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Effects of growth rates on development to metamorphosis in the lubber grasshopper, *Romalea microptera*

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Abstract We tested three developmental models postulating different effects of feeding and growth rates on nymphal development rate in female *Romalea microptera* (Beauvois). We also tested whether assimilation efficiency during the last stadium was affected by earlier feeding in ways that buffer effects of sub-optimal feeding. Hatchling *R. microptera* were raised in nine treatment groups consisting of high food, low food, and switches from low to high food, high to low food, and high to no food after molts to successive instars. High food during the middle three stadia (second through fourth) yielded significantly lower time to adulthood and larger adult size compared to low food during the middle three stadia. Feeding during the first and last stadia had no significant effect on mass at adulthood or total development time; however, some feeding and growth during the last stadium appeared to be required for successful metamorphosis. Feeding rate during one stadium usually had significant effects on durations of subsequent stadia. Assimilation efficiency in the fifth instar was significantly greater when food availability was low during early stadia, then high during the fifth stadium, compared to assimilation efficiency when food availability was high throughout development. Low food availability during the last stadium significantly lowered assimilation efficiency compared to high food availability during the last stadium, regardless of food availability during earlier stadia. Treatments did not significantly affect relative sizes of fore-, mid-, and hindguts. Our results suggest that development rate proceeds relatively independently of feeding during the last stadium. Our results support models that postulate loss of developmental plasticity late in nymphal life, and this constraint on plasticity may limit the ability of late-instar nymphs to respond to a variable environment.

Key words Development rate · Complex life cycles · Feeding efficiency · Growth rate · Plasticity · Metamorphosis

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Introduction

Timing of life history transitions may be affected by a trade-off between the benefits of continued growth and resulting large size (e.g., greater probability of survival, greater fecundity), and the benefits of short development time (e.g., lower cumulative risks of mortality prior to reproduction) (Stearns 1992; Nylin and Gotthard 1998). The resulting trade-off between the size at and time to metamorphosis often leads to simultaneous changes in both traits in sub-optimal environments (Wilbur and Collins 1973; Stearns 1992; Leips and Travis 1994; Bradshaw and Johnson 1995; Nylin and Gotthard 1998). Such phenotypic plasticity in the size at and time to metamorphosis may enable an organism to respond adaptively to environmental changes, or may result primarily from constraints on the ability of any organism to alter development in response to poor environments (Stearns 1992; Gotthard and Nylin 1995; Nylin and Gotthard 1998). The developmental mechanisms that produce the plastic responses to the environment can also place limits on plasticity of size at and time to metamorphosis (Ebert 1994; Leips and Travis 1994; Bradshaw and Johnson 1995; Twombly 1996; Moehrlin and Juliano 1998).

Development rate (the rate of progression through morphological stages) and growth rate (the rate of accumulation of mass) of juveniles determine the size at and time to metamorphosis in organisms with complex life

cycles. A high growth rate results in large size and a low growth rate results in small size at metamorphosis (Wilbur and Collins 1973; Leips and Travis 1994; Bradshaw and Johnson 1995). In contrast, a high development rate results in a short time to metamorphosis and a low development rate results in a long time to metamorphosis. Growth rate may also determine development rate (Wilbur and Collins 1973). The physical and biotic environments affect both growth and development rates (Büns and Ratte 1991; Hensley 1993; Ebert 1994; Leips and Travis 1994; Bradshaw and Johnson 1995), but how and when variation in the environment can affect development remains poorly understood.

There are two broad groups of hypotheses for how development rate and growth rate respond to food availability. "Fixed-development" hypotheses suggest that development rate becomes unresponsive to changing growth rate and food availability at some point in development (Ebert 1994; Leips and Travis 1994; Bradshaw and Johnson 1995). In contrast, "plastic-development" hypotheses postulate that development rate can respond to changes in growth rate and feeding rate at all times and stages (Wilbur and Collins 1973). Models incorporating both types of hypotheses have been tested using arthropods (Ebert 1994; Bradshaw and Johnson 1995; Twombly 1996) and amphibians (Alford and Harris 1988; Hensley 1993; Leips and Travis 1994).

Fixed-development model

Leips and Travis (1994) proposed that development rate is determined during a critical period early in development, and that thereafter, development rate (and, therefore, remaining development time) becomes fixed and no longer plastic in response to food availability. Bradshaw and Johnson (1995), in a similar model specifically based on endocrine control of metamorphosis in insects, postulated that development becomes fixed at a specific juvenile developmental stage, rather than at a particular time, as postulated by Leips and Travis (1994). Bradshaw and Johnson (1995) incorporated Nijhout's (1994) postulate that a target size for initiating metamorphosis is set as some fixed proportional increase in size, implying that initially larger individuals will have a larger target size than will smaller individuals. Bradshaw and Johnson (1995) also postulate that positive growth during the last stadium is necessary for development to proceed to adulthood, because the target size is postulated to be a fixed proportional increase in size beyond the size at the start of the last stadium (Nijhout 1994; Bradshaw and Johnson 1995).

Plastic-development model

Wilbur and Collins (1973; see also Alford and Harris 1988) postulated that development rate will respond to changes in growth rate at any time during juvenile development. Although their model was designed for amphibians, it has been applied to other organisms with complex life cycles, such as arthropods (e.g., Bradshaw and Johnson 1995; Twombly 1996; Moehrlin and Juliano 1998). Their model postulates that individuals with a continuous low growth rate initiate metamorphosis at a minimum mass, and that individuals experiencing a high growth rate will delay metamorphosis and continue to grow until they attain the maximum possible mass.

Efficiency

Plasticity in other characteristics may affect the trade-off between size at and time to metamorphosis by buffering the responses of developing individuals to sub-optimal nutritional environments (Scriber and Slansky 1981; Sibley 1981; Bernays and Barbehenn 1987; Chapman 1988; Ebert 1996; Lepczyk et al. 1998; Krause et al. 1999). Such buffering of growth could result if individuals underfed early in development increase their assimilation efficiency to compensate for the low amount of food. Increased assimilation efficiency with low feeding rate is predicted by optimality models of digestion (Sibley 1981) and has been observed in insects (e.g., Mukerji and Guppy 1970; but see Muthukrishnan and Delvi 1974; Mathavan and Muthukrishnan 1976; Schroeder 1976) and birds (e.g., Lepczyk et al. 1998). Assimilation efficiency may be increased by increasing retention time of meals within the gut as feeding rate declines (e.g., Lawton 1970; Scriber and Slansky 1981; Sibley 1981; Lepczyk et al. 1998). Food retention time and assimilation efficiency could be increased by elongating or otherwise enlarging the gut or the part of the gut that is responsible for absorption (midgut in insects: Chapman 1998). Gut size increases in response to unpredictable or low food quantity in birds (Nir et al. 1978) and fish (Seim and Sikes 1998). In contrast, low food quantity results in reduced gut size in echinoderms (Ebert 1996), presumably because resources are allocated to other tissues. Plasticity in assimilation efficiency and gut morphology in response to low food availability in juveniles has been ignored in most developmental models, though Bradshaw and Johnson (1995) investigated how plasticity may be associated with differences in mass yield per milligram of food. Plasticity in assimilation efficiency may influence effects of feeding rate on size at and time to metamorphosis.

Predictions

Predictions of the developmental models described above differ when food availability changes during the nymphal stages. Predictions of the two models for fixed development (Leips and Travis 1994; Bradshaw and Johnson 1995) primarily involve development time. Both models predict that a change in food level after the critical developmental stage or period will yield adults

that eclose at the same time as individuals receiving constant food levels. Thus two distinct classes of time to eclosion are predicted: individuals fed high food during the critical period will have a short time to eclosion regardless of later feeding rate, while individuals fed low food during the critical period will have a long time to eclosion regardless of later feeding rate. Bradshaw and Johnson (1995) also predict that positive growth during the last stadium is necessary for the transition to adulthood, regardless of how large the individual may be at the start of the last stadium. The Wilbur and Collins (1973) model of continuous plasticity of development predicts that insects switched from low food to high food will attain the maximum size at eclosion, as long as the switch is made before the minimum size necessary for eclosion is reached. A model for insects based upon the Wilbur and Collins (1973) model predicts that if an individual is large enough to become a functional adult at the start of the last stadium, it will reach adulthood, even without further growth of the last instar.

We tested these three alternative models concerning the effect of food availability on growth and, in turn, development. Although none of them incorporates developmental mechanisms for plasticity of assimilation efficiency, we also tested for changes in assimilation efficiency and relative gut size in response to different food levels. We used the eastern lubber grasshopper *Romalea microptera* (Beauvois) as the model organism, manipulating growth rate by manipulating food availability in the juvenile stages.

Materials and methods

Rearing

Romalea microptera hatchlings were third-generation laboratory-reared progeny of individuals originally collected near Copeland, Fla. We maintained eggs in sand containers at 22–24°C until hatching. We sexed hatchlings based on external genitalia (Whitman and Orsak 1985). We randomly assigned females to one of nine treatment groups and placed them in individual 900-ml plastic containers with screen lids. We weighed individuals to the nearest 0.0001 g and provided daily food rations as proportions of the individual's mass (see Treatments, below). All individuals were housed in an environmental chamber with a 14 h:10 h light:dark photoperiod, at temperatures of 32:24°C (day:night). Each day, we recorded body mass, amount of food given, and amount of food left uneaten for each individual. Each day, we removed feces, and gave each individual a mist of water to prevent desiccation. We also removed and weighed all molted exoskeletons and we weighed and froze each female when she eclosed to adulthood.

Treatments

We randomly assigned 72 newly hatched females to nine treatments consisting of high and low feeding regimes, and we switched females from high to low and low to high at different stages during the nymphal life cycle. We chose the daily high food regime to be 300% the mass of the individual in Romaine lettuce and 30% of the mass of the individual in dry oatmeal. Preliminary data indicated that the high food regime was well above ad libitum for *R. microptera*. We chose the low food regime to be 50% of the mass of the individual in Romaine lettuce and 5% the mass of the individual in dry oatmeal. We chose to manipulate food quantity, as opposed to food quality, as the most direct way of manipulating growth rates. Such manipulations have been the standard for testing these models (e.g., Alford and Harris 1988; Leips and Travis 1994; Bradshaw and Johnson 1995; Twombly 1996; Moehrlin and Juliano 1998). Switches in treatment are described in Table 1. Two groups remained on either the high or the low food regime throughout the experiment (Table 1). We switched six other treatment groups from high to low and low to high food regimes at the beginning of the second, third, or fifth (last) stadium (Table 1). We gave the last treatment group water, but no food during the last stadium and then followed their progress until death or eclosion (Table 1). We analyzed the mass of each individual the day before eclosion to adulthood, and the number of days from hatching to eclosion. We also analyzed development times for each instar as functions of feeding treatments up to and including that instar.

Assimilation efficiency

For each fifth-instar female, we determined the amount of food offered and the amount of food left uneaten and converted these to dry masses, based upon the estimated water content for both lettuce (92% by mass) and oatmeal (no measurable water content). We subtracted the dry mass of uneaten food from the estimated dry mass of food offered to each individual to give daily dry mass of food ingested during the fifth stadium, and then summed these daily masses to obtain the amount ingested during the entire stadium (=*I*). During the fifth stadium, we collected feces daily from each individual, and dried them at 60°C for at least 24 h. We determined pooled dry mass of feces produced by each individual during the fifth stadium (=*F*). We expressed assimilation efficiency (Scriber and Slansky 1981) as the $(I–F)/I$, which is equivalent to the coefficient of food utilization (Uvarov 1966) and approximate digestibility (Sibley 1981).

Table 1 Treatments and corresponding food rations with days of food switch. Actual food amounts are calculated daily as a percentage of individual body mass

Treatment	Switch	Day of switch	Food (% of body mass)	
			Before switch (lettuce/oatmeal)	After switch (lettuce/oatmeal)
HHHH	–	Never	300/30	–
HHHL	High to low	Day 1 stadium 5	300/30	50/5
HHLL	High to low	Day 1 stadium 3	300/30	50/5
HLLL	High to low	Day 1 stadium 2	300/30	50/5
HHH0	High to starvation	Day 1 stadium 5	300/30	0/0
LLLL	–	Never	50/5	–
LLLH	Low to high	Day 1 stadium 5	50/5	300/30
LLHH	Low to high	Day 1 stadium 3	50/5	300/30
LHHH	Low to high	Day 1 stadium 2	50/5	300/30

Gut dissection

We thawed each frozen adult and then dissected each one in insect Ringer's solution. We removed the tissues surrounding the gut, and separated the gut from the rest of the body with cuts posterior to the rectum and anterior to the crop. We separated the gut into the foregut, midgut, and hindgut, removed contents of each section of the gut, and rinsed the gut twice in insect Ringer's solution. We dried the non-gut tissues and gut sections for 24 h at 60°C and then weighed them to the nearest 0.0001 g.

Analysis

For size at and total time to eclosion, we log transformed the data in order to meet assumptions of normality and homogeneous variance. We first tested whether log initial femur length or log initial mass as covariates accounted for significant variation in the size at and time to metamorphosis. In both cases, the covariates were not significant, so we analyzed the data by analysis of variance (ANOVA). For durations of each individual instar, raw data met ANOVA assumptions. We log transformed gut data, and we used the log mass at eclosion as a covariate in univariate (ANCOVA) and multivariate (MANCOVA) analysis of covariance, because mass at eclosion accounted for significant variation in gut mass. When treatment effects were significant, we used multiple pairwise comparisons of either least-square means (ANCOVA) or means (ANOVA). We did the pairwise tests with a Ryan-Einot-Gabriel-Welsh test at an experimentwise α=0.05. We conducted all these analyses using SAS 6.12 (SAS 1989). Data in all analyses met assumptions of normality, equal variances, and homogeneous slopes for ANCOVA. For all one-way ANOVAs, we also used a randomization test (Manly 1991a, 1991b) to check whether the conclusions may be influenced by undetected violations of statistical assumptions. In all cases, conclusions of randomization ANOVA were the same as those from parametric ANOVA, hence we report only the latter.

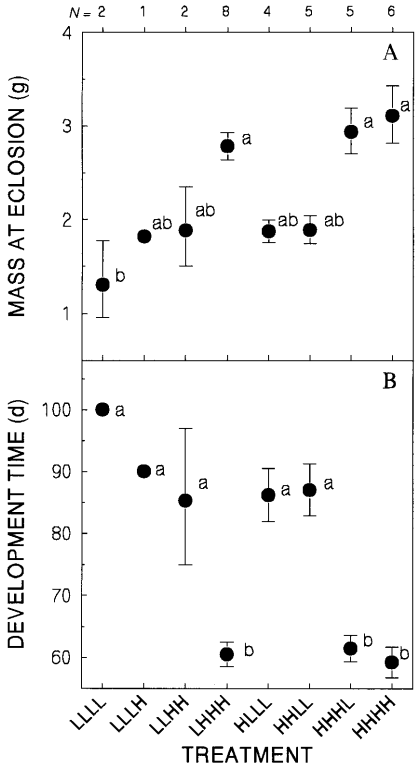

Fig. 1 Mean (±SE) mass at adult eclosion in grams (**A**), and mean (±SE) development time in days (**B**) for the eight treatment groups (see Table 1). Means associated with the same letter are not significantly different (α=0.05). Numbers of surviving adults used in the analysis are listed at the top of the graph

Results

Mortality

Mortality occurred in all but the LHHH treatment groups, and was, predictably, most severe for groups receiving low food for an extended period, resulting in low sample sizes for these groups (Fig. 1, numbers surviving to adulthood given at top). One treatment, LLLH, yielded only one surviving adult (Fig. 1). Exploratory analyses indicated that including or excluding this unreplicated observation had no effect on conclusions regarding treatment differences, so we retained this observation in our analyses.

One female from treatment LLHH underwent an extra juvenile molt, becoming a sixth instar. This female eventually reached adulthood. Exploratory analysis indicated that including or excluding this female had no effect on statistical conclusions about treatment effects on size at and time to metamorphosis. However, because of her abnormal pattern of development, we eliminated this female from our analysis.

Size at adult eclosion

Treatment had a highly significant effect on the size at eclosion ($F_{7,25}$=7.77, P<0.0001, ANOVA). Individuals fed high food during the middle stadia (2–4; HHHH, HHHL, LHHH) had masses at eclosion that did not differ significantly (Fig. 1A). Individuals fed low food throughout development (LLLL) had significantly lower masses at eclosion than individuals in the treatments receiving high food during the middle three stadia (HHHH, HHHL, LHHH; Fig. 1A). Remaining treatments (LLHH, HHLL, LLLH, HLLL) had intermediate masses indistinguishable from all other treatments (Fig. 1A).

Time to adult eclosion

Treatment had a highly significant effect on the time to eclosion (ANOVA, $F_{7,25}$=17.69, P<0.0001). Mean times to eclosion fell into two distinct groups (Fig. 1B). Means within each group were statistically indistinguishable from each other, but differed significantly from means in the other group (Fig. 1B). The first group with short times to eclosion (about 60 days), consisted of individuals receiving high food during the three middle stadia (2–4; HHHH, HHHL, LHHH; Fig. 1B). The second group with much longer times to eclosion (about 85 or more days) consisted of all treatments with low food sometime in the middle instars (LLLL, LLLH, LLHH, HHLL, HLLL; Table 1).

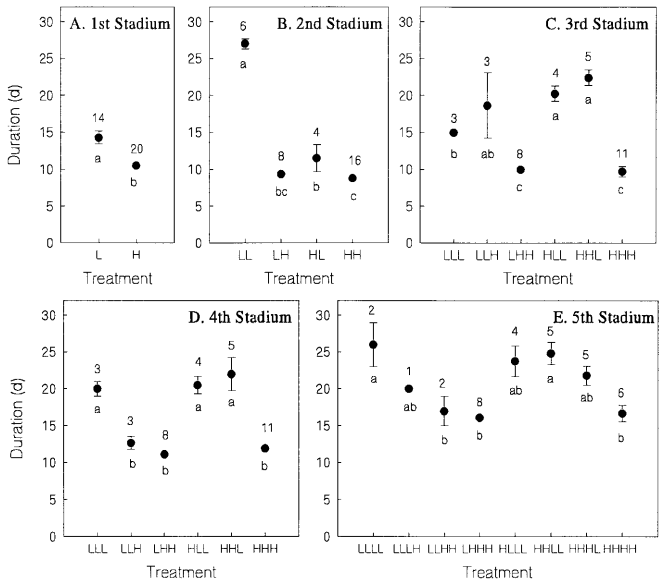

Fig. 2A–E Durations of each stadium (mean±SE). Means associated with the same letter are not significantly different (α=0.05). Numbers of surviving individuals used in the analysis are listed above each group mean. Treatment abbreviations with less than four letters represent pooled treatment groups that are identical up to that point in the experiment (e.g., LL represents pooled data from treatments LLLL, LLLH, and LLHH, through the second stadium, during which time all individuals in those treatments had received low food)

We also analyzed the effects of feeding treatments on durations of individual stadia. The structure of this experiment, with switches in feeding at various points, resulted in different treatment arrangements for each stadium. Food availability during the first stadium significantly affected its duration ($F_{1,32}$=24.23, P=0.0001) and, as expected, low food significantly prolonged the stadium, but only by an average of less than 4 days (Fig. 2A). Duration of the second stadium was significantly affected by treatments (LL, LH, HL, HH; $F_{3,30}$=133.08, P<0.0001). High food during the second stadium significantly shortened development time compared to low food in the second stadium (Fig. 2B), but feeding during the first stadium also affected the duration of the second stadium, particularly when food availability during the second stadium was low (Fig. 2B). Compared to any of the treatments receiving high food (LH, HL, HH; Fig. 2B), low food during both the first and second stadia (LL) dramatically prolonged the duration of the second stadium.

Treatments significantly affected the duration of the third stadium (LLL, LLH, LHH, HLL, HHL, HHH; $F_{5,28}$=23.39, P<0.0001). When food availability during the third instar was high, low food during the second stadium significantly prolonged the duration of the third stadium (LLH vs LHH, HHH; Fig. 2C). When food availability during the third stadium was low, high food during the first stadium significantly shortened the duration of the third stadium (HLL vs LLL; Fig. 1C) but food during the second stadium had no effect (HLL vs HHL; Fig. 2C). Only individuals receiving high food during both the second and third stadia (LHH, HHH) had third stadia of short duration (about 10 days; Fig. 2C).

Duration of the fourth stadium was significantly affected by food treatment ($F_{5,28}$=23.40, P<0.0001), the most obvious effect being that high food during the third and fourth stadia yielded a significantly shorter fourth stadium than low food during the third and fourth stadia (Fig. 2D). Duration of the fourth stadium was not, however, affected by food during the first and second stadia (e.g., LHH vs HHH, LLL vs HLL; Fig. 2D). Finally the duration of the fifth stadium was significantly affected by treatment (LLLL, LLLH, LLHH, LHHH, HHHH, HHHL, HHLL, HLLL; $F_{7,25}$=7.95, P<0.0001). However, in no case did a difference in feeding only during the fifth stadium significantly affect its duration (LLLL vs LLLH, and HHHH vs HHHL; Fig. 2E). For each stadium, its range of maximum to minimum duration is an estimate of the extent of change in stadium duration with feeding treatments. The greatest range was for the second stadium (27–9 days), followed, in order, by the third (22–10 days), fourth (22–11 days), fifth (26–16 days), and first (14–11 days) stadia (Fig. 2).

Growth during the last stadium

None of the nymphs receiving no food during the fifth stadium successfully molted to adulthood (n=7). Mean (±SE) masses of these nymphs at the start of the fifth stadium (1.52±0.10) did not differ significantly from the mean masses the day before adult eclosion from treatments receiving low food during at least two stadia (i.e., HHLL, HLLL, LLHH, LLLH, and LLLL; ANOVA, $F_{5,15}$=1.71, P=0.1930; Fig. 1A). Mean masses at the start of the fifth stadium thus appear to be sufficient for these nymphs to become adults.

Assimilation efficiency

Treatment had a highly significant effect on the proportion of food assimilated during the fifth stadium (ANOVA, $F_{7,25}$=25.14, P<0.0001; Fig. 3). Three distinct groups were identified based upon significant differences among efficiencies. The first group, with the lowest assimilation efficiency, included all treatments with low food availability during the last stadium (LLLL, HLLL, HHLL, HHHL; Fig. 3). The next group consisted of individuals receiving high food during the second through the fifth stadia, and this group (HHHH and LHHH) had greater assimilation efficiency than did the previous group (Fig. 3). Individuals receiving high food during the last stadium but low food during the second through fourth stadia had the greatest assimilation efficiencies, and were indistinguishable from one another (LLLH and LLHH; Fig. 3).

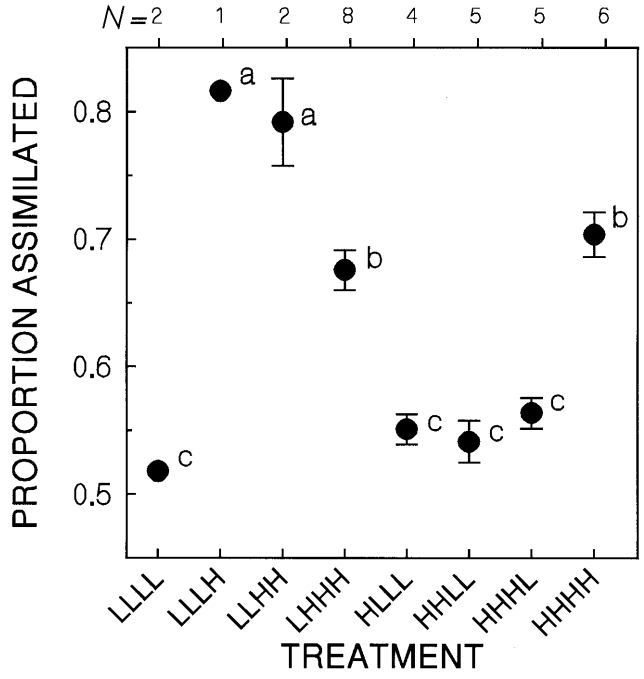

Fig. 3 Mean (±SE) proportion of assimilation efficiency for fifth-instar nymphs in each of the eight treatment groups. Proportion assimilated=(I–F)/I. See Materials and methods for further details. Means associated with the same letter are not significantly different (α=0.05). Numbers of surviving adults used in the analysis are listed at the top of the graph

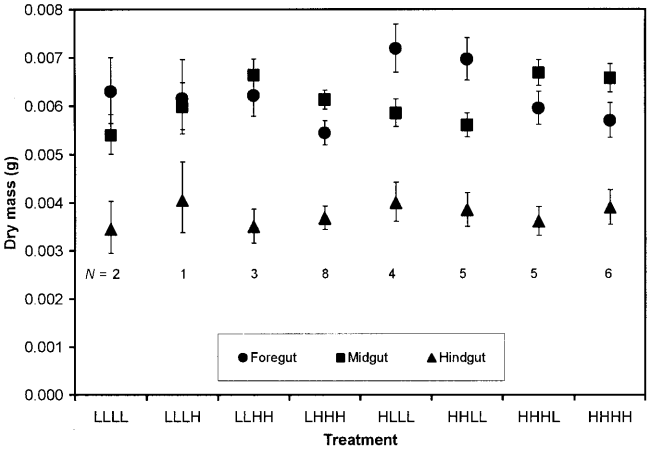

Fig. 4 Least-square mean dry masses (±SE=√mean square error/n) for fore-, mid-, and hindguts at maturity. Least-square means were adjusted for overall total body mass at the end of the experiment (ANCOVA, see text). Numbers of surviving adults used in the analysis for all three variables are listed below each group mean

Gut size

Treatment had no significant effect on masses of the foregut (ANCOVA, $F_{7,25}$=1.86, P=0.1197), midgut ($F_{7,25}$=1.66, P=0.1644), or hindgut ($F_{7,25}$=0.35, P=0.8248). Least-square means for each gut mass component were virtually identical for all treatments (Fig. 4). Log final mass at eclosion, as a covariate, was significant for all three gut sections (foregut: $F_{1,25}$=60.80, P<0.0001; midgut: $F_{1,25}$=38.93, P<0.0001; hindgut: $F_{1,25}$=6.52, P=0.0171). In all cases, the gut mass components increased with an increase in body size. MANCOVA for effects of treatments on all three gut components was also not significant (Pillai's trace=0.760, df=20,75, P=0.2674).

Discussion

In *R. microptera*, development rate responds to food availability primarily during the second through fourth stadia. Differences in food availability only during the first or only during the fifth stadia never yielded a significant effect on overall development time (Fig. 1B). Differences in feeding during the fifth stadium did not produce significant differences in its duration (Fig. 2E) providing further support for the hypothesis that development becomes inflexible by the start of the fifth stadium. The failure of increasing food only in the last stadium to alter significantly stadium duration (Fig. 2E; LLLL vs LLLH) could be explained by low sample size for those treatments, due to mortality, but this explanation is unlikely to account for the lack of a significant effect of decreasing food on last-stadium duration (Fig. 2E; HHHL vs HHHH) where sample sizes were relatively high. The scope for prolongation of different stadia varies (Fig. 2), with the second stadium having the greatest time range (spanning 18 days: compare Fig. 2B to others). The time ranges for the first and fifth stadia are the lowest (first instar spanning 3 days, fifth instar spanning 10 days). These differences in the scope for change in stadium duration no doubt contribute to the greater sensitivity of total development time to feeding during the middle stadia, which have the greatest scope for change in duration.

Growth during the fifth stadium appears to be necessary for successful eclosion to adulthood, even if individuals appear to be sufficiently large at the start of the fifth stadium to become functional adults. Our results thus indicate that after a certain critical period, roughly marked by the end of the fourth stadium, development rate becomes fixed and no longer responds in a major way to changes in food availability. The Bradshaw and Johnson (1995) model for fixed development seems to describe our results most closely, as this model postulates both a period of inflexible development late in the life cycle, and the need for positive growth in the last stadium, whereas the otherwise similar model of Leips and Travis (1994) does not include this latter postulate. These results suggest that the physiological and hormonal events postulated by Bradshaw and Johnson (1995) as being necessary for eclosion to adulthood have already been set into motion by the start of the fifth stadium (Nijhout 1994), though our data cannot provide a test of that specific mechanism.

Feeding during the preceding stadium often had a significant effect on the duration of the subsequent stadium. This implies that resources acquired during previous sta-

dia affect development rate in a later stadium. This kind of a carryover effect is seen in other life history events such as oviposition. For example, McCaffery (1975) showed that feeding early in adult development has a carryover effect on the quality and timing of oviposition in grasshoppers. Despite this evidence for carryover effects from feeding during some stadia, a difference in feeding during the first stadium alone did not affect overall development time or size of *R. microptera*. This is inconsistent with all of the models we tested. In particular, it is inconsistent with the fixed-development models (e.g., Leips and Travis 1994) that predict that development remains flexible early in the juvenile life cycle. Possibly, first-instar nymphs hatch with a supply of yolk or other stored nutrients, which buffer the effects of food availability on growth and development. However, we demonstrated that food during the first stadium affects its duration (Fig. 2A), although the difference in duration is rather small, particularly compared to effects of feeding during the second stadium (compare Fig. 2A,B). The lack of a feeding effect during the first stadium on total development time may simply reflect the limited potential for feeding during the first stadium to have effects on development time in the third, fourth, and fifth stadia. This could be due to the small cumulative mass of food ingested during the first stadium. What is clear, however, is that low food during the second or the third and fourth stadia greatly prolongs total development time. The mechanism by which feeding during one stadium affects development time in a subsequent stadium remains unknown, however Bradshaw and Johnson (1995) documented similar carryover effects of feeding across instars in mosquito larvae, suggesting that such developmental effects may be the rule for insects.

Assimilation efficiency changed in response to food availability. However, these changes were clearly not the result of modified gut size. These grasshoppers do not appear to modify gut size or relative allocation to fore-, mid-, and hindguts in response to low food quantity, and this contrasts with the effects of low food quality on the mass of gut tissue of another grasshopper (Yang and Joern 1994). If assimilation efficiency is not modified via morphological plasticity, it is probably modified via physiological plasticity.

Our results provide, at best, limited support for the hypothesis that changes in assimilation efficiency buffer effects of low feeding rate on growth and development. Low food availability for early instars increased the assimilation efficiency of our grasshoppers during the fifth stadium, but only when food availability was high in the fifth stadium. This effect of early low food may give individuals some ability to compensate for it if they are fortunate enough to encounter greater food availability later. A similar increase in efficiency, when an early low feeding rate is followed by an increased feeding rate is well documented in birds (e.g., Lepczyk et al. 1998). However, early low food does not produce greater assimilation efficiency in the fifth stadium if food remains low, and the strongest effect on efficiency was that low food during the fifth stadium yielded a much lower assimilation efficiency than did high food during the fifth stadium (Fig. 3). Such an effect is inconsistent with optimal-digestion models (Sibley 1981), but similar results have been observed in other insects (Lawton 1970; Mukerji and Guppy 1970). Estimates of efficiencies are subject to high measurement error because of the need to estimate dry masses, and the use of average values (Bernays and Barbehenn 1987), and our most extreme estimates of efficiencies are based on very low sample sizes (LLLL and LLLH; Fig. 3). The apparent effects on efficiency in other treatments (with greater sample sizes) were still strikingly large (Fig. 3). Effects of earlier feeding on efficiency during the fifth stadium may help to buffer grasshopper final size at eclosion against food shortage, but only under specific circumstances: grasshoppers that have been poorly fed throughout development may be more able to exploit flushes of food late in development and thus to compensate, to some extent, for reduced growth earlier in development.

Similar studies on reproductive responses of adult female *R. microptera* also show developmental inflexibility late in the developmental period (Moehrlin and Juliano 1998). These results suggest that similar constraints operated on two phases of the life cycle of *R. microptera* (juvenile growth, development, and maturation and reproductive tactics of females). These two parts of the life cycle are controlled by the endocrine system in insects, and indeed are probably both influenced by juvenile hormone and ecdysteroids (McCaffery and McCaffery 1983; Nijhout 1994; Wheeler 1996; Bellés 1998). The similar constraints present in both life cycle phases may therefore derive from similar endocrine control. Bradshaw and Johnson (1995) found a similar pattern of constraint on development for a mosquito larva, including inflexible development late in the juvenile life, a requirement of growth during the last stadium, and significant carryover effects of feeding during early stadia on the duration of the succeeding stadium. The similarities of these results for different life cycle phases in a single species, and for juvenile stages of a holometabolous mosquito and a hemimetabolous grasshopper suggest that loss of developmental plasticity is a prominent feature of insect life cycles and of the insect developmental system. Similar patterns of developmental constraints on flexibility also seem to be present in other arthropod groups (Ebert 1994; Twombly 1996).

The results imply that development in *R. microptera*, and probably other insects, is constrained by inflexibility late in the life of a juvenile, and these constraints may limit the ability of an individual to respond to a changing environment. Such constraints are likely to arise from the endocrine mechanisms coordinating development, which are products of multiple selective forces and appear to be phylogenetically conservative (e.g., Higgins and Rankin 1996). Our results raise further questions. At present, we do not know the endocrine and physiological events that result in inflexible development, though Bradshaw and Johnson (1995) postulated a mechanism.

We also do not yet know whether the details of these constraints on insect developmental plasticity (e.g., exactly when loss of flexibility occurs) are correlated with environmental differences encountered by different species or populations, as has been documented by Leips and Travis (1994) for anurans.

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References

Alford RA, Harris RN (1988) Effect of larval growth history on anuran metamorphosis. Am Nat 131:91–106

Bellés X (1998) Endocrine effectors in insect vitellogenesis. In: Coast GM, Webster SG (eds), Recent advances in arthropod endocrinology. Cambridge University Press, Cambridge, UK, pp 71–90

Bernays EA, Barbehenn R (1987) Nutritional ecology of grass foliage-chewing insects. In: Slansky F, Rodriguez JG (eds), Nutritional ecology of insects, mites and spiders. Wiley, New York, pp 147–175

Bradshaw WE, Johnson K (1995) Initiation of metamorphosis in the pitcher-plant mosquito: effects of larval growth history. Ecology 76:2055–2065

Büns M, Ratte HT (1991) The combined effects of temperature and food consumption on body weight, egg production and developmental time in *Chaoborus crystallinus* De Geer (Diptera: Chaoboridae). Oecologia 88:470–476

Chapman RF (1988) The relationship between diet and the size of the midgut caeca in grasshoppers (Insecta: Orthoptera: Acridoidea). Zool J Linn Soc 94:319–338

Chapman RF (1998) The insects: structure and function, 4th edn. Cambridge University Press, Cambridge, UK

Ebert D (1994) A maturation size threshold and phenotypic plasticity of age and size at maturity in *Daphnia magna*. Oikos 69:309–317

Ebert TA (1996) Adaptive aspects of phenotypic plasticity in echinoderms. Oceanol Acta 19:347–355

Gotthard K, Nylin S (1995) Adaptive plasticity and plasticity as an adaptation: a selective review of plasticity in animal morphology and life history Oikos. 74:3–17

Hensley FR (1993) Ontogenetic loss of phenotypic plasticity of the age at metamorphosis in tadpoles. Ecology 75:1345–1356

Higgins LE, Rankin MA (1996) Different pathways in arthropod postembryonic development. Evolution 50:573–582

Krause A, Haukioja E, Hanhimäki S (1999) Phenotypic plasticity in foraging behavior if sawfly larvae. Ecology 80:1230–1241

Lawton JH (1970) Feeding and food energy assimilation in larvae of the damselfly *Pyrrhosoma nymphula* (Sulz.) (Odonata: Zygoptera). J Anim Ecol 39:669–689

Leips J, Travis J (1994) Metamorphic responses to changing food levels in two species of hylid frogs. Ecology 75:1345–1356

Lepczyk CA, Caviedes-Vidal E, Karasov W (1998) Digestive responses during food restriction and realimentation in nestling house sparrows (*Passer domesticus*). Physiol Zool [Lond] 71: 561–573

McCaffery AR (1975) Food quality and quantity in relation to egg production in *Locusta migratoria migratoriodes*. J Insect Physiol 21:1551–1558

McCaffery AR, McCaffery VA (1983) Corpus allatum activity during overlapping cycles of oocyte growth in adult female *Melanoplus sanguinipes*. J Insect Physiol 29:259–266

Manly BF (1991a) Randomization and Monte Carlo methods in biology. Chapman & Hall, London

Manly BF (1991b) RT: a program for randomization testing, version 1.02. West Incorporated, Cheyenne, Wyo

Mathavan S, Muthukrishnan J (1976) Effects of ration levels and restriction of feeding durations on food utilization in *Danaus chrysippus* (Lepidoptera: Danaiidae). Entomol Exp Appl 19:155–162

Moehrlin GS, Juliano SA (1998) Plasticity of insect reproduction: testing models of flexible and fixed development in response to different growth rates. Oecologia 115:492–500

Mukerji MK, Guppy JC (1970) A quantitative study of food consumption and growth in *Pseudaletia unipunctata* (Lepidoptera: Noctuidae). Can Entomol 102:1179–1188

Muthukrishnan J, Delvi MR (1974) Effect of ration levels on food utilisation in the grasshopper *Poecilocercus pictus*. Oecologia 16:227–236

Nijhout HF (1994) Insect hormones. Princeton University Press, Princeton, NJ

Nir I, Nitsan S, Dror Y, Shapira N (1978) Influence of overfeeding on growth, obesity and intestinal tract in young chicks of light and heavy breeds. Br J Poultry 39:27–35

Nylin S, Gotthard K (1998) Plasticity in life-history traits. Annu Rev Entomol 43:63–83

SAS (1989) SAS/STAT users guide, version 6, vol 2, 4th edn. SAS Institute, Cary, NC

Schroeder LA (1976) Effects of food deprivation on the efficiency of utilization of dry matter energy, and nitrogen by larvae of the cherry scallop moth *Calocalpe undulata*. Ann Entomol Soc Am 69:55–58

Scriber JM, Slansky F (1981) The nutritional ecology of immature insects. Annu Rev Entomol 26:183–211

Seim DP, Sikes RS (1998) Tradeoffs between growth and reproduction in response to temporal variation in food supply. Evol Biol Fishes 53:319–329

Sibley RM (1981) Strategies of digestion and defecation. In: Townsend CR, Calow P (eds.) Physiological ecology: an evolutionary approach to resource use. Sinauer, Sunderland, Mass, pp 109–139

Stearns SC (1992) The evolution of life histories. Oxford University Press, Oxford

Twombly S (1996) Timing of metamorphosis in a freshwater crustacean: comparison with anuran models. Ecology 65:1155–1160

Uvarov B (1966) Grasshoppers and locusts: a handbook of general acridology. Vol 1: anatomy, physiology, development, phase polymorphism, introduction to taxonomy. Cambridge University Press, Cambridge, UK

Wheeler D (1996) The role of nourishment in oogenesis. Annu Rev Entomol 41:407–431

Whitman DW, Orsak LJ (1985) Biology of *Taeniopoda eques* (Orthoptera: Acrididae) in southeastern Arizona. Ann Entomol Soc Am 78:811–825

Wilbur HM, Collins JP (1973) Ecological aspects of amphibian metamorphosis. Science 182:1305–1314

Yang Y, Joern A (1994) Gut size changes in relation to variable food quality and body size in grasshoppers. Funct Ecol 8: 36–45