{-1}). JH hemolymph titers are also variable in nonbreeding females and depend partly on age and nutritional status (Trumbo, unpublished data). They were also variable among females during breeding. One possible source of error in JH biosynthesis measurements could have resulted from damage to the CC–CA complex during removal or to its incomplete removal. We do not believe that this is a significant problem as the glands are generally easy to excise. Furthermore, damage to the CA would result in low biosynthesis rates whereas the high variation we observed was due to very high production by just a few individuals. Taub-Montemayor et al. (1997) also reported considerable variation in JH biosynthesis in the boll weevil, most likely from a few very active CAs. Alternatively, the peak rate of JH biosynthesis could be fairly brief for all burying beetle females and the variability in measured rates could reflect variability in timing.

Changes in 1-naphthyl acetate esterase activity during a reproductive bout were quite different from those of JH esterase activity. This suggests that burying beetles are specifically regulating the hemolymph JH esterase activity independently of other esterases and that JH esterase is most likely a JH-specific esterase. More detailed studies of JH esterase kinetics and structure–activity relationships for this enzyme would be needed to completely validate this hypothesis. However, the assumption is reasonable considering that reductions in JH esterase activity in the burying beetle were associated with increased JH biosynthesis and increased levels of JH. It was interesting that changes in JH esterase activity were positively correlated with changes in overall hemolymph protein concentration, suggesting that JH esterase activity and protein biosynthesis in general may be regulated by the same mechanism. This positive correlation also may not be significant considering that JH esterase is typically a minor hemolymph protein.

The major hemolymph protein in females was most likely vitellogenin; there was a 2.5-fold increase in ovarian mass in the 12 h following carcass presentation. During the time when the female ovarian mass was increasing, hemolymph protein concentration dropped in both females and males. While this was expected in females as a result of vitellogenin uptake by eggs, this change in hemolymph protein concentration in males is puzzling. However, both males and females produce oral (proteolytic) and anal secretions for preparation of the carcass.

The regulation of JH titers in the hemolymph in some insects during reproduction is determined by the rate of JH biosynthesis (Hammock, 1985; Roe and Venkatesh, 1990; de Kort and Granger, 1996; Gilbert et al., 2000). This mechanism is inferred to be more important than degradation when patterns of JH esterase activity are similar between breeding and non-breeding adults and over a reproductive cycle as is commonly the case, e.g. in the boll weevil (Taub-Montemayor et al., 1997) and in three species of locusts (Okuda et al., 1996). However, in the cockroach, *Diploptera punctata*, JH esterase activity correlates negatively with JH biosynthesis, falls with vitellogenesis and rises with pregnancy (Rotin et al., 1982). Similarly JH esterase activity differs in breeding and non-breeding adults and correlates negatively with JH titers in the Colorado potato beetle, *Leptinotarsa decemlineata* (Kramer and deKort, 1976; Kramer, 1978a). This suggests that JH esterase activity has an important role in JH regulation at least during some periods of the adult reproductive cycle in some species. Apparently, JH esterase regulates JH hemolymph titers in both males and females of *N. orbicollis* during a reproductive bout.

The stimulatory effect of JH on vitellogenin uptake by the developing ovary is very well documented in many species (reviewed by Koeppe et al., 1985; Wyatt and Davey, 1996). For example, ovarian development is positively correlated with JH biosynthesis rates and JH hemolymph titers in both the Colorado potato beetle (Kramer, 1978a,b; Khan et al., 1982) and the boll weevil (Taub-Montemayor et al., 1997). Additional evidence comes from studies of allatectomized adults and subsequent application of JH or its analog. In the Colorado potato beetle, treatment of JH or its analog induced vitellogenin synthesis; removal of both the CA and the CC

was necessary to prevent vitellogenin synthesis and ovarian development in breeding adults (de Loof and de Wilde, 1970). Exceptions are rare. Vitellogenin uptake is independent of JH in the Diptera (Hagedorn, 1985). JH appears to be unnecessary for vitellogenin synthesis and uptake but may facilitate the latter in the stick insect, *Carausius morosus* (Bradley et al., 1995). In spite of showing a positive correlation between JH biosynthesis and ovarian development, methoprene treatment failed to induce ovarian development in non-breeding females of two species of locusts, *Nomadacris japonica* and *N. succincta* (Okuda et al., 1996).

JH may also not directly regulate egg development in the burying beetle. The rapid increase in JH biosynthesis and JH titers and its positive correlation with ovarian development and fall at oviposition suggested a regulatory role. However, we could not induce ovarian development in the absence of a carcass with methoprene or JH III. These results suggest an alternative function for JH in reproduction.

The hypothesis for a gonadotropic role of JH in burying beetles is also undermined by the data from males. The patterns of JH biosynthesis, hemolymph titer and JH esterase activity are very similar for males and females, at least until several days after oviposition. Males would have no need for changes in JH to regulate the physiological event of a reproductive bout but the rise and fall of biosynthesis appears to follow those of their mate. Just as JH has been preempted to serve as a behavioral pacemaker in the highly social honeybee (Robinson and Vargo, 1997), the changing titers of JH in burying beetles may play an important role in providing social cues, most likely through its effects on cuticular hydrocarbons (Singer, 1998). Males and females must recognize their mate to cooperate in nest defense (Scott, 1990; Trumbo, 1991). Reproductive status, signalled by matching JH titers, is the cue used for individual recognition of a mate (Madjid and Scott, unpublished data). If endocrine states do not match (e.g. if one member of a pair is treated with methoprene and both are placed in a box with or without a carcass) injuries and even death are common (Scott, unpublished data). Males perform all the same parental behaviors as females (Fetherston et al., 1990) and many of these behaviors must change during the course of a reproductive bout; aggression towards strangers increases then wanes, larvae are either killed or fed depending on the timing of their appearance (Müller and Eggert, 1990; Scott, unpublished data). Furthermore, male and female must coordinate their behavior with those of their mate and the changing JH titers may mediate synchronization.

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