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Morphology and development of oocyte and follicle resorption bodies in the Lubber grasshopper, *Romalea microptera* (Beauvois)

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Abstract

We describe the development and appearance of Follicle Resorption Bodies (FRBs) and Oocyte Resorption Bodies (ORBs) in the grasshopper *Romalea microptera* (= *guttata*), and demonstrate that these structures can be used to determine the past ovipositional and environmental history of females. In *R. microptera*, one resorption body is deposited at the base of each ovariole following each gonotropic cycle. These structures are semi-permanent, and remain distinct for at least 8 wks and two additional ovipositions. Ovarioles that ovulate a mature, healthy oocyte, produce a cream-colored FRB. Ovarioles that resorb their oocyte, produce an orange-colored ORB. The number of FRBs equals the number of eggs laid, and the number of ORBs equals the number of oocytes resorbed. Healthy, well-fed *R. microptera* resorbed about a quarter of their developing oocytes. Starvation or other environmental or physiological stress increased the rate of oocyte resorption and thus increased the number of ORBs. By counting the number and type of resorption bodies in each ovariole, we determined the number of times a female laid, the number of oocytes resorbed, and the clutch size during each oviposition. These data were used to estimate the relative environmental stress experienced by laboratory females during each gonotropic cycle.

Oocyte resorption occurred frequently in 1° oocytes, and rarely in 2° oocytes. In healthy, well-fed females, oocyte resorption was temporally staggered, and thus the number of resorbing oocytes increased toward the end of each gonotrophic cycle. Adult virgin females maintained at low density with no male contact, resorbed fewer oocytes than females maintained at higher densities with males, suggesting that high densities or continual male harassment stressed females. In comparison to locusts, *R. microptera* were relatively slow to initiate oocyte resorption following starvation, requiring >12 d without food before massive resorption was detected.

Résumé

Nous décrivons l'apparition et le développement des follicules de résorption ovocytaire (trace de ponte)(FRB) et des corps de régression ovocytaire (ORB) chez le criquet *Romalea microptera* et nous démontrons que ces structures peuvent être utilisées pour reconstituer l'historique du fonctionnement ovarien et pour évaluer la qualité de l'environnement où les femelles avaient vécu. Chez *Romalea microptera*, il se forme à la base de chaque ovariole un corps de résorption (folliculaire ou ovocytaire) à la fin de chaque cycle gonotrophique. Ces structures semi-permanentes sont discernables pendant au moins 8 semaines et après 3 pontes. Les ovarioles ayant ovulé un ovocyte sain et mûr, produisent une trace de ponte de couleur blanc crème (FRB) alors que les ovarioles ayant résorbé un ovocyte, produisent un corps de régression de couleur jaune orangé. Le nombre de traces de ponte est égal au nombre d'oeufs pondus et le nombre de corps de régression au nombre d'ovocytes ayant régressé. Les *R. microptera* en bonne santé et nourris en abondance résorbent environ le quart de leur ovocytes en croissance. La privation de nourriture ou le stress physiologique entrainent une augmentation du taux de régression ovocytaire et par conséquent du nombre de corps de régression (ORB). Si l'on compte les traces de ponte et les corps de régression dans chaque ovariole, on obtient le nombre de pontes, le nombre d'oeufs par ponte et dans une certaine mesure le niveau de stress causé par l'environnement durant chaque cycle gonotrophique.

Les régressions ovocytaires se produisent d'abord chez les ovocytes de rang 1, rarement chez les ovocytes de rang 2. Chez les femelles bien nourries et en bonne santé, les régression ovocytaires sont temporairement retardées et le nombre d'ovocytes ayant résorbé augmente vers la fin de chaque cycle ovocytaire. Des femelles adultes vierges, élevées sous faible densité, et sans contact avec des mâles, résorbent moins que celles élevées sous forte densité et en présence de mâles. Ceci suggère que les fortes densités ou le harcèlement permanent des mâles créent des situations de stress. Par rapport à des locustes, les *R. microptera*, lorqu'ils sont privés de nourriture, apparaît comme une espèce qui commence à résorber les ovocytes relativement tardivement.

Key words

Ovary, ovariole, oocyte, resorption body, oviposition, grasshopper, egg pod, fecundity, Acrididae, Romaleidae.

Introduction

Female grasshoppers maintain in their ovaries a record of their ovipositional and nutritional history. This ledger of past life history events exists in the form of cellular debris that remains in the ovarioles following ovulation or oocyte resorption. By examining the ovaries of a grasshopper, a trained researcher can often determine the number of ovipositions, the number of eggs laid during each oviposition, and, in some cases, the relative environmental/nutritional stress experienced by that female during the two previous ovipositional cycles (Descamps & Wintrebert 1961; Farrow 1977, 1982; Launois-Luong 1978, 1979a, 1979b; Lim & Lee

1981; Bellinger & Pienkowski 1985; Foucart & Lecoq 1996; Launois-Luong & Lecoq 1996). This technique is a valuable, yet under-utilized tool for monitoring the eco-physiological and ovipositional history of wild grasshoppers and locusts.

In female grasshoppers, each paired ovary consists of numerous ovarioles (2 to >150, depending on the species), each of which is attached by a short pedicel to a common calyx (Uvarov 1966; Stauffer & Whitman 1997). Each ovariole takes the form of an expanding tube, containing a linear series of progressively developed oocytes. At the narrow, distal end of each ovariole lies the germarium where tiny, new oocytes are produced from oögonia. The new oocytes, interspersed by interfollicular tissue (Lusis 1963), grow and develop as they move down the ovariole toward the broad, proximal end. Hence, the largest and most mature oocyte normally lies adjacent to the pedicel, near the opening to the calyx. In this terminal location, the oocyte completes maturation, including most of the yolk deposition and the production of the vitelline membrane and the chorion. Ovulation occurs when the mature oocyte passes through the pedicel into the calyx, followed by oviposition when the eggs are deposited in the soil.

Oocytes are named by their sequential position in relation to the proximal end of the ovariole. The terminal or primary oocyte (1°) is the most proximal, and rests against the pedicel. The next oocyte in line is the secondary (2°), followed by the tertiary (3°), and so forth, as one moves from the proximal to the distal end of the ovariole. The 1° oocytes across the various ovarioles are usually in developmental synchrony, allowing females to ovulate and oviposit all the 1° oocytes simultaneously in a single clutch. In some cases, every ovariole will contribute a mature oocyte during each ovipositional cycle.

Young oocytes become surrounded by a follicular epithelium. This single layer of cells adheres to the developing oocyte until ovulation, at which time it is sloughed off, remaining in the ovariole while the mature oocyte passes through the pedicel into the calyx on its way to the lateral oviduct. Following ovulation, the empty follicular epithelium collapses, degenerates, and forms, at the base of the pedicel, a clear-, cream-, or yellow-colored Follicle Resorption Body (FRB) (= corpus lutum). In some grasshoppers, the FRB remains visible for months, even through two additional ovipositions (Phipps 1949; Singh 1958; Descamps & Wintrebert 1961; Launois 1972; Launois-Luong 1978; Foucart & Lecoq 1996).

Under optimal environmental conditions, the majority of ovarioles bring their 1° oocyte to maturity. However, during adverse conditions, such as starvation or other physiological stress, female grasshoppers often resorb their developing oocytes (Singh 1958; Descamps & Wintrebert 1961; Phipps 1966; Launois 1972; Farrow 1975, 1977; Lee & Wong 1979; Lim & Lee 1981). Supposedly, in *Schistocerca gregaria* (Forskal), absence of males increases oocyte resorption (Singh 1958; Highnam & Lusis 1962). Resorption usually occurs in the 1°, and rarely in the 2° or 3° oocytes, and can involve a few or all of the 1° ovarioles, either synchronously or progressively, depending on the timing and severity of the stress. In cases of extreme starvation, oocyte resorption can be rapid and complete. For example, during the hot and dry season in southwest Madagascar, migratory locusts, *Locusta migratoria*, often resorb all of their terminal oocytes within a few days (Launois 1972).

During resorption, hydrolytic enzymes produced by the follicle cells penetrate the oocyte, initiating lysis, breaking down first the proteins, then the lipid yolk globules, and, in the case of well developed oocytes, the vitelline membrane and chorion, and finally the follicle cells themselves (Lusis 1963; Hoffmann 1995). This process leaves a red-, orange- or yellow-colored Oocyte Resorption Body (ORB) (= Egg Resorption Body[1]) in the pedicel of the ovariole, composed, in part, of concentrated, ß-carotene pigments from the resorbed oocyte (Singh 1958; Descamps & Wintrebert 1961; Highnam *et al.* 1963a, 1963b; Lusis 1963; Phipps 1966). Like the FRB, the ORB can remain as a distinct and visible structure for several months, through one or two additional ovipositions (Lusis 1963; Launois 1972; Launois-Luong 1978; Foucart & Lecoq 1996). Ovarioles that resorb their 1° oocytes will not contribute an egg to the present clutch. However, given good nutritional/environmental conditions, those ovarioles usually function normally during the next gonotrophic cycle (Phipps 1966; Farrow 1977). In some species, mature chorionated oocytes are not fully resorbed, but instead become an empty, folded, and collapsed oocyte, which usually ends as a dark necrotic body in the ovariole (Launois-Luong, unpublished). Such ovarioles probably remain non-functional for the remainder of the female's life. A third type of pedicel inclusion was noted in *Rhammatocerus schistocercoides* (Rehn) (Acrididae). Termed "pseudocorpora lutea" these pigmented structures resemble ORB's, however their origin is unknown (Launois-Luong & Lecoq 1996).

Thus, at least three different structures can appear in the pedicels of grasshopper ovarioles. These structures can be clear, white, yellow, orange, red, granular, spherical, disk-like, or ring-like (Lusis 1963; Phipps 1966; Launois 1972; Foucart & Lecoq 1996; Launois-Luong & Lecoq 1996). One originates from oocyte resorption, another from follicle resorption, and for the third (pseudocorpora lutea), the origin is unknown (Phipps 1949, 1950; Singh 1958; Lusis 1963; Launois 1972; Launois-Luong 1978, 1979a, 1979b; Roussillon & Laugé 1979; Launois-Luong & Lecoq 1996). Resorption bodies can serve as valuable diagnostic tools because they can provide a record of the past nutritional and reproductive history of individual females. One can dissect a female grasshopper in the field and determine (from the number and state of the oocyte and follicle resorption bodies) whether or not that female had previously laid, how many times she laid, how many eggs were laid during each oviposition, and the nutritional status of that female during the most recent gonotrophic cycles. In this study, we seek to understand the origin, characteristics, developmental history, and diagnostic value of resorption bodies in the grasshopper *Romalea microptera* (Beauvois).

[1]We introduce the new term "Oocyte Resorption Body" to replace the previously used term "Egg Resorption Body," because an oocyte becomes an egg only after it leaves the female's body.

Methods and Materials

Experimental animals originated from a laboratory colony of *R. microptera* maintained at Illinois State University in 1 m^2 wire screen cages at 22-30°C, 14L:10D photoperiod, and fed Romaine lettuce, oatmeal, wheat bran, bean pods, carrot root, and leaves of green onion and carrot. This colony was originally established from wild animals collected in 1996 and 1997 from Copeland, Florida, USA.

Follicle and oocyte resorption in well-fed virgin females.— To study follicle resorption and oocyte resorption in well-fed (= Control Group) females, we reared groups of 10 adult females in 22-l plastic containers with wire screen lids. Containers were kept in environmental chambers at 32:24°C L:D thermoperiod and 14L:10D photoperiod, and animals were fed the same foods as described for the general colony. Females had no contact with males; in this species, virgins readily develop eggs and oviposit (Walker *et al.* 1999). We allowed gravid females to lay into loose, moist sand, and then counted the resulting eggs. We also dissected females at various times before or after their 1st or 2nd oviposition and counted and photographed their FRBs and ORBs using a binocular microscope and a digital camera. We determined the developmental sequence of resorption bodies by comparing FRBs and ORBs from females of different ages and different post-oviposition intervals. We used an optical micrometer to measure the size of oocytes and resorption bodies obtained from one to five females at each age group. We also compared the number of eggs laid with the number of FRBs to determine the degree of correspondence between the two.

Effects of starvation on oocyte resorption in virgin females.— To examine the effects of starvation on oocyte resorption, we removed adult females of various ages from the well-fed containers (above), and placed them into 1-l perforated clear plastic containers (one female/container). These females received only water (no food) for the remainder of the experiment, but were kept in the same environmental chamber as the well-fed females. We dissected females of various ages (and various starvation intervals) in cold physiological saline, measured the lengths of the 1° oocytes with an optical micrometer, counted the number of ovarioles and primary oocytes, counted and photographed the number of resorbing oocytes and ORBs, and photographed the various structures.

Using resorption bodies as diagnostic tools.— We used ORBs to contrast the suitabilities of two different rearing methods for adult females. The Control Group consisted of the well-fed, group-reared females maintained without male contact (above) (~10 females/container). The Treatment Group consisted of females reared under similar conditions, except that each treatment container included 10 adult males (~10 females + 10 males/container). This allowed treatment females to mate repeatedly throughout the experiment. Hence, treatment females differed from control females in both mating status and density. We allowed females to oviposit twice, then dissected each female and counted ORBs and FRBs, and number of eggs in each pod.

Results

Normal ovariole development.— In healthy virgin females, growth and development of the 1° oocytes proceeded in an orderly manner until oviposition. At eclosion, 1° oocytes measured *ca* 0.85 mm in length. At Day 10, the 1° oocytes were *ca* 2.2 mm, and at ovulation (Day 33), they reached their maximum length of *ca* 9.5 mm. The second oviposition cycle was shorter, as the new set of 1° oocytes grew from *ca* 4.9 mm, on the day of the 1st oviposition (~ Day 34), to full size and were laid in about 19 days (~53 days after eclosion). During each gonotropic cycle, most ovarioles were in developmental synchrony (Plate II, A), allowing virtually all the mature 1° oocytes to be ovulated and laid together as a group. However, two pathologies occasionally occurred: (1) rarely, one or more ovarioles remained small and undeveloped, never initiating vitellogenesis, and (2) occasionally, a mature oocyte failed to be ovulated or oviposited, and instead, remained in the ovariole, pedicel, calyx, or lateral oviduct. These "orphans" were sometimes expelled at a later date, but nonetheless, contributed to small discrepancies in our data (*e.g.* eggs laid vs. FRBs).

At adult eclosion, all oocytes were previtellogenic and translucent, and remained so until around Day 9, when vitellogenesis was initiated in the 1° oocytes only. At the start of vitellogenesis, scattered yellow yolk granules first appeared at the ends of the oocyte, but during the next few days, were deposited uniformly throughout the 1° oocytes (Plate II, F3 & G1), until they became filled with yolk (Plate II, A). As females neared ovulation, the eggs became chorionated and then were deposited within the calyx (Plate I, A-C). Secondary oocytes initiated vitellogenesis on ~ Day 26, eight days before the 1° oocytes were laid (Plate I, A, D, & F). Vitellogenic oocytes were a bright yellow color, and thus easily distinguished from previtellogenic oocytes, which were transparent.

When females first eclosed, their ovariole pedicels were semi-transparent and devoid of resorption bodies. Most pedicels remained in this condition until the 1st ovulation. However, even well-fed, healthy females began to resorb some of their 1° oocytes around Day 10, so that near the end of the first oviposition cycle, all females contained at least a few ovariole pedicels with ORBs (Fig. 1; Plate II, B).

Follicle resorption bodies (FRBs).— During ovulation, the follicle cells surrounding the 1° oocytes are sloughed off and remain in the ovarioles as the oocytes pass into the calyx (Plate I, A-E). At this time, the follicle cells resemble a loose, cream-colored, amorphous mass measuring *ca* 2.8 x 1.0 mm (Plate I, F1 & F3). By one day after ovulation, the FRB is reduced to 1.0 x 0.8 mm (Plate I, G & H). By Day 2 it measures 0.8 x 0.6 mm, by Day 7, 0.4 x 0.6 mm, and by Day 10 it has condensed to a yellowish disk *ca* 0.2 x 0.4 mm (Plate I; Fig. 2), where it remains unchanged, in the pedicel, for up to 8 wks and two additional ovulations. Thus, for females who recently have laid, one need only examine the size of the FRB to determine approximately how long ago ovulation occurred.

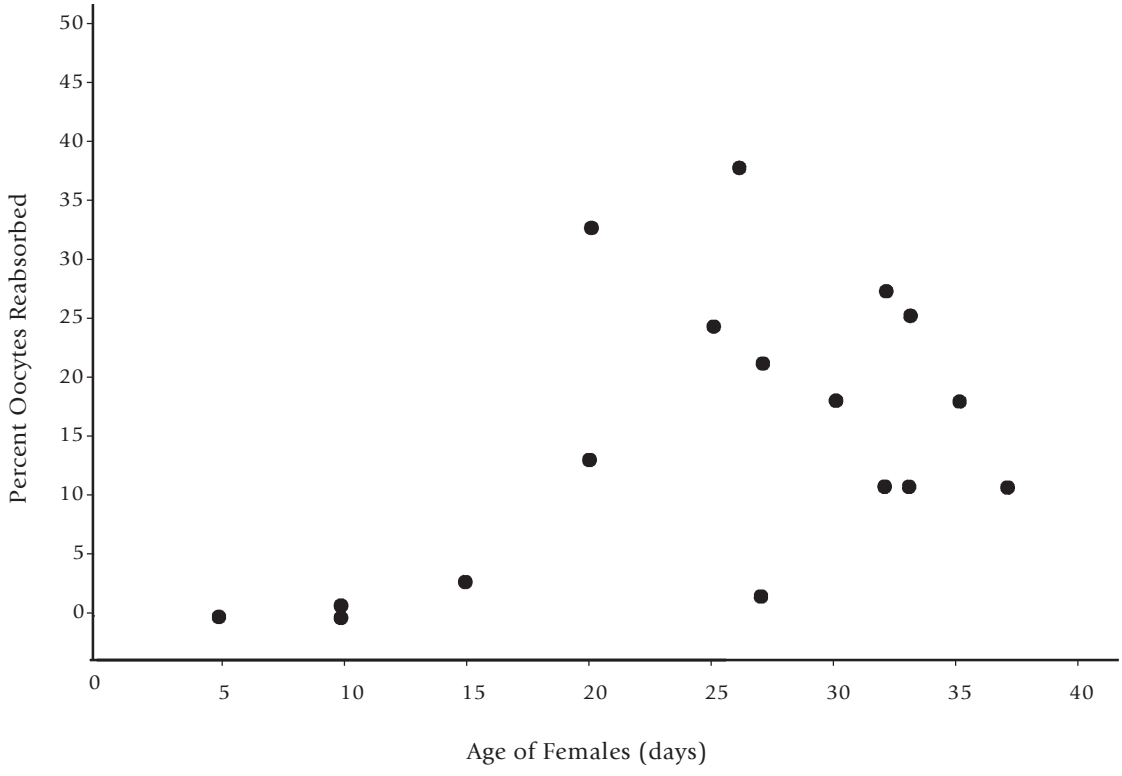

Fig. 1. Rate of resorption in 1° oocytes vs. age during 1st gonotrophic cycle in well-fed virgin female *R. microptera*.

During each oviposition cycle, an additional FRB is added to the pedicel of each ovariole that ovulated. For 8 weeks or longer, these successive FRBs remain distinct (Plate I, G & I; Fig. 2), allowing one to determine the number of times a female has laid and the clutch size at each oviposition. For example, in our study, we found a 96.9% correspondence between eggs laid and FRBs observed in the ovaries during two successive ovipositions (Table 1). Distinguishing individual FRBs becomes increasingly difficult after three ovulations, because additional FRBs crowd together in the short pedicel (as seen in Plate I, I; Fig. 2). Furthermore, each ovulated oocyte usually passes through any existing FRB, stretching it into a ring (like a banana passing through a rubber band) (Plate I, D & E). These successive deformations eventually destroy the integrity of the FRBs, making it difficult to distinguish among them.

Oocyte resorption bodies (ORBs).— We observed moderate levels of oocyte resorption in healthy, well-fed adult females. Oocyte resorption was staggered, hence the number of ORBs increased during the oviposition cycle, and females older than 25 days usually contained some 1° oocytes in various stages of resorption (Fig. 1) (Plate II, B). In well-fed virgin animals, we observed the earliest 1° oocyte resorption on Day 10 of the 1st oviposition cycle, when the 1° oocytes measured *ca* 2.2 mm long. The latest resorption occurred in full sized, chorionated eggs that were ready to ovulate (Plate II, D & K). Occasionally, resorption also occurred in 2° oocytes in healthy, well-fed virgins (Plate 1, H3; Plate II, E2), but never earlier than Day 27 of the 1st oviposition cycle. Overall, we observed 0-37% oocyte resorption in the 17 well-fed females we dissected during the 1st oviposition cycle (Fig. 1). Eleven animals dissected after two ovipositions showed, on average, 26.3% oocyte resorption during the 1st gonotrophic cycle, and 28.3% during the 2nd gonotrophic cycle, based on presence of ORBs (Table 2).

Developmental morphology of ORBs.— Oocyte resorption appeared different in small (undeveloped) *vs* large (mature) oocytes. Young resorbing oocytes (2.2-5.5 mm long), first assumed a uniform opaque deep yellow color that was darker than non-resorbing yolk (Plate II, B). Over time, the contents of these oocytes became translucent. In other small oocytes, the yolk began to dissipate, leaving a patchy, transparent outline of the developing oocyte (Plate II, G2, H, & I). Some resorbing oocytes took on a slight greenish hue. As resorption progressed, the oocyte condensed into a loose, patchy, cylindrical orange mass (Plate II, E2 & E3), and eventually condensed into a disk-like, light orange ORB *ca* 0.6 x 0.22 mm (Plate I, G1; Fig. 2).

In large oocytes (6-8.5 mm), transparent pockets appeared in the yellow yolk. Over time, these transparent patches grew, as the majority of the yolk was resorbed and the oocyte began to lose its cylindrical shape, often displaying a greenish hue. Eventually, the oocyte began to compact and acquired an orange color. Large oocytes terminated in the pedicel as a 0.8 x 0.7 mm orange ORB (Plate I, F2; Fig. 2). Older and larger oocytes produced darker, almost red-colored ORBs.

Oocytes undergoing chorionation were seldom resorbed. However, when they were, they produced a whitish, collapsed oocyte (Plate II, D & K), which usually remained in the ovariole and terminated as a dark necrotic body. Such ovarioles appeared to remain non-functional for the rest of

Table 1. Relationship between number of eggs laid and number of follicle resorption bodies (FRBs) after two ovipositions in well-fed virgin female *R. microptera*.

Female	Clutch size Pod 1	n FRB1	% correspondence eggs vs. FRBs	Clutch size Pod 2	n FRB2	% correspondence eggs vs. FRBs
1	56	54	96.4	55	55	100
2	43	45	95.5	39	40	97.5
3	54	54	100	54	57	94.7
4	59	58	98.3	48	48	100
5	57	56	98.2	47	47	100
6	48	48	100	40	42	95.2
7	35	37	94.6	37	37	100
8	67	65	97	64	65	98.5
9	45	45	100	38	46	82.6
10	41	40	97.6	39	44	88.6
11	34	33	97	41	41	100
Mean	49	48.6	97.7	45.6	47.5	96.1
SE	3.2	2.9	0.5	2.7	2.5	1.7

Table 2. Number of ovarioles and rate of follicle and oocyte resorption for the first two gonotrophic cycles in single-sex, group-reared female *R. microptera*.

Female	n ovarioles	n FRB1	n FRB2	n ORB1	n ORB2	% FRB1	% FRB2	% ORB1	% ORB2
1	75	54	55	21	20	72	73	28	27
2	62	45	40	17	22	73	65	27	35
3	69	54	57	15	12	78	83	22	17
4	62	58	48	4	14	93	77	7	23
5	64	56	47	8	17	87	73	13	27
6	57	48	42	9	15	84	74	16	26
7	69	37	37	32	32	54	54	46	46
8	69	65	65	4	4	94	94	6	6
9	64	45	46	19	18	70	72	30	28
10	68	40	44	28	24	59	65	41	35
11	70	33	41	37	29	47	59	53	41
Mean	66.3	48.6	47.5	17.6	18.8	73.7	71.7	26.3	28.3
SE	1.5	2.9	2.5	3.4	2.4	4.7	3.3	4.7	3.3

the female's life.

Differentiating FRBs *and* ORBs *after multiple ovipositions.*— Females that had laid twice usually contained two resorption bodies in each ovariole pedicel. These two resorption bodies existed in one of four different combinations, corresponding to the outcome (ovulation *vs.* oocyte resorption) of each ovipositional cycle: ORB+ORB, FRB+FRB, ORB+FRB, or FRB+ORB (Plate I, G & I; Fig. 2). These various combinations could occur anywhere in the ovary; however, oocyte resorption appeared to be slightly more common at the anterior and posterior ends of the ovary. Based on their color (FRB = white; ORB = orange), these four combinations were easily distinguished in dissected ovaries (Plate I, F-G; Fig. 2), allowing us to determine the past ovipositional history of females (Table 2). Such determinations were accurate through two ovipositions; however, the existence of three or more resorption bodies/pedicel made identification difficult.

Oocyte resorption in starved females.— In contrast to well-fed virgin females, which always resorbed a moderate percentage of their oocytes, starved virgins resorbed a high percentage of their 1° oocytes (Plate II, C), leaving large numbers of ORBs in the pedicels of their ovarioles (Plate I, D). For example, four females starved for 13, 14, 15 and 18 days, showed resorption in 96, 48, 55 and 76% of their ovarioles, respectively (Table 3). Generally, older females and females starved for longer periods resorbed greater numbers of oocytes (Table 3). Interestingly, a lag period existed between starvation and the onset of resorption; usually, a sharp increase in rate of oocyte resorption was not visibly detectable until at least 13 days starvation. Overall, female *R. microptera* showed a surprising ability to survive starvation, living for 10-18 days when given only water.

Using resorption bodies to assess environmental suitability.— We tested the ability of resorption body analysis to determine the relative suitabilities of two rearing methods for adult females. In virgins maintained in single-sex, 10-member groups, 72.7% of ovarioles contained FRBs (= produced a mature oocyte) (Table 2). In contrast, in adult females maintained in mixed-sex, 20-member groups, and allowed to mate, only 53.0% of ovarioles contained a FRB (Table 4), suggesting that conditions in the latter treatment reduced female fecundity.

Discussion

This study shows that in *R. microptera*, a record of past oviposition events exists in the ovaries in the form of Follicle Resorption Bodies (FRBs). By examining the ovaries, one can determine the number of eggs laid at each of two previous ovipositions with a high degree of accuracy (Table 1). The effects of past environmental conditions on oocyte development are also recorded in the form of Oocyte Resorption Bodies (ORBs): in our experiments, both starvation and the combined factors of high density and male presence, increased the number of ORBs and decreased the number of FRBs, and hence, increased the ORB:FRB ratio. These results suggest that ORBs and FRBs can serve as diagnostic tools to assess the past ovipositional and environmental histories of females.

This technique can be used in the field or the laboratory, and has many advantages over the traditional method of collecting egg pods and counting eggs to assess fecundity. The greatest advantage is speed: one can arrive at a distant field site and, by dissecting an appropriate number of females, quickly determine the current level of ovariole development, the current nutritional/physiological state, and the past ovipositional history and physiological condition of the population (Launois 1972; Farrow 1977, 1982; Launois-Luong 1978, 1979a, 1979b; Foucart & Lecoq 1996; Launois-Luong & Lecoq 1996). FRB and ORB analysis is especially valuable when combined with other measures of female development, fecundity, health, and age, such as body size and mass, degree of fat body and oocyte development, number of cuticular layers (under polarized light), wing and ovipositor wear, presence of parasites or pathogens, etc. (Farrow 1977, 1982; Foucart & Lecoq 1996).

The technique can be used in the laboratory to compare different rearing, social, or environmental conditions (McCaffery 1975; Lee & Wong 1979). For example, we used the technique to compare the effects of density and male presence on the fecundity of grouped females. Our results showed that virgins kept at low density had a higher fecundity (measured as FRBs) than mated females kept at a high density with males. Although mating usually decreases oocyte resorption and increases fecundity in female grasshoppers (Highnam & Lusis 1962; Stauffer & Whitman 1997; Walker *et al.* 1999), negative effects of male presence are known (Schmidt & Osman 1988; Reinhardt & Köhler 1999; Reinhardt 2000), and may derive from increased stress experienced by females exposed to constant male attention (Walker *et al.* 1999).

In lubber grasshoppers, individual ovarioles exhibited a high degree of flexibility. In well-fed females, most ovarioles produced a mature oocyte during each gonotrophic cycle. A few ovarioles produced an egg during the 1st cycle, but not during the 2nd, others ovulated during the 2nd, but not the 1st, and a few failed to produce eggs during either cycle. These four combinations can be found anywhere in the ovary; however, their frequencies change dramatically under different social and environmental conditions. Indeed, an ovariole that has failed twice may succeed during the 3rd oviposition cycle, if conditions improve. Flexibility in the reproductive physiology of *R. microptera* from one gonotrophic cycle to the next is clearly observed when resorption bodies from successive ovulations are compared, providing a convenient diagnostic tool to measure the impact of the environment on egg production *vs.* oocyte resorption.

Comparison to other grasshoppers.— Overall, the development and appearance of follicle and oocyte resorption bodies in *R. microptera* are similar to that of other grasshoppers, but with differences in timing. In all grasshoppers studied thus far, ovulation results in the deposition of a FRB in the pedicel (Singh 1958; Farrow 1975; Launois-Luong 1978; Lim & Lee 1981). FRBs are usually white and sometimes

Table 3. Percentage of 1° oocytes resorbing for adult female *R. microptera* starved at different ages and for different durations during 1st gonotrophic cycle.

Female	Age start starvation	Age dissected	n days starved	n ovarioles	% of 1° oocytes resorbing
1	20	24	4	70	0
2	10	15	5	70	3
3	20	25	5	74	24
4	23	28	5	72	0
5	16	25	9	64	0
6	20	30	10	60	22
7	25	35	10	61	11
8	12	23	11	68	0
9	12	24	12	58	0
10	14	26	12	70	0
11	14	27	13	58	0
12	17	30	13	70	96
13	20	34	14	70	48
14	10	25	15	59	3
15	15	30	15	64	55
16	16	34	18	58	76

yellow, and are easily distinguished from ORBs, which are usually red or orange (Descamps & Wintrebert 1961). However, in some cases, confusion can occur.

In *Rhammatocerus schistocercoides* from Brazil, pseudovitellogenesis leads to the formation of yellowish ring-like pseudocorpora lutea in ovariole pedicels, which can be confused with FRBs (Launois-Luong & Lecoq 1996). In *Oxya japonica willemse*, both the ORB and FRB are reported to be bright orange and similar in appearance (Lim & Lee 1981). In other species, empty (post-ovulation) follicles occasionally are not immediately resorbed, but continue to deposit carotene-containing lipids for a few days. When these follicles are eventually resorbed, they produce an orange or red FRB that can be confused with an ORB (Lusis 1963). Finally, a few species with colorless yolk produce transparent, light-orange ORBs that can be confused with a FRB (Launois-Luong 1979). However, such confusion would never happen in *R. microptera*, because its dark yellow-colored yolk produces a bright orange ORB.

We observed relatively high rates of oocyte resorption (27.3% and 47.1%) in well-fed, seemingly healthy virgins and mated females respectively. This discrepancy between ovariole numbers and clutch size appears to be common in large grasshoppers with numerous ovarioles (Phipps 1959). For example, under normal laboratory conditions, desert locust, *S. gregaria* resorb about 23% of their 1° oocytes (Highnam & Lusis 1962). Similar or greater levels of oocyte resorption are noted in the field (Viado 1950; Phipps 1959, 1966; Farrow 1975, 1977, 1982; Launois-Luong & Lecoq 1996). For example, Bellinger & Pienkowski (1985) recorded 17%, 26% and 40% oocyte resorption in wild females of three grasshopper species from Virginia, USA. Why medium and large grasshoppers routinely possess more ovarioles than the maximum number of eggs that can successfully develop, and why they produce oocytes, only later to resorb them is unknown. It seems energetically wasteful. Physiologically, selective resorption has been explained as a "contest" type of competition among competing oocytes for scarce vitellogenin; some oocytes win and develop into eggs; others lose and are resorbed (Highnam *et al.* 1963a). Evo-

Table 4. Number of ovarioles and rate of follicle and oocyte resorption for the first two gonotrophic cycles in mixed-sex, group-reared female *R. microptera*.

Female	n ovarioles	n FRB1	n FRB2	n ORB1	n ORB2	% FRB1	% FRB2	% ORB1	% ORB2
1	60	30	50	30	10	50	83	50	17
2	70	30	32	40	38	43	46	57	54
3	68	32	30	36	38	47	44	53	56
4	64	12	24	52	40	19	38	81	63
5	58	32	50	25	8	55	86	45	14
6	72	30	32	42	40	42	44	58	56
7	70	48	54	22	16	69	77	31	23
8	70	28	44	42	26	40	63	60	37
9	60	16	34	44	26	27	57	73	43
10	60	40	44	20	16	67	73	33	27
11	60	26	24	34	36	43	40	57	60
12	68	34	44	34	24	50	65	50	35
13	64	32		32		50		50	
Mean	64.9	30	38.5	34.9	26.5	46.3	59.7	53.7	40.4
SE	1.4	2.5	3	2.5	3.4	3.8	5	3.8	5

lutionarily, supplementary ovarioles may serve as a backup in case disease, parasites, or developmental or genetic abnormalities disable a percentage of the ovarioles or oocytes. Interestingly, small grasshoppers, or those with few ovarioles, often have little or no resorption (Phipps 1959; Foucart & Lecoq 1996), and under optimal conditions, a small percentage of females in some large species can achieve a 100% ovariole success rate. For example, during the rainy season in Madagascar, up to 10% of female migratory locusts manage to produce an egg from each ovariole (Launois1972).

Starved grasshoppers resorb large numbers of developing oocytes (Highnam *et al.* 1966; McCaffery 1975; Lim & Lee 1981). In *R. microptera*, females starved for more than 12 days resorbed up to 96% of their 1° oocytes. During the harsh dry season in Madagascar, 45% of migratory locusts, *L. migratoria*, resorbed all their 1° oocytes and some of their 2° oocytes as well (Launois 1972). Not only does resorption increase during starvation, but, under stressful conditions, the growth of the oocytes can become heavily desynchronized. For example, one *L. migratoria* female from Madagascar, laying for the first time, produced eggs belonging to the 1°, 2° and 3° oocytes. Out of 36 eggs, 29 came from the 1° oocyte, six from the 2° and one from the 3° oocyte (Launois1972). This mixed egg origin was attributed to stress-induced hormone disturbance. Hormones control oocyte development (see Pener 1997), and are thought to influence resorption by regulating protein (vitellogenin) availability (Highnam *et al.* 1963a, 1963b).

Normally only the terminal oocytes grow because there is only enough protein available for one gonotrophic cycle, the larger, 1° oocytes competing more successfully for this protein (Highnam *et al.* 1963a). However, in some small grasshoppers, vitellogenesis can occur in both the 1°, 2°, and even 3° simultaneously (Phipps 1959, 1966; Foucart & Lecoq 1996). Many acridids can resorb their terminal oocytes at any stage in their adult development, even before yolk deposition (Phipps 1959; Descamps & Wintrebert 1961; Lusis 1963; Farrow 1975; Launois-Luong & Lecoq 1996). In well-fed *R. microptera*, we never observed resorption earlier than Day 10; however, we suspect that earlier resorption may occur in females starved immediately after eclosion. Similar to other grasshoppers, oocyte resorption occurred preferentially in the apical and distal ends of the ovary, as opposed to the middle region (Phipps 1959; Lusis 1963; Bellinger & Pienkowski 1985).

Lubbers differed from locusts in the speed in which starvation affected resorption. Many locusts show very rapid physiological responses to environmental changes. For example, *Locusta migratoria capito* Saussure, under the harsh conditions of the hot dry season in the southwestern part of Madagascar, start to resorb their terminal oocytes in as little as 5 d after starvation (Launois 1972). During only 4 d without food, resorption of 1° oocytes in *Oxya japonica* grasshoppers increased from 24% to 86%, and dry mass of ovaries decreased from 19 to 5 mg (Lim & Lee 1981). The African acridine *Trilophidia conturbata* (Walker) responded even faster, with oocyte resorption increasing from 1.3%-6.6% after only 36 h of starvation (Phipps 1959). In contrast, lubbers responded slowly to starvation, and usually required 13 or more days without food until massive resorption could be detected. This slow physiological response in lubbers may be a consequence of their longer oogenic cycle when compared to most other acridids (Uvarov 1966). It could also be due to the massive size of female lubbers (3-12 g), which may afford them greater reserves, or perhaps because our lubbers originated from a very predictable, warm, humid, and lush environment (south Florida), where there is little need to evolve rapid resorption.

We could accurately identify and count ORBs and FRBs through two successive ovulations. After three ovulations, accuracy was reduced, and after four ovulations, it became difficult to distinguish among the many resorption bodies crowded together in the short ovariole pedicels. This is the case with other grasshoppers as well (Launois-Luong 1978). However, histologically, it is possible to distinguish three successive resorption bodies in *L. migratoria* and *S. gregaria* (Singh 1958).

It is clear that female grasshoppers show a high degree of reproductive plasticity. During optimal conditions, the majority of ovarioles will produce and ovulate a mature 1° oocyte. Under poor conditions, a portion of the 1° oocytes will be resorbed, and under extremely stressful conditions all 1° oocytes, and occasionally 2° oocytes, will be resorbed (Launois 1972, 1974; Launois-Luong 1979a, 1979b). Such plasticity is probably adaptive for grasshoppers that survive in highly variable and unpredictable environments. Under suboptimal conditions, resorption decreases the number of developing oocytes competing for limited nutrients, thus allowing at least some to mature. Under conditions of total starvation, the massive and complete resorption of 1° oocytes, which is observed in some species, probably redirects nutrients from oocytes to somatic maintenance, allowing the insect to survive to breed later when environmental conditions improve. In our study, some starved *R. microptera* lived for as long as 18 days, possibly surviving on nutrients released from resorbing eggs. Also, some locust species, such as the desert locust, are notorious for their ability to quickly restart dormant reproductive systems after rains or the appearance of new vegetation (Carlisle *et al.* 1965). The ability to resorb and recycle nutrients from developing oocytes undoubtedly contributes to the success and subsequent pest status of grasshoppers and locusts.

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Plate Legends

Plate I. Ovulation and Formation of Follicle Resorption Bodies in *R. microptera*.

A. Ovariole showing a mature oocyte that is ready to be ovulated into the calyx. The orange ORB indicates a previous oocyte resorption. Note that the 2° oocyte has already begun vitellogenesis. **B**. Ovulation: the oocyte is passing through the pedicel. **C**. Ovulation: oocyte has passed through the pedicel, leaving behind the empty ovariole sheath and follicle tissue. **D & E**. Ovulation during 2nd gonotropic cycle: oocyte is being deposited into the calyx as it slides through the pedicel. During ovulation, oocytes pass through any existing resorption bodies as a finger passing through a ring. In this case, the oocyte is passing through an orange ORB, stretching it into a ring. Note high number of ORBs, indicating that this female was starved during the 1st oviposition cycle. **F**. Three ovarioles, hours after the 2nd ovulation. F1: this ovariole had two successful ovulations, indicated by the yellow FRB and the empty ovariole sheath. F2: this ovariole resorbed oocytes during both gonotrophic cycles, as indicated by the two orange ORBs. F3: this ovariole resorbed its oocyte during the 1st cycle, as indicated by the orange ORB. It successfully ovulated during the 2nd cycle, as indicated by the empty ovariole sheath. See also Fig. 2. **G**. Ovarioles from a female ~ 1 day after laying her 2nd clutch; 1° oocytes are vitellogenic. G1: note two orange ORBs in pedicel, indicating two resorptions. G2: note two yellow FRBs in pedicel, indicating two successful ovulations. G3: note one orange ORB and one yellow FRB, indicating one resorption and one successful ovulation. G4: note one yellow FRB and one orange ORB, indicating a successful ovulation followed by an oocyte resorption. Note interfollicular tissue separating successive oocytes. See also Fig. 2. **H**. Three ovarioles, 1 day after first clutch. Note that 1° oocytes in H1 & H2 are undergoing vitellogenesis, but that H2 is slightly more developed (larger with granular yolk). The 1° oocyte in H3 is being resorbed. See also Fig. 2. **I.** Ovarioles from female that has laid twice and is well into vitellogenesis for her 3rd clutch. I1: ORB/ORB for cycles 1 & 2. Her 1° is currently being resorbed. I2: ORB/FRB for cycles 1 & 2. I3: FRB/FRB for cycles 1 & 2. I4: ORB/FRB for cycles 1& 2; her 1° oocyte is being resorbed. See also Fig. 2.

Plate II. Formation of Oocyte Resorption Bodies.

A. Ovary of well-fed female at Day 17 (compare with C). Note large, yellow, vitellogenic 1° oocytes, and transparent, non-vitellogenic 2° and 3° oocytes. **B**. Ovary of healthy, well-fed female at Day 25, showing different stages of resorption. Resorbing oocytes are smaller, darker, and more opaque. **C**. Ovary of female starved from Day 16 to Day 34; 76% of 1° oocytes are being resorbed. Ooctyes to

the far right are half resorbed and are orange. Oocytes in the middle are developing, but are much smaller than in well-fed females (compare with A). Oocytes to the far left are smaller and starting to be resorbed. Note contrast between yellow vitellogenic 1° oocytes and transparent, non-vitellogenic 2° and 3° oocytes. **D**. Pathogenic ovariole containing a mature, partly chorionated oocyte which failed to ovulate, and instead lies adjacent to the 2° oocyte and a previous orange-colored ORB. Its whitish color indicates the first stages of resorption. **E**. Different stages of oocyte resorption in a starved female. E1: early oocyte resorption, characterized by dark, patchy, granular yolk. E2: middle stage of resorption, characterized by very patchy, somewhat orange yolk. Note that the previtellogenic 2° oocyte is also resorbing. E3: advanced resorption, characterized by small size and orange color. **F**. Normal ovariole (F3) vs. resorbing ovarioles (F1 & F2) in 16-day-old adult. In F1 and F2, resorption began near onset of vitellogenesis, hence there is little pigment. Note interfollicular tissue separating oocytes in F3. **G**. Ovarioles in 16-day-old female showing normal (G1) and resorbing (G2) 1° oocytes. In G2, resorption began well after vitellogenesis. **H**. Mature 1° oocyte in advanced resorption. The oocyte has retained its shape, however nearly all the yolk has been resorbed, and oocyte periphery is becoming orange. **I**. Most of the yolk has been resorbed in this 1° oocyte, leaving a patch along one side. **J**. Advanced resorption in oocyte to the right, which has collapsed to form an orange sphere. Secondary oocyte, to the left, is undergoing vitellogenesis. **K**. Resorption in a chorionated oocyte produces a whitish, collapsed mass.

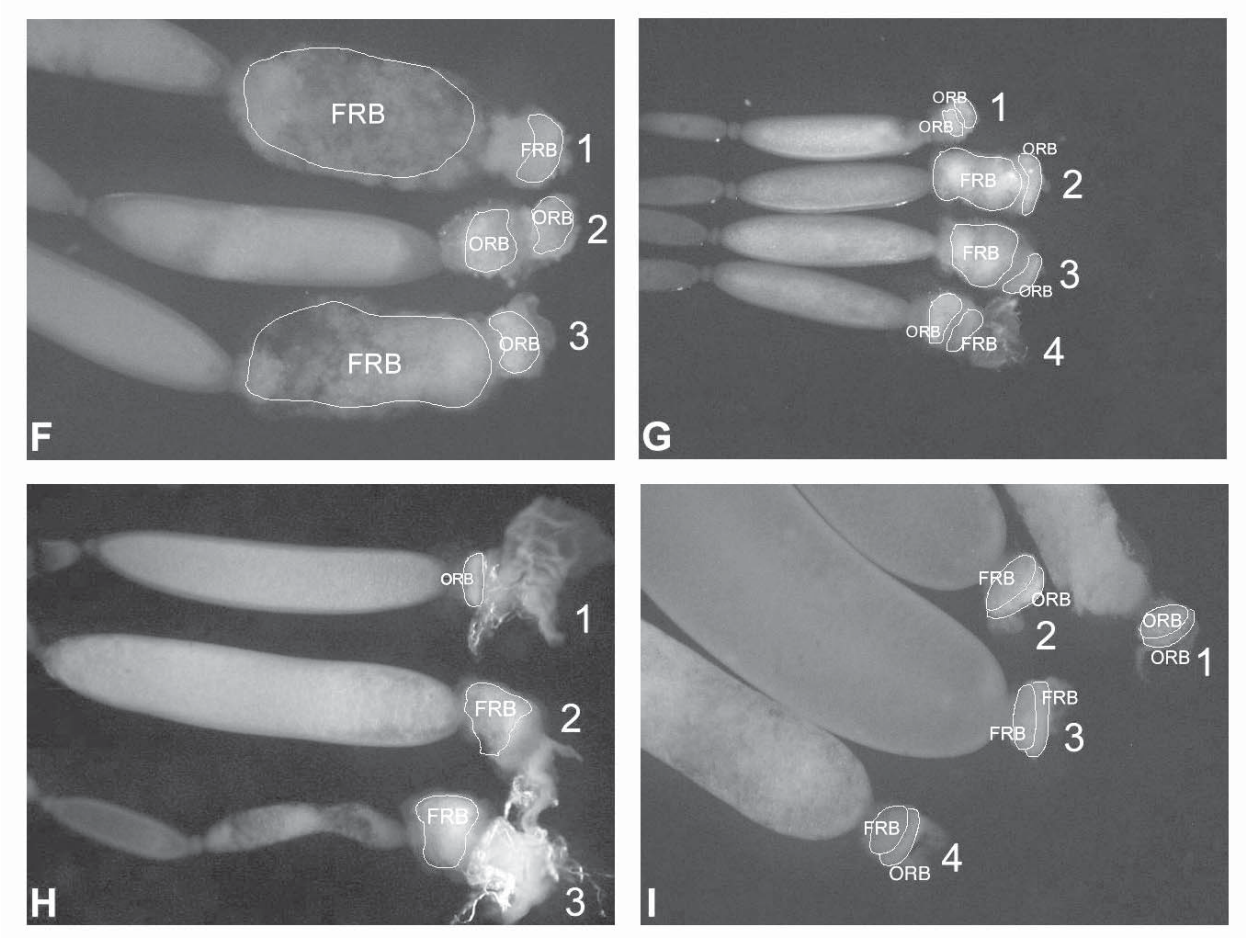

Fig. 2. Diagram of Plate I, F-I, which follows, delineating resorption bodies.

Plate I. Ovulation and formation of follicle resorption bodies in *R. microptera*.

Plate II. Formation of oocyte resorption bodies.