



Pergamon

Journal of Insect Physiology 49 (2003) 73–80

Journal
of
Insect
Physiology

www.elsevier.com/locate/jinsphys

Canalization of development and ecdysteroid timing during the last instar in lubber grasshoppers

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Received 30 July 2002; received in revised form 18 October 2002; accepted 20 October 2002

Abstract

Development in many phytophagous, holometabolous insects is flexible at the beginning but inflexible at the end of the last larval instar. A prominent feature of the inflexible period is a peak in hemolymph levels of ecdysteroids. We tested whether this pattern holds true for the final molt of a phytophagous, hemimetabolous insect, *Romalea microptera* (the Eastern lubber grasshopper). We fed one group of grasshoppers a high quantity diet (H) throughout the 5th (final) instar and a second group a low quantity diet (L) throughout the instar. Three other diet treatments involved starting the instar on the high diet and then abruptly switching to the low diet at 3, 8, or 13 days (H3L, H8L, and H13L respectively) and continuing the low diet until adult molt. Diet treatment did not affect the maximum hemolymph level of ecdysteroids (E_{\max}); this peak typically reached ~4000 ng/ml. Ecdysteroid levels were elevated for ~4 days in all groups. In contrast, diet significantly affected age at adult molt and age at E_{\max} such that $H = H13L = H8L < H3L = L$. We identified estimates of thresholds for weight gain (20% initial weight) and hemolymph ecdysteroids (100 ng/ml), after which diet did not affect the time to the adult molt. The weight gain threshold was less precise than the ecdysteroid threshold. These results suggest that *R. microptera* has an extended period of inflexible (canalized) development during the final instar that includes a peak of ecdysteroids. We hypothesize this pattern holds for many phytophagous, hemimetabolous insects.

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Keywords: Hemimetabolous; Molt; Phenotypic plasticity; Canalization; Critical weight

1. Introduction

The ability of animals to respond developmentally to changes in food availability has been an intense area of research (Schlichting and Pigliucci, 1998; West-Eberhard, 2002). Phenotypic plasticity is the ability of developmental processes to respond to environmental changes. The opposite of plasticity is canalization, the developmental inability to respond to environmental changes (Schlichting and Pigliucci, 1998). Some models of development posit that animals can continuously adjust their development, even after attaining a developmental threshold (Wilbur and Collins, 1973; Alford and Harris, 1988; Reznick, 1990; Day and Rowe, 2002). These *plasticity* models explicitly predict that removing

food from an animal that has attained its threshold will speed its rate of development in comparison to well-fed controls. Other models of development hold that animals cannot adjust their development rates after attaining a threshold (Reznick, 1990; Leips and Travis, 1994). These *canalization* models predict that removing food from an animal that has attained its threshold will not change its rate of development in comparison to well-fed controls (see Bradshaw and Johnson 1995 for a canalization model specifically for insects).

A major developmental event in arthropods is molting. In many phytophagous, holometabolous insects development toward the last larval molt is canalized at the end of the instar (e.g., Allegret 1964; Nijhout and Williams, 1974a,b; Sparks et al., 1983). For example, once the tobacco hornworm (*Manduca sexta* [Lepidoptera: Sphingidae]) attains a threshold weight (i.e., critical weight), starvation does not change the time to molting (Nijhout and Williams, 1974a). The endocrinological and physiological events underlying the canalized phase

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of development in phytophagous, holometabolous insects have been described in detail (see Truman, 1985; Richter, 1999 and references therein). Included in this progression of physiological events is the appearance of high levels of ecdysteroids (includes the arthropod molting hormones, their precursors, and metabolites) in the hemolymph. Elevated ecdysteroid titers are correlated with processes involved in molting, such as the deposition of the epicuticle (Riddiford, 1985; Nijhout, 1994). These results for *Manduca sexta* are consistent with canalization models of development, as are other developmental events in most insects that have been studied (reproduction, Moehrlin and Juliano, 1998; development from hatchling to adult, Flanagin et al., 2000). However, canalization models of development do not explain molting in all insects. At least one insect speeds its development upon starvation. After reaching a threshold mass, starvation actually decreases the time to molting in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae) (Shafiei et al., 2001). This result is consistent with plastic models of development (Wilbur and Collins, 1973).

The developmental events of the last larval molt in phytophagous, hemimetabolous insects are not understood as completely as those for phytophagous, holometabolous insects. However, most endocrinological evidence suggests that the hormonal mechanisms underlying the last larval molt in these two groups are similar (Riddiford, 1985; Truman, 1985; Gelman et al., 2002). The canalization of the molt cycle in blood-feeding, hemimetabolous insects is perhaps best known in *Rhodnius prolixus* (Hemiptera: Reduviidae). This bug clearly has a canalized phase at the end of the molt cycle, because feeding is required for molting to occur, yet it normally feeds only once per instar (Wigglesworth, 1934). For phytophagous, hemimetabolous insects, canalized development toward molting has been reported only for *Oncopeltus fasciatus* (Hemiptera: Lygaeidae). Both of these hemipterans appear to use abdominal stretching as a cue for molting (Nijhout, 1979), which may not be true for Orthoptera and other Hemimetabola.

Romalea microptera Beavois (= *R. guttata* Houttuyn), the Eastern lubber grasshopper, is a phytophagous, hemimetabolous insect (Jones et al., 1987, 1989; Whitman, 1988). In one recent study it was demonstrated that nymphs showed plasticity in response to food availability during the entire developmental period from hatching to the adult molt (i.e., across instars), which varied from 60 (for well-fed grasshoppers) to 90 days (for poorly-fed grasshoppers) (Flanagin et al., 2000). Furthermore, individuals that were well-fed through the first four instars, but then starved during the 5th instar, died before adulthood. This demonstrates that nutrition during the 5th instar is necessary for development (Flanagin et al., 2000). Neither plasticity within an instar

nor ecdysteroid titers in juveniles have been reported for this grasshopper.

We examined the duration of the 5th (last) instar, grasshopper mass, and ecdysteroid levels in *R. microptera* males maintained on different diet treatments. Our predictions were that: (1) an extended canalized phase of development would exist during the 5th instar [i.e. development would fit canalization models]; (2) a critical weight would occur at the beginning of this canalized phase, after which changes in diet would not affect the time to the adult molt (as predicted by Nijhout, 1981); and (3) there would be a threshold titer of ecdysteroids that would mark the beginning of the canalized phase.

2. Materials and methods

2.1. Experimental animals

We used 5th instar male *R. microptera* from our laboratory colony. This colony originated with individuals collected near Copeland, FL, USA in 1996 and 1997. We isolated males (weighing between 0.8 g and 1.8 g) on the day of ecdysis to the 5th instar (= 5th instar ecdysis) using wing size and body shape as described for Western lubber grasshoppers (Whitman and Orsak, 1985). Females were not used to avoid effects that might result from their preparation for vitellogenesis. All grasshoppers were housed individually in 1-liter, ventilated plastic cups. A rectangle of wire mesh ~40 × 200 mm (that served as molting substrate) was hung from the interior lid of each cup. Grasshoppers were kept on a 14L:10D photoperiod with a corresponding 32:24°C thermocycle and weighed daily throughout the experiment.

2.2. Diet treatments

We assigned each grasshopper to one of five diet treatments: 5 g Romaine lettuce and 20 oat flakes daily from 5th instar ecdysis to adult molt (high-fed = H); 0.5 g Romaine lettuce and 2 oat flakes daily from 5th instar ecdysis to adult molt (low-fed = L); H rations from 5th instar ecdysis to day 13 and then L rations until adult molt (H13L); H rations from 5th instar ecdysis to day 8 and then L rations until adult molt (H8L); H rations from 5th instar ecdysis to day 3 and then L rations until adult molt (H3L). For example, H3L grasshoppers received a high ration on the day of ecdysis to the 5th instar (i.e., day 0), a high ration on each of the next 2 days (i.e., days 1 and 2), and low rations on each subsequent day until the adult molt. Grasshoppers fed a high ration never completely consumed their meals; in contrast, grasshoppers fed a low ration almost always completely con-

sumed their meals, except for the last 4 days before adult molt.

2.3. Test of plasticity vs. canalization

The ages at adult molt (reciprocal transformed) across treatment groups were compared with ANOVA. Because these data fit developmental models of canalization (see Results and Discussion), we sought thresholds of weight gain and ecdysteroid titers.

2.4. Test of critical weight

We tested whether there was a critical (threshold) weight after which changes in diet would not affect the time remaining to the adult molt. The weight gained (as percent initial) for each individual was calculated, and the average weight gains for each group were plotted (Fig. 1). We chose to use percent weight gain as the metric of critical weight because it seemed to reduce some of the variance within diet groups, even though initial weight was not a significant cofactor using ANCOVA. From the plot (Fig. 1), it appeared that the best estimate of the critical weight gain was between 10 and 40% (i.e., the time from these weight gains to adult molt did not appear to vary across diets). We calculated the time required by each grasshopper ($n = 8$ per treatment group for this analysis) to reach the adult molt after crossing the putative thresholds of 10%, 20%, 30%, and 40% weight gain, and maximum weight. Because all these thresholds were determined by individual, there was a distinct age on which the threshold is breached (Fig. 1). After determining the age at which each threshold was reached, we tested whether the times to the

adult molt from each putative critical weight differed across diet treatments. Because the groups did not have equal variances and were not easily transformed to meet this assumption, the data were analyzed with Kruskal–Wallis tests (i.e., non-parametric ANOVAs) with Dunn's post-test. Because this analysis used five iterative tests, $\alpha = 0.01$ (Bonferroni's correction). In addition, we also tested whether final body size was affected by diet using ANOVA.

2.5. Analysis of hemolymph ecdysteroids

Beginning five days after 5th instar ecdysis and continuing until adult molt, we collected 5 μ l of hemolymph from these same grasshoppers every day. Sample sizes for ecdysteroid analysis were: H = 6; H13L = 8; H8L = 9; H3L = 9; and L = 9. Each hemolymph sample immediately was added to an aliquot of methanol and stored at 4°C until analyzed by radioimmunoassay (RIA) for ecdysteroids (Borst and O'Conner, 1972). At the time of analysis, each sample was dried and resuspended in 500 μ l methanol. A 50 μ l aliquot (equivalent to 0.5 μ l hemolymph) of this resuspension was dried in an assay tube and resuspended in 100 μ l of a borate buffer (0.05 M H_3BO_3 ; 0.9% NaCl; 0.1% gelatin; 0.05% Triton X-100; pH 8.4) containing ~5000 DPM [^3H]ecdysone (α -[23,24,- ^3H (N)]ecdysone; NEN; Boston, USA; specific activity = 1.9 TBq/mmol). Then we added 100 μ l of borate buffer containing a mouse monoclonal anti-ecdysteroid antiserum (final dilution 1:20 000). This antiserum binds ecdysone (i.e. α -ecdysone) about twice as strongly as 20-hydroxyecdysone (i.e., β -ecdysone or ecdysterone). The reaction mixture was vortexed, incubated for 2 h at room temperature, and chilled on ice for 10 min. Then 0.5 ml of cold, stirred dextran-coated charcoal (0.1 g H_3BO_3 ; 0.045% NaCl; 12.5 mg dextran; 1.4 mg EDTA; 0.5 mg sodium azide; 1 g charcoal; 100 ml dH_2O) was added to each tube. The tubes were incubated for 3 min and then centrifuged at 2000 g at 4°C for 5 min. The supernatant (containing [^3H]ecdysone bound to the antibody but not free [^3H]ecdysone) was counted in a liquid scintillation counter. The radioactivity in each assay tube was compared to the standard curve to determine the amount of ecdysteroids in the sample.

For our standard curve, we used a series of methanolic dilutions of 20-hydroxyecdysone (Sigma Chemical Co., St. Louis, USA) ranging from 0 (i.e. total bound) to 10 ng. The radioactivity from each assay tube was expressed as a percentage of the radioactivity in the total bound tubes (= %TB). The standard curve was plotted as log ecdysteroids vs. %TB, and an equation fit to this curve was used to estimate the amount of ecdysteroids in each sample. All data in this paper are reported as 20-hydroxyecdysone equivalents and are referred to simply as 'ecdysteroids'. Our detection limit (mean \pm SE)

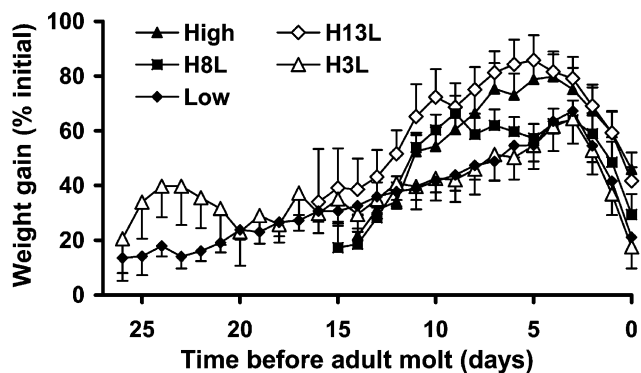


Fig. 1. Diet treatment significantly affected weight gain in last instar lubber grasshoppers. The figure shows mean weight gains (+SE) of 5th instar male *Romalea microptera* before adult molt. See text for description of diets. Only those days that included at least half the grasshoppers from the treatment group are plotted. Hence, the data for H3L and L grasshoppers (some of which required >30 days to molt) are truncated at 26 days before the adult molt. Including only days with at least half the grasshoppers from each treatment group also results in each group appearing to begin the instar at greater than 0% weight gain.

for this experiment was 10 pg/assay tube or ~20 ng ecdysteroids/ml hemolymph.

2.6. Test of ecdysteroid threshold for molting

We tested whether there was a threshold titer of ecdysteroids, after which changes in diet would not affect time to adult molt. Putative ecdysteroid thresholds of 40 ng/ml, 100 ng/ml, and the maximum titer were tested. Similar to the analysis of the critical weight, we calculated the time required to reach the adult molt after reaching each putative threshold titer. Statistical tests were analogous to those for critical weight tests. Because three iterative tests were used, $\alpha = 0.0167$ (Bonferroni's correction).

3. Results

3.1. Time to adult molt

Diet treatment significantly affected the time from the 5th instar ecdysis to the adult molt (Fig. 2; ANOVA; $F_{4,40} = 10.00$; $P < 0.0001$). High-fed, H13L, and H8L grasshoppers did not differ significantly in time to adulthood. H3L and L grasshoppers molted significantly later than H, H13L, and H8L. Thus, limiting the diet for at least the last 50% of the 5th instar did not change the age at adult molt, whereas limiting the diet from days 3 to 8 of the cycle delayed age at adult molt.

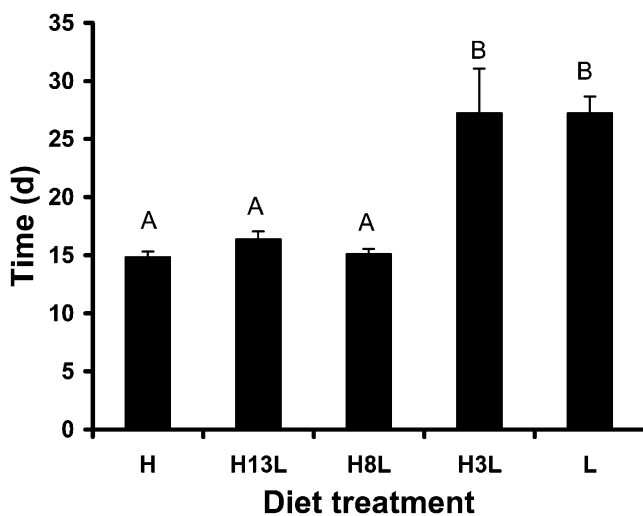


Fig. 2. Time to the adult molt initially is affected by diet but is canalized after attainment of a threshold. The figure shows the age (mean + SE) at adult molt in 5th instar male *Romalea microptera* in response to diet treatments. See text for description of diets. Letters indicate significant differences across treatment groups.

3.2. Test of critical weight

The average weight at the 5th instar ecdysis for these grasshoppers was 1.45 ± 0.03 g. Diet significantly affected the time from reaching 10% weight gain to the adult molt (Figs. 1 and 3; Kruskal–Wallis test; $KW = 32.0$). In contrast, diet did not significantly affect the time from reaching 20% ($KW = 9.61$), 30% ($KW = 7.08$), and 40% ($KW = 8.23$) weight gain to the adult molt. Diet significantly affected the times from reaching maximum weight gain to the adult molt ($KW = 25.2$). In addition, diet significantly affected adult size (see weight gain at zero days before adult molt in Fig. 2; ANOVA; $F_{4,38} = 3.56$; $P = 0.0157$). High-fed grasshoppers had a greater percent increase in weight than H3L grasshoppers, but no other pairwise comparisons were significantly different. Weight gains (percent initial) at adult molt (mean ± SE) were: H = 46.9 ± 6.2 ; H13L = 41.7 ± 5.0 ; H8L = 29.4 ± 7.5 ; H3L = 17.6 ± 7.9 ; L = 21.1 ± 6.0 .

3.3. Test of ecdysteroid threshold for molting

All grasshoppers had developmental profiles of total RIA-detectable ecdysteroids that were initially low and then showed a single, broad peak during the final ~8 days of the cycle (Fig. 4). Diet significantly affected the time from reaching an ecdysteroid titer of 40 ng/ml to the adult molt, such that $H8L < L$ but no other pairwise comparisons were significantly different (Fig. 5; $KW = 18.1$). In contrast, diet had no significant effect on the time to the adult molt from either reaching 100 ng/ml ($KW = 8.78$) or reaching the maximum titer of

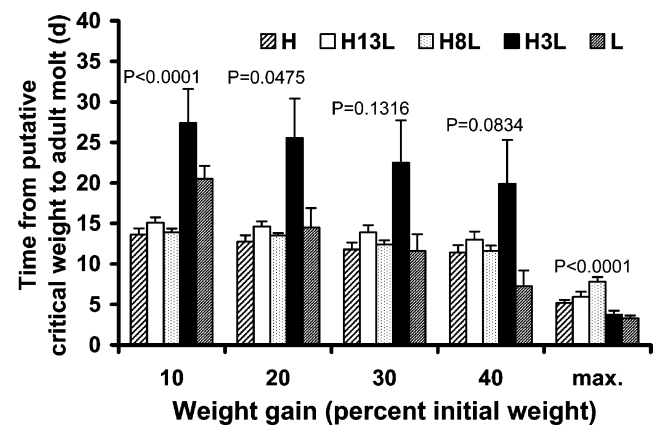


Fig. 3. The best estimate of the weight gain threshold for lubber grasshoppers is 20% initial weight. The figure shows the time to the adult molt (mean + SE) after reaching putative critical weight gain thresholds for 5th instar male *Romalea microptera* on five diet treatments. See text for description of diets. Diet treatment significantly affected time to the adult molt after grasshoppers had gained 10% of their initial weight but did not affect time to the adult molt after grasshoppers had gained 20%, 30%, and 40% of their initial weight ($\alpha = 0.01$; Bonferroni's correction for five iterative tests).

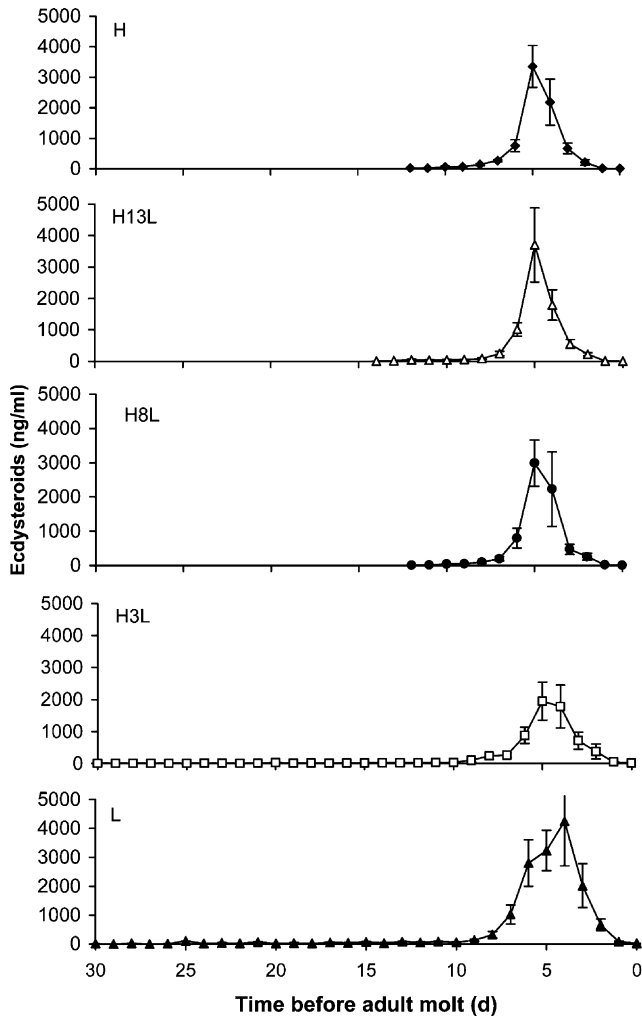


Fig. 4. The time before the adult molt at which hemolymph ecdysteroids peak was unaffected by diet treatment in lubber grasshoppers. Developmental profiles of hemolymph ecdysteroid levels in 5th instar male *Romalea microptera* fed five diet regimes. See text for description of diets. Detection limit for the radioimmunoassay was ~ 20 ng/ml.

ecdysteroids (E_{\max} ; KW = 3.02). Diet also had no significant effect on the level of E_{\max} (ANOVA; $F_{4,40} = 1.11$; $P = 0.3665$). The E_{\max} titers reached during the 5th instar for each diet group were: H = 4260 ± 350 ng/ml; H13L = 4290 ± 1100 ng/ml; H8L = 4150 ± 950 ng/ml; H3L = 2560 ± 490 ng/ml; L = 5340 ± 1370 ng/ml.

4. Discussion

4.1. Development during the last instar fits models of canalization

We have identified a canalized phase of development in *R. microptera* that begins after crossing a developmental threshold. This inflexible phase is at least 8 but less than 13 days in duration. Our results are consistent

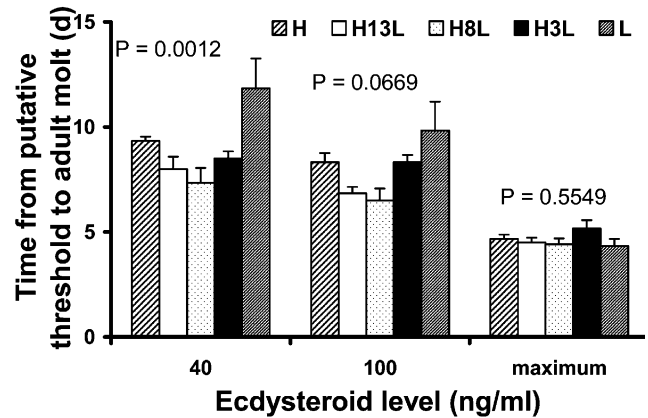


Fig. 5. The best estimate of the ecdysteroid titer threshold for lubber grasshoppers is at least 100 ng/ml. The figure shows the time to the adult molt (mean + SE) after reaching putative ecdysteroid thresholds for 5th instar male *Romalea microptera* on five diet treatments. See text for description of diets. The time from attaining 40 ng/ml ecdysteroids to adult molt was affected by diet, whereas the time from attaining 100 ng/ml or the maximum titer of ecdysteroids to adult molt was not affected by diet ($\alpha = 0.0167$; Bonferroni's correction for three iterative tests).

with canalization models of development, which predict that reducing food availability of a grasshopper after it attains a developmental threshold will not change the time to its adult molt (Leips and Travis, 1994; Bradshaw and Johnson, 1996). At least three results lead to this conclusion. First, our low diet was limiting because it significantly delayed the adult molt when fed throughout the entire 5th instar (compare H and L grasshoppers in Fig. 2). Second, H3L grasshoppers molted significantly later than H grasshoppers, demonstrating that the low diet does delay molting when initiated at age 3 days. These two results show that development toward the adult molt is initially plastic. Third, H8L grasshoppers molted at the same age as H grasshoppers, demonstrating that low rations beginning at age 8 days neither accelerated nor delayed molting. This unresponsive phase is at least twice as long as the 4-day period at the end of the molt cycle during which *R. microptera* does not feed (data not shown). These observations are inconsistent with developmental models of plasticity (see Introduction; Wilbur and Collins, 1973). The canalized phase that we detected at the end of the last larval instar is analogous to the canalized phase observed at the end of the oviposition cycle in *R. microptera* (Moerhlin and Juliano, 1998; Hatle et al., 2000). Importantly, our data demonstrate canalization of molt development in a phytophagous, hemimetabolous insect with a long evolutionary history of solid-plant feeding (*sensu* Dow, 1986), in contrast to hemipterans, most of which are liquid feeding. This study suggests that phytophagous, hemimetabolous insects may generally lack responsiveness to feeding during the final development to molt, as do phytophagous, holometabolous insects.

4.2. Test of critical weight

Because our results for age at adult molt imply that lubber grasshoppers attain a threshold and then enter a canalized phase of development, we sought a weight threshold that marks entrance into the canalized phase. Our data suggest that *R. microptera* does have a critical weight (as predicted by Nijhout, 1981), after which diet does not influence the time until the adult molt (Fig. 3). Our best estimate of this critical weight gain is 20% of the initial weight. In contrast, diet strongly affected the amount of time required to reach the adult molt after a grasshopper gained 10% of its initial weight. The estimate of 20% weight gain as the critical weight is not ideal; half the individuals in the H3L group reached 20% initial weight over 20 days before the adult molt. These individuals do not seem to be canalized upon reaching 20% weight gain, and perhaps even 30% weight gain (see below for other weaknesses of critical weights). It is likely that the critical weight for a population is a distribution of weight gains instead of an exact weight gain for each individual. In this light, it could be expected that some individuals are not canalized upon reaching the mean critical weight of 20% weight gain.

Interestingly, diet significantly affected the time from maximum weight gain to adult molt. This observation is not inconsistent with a critical weight. Instead, H3L and L grasshoppers simply continued to gain weight (and presumably continued to feed) further into the molt cycle than H8L grasshoppers. H8L grasshoppers had the longest period from maximum weight gain to the adult molt, which may reflect the fact that food availability was reduced about this time.

4.3. Test of ecdysteroid threshold for molting

Similar to the weight threshold, we sought a threshold ecdysteroid titer that marks the canalized phase. Lubber grasshoppers appear to have an ecdysteroid threshold of approximately 100 ng/ml, because after reaching this level the time to adult molt is unresponsive to diet (Fig. 5). This titer is <5% of the mean E_{\max} titer; in other words, it is a very low level of ecdysteroids that marks the canalized phase. The physiological actions of ecdysteroids in molting processes are well-characterized (Truman, 1985; Richter, 1999 and references therein). That the canalized phase is marked by a threshold ecdysteroid titer suggests that canalization is due to physiological processes involved with molting. That is, it suggests the canalized phase is the time needed for gene activation, protein synthesis, and protein function (Truman, 1985; Richter, 1999 and references therein) to proceed.

4.4. Comparison of critical weight vs. ecdysteroid titer as the canalization threshold

We feel ecdysteroid titers are a more conservative and more believable threshold than the critical weight. Ecdysteroid titers appear to provide more consistent mean times from the threshold level to the adult molt. Most notably, the mean time from a 20% weight gain to the adult molt in H3L grasshoppers was about twice that observed in four other diet treatments. Perhaps more important, there was high variance in age at adult molt among the H3L grasshoppers (see error bars in Fig. 2). In contrast, the mean time after reaching the ecdysteroid threshold had a lower variance than the mean time after reaching the weight threshold (compare error bars in Figs. 3 and 5). Further, it seems much more likely to us that the mechanism(s) underlying the canalization of development to molting involves ecdysteroids and not weight gain per se. Nevertheless, the weight gain threshold may be better at identifying the initiation of the canalized phase. The time from the ecdysteroid threshold to adult molt was ~8 days, whereas the time from the critical weight to the adult molt was ~13 days (cf. Figs. 3 and 5). The 5-day difference between reaching the thresholds raises the question of what happens between the weight gain threshold and the initiation of ecdysteroid synthesis. Although we have no data for lubber grasshoppers to directly address this question, it seems likely that the release of prothoracicotropic hormone is one event that occurs between these two thresholds (Nijhout, 1994).

4.5. Plasticity in weight gain while timing of molting is canalized?

Large body size has been shown in males of many species to have benefits, including fighting ability, mate choice, and fecundity (Alcock, 1993). It could be predicted that selection pressures for large body size have favored grasshoppers that grow as much as possible, even when the timing of development to the adult molt is fixed. This would be analogous to the canalization of timing to oviposition before the determination of the number of eggs in lubber grasshoppers (Moehrlin and Juliano, 1998). Hence, we tested whether weight gain was responsive to diet during the period when time to the adult molt is not responsive to diet. The critical comparison is H vs. H8L grasshoppers, which had similar developmental timing despite different diets for the last half of the instar. The weight gain at the adult molt was not significantly different in these two groups (ANOVA, Tukey–Kramer $q = 2.57$; $P > 0.05$), so our data do not support plasticity in weight gain while timing of molting is canalized. However, there was a trend in the predicted direction, because the weight gained by H grasshoppers (46.9 ± 6.2) was 1.6-fold that in H8L animals

(29.4 ± 7.5). Further studies in this direction may be profitable.

4.6. Comparison of molting in hemimetabolous and holometabolous phytophagous insects

Most hormonal mechanisms underlying molting in hemimetabolous insects appear to be similar to those for holometabolous insects (Truman, 1985). However, whether these endocrinological events create a lengthy canalized phase in the molt cycle was unclear and has to our knowledge only been investigated for a single hemipteran (Nijhout, 1979). Our data indicate that a major peak of ecdysteroids occurs during the canalized phase before the adult molt in *R. microptera* (Fig. 4). These results are comparable to phytophagous, holometabolous insects before the pupal molt (Nijhout, 1994). We suspect that development during the last instar in other phytophagous, hemimetabolous insects might also be canalized when ecdysteroid titers peak, or even at low, threshold levels of ecdysteroids.

These results may be applicable to non-ultimate instars of phytophagous, hemimetabolous insects. Most studies suggest that the mechanisms underlying the progression toward molting are similar for each instar (Nijhout, 1981; Riddiford, 1985). We hypothesize that development toward molting is canalized in non-ultimate instars of the lubber grasshopper and this development is marked by both weight and ecdysteroid thresholds.

Understanding the physiological steps that occur before and during the canalized phase of insect molting may lead to a more general understanding of why canalized phases exist. Phases of plasticity and canalization (e.g., sensitive phases and critical weights), and much of the physiology underlying these phases, have been well studied for development during the last larval instar in several holometabolous insects. For this reason, insect molting may be the best experimental system with which to address why canalized phases exist.

Acknowledgements

We thank members of the Collaborative-Research at Undergraduate Institutions program at Illinois State University, particularly Steve Juliano and Doug Whitman, for constructive criticism throughout the course of this project, and two anonymous reviewers for constructive criticism. This research was funded by National Science Foundation grant DBI-9978810 to DWB.

References

Alcock, J., 1993. *Animal Behavior*, 5th ed. Sinauer Assoc, Inc, Sunderland, MA, USA.

- Alford, R.A., Harris, R.N., 1988. Effect of larval growth history on anuran metamorphosis. *American Naturalist* 131, 91–106.
- Allegret, P., 1964. Interrelationship of larval development, metamorphosis and age in a pyralid lepidopteran, *Galleria mellonella* (L.), under the influence of dietetic factors. *Experimental Gerontology* 1, 49–66.
- Borst, D.W., O'Conner, J.D., 1972. Arthropod molting hormone: radioimmune assay. *Science* 178, 418–419.
- Bradshaw, W.E., Johnson, K., 1996. Initiation of metamorphosis in the pitcher-plant mosquito: effects of larval growth history. *Ecology* 76, 441–443.
- Day, T., Rowe, L., 2002. Developmental thresholds and the evolution of reaction norms for age and size at life-history transitions. *Am Nat* 159, 338–350.
- Dow, J.A.T., 1986. Insect midgut function. *Advances in Insect Physiology* 19, 187–328.
- Flanagin, V.L., Hasse, S.P., Juliano, S.A., 2000. Effects of growth rates on development to metamorphosis in the lubber grasshopper, *Romalea microptera*. *Oecologia* 125, 162–169.
- Gelman, D.B., Blackburn, M.B., Hu, J.S., 2002. Timing and ecdysteroid regulation of the molt in last instar greenhouse whiteflies (*Trialeurodes vaporariorum*). *Journal of Insect Physiology* 48, 63–73.
- Hatle, J.D., Juliano, S.A., Borst, D.W., 2000. Juvenile hormone is a marker of the onset of reproductive canalization in lubber grasshoppers. *Insect Biochemistry and Molecular Biology* 30, 21–27.
- Jones, C.G., Hess, T.A., Whitman, D.W., Silk, P.J., Blum, M.S., 1987. Effects of diet breadth on autogenous chemical defense of a generalist grasshopper. *Journal of Chemical Ecology* 13, 283–297.
- Jones, C.G., Whitman, D.W., Compton, S.J., Blum, M.S., 1989. Reduction in diet breadth results in sequestration of plant chemicals and increase of chemical defense in a generalist grasshopper. *Journal of Chemical Ecology* 15, 1811–1822.
- Leips, J., Travis, J., 1994. Metamorphic response to changing food levels in two species of hylid frogs. *Ecology* 75, 1345–1356.
- Moerhlin, G.S., Juliano, S.A., 1998. Plasticity of insect reproduction: testing models of flexible and fixed development in response to different growth rates. *Oecologia* 115, 492–500.
- Nijhout, H.F., 1979. Stretch-induced moulting in *Oncopeltus fasciatus*. *Journal of Insect Physiology* 22, 453–463.
- Nijhout, H.F., 1981. Physiological control of molting in insects. *American Zoologist* 21, 631–640.
- Nijhout, H.F., 1994. *Insect hormones*. Princeton University Press, Princeton.
- Nijhout, H.F., Williams, C.M., 1974a. Control of moulting and metamorphosis in the tobacco hornworm *Manduca sexta* (L.): growth of the last-instar larva and the decision to pupate. *Journal of Experimental Biology* 61, 481–491.
- Nijhout, H.F., Williams, C.M., 1974b. Control of moulting and metamorphosis in the tobacco hornworm *Manduca sexta* (L.): cessation of juvenile hormone secretion as a trigger for pupation. *Journal of Experimental Biology* 61, 493–501.
- Reznick, D.N., 1990. Plasticity in age and size at maturity in male guppies (*Poecilia reticulata*): an experimental evaluation of alternative models of development. *Journal of Evolutionary Biology* 3, 185–203.
- Richter, K., 1999. XIIIth ecdysone workshop. *Archives of Insect Biochemistry and Physiology* 41, i–iv.
- Riddiford, L.M., 1985. Hormone action at the cellular level. In: Kerkut, G.A., Gilbert, L.I. (Eds.), *Comprehensive Insect Physiology, Biochemistry, and Pharmacology*, vol. 8. Pergamon Press, Oxford, pp. 37–84.
- Schlichting, C.D., Pigliucci, M., 1998. *Phenotypic evolution: a reaction norm perspective*. Sinauer, Sunderland, MA, USA.
- Shafiei, M., Moczek, A.P., Nijhout, H.F., 2001. Food availability controls the onset of metamorphosis in the dung beetle *Onthophagus*

- taurus* (Coleoptera: Scarabaeidae). *Physiological Entomology* 26, 173–180.
- Sparks, T.C., Hammock, B.D., Riddiford, L.M., 1983. The haemolymph juvenile hormone esterase of *Manduca sexta* (L.) -inhibition and regulation. *Insect Biochemistry* 13, 529–541.
- Truman, J.W., 1985. Hormonal control of ecdysis. In: Kerkut, G.A., Gilbert, L.I. (Eds.), *Comprehensive Insect Physiology, Biochemistry, and Pharmacology*. Pergamon Press, Oxford, pp. 413–440.
- West-Eberhard, M.J., 2002. *Developmental Plasticity and Evolution*. Oxford University Press, Oxford, UK.
- Whitman, D.W., Orsak, L., 1985. The biology of *Taeniopoda eques* (Orthoptera: Acrididae) in southeastern Arizona. *Annals of the Entomological Society of America* 77, 811–825.
- Whitman, D.W., 1988. Allelochemical interactions among plants, herbivores, and their predators. In: Barbosa, P., Letourneau, D. (Eds.), *Novel Aspects of Insect–Plant Interactions*. John Wiley & Sons, Inc, New York, pp. 11–64.
- Wigglesworth, V.B., 1934. The physiology of ecdysis in *Rhodnius prolixus* (Hemiptera). II. Factors controlling moulting and ‘metamorphosis’. *Quarterly Journal of Microscopic Science* 77, 191–222.
- Wilbur, H.M., Collins, J.P., 1973. Ecological aspects of amphibian metamorphosis. *Science* 182, 271–283.