Ovarian physiology and age-grading in the rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae)☆

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Abstract

Physiological and morphological changes in the ovarian system in rice weevils, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), reared on wheat seeds were studied as a function of adult age, mating status, and nutrient availability. X-ray analysis was used to determine time of adult eclosion and the duration of development of pre-emergent weevils within the seeds, a process that lasted almost 4 days at 25°C and 60% r.h. There was no follicular differentiation in pre-emergent weevils. Oocyte maturation began after adults emerged from the seeds and started to feed. There was a significant increase in mean germarium length and the size of proximal follicles within the first 5 days when newly emerged weevils were mated and fed *ad lib*. Maximum number of follicles and mature eggs per ovariole in mated females occurred at 5–30 days of age. The number of mature eggs decreased in 60-days-old weevils, at the same time that adult mortality increased. Development of the ovarian system was much slower in unmated females than in mated females. Although there was follicle development in unmated females, ovulation never occurred and no eggs were laid. Starvation of mated females resulted in a rapid reduction in numbers of follicles and mature eggs, probably as a result of oosorption. Females were categorized into two nulliparous and three parous stages according to ovarian development and the degree of accumulation of follicular relics. Parity was directly correlated with both weevil age and the number of progeny produced and was the physiological basis used to construct an age-grading model for this species. The method will be useful for determining the age structure and reproductive potential of rice weevil populations in the field.

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Keywords: *Sitophilus oryzae*; Ovarian development; Follicular relics; Mating status; Starvation; Age-grading

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1. Introduction

Determination of the age structure of adult insect populations can improve our understanding of the ecology and population dynamics of a species, particularly one with overlapping generations. Age structure can be used to construct time-specific life-tables to characterize factors that regulate fluctuations in population size and dispersal rate, as well as to monitor fertility and mortality. Furthermore, these data can be included in simulation models developed to predict populational responses to control programs or to predict outbreaks of pests in response to environmental changes (Tyndale-Biscoe, 1984). Methods for age determination of insects are based primarily on changes in the reproductive system, changes in cuticle structure, or somatic changes (Hayes and Wall, 1999).

Changes in the ovarian system can give a measure of physiological age of the insect. However, in many species, particularly Diptera of medical and veterinary importance, physiological age does not always equal chronological age because ovarian development is regulated not only by temperature but also by nutrient availability, both of which can fluctuate dramatically (Tyndale-Biscoe, 1978). In contrast to these species, insects associated with stored grain live in a relatively stable environment in which changes in abiotic and biotic conditions occur very slowly (Bailey, 1992). In particular, nutrients are usually available in excess and can extend through one or two storage seasons. In this ecosystem, and under these conditions, the physiological age of an insect can closely approximate to its chronological age.

Age-grading techniques based on physiological changes in the reproductive system include physical changes in ovaries and in the tracheoles supplying the ovaries, a decline in fecundity, and the accumulation of follicular relics or “yellow bodies” (Tyndale-Biscoe, 1984; Hayes and Wall, 1999). Follicular relics are fragments of follicular tissue that accumulate at the base of the ovariole during ovulation. Follicular relics can also form as a result of egg resorption (Tyndale-Biscoe and Watson, 1977). Through successive ovulations, the follicular relics become compressed by the action of follicles passing through the constricted opening into the lateral oviduct. As more ovulations occur, distinctly darker particles become apparent within the follicular relics. Hence, changes in the appearance of follicular relics (i.e., darkening) typically indicate a greater number of ovulations (Grodowitz et al., 1997).

Follicular relics occur in a wide range of insects and have been used in age determination of several flies, including the Australian sheep blow fly, Lucilia cuprina (Wied.) (Vogt et al., 1974); face flies, Musca autumnalis De Geer (Van Geem et al., 1983); stable flies, Stomoxys calcitrans (L.) (Lehane et al., 1986); Australian bush fly, Musca vetustissima Walker (Vogt and Walker, 1987); and the blow fly, Lucilia sericata Meigen (Wall et al., 1991). In addition to these Diptera, coleopterans such as the dung beetle, Euoniticellus intermedius (Reiche) (Tyndale-Biscoe, 1978); the Australian dung beetle, Onthophagus granulatus Boheman (Tyndale-Biscoe et al., 1981); the boll weevil, Anthonomus grandis Boheman (Grodowitz and Brewer, 1987); the weevil, Neochetina eichhorniae (Warner) (Grodowitz et al., 1997); and the larger grain borer, Prostephanus truncatus (Horn) (Scholz et al., 1998), have been aged using this method.

The rice weevil, Sitophilus oryzae (L.) (Coleoptera: Curculionidae), is a long-lived pest of stored cereals in the United States (Coombs and Porter, 1986) and in tropical and warm temperate regions of the world (Champ and Dyte, 1976). The female reproductive system in S. oryzae is of the meroistic/telotrophic type, in which nurse cells, or trophocytes, are present in the germarium
and are connected to oocytes in early stages of their development by trophic filaments called nutritive cords (King and Bünning, 1985). There is a pair of ovaries, each composed of two ovarioles (Fig. 1), in which the oocytes develop and mature (Murray and Tiegs, 1935). The ovarioles are divided into two main regions: the more distal and somewhat enlarged germarium in which oocytes are produced from oogonia, and a more proximal vitellarium in which oocytes grow and mature. In addition, the gerarium contains an apical cell mass (bacteriome) that harbors symbiotic bacteria (Mansour, 1930; Nardon, 1971). The vitellarium contains a series of follicles (oocytes with a surrounding follicular epithelium) in successive stages of development. A lateral oviduct connects the two ovarioles per ovary to each other. As the follicle passes through the constricted opening of the lateral oviduct, the follicular epithelium is stripped from the follicle and the egg passes into the lateral oviduct. The lateral oviducts from the two ovaries unite to form the common oviduct that joins the bursa copulatrix. Sperm stored within the spermatheca pass through the spermathecal duct into the bursa copulatrix where the eggs are fertilized (Khan and Musgrave, 1969).

We describe below a method of age grading weevils that is based on physiological changes in the reproductive system as a function of age, mating status, and nutrient availability. In addition, we present data on ovarian anatomy and development in the pre-emergent adult stage (newly eclosed adults that remain inside the kernels) prior to their emergence from the seeds.

Fig. 1. Reproductive organs of female Sitophilus oryzae showing general structure of the ovaries (ov, ovary; ovl, ovariole; v, vitellarium; ge, germarium; bovl, base of ovariole; lovid, lateral oviduct; covid, common oviduct).
2. Materials and methods

2.1. Insect cultures

Adult *S. oryzae* used in this study were obtained from a laboratory strain reared on whole kernel, hard red winter wheat, *Triticum aestivum* L. Weevil cultures were initiated by introducing 200 unsexed adults into 200 g of wheat (13.5% moisture content) in 800 ml glass jars that had screen/filter paper lids. Jars were held at 30 ± 1°C and 70 ± 5% r.h. with a 12:12 L:D photoperiod. All founding adults were removed after 4 days. Seeds containing weevil larvae and pupae were detected by X-ray analysis (Throne, 1994) of 21-day-old cultures. Infested seeds were placed in a single layer on sheets of cellulose (previously exposed 13 × 18 cm² radiographs) that were coated with double-stick tape. A sheet of wheat kernels was placed on a sheet of film (Kodak Industrex M X-ray film in Ready Pack II foil packs, Eastman Kodak, Rochester, NY) placed 56 cm below an X-ray source (Model 43855A Faxitron, Hewlet-Packard, McMinnville, OR), and exposed for 3 min at 18 kV and 3 mA. Negatives were examined under a stereomicroscope at a minimum of 12 × magnification for the presence of weevil larvae or pupae.

2.2. Morphology and changes in the reproductive system of pre-emergent females inside the seed

Development of rice weevil ovaries in pre-emergent adults was studied. X-ray analysis was used to obtain seeds containing pupae. These seeds were held at 25 ± 1°C and 60 ± 5% r.h. with a 12:12 L:D photoperiod and were X-rayed twice a day to determine when adult eclosion occurred. Adults were removed from the seeds by cutting seeds with a razorblade at 0, 24, 48, and 72 h after eclosion. Ovarian development was determined for 20 females at each age interval (see next section for methods). We also determined ovarian development in 20 females within 12 h after emergence from the seed, and in 20 females each at 24 and 48 h post-emergence. Time spent as a pre-emergent adult was determined in separate groups of 50 males and 50 females.

2.3. Morphology and changes in the reproductive system of mated and unmated females during adult life

Total progeny production and follicular relic formation were determined for females that were allowed to oviposit for different time periods up to 60 days. X-ray analysis was used to obtain seeds containing large larvae and pupae. These infested seeds were placed individually in 13 mm × 100 mm glass tubes and checked daily for adult emergence. Newly emerged adults from seeds were sexed (Halstead, 1962) and isolated in individual tubes. Virgin males and females that had emerged during the previous 24 h were paired in tubes that contained 5–8 wheat seeds. After 24 h, two pairs were placed in each of 70 plastic vials (3.2 cm × 8 cm) with snap-cap screen lids, that contained 20 g of uninfested wheat. These vials were arranged into groups of ten, and females were allowed to oviposit for 5, 10, 20, 30, 40, 50, or 60 days at 25 ± 1°C and 60 ± 5% r.h., with a 12:12 L:D photoperiod. We placed two females in each vial to ensure that we had enough females for dissection while also reducing the number of vials that had to be set up and maintained, as compared to having one female per vial. After each time period (including 0 time or newly mated females), the 20 parental females were removed from the vials (10 reps × 2♀/rep). There was no
mortality among these females, even during the longer oviposition periods. These females were immediately dissected to determine morphological changes in the ovarian system. In addition, the number of adult progeny was determined (see below).

Weevils were dissected under cold Ringer’s solution (Presnell and Schreibman, 1997) by using a stereomicroscope equipped with an ocular micrometer (25 units = 1 mm at 25 × ). The elytra and metathoracic wings were excised, and the reproductive system was removed with fine forceps. Ovarian development was assessed at specific time intervals by measuring the length of one germarium and estimating the size of the largest proximal follicle from each female dissected at each specific age. The size estimate was based on the formula for the area of an ellipse, \( \pi ab \), where \( a = \text{length}/2 \) and \( b = \text{width}/2 \). In addition, we counted the number of follicles and eggs present in each ovariole and determined the degree of follicular relic formation. Follicular relic formation and extent of ovarian development were used to characterize degree of parity.

Number of adult progeny produced during a time interval was determined by incubating the wheat in each vial until adult emergence was complete. Total adult progeny numbers were expressed on a per parental female basis. Although there was no parental mortality, we did not determine the extent, if any, of immature mortality in these tests, and make the assumption that immature mortality was similar within each ovipositional time period. Emerged adults were counted and removed every 5 days. For weevils held longer than 30 days, the parent weevils were transferred to new wheat after 30 days, so that emerging progeny could be differentiated from the parents.

Follicular relic formation resulting from egg resorption, as a function of age of unmated females, was also studied. Unmated females were obtained as above. Two unmated females that had emerged from seeds during the previous 24 h were placed in each of 80 vials that contained 20 g of uninfested wheat as above. These vials were divided into groups of ten, and females were allowed to feed for 1, 5, 10, 20, 30, 40, 50, 60, or 70 days. At appropriate intervals, 20 virgin females were removed (10 reps × 2 \( \frac{g}{\text{rep}} \)) and immediately dissected to determine morphological changes in the ovarian system.

2.4. Effects of starvation on egg resorption

To determine if starvation and subsequent oosorption affected ovarian development and formation of follicular relics, seven groups of females were isolated from a culture containing 3-week-old adults. One group of 20 females was dissected immediately to determine ovarian development. Three groups of 20 females were placed in Petri dishes (15 × 100 mm) without food, and three groups of 10 females were kept with food (wheat seeds) as controls. The dishes were maintained at 25 ± 1°C and 60 ± 5% r.h., with a 12:12 L:D photoperiod. Females (20 starved and 10 fed per time period) were removed from the dishes after 24, 48, and 72 h and dissected to determine the extent of follicular relic formation and ovarian development.

2.5. Statistical analysis

We analyzed measurement data by using analysis of variance (ANOVA) (PROC ANOVA and GLM, SAS Institute, 1998). The relationship between physiological age and progeny production was determined by linear regression (PROC REG, SAS Institute, 1998).
3. Results

3.1. Reproductive system of pre-emergent and newly emerged females

All females dissected in this study had two ovaries, each with two ovarioles. The ovarioles of pre-emergent females (newly eclosed but still inside the seed) were small, 0.74 ± 0.14 mm in length, relative to those of older females. The germarium was the most prominent feature of the ovariole and, unlike older females, there was no clear differentiation between the germarium and the vitellarium. The ovaries were almost transparent with no apparent follicular differentiation (Figs. 2A and B).

Ovarioles of newly emerged females (adults that have just exited the seeds) had no detectable follicular differentiation, but the germarium had begun to show prefollicular tissue (Fig. 2C). Ovaries from 24-h-old emerged females began to show clear differentiation between the germarium and the vitellarium, with distinct follicles clearly visible (Fig. 2D). The most proximal follicle was transparent, and the ovarioles were more than 2-fold larger than those of pre-emergent females.

The length of the pre-emergent adult stage for males (i.e., the time from adult eclosion until emergence from the seed) lasted an average of 96 h (range 76–122 h), and more than 80% of males emerged from seeds between 85–110 h of eclosion. The length of the pre-emergent adult stage for females lasted an average of 108 h (range 83–166 h), and more than 80% of females emerged from seeds between 90–130 h of eclosion.

3.2. Reproductive system of emerged mated and unmated females

Ovaries from young females (10- to 20-d-old) contained more follicles and mature eggs than ovaries from older females (Fig. 3). Germarium length differed with age ($F = 37.7; df = 7, 291; P < 0.01$) and mating status ($F = 123.8; df = 1, 291; P < 0.01$), and there was interaction between age and mating status ($F = 30.1; df = 7, 291; P < 0.01$). There was a dramatic increase in mean germarium length in mated females within the first 5 days; germarium length increased more slowly in non-mated females (Fig. 4A). After attaining a maximum size within several days after emergence and immediate mating, germarium length did not change or was slightly smaller through 60 days. In contrast, unmated females did not have the same dramatic increase in germarium length subsequent to emergence from the seed, but had a more gradual increase in germarium length throughout development. Nevertheless, after about 40 days, the mean length of germaria of both mated and unmated weevils was similar.

We measured the size of the proximal follicle because it contains the most mature oocyte. The size of proximal follicles differed with age ($F = 29.4; df = 7, 291; P < 0.01$) and mating status ($F = 96.5; df = 1, 291; P < 0.01$), and there was interaction between age and mating status ($F = 14.8; df = 7, 291; P < 0.01$). Mean size of proximal follicles of mated females increased ca. 2.4-fold within the first 5 days after emergence from seeds and mating (Fig. 4B). This period of rapid growth was followed by a steady reduction in proximal follicle size throughout the remainder of adult life. There was a similar pattern of follicle growth in unmated females except development was delayed about 1 week, compared with that of mated females, and the maximum mean size of proximal follicles was significantly smaller ($F = 65.2; df = 1, 291; P < 0.01$).
Fig. 2. Ovaries of newly eclosed and 72-h-old pre-emergent and newly emerged and 24-h-old female adults of *Sitophilus oryzae*.
Fig. 3. Effect of age on the condition of the ovarian system of mated female Sitophilus oryzae.
The number of immature follicles (defined as those present in the ovarioles but not including those in the lateral and common oviducts) differed with age \((F = 18.9; \text{df} = 7, 231; P < 0.01)\) and mating status \((F = 13.7; \text{df} = 1, 231; P < 0.01)\), and there was interaction between age and mating status \((F = 6.5; \text{df} = 7, 231; P < 0.01)\) (Fig. 4C). The maximum mean number of immature follicles was found in 5 to 30-d-old mated females. Mated females had significantly more immature follicles than unmated females. During maximum production of follicles, two to four follicles were found in each ovariole of mated females and two to three in ovarioles of unmated females.

The mean number of mature eggs (defined as those present in the lateral and common oviducts) also differed with age \((F = 63.6; \text{df} = 7, 218; P < 0.01)\) and mating status \((F = 425.6; \text{df} = 1, 218; P < 0.01)\), and there was interaction between age and mating status \((F = 33.9; \text{df} = 7, 218; P < 0.01)\) (Fig. 4D), with maximum numbers found in mated females from about 5 to 30-days of age. The age-related production of mature follicles paralleled the number of immature follicles in mated females. Mature follicles were present in packets of two or three not only in the lateral oviducts, but also in the common oviduct and in the bursa copulatrix. However, this was not the case for unmated females. Although immature oocytes were always present in unmated females, ovulation (the act of passing follicles from the vitellarium to the lateral oviducts) rarely occurred.

Fig. 4. Effect of age on germarium length, size of proximal follicle, and number of follicles and eggs in mated and unmated female *Sitophilus oryzae*.
As a result, there was no significant age effect \((F = 2.3; \text{df} = 7, 81; P = 0.16)\) on numbers of mature eggs in unmated weevils.

### 3.3. Follicular relic accumulation in mated and unmated females

Follicular relics were present in both mated and unmated *S. oryzae* females. In mated females, follicular relics appear initially as a pale yellow tissue ring at the base of the ovarioles. As the female lays more eggs, grayish or blackish granular particles appear. As females become older, the ring of yellow tissue becomes thicker and dense. Unlike *P. truncatus*, in which the same number of ovulations occurred from each ovariole (Scholz et al., 1998), follicular relics of individual *S. oryzae* females were not always of similar size and coloration, suggesting that different numbers of ovulations occurred in each ovariole. In unmated females, follicular relics appeared as a yellow tissue ring at the base of the ovarioles, but their rate of accumulation was significantly slower than that in mated females.

### 3.4. Age-grading of mated and unmated females

A physiological age-grading system based on that described by Tyndale-Biscoe (1984) was developed to separate ovarian development into two distinct nulliparous and three parous stages. Criteria used to categorize follicular relics in the ovarioles of female *S. oryzae* were similar to those described for the dung beetle, *E. intermedius*, by Tyndale-Biscoe (1978). Two nulliparous stages, N1 and N2, and three parous stages, P1, P2, and P3, were designated (Table 1). The nulliparous stages were easily differentiated from the parous stages by the absence of mature eggs in the lateral or common oviducts, although mature eggs were also lacking in some senile individuals (see below). N1 was characterized by little detectable follicular differentiation, no distinguishable follicles present within the ovarioles, large quantities of fat globules occupying the entire abdominal cavity so that ovaries were hard to distinguish from the fat globules, the cuticle was very soft, and melanization was still in progress in most females. All pre-emergent females (inside seeds) and all females newly emerged from seeds were N1.

<table>
<thead>
<tr>
<th>Age class</th>
<th>Parous stage</th>
<th>Adult stage(^a)</th>
<th>Follicular differentiation</th>
<th>Mature eggs(^b)</th>
<th>Follicular relics</th>
<th>Fat globules(^c)</th>
<th>Cuticle hardness(^d)</th>
</tr>
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<tr>
<td>N1</td>
<td>Nulliparous</td>
<td>Pre</td>
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<td>No</td>
<td>Absent</td>
<td>H</td>
<td>S</td>
</tr>
<tr>
<td>N2</td>
<td>Nulliparous</td>
<td>Post</td>
<td>Yes</td>
<td>No</td>
<td>Absent</td>
<td>H/M</td>
<td>S/H</td>
</tr>
<tr>
<td>P1</td>
<td>Parous</td>
<td>Post</td>
<td>Yes</td>
<td>Yes</td>
<td>Absent/present</td>
<td>M/L</td>
<td>H</td>
</tr>
<tr>
<td>P2</td>
<td>Parous</td>
<td>Post</td>
<td>Yes</td>
<td>Yes</td>
<td>Present</td>
<td>L</td>
<td>H</td>
</tr>
<tr>
<td>P3</td>
<td>Parous</td>
<td>Post</td>
<td>Yes</td>
<td>No/Yes</td>
<td>Present</td>
<td>L</td>
<td>H</td>
</tr>
</tbody>
</table>

\(^{a}\)Pre, newly eclosed adults still inside seeds; Post, adults emerged from seeds.

\(^{b}\)In lateral and/or common oviducts.

\(^{c}\)Number of fat globules was estimated in each female as: H, high; M, medium; L, low.

\(^{d}\)S, soft; H, hard. Cuticle hardness was estimated based on resistance to pressure from forceps.
The N2 ovarian development stage differed from the N1 stage in that there was noticeable
differentiation of the ovarioles, distinct follicles were present in the vitellarium, and the ovarioles
were larger than in N1 females. In general, there was a decrease in the number of fat globules so
that the fat body did not completely fill the abdominal cavity, cuticular hardness had increased,
and melanization was complete.

Three parous stages were detected for the rice weevil, and these were most easily distinguished
by the degree of follicular relic accumulation. P1 was characterized by the presence or absence of
follicular relic tissue. A visible yellowish band was present in individuals with follicular relics
(early parous). Fat body was normally scattered throughout the abdominal cavity. Individuals in
the P2 stage were characterized by the presence of more follicular relic tissue than in P1 females,
and the presence of small grayish particles of follicular relic tissue (mid-parous). The P3 stage was
distinguished by the presence of small dark brownish/black clumps of follicular tissue that
surrounded the base of the ovariole, and by the presence or absence of mature eggs in the lateral
and common oviducts (advanced-parous). Some individuals in this stage had reached senility in
terms of egg laying capacity, so ovaries of these individuals normally had no distinct follicles in
the ovarioles.

Definitions of physiological-age grading for unmated females were based only on the
differentiation of follicles in the ovarioles (N1 and N2) and in the accumulation of follicular relics
at the base of the ovarioles (P1–P3) because these females had no eggs present in the lateral or
common oviducts.

Parous stages differed with the chronological age of weevils \( F = 108.2; \text{ df} = 13, 422; \text{ P}<0.01 \)
and between mated and unmated females \( F = 99.6; \text{ df} = 1, 422; \text{ P}<0.01 \). Age by mating status
interaction was also significant \( F = 15.7; \text{ df} = 13, 422; \text{ P}<0.01 \). Therefore, parous stage was
regressed on age for mated and unmated females separately. For mated females, follicular relics
first became visible 5–10 days after emergence from the seed (Fig. 5A), whereas follicular relics
were not visible in unmated females until they were 30 days old (Fig. 5B).

Parous stages correlated well with chronological age of both mated and unmated rice weevil
females. The relationship between parous stage and chronological age for mated females was (SE
shown in parentheses):

\[
y = 1.37(0.05) + 0.060(0.002)x \quad (1)
\]

\( (F = 1196.6; \text{ df} = 1, 271; \text{ P}<0.01; \ r^2 = 0.82) \) and for unmated females was:

\[
y = 1.16(0.05) + 0.051(0.001)x \quad (2)
\]

\( (F = 1261.5; \text{ df} = 1, 150; \text{ P}<0.01; \ r^2 = 0.89) \), where \( y = \) parous stage (N1 = 1; N2 = 2; P1 = 3;
P3 = 4; and P3 = 5) and \( x = \) age (days).

Few follicular relics were observed in young females, whether mated (5-days-old) or unmated
(0- to 20-days-old), whereas follicular relics grew larger and darker with increasing age in both
mated and unmated females. Although the age-dependent relationship is very clear, there was a
significant overlap among the parous stages (P1–P3). Under the conditions of this study
(unlimited nutritional resource and constant temperature), confidence limits on predicted ages
from each parous stage varied from 17 to 19 days for unmated and mated females. Thus, one can
predict the chronological age of a female in each parous stage with confidence limits of \( \pm 3 \) weeks
by using this method.
Fig. 5. Follicular relic formation in relation to age and mating status in female *Sitophilus oryzae*.

### 3.5. Progeny production of mated and unmated females

Progeny production varied among parous groups \((F = 92.4; \text{df}=3, 75; P<0.01)\) (Fig. 6), suggesting that oocyte development rates also differed and consequently influenced follicular relic accumulation. Newly emerged females classified as nulliparous (N1 and N2) produced no
Fig. 6. Relationship between parous stage and total progeny produced by mated female *Sitophilus oryzae*.

Fig. 7. Effect of starvation on reproductive organs of female *Sitophilus oryzae*.
progeny. Several young mated females classified as P1 produced progeny without showing any sign of follicular relic accumulation. However, the presence of mature eggs in the lateral oviducts can help to classify these individuals. P1 individuals averaged 51.6 ± 6.9 progeny with a range of 5–120, and more than 70% of P1 individuals produced 25–95 progeny. P2 individuals averaged 123.1 ± 7.1 progeny with a range of 47–160, and the majority (79%) of P2 individuals produced 95–160 progeny. P3 individuals averaged 181.9 ± 7.7 progeny with a range of 159–265, and more than 90% of these produced 160–186 progeny (Fig. 6).

3.6. Effects of starvation on egg resorption

Egg production in young female *S. oryzae* occurs continuously when food is present. Starvation had a rapid impact on the reproductive physiology of weevils (Fig. 7). When wheat seeds were removed, germarium length ($F = 16.8; \text{df}=3, 106; P < 0.01$) (Fig. 8A), size of proximal follicles ($F = 449.8; \text{df}=3, 106; P < 0.01$) (Fig. 8B), and the number of follicles ($F = 421.4; \text{df}=3, 106; P < 0.01$) and eggs ($F = 52.5; \text{df}=3, 106; P < 0.01$) (Figs. 8C and D) decreased rapidly, compared with fed females. Ovaries of fed females had an average of 3.5 follicles per ovariole and almost 5
eggs (Figs. 8C and D) ready for oviposition. After 24 h, no follicles or eggs were found in ovaries of starved females.

4. Discussion

The general anatomy of female rice weevil reproductive organs described in this study was similar to that of the granary weevil, S. granarius (L.) (Richards, 1947); the boll weevil, A. grandis (Grodowitz and Brewer, 1987); and the weevil, Neochetina eichhorniae (Grodowitz et al., 1997). Four ovarioles were present in all females dissected in this study, regardless of experimental treatment. Some atypical individuals of S. oryzae may have only one, two, or three ovarioles, an anomalous trait that is genetically controlled and temperature sensitive (Grenier and Nardon, 1994).

In this study, germarium length was greatest in reproductive females that were at their peak reproductive rate. Maximum germarium length also occurred in similar physiological stages in females of the larger grain borer (Scholz et al., 1998). The size of proximal follicles, the number of follicles inside the ovarioles, and the number of eggs in the lateral and common oviducts, varied with age and mating status. A rapid growth in proximal follicles had occurred by 5 days in mated and by 10 days in unmated females. However, after this period of growth, the size of proximal follicles tended to decrease as females became older. This pattern was also found in proximal follicles of the larger grain borer (Scholz et al., 1998). Young (5- to 20-days-old) female S. oryzae had more follicles than older mated and unmated females. Mated females tended to have more follicles than unmated females. Contrary to this pattern, Scholz et al. (1998) found that unmated females of the larger grain borer had more follicles than did mated females. Among mated S. oryzae females, greater numbers of eggs were present in 5- to 20-days-old females. The number of eggs decreased as females became older. No eggs were found in lateral and common oviducts of unmated females because ovulation never occurred in these females. Ovulation was also inhibited in virgin female Rhodnius prolixis (Davey, 1985).

Starvation had a significant effect on the size of the germarium and proximal follicle, and on the number of follicles and eggs. Ovaries of females starved for 24 h had smaller germaria and proximal follicles compared with ovaries from fed females. Neither follicles nor eggs were found in ovaries from females starved for 24 h, suggesting that eggs were resorbed rapidly after the weevils were removed from the wheat. Oosorption is a common phenomenon in insects and represents a reproductive strategy that occurs while the egg is still in the ovariole (Chapman, 1998). The process is characterized by the interruption of vitellogenesis, and this eventually results in the destruction of the oocyte while still enveloped in the follicle (Retnakaran and Percy, 1985). Typically, oosorption occurs when food reserves are depleted. However, oosorption also occurs in virgin females, or when conditions are unsuitable for oviposition (e.g., at temperature extremes, when changes in photoperiod occur, or during diapause initiation) (Bell, 1971; Tyndale-Biscoe and Watson, 1977). Oosorption has been described in several insect orders, including Orthoptera (Lusis, 1963; Bell, 1971), Lepidoptera (Lum, 1979), and Coleoptera (Tyndale-Biscoe and Watson, 1977; Scholz et al., 1998).

Follicular relics were present at the bases of ovarioles in both mated and unmated female S. oryzae, and accumulation of follicular relics correlated well with chronological age under our
controlled conditions. The criteria used to categorize the presence of follicular relics at the bases of ovarioles in this study included two nulliparous stages and three parous stages. Three nulliparous stages have been observed in other Coleoptera (Tyndale-Biscoe et al., 1981; Grodowitz and Brewer, 1987; Grodowitz et al., 1997). However, we categorized nulliparity into two stages in *S. oryzae*. N1 stage occurred mainly in pre-emergent females that remained inside the seeds for 3–6 days after eclosion and before emerging from the seeds. The duration of this pre-emergent period varies with temperature (Birch, 1945; Longstaff, 1981). Sharifi and Mills (1971) also reported that pre-emergent weevils from this species remained inside seeds an average of 4.6 days with a minimum of 3 to a maximum of 6 days at 27°C and 69% r.h. When weevils first emerged from the seeds, follicles were not visible in the ovarioles. However, follicles started to develop in the distal regions of the ovarioles in N2 females that began to feed within 24 h after emergence. These females were ready to mate, and our results provide evidence that mating is necessary for ovulation to occur.

We did not see any evidence from preliminary observations of weevil midguts that pre-emergent females fed while they were inside the seeds. In addition to utilization of fat body reserves, it may be that emergent females need to feed to begin oocyte production.

The presence of follicular relics in unmated females occurs when oocytes fail to mature and are resorbed. Follicular relics from these resorbed oocytes accumulate at the bases of the ovarioles, and they cannot easily be differentiated from relics accumulated during ovulation (Grodowitz et al., 1997). However, mated females accumulated follicular relics faster than unmated females. Similar results were found in female larger grain borers (Scholz et al., 1998). Unmated females can easily be distinguished from mated females by presence of sperm in the spermatheca. Thus, the presence of unmated females would not confound physiological age-grading of female weevils in field populations.

The utilization of this age-grading technique may help our knowledge of a specific population age structure. By sampling female weevils in populations infesting stored grain and determining their parous state, information can be obtained concerning their physiological age and reproductive potential. These data can improve the accuracy of population models. In addition, changes in overall age structure through time can indicate those periods when control tactics would be most successful.

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**References**


Throne, J.E., 1994. Life history of immature maize weevils (Coleoptera: Curculionidae) on corn stored at constant temperatures and relative humidities in the laboratory. Environmental Entomology 23, 1459–1471.