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Plasticity in the length of the ovulation-oviposition interval in the lubber grasshopper *Romalea microptera*

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Abstract

In female *Romalea microptera* grasshoppers, the interval between ovulation and oviposition is flexible. Females kept without sand laid their 1st egg clutch an average of 7 d later (Day 36) than females maintained on moist sand (Day 29). When we examined the ovaries of females who had recently laid, females denied sand had longer primary oocytes (5.7 *vs* 3.3 mm) and smaller follicle resorption bodies (0.61 *vs* 0.83 mm diameter) than females kept on sand, confirming that mature oocytes had been retained in the lateral oviducts of females denied sand. Some females kept without sand retained their 1st clutch until the 2nd clutch was ready to be ovulated. This suggests that grasshoppers exhibit adaptive flexibility in their ability to retain ovulated oocytes in the calyses of their lateral oviducts until a suitable oviposition substrate is found. The results also imply that using oviposition as a marker for underlying physiological/hormonal events or treatment/environment effects is problematic, because oviposition may not reliably indicate time of oocyte maturation or ovulation.

Key words

Acrididae, grasshopper, Romaleidae, *Romalea microptera*, oviposition, egg, egg laying, ovulation, ovary, oocyte, phenotypic plasticity

Introduction

In grasshoppers, the length of time between ovulation (the passage of oocytes from the ovarioles to the calyx region of the lateral oviducts) and oviposition (passage of eggs from the body to the environment) is unknown (Stauffer & Whitman 1997). Information on this subject is anecdotal and contradictory. Phipps (1950) believed that in *Locusta migratoria*, oviposition followed ovulation by a few hours. In contrast, Okelo (1971) suggested that in *Schistocera gregaria*, the oocytes normally remained in the lateral oviducts for about a week. In addition, female grasshoppers may be flexible and show adaptive retention of oocytes if suitable oviposition substrates are unavailable. For example, *S. gregaria* retained their oocytes for up to 4 d when denied moist sand, but eventually laid them on the floor of the cage (Norris 1968). Okelo (1971) suggested a 2 to 3 d flexibility for this species.

Knowing the ovulation-oviposition interval is important for several reasons. It aids our understanding of the underlying physiological/hormonal/neural processes that regulate oogenesis and oviposition. For example, one cannot correlate ovulation with endocrine events, without knowing the time of ovulation. Likewise, it is difficult to understand environmental effects (temperature, nutrition, disease, pesticides, *etc*.) on fecundity, without knowing when oocyte growth stops and ovulation occurs; presumably, a treatment applied after ovulation would have little effect on the oocytes. Finally, it is valuable to know if females are flexible in their oviposition response. Can females hold their ovulated ooctyes for long periods while they search for suitable oviposition substrates or when conditions are unfavorable for laying (high soil temperatures, presence of predators, *etc.*) or when they are unmated, or must females oviposit soon after ovulation?

In this paper we describe an experiment investigating the length of time that female grasshoppers can retain their ovulated oocytes. We kept mated females with or without moist sand. We assumed that females kept on sand would lay as soon as they were ready, whereas females without an oviposition substrate might retain their oocytes for an unknown duration.

Materials and Methods

Insects.—Eastern Lubber grasshoppers, *Romelea microptera* (Beauvois) were obtained from the Illinois State University colony, maintained in 1 m^3 wire-mesh cages at 23 to 34°C and L:D 14:10 photoperiod, and fed Romaine lettuce, wheat bran, and oatmeal *ad libitum*, with supplements of green onion, green bean pods, and carrot leaves and roots, 3 times per week (Chladny & Whitman 1997, Matuszek & Whitman 2001). The colony was established from wild animals captured in Copeland, Florida, in 1997.

Forty 1-d-old adult females were individually marked on the wings with a permanent marker, weighed, and assigned to either a control group or a treatment group, so that each group had an equal number of light and heavy animals. Each group was maintained communally in its own 0.1 m^3 wire-screen cage, and the 2 cages kept in a single incubator at 34:25°C Light:Dark temperatures, and 14:10 LD photoperiod. The insects were fed daily the diet described above. When females were between 15 and 24 d old as adults, they were allowed to mate with males. When females were 24 d old, they were transferred to individual plastic containers that differed in the presence or absence of a 2000-ml cup, filled with moist sand. Hence, from age 24 d onward, control females were kept above sand, a good substrate for oviposition, whereas treatment females were kept in containers lacking an oviposition substrate. Eggs laid by both groups were compared for size, shape, and color. We dissected the last 6 control females that oviposited into sand, and the last 7 treatment females that oviposited onto the floor of their container, and, 1) measured the length of the primary oocytes, 2) measured the length and width of the follicle resorption bodies, 3) looked for resorbing, necrotic, or malformed eggs in the lateral oviducts, and 4) confirmed successful mating by noting the presence or absence of sperm in the spermatheca.

Results

Females with sand laid significantly earlier ($p < 0.001$, $t = 6.25$; $n = 20$; $\bar{x} \pm s = 28.7 \pm 1.7$ d; range = 26 to 32 d) than females without sand ($n = 20$; 35.7 ± 4.7 d; range = 28 to 49 d), and had smaller primary oocytes [$p < 0.05$, $t = 3.15$; $\bar{x} \pm s = 3.3 \pm 0.22$ mm ($N = 6$) vs. 5.7 ± 2.00 mm ($N = 7$)] and larger follicle resorption bodies [length × width = 0.83 × 0.38 mm ($N = 6$) vs 0.61 × 0.35 mm ($N = 7$)] than females kept without sand, suggesting that females hold their eggs in the calyx portion of their lateral oviducts when a suitable oviposition substrate is not present. One treatment female did not lay until age 49 d, when her primaries had already reached 8.6 mm, and (based on chorion deposition) were within 2 d of being ovulated. Eggs laid by treatment animals were normal in size, shape, and color, indicating that there was no resorption as the mature oocytes sat in the lateral oviducts. We also observed no resorbing or discolored eggs in the lateral oviducts during our dissections. The spermathecae of all dissected females contained ample sperm, suggesting that late oviposition was not due to lack of mating.

Discussion

The timing of oviposition is flexible in female *Romalea microptera* grasshoppers. Females lacking a suitable oviposition site hold their eggs an average of 7 d longer than females given sand, and some females can hold their eggs in their lateral oviducts for up to 20 d beyond the average oviposition time, and well into the 2nd gonotrophic cycle (Sundberg *et al*. 2001). Indeed, in *R. microptera*, the 2nd egg pod is usually laid about 18 d after the 1st (Walker *et al*. 1999). Retention of mature oocytes when conditions are unfavorable for oviposition may be adaptive, allowing grasshoppers the flexibility to search for suitable substrates, to avoid predators, or to wait-out unfavorable hot, cold, dry, rainy, or flooded conditions. The latter factor may be important for *R. microptera*, which, in south Florida, survives in the Everglades marsh, and can easily find itself living above water on emergent plants, 100 m or more from dry land. An ability to retain eggs would be highly adaptive in such situations. Previous research suggests that female *R. microptera* can delay oviposition by a few days when unmated, thereby increasing opportunities for fertilization (Walker *et al.* 1999). However, it is unknown if this delay occurs pre- or post-ovulation.

We detected no immediate costs to delayed oviposition: There was no resorption of mature oocytes as they sat for days in the lateral oviducts of treatment females. Likewise, we could not detect any differences in eggs laid by control *vs* treatment females. Finally, the eggs retained by treatment females did not seem to influence the growth of the new primary oocytes as they developed in the ovarioles.

In our experiments, we assume that there was no difference in mean ovulation times between treatments. Thus, the differences in time to lay are presumably due to differences in length of retention after ovulation (= the ovulation-oviposition duration), as the mature oocytes sat in the calyces of the lateral oviducts. This is confirmed by our measurements of primary oocytes and follicle resorption bodies (**FRB**). Grasshopper ovaries consist of a series of ovarioles, each containing a linear sequence of developing oocytes of decreasing size, designated primary oocyte, secondary oocyte, tertiary oocyte, etc. (Stauffer & Whitman 1997). The primary oocytes are the largest; they mature and are ovulated in unison, after which the small secondary oocytes move forward in the ovariole, becoming the new primaries, and begin to grow. After ovulation, the follicle tissue that once surrounded the old primary oocyte remains in the ovariole. It slowly condenses to form a small, pigmented, disk-shaped FRB that remains at the base of the ovariole for weeks (Sundberg *et al.* 2001). One can determine how long ago a female ovulated by measuring the lengths of the new primaries and the size of the FRB (Stauffer & Whitman 1997, Sundberg *et al.* 2001); recently ovulated females have small new primaries and large FRB. In our study, Treatment females had large new primaries and small FRB, suggesting that they had ovulated long ago.

Scientists working with insects often use oviposition as a temporal marker for treatment effects, hormonal events, and life history strategy. However, if age at oviposition is flexible, then care must be taken in using and interpreting this metric. This problem is confounded by the fact that fully provisioned oocytes may require additional days for deposition of the vitelline envelope and chorion, and for ovulation (Kimber 1980, Okelo 1985). Hence, provisioning of oocytes, and the ability of internal and external factors to influence oocyte mass, size, and composition, may end a week or more *before* oviposition. Finally, this paper concerns only one species. It would be interesting to examine egg retention in grasshoppers from harsh, unpredictable, and highly heterogeneous environments, where flexibility in timing of oviposition would be favored.

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