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Quantification of vitellogenin–mRNA during maturation and breeding of a burying beetle

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Abstract

Burying beetles (*Nicrophorus orbicollis*) are unusual in that to breed they require an unpredictable and valuable resource, a small carcass. Thus the timing of reproduction is unpredictable and beetles' physiological response must be fast. We hypothesized that their pattern of vitellogenin (Vg) synthesis might reflect these requirements. We examined the expression of two Vg genes (sequenced for this study) during sexual maturation and through a reproductive bout. Vg–mRNA, juvenile hormone (JH) titers, ovarian development, and hemolymph concentrations of Vg were quantified in the same individuals. All four variables gradually increased during maturation to peak 15–20 days after eclosion. Twelve hours after the discovery of a carcass, a few hours before oviposition, mRNA was high, hemolymph Vg had decreased, JH and ovarian weight had increased. After oviposition, mRNA was low, hemolymph Vg concentrations and JH were high. This is consistent with our hypothesis that beetles produce and store Vg in the hemolymph prior to the discovery of a breeding resource and replace it quickly. Partial regression of these variables (with the effect of time removed) indicated that JH was not correlated with mRNA, hemolymph Vg, or ovarian weight at any time. Thus the role of JH as a gonadotropin remains unclear.
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Keywords: *Nicrophorus orbicollis*; Burying beetle; Vitellogenin genes; Vitellogenin mRNA; Juvenile hormone

1. Introduction

Reproductive behavior of insects is extremely varied. While some emerge as adults and do not require environmental or social cues to breed, others undergo regular or opportunistic reproduction that depends on the location of specific resources. In some species, females have short or even long periods of parental care in their reproductive cycles (Trumbo, 1996, 2002). The hormonal control of reproductive physiology of females has been studied in dozens of species but few general rules have emerged (Nijhout, 1994; Wyatt and Davey, 1996; Bellés, 1998). In long-lived insects with multiple reproductive bouts, juvenile hormone (JH) is the common, but not universal, gonadotropin regulating the synthesis of vitellogenin (Vg), egg maturation and oviposition (Koeppe et al., 1985; Wyatt and Davey, 1996; Gilbert et al., 2000). However species differ in which aspect of reproduction is regulated. 20-hydroxyecdysone along with various neuroendocrine hormones have also been shown to play gonadotropic roles. In most species, Vg synthesis is promoted by JH. A notable exception are the Diptera, in which Vg synthesis is stimulated by ecdysteroids, although previous exposure to JH is required.

Burying beetles (Silphidae: *Nicrophorus*) undergo cyclical reproduction cued by a complex set of factors. To breed they require a small vertebrate carcass (typically 10–60 g), a valuable but unpredictable resource. A male and female cooperate to bury and prepare it. They are unusual in a having a hormonal surge (JH) triggered by behavioral cues associated with the discovery and assessment of the carcass (Trumbo et al., 1995). Neither a mate without a carcass, nor the

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nutritional content of the carcass is sufficient for ovarian development (Scott and Traniello, 1987). JH hemolymph titers drop about the time of oviposition (Trumbo, 1997) and rise again to peak when larvae hatch and parental care is most intense. Three days later JH titers fall to pre-breeding levels. Both parents feed and defend the young although typically, females remain longer with the brood than males (Scott and Traniello, 1990). In spite of these dramatic changes in JH hemolymph titers that are correlated with reproductive events, the application of JH III or its analogue, methoprene, is not sufficient to cause Vg up-take by the ovaries (Scott et al., 2001). This raises the question of the role of JH in the reproductive physiology of burying beetles.

This study is a first step in addressing this question. Here we examine the transcription of Vg–mRNA by qPCR, and look for correlations with hemolymph titers of JH, ovarian development and hemolymph concentrations of Vg which we measure in the same individuals over the 20 days after eclosion that is required for sexual maturation and during a breeding bout. To do this we first identified two vitellogenin genes in burying beetles by sequencing a portion from the conserved GL/ICG region to the C-terminus (Lee et al., 2000).

2. Materials and methods

2.1. Animals and breeding manipulations

All beetles were laboratory-reared from a colony derived from *Nicrophorus 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 20C and 14:10 L:D. To breed, a male and female were placed in a plastic box ($19 \times 14 \times 10$ cm) of autoclaved soil with a previously frozen mouse (laboratory culls from the Dana Farber Cancer Institute and the Forsyth Institute, Boston, MA). On the day that females eclosed and every five days thereafter, three females were bled (two samples of hemolymph were taken, one for SDS PAGE analysis for Vg concentration and one for JH RIA), fat body was removed and their ovaries dissected and the wet weight taken. Females from another cohort were given a mate and a carcass to breed when they were about 35 days post-eclosion. Three females were removed at each of 12 h, 3, 5 and 8 days after discovery of a carcass and tissue and hemolymph samples taken as described above.

2.2. Identification of vitellogenin genes

The fat bodies of four never-mated females five weeks post-eclosion were dissected in saline made from RNase free water. Two fat bodies were placed in 0.5 ml Trizol (Invitrogen). These were sheered by drawing them through a 22 gauge syringe several times and another 0.5 ml Trizol was added. Total RNA was extracted and quantified by spectral absorption at 260/280 nm. Reverse transcription of 1 μl total RNA was primed with 50 pmol poly-T primer using Superscript II (Invitrogen) and incubated at room temperature for 10 min and then 42 °C for 50 min.

Amplification of 3′ cDNA ends (Frohman et al., 1988; Ohara et al., 1989) was performed with forward primers (Qiagen) suggested by Lee et al. (2000). These primers contain an EcoR1 cloning site. The primer nucleotide sequences (and their corresponding amino sequences) were:

1. GCGCGGAATTCGGNYTNTGYGG (GLCG)
2. GCGCGGAATTCGGNATHTGYGG (GICG).

The reverse primers were poly-Tamp (GCGAATTCGTCGACAAGGCT$_{17}$) and Tamp (GCGAATTCGTCGACAAGGC). The reaction was done with 1 μl cDNA in a final concentration of 1 × Taq buffer, 1 μM primer, 0.06 μM poly-Tamp, 2.6 μM Tamp, 0.2 mM dNTP, and 1 μl Taq in a final volume of 50 μl. PCR was run for 50 cycles of 94 °C for 30 s, 52 °C for 45 s, and 72 °C for 1 min 30 s. The poly-Tamp and Tamp were added after the first 10 cycles (Frohman et al., 1988). Primer 1 yielded the best results with a 650 bp fragment. Products were run on a 1% SeaPlaque gel, cut out, purified with a QIAquick gel extraction kit and resuspended in 30 μl buffer. DNA was quantified with a spectrofluometer.

DNA was cloned into pGEM-T vector (Promega) and transformed into DH10B bacteria and plated. Twenty-four colonies were picked and tested for insert size by PCR using vector primers. Ten of the resulting PCR products were directly sequenced using DYEnamic Terminator ET chemistry (Amersham) and run on an ABI 377.

To improve the resolution of the sequences at the 3′end, internal primers were designed 125 bp from the end (GCCCGTCGAAGTCCACTGT and CATCAAATTCCATTGCGTTCCA). Sequences were aligned using Sequencher. The deduced amino acid sequence (177 aa) was aligned with other insect vitellogenin genes using ClustalW. A distance tree was constructed using PAUP 4.0b10 (Swofford, 2003) and tested for significance with 1000 bootstrap replicates.

2.3. Measurement of vitellogenin mRNA by qPCR

Primers and probes (Qiagen) for Taqman qPCR were designed for each Vg gene using Primer Express (ABI). Vg1: Forward primer: TTGAGCGCAAGGGACACA; Reverse primer: TTGGCTGTAGCATGGAGACTTC; Probe: CCAAGAATTGCATTCCCGTGCCC Vg2:

Forward primer: GACGATTTCCTCACCCCAAA; Reverse primer: GGCGTACGTAGCAGCAAATT; Probe: AACTGCATCCTACGCAACCCACACG. Probes were 5′ labeled with 6′FAM and 3′ labeled with TAMRA.

Hemolymph was taken and fat body dissected from females (three each sample point) on designated days during sexual maturation and during a breeding bout: the day they eclosed, 5, 10, 15 and 20 days after (when they are sexually mature), and at 12 h after the discovery of a carcass, and 3, 5, and 8 days after discovery. Total RNA was isolated and preliminarily quantified by spectral absorption as described above. A portion (about 4 μg) was treated with RNase-free DNase (RQ1Promega) in a final volume of 10 μl. Total RNA was then precisely quantified with RiboGreen (Molecular Probes) by a CytoFluor 2300 (Millipore). Reverse transcription of exactly 1 μg total RNA was performed as described above. Rather than use a "housekeeping" gene to compare and correct for sample to sample variation, we chose to relate all copy numbers to total RNA concentration (Bustin, 2000, 2002), and therefore measured it accurately. RiboGreen can detect as little as 5 ng/ml RNA.

qPCR reactions were performed for each Vg gene with 1 μl cDNA, 3 pmol each primer and 2 pmol probe with Taqman mix in 30 μl. PCR parameters were 40 cycles of 95 °C for 15 s, 55 °C for 30 s, 65 °C for 60 s. The qPCR threshold was set at 0.05. Each cDNA was run in three different reactions and the average used for analysis. The PCR efficiencies for qPCR were calculated from making a thousand-fold dilution series and plotting ln(T) vs. C_t where T is template concentration and C_t is the number of cycles to reach the threshold. R^2 for each gene were 0.99 and 0.98. Efficiency (E) for Vg1 was 100% and for Vg2 was 87%. The relative amount of Vg mRNA (per μg of total RNA) was calculated as

$$Vg_i = \frac{1}{(1+E_i)^{C_{ti}}},$$

where C_{ti} and E_i are the critical threshold and efficiency for Vg gene i. mRNA of pre-breeding females and that of females during a breeding cycle were analyzed separately because they were from different cohorts of beetles. Statistical tests were single-factor ANOVA.

2.4. Radioimmunoassay for juvenile hormone

Hemolymph (5–10 μl, usually 10 μl) was taken by puncturing the cuticle beneath the first pair of legs with a calibrated micro-capillary tube and placed in 0.5 ml chilled acetonitrile. Extraction and RIA followed the methods of Trumbo et al. (1995), Scott and Panaitof (2004), and Panaitof et al. (2004) using the chiral-specific antiserum to 10R-JH III, the naturally occurring enantiomer (Hunnicutt et al., 1989). JH concentrations in the hemolymph were calculated from the standard curve, the volume of hemolymph taken, the extraction efficiency, its concentration in MeOH and multiplied by 0.5 since the standards were of racemic JH and the antibody is chiral specific. Data were log-transformed for statistical analysis. Statistical tests were single-factor ANOVA.

2.5. SDS PAGE assay for hemolymph concentrations of vitellogenin

Samples of hemolymph and egg homogenate were subjected to SDS PAGE to quantify relative Vg. Ten microliter of hemolymph were taken and an equal volume of 2 × SDS mercaptoethanol sample buffer was added. Samples were boiled then frozen in liquid nitrogen. After thawing, samples were solubilized in 4 M urea. Five microliter of a 1:20 dilution of each sample were electrophoresed on a 4–15% gradient gel (Criterion). High molecular weight standards for SDS PAGE (Bio-Rad) were run alongside the test sample. Electrophoresis was performed at constant voltage (200 V) for 50 min. Following the electrophoresis, the gel was stained with Coomassie blue to detect the proteins. Two bands, of 192 and 38 kDa, were present in eggs and female hemolymph but not in male hemolymph. This is similar in size to Vg in the Colorado potato beetle (199 and 162 kDa, Koopmanschap et al., 1992) and the boll weevil (160 and 47 kDa, Heilmann et al., 1993). Gels (Fig. 1) were scanned and differences in Vg concentration in the hemolymph were quantified by QuantiScan 3.0 (Biosoft). The areas under the peak (above the background) of the 38 kDa band were compared giving us relative concentrations of Vg in the 10 μl hemolymph. Analyses of the two bands were very similar; we chose the smaller subunit to report because, being smaller, the band was slightly less intense, less likely to have saturated and results were more linear.

2.6. Statistical analyses of relationships between variables of individuals

Because we measured Vg mRNA, JH, ovarian development and hemolymph concentration of Vg in the same individuals we could examine the relationship of these variables with the effect of time (age or breeding stage) statistically removed. Because all change with time, we analyzed variables with partial correlations. We therefore calculated the residuals of each variable regressed on time and looked for correlations among the residuals (Systat 10.0). This enabled us to test if the individual with the highest x of any one sampling period also had the highest y.

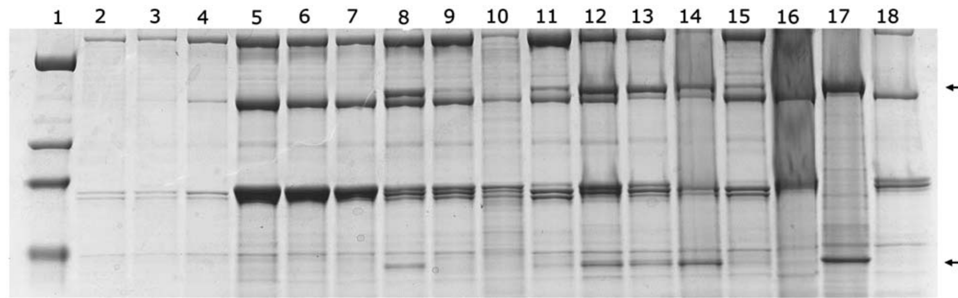

Fig. 1. SDS PAGE gel of female hemolymph during sexual maturation. Lanes are: 1 standard, 2–4 the day females eclosed, 5–7, the 5th day after eclosion, 8–10 the 10th day after eclosion, 11–13 the 15th day after eclosion, 14–16 the 20th day after eclosion, 17 egg (positive control), 18 male (negative control). Arrows point to proteins of 192 and 38 kDa thought to be the two subunits of Vg. Densitometry quantified the protein of the smaller subunit.

3. Results

3.1. Identification of vitellogenin genes

Based on the sequences from the conserved GL/ICG motif to the C-terminus, we identified two vitellogenin genes in *N. orbicollis* (AY728384, AY728385), six clones were of Vg1 and 4 clones were of Vg2. The deduced amino acid sequences were 59.3% similar. Both fragments were about 531 bp. The phylogenetic distance tree constructed by aligning *Nicrophorus* aa sequences with those of other insects (Fig. 2) is in general agreement with similar studies (Chen et al., 1997; Nose et al., 1997; Lee et al., 2000; Piulachs et al., 2003). We constructed the distance tree in order to examine the similarity of the two *Nicrophorus* genes relative to the similarity of multiple Vg genes reported for other species. The three Vg genes of *Plautia stali*, the two Vg genes of *Periplaneta americana* and the two genes of *N. orbicollis* are all monophyletic which strongly suggests these insects have repeatedly duplicated this gene through separate duplication events. The distance between the two *N. orbicollis* Vg genes was about the same as among the three Vg genes of *P. stali* and less than the distance between the two Vg genes of *P. americana*.

3.2. Measurement of vitellogenin mRNA

There was low variation in our triplicate qPCR measures for each individual. The average coefficient of variation for Vg1 was 16% and for Vg2 was 14%. There was a significant correlation of expressed mRNA between Vg1 and Vg2 for pre-breeding females ($y = 0.4617x + 6^{e-9}$, $r = 0.94$, $p < 0.001$), females during a breeding cycle ($y = 0.3425x + 6^{e-7}$, $r = 0.83$ $p < 0.01$), and overall ($y = 0.4219x + 2^{e-7}$, $r = 0.91$, $p < 0.001$) (Fig. 3a and b) but on average, there was 2.4 times as much mRNA/μg total RNA of Vg2 as of Vg1. There were significant differences in mRNA expression over time of Vg1 (Fig. 4a) and Vg2 (Fig. 4b) pre-breeding ($p = 0.001$ and 0.005, respectively) and during a breeding bout ($p = 0.03$ and 0.005, respectively) but there was also considerable variation among the three individuals at each sampling period, especially later during sexual maturation (15 and 20 days post-eclosion). mRNA of both Vg1 and Vg2 were low in all females for the first five days after eclosion. Expression was high 15–20 days after eclosion when they have become sexually mature. At this time eggs are at "resting stage" and Vg uptake must occur rapidly upon discovery of a breeding resource. Expression of both genes was also high at 12 h after the discovery, just before oviposition, and was then lower at 3, 5, and 8 days after discovery.

3.3. The relationship of mRNA to JH hemolymph titers, ovarian development, and relative vitellogenin concentrations in the hemolymph

JH hemolymph titers, ovarian weight and hemolymph concentrations of Vg were measured in the same individuals as those for which we measured the mRNA expression of the two Vg genes. There was a gradual increase in JH (Fig. 5a) and ovarian weight (Fig. 5b) during sexual maturation as previously described (Trumbo et al., 1995). Also in agreement with previous studies, JH increased sharply on the 5th day of a breeding bout (Trumbo, 1997; Panaitof et al., 2004) and ovarian weight increased after the discovery of the breeding resource and decreased after oviposition (Wilson and Knollenberg, 1984; Scott and Traniello, 1987; Trumbo et al., 1995; Trumbo, 1997). Hemolymph concentration of Vg (Fig. 5c) was almost below the level of detection at eclosion and rose steadily during sexual maturation; concentrations were lower when Vg was being incorporated into the eggs just prior to oviposition and rose again soon after. This pattern was expected as females must be ready to oviposit a second clutch almost immediately if the first fails. However, due to the small sample sizes and large variation, these differences were not statistically significant.

When the effect of time was statistically removed, few significant relationships were uncovered by the partial

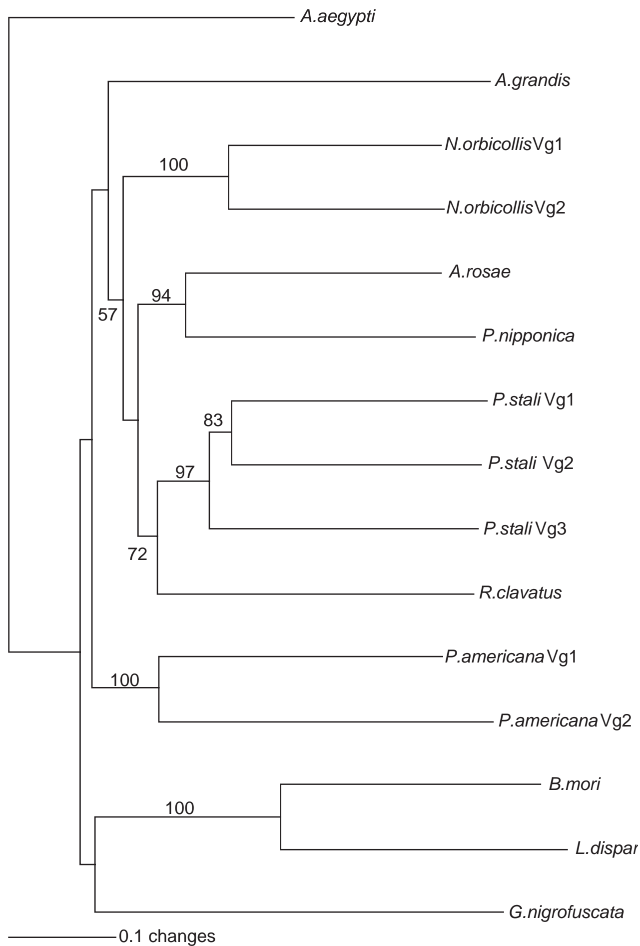

Fig. 2. A molecular phylogenetic distance tree based on the sequence from the conserved GL/ICG to the C-terminus of known insect vitellogenin genes constructed with 1000 bootstrap replicates using PAUP. The scale indicates distance (% changes) and the number at various nodes indicate the percent of bootstrap replicates that are consistent with these groupings. Species (and database accessions numbers) are: mosquito, *Aedes aegypti* (L41842) (Diptera); boll weevil, *Anthonomus grandis* (M72980) (Coleoptera); burying beetle *Nicrophorus orbicollis* (AY728384, AY728385) (Coleoptera); sawfly, *Athalia rosae* (AB007850) (Hymenoptera); wasp, *Pimpla nipponica* (AF026789) (Hymenoptera); bean bug, *Plautia stali* (AB033498, AB033499 and AB033500) (Heteroptera); bean bug *Riptortus clavatus* (U97277) (Heteroptera); cockroach, *Periplaneta americana* (AB034804, AB047401) (Dictyoptera); silk moth, *Bombus mori* (D13160) (Lepidoptera); gypsy moth, *Lymantria dispar* (U60186) (Lepidoptera); and cicada, *Graptopsaltria nigrofuscata* (AB026848) (Homoptera).

correlations (Table 1), however, because of the small sample size and high variance, the power of these tests was low. During sexual maturation, mRNA of Vg1 and Vg2 were significantly positively correlated but were not significantly correlated during breeding. During sexual maturation hemolymph concentration of Vg and ovarian weight were significantly positively correlated, i.e. for any given time, those individuals with the highest Vg concentration in the hemolymph also had the heaviest ovaries. We might have anticipated that

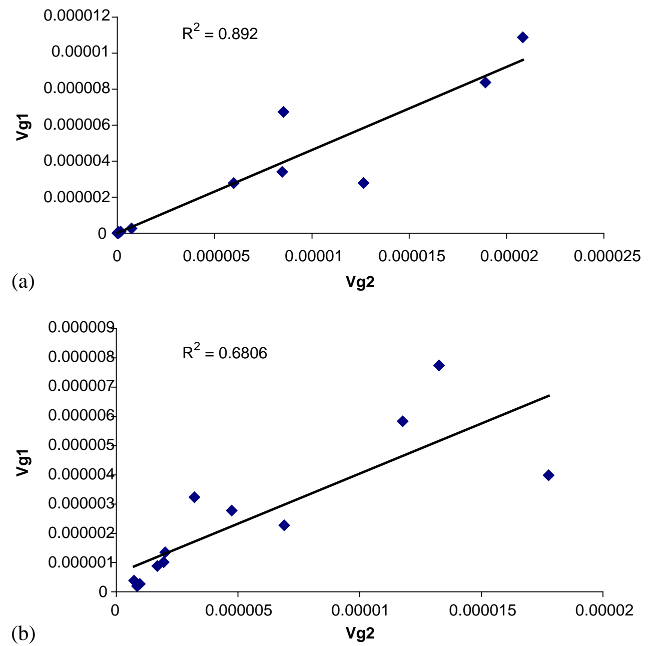

Fig. 3. Correlation of template mRNA/μg total RNA for vitellogenin genes 1 and 2 of burying beetles: (a) pre-breeding females sampled at 0, 5, 10, 15 and 20 days post-eclosion and (b) females during a breeding cycle sampled at 12 h, 3, 5 and 8 days after the discovery of a carcass.

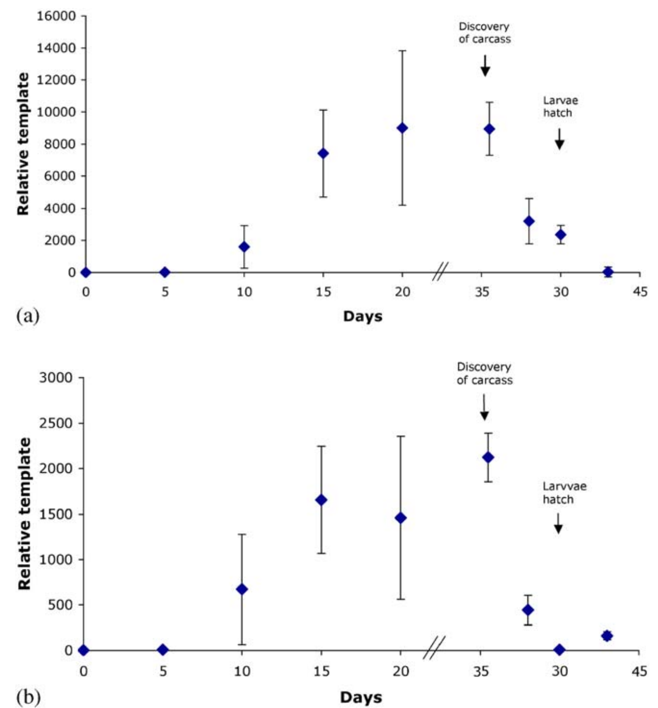

Fig. 4. Relative vitellogenin expression (mRNA) of Vg1 (a) and Vg2 (b) of burying beetle females in the first 20 days after eclosion and at 4 times during a breeding bout. The relative template was normalized by dividing T by the lowest T measured for that gene (for one of the females on the day of eclosion). ANOVAs revealed significant changes over time in pre-breeding mRNA in both Vg1 and Vg2 ($p = 0.001$ and 0.005, respectively) and in mRNA during a breeding cycle in both genes ($p = 0.03$ and 0.005, respectively). Means ± standard error are shown.

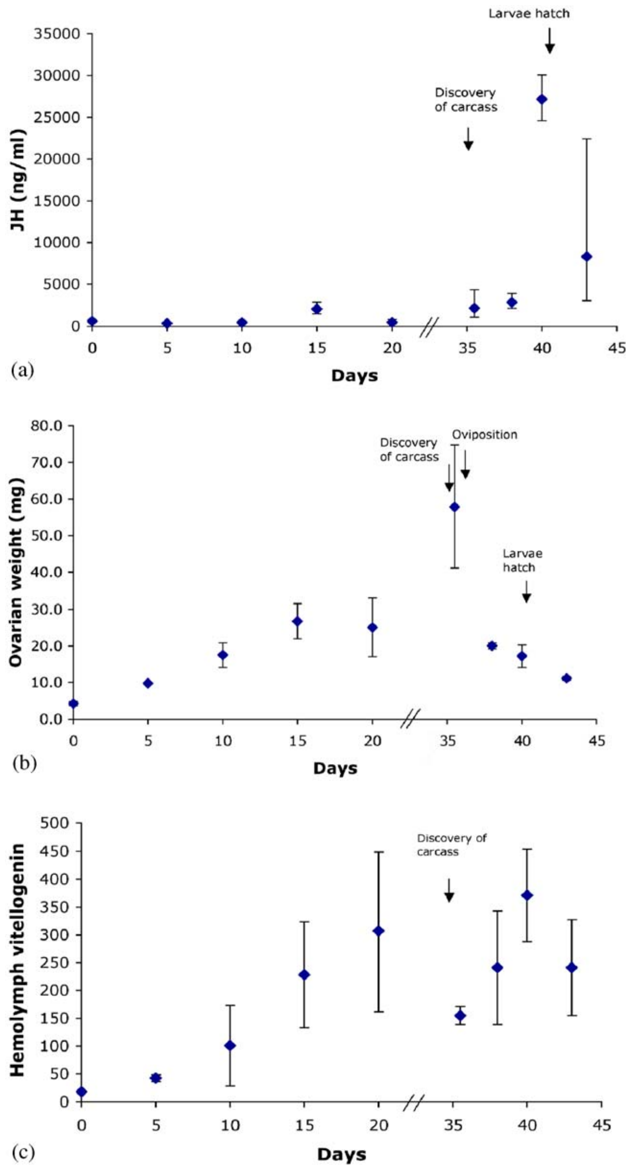

Fig. 5. Juvenile hormone hemolymph titer (a), weight of wet ovaries (b), and relative vitellogenin hemolymph titers (c) of *Nicrophorus orbicollis* pre-breeding and during a breeding bout. The units of vitellogenin hemolymph concentration are arbitrary and represent the relative intensity of the 38 kDa band. Results for individuals are comparable because the same volume of hemolymph was taken and the same proportion was loaded on the gel. Hemolymph and ovaries were sampled every five days after females eclosed and at 12 h, 3, 5, and 8 days after the discovery of a carcass. There was a significant difference in JH titers pre-breeding but not during breeding ($p = 0.03$ and 0.08, respectively) and significant differences in ovarian weight both pre-breeding and during breeding ($p = 0.02$, 0.02, respectively). Differences in hemolymph Vg were not significant either pre-breeding or during breeding. The same three females as those used for qPCR were sampled for each data point in each figure. Means ± standard error are shown.

individuals with the highest mRNA expression would also have the highest hemolymph concentration of Vg. There was a hint of this relationship during maturation

Table 1
Partial correlation coefficients (with the effect of time removed) for mRNA of the 2 Vg genes, hemolymph titers of JH, ovarian weight, and hemolymph concentrations of Vg

	Vg1	Vg2	JH	Ovaries
Pre-breeding				
Vg1				
Vg2	0.860*			
JH	0.145	0.168		
Ovaries	0.175	0.384	0.167	
Hemolymph Vg	0.395	0.500	0.190	0.952*
Breeding				
Vg1				
Vg2	0.513			
JH	0.049	0.134		
Ovaries	0.064	0.496	−0.125	
Hemolymph Vg	0.008	−0.060	0.482	−0.057

*indicates $p<0.001$. (Pre-breeding: $\alpha = 0.05$ critical value for correlation coefficient $r = 0.532$; breeding: $\alpha = 0.05$ critical value for $r = 0.576$.)

($r = 0.395$ and 0.5 with Vg1 and Vg2, respectively) but the lack of a correlation is not surprising as the protein is stable and the mRNA is not, and they have different turnover dynamics. Although not significant, the partial correlations between Vg2 and hemolymph Vg and ovarian development do suggest a potentially important biological relationship; partial correlations with Vg1 were lower but Vg1 expresses less than 1/3 of the combined Vg–mRNA. There was no evidence for a relationship between JH and mRNA at any time nor between JH and ovarian development. The relatively high coefficient of correlation between JH and hemolymph Vg during breeding, although suggestive, is undermined by the lack of strong relationship between JH and mRNA at that time.

4. Discussion

The eggs of most animals are provisioned by vitellogenins that are synthesized, by insects, primarily in the fat body and transported to the developing oocytes via the hemolymph (Wyatt and Davey, 1996). The primary function of Vg is to provide amino acids and nutrition (carbohydrates, lipids, etc.) for the developing embryo. Because of its nutritive function selective constraints on Vg are low and it tends to be very variable among taxa (Chen et al., 1997; Hagedorn et al., 1998; Sappington and Raikhel, 1998). Nonetheless, Vg genes of vertebrates and invertebrates are homologous and, in insects, are about 50% similar (Romans et al., 1995; Hagedorn et al., 1998). Our study reports partial sequences for two Vg genes in burying beetles that are similar to Vg genes of other insects. The

two amino acid sequences are 59% similar. This divergence is consistent with duplicate Vg genes which have been reported and sequenced in other insects: the cockroach *Periplaneta americana*, has two genes that are only 30% similar (Tufail et al., 2001); the bean bug, *P. stali*, has three genes that are 52%, 44% and 45% similar (Lee et al., 2000); two genes have been reported for the bean bug, *Riptortus clavatus* (Hirai et al., 1998); and five have been reported for the mosquito, *Aedes aegypti* (Romans et al., 1998).

The pattern of transcription of the two Vg genes during sexual maturation and during a breeding bout was expected from the natural history of these beetles. Burying beetles require 15–21 days after eclosion (depending on their nutritional state) for their ovaries to develop to resting stage (Wilson and Knollenberg, 1984; Trumbo and Robinson, 2004). Ovaries remain in this state until beetles discover a carcass when development resumes very rapidly (Scott and Traniello, 1987; Trumbo et al., 1995). The pattern of Vg–mRNA during sexual maturation, which increased until 15–20 days after eclosion, corresponds to the requirement for ample Vg to be available so that it can be quickly incorporated into the oocytes when a resource is discovered. Vg–mRNA is also high immediately following the discovery of a carcass which suggests either that final Vg synthesis occurs after the resource is discovered or that additional Vg must be synthesized in case a replacement clutch is necessary; females often need to produce a replacement clutch for this rare and valuable resource if her first is lost as a result of infanticide or poor hatching (Müller, 1987; Scott, 1997). The latter explanation, preparation for a possible replacement clutch, is supported by the high hemolymph Vg but low expression towards the end of the breeding bout when the ovaries are again at resting stage and females are physiologically capable of producing another clutch immediately if they find another resource.

An increase in mRNA expression of Vg in the days following eclosion has been reported for many insects that do not depend on an unpredictable resource to breed: spruce terminal weevil (Leal et al., 1997), tobacco horn worm (Ismail et al., 1998), grasshopper (Borst et al., 2000), locust (Zhou et al., 2002), German cockroach (Martin et al., 1998), honeybee queen (Piulachs et al., 2003). The mosquito does depend on the unpredictable resource of a blood meal both for breeding and for activation of the Vg genes (Kokoza et al., 2001). Similarly, we might have expected burying beetles to also require a resource for activation of the genes. However, during sexual maturation, they are more like insects that do not require an unpredictable resource. In the mosquito, Vg expression yields products necessary for the upcoming oviposition. In burying beetles up regulation of the Vg genes following oviposition is more likely to be a bet-hedging measure. Unlike mosquitoes, burying beetles must exploit the resource quickly and have many physiological and behavior adaptations to do so.

The regulation by JH of Vg synthesis and uptake is common in other beetle species. In the boll weevil, JH is necessary and sufficient to stimulate Vg synthesis but JH and a brain factor are required for Vg uptake by the ovary (Taub-Montemayor and Rankin, 1997). The same pattern is seen in three species of lady beetle (reviewed in Taub-Montemayor and Rankin, 1997). The Colorado potato beetle shows a slightly different pattern but results from manipulations vary with the age of the female. If the corpora allata (the source of JH) are removed at ecdysis, Vg synthesis is not prevented; but if the corpora cardiaca (a neurohemal organ from which neurosecretory products from the brain are released into the hemolymph) are removed along with the corpora allata at ecdysis, Vg synthesis is prevented and can be restored by treatment with JH (de Loof and de Wilde, 1970). If the corpora allata–corpora cardiaca are removed in older females, Vg synthesis is not affected (Dortland, 1979). JH promotes the uptake of Vg into the ovary but it is not known if a brain factor is also required (de Loof and de Wilde, 1970).

We are not able to address directly the relationship of JH to Vg transcription or to Vg concentration in the hemolymph in burying beetles. Past studies have indicated that JH, on its own and in the absence of a carcass, does not promote the uptake of Vg by the ovaries (Scott et al., 2001) but JH, on its own or in conjunction with 20-hydroxyecdysone and/or various neurosecretory hormones from the brain, might stimulate synthesis of Vg. Certainly JH, Vg–mRNA, hemolymph Vg concentrations and ovarian development increase in parallel during sexual maturation. However, when JH titers are highest, during the first two days of parental care, Vg transcription is very low. Furthermore, the partial regressions, which examine the correlation of variables within single individuals, do not hint at a gonadotropic role for JH. All partial correlation coefficients of variables to JH, except that of JH and hemolymph Vg during breeding, were extremely low. However, low or intermediate levels of JH may be necessary to prime the fat body to synthesize Vg or during sexual maturation low levels of JH may stimulate Vg–mRNA expression directly. These hypotheses await further study.

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