Bulletin of Entomological Research (2016) **106**, 642–650
doi:10.1017/S0007485316000353

Molecular cloning and expression of the vitellogenin gene and its correlation with ovarian development in an invasive pest *Octodonta nipae* on two host plants

Jin-Lei Li[1,2], Bao-Zhen Tang[1,2], You-Ming Hou[1,2]* and Yi-Xing Xie[1,2]

[1]Fujian Provincial Key Laboratory of Insect Ecology, Department of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou, Fujian, China: [2]Key Laboratory of Integrated Pest Management on Crops in Fujian-Taiwan, Ministry of Agriculture, Fuzhou, China

Abstract

There is an ongoing relationship between host plants and herbivores. The nutrient substances and secondary compounds found in the host plant can not only impact the growth and development process of herbivores, but, more importantly, may also affect their survival and reproductive fitness. Vitellogenesis is the core process of reproductive regulation and is generally considered as a reliable indicator for evaluating the degree of ovarian development in females. Vitellogenin (Vg) plays a critical role in the synthesis and secretion of yolk protein. In this study, the full-length cDNA of the Vg gene in an alien invasive species, the nipa palm hispid beetle *Octodonta nipae* Maulik (Coleoptera: Chrysomelidae) (*OnVg*) was cloned and, the effect of host plant on the *OnVg* expression level and ovarian development was investigated. The results revealed that the *OnVg* was highly and exclusively expressed in adult females, but barely detectable in larvae, pupae and adult males. The relative expression level of *OnVg* and egg hatchability were much higher in females fed on *Phoenix canariensis* (their preferred host) than those fed on *Phoenix roebelenii*. A positive correlation relationship between *OnVg* expression and egg hatchability was also detected. Additionally, the anatomy of the female reproductive system showed that the ovaries of individuals fed on *P. canariensis* were considerably more developed than in females fed on *P. roebelenii*. The results may be applicable to many pest management situations through reproductive disturbance by alternating host plant species or varieties or by reproductive regulation through vitellogenesis mediated by specific endocrine hormones.

Keywords: egg hatchability, expression level, host plant, *Octodonta nipae*, ovarian development, vitellogenin

(Accepted 20 April 2016; First published online 24 May 2016)

Introduction

It is well established that vitellogenesis is associated with significant accumulation of yolk proteins. These are usually phospholipoglycoproteins and play a key role in embryogenesis by providing nutrient sources such as lipids, free amino acid, carbohydrates, carotenoids, and minerals (Adams *et al*., 2002; Khalaila *et al*., 2004) to the growing oocytes, embryos (Lee *et al*., 1997; Tsukimura *et al*., 2000), and even larvae (Yang *et al*., 2005). As a precursor of vitellin (Vt), the common form of yolk proteins, vitellogenin (Vg) plays a vital role in the synthesis and secretion of yolk protein (Tufail & Takeda, 2009; Guo *et al*., 2012). The quality and quantity of the yolk proteins

*Author for correspondence
Tel.: + 86 591 8375 0663
E-mail: ymhou@fafu.edu.cn

synthesized and accumulated in eggs are crucial for embryonic survival (Harrison, 1990) and development (Yang *et al*., 2005). The synthesis of Vg/Vt is, therefore, considered to be a reliable indicator for evaluating female ovarian development and reproductive fitness (Tsukimura, 2001).

Reproductive regulation is essential for living organisms. This is especially critical in invasive species allowing them to achieve their reproductive potential and to successfully colonize and adapt to new environments. As a major component of oogenesis (Shapiro *et al*., 2000), vitellogenesis is considered to be the core process of reproductive regulation (Guidugli *et al*., 2005). The reproductive success of all the oviparous animals including insects is heavily dependent on the synthesis and accumulation of Vg/Vt in oocytes. Adequate Vg/Vt production, however, requires sufficient nutrient availability, and their circulating titers are, therefore, closely associated with nutrition intake (Bitondi & Simoes, 1996). It has been suggested that nutritional condition is an important factor for reproductive success (Awmack & Leather, 2002; Barone & Frank, 2003) and that Vg synthesis and protein production in the fat body can be affected by food availability (Fei *et al*., 2005). On the other hand, the ability to rapidly expand and populate a new territory in the shortest time possible is indispensable for successful colonization by certain species especially those invasive ones. The close relationship between host plant and herbivore has been extensively studied both from the nutritional and co-evolutionary aspects (Awmack & Leather, 2002; Mello & Silva-Filho, 2002; Utsumi, 2011). The nutrient substances and secondary compounds inside host plants can impact the growth and development process as well as survival of herbivores, which may ultimately lead to variability of reproductive success and offspring fitness for these species (Awmack & Leather, 2002). Previous studies have documented the molecular characteristics (Tufail & Takeda, 2005, 2008; Shu *et al*., 2011), regulatory mechanisms (Guidugli *et al*., 2005; Nilsen *et al*., 2011), and potential roles of Vg in oogenesis (Tufail & Takeda, 2005, 2009) and social organization (Nelson *et al*., 2007), however, few reports focus on combining the insect Vg accumulation and ovarian development or egg quality from a host nutrition-dependent aspect, especially in those invasive species with tremendous reproductive capacity. In the present study, an invasive species, the nipa palm hispid beetle, *Octodonta nipae* Maulik (Coleoptera: Chrysomelidae) was used to determine the effect of host plant on ovarian development. This pest is native to Malaysia (Sun *et al*., 2003) and feeds on a wide range of host plants, causing serious damage to palm trees in southeast China (Hou & Weng, 2010). The full-length cDNA of the Vg gene in *O. nipae* (*OnVg*) was cloned and the relative expression level for different developmental stages of the beetle reared on two different host plants was also analyzed. The results determined the sex- and developmental stage-specific expression pattern of *OnVg*, and demonstrated the significant impact the host plant has on ovarian development and offspring quality.

Materials and methods

Insect rearing and sampling

The laboratory population of *O. nipae* was established from individuals collected from a nursery (25°43′42″N, 119°20′35″ E) in Fuqing City, Fujian Province of China (Hou & Weng, 2010). Two different species of host plants, Canary Island date palm *Phoenix canariensis* Hort. ex Chabaud and pygmy date palm *P. roebelenii* O′ Brien, were used as hosts to rear *O. nipae* over 12 generations beginning in 2012. Insect rearing was carried out under laboratory conditions (see Li *et al*., 2014). *O. nipae* female adults fed on *P. canariensis* were sampled for cloning *OnVg*. To identify possible variations caused by selection of host plant, insects that were reared on each of the two host plants were sampled for developmental stage-specific expression analysis at different developmental stages (i.e., 4th instar larvae, 3 day-old pupae, 15 day-old virgin females and males; 15 days are needed for *O. nipae* to be sexually mature). Five individuals were pooled in each sample with four replications for the *OnVg* expression profile analysis.

Cloning the full-length cDNA of Vg gene

The Trizol reagent (Invitrogen) was used for total RNA extraction following the manufacturer's protocol. The total RNA samples were assayed for RNA concentration using the NanoDrop 2000 (Thermo Fisher Scientific Inc., Waltham MA, USA) and was subsequently stored at −80°C. The Thermo Scientific Verso cDNA Synthesis Kit (Thermo Fisher Scientific Inc., Waltham MA, USA) was used for cDNA synthesis. One microgram of total RNA was added as template and then the Verso Enzyme Mix as reverse transcriptase and anchored Oligo dT as primers following the manufacturer's protocol.

The potential *OnVg* fragments were first screened from the *O. nipae* transcriptome database (Tang *et al*., 2014). The primers were then designed by Primer3 (version 4.0.0 http://primer3.ut.ee/, see Koressaar & Remm, 2007; Untergasser *et al*., 2012). Polymerase Chain Reaction (PCR) amplifications were proceeded in a total reaction volume of 50 µl, consisting of 25 µl of 2 × Taq Plus MasterMix (Tiangen Biotech Co., Ltd., Beijing, China), 2 µl of each primer (VgF and VgR, 10 µM), 2 µl of cDNA template and 19 µl of PCR-grade water. The amplification conditions used were as follows: 94°C for 3 min, 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 5 min. The obtained PCR products were purified using a PCR purification kit (Tiangen Biotech Co., Ltd., Beijing, China). The purified DNA fragment was ligated with the pMD 18-T vector (TAKARA Biotech Co., Ltd., Dalian, China) and transformed into competent *Escherichia coli* cells (DH5α, Tiangen Biotech Co., Ltd., Beijing, China). The recombinants were screened for subsequent sequencing (Sangon Biotech Co., Ltd., Shanghai, China) through blue-white plaque selection on LB plates containing ampicillin (final concentration: 100 µg ml^{-1}).

After obtaining *OnVg* fragments, the 3′ and 5′ rapid amplification of cDNA ends (RACE) PCRs were performed to obtain the 3′ and 5′ cDNA ends of *OnVg* using the SMARTer™ RACE cDNA Amplification Kit (Clontech Laboratories, Inc.). The gene-specific primers Vg3F, Vg5R, and nested primers NVg3F, NVg5R were designed for the 3′ and 5′ RACE, respectively. The cDNA template synthesis and amplification conditions were conducted according to the user manual. The subcloning and sequencing of RACE-PCR products were performed as described above except the cloning vector and competent *E.coli* cells used for those procedures were *pEASY*-T5 (TransGen Biotech Co., Ltd., Beijing. China) and *Trans1*-T1 Phage Resistant Chemically Competent Cell (TransGen Biotech Co., Ltd., Beijing. China), respectively. The full-length cDNA of Vg gene in *O. nipae* was then obtained by contig alignment of three cDNA fragments according to the

https://doi.org/10.1017/S0007485316000353 Published online by Cambridge University Press

overlapping region. All of the primers used for molecular cloning are available in Supplementary Table S1.

Sequence analysis

The homology of *OnVg* sequences with other *Vgs* were checked using the National Center for Biotechnology Information (NCBI) Translated BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The Expasy-Translation tool (web.expasy.org/translate/) was used to translate the full-length *OnVg* into the deduced amino acid sequence. The signal peptide position was detected using SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/) and the molecular weight and isoelectric point of deduced amino acid sequence were detected using the ExPASy-Compute pI/Mw tool (http://web.expasy.org/compute_pi/). The putative phosphorylation and glycosylation sites were checked through the NetPhos 2.0 Server (http://www.cbs.dtu.dk/services/NetPhos/) and NetNGlyc 1.0 Server (http://www.cbs.dtu.dk/services/NetNGlyc/), respectively. The NCBI Conserved Domain Search (CDD, http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) was used for detecting the conserved domains of the deduced amino acid sequence.

Construction of phylogenetic tree

Multiple alignments were performed using the ClustalX program (Thompson *et al.*, 1997) with gap opening penalty 10, gap extension penalty 0.05, and gap separation penalty range 8. The phylogenetic tree based on the *OnVg* deduced amino acid sequence was constructed by MEGA 5 (Tamura *et al.*, 2011) using the neighbor-joining (NJ) algorithm, the reliability of the clusters within the tree was tested using bootstrap resampling with 1000 replicates. The Vg sequences used for comparison and phylogenetic tree construction were available in Supplementary Table S2.

Expression profile of OnVg *across different developmental stages reared on two different host plant species*

Samples were collected from different developmental stages (i.e., 4th instar larvae, 3 day-old pupae, and 15 day-old virgin females and males) of *O. nipae* individuals fed on either *P. canariensis* or *P. roebelenii*. The total RNA extraction of the whole body was performed as described above and cDNA synthesis was achieved using the Thermo Scientific Verso cDNA Synthesis Kit (Thermo Fisher Scientific Inc., Waltham MA, USA). SYBR Green fluorescence quantitive real-time PCR (qRT-PCR) was carried out for relative expression analysis of *OnVg* with ribosomal protein S3 gene (*OnRPS3*) being chosen as the internal reference gene. Primers qVgF and qVgR were used for *OnVg* amplification while RPS3F and RPS3R were used to amplify *OnRPS3* (see Supplementary Table S1). The qRT-PCR was conducted in an ABI 7500 real-time PCR system and the reaction procedure consisted of the following: one cycle of 95°C for 10 min, 40 cycles of 95°C for 15 sec, 60°C for 1 min, followed by one cycle of 95°C for 15 sec, 60°C for 1 min, 95°C for 30 sec, 60°C for 15 sec (melting-curve stage for a specific PCR product verification). Each sample was assayed in triplicate and the amplification data were normalized to *OnRPS3* expression. The $2^{-\Delta\Delta CT}$ method was used to calculate the real-time data (Livak & Schmittgen, 2001).

Ovarian development evaluation

Females (15 day-old) fed on *P. canariensis* and *P. roebelenii* were collected for ovarian dissection. Prior to dissection, the females were anesthetized on ice and then disinfected with a 75% alcohol solution. The elytra were removed using a McPherson-Vannas scissors (World Precision Instruments, Inc., Sarasota, FL, USA). Female bodies were placed on a glass slide containing a drop of phosphate buffer saline (PBS) (NaCl 8 g, KCl 0.2 g, Na_2HPO_4 1.42 g, KH_2PO_4 0.27 g, in ultrapure water 1000 ml, pH 7.4). The insect body was cut open from the flank to the end of abdomen using the McPherson-Vannas scissors. The *O. nipae* female reproductive systems were isolated from the body using two Dumont forceps (World Precision Instruments, Inc., Sarasota, FL, USA). The reproductive systems were then immersed into Giemsa staining solution for 5 min followed by three washings of PBS. Each complete reproductive system was placed under a stereoscopic microscope (Nikon SMZ745 T, Japan), which connected to a digital camera (Digital Sight DS-Fi2, Nikon, Japan) for photographing. The digitalized images of the reproductive systems were then analyzed using NIS Elements D software (version 4.30, Nikon, Japan). The length, width and cross-sectional area of each *O. nipae* ovariole were calculated and recorded through measuring tools attached to the software.

Egg hatchability assessment

An egg hatchability study was conducted in order to evaluate the quality of eggs laid by the females. Twenty-five virgin females and an equivalent number of males fed on *P. canariensis* and *P. roebelenii* were paired and kept in a sterile transparent plastic container for mating under laboratory conditions (25 ± 0.5°C, RH: 75 ± 5%, L:D = 14:10). After 7 days, all eggs were collected and 50 eggs were added to each jar containing the corresponding host plant. The containers were kept in an incubator (25 ± 0.5°C, RH: 75 ± 5%, L:D = 14:10) for 10 days. Palm leaves of the two species were replaced daily and the newly hatched larvae were counted to determine egg hatchability. There were a total of 33 replicates of *P. canariensis*-fed beetles and 30 replicates of *P. roebelenii*-fed beetles being carried out. Among these groups, two replicates fed on *P. canariensis* and three replicates fed on *P. roebelenii* were excluded from later analysis due to the escape of newly hatched larvae.

Statistical analysis

Data were tested for normality and homogeneity of variance using the Kolmogorov–Smirnov test and Levene's test, respectively. The egg hatchability data were analyzed using an unpaired t-test. Because they did not meet the homogeneity of variance assumption, ovarian development (ovariole length, ovariole width and ovariole cross-section area) was analyzed using the Mann–Whitney test. The data for relative expression of *OnVg* across different developmental stages also did not confirm to homogeneity of variance, therefore, a Kruskal–Wallis test was used to examine the expression level of *OnVg* among developmental stages. For the expression analysis of *OnVg* at a particular developmental stage on each of the two host plants, the data for the pupal and male adult stages were analyzed using an unpaired t-test while the larval and female adult stage data were analyzed using the Mann–Whitney test due to their heteroschedasticity. In addition, the linear regression analysis was used to test the potential

https://doi.org/10.1017/S0007485316000353 Published online by Cambridge University Press

correlation between *OnVg* expression and egg hatchability. To minimize the impact of sampling on correlation analysis, females from the same batch were collected and then divided into two groups, one group was used for the expression analysis of *OnVg* and the other one for the egg hatchability assay. All statistical analyses were conducted using SPSS, version 17.0 (SPSS Inc., Chicago, Illinois). The figures were mapped using SigmaPlot for Windows version 12.0 (Systat Software Inc., San Jose, California).

Results

Ovarian development assessment and egg hatchability

The host plants significantly influenced the ovarian development of female *O. nipae* (fig. 1a, b). Females fed on *P. canariensis* showed significantly higher ovariole length (Mann–Whitney test: $Z = -7.429$, $P < 0.001$), width (Mann–Whitney test: $Z = -8.355$, $P < 0.001$) and cross-sectional area (Mann–Whitney test: $Z = -7.918$, $P < 0.001$) than those fed on *P. roebelenii* (fig. 1d–f).

Moreover, the hatchability of eggs laid by females fed on different host plants was significantly different (unpaired *t*-test, $t_{1,52} = 4.935$, $P < 0.001$). The hatch rate of eggs laid by females fed on *P. canariensis* (84.29 ± 1.48%) was significantly higher than that of females fed on *P. roebelenii* (70.92 ± 2.32%) (fig. 1c).

*Analysis of the full-length cDNA of Vg gene (*OnVg*)*

The complete *OnVg* was 5451-bp long, including a 12 bp 5′-untranslated region (UTR), a 69 bp 3′-UTR and a 5370 bp opening reading frame (ORF), which encoded 1789 amino acid residues (accession number: KR736347 and Supplementary Figure S1). The first 18 amino acid residues of ORF were predicted signal peptide through the SignalP program (Supplementary Figure S1). The predicted molecular weight and isoelectric point of the mature protein were 204.27 kDa and 4.72, respectively. A Vg-N domain (amino acid position: 22–717) with a gap (amino acid position: 325–387), a DUF 1943 domain (amino acid position: 774–1042) and a von Willebrand factor type D domain (amino acid position: 1461–1645), were identified using a NCBI CCD search. In addition, six potential cleavage recognition sites RXXR and two conserved amino acid motifs GL/ICG (here GICG) and DGXR (here DGQR) were identified. Additionally, the putative glycosylation site (NXS/T) and potential phosphorylation residues were also found in the *Vg* sequence in *O. nipae* (Supplementary Figure S1).

A NJ tree construction was used to illustrate the evolutionary relationship among different *Vgs* (fig. 2). The dendrogram showed that the *OnVg* was placed with other insects especially other Coleoptera *Vgs* (i.e., *Anthonomus grandis*, *Tenebrio molitor*, *Tribolium castaneum*) into a single cluster. It also indicated that the coleopteran *Vgs* appear to have a closer evolutionary relationship with the hymenopteran *Vgs* than with the *Vgs* of other insects. The phylogenetic tree divided the *Vgs* from different orders into separate clusters reflecting their phylogeny. Moreover, the dendrogram clustered different insect orders into one homogeneous group, showing the existence of a greater sequence similarity that extends beyond the insect order level. Additionally, it seems to imply that the hymenopteran *Vgs* are the sister group of coleopteran *Vgs* while the dipteran *Vgs* are the sister group of hemipteran *Vgs*. However, the lepidopteran *Vgs* showed a distant phylogenetic relationship with other insect *Vgs*.

Expression profile of OnVg

The *OnVg* was specifically expressed in female adults regardless of host plant (*P. canariensis*: Kruskal–Wallis test, d.f. = 3, $\chi^2 = 8.824$, $P = 0.032$; *P. roebelenii*: Kruskal–Wallis test, d.f. = 3, $\chi^2 = 8.500$, $P = 0.037$). *OnVg* expression was barely detected in the larvae, pupae and male adults (fig. 3). In addition, no significant differences in terms of *OnVg* expression were found in the larval (Mann–Whitney test: $Z = 0.00$, $P = 1.00$), pupal (unpaired *t*-test: $t_{1,6} = 0.214$, $P = 0.838$) and male adult stages (unpaired *t*-test: $t_{1,6} = -0.338$, $P = 0.747$). However, the *OnVg* expression in the female adult stage was significantly affected by host plants (Mann–Whitney test: $Z = -2.309$, $P = 0.021$). Females fed on *P. canariensis* showed a much higher *OnVg* expression level than those fed on *P. roebelenii* (fig. 3).

Relationship between expression profile of OnVg *and offspring quality*

Regression analysis was used to illuminate the potential relevance between *OnVg* expression and offspring quality. Despite the host plant, a significant positive correlation between *OnVg* expression and egg hatchability was detected in females fed on either *P. canariensis* ($F_{1,29} = 56.836$, $P < 0.001$, fig. 4) or *P. roebelenii* ($F_{1,25} = 67.820$, $P < 0.001$, fig. 4). Those females with a higher *OnVg* expression level were inclined to lay higher-quality eggs (with high egg hatching rates).

Discussion

Numerous *Vg* cDNAs including several complete *Vg* cDNAs have been sequenced in hymenopteran insects (Pinto *et al.*, 2000; Guidugli *et al.*, 2005; Nelson *et al.*, 2007), dipteran insects (Ahmed *et al.*, 2001; Dana *et al.*, 2006), hemipteran insects (Lee *et al.*, 2000; Guo *et al.*, 2012), dictyopteran insects (Tufail & Takeda, 2005; Ciudad *et al.*, 2006) and lepidopteran insects (Meng *et al.*, 2008; Shu *et al.*, 2011) while only a limited number of *Vgs* in coleopteran insects have been reported. In the present study, the full-length *Vg* in *O. nipae* was cloned and its evolutionary relationship with other *Vgs* was investigated. The deduced amino acid sequence of *OnVg* contained typical domains and conserved motifs that are commonly found in insect *Vg*. In addition, a close molecular evolutionary relationship in the sequence similarity of Vg genes between *O. nipae* and its subordinative insect order was also verified through multiple alignment. Furthermore, our results showing the close relationship between *Vg* expression level and offspring quality should be applicable to many pest management situations through reproductive regulation in insects especially those invasive ones.

In line with previous studies (Lee *et al.*, 1997; Dong *et al.*, 2008), the results in this study demonstrate the characteristic female-specific expression of *Vg*. The specific expression of *OnVg* in the female adult stage also suggests a developmental stage-specific expression pattern of *Vg* in *O. nipae*, which may be attributable to the variation in reproductive physiology resulting from peculiarities in an individual's life history. In most insect species, the degree of ovarian maturation directly contributes to the oviposition and egg quality (Papaj, 2000). Since nutrition both in the larval and adult stages is important for the realization of reproductive fitness, and feeding is an

https://doi.org/10.1017/S0007485316000353 Published online by Cambridge University Press

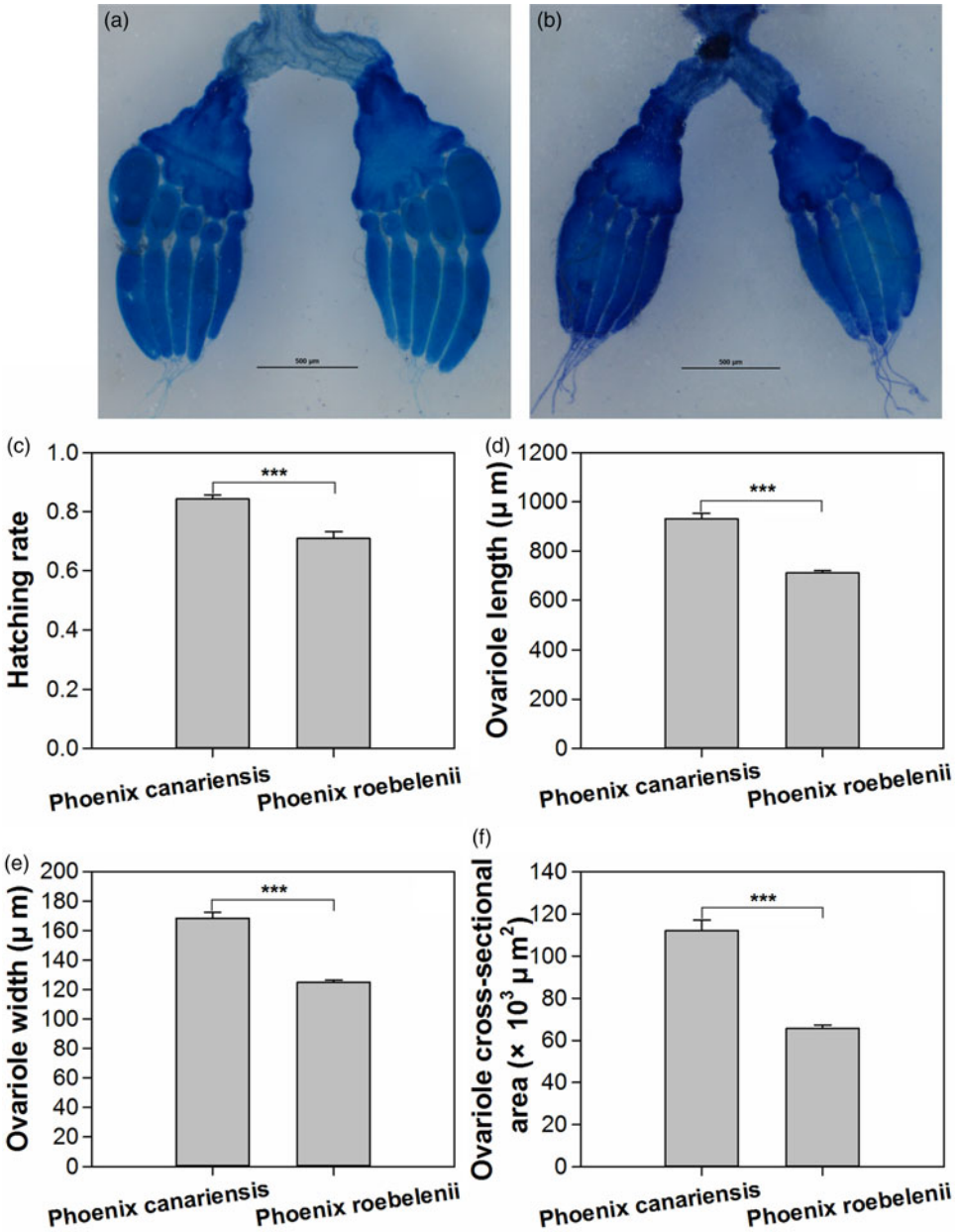

Fig. 1. Ovarian dissection of female *Octodonta nipae* fed on *P. canariensis* (a) and *P. roebelenii* (b), and effects of host plants on the egg hatchability (c), ovariole length (d), ovariole width (e) and ovariole cross-sectional area (f). Data were shown as Mean ± SE. Asterisks above the bars indicate statistically significant difference (***$P < 0.001$).

obvious prerequisite of egg production, food quality and availability would strongly influence reproductive capability. Therefore, the reproductive efficiency of herbivores would be expected to decrease when fed on low host quality or availability and vice versa. In the present study, *P. canariensis* has a higher crude fat, soluble protein, and free amino acid content than *P. roebelenii* (data not shown here), which coincides with the above prediction. Oviposition is a dynamic consequence in which the female individual in respond to the variation of host quality and availability from a functional way (Papaj, 2000). Egg production is therefore generally considered to be a reliable indicator of female reproductive fitness in response to differing potential factors (e.g., abiotic factors such as temperature and humidity or biotic factors such as host quality and prey availability, etc.). On the other hand, dissection of the reproductive system provides a direct and visual assessment of ovarian development from the aspect of developmental biology. In the present study, both the higher egg hatchability and the more developed reproductive system found in female *O. nipae* fed on *P. canariensis* indicated a higher offspring fitness and an increased female reproductive efficiency, which corresponds to the beetle's host preference

https://doi.org/10.1017/S0007485316000353 Published online by Cambridge University Press

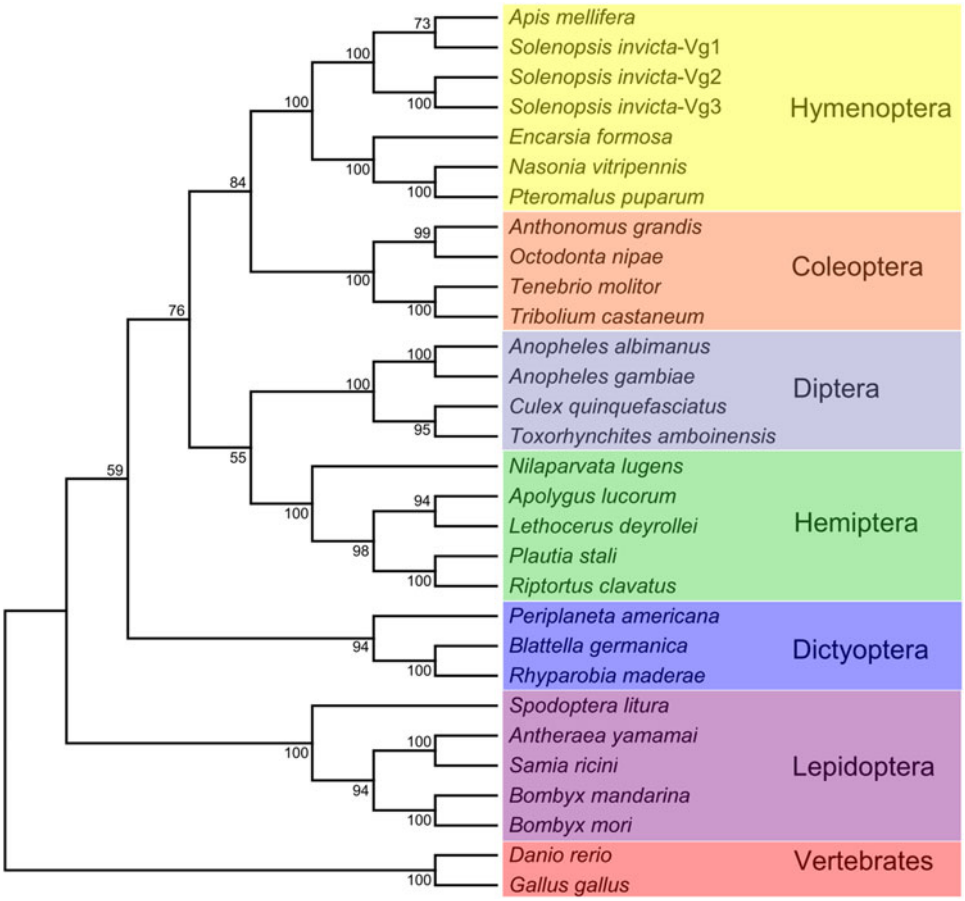

Fig. 2. Molecular phylogenetic tree comparing the deduced amino acid sequences of *Octodonta nipae Vg* with other *Vgs*. The phylogenetic analysis was initially performed using the ClustalX program for multiple sequence alignment and followed by MEGA5 for a neighbor-joining tree construction. The numbers at each node are the percentage of 1000 bootstrap resampling.

(*O. nipae* prefers to feed on *P. canariensis* rather than *P. roebelenii*, personal observation). Finally, being the most important nutritional source, Vg provides the growing oocytes, embryos, and even larvae with various nutrients (see the section Introduction). The significant correlation between the *OnVg* expression level and ovarian development in the present study provides us with robust evidence regarding the promotion of Vg accumulation on reproductive success.

It has been suggested that Vg has comprehensive regulatory functions and relates to some life history traits in honeybees (Guidugli *et al.*, 2005; Nelson *et al.*, 2007). The pleiotropic effects of the Vg gene on various physiological processes have also been demonstrated in honeybees (Nelson *et al.*, 2007). However, in most insects, hormonal regulation especially juvenile hormone (JH) is an important endocrine and metabolic regulator in vitellogenesis, including Vg biosynthesis and its absorption and translocation into growing oocytes (Raikhel *et al.*, 2005; Dong *et al.*, 2008). For example, JH inhibits the Vg synthesis and accumulation in honeybees when treated with high doses of pyriproxyfen (a potent JH analogue) (Pinto *et al.*, 2000), and the hemolymph titer of JH is sensitive to life stage activities such as foraging and reproduction (Robinson *et al.*, 1992). Besides, in response to the accumulation of ecdysteroids, the expression of Vg, vitellogenic carboxypeptidase and vitellogenic cathepsin B genes are increased during vitellogenesis in *Aedes aegypti* (Raikhel *et al.*, 2005). In the present study, host plants significantly affect the Vg expression level and ovarian development. Despite the factors of hormones and host plant nutrition influencing the Vg gene expression, the presence of the insulin/insulin-like signaling (IIS) pathway (Corona *et al.*, 2007) and the target of rapamycin (TOR) pathway (Patel *et al.*, 2007) drive the complex relationship that exists between nutrition status, Vg expression, hormonal regulation and social behavior, and are responsible for even more complex interactions in honeybees (Nilsen *et al.*, 2011). For example, some key components of IIS pathway such as insulin-like peptides, insulin-like receptors and transcription factor FOXO have been reported to play a part in Vg synthesis by a feedback loop mechanism (Corona *et al.*, 2007). And a positive effect of TOR on Vg expression has been verified in both honeybees (Patel *et al.*, 2007) and mosquitoes (Hansen *et al.*, 2004). Therefore, the functional verification of *OnVg* on ovarian development by means of RNA interference and the correlation between hormones and Vg expression and their interaction with other nutrient-sensitive target genes are necessary in the future for a broad understanding of insect Vg.

In summary, the present study clones the complete Vg gene in *O. nipae*, analyzes the expression profile of *OnVg*

https://doi.org/10.1017/S0007485316000353 Published online by Cambridge University Press

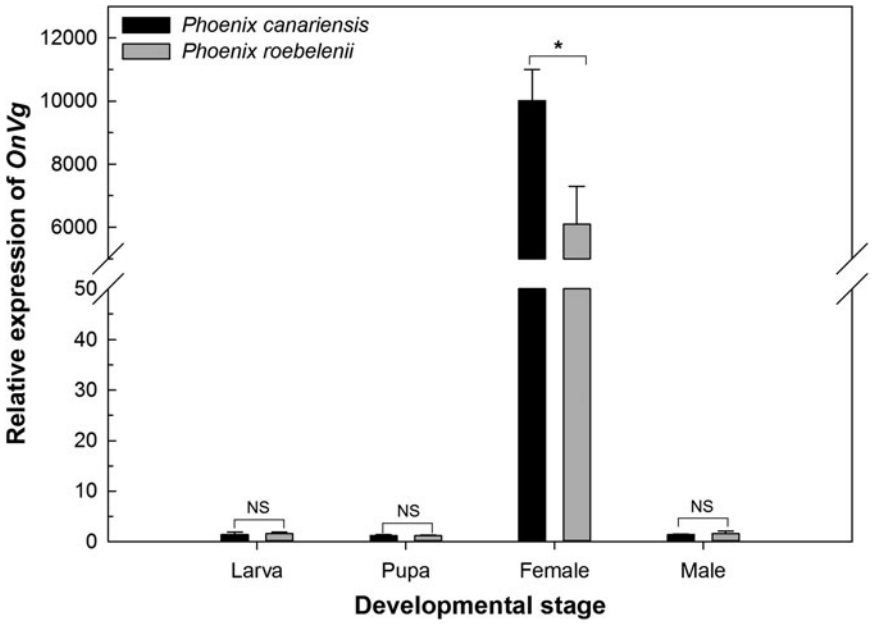

Fig. 3. Relative expression level of *OnVg* in different developmental stages fed on two different host plants. Asterisks above the bars indicate statistically significant difference (*$P < 0.05$), NS above the columns denote non-significant difference. Data were shown as Mean ± SE.

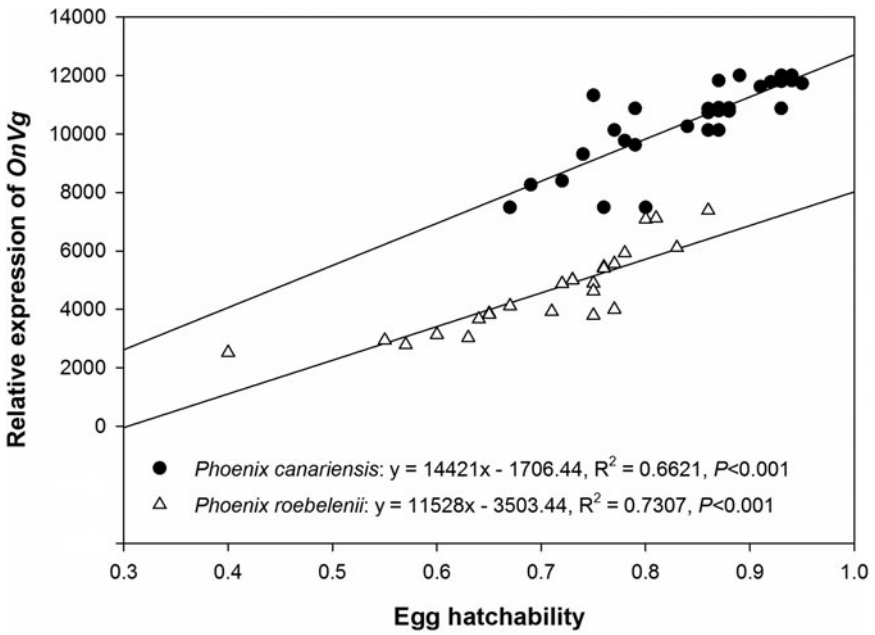

Fig. 4. Regression analysis between *OnVg* expression level and offspring quality. Females from the same batch were used for the gene expression analysis and the egg hatchability assay.

and verifies its correlation with ovarian development and subsequent egg quality, enhancing our understanding regarding Vg expression levels and the effects of differing host plant environments on ovarian development. In addition, data from this study may also have practical applications in pest management programs through the use of artificial reproductive disturbance by alternating host plant species or varieties or by reproductive regulation through vitellogenesis mediated by specific endocrine hormones.

Supplementary Material

The supplementary material for this article can be found at http://dx.doi.org/10.1017/S0007485316000353.

Acknowledgements

We gratefully thank Xiaoqiang Yu (University of Missouri-Kansas City) and Cecil L. Smith (Georgia Museum of Natural History) for their critical comments on the manuscript. This work was supported by the National Natural Science Foundation of China (31272108, 31471829).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Adams, T.S., Filipi, P.A. & Yi, S.X. (2002) Effect of age, diet, diapause and juvenile hormone on oogenesis and the amount of vitellogenin and vitellin in the twospotted stink bug, *Perillus bioculatus* (Heteroptera: Pentatomidae). *Journal of Insect Physiology* **48**, 477–486.

Ahmed, A.M., Maingon, R., Romans, P. & Hurd, H. (2001) Effects of malaria infection on vitellogenesis in *Anopheles gambiae* during two gonotrophic cycles. *Insect Molecular Biology* **10**, 347–356.

Awmack, C.S. & Leather, S.R. (2002) Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology* **47**, 817–844.

Barone, M. & Frank, T. (2003) Habitat age increases reproduction and nutritional condition in a generalist arthropod predator. *Oecologia* **135**, 78–83.

Bitondi, M.M.G. & Simoes, Z.P. (1996) The relationship between level of pollen in the diet, vitellogenin and juvenile hormone titres in Africanized *Apis mellifera* workers. *Journal of Apicultural Research* **35**, 27–36.

Ciudad, L., Piulachs, M.D. & Bellés, X. (2006) Systemic RNAi of the cockroach vitellogenin receptor results in a phenotype similar to that of the *Drosophila* yolkless mutant. *The FEBS Journal* **273**, 325–335.

Corona, M., Velarde, R.A., Remolina, S., Moran-Lauter, A., Wang, Y., Hughes, K.A. & Robinson, G.E. (2007) Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 7128–7133.

Dana, A.N., Hillenmeyer, M.E., Lobo, N.F., Kern, M.K., Romans, P.A. & Collins, F.H. (2006) Differential gene expression in abdomens of the malaria vector mosquito, *Anopheles gambiae*, after sugar feeding, blood feeding and *Plasmodium berghei* infection. *BMC Genomics* **7**, 119.

Dong, S.Z., Ye, G.Y., Yao, P.C., Huang, Y.L., Chen, X.X., Shen, Z. C. & Hu, C. (2008) Effects of starvation on the vitellogenesis, ovarian development and fecundity in the ectoparasitoid, *Nasonia vitripennis* (Hymenoptera: Pteromalidae). *Insect Science* **15**, 429–440.

Fei, H., Martin, T.R., Jaskowiak, K.M., Hatle, J.D., Whitman, D. W. & Borst, D.W. (2005) Starvation affects vitellogenin production but not vitellogenin mRNA levels in the lubber grasshopper, *Romalea microptera*. *Journal of Insect Physiology* **51**, 435–443.

Guidugli, K.R., Nascimento, A.M., Amdam, G.V., Barchuk, A.R., Omholt, S., Simões, Z.L. & Hartfelder, K. (2005) Vitellogenin regulates hormonal dynamics in the worker caste of a eusocial insect. *FEBS Letters* **579**, 4961–4965.

Guo, J.Y., Dong, S.Z., Yang, X.L., Cheng, L., Wan, F.H., Liu, S.S., Zhou, X.P. & Ye, G.Y. (2012) Enhanced vitellogenesis in a whitefly via feeding on a begomovirus-infected plant. *PLoS ONE* **7**, e43567.

Hansen, I.A., Attardo, G.M., Park, J.H., Peng, Q. & Raikhel, A.S. (2004) Target of rapamycin-mediated amino acid signaling in mosquito anautogeny. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 10626–10631.

Harrison, K.E. (1990) The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review. *Journal of Shellfish Research* **9**, 1–28.

Hou, Y.M. & Weng, Z.Q. (2010) Temperature-dependent development and life table parameters of *Octodonta nipae* (Coleoptera: Chrysomelidae). *Environmental Entomology* **39**, 1676–1684.

Khalaila, I., Peter-Katalinic, J., Tsang, C., Radcliffe, C.M., Aflalo, E.D., Harvey, D.J., Dwek, D.A., Rudd, P.M. & Sagi, A. (2004) Structural characterization of the N-glycan moiety and site of glycosylation in vitellogenin from the decapod crustacean *Cherax quadricarinatus*. *Glycobiology* **14**, 767–774.

Koressaar, T. & Remm, M. (2007) Enhancements and modifications of primer design program Primer3. *Bioinformatics* **23**, 1289–1291.

Lee, F.Y., Shih, T.W. & Chang, C.F. (1997) Isolation and characterization of the female-specific protein (vitellogenin) in mature female hemolymph of the freshwater prawn, *Macrobrachium rosenbergii*: comparison with ovarian vitellin. *General and Comparative Endocrinology* **108**, 406–415.

Lee, J.M., Hatakeyama, M. & Oishi, K. (2000) A simple and rapid method for cloning insect vitellogenin cDNAs. *Insect Biochemistry and Molecular Biology* **30**, 189–194.

Li, J.L., Zhang, X., Hou, Y.M. & Tang, B.Z. (2014) Effects of multiple mating on the fecundity of an invasive pest (*Octodonta nipae*): the existence of an intermediate optimal female mating rate. *Physiological Entomology* **39**, 348–354.

Livak, K.J. & Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25**, 402–408.

Mello, M.O. & Silva-Filho, M.C. (2002) Plant-insect interactions: an evolutionary arms race between two distinct defense mechanisms. *Brazilian Journal of Plant Physiology* **14**, 71–81.

Meng, Y., Liu, C.L., Shiomi, K., Nakagaki, M. & Kajiura, Z. (2008) Purification and cDNA cloning of vitellogenin of the wild silkworm, *Saturnia japonica* (Lepidoptera: Saturniidae). *Journal of Insect Biotechnology and Sericology* **77**, 1_35–1_44.

Nelson, C.M., Ihle, K.E., Fondrk, M.K., Page, R.E. & Amdam, G. V. (2007) The gene vitellogenin has multiple coordinating effects on social organization. *PLoS Biology* **5**, e62.

Nilsen, K.A., Ihle, K.E., Frederick, K., Fondrk, M.K., Smedal, B., Hartfelder, K. & Amdam, G.V. (2011) Insulin-like peptide genes in honey bee fat body respond differently to manipulation of social behavioral physiology. *The Journal of Experimental Biology* **214**, 1488–1497.

Papaj, D.R. (2000) Ovarian dynamics and host use. *Annual Review of Entomology* **45**, 423–448.

Patel, A., Fondrk, M.K., Kaftanoglu, O., Emore, C., Hunt, G., Frederick, K. & Amdam, G.V. (2007) The making of a queen: TOR pathway is a key player in diphenic caste development. *PLoS ONE* **2**, e509.

Pinto, L.Z., Bitondi, M.M. & Simões, Z.L. (2000) Inhibition of vitellogenin synthesis in *Apis mellifera* workers by a juvenile hormone analogue, pyriproxyfen. *Journal of Insect Physiology* **46**, 153–160.

Robinson, G.E., Strambi, C., Strambi, A. & Huang, Z.Y. (1992) Reproduction in worker honey bees is associated with low juvenile hormone titers and rates of biosynthesis. *General and Comparative Endocrinology* **87**, 471–480.

Raikhel, A.S., Brown, M.R. & Bellés, X. (2005) Hormonal control of reproductive processes. pp. 433–491 *in* Gilbert, L.I., Iatrou, K. & Gill, S.S. (*Eds*) *Comprehensive Molecular Insect Science*. Oxford, Elsevier Ltd.

Shapiro, J.P., Wasserman, H.A., Greany, P.D. & Nation, J.L. (2000) Vitellin and vitellogenin in the soldier bug, *Podisus maculiventris*: identification with monoclonal antibodies and reproductive response to diet. *Archives of Insect Biochemistry and Physiology* **44**, 130–135.

Shu, Y.H., Wang, J.W., Lu, K., Zhou, J.L., Zhou, Q. & Zhang, G.R. (2011) The first vitellogenin receptor from a Lepidopteran insect: molecular characterization, expression patterns and RNA interference analysis. *Insect Molecular Biology* **20**, 61–73.

Sun, J.H., Yu, P.Y., Zhang, Y.Z. & Wang, X.J. (2003) A new invasive coconut pest in Hainan Province. *Entomological Knowledge* **40**, 286–287.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**, 2731–2739.

Tang, B.Z., Chen, J., Hou, Y.M. & Meng, E. (2014) Transcriptome immune analysis of the invasive beetle *Octodonta nipae* (Maulik) (Coleoptera: Chrysomelidae) parasitized by Tetrastichus brontispae Ferrière (Hymenoptera: Eulophidae). *PloS ONE* **9**, e91482.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**, 4876–4882.

Tsukimura, B. (2001) Crustacean vitellogenesis: its role in oocyte development. *American Zoologist* **41**, 465–476.

Tsukimura, B., Bender, J.S. & Linder, C.J. (2000) Development of an anti-vitellin ELISA for the assessment of reproduction in the ridgeback shrimp, *Sicyonia ingentis*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **127**, 215–224.

Tufail, M. & Takeda, M. (2005) Molecular cloning, characterization and regulation of the cockroach vitellogenin receptor during oogenesis. *Insect Molecular Biology* **14**, 389–401.

Tufail, M. & Takeda, M. (2008) Molecular characteristics of insect vitellogenins. *Journal of Insect Physiology* **54**, 1447–1458.

Tufail, M. & Takeda, M. (2009) Insect vitellogenin/lipophorin receptors: molecular structures, role in oogenesis, and regulatory mechanisms. *Journal of Insect Physiology* **55**, 88–104.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M. & Rozen, S.G. (2012) Primer3 – new capabilities and interfaces. *Nucleic Acids Research* **40**, e115.

Utsumi, S. (2011) Eco-evolutionary dynamics in herbivorous insect communities mediated by induced plant responses. *Population Ecology* **53**, 23–34.

Yang, F., Xu, H.T., Dai, Z.M. & Yang, W.J. (2005) Molecular characterization and expression analysis of vitellogenin in the marine crab *Portunus trituberculatus*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **142**, 456–464.