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Juvenile Hormone and Reproductive Tactics in Romalea Microptera, the Eastern Lubber Grasshopper

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Juvenile hormone and reproductive tactics in *Romalea microptera*, the eastern lubber grasshopper

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CHAPTER 1

Interpopulation variation in the trade-off between body mass gain and age at oviposition in the eastern lubber grasshopper, *Romalea microptera*

Abstract

Isolated populations that inhabit various geographic and climatic ranges tend to diverge in their life history tactics. When development time is constrained by unfavorable seasons, often an organism must trade-off the investment of resource allocation between somatic and reproductive growth. The variation in reproductive tactics and juvenile hormone titers were studied among three populations of *Romalea microptera* from Athens, GA, Jacksonville, FL, and Miami, FL, all of which exist on a latitudinal cline. The Athens population was significantly younger at oviposition and gained significantly less body mass than both the Jacksonville and Miami populations, which did not differ from each other. Clutch mass did not differ across populations. With respect to both body size and oviposition age, Athens invested significantly more (measured by clutch size) to their first clutch than either Jacksonville or Miami, which did not differ from each other. Juvenile hormone and lipid profiles did not differ among populations. In response to the markedly reduced season length, results suggest that Athens grasshoppers respond with reproductive tactics that support terminal investment by investing more energy in less time to reproduction, at the expense of future reproduction.

Introduction

Studying interpopulation variation in common garden experiments can provide evidence of differences due to natural selection (Futuyma 1998). Populations from localities with shorter growing seasons may exhibit earlier life history transitions at lower body masses (Forrest 1987; Rowe & Ludwig 1991; Temte 1993; Berkenbusch & Rowden 2000; Hatle et al. 2002; Luker et al. 2002). This trade-off between development time and body mass is most critical when time constraints on growth and reproduction are imposed by seasonality (e.g., onset of winter). Models of life history evolution speculate that a decrease in lifespan will result in earlier development and an increased reproductive investment at early ages (e.g., Williams 1966; Charlesworth 1980; Reznick et al.1990; Roff 1992; Partridge et al 1995).

Romalea microptera, the eastern lubber grasshopper, inhabits a wide geographic range that includes distinctly different climates. Lubber grasshoppers are flightless and disperse little (~50 m / lifetime, Whitman 1990). In addition, there are distinct differences in the size and color of distant populations of lubber grasshoppers. Sequencing of the mtDNA cytochrome-b gene showed that nearly all populations are distinguishable (Mutun & Borst 2004). Previous work suggested a latitudinal trend in the trade-off among age at oviposition, clutch mass, and body mass gain (Hatle et al. 2002). This study, which included populations from Miami, Florida (FL), Lydia, Louisiana (LA), and Athens, Georgia (GA), was confounded by longitudinal variation. To address this weakness, I compared a population from Jacksonville, Florida with Miami and Athens grasshoppers.

The Jacksonville population of lubber grasshoppers is of particular interest. First, it falls directly on a latitudinal cline between previously studied Athens and Miami populations, and is almost exactly in between them. Second, the Jacksonville population exists in a remarkably different ecosystem than the Miami and Athens populations. Miami and Jacksonville are separated by the "frost line", which marks the transition from mangroves in the south (Myers & Ewel 1990) to pines in north Florida. Athens is in the primarily deciduous Southern Piedmont, distinct from both Jacksonville and Miami (Narsal 2007).

In all major insect orders except *Diptera,* juvenile hormone (JH) is a major gonadotropin. It stimulates vitellogenin synthesis and mediates patency (Nijhout 1994). In the lubber grasshopper, JH is required for vitellogenin-mRNA production (Fei et al., 2005). Further, JH levels are associated with the timing of oviposition; low fed grasshoppers reach the maximum level of JH later and oviposit later than highly fed grasshoppers (Hatle et al. 2000).

Variation in reproductive tactics and JH titers among Athens, Jacksonville, and Miami populations existing on a latitudinal cline were examined. I predicted that age at oviposition will vary such that Athens < Jacksonville < Miami. A cost for early oviposition should be observed in the Athens population, perhaps as a reduction in clutch mass or somatic growth. Further, I predict that the age at which the maximum level of JH is attained will vary such that Athens < Jacksonville < Miami.

Methods

Juvenile lubber grasshoppers were field-collected from Athens, Jacksonville, and Miami and shipped to the laboratory in Jacksonville. Latitudes for these locations are approximately 25° N, 30° N, and 33° N respectively. Athens has 249 frost-free days; Jacksonville has 345 frost-free days; and Miami is frost-free year round (NCDC 2007).

Juveniles were reared *en masse* on *ad libitum* Romaine lettuce and oats and under heat lamps at 24±2 ° C on a 14L:10D photoperiod. On the day of adult molt, females were weighed, isolated and reared individually in 500 ml ventilated containers, at a 14L:10D photoperiod and a corresponding 32:24°C thermocycle. Previous research suggests that variable reproductive plasticity among interpopulations is absent; i.e., Athens, Louisiana, and Miami populations all responded to low food similarly (Hatle et al. 2002). Therefore, individuals were offered the same relative diet adjusted for body size. To determine the amount of feed to be given to each insect, the femur length of each individual was multiplied by a constant (0.12) yielding the total mass of Romaine lettuce in grams.

Hemolymph samples were taken twice a week and stored in hexane at -20 °C for later analysis of JH titers (Hatle et al. 2000) and total hemolymph lipids. Beginning at day 24, females were tested for oviposition (Hatle et al. 2000). Once a female oviposited, she was weighed and retired from the study. Eggs from each female were counted and dried (Athens n = 9, Jacksonville n = 12, Miami n =11). Egg weight for each

individual was obtained by averaging the weight of ten eggs. Clutch mass (n = 9 for all populations) was calculated by multiplying an individual's average egg weight by the total number of eggs oviposited.

Juvenile hormone titers over the course of each individual's reproductive cycle were analyzed via radio-immunoassay (Hatle et al. 2000). All of the samples from a single individual were analyzed simultaneously to avoid any effects of interassay variation. The order of analysis of individuals was randomized. The maximum level of JH (Athens n = 9, Jacksonville n = 12, Miami n = 6) was determined by comparing all samples for an individual and identifying the sample with the highest JH titer. The age at which that sample was collected was defined as the age at maximum level of JH (Athens n = 9, Jacksonville n = 12, Miami n = 6)

The total hemolymph lipids of seven individuals from each population were also measured from the same hemolymph samples. The transportation of lipids occurs via the hemolymph. Approximately 40% of the egg is lipid (Chapman 1998). Therefore, a minimum rate of lipid transport may be required to complete vitellogenesis. If lipids play an important role in the timing of oviposition, I predict a peak of lipid transport during the period of greatest growth of oocytes. Lipids were measured as vanillin-positive material using vegetable oil standards (Hatle & Spring 1998).

All data were statistically analyzed to determine the effects of population. A MANCOVA with initial weight as a covariate was used to analyze the three-way trade-off among age at oviposition, clutch mass and body mass gain after oviposition

(Athens n = 9, Jacksonville n = 10, Miami n = 12). Because initial body mass was used as a covariate (Pillai's Trace = 0.337; $F_{3, 20} = 3.38$; P = 0.038), body mass after oviposition estimates the somatic mass gained from adult molt to oviposition. A second MANOVA was used to analyze data on maximum level of JH, age at maximum level of JH and time from maximum level of JH to oviposition (Athens n = 9, Jacksonville n = 12, Miami n = 6). A one-way ANOVA was used to analyze lipid data. SAS PROC GLM was used for all analysis (SAS 1989).

Results

Reproductive tactics

Both body mass after oviposition ($F_{3, 22} = 17.02$, $P < 0.0001$) and age at oviposition ($F_{3, 22} = 5.81$; P = 0.004) were significantly affected by population (Fig. 1; MANCOVA; Pillai's Trace = 0.598; $F_{6, 42} = 2.99$; P = 0.016). Multivariate pairwise contrasts indicated that the Athens population differed significantly from both the Jacksonville (P = 0.0005) and Miami (P = 0.0258) populations, which did not differ from each other (P = 0.3196).

Standardized canonical coefficients (age at oviposition = 0.819; body mass after oviposition = 1.48; clutch mass = -0.061) indicate that the greatest effect of population was due to the mass after oviposition followed by the age at oviposition. Clutch mass had no contribution to population differences. Athens age at oviposition was significantly less than both Jacksonville (pairwise contrast statement P = 0.0113) and Miami (P = 0.0446) populations, which did not differ from each other (P = 0.6009).

Similarly, body mass after oviposition (adjusted for body mass at molt as a MANCOVA co-variate) for Athens was significantly less than both Jacksonville (P = 0.0003) and Miami (P = 0.0243) populations, which did not differ from each other (P = 0.0847).

The ratio of clutch size to body size (as femur length) was significantly affected by population (ANOVA; $F_{2, 25} = 8.932$; P = 0.001). Pairwise comparisons indicate that the Athens population produced a larger clutch with respect to body size than both the Jacksonville (P = 0.001) and Miami (P = 0.015) populations, which did not differ from each other (P = 0.497).

The ratio of clutch size to oviposition age was significantly affected by population (ANOVA; $F_{2, 18} = 10.744$; P = 0.001). Pairwise comparisons indicate that the Athens population produced a larger clutch with respect to age than both the Jacksonville (P = 0.001) and Miami (P = 0.011) populations, which did not differ from each other (P = 1.000).

Juvenile Hormone attributes

There was no significant population effect on JH attributes (Fig. 2; MANOVA; Pillai's Trace = 0.338; $F_{6, 46} = 1.56$; P = 0.180). Populations did not differ statistically in their age at maximum level of JH ($F_{2, 24} = 1.51$; P = 0.242), time from maximum level of JH to oviposition ($F_{2, 24} = 1.60$; P = 0.222), or maximum titer of JH ($F_{2, 24} = 0.22$; P = 0.807).

Hemolymph Lipids

No distinct peaks among the hemolymph lipid profiles existed for any of the populations (Fig. 3). The grand means of each population were compared by ANOVA. There was no significant population effect on mean hemolymph lipid concentration ($F_{2, 14} = 0.44$; $P = 0.653$).

Discussion

In this study, I examined interpopulation variation in a three-way trade-off among body mass gain, age at oviposition, and clutch mass. I also tested interpopulation variation in JH titers and lipid transport. Populations significantly differed in the three-way trade-off among body mass gain, age at oviposition, and clutch mass. The body mass gain and age at oviposition were the only variables that contributed significantly to the interpopulation variation (Fig. 1).

Across populations, early oviposition correlated with less somatic growth. It has been hypothesized that somatic mass gained during egg production is directed toward reproduction of subsequent clutches (Hatle et al. 2002). This implies that the cost for early reproduction does not appear in the current reproductive event, but rather at the cost of future reproduction. The current reproduction over future reproduction trade-off, known as the terminal investment hypothesis, has been observed previously in various organisms faced with the threat of a reduced lifespan (Pianka & Parker 1975). Most previous research on terminal investment involves *individual* plasticity in responding to life-reducing events such as exposure to bacteria, parasites, or viruses (Adamo 1999; Bonneaud et al. 2004), injury (Javois &

Tammaru 2004), or senescence (Tatar & Carey 1995). My research involves adaptive responses due to *population* variation. Further studies would be needed to determine if the terminal investment theory might apply to populations as an adaptive response to environments that shorten lifespan.

Athens was the only population that differed in body mass gain and age at oviposition with no difference in clutch mass among populations. Jacksonville populations are intermediate and equidistant from Athens and Miami both latitudinally and climatologically. However, the results indicate that biologically Jacksonville populations are more similar to Miami than to Athens. The results suggest that among the three populations, Athens is the only one that has undergone evolutionary divergence in reproductive tactics. The similarities between the Jacksonville and Miami populations are consistent with a linear relationship among populations, yet are not consistent with the predicted latitudinal cline. The absence of a distinction between Jacksonville and Miami populations could be due to a balance between the costs and benefits that early reproduction yields upon fitness. In this case, it might suggest that the Jacksonville population does not sustain a sufficient reduction in lifespan to warrant the cost of reduction in somatic growth during oviposition.

Investment into each reproductive event can be measured via clutch mass. Similarity in clutch mass alone among the populations implies that each population invested the same amount in their first clutch. However, when taking body size and oviposition timing into consideration, these populations differ in investment. All three

populations significantly differ in body size (ANOVA; $F_{2, 30} = 39.370$; $P < 0.001$) such that Athens < Miami < Jacksonville. As well as being smaller, Athens grasshoppers oviposited in significantly less time with no difference in clutch mass. Effectively, this indicates that in comparison to Jacksonville and Miami, Athens invests more energy and acquired resources to produce larger clutches with respect to bony size and age at oviposition. This accounts for the minimal body mass gain of Athens and is consistent with the current over future reproduction strategy.

Maternal environments have the ability to influence the expression of traits in their offspring (Mousseau & Fox 1998). Therefore, due to potential environmental maternal effects, the differences found between these populations may not be genetic. Some life history traits of lubber grasshoppers suggest that any potential maternal effects on reproduction would only have a minor contribution to the observed interpopulation variation. Varying egg size is a common mechanism used by mothers in response to different environments (Parker & Begon 1986). For example, in unfavorable environments with limited food, a mother may lay fewer but larger eggs in order to provide offspring with a better chance of survival. However, maternal diet in lubber grasshoppers has no effect on egg size (Moehrlin & Juliano 1998; Hatle et al. 2000). In addition, juvenile diet does not seem to affect reproductive tactics. In fact, unlike many insects, following adult molt lubber grasshoppers undergo a period of further somatic growth prior to any reproductive growth (Hatle et al. 2004). These tactics employed by lubber grasshoppers make maternal effects seem less likely.

The maximum level of JH occurs during the fixed phase of oviposition, indicating commitment to oviposition (Hatle et al. 2000). Vitellogenin and JH profiles have been shown to have similar developmental patterns (Borst et al. 2000; Hatle et al. 2000, 2001). Maximum vitellogenin levels among populations corresponded to oviposition timing (Hatle et al. 2004). Specifically, Athens oviposited earlier than the other populations, and exhibited a younger age at vitellogenin maximum. Hence, I predicted that the maximum level of JH would vary across populations in concert with age at oviposition. However, the JH titers between populations did not differ. In previous studies, JH profiles exhibited distinct maxima (Hatle et. 2000), but profiles from this study did not reveal distinct maxima. It is possible that I have not obtained an accurate picture of the JH profiles, perhaps because hemolymph samples were taken only bi-weekly, or samples may have degraded.

There were no distinct peaks in the lipid profiles and populations did not vary in the magnitude of hemolymph lipids. Hemolymph lipids appear to remain stable throughout the somatic and reproductive growth phases in lubber grasshoppers, suggesting that oocyte production does not yield an increase in hemolymph lipid transport.

Appendix I

Figure Legends

Figure 1. Bi-variate plots of reproductive tactics (means ± SE). Data analyzed using a MANCOVA with initial weight as a significant covariate. The Athens population differed significantly from the Jacksonville and Miami populations in body mass after oviposition and oviposition age but not clutch mass. Somatic growth indicates the somatic storage retained after laying the first clutch, adjusted for mass at molt.

Figure 2. Hemolymph juvenile hormone profiles (mean ±SE) for adult female lubber grasshoppers from three populations (Athens n = 9; Jacksonville n = 12; Miami n = 6). Hemolymph samples were collected bi-weekly. Profiles end at the median age of oviposition for each population.

Figure 3. Hemolymph lipid profiles (mean ±SE) for adult female lubber grasshoppers from three populations (n = 7 for all populations). Hemolymph samples were collected bi-weekly. Profiles end at the median age of oviposition for each population.

Appendix II

Figures

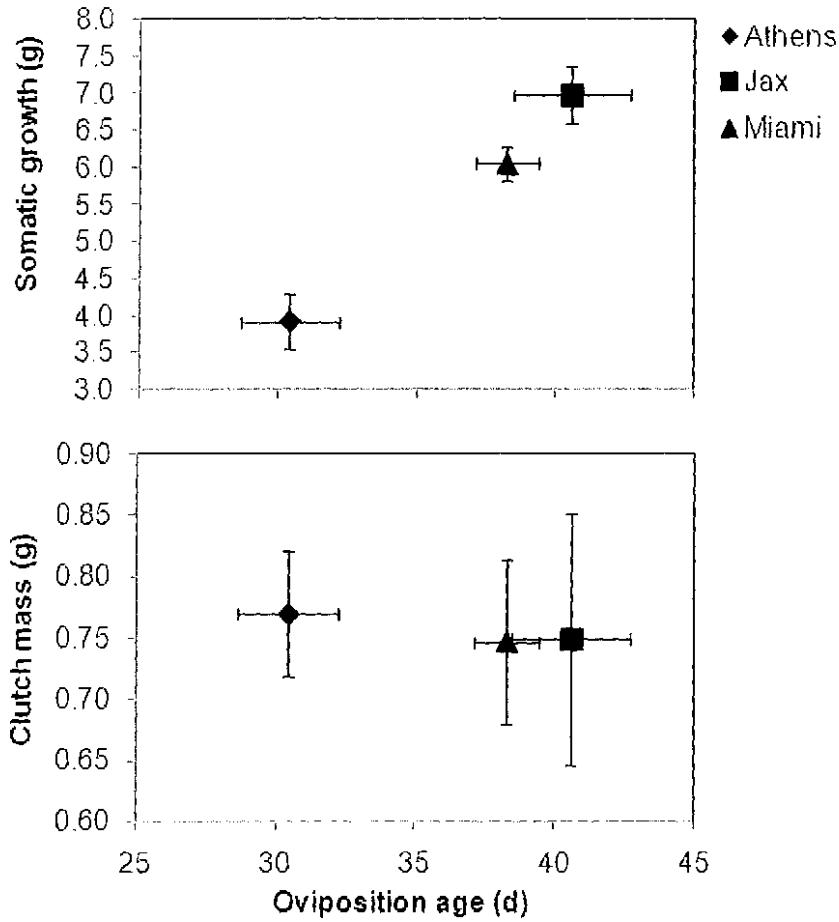

Figure 1

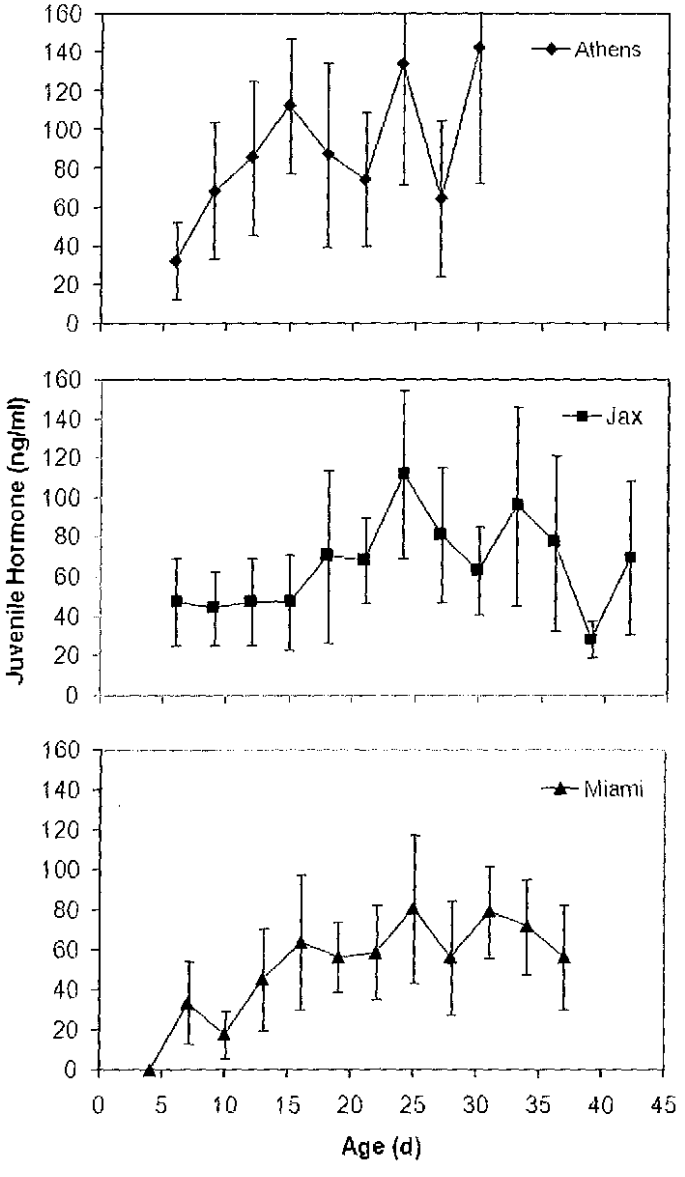
Athens
Jax
Miami
Juvenile Hormone (ng/ml)
Age (d)
0
20
40
60
80
100
120
140
160
0
5
10
15
20
25
30
35
40
45

Figure 2

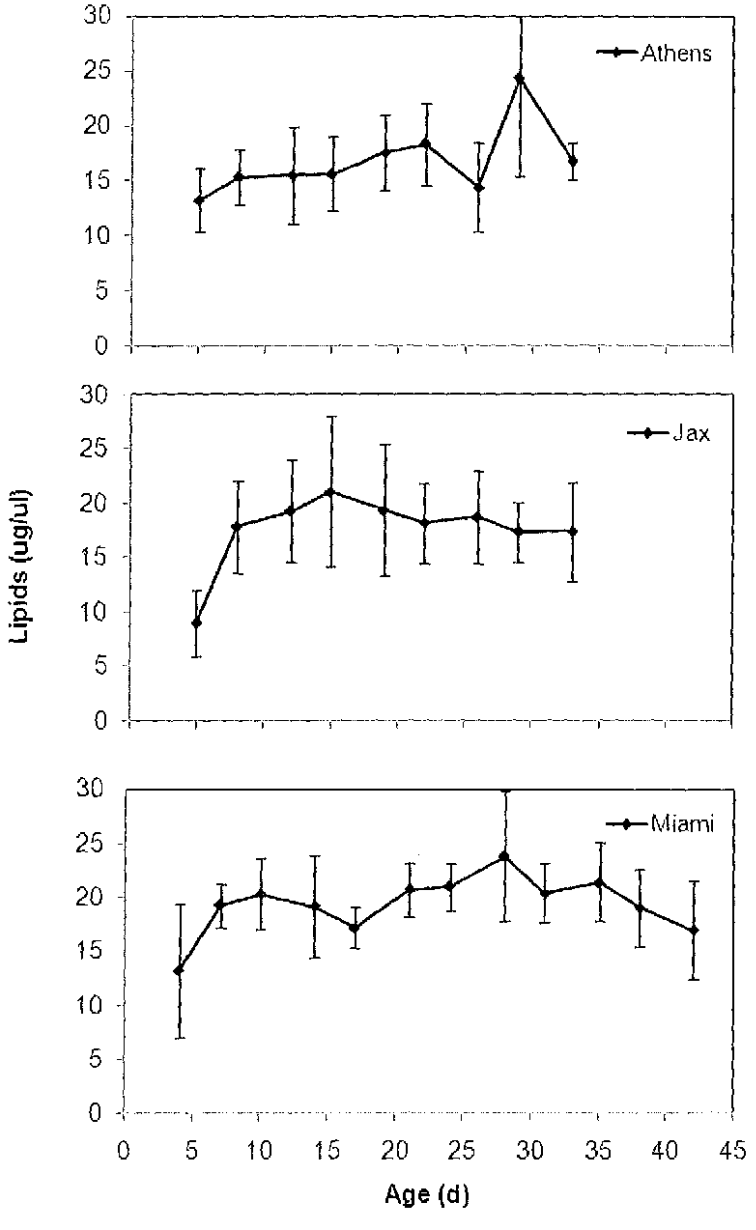

Figure 3

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Chapter 2

A cumulative feeding threshold required for vitellogenesis can be obviated with juvenile hormone treatment in lubber grasshopper

Abstract

Developmental thresholds can ensure adequate condition has been attained to proceed through major transitions (e.g., initiation of reproduction, metamorphosis). Nutrition is critical to attaining most thresholds, because it is needed for both growth and storage. Attaining a threshold may stimulate the release of hormones that commit the animal to the developmental transition, yet the relationships between developmental thresholds and these endocrine signals are poorly understood. Lubber grasshoppers require a cumulative feeding threshold to initiate vitellogenesis and potentially commit to oviposition. I tested the relative roles of the major gonadotropin (juvenile hormone; JH) and the nutritional threshold in initiating vitellogenesis and committing to oviposition. The source of JH was removed from all females, and then JH analog was applied after different levels of feeding. Threshold feeding was not required to initiate vitellogenesis, suggesting that sub-threshold grasshoppers are competent to JH. Hence, threshold feeding is required only to cause the release of JH. The present experiment suggests that JH is more important than a nutritional

threshold. In addition, individuals that were restored with JH late in life tended to favor current reproduction, at the expense of future reproduction, supporting the terminal investment hypothesis; both time to oviposition and vitellogenin profiles were consistent with the hypothesis. Taken together, the results suggest that lubber grasshoppers can adjust reproductive tactics depending on their age, but this control is secondary to JH, which is in turn subject to nutrition.

Introduction

Major post-embryonic developmental transitions (e.g., initiation of reproduction, metamorphosis, cessation of growth) are critical stages in an organism's life. Phenotypic plasticity in timing (e.g., age at oviposition) or resource allocation (e.g., size of clutch) at these developmental transitions is common (Stearns, 1992; Morey and Reznick, 2000; Schoech et al., 2004; Davidowitz and Nijhout, 2005; this plasticity can have important effects on fitness (Denver et al. 1998). An individual's physiological condition often influences the plasticity at transitions, particularly in the timing of transitions (Zera and Harshman, 2001). Prior to a developmental transition, a threshold can ensure adequate condition has been attained to proceed through the transition (Wilbur and Collins, 1973; Frisch, 1994; Reynolds, 2003a; 2003b; Moczek, 2003; Davidowitz et al, 2004; Nijhout et al., 2006),

A developmental threshold describes the status needed to proceed through a developmental transition (Nijhout 2003). Thresholds can be described: 1) morphologically as a critical body or organ size (e.g., Nijhout and Williams. 1974a,b; Davidowitz and Nijhout 2004; Mirth et al. 2005), 2) physiologically as a level of storage (Frisch 1994), or 3) nutritionally as an amount ingested (Nijhout, 1979; Klowden, 1987; Juliano et al. 2004). First, critical size thresholds are most common in the literature. For example, male dung beetles must attain a critical body size to grow huge horns for aggressive male-male interactions; small males produce tiny horns (Emlen et al., 2005). More precisely, in *Drosophila* a critical organ size (i.e., an enlarged prothoracic gland) stimulates metamorphosis at a body size smaller than the normal critical size

for metamorphosis (Mirth et al., 2005). Second, a storage threshold for human ovulation has been identified, with 22% body fat required (Frisch 1994). Third, mosquitoes and blood sucking bugs require a single, large meal to stretch the gut and release a developmental transition (Nijhout 1979; Bowden 1987). Similarly, lubber grasshoppers must ingest a cumulative amount of lettuce over many meals to initiate vitellogenesis and later commit to oviposition (Juliano et al. 2004). Clearly, nutrition is vital to attaining all of these thresholds, because it is needed for both growth and storage.

Hormones are likely to be essential to translating this growth or storage (via nutrition) into a developmental transition (Hatle 2003). The association between a threshold and a developmental transition does not infer direct control; rather the threshold must be translated to endocrine signals that mediate the life history transition (Nijhout and Williams, 1974a; 1974b; Day and Rowe, 2002; Juliano et al., 2004; Moczek and Nijhout, 2002; Davidowitz and Nijhout, 2004; Nijhout et al., 2006). The relationships between developmental thresholds and these endocrine signals are poorly understood, yet they are a critical link in how environmental conditions produce variation in life histories (Emlen and Nijhout 1999; Zera and Harshman 2001; Davidowitz and Nijhout, 2005; Shingleton et al. 2007).

Studies that simultaneously manipulate both nutrition and hormones provide excellent approaches to determining the relationships of thresholds and endocrine signals in development. For example, imaginal discs in larvae of *Manduca sexta* continue to grow in the absence of JH despite starvation. However, both nutrition and

JH are required for normal larval growth to the critical size for metamorphosis (Truman et al., 2006). In contrast, eye development in *Manduca sexta* is activated by nutritional cues and is independent of JH levels (MacWhinnie et al., 2005).

In lubber grasshoppers (*Romalea microptera*), a cumulative feeding threshold of 4.0 dry g of Romaine lettuce is required to initiate the transition from somatic growth to reproductive development, and ultimately oviposition (Juliano et al., 2004; see Fig. 1). Diet prior to adult molt has little effect on the timing of reproduction (Moehrlin and Juliano, 1998). The developmental model that best described reproduction in lubber grasshoppers is a fixed threshold model (Reznick, 1990), in which a single developmental program produces different phenotypes simply due to its expression in different environments (e.g., nutritional levels). That is, the nutritional threshold did not change with the rate of feeding, but instead the threshold was fixed at 4.0 dry g of cumulative ingestion. Plasticity of reproductive timing was thus dependant only on the time to attain that threshold.

In well-fed lubber grasshoppers (offered 0.77 dry g Romaine lettuce / day of adulthood), oviposition occurs ~ 35 days after adult molt. In contrast, in low fed animals (0.12 dry g/day), oviposition occurs ~ 65 days after adult molt (Hatle et al., 2000; 2003). Mature females generally lay 2 to 3 clutches during their life (Hatle et al., 2006). Lubber grasshoppers are univoltine and over-winter as eggs; therefore, reproduction is potentially time constrained with the onset of freezing or plant senescence (Luker et al., 2002; Homeny and Juliano, in press). The large size of lubber grasshoppers enables the collection of multiple hemolymph samples with very little

interference of the reproductive cycle (cf. Hatle et al., 2002; Hatle et al., 2004).

Nutrition is clearly needed to stimulate the production of JH. But whether nutrition is needed to attain the status needed for JH response (i.e., competence) is unknown. During the oviposition cycle, JH levels start low, rise to a maximum around mid-vitellogenesis, and fall before oviposition. Low levels early in the cycle are involved in vitellogenin production. Higher levels later in the cycle coincide with oocyte growth and patency. The maximum titer of JH (the point at which JH degradation or export is favored over JH synthesis) always occurs about 12 d before oviposition, regardless of diet (Hatle et al. 2000; 2003). These studies on JH levels suggest that JH is sufficient for initiating vitellogenesis and the later commitment to oviposition, regardless of diet. That is, threshold feeding (see Juliano et al. 2004) is only needed to allow production of JH, and the female is competent to JH prior to attaining the threshold.

Production of vitellogenin-mRNA requires JH (Fei et al., 2005). In starved grasshoppers, the infusion of JH increases vitellogenin-mRNA, but feeding is required for synthesis of vitellogenin protein by the fat body (Fei et al., 2005). These results suggest that JH may not be the only factor involved in the regulation of vitellogenin production, but instead some other nutrition-dependent change is needed. Similarly, Hatle et al. (2006a) examined the relative importance of stimulation of the fat body vs. total fat body mass in total vitellogenin production. They found that the total fat body mass was much more important than mass-specific tissue stimulation (typically by JH). This suggests that growth factors

affecting the fat body, in addition to JH, might also be critical to promoting vitellogenesis (Hatle et al., 2006a). Such growth factors are likely to be nutrition dependent. Hence, in contrast to studies on the levels of JH (see previous paragraph), studies on vitellogenesis suggest that feeding to the threshold is required in addition to JH. That is, threshold feeding is needed both to initiate production of JH *and* to bring about competence to JH.

I manipulated both the timing of initial treatment and feeding to test whether JH is solely responsible for vitellogenesis, or if the feeding threshold must also be met for competence to JH (Fig. 2). I predicted that the feeding threshold must be met (after Fei et al., 2005; Hatle et al., 2006). Specifically, I hypothesized that individuals which are sub-threshold at the start of JH analog treatment will delay vitellogenesis, and ultimately oviposition, in comparison to individuals that are supra-threshold at the start of JH analog treatment. In other words, I predict a statistically significant interaction of diet and timing of JH initiation on the onset of vitellogenesis and timing of oviposition. Alternatively, if attainment of the feeding threshold is not required along with JH, sub-threshold females treated with JH should undergo vitellogenesis in concert with supra-threshold females treated with JH.

Methods

Experimental design

This experiment employed a 2 * 2 factorial design, manipulating both

cumulative feeding amount and age at initial analog application (JHAi). New adult female grasshoppers were serially assigned into two groups: low or high diet. Within both of these two diet groups, individuals were later assigned to either early or late JHAi The four treatment groups were: low food-early JHAi (n = 14), low food-late JHAi (n = 5), high food-early JHAi (n = 9), and high food-late JHAi (n = 2). Only individuals that ultimately oviposited were included in the study. Individuals in the late JHAi groups took longer to oviposit; therefore, their rates of survival to oviposition were lower, resulting in low sample sizes. Fortunately, the high food-late JHAi is the least important group for addressing the hypothesis.

The timing of early JHAi was chosen to ensure that, when fed ad libitum, the high food-early JHAi group had consumed a supra-threshold quantity of lettuce at JHAi (i.e., greater than 4.0 dry g as determined by Juliano et al., 2004). The timing of late JHAi was chosen to ensure that the low food-late JHAi group also had consumed a supra-threshold quantity of lettuce at JHAi when fed at the same rate as the low food-early JHAi group. The low food-early JHAi group was the only sub-threshold feeding group at the start of hormone treatments (Fig. 2; Table 1).

Animal rearing

Lubber grasshoppers were shipped from a lab colony at Illinois State University in Normal, IL, USA. The colony was founded with grasshoppers from Copeland, FL, USA. Juveniles were reared en masse in screen cages with a 14L: 10 D photoperiod at 32°C. Juveniles were fed Romaine lettuce and oatmeal ad libitum. Newly molted females

were isolated and reared individually in 500 ml ventilated containers at a 14L:10 D photoperiod and a corresponding 32:24° C thermocycle.

Allatectomy procedure

The corpora allata (the sole source of JH; TO Barry, JD Hatle and DW Borst, unpublished data) of all individuals were surgically removed 4 to 6 d after adult molt. The day before surgery, food was withheld. Grasshoppers were cold-anesthetized for ≥1 hr, fastened to the dissecting dish with modeling clay, and the intersegmental neck membrane was opened with a U-shaped incision. Two air sacs were removed, both corpora allata were excised, a 25 µg dose of gentamicin sulfate (ICN Biomedicals, Irvine, CA, US) was placed in the open wound, and the neck membrane was folded back into place.

Diet treatments and timing of juvenile hormone analog initiation

The experimental goal was to test whether each group was competent to respond to JH at the initiation of hormone treatment. Using this design, I predicted that individuals that individuals not competent to respond to JH at the moment of JHAi would have later times of vitellogenin onset (Vg onset; the first sampling date with detectable Vg) or oviposition.

Daily food rations were weighed fresh and all grasshoppers were always fed fresh lettuce. The previously determined threshold was described as dry mass (Juliano et al. 2004), so the dry mass ingested at each meal was determined. Several 5.0 wet g controls were dried and weighed to obtain a fresh-to-dry conversion factor.

Using this conversion factor, the dry mass offered was calculated. Daily, each individual was offered a specific amount of fresh lettuce. The next day, each individual's uneaten food was collected, dried at 55° C, and weighed. The dry mass uneaten was subtracted from the dry mass offered to determine the dry mass eaten.

Juliano et al. (2004) found cumulative feeding, and not the feeding rate, to be critical for commitment to oviposition. Therefore, the important feeding variable to manipulate is the amount that has been ingested when JH is restored. From adult eclosion to the day before surgery, all individuals were fed 0.15 dry g of Romaine and 3-5 oatmeal flakes daily. Immediately following surgery, the grasshoppers began their assigned diets. Allatectomized females eat low amounts of food (about 1/3 of unmanipulated females), and therefore take a longer time to reach the feeding threshold. The feeding schedules were designed to produce a sub-threshold feeding group (low food-early JHAi) and three supra-threshold feeding groups at JHAi (Fig. 2).

Hormone analog treatments and hemolymph sampling

Hormone replacement was achieved by applying methoprene (Sigma Chemical, St. Louis, MO, USA), an analog of JH (Nijhout, 1994; Flatt and Kawecki 2007). Once the designated age was reached, a 5 µl hemolymph sample was collected from each grasshopper and a topical application of 500 µg of methoprene in 10 µl of ethanol was applied to the neck membrane. This is approximately the same dosage per body mass used by Chizei and Wyatt (1985) for locusts. Hemolymph samples were acquired once a day for the first 5 days after methoprene treatment initiation and twice a week

thereafter. All hemolymph samples were placed in 250 µl of hemolymph buffer (Hatle et al., 2001) and stored at -20°C for later analysis of vitellogenin and total protein. Twice a week until oviposition, methoprene was applied immediately before hemolymph sampling. I repeatedly dosed grasshoppers with 500 µg methoprene to force all individuals into the same hormonal status once hormone replacement was begun. This design has low power to separate the requirements for vitellogenesis from the requirements for oviposition. However, it is excellent for testing the primary experimental goal, namely isolating the competence of the individual at JHAi. By artificially maintaining high levels of JH in all groups regardless of past or current diet, effects from the individual's status at the time of JHAi (i.e., feeding level) could be identified.

Oviposition

Females were allowed to oviposit in their cages as virgins. Lubber grasshoppers will lay eggs without mating; if oviposition substrate is not available, egg laying is delayed ~ 7 d but still occurs (Mefferd et al. 2005). At oviposition, the individual's age was recorded and it was removed from the study. Laid eggs were counted; because individual egg size is largely fixed, this is a good estimate of clutch mass (Moerhlin and Juliano 1998; Hatle et al. 2000). Grasshoppers were dissected to measure the number of retained eggs, the number of secondary oocytes, and the size of secondary oocytes. Laid eggs and fully developed retained eggs were combined as the total number of developed eggs.

Together, secondary oocyte size and number indicate the investment in future reproduction. The number of secondary oocytes implies the potential for the mass of the ensuing clutch. The size of secondary oocytes implies the probable timing of the ensuing clutch, because oocytes need to grow to 1.0 cm to be ready for oviposition.

Hemolymph vitellogenin

Vitellogenin was measured by ELISA (modified from Borst et al. 2000). All samples from an individual were analyzed concurrently, and groups were analyzed alternately. The time of Vg onset for each individual was determined by identifying the age at the first sample in which vitellogenin was detectable. The time of maximum vitellogenin titer was determined by identifying the age at the sample with the highest amount of vitellogenin for each individual, throughout the oviposition cycle. The maximum vitellogenin titer is the point at which sequestering of vitellogenin into the oocytes becomes favored over synthesizing vitellogenin and exporting it into the hemolymph (Hatle et al. 2001). Individuals that showed detectable vitellogenin prior to JHAi (likely due to failed allatectomy) were removed from the study (n = 5).

Hemolymph storage proteins

Total hemolymph protein was measured using the Bradford (1976) assay with bovine serum albumin standards. The amount of vitellogenin in the same sample was subtracted from this measure of total protein. Total non-vitellogenin hemolymph protein is an estimate of storage proteins, because ~ 80 % of nonvitellogenin hemolymph protein exists as 3 hexamerin storage proteins throughout the first

oviposition cycle (Hatle et al. 2001). Hexamerins are a conserved family of storage proteins in insects (Haunerland 1996). The time to storage protein maximum and the storage protein maximum were calculated in the same way as in the vitellogenin analysis.

Statistical analysis

All data were tested for the effects of food, JHAi, and the interaction of food and JHAi. Data were analyzed primarily by MANOVA. I used 3 MANOVAs: 1) number and size of secondary oocytes; 2) time of Vg onset, time of maximum Vg, time from Vg maximum to oviposition, and Vg maximum titer; and 3) initial storage protein titer and storage protein maximum. Data were transformed to meet assumptions of normality and homogeneity of variances as needed. Due to the inability to transform multiple variables to meet the assumptions of the test, data on oviposition timing and numbers of eggs were analyzed using separate ANOVAs.

RESULTS

Diet treatment

The treatments were successful at producing groups that differed in cumulative feeding but not timing of JHAi (see Table 1). At JHAi, the low food/early JHAi group (57% of the 4.0 g dry mass threshold) was well below the threshold, whereas all other groups were above the threshold (low food/late JHAi = 135% of threshold; high food/early JHAi

= 175% of threshold; high food/late JHAi = 278% of threshold). Body mass gains through adulthood supported the efficacy of the feeding treatments (Fig. 2).

Oviposition

The time from JHAi to oviposition was significantly affected by timing of JHAi (ANOVA; $F1 = 7.184$; $P = 0.013$) but not by diet ($F1 = 2.311$; $P = 0.141$) or the interaction of JHAi and diet ($F1 = 2.468$; $P = 0.129$). Early JHAi groups had a longer period from JHAi to oviposition than did late JHAi groups (Fig. 3). Notably, the low food/early JHAi (sub-threshold) group did not have a longer period from JHAi to oviposition than all three other groups.

Egg and oocyte production

The number of eggs was significantly affected by the timing of JHAi (ANOVA; $F_1 = 5.488$; $P = 0.027$) but not by diet ($F_1 = 1.081$; $P = 0.308$) or interaction ($F_1 = 0.064$; $P = 0.802$). Early JHAi groups produced fewer eggs than the late JHAi groups (Fig. 4). Secondary oocyte characteristics (i.e. number and size of secondary oocytes) were significantly affected by diet (MANOVA; Pillai's trace $F_{2,25} = 7.454$; $P = 0.003$) and the timing of JHAi ($F_{2,25} = 9.417$; $P = 0.001$) but not by their interaction ($F_{1,27} = 1.020$; $P = 0.375$). Canonical coefficients (number of secondary oocytes = 1.095; secondary oocyte length = 0.437) suggested that the main effect was due mostly to the number of secondary oocytes, with the size of the oocytes being less important. Upon dissection immediately following oviposition, the low food groups had fewer secondary oocytes than the high food groups (Fig.5) ($P = 0.001$). This was the only significant effect of diet

in the entire experiment. The number of secondary oocytes was not affected by the timing of JHAi ($P = 0.732$) or the interaction of diet and JHAi timing ($P = 0.173$). By contrast, early JHAi groups had larger secondary oocytes than the late JHAi groups (Fig. 5) ($P < 0.001$). Yet, secondary oocyte length was not significantly affected by diet ($P = 0.968$) or interaction ($P = 0.471$).

Analysis of vitellogenin

Vitellogenin profile characteristics were significantly affected by the timing of JHAi (MANOVA; Pillai's trace $F_{4,21} = 6.918$; $P = 0.001$) but not by diet ($F_{4,21} = 2.754$; $P = 0.055$) or interaction ($F_{4,21} = 0.541$; $P = 0.708$). For diet, all univariate $P > 0.10$. Because diet did not have a significant effect on Vg parameters, we combined the Vg parameter data by diet groups for clearer graphical presentation (Fig. 6).

Canonical coefficients (maximum level of Vg = 0.351; time of Vg maximum = 0.219; time from Vg maximum to oviposition = 1.024) suggest that the effect on Vg timing was due primarily to the time from Vg maximum to oviposition and secondarily to timing from JHAi to Vg maximum and the maximum level of Vg, and Vg onset was not significant. Compared with late JHAi groups, early JHAi groups had a longer period from JHAi to Vg maximum, a shorter time from Vg maximum to oviposition, and a lower maximum level of Vg (Fig. 6). The primary prediction (see last paragraph of Introduction) was that vitellogenesis would be delayed in females with sub-threshold food intake that were treated with JH (i.e. low food/early JHAi). Hence, the Vg onset data are particularly relevant to the hypothesis. The time from JHAi to Vg onset was not significantly affected

by JHAi ($P = 0.051$), diet ($P = 0.130$) or interaction ($P = 0.493$). The mean (± s.e.m.) times of Vg onset were: low food/early JHAi = 15.5 ± 2.1 days; low food/late JHAi = 7.2 ± 1.8 days; high food/early JHAi = 16.0 ± 3.9 days; and high food/late JHAi = 10.2 ± 2.9 days. The non-significant trend was for Vg onset to be delayed in all early JHAi groups, not only the low food/early JHAi group.

Storage proteins

Storage protein profiles were not significantly affected by the timing of JHAi (Fig. 7) (MANOVA; Pillai's trace $F_{2,26} = 1.144$; $P = 0.334$), diet ($F_{2,26} = 0.175$; $P = 0.841$) or the interaction ($F_{2,26} = 0.202$; $P = 0.819$).

Discussion

Developmental thresholds are important indicators of body condition that stimulate life-history transitions, but the relative roles of diet and hormones in these transitions are not well understood. I tested whether JH treatments are sufficient to initiate vitellogenesis and the commitment to oviposition in sub-threshold lubber grasshoppers. The prediction that females with sub-threshold feeding would not be competent to respond to the hormone was wrong; a significant statistical interaction of JHAi and diet is needed to confirm this prediction, but there were no significant interactions in the entire study. From the results, it is clear that vitellogenesis depended only on the presence of JH. In addition, by controlling the timing of JHAi, we identified a developmental shift in the trade-off between current and future reproduction. Individuals that initiated first reproduction early in life favored future reproduction,

relative to individuals that initiated first reproduction late in life, which favored current reproduction.

Juvenile hormone was sufficient for vitellogenesis, even with sub-threshold feeding

Due to differences in the timing of JHAi, the low food-late JHAi group consumed 137% more food before JHAi than the low food-early JHAi group. Similarly, the high food-late JHAi group consumed 59% more food before JHAi than the high food-early JHAi group. Despite these large differences in cumulative consumption, when JH was controlled, diet only had an effect on the number of secondary oocytes. No other variables measured in this experiment were affected by diet. Previous work has repeatedly found strong effects of diet on the timing of first oviposition, age at Vg maximum, and the number of eggs (Moehrlin and Juliano 1998; Hatle et al. 2000; 2001; 2003a,b; 2004; Juliano et al. 2004). By controlling JH levels, in the present paper I determined that diet, when analyzed separately from JH, did not affect any of these three reproductive tactics. This suggests that feeding for vitellogenesis is required only to produce adequate JH.

This demonstration of the dominance of JH over diet in vitellogenesis has been conducted in a species for which a feeding threshold has been explicitly established by two distinct approaches. Juliano et al. (2004) used constant feeding rates and mathematical modeling to estimate the threshold as 4.0 dry g cumulative feeding. Further, Moehrlin and Juliano (1998) used abrupt switches in food availability to show

that the timing of oviposition is unaffected by diet level (short of starvation) after 14 d of full feeding. This later result was repeated in an independent experiment which demonstrated that the maximum titer of JH during adulthood always occurs ~12 d before oviposition regardless of diet (Hatle et al. 2000; 2003). Therefore, in intact grasshoppers the requirement for feeding to initially increase levels of JH is clear, but this dietary requirement is eliminated when JH reaches high levels. Further, JH titers have been directly measured in lubber grasshoppers (Borst et al. 2000; Hatle et al. 2000), and the requirement of JH for Vg-mRNA production is clear (Hatle et al. 2000; Fei et al. 2005). Hence, it is appropriate to use an analog of JH (Zera 2006).

The experiment focused on the ability to commit to vitellogenesis, and perhaps ultimately oviposition, after certain levels of feeding. Hence, once I restored gonadotropin (i.e., JH), I continued hormone treatments until oviposition. This design allowed me to test the main hypothesis, namely the competence of females before the feeding threshold was attained. However, a weakness of this design was the low probability of identifying developmental plasticity between the initiation of vitellogenesis and oviposition. Indeed, I failed to find effects of post-JHAi diets on reproductive tactics, as could be expected with repeated methoprene applications. At least a low level of developmental plasticity between the initiation of vitellogenesis and oviposition seems likely. Indeed, complete starvation starting at 20 d (i.e., after Vg onset but 2 weeks before oviposition in well-fed grasshoppers) prevents oviposition (DW Whitman, personal communication). Similarly, in Fei et al. (2005), complete starvation reduced the rate of vitellogenin production over 48 h, even when

JH was infused. Nonetheless, this experiment demonstrates that even sub-threshold females, maintained on a low diet throughout adulthood, have the hormonal competence and resources needed to initiate vitellogenesis and commit to oviposition if JH is provided and maintained.

Undergoing vitellogenesis in the absence of adequate nutrition (as done by the low diet-early JHAi females in this experiment) implies that some cost would be incurred. In grasshoppers the investment presently allocated for future reproduction can be observed at any point by measuring the size and number of secondary oocytes (Sandberg et al. 2001). Low diet groups had significantly fewer secondary oocytes than high diet groups, suggesting a reduced number of eggs possible for the second clutch. However, both low diet-early JHAi and low diet-late JHAi had fewer secondary oocytes, and low diet-late JHAi grasshoppers had supra-threshold feeding. Hence, the reduced number of secondary oocytes in low diet-early JHAi females likely does not represent a cost of reproduction without sufficient nutrition. It is possible that the number of secondary oocytes responds to the feeding *rate* early in production of the first clutch.

The time from JHAi to Vg onset was statistically indistinguishable across groups; however, the low probability ($P = 0.051$) suggests that a trend might exist. This trend was for Vg onset to occur later in both early JHAi groups, not only in the low diet-early JHAi group as I predicted. It is clear that low diet-early JHAi did not take longer for Vg onset than other groups, because their mean time of Vg onset was

actually less than that for the high diet-early JHAi group. These data on Vg onset are consistent with the notion that threshold feeding is unneeded for competence to JH. Instead, they are consistent with current reproduction being favored by late JHAi groups.

Current reproduction was favored by late JHAi groups

The terminal investment hypothesis suggests that as life expectancy decreases (e.g., with increasing age), favoring of current reproductive investment increases, at the cost of future reproduction (Hirshfield and Tinkle 1975; Clutton-Brock 1984). Indeed, patterns of contributions to current vs. future reproductive investment have been observed in response to age (Williams 1966; Langley and Clutton-Brock 1998) and to reductions of life expectancy (i.e., illness or environmental conditions).

The present results are consistent with the terminal investment hypothesis. Because lubber grasshoppers are univoltine, their life history may be constrained by time, creating a pressure to reproduce early (see Rowe and Ludwig 1991; Rowe et al. 1994). I observed a trade-off between the timing of the first clutch (i.e., time from JHAi to oviposition) and the timing of the second clutch (as estimated by the length of secondary oocytes). At the expense of ovipositing the second clutch later, late JHAi individuals allocated more resources in less time to their first clutch. By manipulating JH, I have demonstrated a developmental shift from initial relative favoring of future reproduction to later favoring current reproduction. This effect of

age on reproductive tactics was previously undetected in experiments manipulating only diet (e.g., Juliano et al. 2004; Hatle et al. 2006).

Vitellogenin profiles also tended to fit the predictions of the terminal investment hypothesis. The late JHAi groups had significantly earlier and higher Vg maxima than the early JHAi groups, but a longer period between Vg maximum and oviposition. In other words, late JHAi females had a faster rate and greater magnitude of vitellogenin production. Further, as estimated by the mean slopes after Vg maximum (late JHAi = 1.38±0.44 mg/day; early JHAi = 0.86±0.34 mg/day), the rate of Vg transport into the oocytes appears to be greater in late JHAi females (Fig. 7). Both of these are consistent with the terminal investment hypothesis. Taken together, the results suggest that lubber grasshoppers can adjust reproductive tactics depending on their age, but this control is secondary to JH, which is in turn subject to nutrition.

It was previously hypothesized that a threshold level of hemolymph storage protein would serve as a physiological manifestation of the feeding threshold (Hatle et al. 2003 Juliano et al. 2004). The present data are inconsistent with this hypothesis. Sub-threshold feeding did not affect initial storage protein titers or response to JH. In fact, Hatle et al. (2006a) found a tighter association between changes in fat body mass and reproductive plasticity than between changes in storage protein titers and reproductive plasticity. Indeed, it is the fat body that produces the storage proteins and vitellogenin. In *Drosophila,* the fat body serves as a nutrient sensor, regulating body growth (Colombani et al. 2003). From the present data, I hypothesize that the fat body is more critical to pre-reproductive development than are hemolymph storage

proteins. Further studies on the regulation of fat body growth are needed to fully comprehend the initiation of vitellogenesis and commitment to oviposition.

Hormonal cue exceeds nutritional threshold

In other animals that exhibit growth dependent thresholds for development, the nutritional state or critical size induces development via endocrine cues, rather than a direct response to the dietary nutrient (e.g., Emlen and Nijhout 1999). This suggests that endocrine-producing tissues would respond to some signal to make the hormonal signal and stimulate the commitment to the next developmental stage. However, it does not yield insight into whether or not the subsequent events will be followed through without the actual nutritional state or critical size. In other words, is the hormone alone sufficient, or is attaining the storage threshold necessary for competence? The present experiment suggests that hormones are more important than growth or size thresholds, and individuals early in development are competent to developmental hormones, but simply have not yet attained sufficient levels of these hormones. Further studies on other experimental systems are needed to test the generality of this conclusion.

Appendix I

Table and Figure Legends

Table 1. Mean ± SE feeding rates and cumulative amounts eaten for lubber grasshoppers. Feeding treatments were designed to create one sub-threshold feeding group at initiation of gonadotropin treatment and three supra-threshold feeding groups at initiation of gonadotropin treatment. The cumulative feeding threshold for initiation of vitellogenesis and ultimately oviposition is 4.0 dry g (Juliano et al. 2004). The gonadotropin is juvenile hormone, and methoprene was used as an analog. JHAi refers to the timing of initiation of the JH analog. By manipulating food amount and hormone timing, I tested whether JH alone was sufficient to undergo vitellogenesis or if a cumulative feeding threshold was also necessary.

Figure 1. The experimental design was a 2 * 2 manipulation of cumulative feeding and timing of initiation of juvenile hormone (JH) analog (methoprene). The timing of the initiation of JH treatments is referred to as JHAi. Lubber grasshoppers have a cumulative feeding threshold of 4.0 dry g needed to initiate vitellogenesis and ultimately oviposition (Juliano et al. 2004). The low diet-early JHAi group had not attained the feeding threshold at JHAi, whereas all three other groups had attained the threshold at JHAi. Hence, the low diet-JHAi group was used to test whether JH is sufficient for reproduction, or if the feeding threshold is also required.

Figure 2. Body mass profiles of lubber grasshoppers on low or high diets and early or late juvenile hormone analog initiation (JHAi). The body mass profiles imply responses to feeding levels.

Figure 3. Early juvenile hormone analog initiation (JHAi) reduces the time from JHAi to oviposition in lubber grasshoppers, regardless of whether the feeding threshold for vitellogenesis has been attained. See Fig. 2 for experimental design. A cumulative feeding threshold of 4.0 dry g for the initiation of vitellogenesis and ultimately oviposition has been demonstrated for lubber grasshoppers (Juliano et al. 2004).

Figure 4. The number of eggs laid by lubber grasshoppers was significantly decreased by early juvenile hormone analog initiation (JHAi), but was not affected by diet. See Fig. 2 for experimental design. A cumulative feeding threshold of 4.0 dry g for the initiation of vitellogenesis and ultimately oviposition has been demonstrated for lubber grasshoppers (Juliano et al. 2004).

Figure 5. The number of secondary oocytes was significantly greater on high diet than on low diet in lubber grasshoppers, but was not affected by the timing of juvenile hormone analog initiation (JHAi). In contrast, the length of secondary oocytes was greater in grasshoppers subjected to early JHAi than late JHAi, but was not affected by diet. See Fig. 2 for experimental design. A cumulative feeding threshold of 4.0 dry g for the initiation of vitellogenesis and ultimately oviposition has been demonstrated for lubber grasshoppers (Juliano et al. 2004).

Figure 6. Vitellogenin profile characteristics in lubber grasshoppers treated with juvenile hormone early or late in life. Females subjected to juvenile hormone analog initiation (JHAi) later had vitellogenin profiles consistent with favoring current reproduction at the expense of future reproduction, relative to females on early JHAi. Numbers on the graphs label, from left to right: time of vitellogenin onset; time from JHAi to vitellogenin maximum; maximum level of vitellogenin; and time from vitellogenin maximum to oviposition. See Fig. 2 for experimental design. A cumulative feeding threshold of 4.0 dry g for the initiation of vitellogenesis and ultimately oviposition has been demonstrated for lubber grasshoppers (Juliano et al. 2004).

Figure 7. Hemolymph storage protein parameters were not affected by timing of juvenile hormone analog initiation (JHAi) or diet. See Fig. 2 for experimental design. A cumulative feeding threshold of 4.0 dry g for the initiation of vitellogenesis and ultimately oviposition has been demonstrated for lubber grasshoppers (Juliano et al. 2004).

Appendix II

Tables and Figures

Table 1

	Low food-early JHAi (sub-threshold)	Low food-late JHAi (supra-threshold)	High food-early JHAi (supra-threshold)	High food-late JHAi (supra-threshold)
Consumption rate before JHAi (g/day)	0.058±0.001	0.061±0.002	0.184±0.016	0.131±0.033
Age at JHAi (d)	40.000±0.000	40.000±0.000	40.000±0.000	40.000±0.000
Cumulative amt. eaten at JHAi (dry g)	2.282±0.033	5.400±0.399	7.001±0.367	11.127±2.778
Consumption rate after JHAi (g/day)	0.059±0.001	0.060±0.005	0.104±0.011	0.099±0.017
Cumulative amt. eaten at oviposition (dry g)	5.176±0.175	7.843±0.495	12.692±0.592	15.347±3.430

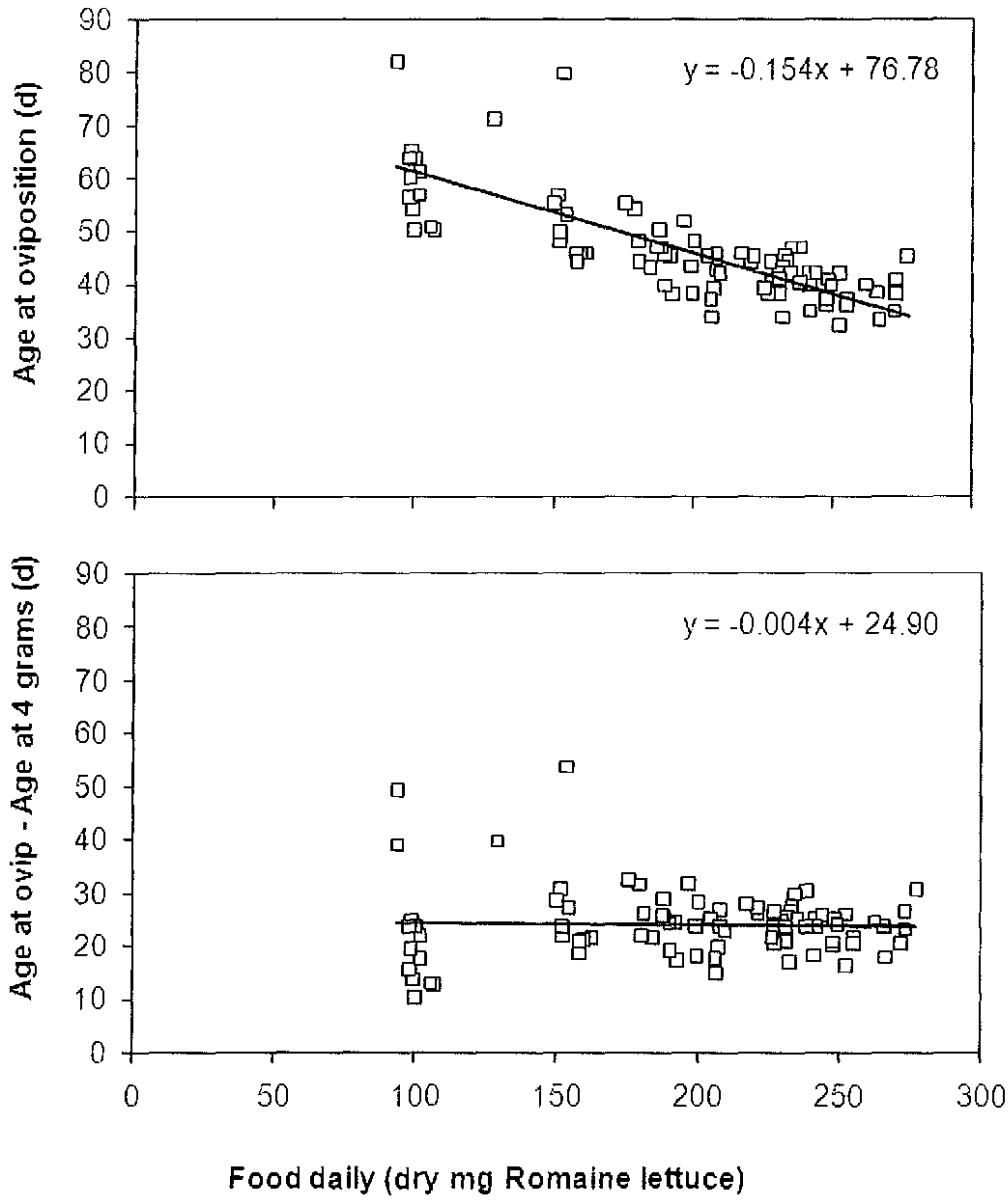
Age at oviposition (d)
y = -0.154x + 76.78
Age at ovip - Age at 4 grams (d)
y = -0.004x + 24.90
0
10
20
30
40
50
60
70
80
90
0
50
100
150
200
250
300
Food daily (dry mg Romaine lettuce)

Figure 1

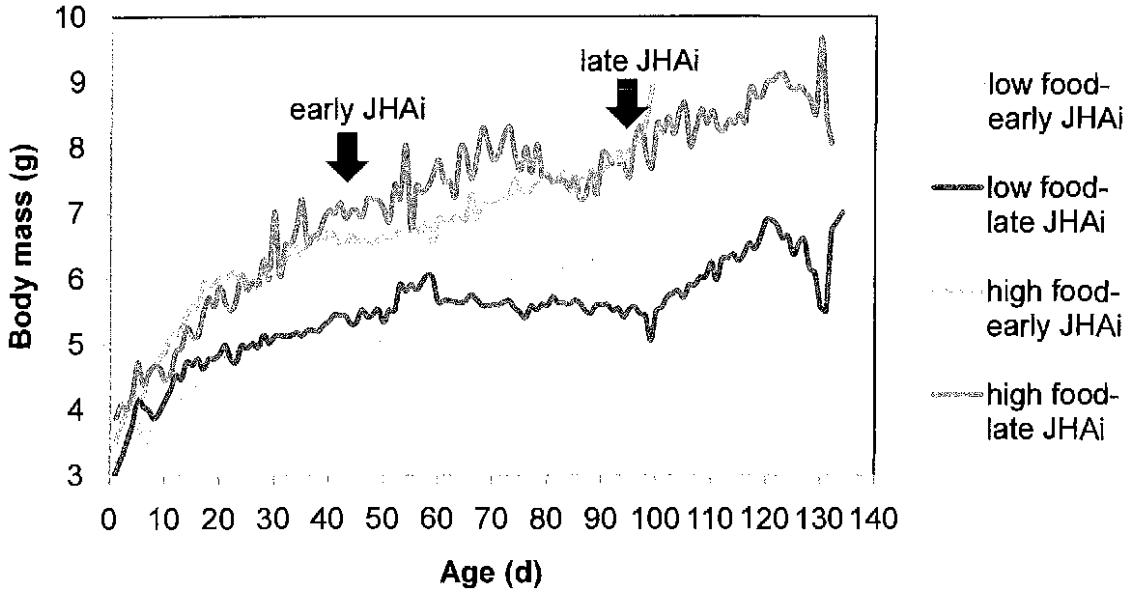

Figure 2

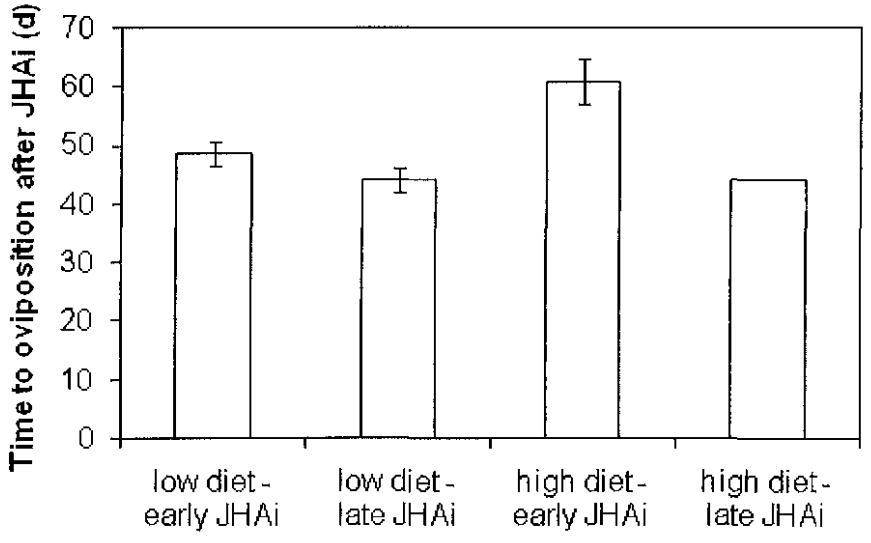

Figure 3

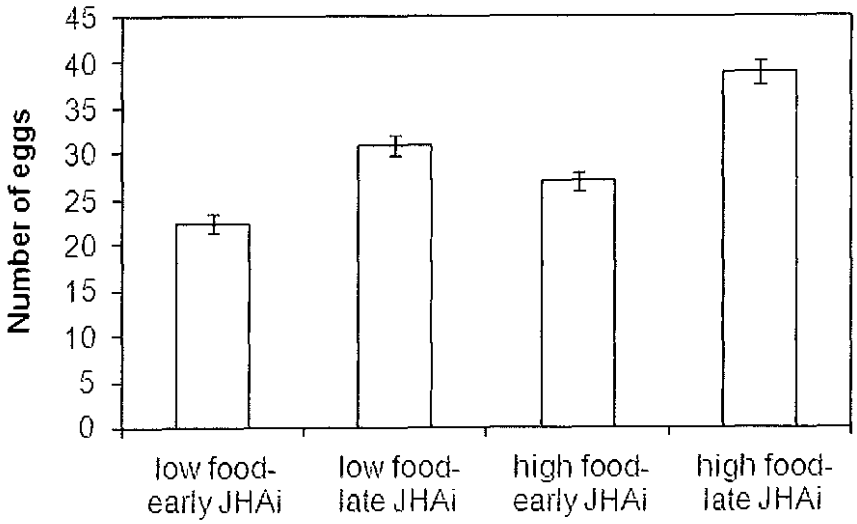
Number of eggs
45
40
35
30
25
20
15
10
5
0
low food-early JHAi
low food-late JHAi
high food-early JHAi
high food-late JHAi

Figure 4

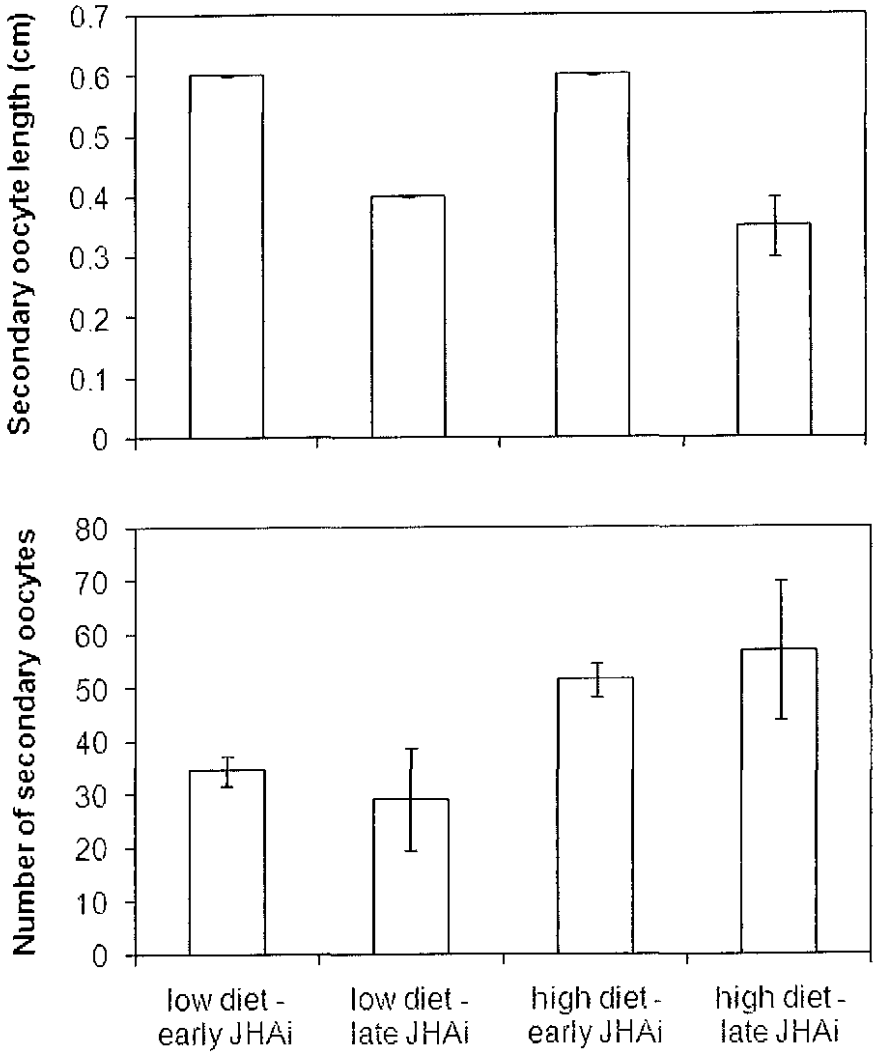
Secondary oocyte length (cm)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Number of secondary oocytes
80
70
60
50
40
30
20
10
0
low diet - early JHAi
low diet - late JHAi
high diet - early JHAi
high diet - late JHAi

Figure 5

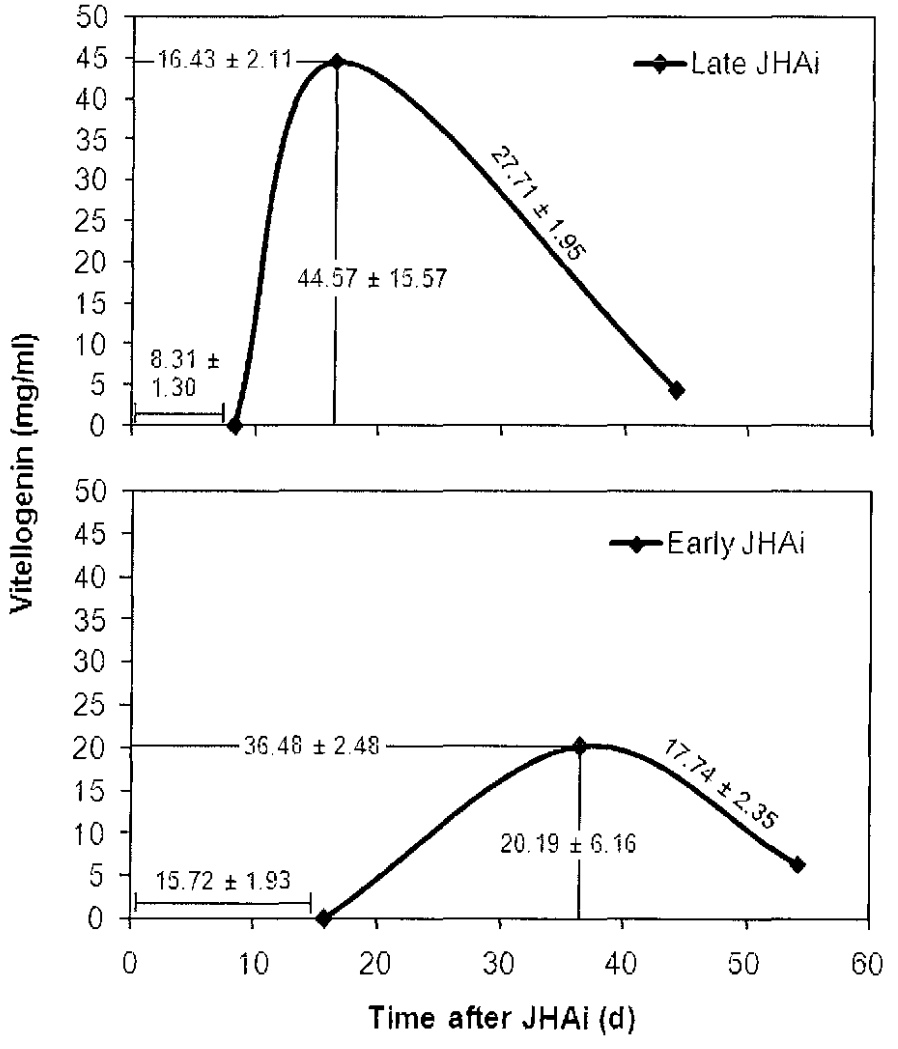
Late JHAi
16.43 ± 2.11
27.71 ± 1.95
44.57 ± 15.57
8.31 ± 1.30
Early JHAi
36.48 ± 2.48
17.74 ± 2.35
20.19 ± 6.16
15.72 ± 1.93
Vitellogenin (mg/ml)
Time after JHAi (d)
0
5
10
15
20
25
30
35
40
45
50
60

Figure 6

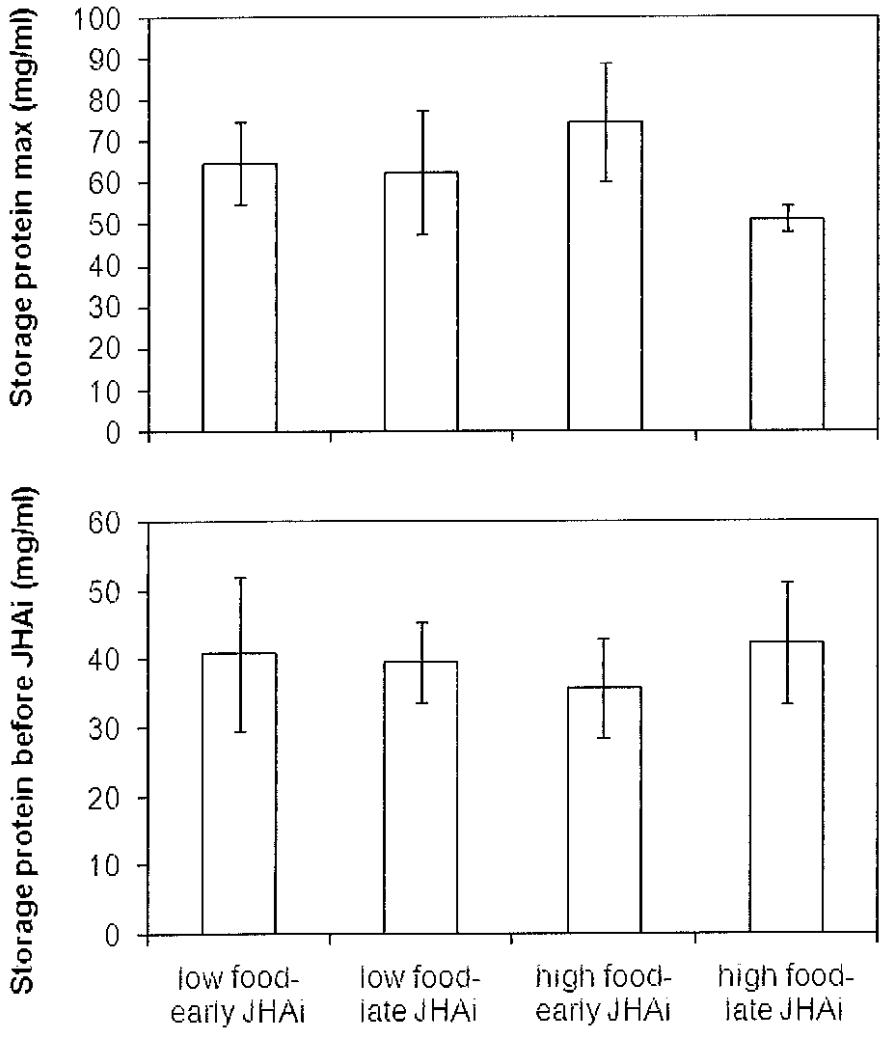
Storage protein max (mg/ml)
100
90
80
70
60
50
40
30
20
10
0
Storage protein before JHAi (mg/ml)
60
50
40
30
20
10
0
low food-early JHAi
low food-late JHAi
high food-early JHAi
high food-late JHAi

Figure 7

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Vita

Raime Blair Fronstin

Education:

University of North Florida, Jacksonville, FL
M.S. in Biology **2007**
Graduated with a 3.9 GPA.
Graduate Research: Juvenile Hormone in variation in grasshopper egg production.
Major Advisor: Dr. John Hatle

University of Florida
B.S. in Zoology **2003**
Graduated with a 3.0 GPA.
Undergraduate Research: Evaluating the suitability of using the protein content of colobus monkey faecal samples as an index of the protein content in foods eaten in order to assess habitat quality.
Research Advisor: Dr. Colin Chapman

Work Experience:

University of North Florida, Jacksonville, FL
Teaching Assistant **2004-2007**
Lab instructor for General Biology and Advanced Physiology. Responsible for organizing and implementing lectures and quizzes. Supervised, graded, and guided students through laboratory exercises and lab safety.

University of North Florida, Jacksonville, FL
Research Assistant **2004-2007**
Composed and implemented my master's research studying reproductive physiology, thresholds, and interpopulation variation. I also assisted in a study observing the effects of ovariectomy on lifespan in lubber grasshoppers. I employed numerous biological assays including the use of radioisotopes.
Contact: Dr. John Hatle

University of North Florida, Jacksonville, FL
Research Assistant **2007**
Assisted in research comparing the ecological impact on stream ecosystems with varying levels of anthropogenic disturbance. This involved transect sampling of soil and water, and the collection and identification of insects and spiders.
Contact: Dr. Daniel Moon

University of North Florida, Jacksonville, FL
Research Assistant **2007**
Assisted in a study to determine and map (using GIS) potential, reported, and observed occurrences of hognose snakes within the coastal areas and bordering regions of northern FL and southern GA.
Contact: Dr. Cathy Paterson

Dial Cordy and Associates, Jacksonville, FL
Field Surveyor **2004-2005**
Independent contractor; surveyed submerged aquatic vegetation in the lower St. Johns River in an ongoing water management study. This job included measuring transects, knowledge of aquatic vegetation, measuring vegetation density, diversity, and height to determine water quality.
Contact: Jason Evert

Other Experience:

Tutor
Worked independently tutoring college level Anatomy and Physiology.

Volunteer Work:

Florida Springs Turtle Survey **2005 – present**
This is an ongoing study capturing, marking and recapturing turtles in several FL springs. The study focuses on turtle diversity among springs and the effects of water loss on turtle populations.

We also remove invasive fish from the springs.
Contact: Eric Munscher

Gopher Tortoise Conservation **2006 –present**
Several ongoing studies involving habitat restoration and surveying of gopher tortoises in Pumpkin Hill Creek Preserve State Park.
Contact: Dr. Joseph Butler

Publications:

Fronstin RB and Hatle JD (in press) A cumulative feeding threshold required for vitellogenesis can be obviated with juvenile hormone treatment in lubber grasshoppers. *Journal of Experimental Biology.*

Fronstin RB and Hatle JD (in press) Interpopulation variation in body mass after laying and age at oviposition, but not clutch mass, in eastern lubber grasshoppers. *Journal of Orthoptera Research* 17:## (special issue on body size).

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Chapman CA, Webb **T**, **Fronstin RB**, Wasserman MD, Santamaria AM. 2005. Assessing dietary protein of colobus monkeys through faecal sample analysis: a tool to evaluate habitat quality. *African Journal of Ecology.* 43: 276-278

Achievements:

Vice president and co-founder of the UNF Biology Club

Top ten percent of my graduate class

Professional Societies:

Sigma Xi- The Scientific Research Society

Florida Entomological Society

Gopher Tortoise Council

The Honor Society of Phi Kappa Phi