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# Female Reproductive System Morphology and the Development of a Physiological Age-Grading System for *Bagrada hilaris* (Hemiptera: Pentatomidae)

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## Abstract

In this paper, we describe the morphology of the female *Bagrada hilaris* (Burmeister) reproductive system and develop a physiological age-grading system related to egg production. The female reproductive system is composed of two meroistic and telotrophic ovaries each containing 5–6 tubular ovarioles. The ovarioles unite into the lateral oviduct which combine to form the common oviduct. The ovarioles are composed of two regions; the distal germarium and the tubular vitellarium which contains maturing follicles. Each follicle is surrounded by a layer of cells; the follicular epithelium. As the follicle passes from the ovariole to the lateral oviducts the follicular epithelial cells slough off and accumulate in the base of the ovarioles and are known as follicular relics. The continuum of ovarian development is divided into two categories: nulliparous ('nonreproducing') and parous ('reproducing'). The nulliparous category is characterized by the absence of follicular relics or eggs in the oviducts as opposed to the parous category where follicular relics and, in many cases, eggs occurred in the oviducts. The nulliparous category is divided further into two stages; N1 and N2 based on ovariole differentiation. The parous category is divided into three stages; P1, P2, and P3, based on the quantity and appearance of follicular relics. Females characterized as P3 produced three times more eggs (79.2 eggs  $\pm$  5.7) than females characterized as P1 (27.1 eggs  $\pm$  6.0).

**Key words:** follicular relics, stink bug, egg production

*Bagrada hilaris* (Burmeister), a polyphagous herbivore native to Africa, India, and Asia is an invasive pest in the United States. It first was detected in 2008 in southern California and its distribution has expanded rapidly. Currently, the northern boundary includes central California and southern Nevada, the eastern boundary reaches to western and central Texas, and the southern boundary includes northern areas in Mexico (Reed et al. 2013, Sánchez-Peña 2014, Torres-Acosta and Sánchez-Peña 2016). It possesses a relatively large host range (Bundy et al. 2018) but prefers wild and cultivated plants in the Brassicaceae. Interestingly, field populations of *B. hilaris* in the United States have declined recently in several areas, likely due to parasitism by native and introduced parasitoids as well as predation by generalist predators (Ganjisaffar et al. 2018).

*Bagrada hilaris* biology is similar to other pentatomids; however, significant differences have been observed in mating and oviposition (Palumbo et al. 2016, Bundy et al. 2018). Adults are active whenever temperature conditions are favorable, even during the winter months where they can be found congregating on plants during warm days (Reed et al. 2013). Eggs are laid singly or in small clusters in a variety of environments including the undersides of leaves,

stems, and in loose soil (Taylor et al. 2014). This soil-oviposition behavior is unique among pentatomids which typically deposit their eggs on leaves of plants in clusters or distinct rows (Bundy et al. 2018). Five instars have been reported for *B. hilaris*. Time from egg to adult varies with temperature averaging 36.4 d at 24°C to 15.6 d at 35°C (Reed et al. 2017). Females oviposit up to 217 eggs during their life span and a pre-ovipositional period of 2–14 d has been reported (Bundy et al. 2018). Adults spend a majority of their time *in copula* (Huang et al. 2013), possibly a behavior to limit or prevent other males from transferring sperm to the female as hypothesized for other insect species (McLain 1980, 1989; García-González and Gomendio 2004).

Much of the recent scientific literature on *B. hilaris* addresses its establishment and impact on crops in the United States and studies on management. For example, several studies have been published detailing the impact to broccoli and other related *Brassica* species (Reed et al. 2013, Huang 2014a, Palumbo and Carrière 2015, Joseph et al. 2017). Information on establishment and changes in distribution is also available (Bundy et al. 2012, 2018; Sánchez-Peña 2014; Palumbo et al. 2016). In addition, research has been conducted on

the efficacy of various management techniques (Ahuja et al. 2008; Obopile et al. 2008; Palumbo 2011, 2015; Reed et al. 2013; Palumbo and Carrière 2015; Palumbo et al. 2016; Joseph 2017).

Few studies have been published on *B. hilaris* behavior, physiology, and, to some extent, ecology. Host selection behavior on agricultural crops has been examined (Huang et al. 2014b) along with diel activity related to feeding based on temperature and humidity (Huang et al. 2013). The relationship between temperature and immature development was detailed by Reed et al. (2017). Interesting work on cuticular hydrocarbons and volatile emissions (De Pasquale et al. 2007, Guarino et al. 2008) provide first steps for understanding mate finding and selection. Research on reproductive physiology has concentrated on oviposition behavior (Taylor et al. 2014).

Physiological age-grading systems have been developed for a variety of insect species and use different characteristics to define physiological status (Tyndale-Biscoe 1984, Hayes and Wall 1999). Recognition of morphological changes in the reproductive system has been used to define physiological status. Characters used in these analyses include presence or absence of follicular relics and their appearance (i.e., sloughed-off cellular debris accumulating at the base of the ovarioles sometimes termed yellow bodies), bead-like dilations at the base of the ovarioles corresponding to each ovulation cycle, appearance of the follicles, and tracheation of the ovaries. Previous insects that have been studied include the boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae) (Grodowitz and Brewer 1987), the mottled water hyacinth weevil, *Neochetina eichhorniae* (Warner) (Coleoptera: Curculionidae) (Grodowitz et al. 1997), the rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) (Perez-Mendoza et al. 2004), the salvinia weevil, *Cyrtobagous salviniae* Calder & Sands (Coleoptera: Curculionidae) (Eisenberg et al. 2018), the dung beetle, *Euoniticellus intermedius* (Reiche) (Coleoptera: Scarabaeidae) (Tyndale-Biscoe 1978), the Asian hydrilla leaf-mining fly, *Hydrellia pakistanae* Deonier (Diptera: Ephydriidae) (Lenz et al. 2007), the face fly, *Musca autumnalis* De Geer (Diptera: Muscidae) (Van Geem et al. 1983), and the tsetse fly, *Glossina morsitans centralis* Machado (Diptera: Glossinidae) (Davies 1978). The research presented here describes the morphology of the female reproductive system of *B. hilaris* and compares structures to descriptions for other stink bugs. In addition, we characterized the continuum of ovarian development and used this information to develop a physiological age-grading system that correlates egg number to age classes based on distinct changes in the morphology of the reproductive system.

## Materials and Methods

### Insect Colonies and Sources

*Bagrada hilaris* adults of various ages were collected near the University of California (UCR) (Riverside, CA) from London rocket (*Sisymbrium irio* L.) and shortpod mustard (*Hirschfeldia incana* (L.) Lagr.-Foss.) in the fall of 2010. Insects were reared on a mixture of brassicaceous plants including sweet alyssum (*Lobularia maritima* (L.) Desvieux), broccoli (*Brassica oleracea* L. variety Italica), canola (*Brassica napus* L.), and mustard greens (*Brassica juncea* (L.)) grown in 4-inch pots in insect cages (BugDorm-2120, MegaView Science Co., Taichung, Taiwan) held within a greenhouse set at 30°C with natural light. With egg to adult development in 18.5 d at this temperature (Reed et al. 2017), approximately 20 generations developed per year. To maintain genetic diversity, the colony was introgressed with field-collected insects on a routine basis. Insects reared at UCR were sent to the USDA-ARS National Biological Control Laboratory (NBCL) in Stoneville, MS, to start another colony. For the colony at the NBCL, insects were

fed mustard seedlings grown in plastic trays with the addition of organic cabbage and broccoli florets. At the NBCL, insects were held in screened cages in an incubator (Percival-Scientific, Perry, IA) at 28°C with a 14:10 (L:D) h light cycle, and 60% humidity.

For determining the relationship between physiological age and egg production, adult mating pairs from the UCR colonies were collected and sent to the NBCL where they were reared in round plastic containers (15 cm diameter × 6.5 cm height, Tri-State Plastics, Inc., Covington, KY) which were modified by boring four 2.5 cm holes in the sides and covering them with small mesh brass screen to provide ventilation. Each container was lined with white paper toweling as a substrate for oviposition. Organic broccoli florets were used as food and dietary moisture; florets were replenished every 24 h. The mating pair samples at the NBCL were held in an incubator (Percival-Scientific) maintained at 25°C day:23°C night, 14:10 (L:D) h light cycle, and 80% RH.

In addition to insects reared in colonies, we also collected *B. hilaris* from the field near UCR and used them directly in experiments used to define the relationship between egg production and physiological age. For these studies, fifth instar nymphs were collected from the field, and their sex was determined upon adult emergence. Newly eclosed females were paired with wild males and maintained in 15 × 6.5 cm containers previously described.

### Insect Dissections

One hundred and thirty adult female *B. hilaris* were selected randomly from the NBCL laboratory colony. These insects were pinned through the thorax, dorsal side up to wax coated SEM stubs. The wings and pronotum were removed using iridectomy scissors exposing the dorsal abdominal cuticle. The entire individual was covered with phosphate-buffered saline (P. No. P4417, Sigma-Aldrich, Saint Louis, MO). An incision was made along the entire lateral margins of the abdomen to remove the dorsal abdominal cuticle and to allow the extraction of reproductive organs using fine-bladed forceps. Reproductive organs were placed on a microscope slide containing phosphate-buffered saline. The tissues were examined using a stereo microscope (either a Model No. M165C, Leica Microsystems, Buffalo Grove, IL, or a Model No. SZX12, Olympus, Center Valley, PA) at magnifications ranging from 7× to 63×. A variety of morphological characteristics of the reproductive system were observed including presence and appearance of follicular relics, differentiation of the follicles, and tracheation of the ovaries. Based on experience and published literature on physiological age grading, tentative physiological ages were defined.

### Physiological Age-Grading System

To study the relationship between the number of eggs produced and physiological age reflected in the morphology of the reproductive tract, fifth instar nymphs were collected from the Riverside colony or from the field at UCR. These nymphs were placed individually into small (5 cm diameter), paper-lined, ventilated Petri dishes for daily observation of adult eclosion. Upon eclosion, individuals were sexed and one female and two males of similar age were held in 5 cm Petri dishes. Mating groups were fed broccoli florets daily and observed for egg deposition and adult mortality. Approximately every other day, the number of eggs for each female was counted and the total number of eggs oviposited was determined. During the pre-oviposition and early ovipositional period, the insects were maintained in an environmentally controlled incubator (Percival-Scientific) set at 30 ± 1°C, 25–35% RH, with a 14:10 (L:D) h photoperiod. After the females started oviposition, they were sent to the NBCL for further oviposition and selection for dissection; only females that

survived the shipment were dissected. From these insects, many were dissected and ultimately 17 of the lab-reared insects and 14 of the field-collected insects were used in the age-grading analysis. These 31 insects were selected based on the range of their total number of eggs produced, which was the sum of the eggs laid and eggs in the oviducts. It is important to include eggs in the oviducts as well as eggs oviposited since they both contribute to morphological changes in the ovaries because they have passed through the lumen of the ovariole causing epithelial cells to slough off.

### Statistical Analysis

Analysis of variance (ANOVA), assumption tests including Levene test of homogeneity of variances and Brown–Forsythe test of homogeneity of variances, and post hoc test Newman–Keuls were performed using Statistica version 13 (TIBCO 2017) examining differences in mean number of eggs for each parous stage. Unless noted otherwise, significant differences were determined at  $P = 0.05$ . In addition, Pearson product-moment correlation analysis was used to further define the relationship between the number of eggs produced and the chronological age of the females.

### Results and Discussion

This study showed that the morphology of the female reproductive system of *B. hilaris* is similar to other pentatomids (Pendergrast 1957; Adams 2000, 2001). It is composed of two ovaries each containing from 5, to more commonly, 6 tubular ovarioles per ovary ( $5.9 \pm 0.08$  SE), where the follicles (i.e., immature eggs) develop and mature (Fig. 1). The ovarioles unite into the lateral oviduct which then combines with the lateral oviduct from the other ovary forming the common oviduct (Fig. 1). The ovaries are dorsally located, and each is somewhat opposed laterally to the medial line.

The ovarioles are composed of two regions: the distal germarium containing trophic tissue, oogonia, and oocytes (Chapman 1998), and the tubular vitellarium which houses the maturing follicles (Fig. 2). For ovaries which are fully reproductive, the mean length of the germarium was  $0.404 \text{ mm} \pm 0.051$  SE ( $n = 11$ ) while the vitellarium was twice that length at  $0.92 \text{ mm} \pm 0.080$  SE ( $n = 12$ ).

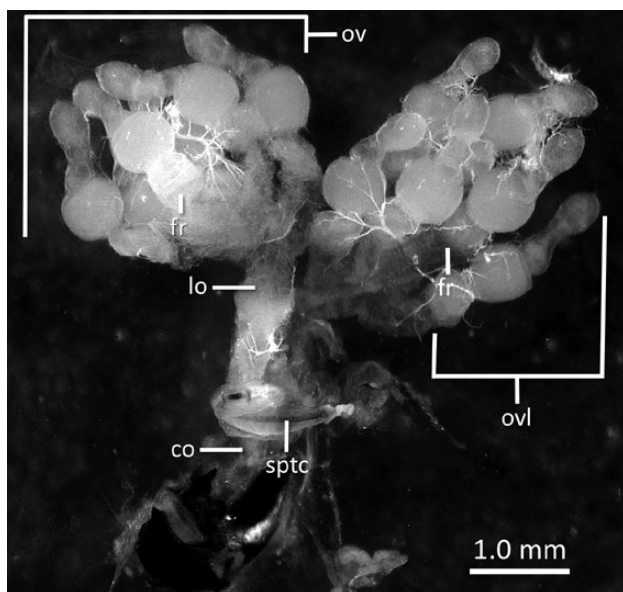


Fig. 1. Ovarian morphology of *Bagrada hilaris* (ov, ovary; fr, follicular relics; lo, lateral oviduct; co, common oviduct; sptc, spermathecal complex).

There was some variation in the size of these structures largely due to sample preparation and reproductive status. In addition, the size of the vitellarium is dependent on the maturity of the most proximal follicle which increases in length as it matures. A thin strand of tissue located at the apex of each ovariole, known as the terminal filament, along with a high number of tracheae hold the ovarioles from a single ovary together as one unit as well as anchor it to the interior wall of the cuticle. The ovarioles are enclosed in a thin layer of tissue known as the ovariole sheath (Chapman 1998) and the material between developing follicles connecting adjacent follicles is the interfollicular tissue (Fig. 3).

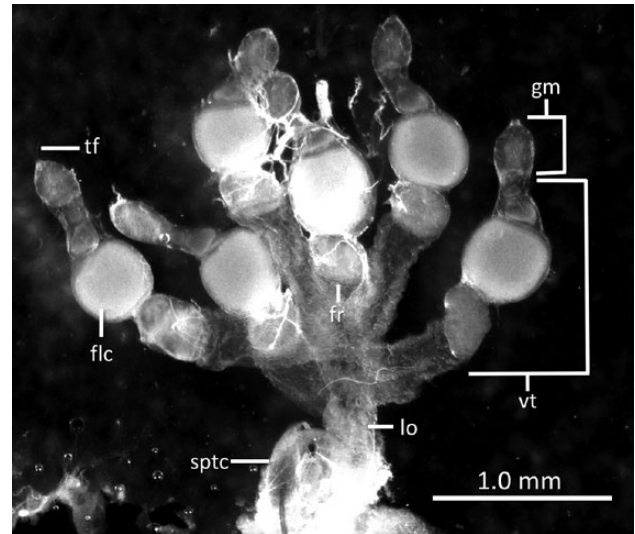


Fig. 2. Morphology of a single ovariole of *Bagrada hilaris* (gm, germarium; vt, vitellarium; tf, terminal filament; flc, follicle; sptc, spermathecal complex; lo, lateral oviduct; fr, follicular relic).

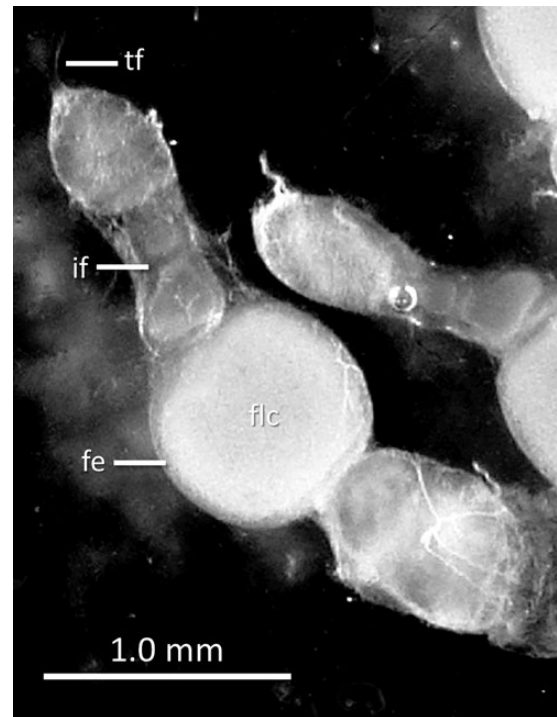


Fig. 3. Morphology of a single ovariole of *Bagrada hilaris* (tf, terminal filament; if, interfollicular tissue; fe, follicular epithelium; flc, follicle).

Each follicle is surrounded by a layer of epithelial cells or follicular epithelium. A gradient of follicle maturities can be found along the length of the ovarioles, with the more mature follicles situated just distal from the lateral oviducts. The follicles are nearly transparent when first formed but become more opaque as they mature and fill with yolk. As the follicle passes from the ovariole to the lateral oviducts, the follicular epithelial cells slough off and accumulate at the base of the ovarioles and are collectively known as follicular relics or yellow bodies (Figs. 1, 2, and 6) (Tyndale-Biscoe 1984). A follicle becomes an egg once it passes from the ovariole and enters the lateral oviducts, a process known as ovulation.

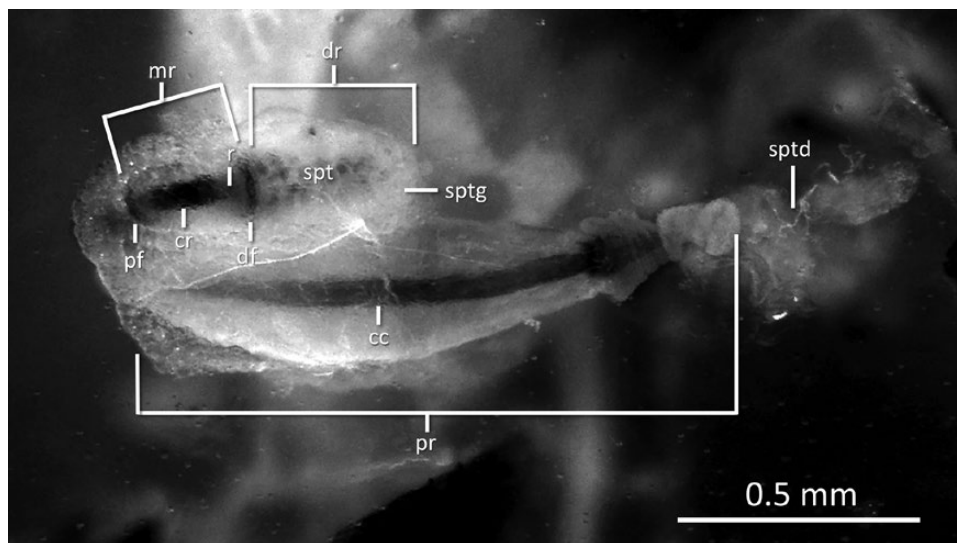
The ovarioles in *B. hilaris* are both meroistic and telotrophic. The follicles are meroistic since specialized nurse cells are present (Chapman 1998) and contained in the germarium. In comparison, panoistic ovarioles occur in the more primitive insect orders (e.g., Odonata, Plecoptera) and do not have specialized nurse cells. In *B. hilaris*, the ovarioles are considered telotrophic since specialized nurse cells, which transport nutrients to the follicles, remain in the germarium and are connected to each follicle via a nutritive chord (Chapman 1998). This is opposed to polytrophic ovarioles, where specialized nurse cells remain with each developing follicle and are discarded and accumulate in the ovariole base once the follicle is ovulated as in *H. pakistanae* (Lenz et al. 2007).

As eggs pass through the common oviduct they are fertilized by sperm contained within the spermatheca, which is part of an elaborate spermathecal complex. The spermathecal complex in *B. hilaris* is similar to other pentatomids (Candan et al. 2015, Souza et al. 2015). It arises from the medial or distal portion of the common oviduct via the spermathecal duct (Figs. 4 and 5). It is composed of three main regions including the distal, median, and proximal regions with the spermathecal duct arising from the common oviduct and connecting to the proximal region. The proximal region appears as an oblong sac that expands in girth. Differences in the size of the proximal region can be seen by comparing Fig. 5a to Fig. 5b with the proximal region in Fig. 5b nearly twice the size as that shown in Fig. 5a. Since this expansion was present only in mated females, we conclude that the expansion is related to mating. It is unknown

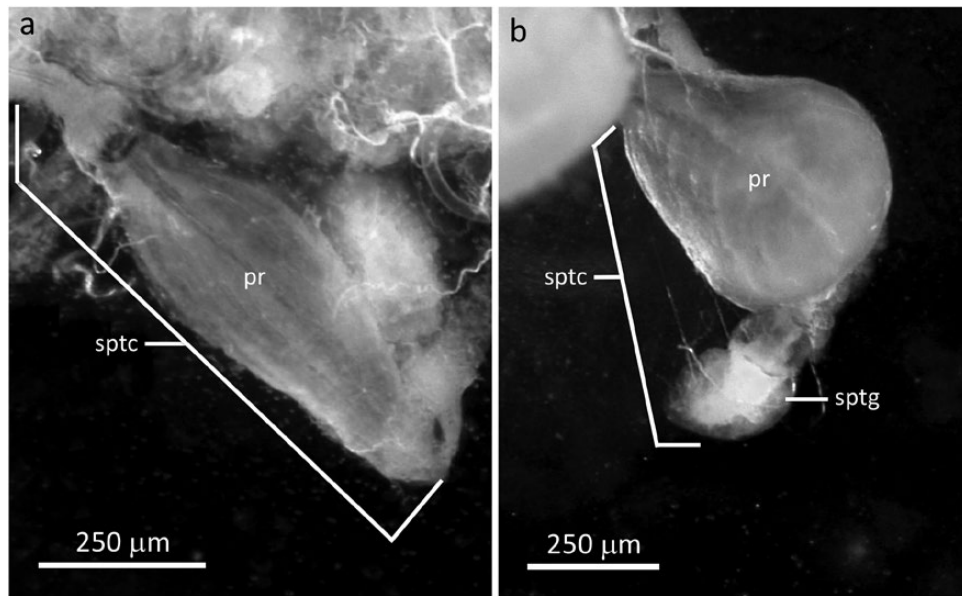
what material allows the expansion of the proximal region since we never observed sperm in the sac. Medially, along the entire length of the interior of the proximal region is the cuticular channel which appears to be sclerotized and evidently involved in sperm delivery. The medial and distal regions are complex, being composed of a series of flanges and an interesting structure between the distal and proximal flanges; the coiled region (Fig. 4). The most distal portion of the spermathecal complex contains the spermathecal bulb which houses the sperm. The spermathecal bulb is covered by glandular tissue presumed to be the spermathecal gland and can barely be discerned in Fig. 4. The only structure in the spermathecal complex observed to contain sperm was the spermathecal bulb.

As discussed previously, cellular debris that accumulates at the base of the follicles as they pass from the ovarioles to the lateral oviducts are collectively known as follicular relics. Follicular relics are important structures that have been shown to be instrumental in determining number of eggs produced in various insect species (Tyndale-Biscoe 1984, Grodowitz and Brewer 1987, Grodowitz et al. 1997, Hayes and Wall 1999, Perez-Mendoza et al. 2004, Lenz et al. 2007). While follicular relics can be bright orange in some species, such as *Oxyops vitiosa* (Pascoe) (Coleoptera: Curculionidae) (Pratt et al. 2018), they are pale to a very dark brownish yellow in *B. hilaris* (Fig. 6a and b). Follicular relics in *B. hilaris* are typically expressed as a narrow band that can completely encircle the follicle base. The yellow to orange color of the follicular relics is thought to arise from an accumulation of beta-carotenes (Tyndale-Biscoe 1984, Hayes and Wall 1999).

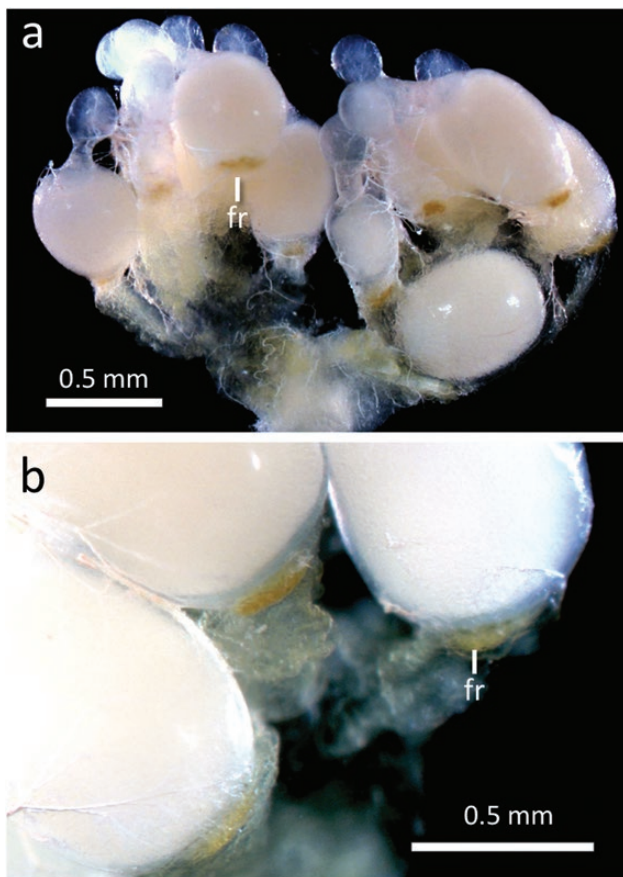
The continuum of ovarian development was separated into two broad categories; nulliparous ('not reproducing') and parous ('reproducing'). The nulliparous category is characterized by no follicular relics at the base of the ovarioles or eggs present in either oviduct as opposed to the parous category where follicular relics and, in many cases, eggs were present in the oviducts. However, the defining characteristic is the presence or absence of follicular relics. Each of the main categories was further subdivided into different stages or physiological ages using characteristics based on those provided in Grodowitz and Brewer (1987), Grodowitz et al. (1997), Perez-Mendoza et al. (2004), and Lenz et al. (2007). For the nulliparous



**Fig. 4.** Morphology of the spermathecal complex of *Bagrada hilaris* (pr, proximal region; mr, medial region; dr, distal region; sptg, spermathecal gland; sptd, spermathecal duct; df, distal flange; r, deformable ring; cr, coiled region; pf, proximal flange; spt, spermatheca; cc, cuticular channel). Note that the spermathecal gland covers the actual spermatheca in the distal region of the spermathecal complex almost completely obscuring the spermathecal bulb where sperm is stored.



**Fig. 5.** Spermathecal complex of *Bagrada hilaris* showing changes in the proximal region apparently associated with mating and in some cases actual sperm transfer (pr, proximal region; sptc, spermathecal complex; sptg, spermathecal gland). Note the swollen appearance of the proximal region of the complex (b) as compared to that shown in (a). The swollen appearance of the proximal region of the spermathecal complex is often observed after mating. No sperm was observed in the proximal region only a gelatinous sometimes hardened material filling the sac surround the cuticular channel.



**Fig. 6.** Accumulation of cellular debris known as follicular relics located at the base of the ovarioles of *Bagrada hilaris* (a and b) which typically arise from epithelial cells surrounding the ova that are sloughed off during ovulation (fr, follicular relic).

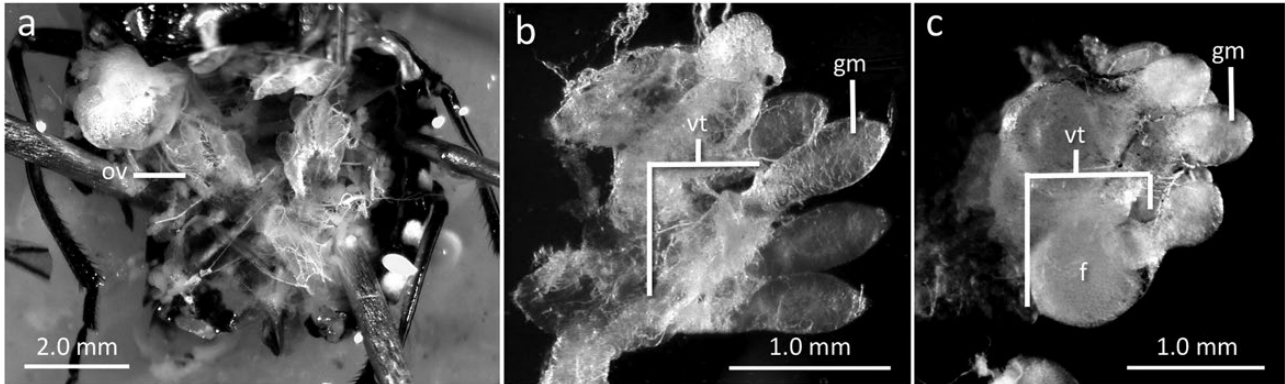
category, two physiological ages are recognized and include N1 and N2 (Table 1; Fig. 7a and b). In the N1 stage, the ovarioles are small and undifferentiated, appearing as simple tubes that are often highly tracheated. No follicular relics are present in the N1 stage. In comparison, the N2 stage is characterized by the ovarioles being differentiated into distinct follicles with the more proximal follicles appearing semitransparent to opaque depending on yolk quantity. As in the N1 stage, no follicular relics are present in the ovariole bases and no eggs are observed in the oviducts.

The parous category is subdivided into three stages: P1, P2, and P3 (Table 1; Fig. 8). These stages are characterized mainly by differences in follicular relic appearance and in some cases quantity. For all stages, eggs may or may not be present in the oviducts. For the P1 stage, follicular relics are present and appear pale yellow in coloration and do not surround the ovariole base (Fig. 8a and b). In comparison, females in the P2 stage have follicular relics surrounding the ovariole base that range from a pale yellow to dark yellow in coloration (Fig. 8c and d). The final stage, P3, had follicular relics that are bright to dark yellow that may or may not surround the ovariole base. In addition, distinct dark particles are contained within the follicular relics (Fig. 8e and f). It is thought that the dark particles arise from compression of the follicular relics as high numbers of follicles move through the small lumen of the ovarioles into the lateral oviducts (Grodowitz and Brewer 1987, Grodowitz et al. 1997, Lenz et al. 2007).

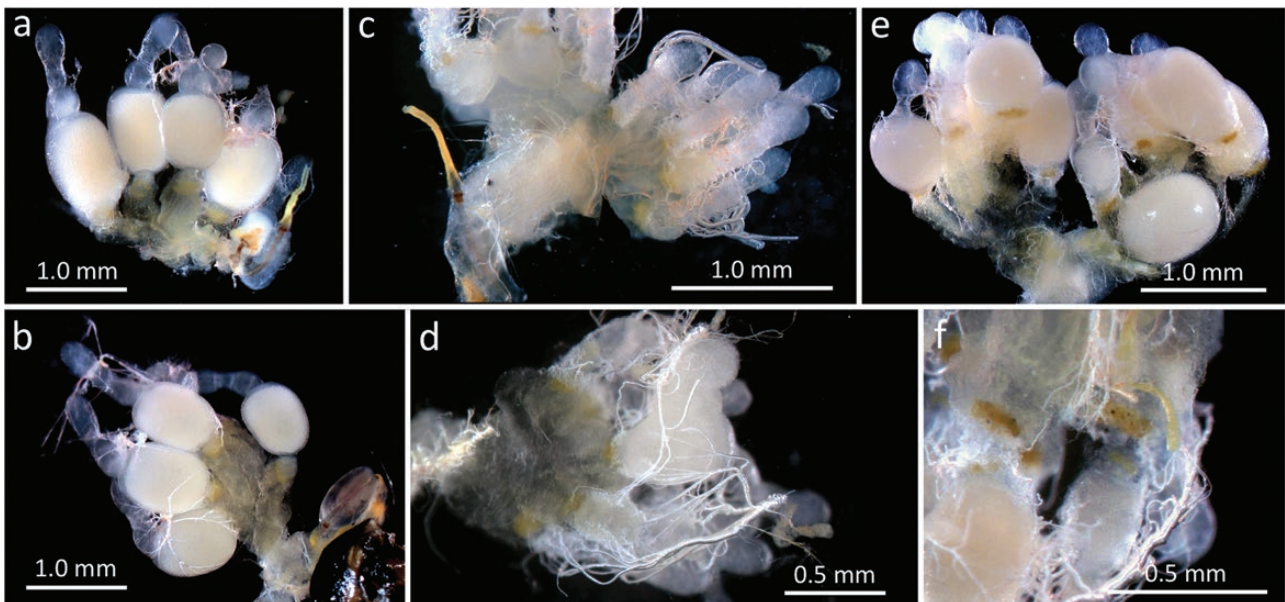
Based on dissections of numerous females, we were able to use follicular relics to create distinct categories in the reproductive system that were related to number of eggs produced. Similar separations based on the appearance and to some extent quantity of follicular relics have been shown to be valid for other insect species (Grodowitz and Brewer 1987, Grodowitz et al. 1997, Hayes and Wall 1999, Perez-Mendoza et al. 2004, Lenz et al. 2007). For females that ovulated a small number of eggs, a thin pale-yellow band not completely encircling the ovariole base was present. As more and more eggs are ovulated one would expect higher amounts of

**Table 1.** Characteristics associated with each physiological age for *Bagrada hilaris*

|   | N1  | N2     | P1          | P2                             | P3                |
|---|-----|--------|-------------|--------------------------------|-------------------|
| Ovarioles differentiated                    | No  | Yes    | Yes         | Yes                            | Yes               |
| Follicles with yolk                         | No  | Yes/no | Yes         | Yes                            | Yes               |
| Follicular relics present                   | No  | No     | Yes         | Yes                            | Yes               |
| Follicular relic color                      | N/A | N/A    | Pale yellow | Yellow to dark brownish yellow | Dark brown yellow |
| Follicular relics surrounding ovariole base | N/A | N/A    | No          | Yes                            | Yes/no            |
| Dark particles present in follicular relics | N/A | N/A    | No          | No                             | Yes               |
| Eggs present in oviducts                    | No  | No     | Yes/no      | Yes/no                         | Yes/no            |



**Fig. 7.** Nulliparous stages, N1 (a) and N2 (b and c) of ovarian development for *Bagrada hilaris* (ov, ovary; gm, germarium; vt, vitellarium; f, follicle). In the N1 stage, the ovarioles are small and undifferentiated, appearing as simple tubes that were often highly tracheated. No follicular relics are present in the N1 age. In comparison, the N2 age is characterized by the ovarioles being differentiated into distinct follicles with the more proximal follicles appearing semitransparent to opaque depending on yolk quantity.



**Fig. 8.** Parous stages P1 (a and b), P2 (c and d), and P3 (e and f) of ovarian development for *Bagrada hilaris*. For the P1 stage, follicular relics are present and appeared pale yellow in coloration and did not completely surround the ovariole base. In comparison, females in the P2 stage the follicular relics completely surrounded the ovariole base and ranged from a pale yellow to dark yellow in coloration. The final stage, P3, had follicular relics that are bright to dark yellow that may or may not surround the ovariole base, with distinct darkened particles contained within the follicular relics (Fig. 8e and f).

follicular relics to accumulate in the ovariole base hence the complete encircling of the ovariole base often with a darker yellow color. With successive ovulations compression of the cellular debris would occur causing the darker yellow coloration and formation of darkened particles. Thus it is logical to assume that the quantity of the follicular relics would increase with successive ovulations such that

determining their volume would suffice in relating eggs ovulated to follicular relic deposition. However, we must be cautious here because it has been shown for other insects that follicular relics can be flushed out of the ovariole base during the process of ovulation or can be reabsorbed during periods of nutrient insufficiency (Tyndale-Biscoe 1984).

Another factor that validates the selected morphological separations into distinct physiological stages is the association between number of eggs and the parous stages. Significantly more eggs were associated with the later physiological ages ( $F_{(2,28)} = 19.734$ ,  $P < 0.000004$ ; Fig. 9). The mean number of eggs for the P3 stage was almost threefold higher than that found for the P1 stage. There was some overlap in the number of eggs between each physiological age, but such overlap is common for physiological age-grading systems using female ovarian morphology as demonstrated for the boll weevil, *A. grandis grandis* (Grodowitz and Brewer 1987), the mottled water hyacinth weevil, *N. eichhorniae* (Grodowitz et al. 1997), and the Asian hydrilla leaf-mining fly, *H. pakistanae* (Lenz et al. 2007).

There was a significant positive correlation ( $n = 31$ ,  $P < 0.05$ ,  $r = 0.435$ ) between the number of eggs produced and chronological age (i.e., number of days from adult eclosion; Fig. 10). While this

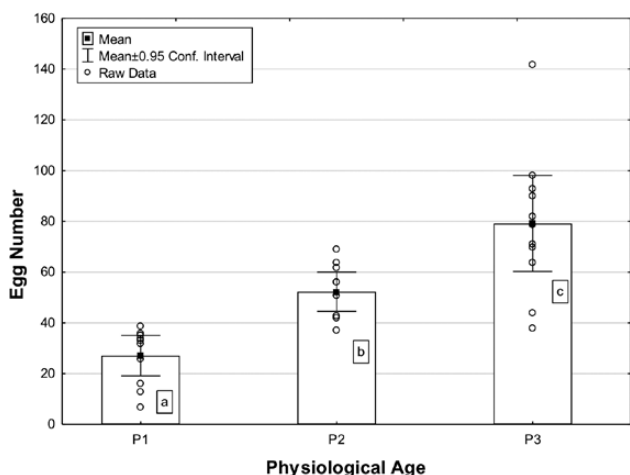


Fig. 9. Relationship between number of eggs produced and parous physiological stages for all females examined. Note bars followed by the same letter are not significantly different ( $F_{(2,28)} = 19.734$ ,  $P = 0.000004$ ).

relationship was statistically significant, it explained only 43.5% of the variation, indicating that there are other sources of variation impacting the relationship. These might include substandard rearing conditions in the colonies from which the insects used in this study were collected, past nutritional exposure especially for wild individuals, or interruption in egg production that occurred during the shipping of insects from Riverside to Stoneville.

We did not observe high numbers of eggs in the lateral and common oviducts for any parous physiological age nor were there any significant differences ( $F_{(2,28)} = 2.11$ ,  $P > 0.1402$ ) noted for eggs in the oviducts between the parous stages. Overall number of eggs in the oviducts for all parous stages averaged 1.8 eggs  $\pm$  0.602 SE. This coincides with the ovipositional behavior of *B. bilaris*, which prefers to lay eggs singly or in small batches. This is an unusual ovipositional strategy compared to other pentatomids that typically oviposit high number of eggs in single batches or clusters (McPherson 2018). For example, *Nezara viridula* (L.) (Hemiptera: Pentatomidae) oviposits clusters of eggs that can exceed 80 eggs per cluster yet only possess 7 ovarioles per ovary for a grand total of 14 ovarioles. It appears that *N. viridula* matures the most proximal follicles simultaneously across all ovarioles. Hence, they can only ovulate 14 eggs per cycle and subsequently hold the mature eggs till suitable numbers are ready to oviposit. The eggs are held in the oviducts where greater than 100 eggs have been observed (M. J. Grodowitz, unpublished data).

The determination of physiological age based on changes in the reproductive system can provide important information on past, present, and to some extent, future reproductive activity. In comparison, while chronological age (number of days since adult emergence) can be used to infer information on reproductive status, other factors like nutrition can have a profound impact on reproductive activity. Therefore, chronological age typically does not accurately reflect reproductive status (Hayes and Wall 1999). Importantly, physiological age-grading has been used with success for many insect species though primarily for insects of medical and veterinary importance. Hayes and Wall (1999) discuss the importance of vector potential for such insect species with implications for disease spread. Dethier

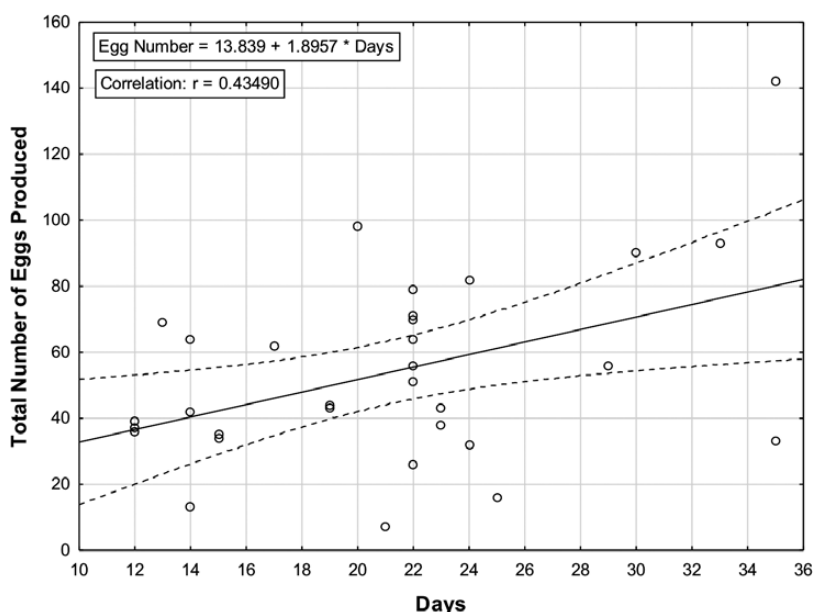


Fig. 10. Relationship between number of eggs produced and age (days) of the adults from time of eclosion. Correlation is significant at  $P < 0.05$  with an  $r$ -value = 0.435.



(1976) found that, for some species of Diptera, their location in different environments was related to the availability of protein sources needed for egg development. Similarly, Van Geem et al. (1983) and Van Geem and Broce (1986) found that physiological age in female *M. autumnalis* was correlated to physiological demands for protein and hence their location in the environment (i.e., feeding on the eye to obtain significant proteins for egg development).

The determination of reproductive status based on the condition of the female ovaries in stink bugs also has been used with some success. For example, Herbert and Toews (2011) assessed reproductive condition for *Euschistus servus* (Say) (Hemiptera: Pentatomidae) seasonally and for different crops. They found that the occurrence of late instars and nonreproductive adults was low for corn, cotton, and peanut, and speculated that these hosts may not be suitable for reproduction in this species. In addition, it has been shown that insect behavior often is driven by its physiology. Thus, understanding the current reproductive state of *B. hilaris* may help explain its movement from one host plant to another as well as behavioral activity on and around the plant, especially in relation to reproduction and mating activity. *Bagrada hilaris* is known to shift from cruciferous weeds during the winter months to brassicaceous agricultural crops during the spring. These host switches at certain times of the year may be related to nutritional requirements relative to reproduction, and the physiological age-grading system presented in this paper may be a useful tool to investigate these relationships. Finally, developing reproductive life tables based on physiological age for field populations of *B. hilaris* should provide information on past reproductive activity (including a quantitative estimate of egg production), as well as future reproductive potential. For example, field populations consisting mainly of females in the early physiological ages may indicate the potential for substantial increases in the population as opposed to populations consisting of older physiological ages which would indicate a population in decline.

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