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Plasticity of insect reproduction: testing models of flexible and fixed development in response to different growth rates

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Abstract We tested alternative developmental hypotheses describing when during an insect oviposition cycle reproductive tactics are determined. Newly eclosed adult females of the grasshopper *Romalea guttata* were raised on eight different feeding treatments consisting of a low food diet, a high food diet, and changes from high to low food, or low to high food, at different times during the first oviposition cycle. When initial food availability was high, a decline in food availability >7 days after adult eclosion produced no significant increase in time to oviposition compared to constant high food. In contrast, when initial food availability was low, an increase in food availability as late as day 14 produced a significant decrease in time to oviposition compared to constant low food. Thus, time to oviposition is determined by feeding rate early in the oviposition cycle, but the time of this determination is later when food availability is lower. Masses of individual eggs were unaffected by these treatments. When initial food availability was high, a decrease in food availability on day 21 produced no significant change in numbers of eggs in a clutch compared to constant high food. In contrast, when initial food availability was low, an increase in food availability after day 7 produced no significant change in number of eggs in a clutch compared to constant low food. Changes in egg production resulted from oocyte resorption, which appeared to become unresponsive to food availability between day 14 and day 21. Our results refute the hypothesis that reproductive tactics are continuously flexible. Development toward oviposition seems to be structured so that reproductive tactics become independent of feeding late during the first oviposition cycle. Reproductive tactics become unresponsive to food at different times for groups initially receiving low or high food, suggesting that a particular developmental state, rather than some absolute time, marks the shift to development that is unresponsive to food. Plasticity in reproductive tactics appears to be controlled by hormones in a manner similar to the hormonal control of plasticity of metamorphosis in other insects.

Key words Development rate · Egg production · Growth rate · Reproductive tactics · *Romalea guttata*

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Introduction

We investigated the pattern of plasticity of reproductive tactics in insects by testing alternative developmental hypotheses that describe how female reproductive tactics vary with resource acquisition during an oviposition cycle. Our goal was to determine when during an oviposition cycle tactics such as time to oviposition, egg mass, egg number, and oocyte abortion (= failure to provision oocytes) or resorption (= withdrawal of resources from a provisioned oocyte) are determined. To do this we employed models originally devised to describe plasticity of another developmental event, metamorphosis.

Reproductive allotment (i.e., mass invested in a clutch, or the product of number of eggs and mass per egg) and the time to reach reproduction are life-history variables that can have profound effects on an organism's fitness (Stearns 1992). These important traits are not, however, static properties of organisms. Rather, they change in response to the environmental conditions experienced by an individual (Stearns 1992). For example, differences in food availability (McCaffery 1975; Tobe and Chapman 1979; Juliano 1986; Wiedenmann and O'Neil 1990; Boggs and Ross 1993; Wheeler 1996), ambient temperature (Whitman 1986, 1988), season (Landa 1992a,b), and food quality (McCaffery 1975; Yang and Joern 1994) all may influence number or total

mass of eggs, or timing of egg production. We seek to understand the common pattern that when an adult female has a lower growth rate (= rate of accumulation of mass), two reproductive tactics change in concert: reproductive allotment is reduced and the time to produce a clutch is prolonged (e.g., McCaffery 1975; Matsura and Morooka 1983; Juliano 1986; Whitman 1986; Wall and Begon 1987; Wiedenmann and O'Neil 1990; reviewed by Wheeler 1996). This observation implies that development rate (= rate of progression through events in the adult's life leading up to oviposition) is in some way dependent on growth rate.

We are explicitly considering growth (= mass accumulation) during the adult stage only. Among insects in which egg production is dependent on resources acquired by the adult female (e.g., many Orthoptera, blood-feeding Diptera, muscoid Diptera – Wheeler 1996), differences in food availablity obviously influence rate of accumulation of mass that can be allotted to eggs (e.g., Ives 1981; Matsura and Morooka 1983; Juliano 1986; Wiedenmann and O'Neil 1990). Less obviously, differences in food availablity also may affect neuroendocrine tissues that control the progression though different events or changes leading up to oviposition (e.g., Tobe and Chapman 1979; McCaffery and McCaffery 1983; Wheeler 1996; Stauffer and Whitman 1997) and so affect time to oviposition (e.g., Ives 1981; Matsura and Morooka 1983; Weaver 1984; Wiedenmann and O'Neil 1990). Insect egg production is under hormonal control (reviewed by Wheeler 1996). Growth of adults, oogenesis, and oviposition are controlled by the same system of hormones (neuropeptides, ecdysteroids, juvenile hormone) and secretory tissues (brain, corpus allatum, corpus cardiacum) that control growth of juveniles, molting, and metamorphosis (Tobe and Chapman 1979; McCaffery and McCaffery 1983; Schwartz et al. 1985; Wheeler 1996).

There have been a number of theoretical and empirical studies of how food intake influences growth, how growth influences development, and ultimately how these effects influence plasticity of metamorphosis in both vertebrates and arthropods (e.g., Bakker 1959; Wilbur and Collins 1973; Travis 1984; Alford and Harris 1988; Hensley 1993; Leips and Travis 1994; Ebert 1994; Bradshaw and Johnson 1995; Gravel 1996; Twombly 1996). The ideas, models, mechanisms, and methods involved in these studies focus on plasticity of metamorphosis in response to feeding rate, and thus should be applicable to another developmental event like oviposition. Many of the models employed in these studies fall into two distinct groups:

1. Models that postulate that development rate responds to growth rate continuously, and development to the end point (metamorphosis) is inhibited as long as growth rate is above some threshold (Wilbur and Collins 1973; Alford and Harris 1988; Bradshaw and Johnson 1995)

2. Models that postulate that development rate is responsive to growth rate only during a certain period early in development (Travis 1984; Hensley 1993; Ebert 1994; Leips and Travis 1994; Bradshaw and Johnson 1995). After that period, development to the end point (metamorphosis) becomes fixed and non-plastic. Thus, in translating these models to another developmental event like oviposition, the major question is: do development rate and time to oviposition become unresponsive to feeding rate at some time in the oviposition cycle, and if so, when? An additional question is: when during the oviposition cycle does reproductive allotment become unresponsive to food availability?

Developmental models applied to oviposition

Continuously flexible development

Models postulating continuously flexible development (Wilbur and Collins 1973) can be translated into a description of oogenesis and oviposition as follows. Development rate responds to changes in growth throughout the oviposition cycle. There is a minimum mass of stored resources that must be available to allot to eggs in order for oviposition to occur. Accumulation of nutritional resources (growth) after this minimum amount is reached determines the time at which oviposition occurs, and the final mass of eggs produced. Individuals that are growing rapidly when they reach the minimum reproductive allotment will continue to accumulate resources until a maximum reproductive allotment is reached, and oviposit soon after. However, individuals that are accumulating resources slowly will produce a minimal mass of eggs and take a long time to reach oviposition. If, at any time during the oviposition cycle, feeding rate and growth rate change, the female's development rate toward oviposition also changes. A decline in growth rate, which may indicate poor potential for attaining greater reproductive allotment, is predicted to result in rapid development to oviposition with a less-than-maximal reproductive allotment. In contrast, an increase in growth rate is predicted to result in delayed development to oviposition and a maximal reproductive allotment (see also Wilbur and Collins 1973; Alford and Harris 1988; Bradshaw and Johnson 1995 for further details on this model).

Fixed development

Models postulating that development becomes unresponsive to feeding (Travis 1984; Ebert 1994; Leips and Travis 1994; Bradshaw and Johnson 1995) can be translated into a description of oogenesis and oviposition as follows. There is a period early in development toward oviposition during which growth rate determines development rate, possibly by setting in motion hormonal events that control oogenesis and oviposition. By the end of this critical period, time to oviposition becomes fixed and no longer responds to changes in growth. All indi-

viduals that grow rapidly during the critical period will have a high development rate, and oviposit at the same early time, regardless of growth after the critical period. If growth declines after the critical period, a lower mass will be invested in the clutch of eggs, but oviposition will occur at the same early time. Alternatively, individuals that grow slowly during the critical period will have a slow development rate, and will take a long time to reach oviposition, regardless of growth after the critical period. If growth increases after the critical period, a greater mass will be invested in a clutch of eggs, and oviposition will occur at the same late time (see also Travis 1984; Alford and Harris 1988; Leips and Travis 1994; Bradshaw and Johnson 1995 for further details on this type of model). In one description of this type of model (Travis 1984), the critical period was defined by an absolute (i.e., clock or calendar) time that was independent of feeding rate. Other versions of this type of model (Ebert 1994; Leips and Travis 1994; Bradshaw and Johnson 1995) differ in postulating that development rate becomes fixed only after attaining a particular mass or a particular developmental stage, implying that the absolute time at which development becomes fixed may differ for individuals initially experiencing high growth (that reach that state sooner) as opposed to low growth (that reach that stage later). Thus, the time at which development toward oviposition becomes unresponsive to feeding may depend on initial feeding and development rates (for further details, see Bradshaw and Johnson 1995).

Though these models may be applicable to oviposition, there are differences between metamorphosis and reproduction that must be considered before generating predictions. Metamorphosis is an event that occurs once during the life of an individual, whereas reproduction occurs repeatedly in many insects, including grasshoppers (Stauffer and Whitman 1997). One consequence of the iterative nature of oviposition is that growth occurring during one oviposition cycle may be allocated to future reproduction (i.e., to eggs in later oviposition cycles). There may be a point in one cycle where accumulated resources are no longer allocated to current reproduction, but rather are allocated to the next cycle. This leads to the prediction that at some point during the cycle, number of eggs or reproductive allotment also might become fixed and unresponsive to growth rate.

Predictions

Both classes of models predict that reproductive allotment should increase, and time to oviposition should decrease with food availability. However, these models make distinct predictions about reproduction when food availability changes during an individual's life. Predictions of the continuously flexible model based on Wilbur and Collins (1973) focus primarily on reproductive allotment. The continuously flexible model predicts that individuals switched from a low diet to a high diet will achieve the maximum reproductive allotment, as long as the switch is made prior to reaching the minimum reproductive allotment. It also predicts that individuals switched from a high diet to a low diet after the minimum reproductive allotment is reached will oviposit soon after the switch, and attain a lower reproductive allotment than those kept on a high diet throughout. In contrast, predictions of the fixed development model based on Travis (1984), Ebert (1994), Leips and Travis (1994) or Bradshaw and Johnson (1995) focus primarily on time to oviposition. The fixed development model predicts that individuals switched from a high diet to a low diet will oviposit at the same time as those kept on a high diet throughout, provided that the switch comes after the critical period (or critical development state). Similarly, individuals switched from a low diet to a high diet will oviposit at the same time as those kept on a low diet throughout, provided that the switch occurs after the critical period (or critical development state). Models of fixed development (Travis 1984; Ebert 1994; Leips and Travis 1994; Bradshaw and Johnson 1995) vary in their details, particularly about whether it is a development time vs. a developmental state that determines time to metamorphosis. In this paper, we test the common element of those hypotheses – development rate late in the gonotrophic cycle that is unresponsive to feeding.

We conducted an experiment designed to test the two alternative hypotheses represented by these two classes of models (continuously flexible development vs. fixed development late), using the lubber grasshopper *Romalea guttata* as a model organism. In grasshoppers, as in other insects, developing oocytes are provisioned with proteins produced in the fat body (Stauffer and Whitman 1997). This process, known as vitellogenesis, is under hormonal control (Tobe and Chapman 1979). Adults have a previtellogenic stage of resource acquisition and somatic growth during which material for eggs accumulates in the fat body. This is followed by a vitellogenic stage, during which vitellogenin, the precursor of yolk protein, is transferred from the fat body to the developing oocytes (Stauffer and Whitman 1997). Under some conditions, such as low food intake, some developing oocytes may be resorbed, resulting in reduced clutch size (Bellinger and Pienkowski 1985; Stauffer and Whitman 1997). To our knowledge, no one has attempted to test models of continuously flexible vs. fixed development toward reproduction for insects, as we do here. We raised *R. guttata* females on eight different food treatments involving switching from high-to-low or low-to-high feeding regimes. Time to oviposition, number of eggs, number of resorbed oocytes, and individual egg mass were analyzed.

Methods

Rearing of individuals

R. guttata nymphs were obtained from their natural habitat near Everglades City, Florida, raised in groups at 26–30°C, and fed *ad libitum* on fresh Romaine lettuce, green onion, carrot root and

foliage, and oatmeal, an adequate laboratory diet for lubber grasshoppers (Whitman 1986). Fifth-instar females were transferred to an environmental chamber with a 14:10 L:D photoperiod, and 32°C:24°C day:night temperature. Upon adult eclosion, females were randomly assigned to one of eight feeding treatments and placed in individual oviposition containers consisting of a 1.32-l plastic container with a screen lid and a hole cut in the bottom. The hole was positioned over a 1-l plastic oviposition cup filled with moist sand, and held in place by wooden applicator sticks inserted into the sand. Individuals were fed and containers were cleaned daily. Males were placed with females on day 21 of adulthood for mating. Prior to day 21, females appear to be unreceptive to mating (unpublished data). Males used for mating were well fed, and were placed with females while containers were being cleaned, thus avoiding consumption of the females' food rations by the males. Pairs were observed periodically to verify mating. This continued daily until all females had mated once.

Treatments

Forty newly-eclosed females were randomly assigned to eight treatments that consisted of combinations of high and low food, with feeding rate changed at different times during the first oviposition cycle. The high food diet consisted of 15 g of fresh Romaine lettuce and 0.15 g of oatmeal daily. Preliminary experiments determined that an *ad libitum* ration for a female during this phase of adult life averaged about 6 g of lettuce consumed. Our high food diet was never fully consumed by any female during the experiment. The low food diet consisted of 2 g of lettuce and 0.02 g of oatmeal daily, which was clearly less than an *ad libitum* ration, as this ration was always eaten within 1 day. Treatments are described in Table 1. Two groups of females remained on the high food and low food diets, respectively, for the duration of the experiment (Table 1). The other six groups were switched from one diet to the other on days 7, 14, and 21 (Table 1). These days were chosen so that they were roughly equally spaced across the duration of the first oviposition cycle of a female fed *ad libitum*.

Data collection

For each female we determined femur length as a measure of initial body size. Mass of each female grasshopper and food intake were determined daily, making oviposition obvious, and indicated by a large decline in female mass between days. Upon oviposition, number of days to oviposition was recorded, egg pods were removed from the sand, dissected, and number of eggs determined. Egg pods were recovered from the sand within about 12 h of oviposition, soaked in warm water, and eggs were easily separated using a small metal spatula. Ten eggs from each pod were dried for at least 24 h at 55°C, weighed as a group, and mean dry mass per egg (group mass/10) was determined to the nearest 0.0001 g. After oviposition, each female was dissected, and number of ovarioles and number of egg resorption bodies were counted. Egg resorption bodies, which form when developing oocytes are resorbed (Stauffer and Whitman 1997), appeared as small orange-red spots between the secondary oocyte and the calyx. For analysis, oocyte resorption was quantified as both absolute numbers resorbed (by direct count) and proportion of oocytes resorbed (number resorbed/ovariole number). For all individuals, the difference between direct counts of ovarioles and the sum of eggs laid and resorption bodies did not differ significantly from 0 (mean difference = −0.72, SE = 0.56, paired $t = 1.29$, $df = 35$, $P = 0.2057$). This result indicates that we attained a very accurate accounting of fates of the functional primary oocytes.

Analysis

When analyzing number of eggs, individual egg mass, egg resorption, or time to oviposition, we first tested whether female size (femur length at eclosion) as a covariate accounted for significant variation in the response variable. When it did, we used analysis of covariance (ANCOVA) to analyze the response variables. When female size was not significantly related to a response variable, we simply analyzed responses by one way analysis of variance (ANOVA), ignoring the covariate. In both cases, when treatment effects were significant, we used multiple pairwise comparisons of either least-squares means from ANCOVA, or of means from ANOVA. Pairwise tests were done with an experimentwise $\alpha = 0.05$. The sequential Bonferroni method (Rice 1989) was used to compare least-squares means from ANCOVA. Ryan's Q (SAS Institute 1989) was used to compare means from ANOVA. All analyses were conducted using SAS 6.11 (SAS Institute 1989). Data for all analyses were checked for normality, equal variances (and homogeneous slopes in the case of ANCOVA), and no transformations were necessary.

Results

Time to oviposition

Treatment had a significant effect on time to oviposition (Table 2), but initial size did not ($F_{1,30} = 0.52$, $P = 0.4746$). For individuals initially receiving the high food diet, a switch to the low food diet on either day 21 (HL21) or day 14 (HL14) did not significantly increase time to oviposition relative to the high food diet (Fig. 1). Indeed, means for days to oviposition were very similar (29.0 to 30.5 days) for treatments High, HL21, and HL14 (Fig. 1). In contrast, females that were switched to the low food diet on day 7 (HL7) took significantly longer to oviposit (Fig. 1), and also did not differ in time to oviposition from individuals fed continuously on the low food diet (Fig. 1). Of the treatments that originally started on the low food diet, a switch to the high food

Table 1 Treatments used in experimental manipulation of feeding of female *Romalea guttata* during the first reproductive cycle

Treatment	*n*	Initial food Lettuce + oatmeal	Switch	Food after switch Lettuce + oatmeal
High	5	15 g + 0.15 g	Never	–
HL21	4	15 g + 0.15 g	Day 21	2 g + 0.02 g
HL14	5	15 g + 0.15 g	Day 14	2 g + 0.02 g
HL7	5	15 g + 0.15 g	Day 7	2 g + 0.02 g
LH7	5	2 g + 0.02 g	Day 7	15 g + 0.15 g
LH14	5	2 g + 0.02 g	Day 14	15 g + 0.15 g
LH21	5	2 g + 0.02 g	Day 21	15 g + 0.15 g
Low	5	2 g + 0.02 g	Never	–

Table 2 Analysis of variance (ANOVA) and analysis of covariance (ANCOVA) for reproductive responses of female *R. guttata* to food manipulations. In all cases where ANCOVA was used, body size, as measured by femur length (mm), was used as the covariate. In all cases, treatment-covariate interactions were nonsiginificant, indicating homogeneous slopes. When the covariate was not significant at $P < 0.10$, one way ANOVA was used

Source	*df*	Days to oviposition ANOVA $r^2 = 0.658$		Mean egg mass ANOVA $r^2 = 0.202$		Number of eggs laid ANCOVA $r^2 = 0.768$		Number of oocytes resorbed ANCOVA $r^2 = 0.788$	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatment	7	9.65	0.0001	1.13	0.3716	8.27	0.0001	9.41	0.0001
Femur	1					6.92	0.0133	9.14	0.0051
Error		*df* = 31		*df* = 31		*df* = 30		*df* = 30	
Slope ± SE						2.25 ± 0.85		−1.80 ± 0.59	

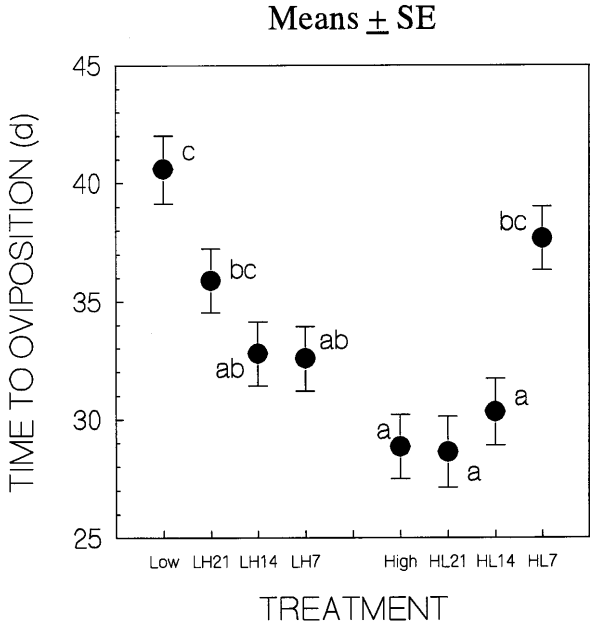

Fig. 1 Time to oviposition (days) for the first reproductive cycle of *Romalea guttata*. Means (±SE) associated with the same *letter* are not significantly different at an experimentwise $\alpha = 0.05$, using Ryan's *Q* criterion (SAS Institute 1989). Means are based on $n = 5$ individuals for all treatments except HL21, in which there were $n = 4$ individuals

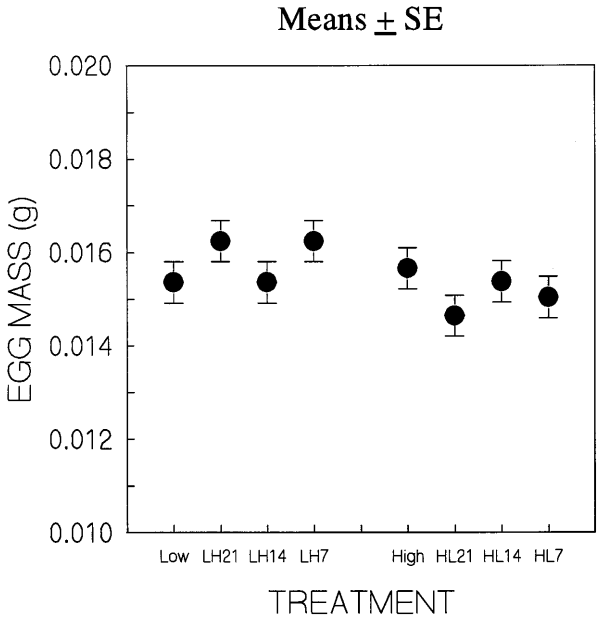

Fig. 2 Masses of individual eggs (g) laid in the first reproductive cycle of *R. guttata*. There were no significant differences among means (see Table 2). Means (±SE) are based on $n = 5$ individuals for all treatments except HL21, in which there were $n = 4$ individuals

diet on day 21 (LH21) did not significantly alter time to oviposition relative to a constant low food regime (Fig. 1). Females switched from the low food diet to the high food diet on days 14 (LH14) or 7 (LH7) reached oviposition significantly sooner than did females on the low treatment (Fig. 1). Females from the high, HL21, HL14, LH14, and LH7 treatments were all indistinguishable, and had low times to oviposition (Fig. 1).

Individual egg mass

Treatment had no significant effect on the mass of individual eggs (Table 2). The mass of individual eggs was also not significantly related to the covariate, female femur length ($F_{1,30} = 1.49$, $P = 0.2323$). The mean masses of individual eggs for all treatment groups were virtually identical (Fig. 2).

Egg number

Initial size was significantly and positively related to number of eggs produced (Table 2). Treatment had a significant effect on the number of eggs produced (Table 2). Of the treatments that started on the high food diet, high and HL21 yielded virtually identical least-squares mean numbers of eggs that were significantly greater than least-squares mean numbers of eggs from treatments HL7, low, LH21 (Fig. 3). Treatment HL14 yielded a substantially lower least-squares mean number of eggs (43.2 eggs) than did high and HL21 (56 to 57), but this difference was not statistically significant (Fig. 3). Treatment HL7 yielded the lowest least-squares mean number of eggs of those started on the high food

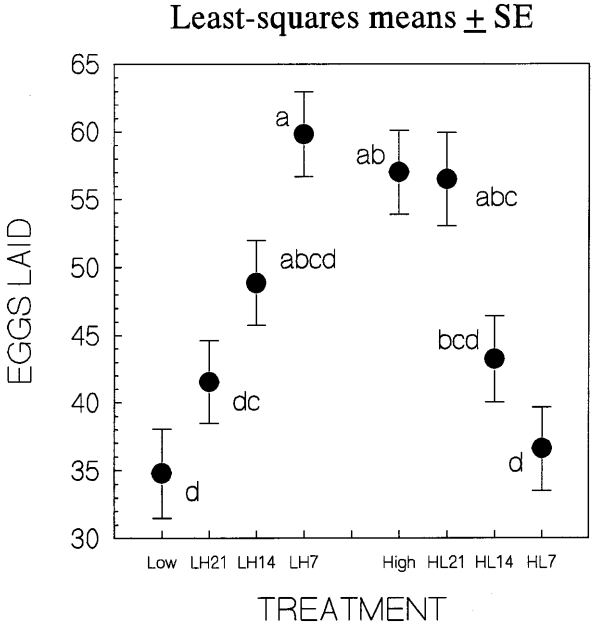

Fig. 3 Number of eggs produced in the first reproductive cycle of *R. guttata*. Least squares means (±SE) associated with the same *letter* are not significantly different at an experimentwise $\alpha = 0.05$, using a sequential Bonferroni criterion (Rice 1989). Least squares means are adjusted for the covariate femur length and are based on $n = 5$ individuals for all treatments except HL21, in which there were $n = 4$ individuals

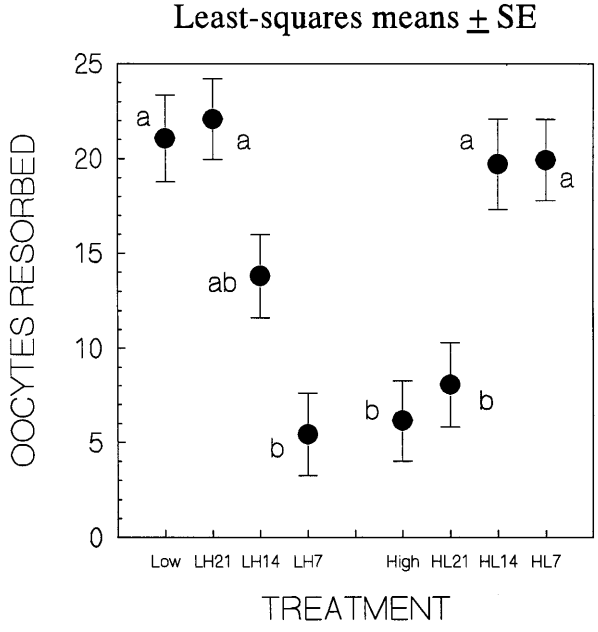

Fig. 4 Number of oocytes resorbed during the first reproductive cycle of *R. guttata*. Least squares means (±SE) associated with the same letter are not significantly different at an experimentwise $\alpha = 0.05$, using a sequential Bonferroni criterion (Rice 1989). Least squares means are adjusted for the covariate femur length and are based on $n = 5$ individuals for all treatments except HL21, in which there were $n = 4$ individuals

diet, and did not differ significantly from the least-squares mean for the low treatment (Fig. 3). Of the treatments that started on the low food diet, low yielded the lowest least-squares mean number of eggs, and LH21 did not differ significantly from low (Fig. 3). Treatment LH14 produced a least-squares mean number of eggs (48.9) that was substantially greater than that for the low treatment (34.8), but these values did not differ significantly (Fig. 3). Treatment LH7 yielded the greatest least-squares mean number of eggs in the experiment (59.8), and this was indistinguishable from that for the high treatment (Fig. 3).

Number and proportion of oocytes resorbed

Initial size was significantly and negatively related to number of oocytes resorbed (Table 2), indicating that larger females resorb fewer eggs. Number of oocytes resorbed was significantly affected by treatment (Table 2). Least-squares mean numbers of eggs resorbed for the eight treatments fell into two distinct groups that differed significantly: high, HL21, and LH7 had low mean numbers resorbed that were statistically indistinguishable; LH21, low, HL7, and HL14 had high least-squares mean numbers resorbed that were statistically indistinguishable (Fig. 4). The least-squares mean for LH14 was intermediate between the two groups, and was indistinguishable from all other least-squares means (Fig. 4). High levels of oocyte resorption are roughly three times greater than observed low levels of oocyte resorption (Fig. 4). Proportion of oocytes resorbed was also analyzed, and yielded virtually identical results concerning statistical significance of treatment ($F_{7,27} = 12.70$, $P = 0.0001$) and femur length ($F_{1,27} = 13.34$, $P = 0.0011$), and patterns of differences among treatments (results not shown). Finally, we also tested for treatment effects on number of functional ovarioles, and found no significant effect of treatment ($F_{7,28} = 1.94$, $P = 0.1001$, means omitted in the interest of brevity).

Discussion

Plastic and fixed reproductive tactics

Our results demonstrate that three of the four reproductive tactics we tested are plastic in response to food availability: time to oviposition, number of eggs, and oocyte resorption. These results are consistent with other studies of grasshopper reproduction that show time to reproduction increases (McCaffery 1975; Whitman 1986), oocyte resorption increases (McCaffery 1975), and egg production decreases (Wall and Begon 1987), in response to low food intake. Hence, females may respond to food availability by modification of three reproductive tactics. However, not all reproductive tactics are plastic in response to food availablity. Mean egg mass for *R. guttata* is unaffected by food treatments.

Critical periods and fixed or flexible development

Our results indicate that there are points in the reproductive cycle after which some reproductive traits become fixed and no longer responsive to differences in feeding and growth rates of adult females. Days to oviposition, number of eggs, and oocyte resorption are all determined well before oviposition.

Our results show that time to oviposition (Fig. 1) is determined between days 7 and 14 for females initially on the high food diet. Only a switch to the low food diet at day 7 caused time to oviposition to be lengthened. Indeed, means for high, HL21, and HL14 were all very similar at about 28–30 days (Fig. 1). Results for females initially on the low food diet also suggest that development time to oviposition becomes fixed, but the pattern of response differed from that for females initially on the high food diet. Time to oviposition was not significantly shortened by switching to the high food diet at 21 days, though the actual mean value was reduced by about 4.5 days compared to that continuous low food (Fig. 1). Females that were switched at day 14 oviposited significantly earlier than those maintained on the low food diet throughout the experiment. Thus, it appears that the point at which development rate becomes fixed and unresponsive to food is not an absolute time, but rather some developmental state that must be attained. Such a developmental state is apparently attained later for those females initially given the low food diet compared to those females initially given the high food diet.

Reproductive allotment, in units of mass, is the product of number of eggs produced and the mass of each egg. Because the mass of *R. guttata* eggs does not appear to be affected by feeding treatments, analysis of number of eggs produced gives an accurate assessment of reproductive allotment for the first clutch. Number of eggs laid appears to be determined before day 21 and perhaps as early as day 14 of the first oviposition cycle (Fig. 2). This pattern is even more apparent when examining oocyte resorption (Fig. 4). For females initially receiving the high food diet, oocyte resorption is unaffected by reducing food on day 21 (Fig. 4). For females initially receiving the low food diet, the pattern is somewhat more ambiguous because an increase in food on day 14 produces an intermediate level of oocyte resorption (Fig. 4). Because an increase in food on day 14 yields oocyte resorption that is statistically indistinguishable from that produced by continuously high food (Fig. 4), we conclude that the final commitment to resorb oocytes occurs some time between days 14 and 21. Thus, both number of eggs and reproductive allotment become fixed and unresponsive to changes in feeding at day 21. This conclusion is also consistent with the equivocal results for eggs laid (Fig. 2) in that changing food levels on day 21 never yielded a significantly different number of eggs compared to constant low or high food. Indeed, least-squares means for eggs from high and HL21 treatments were virtually identical at about 57 eggs.

In our experiment, females continued to feed after day 21 with the only noticeable decline in feeding occurring on the day of oviposition. If feeding from day 21 onward has no significant effect on number of eggs produced or oocyte resorption (see Figs. 3 and 4), but feeding continues almost until the day of oviposition (day 28–30 for well-fed females), what is the fate of resources accumulated after day 21? It seems likely that resources acquired late in the oviposition cycle are stored and used in future oviposition cycles. These results reemphasize one difference in the nature of oviposition vs. metamorphosis as developmental events. Mass at the single event of metamorphosis remains continuously flexible in response to food intake (e.g., Bradshaw and Johnson 1995; Gravel 1996), but mass allocated to reproduction or number of eggs become canalized and unresponsive to changing food intake after a certain point in development toward oviposition.

Models of flexible and fixed development

These results for time to oviposition and number of eggs produced clearly refute the continuously flexible development model based on Wilbur and Collins (1973). The continuously flexible model predicts that individuals that are switched from low to high food will continue to accumulate resources until the maximum reproductive allotment is reached. Thus, egg production for LH14 or LH21 treatments should be as high as that of the high treatment. Clearly this was not the case (Fig. 3). The continuously flexible model also predicts that a switch from high to low food will induce oviposition soon after the switch. Our results clearly show that HL21 and HL14 treatments oviposited no sooner than did the high treatment (Fig. 1).

Our results, like data from similar experiments on insect metamorphosis (Bakker 1959; Bradshaw and Johnson 1995) are consistent with predictions of the fixed-development models (Ebert 1994; Leips and Travis 1994; Bradshaw and Johnson 1995). Such models predict that females that are switched from the high food diet to the low food diet late in the cycle will oviposit at the same time as those kept on the high food diet throughout. Likewise, they predict that females switched from the low food diet to the high food diet late in the cycle will oviposit at the same time as those kept on the low food diet throughout. These models specify a period of fixed development rate (and therefore fixed development time) late in development. Several of these original models (Ebert 1994; Bradshaw and Johnson 1995) explicitly postulate that the period of fixed development is initiated when the organism has attained a defined developmental state, typically some particular size, rather than after a particular period. This postulate is consistent with one aspect of our data – the number of days required for females to enter the period of unresponsiveness to feeding differs for individuals initially on the high diet vs. initially on the low diet (see Fig. 1). The

Bradshaw-Johnson model seems to us to be the most useful because it was specifically designed for insects, and is consistent with hormonal mechanisms known to operate in insect development.

The Bradshaw-Johnson model must be further modified to account for features of insect reproduction that are absent in insect metamorphosis. It appears that at a certain point in the oviposition cycle, the number of developing oocytes becomes fixed via oocyte abortion or resorption. This phenomenon is consistent with some previous work on orthopteran reproduction (e.g., McCaffery 1975; Whitman and Stauffer 1997). However, our results imply that oocyte resorption becomes canalized and unresponsive to feeding after a certain point in development, and thus cannot occur in response to changing food intake after that developmental point during oogenesis. This result contradicts previous claims that oocyte resorption can occur at any time during a cycle (e.g., Bellinger and Pienkowski 1985). Thus, development toward oviposition in *R. guttata* appears to be a complex process with at least two critical events that serve to constrain the rest of development. We propose that these events are:

1. Determination of development rate. Initial rate of mass increase determines when a critical morphological state will be attained. That morphological state could be a particular mass or a particular concentration of some stored nutrient (e.g., protein) necessary for oocyte development. Attaining that critical morphological state triggers developmental and hormonal events that determine remaining time of development, which is then independent of further changes in food intake.

2. Determination of egg number. Some time after the determination of development rate, the number of oocytes that will mature is determined via oocyte abortion and resorption. This event may be triggered when the individual attains some proportional increase in mass or concentration of a stored nutrient beyond that at the determination of development rate (Bradshaw and Johnson 1995). Changes in food intake, particularly increases, after the determination of egg number affect the amount of stored resources available for future reproduction. It seems likely that both events are controlled by hormones, with the most likely candidate being juvenile hormone, which shows at least one conspicuous peak during the oviposition cycle of orthopterans (Dale and Tobe 1986; Stauffer and Whitman 1997).

These results have important implications for the ecology and evolution of insect reproduction. Our results imply that reproduction by *R. guttata*, and probably by other insects, is significantly constrained by the developmental system, which yields reproductive tactics that are unresponsive to energy intake, at least after a certain point in development. Such inflexibility may limit an individual's ability to respond adaptively to a variable or unpredictable environment, and therefore could affect distribution and abundance across a range of environments. Because our data show that reduced egg production is a product of oocyte resorption, rather than reduction of the number of functional ovarioles, which is likely to be permanent, the inflexibility induced late in one oviposition cycle may not carry over to the next oviposition cycle. This implies that though the insect has limits on its flexibility in a single oviposition cycle, it may retain considerable flexibility in lifetime reproductive output. It remains to be determined whether this pattern of limitation of flexibility is peculiar to *R. guttata*, and perhaps a product of evolution in its particular subtropical environment, or, alternatively, is typical of most insects. We need to know, for example, when development rate becomes unresponsive to feeding for other populations of *R. guttata*, or for related species. We also need to know whether reproductive plasticities are correlated with environmental variables such as the length of the active season. Such comparative data on the organization of reproductive plasticity and the relationships of differential plasticity to different natural environments would be extremely useful in understanding the ecological and evolutionary roles of reproductive plasticity and developmental constraints.

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