Interpopulation Variation in Developmental Titers of Vitellogenin, but Not Storage Proteins, in Lubber Grasshoppers

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ABSTRACT

We examined simultaneous plastic and latitudinal interpopulation variation in the time course of hemolymph protein titers during egg production in the lubber grasshopper. Our goal was to gain insight into possible evolutionary changes in the physiology underlying reproductive plasticity. We used lubbers from three locations in the United States (Florida [FL], Louisiana [LA], and Georgia [GA]), each offered three daily food rations. Previous genetic analysis indicated that grasshoppers from FL (the low-latitude population) and GA (the high-latitude population) were phylogenetically closer to each other than to LA grasshoppers (the intermediate-latitude population). The ages at maximum titers of vitellogenin (Vg_{max}) and three storage proteins that were referred to as major hemolymph proteins (MHP_{max}) were used as indices of the progress of oocyte development. Age at Vg_{max} was affected significantly both by diet and by population. Perhaps most importantly, age at Vg_{max} was less for GA grasshoppers than for FL and LA grasshoppers; this pattern differs from the phylogenetic relationships of the populations. Age at MHP_{max} was significantly affected only by diet and not by population. Hence, the regulation of these proteins may differ across populations. Finally, we found no evidence that plasticity of reproductive investment in response to food availability differs across populations (as indicated by nonsignificant interactions of population and feeding environment).

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Introduction

A major question in evolutionary physiology is how intraspecific variation in phenotypes is produced (Travis et al. 1999; Hodin 2000). Such phenotypic variation can arise either by developmental plasticity (i.e., among individuals of similar genotype due to development in different environments) or by interpopulation variation (i.e., among different genotypes). A growing number of studies have investigated plastic and interpopulation variation simultaneously, with the goal of understanding the physiological mechanisms underlying that variation (Brown 1985; Winn and Evans 1991; Rountree and Nijhout 1995*a*, 1995*b*; Peric-Mataruga et al. 1997; Cordell et al. 1998; Dahlhoff and Rank 2000; Sokolova et al. 2000; Balaguer et al. 2001; Lee and Petersen 2002; Patrick et al. 2002); only a few of these studies addressed reproduction (Hodin and Riddiford 2000; Seigel and Ford 2001).

Related questions are how phenotypic plasticity and the physiology underlying this plasticity evolve. Despite predictions by evolutionary biologists that developmental plasticity will prove to be labile (West-Eberhard 1989, 2003; Hodin 2000), convincing demonstrations of plasticity evolving in a presumably adaptive way are rare (e.g., Donohue et al. 2000). Addressing plasticity and interpopulation variation simultaneously provides a comparative perspective and therefore insight into possible evolutionary changes. In this article, we examine plastic and interpopulation variation in the time courses of hemolymph protein titers during oocyte development.

The Eastern lubber grasshopper (*Romalea microptera* [= *guttata*]) is an excellent model for studies of reproductive plasticity. For a laboratory colony of lubbers from south Florida, the first oviposition cycle (~35 d) involves first somatic growth and then reproductive growth. During the first ~10 d, the primary oocytes are not vitellogenic, despite an ~50% increase in somatic mass (Sundberg et al. 2001). Hence, we suspect that nutritional resources acquired during the nymphal stages are relatively unimportant for oocyte development. This helps justify our common garden design in which adult nutrition is manipulated (see "Material and Methods" section). The large size of lubber grasshoppers (4–8 g) makes it possible to take repeated hemolymph samples throughout the oocyte development of a single individual, allowing detailed analyses of developmental trajectories of protein titers. Finally, lubbers lay large clutches, with most females laying only two or three clutches per lifetime. Thus, each clutch is a major developmental event, requiring the allocation of up to 1 g of protein.

Lubber grasshoppers are equally good models for studies of interpopulation variation but for different reasons. Lubbers are flightless, gregarious, and univoltine, exhibiting patchy distributions. The lifetime movement of single individuals is estimated at 50 m (Whitman 1990). Hence, populations are largely isolated from each other. We investigated lubbers from south Florida (FL; 25.8°N, 80.3°W), south Louisiana (LA; 29.9°N, 91.2°W), and north Georgia (GA; 34.0°N, 83.4°W), all in the United States. The phylogenetic relationships of 12 populations of lubber grasshoppers (including the three in this study) have been examined by sequencing the mtDNA cytochrome-b gene (Mutun 1999). Mutun (1999) found a 69% probability that grasshoppers from FL and GA are more closely related to each other than to LA grasshoppers.

Reproductive tactics in lubber grasshoppers show both phenotypic plasticity in response to food availability (Moehrlin and Juliano 1998; Hatle et al. 2000) and interpopulation variation (Hatle et al. 2002). Early in the oviposition cycle, the timing of oviposition is plastic across diet levels. This timing of first oviposition is important because it could affect the total number of clutches laid by a female (and her lifetime fecundity). Later in the oviposition cycle, reproduction enters a canalized phase; the timing of oviposition becomes unresponsive to variation in diet quantity at least 14 d before oviposition, and the number of eggs laid becomes unresponsive at least 7 d before oviposition (Moehrlin and Juliano 1998). Across populations, reproductive tactics also vary. The FL, LA, and GA populations each show a distinct combination of age at oviposition, clutch mass, and somatic storage remaining after laying (Hatle et al. 2002). Florida grasshoppers make smaller clutches, oviposit later, and retain more somatic mass after laying than do GA grasshoppers. LA females are intermediate between FL and GA females in age at oviposition, similar to FL in somatic storage, and similar to GA in clutch size. These populations represent a latitudinal cline with active seasons of varying duration. The precise duration of the active seasons has not been investigated, but one indicator of active season length is the mean duration of the frost-free period: FL = 365 d, LA = 280 d, and GA = 224 d (Koss et al. 1988). Therefore, reproductive tactics, particularly age at oviposition, vary among these three populations in ways that appear to be correlated with their local climates (Hatle et al. 2002).

We have previously investigated the roles of the hemolymph precursor of egg yolk protein (vitellogenin [Vg]) and storage proteins during egg production in lubber grasshoppers. These storage proteins are total non-Vg hemolymph proteins that we collectively refer to as major hemolymph proteins (MHPs). In a laboratory population from Florida, Vg is first detectable ~10 d after adult molt; levels increase steadily until a peak at age ~22 d, and then levels fall steadily until oviposition (Borst et al. 2000). Juvenile hormone, the central hormone in egg production for most insects (Nijhout 1994), is necessary for vi-

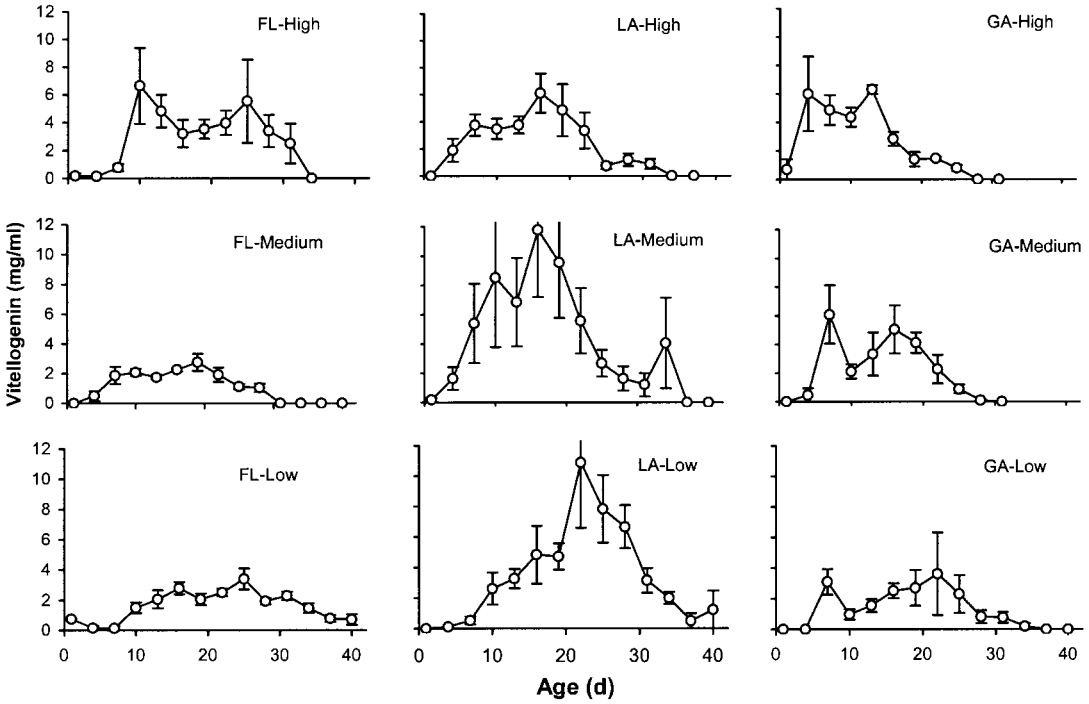

Figure 1. Developmental profiles of vitellogenin. Lubber grasshopper populations each were raised from adult molt to first oviposition on three diet levels. Hemolymph samples were collected from each individual every third day. Error bars represent 1 SEM.

Table 1: Reproductive tactics across diets and populations in lubber grasshoppers

	Age at Oviposition (d)	Clutch Mass (g)	Mass after Oviposition (g)
Diet:			
High	33.3 (32.2–34.3)[a]	.96 (.90–1.02)[a]	5.22 (4.96–5.50)[a]
Medium	35.2 (34.4–36.1)[a]	1.02 (.96–1.07)[a]	5.28 (5.07–5.51)[a]
Low	40.7 (39.6–41.9)[b]	.70 (.66–.74)[b]	5.01 (4.79–5.24)[a]
Population:			
GA	33.3 (32.2–34.5)[A]	.91 (.85–.98)[A]	4.95 (4.68–5.25)[A]
LA	38.2 (37.2–39.2)[B]	.83 (.79–.88)[A]	5.16 (4.95–5.38)[A]
FL	36.6 (35.6–37.6)[B]	.87 (.82–.92)[A]	5.38 (5.15–5.63)[A]

Note. Lubber grasshopper populations each were raised from adult molt to first oviposition on three diet levels. Because the data are backtransformed from log transformations, we present them as means and asymmetrical upper and lower bounds of SE intervals. For each variable, means for diets or means for populations that are associated with the same letter are not significantly different at experimentwise $\alpha = 0.05$. See "Results" for comparison with previously published data (Hatle et al. 2002).

tellogenesis in lubbers (Barry et al. 2002). Nonetheless, in this study, we chose to monitor reproductive physiology by tracking proteins. Titers of Vg and MHPs are more easily and accurately measured than are juvenile hormone levels, and Vg levels have similar patterns to juvenile hormone (Borst et al. 2000; Hatle et al. 2000, 2001) and hemolymph ecdysteroid (Hatle et al. 2003) levels. Our initial characterization of MHPs demonstrates they are immunologically distinct from Vg (Hatle et al. 2001) and suggests they are members of the family of insect storage proteins (J. D. Hatle and D. W. Borst, unpublished data). Gel electrophoresis shows that throughout egg production, 80% of total non-Vg hemolymph protein is made up of only three proteins, collectively called the MHPs. Similar to Vg, MHP levels peak at an age of ~22 d and then fall until oviposition. The maximum level of MHPs predicts the number of eggs (Hatle et al. 2001). These MHPs may serve as a reservoir of amino acids for major developmental events (Pan and Telfer 1996), such as oocyte development.

We studied the relationships of developmental titers of Vg and MHP to the transition from plastic to canalized oocyte development (Hatle et al. 2001). The timings of the maximum titers of Vg (Vg_{max}) and MHP (MHP_{max}) between adult molt and first oviposition can be used as indices of the progress of oocyte development. These maximum titers indicate the moment in the oviposition cycle at which import of the protein (e.g., synthesis) is overtaken by export (e.g., degradation). This is an important point in development because it is the transition from favoring storage of protein (in the hemolymph) to favoring oocyte growth. In addition, Vg_{max} and MHP_{max} both occur during the canalized phase at the end of egg production (Hatle et al. 2001). Times from adult molt to both Vg_{max} and MHP_{max} are flexible in response to food availability, whereas times from Vg_{max} and MHP_{max} to oviposition are unaffected by food availability before or after the maxima (Hatle et al. 2001). Hence, the timings of Vg_{max} and MHP_{max} are developmental landmarks in grasshopper egg production.

We tested whether Vg and MHP titers vary among diets and among populations and predicted the following: (1) ages at

Table 2: MANOVA results for the timing of maximum protein titers in lubber grasshoppers from three populations, each raised from adult molt to first oviposition on three diet levels

					Standardized Canonical Coefficients	
	Pillai's Trace	*F*	df	*P*	Age at Maximum	Time from Maximum to Oviposition
Vg timing:						
Diet	.522	7.24	4, 82	.0001	2.169	1.015
Population	.404	5.19	4, 82	.0009	2.106	.860
Diet × population	.189	1.07	8, 82	.3942	…	…
MHP timing:						
Diet	.406	5.22	4, 82	.0008	1.255	.882
Population	.270	3.20	4, 82	.0170	.869	1.224
Diet × population	.131	.72	8, 82	.6730	…	…

Note. Developmental titers of both vitellogenin (Vg) and major hemolymph proteins (MHP) were measured in individuals. For significant main effects, standardized canonical coefficients are included.

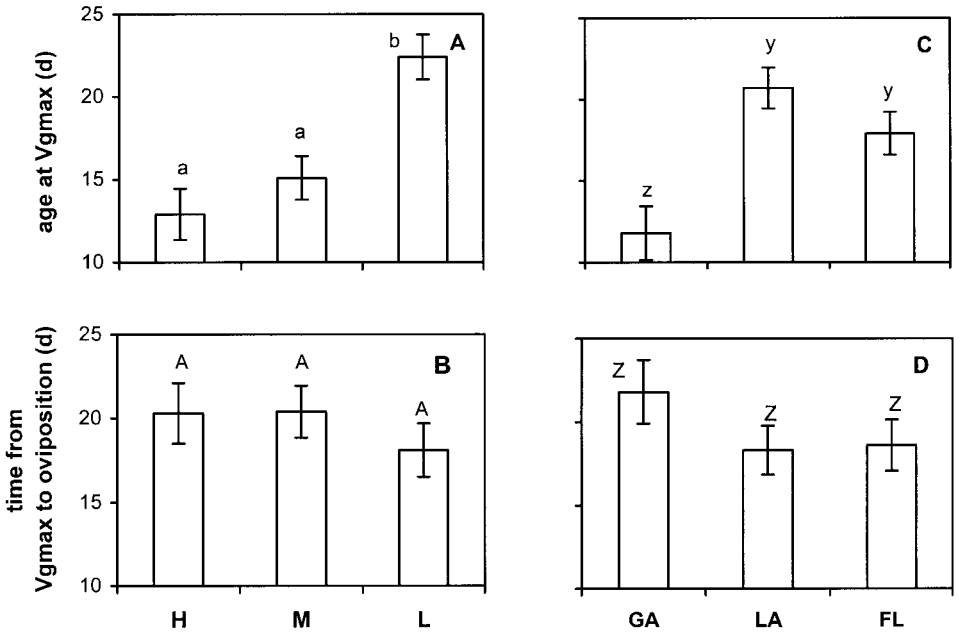

Figure 2. Both diets and populations differ in age at maximum titer of vitellogenin (Vg). Neither diets nor populations differ in time from maximum titer of Vg to oviposition. Three populations of lubber grasshoppers each were raised from adult molt to first oviposition on three diet levels. For each variable, means for diets or means for populations that are associated with the same letter are not significantly different at experimentwise $\alpha = 0.05$. Error bars represent 1 SEM.

Vg_{max} and MHP_{max} will occur significantly earlier in grasshoppers fed a high-quantity diet than in grasshoppers fed a low-quantity diet (as shown by Hatle et al. 2001 for a laboratory population of Florida grasshoppers); (2) ages at Vg_{max} and MHP_{max} will occur significantly earlier in grasshoppers from locations with shorter growing seasons (and younger ages at oviposition) than in grasshoppers from locations with longer growing seasons (and older ages at oviposition); specifically, GA grasshoppers will be significantly younger when they reach Vg_{max} and MHP_{max} than will FL grasshoppers; and (3) ages at Vg_{max} and MHP_{max} will be least flexible in GA grasshoppers and most flexible in FL grasshoppers. That is, a significant interaction of diet and population will exist, suggesting that the degree of plasticity differs among these populations.

Material and Methods

Grasshoppers from all three populations were collected in the wild as juveniles and shipped to our laboratory in Normal, Illinois. They were raised to adulthood in communal cages (by population) with heat lamps on a 14L : 10D photoperiod. They were fed ad lib. romaine lettuce and oats. All juveniles were housed in the same room to minimize differences in rearing temperature and photoperiod. This design did not eliminate the possibility of maternal or nymphal environmental effects producing different results in each of the three populations. However, the fact that a phase of somatic growth precedes reproductive growth in adult females suggests that resources allocated toward reproduction are ingested as adults and therefore that a nymphal environment may have a limited effect on reproduction.

On the day of adult molt, the femur length of each grasshopper was measured (as an estimate of body size). Grasshoppers were placed individually into ventilated 500-cm^3 plastic containers in an environmental chamber (14L : 10D photoperiod and a corresponding 32°C : 24°C thermocycle). Grasshoppers were assigned to one of three feeding treatments in which food rations were scaled to the size of each individual (as done by Hatle et al. [2002] and Luker et al. [2002]). Low-fed grasshoppers were offered (femur length [mm] × 75) mg romaine lettuce + (femur length [mm] × 0.75) mg oats daily. This diet typically consisted of between 2,000 and 2,500 mg of lettuce daily, and the grasshoppers almost always completely consumed this ration. Medium-fed grasshoppers were offered twice the food of low-fed grasshoppers, and high-fed grasshoppers were offered twice the food of medium-fed grasshoppers. In population × diet treatment combinations, mean $n = 5.4$ and range = 3–8. This experiment was conducted in parallel with an experiment on the reproductive tactics of these three populations (Hatle et al. 2002), with the only difference in rearing being that the grasshoppers in this study were bled

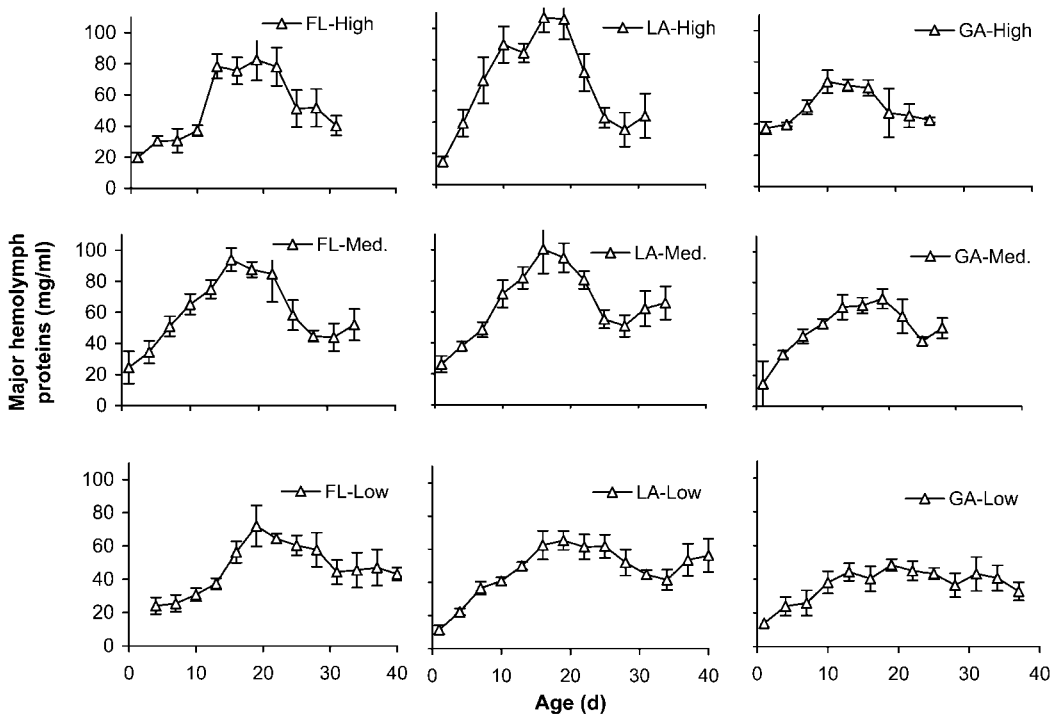

Figure 3. Developmental profiles of major hemolymph proteins. Lubber grasshopper populations each were raised from adult molt to first oviposition on three diet levels. Hemolymph samples were collected from each individual every third day. Error bars represent 1 SEM.

every third day. For all grasshoppers, age at oviposition, clutch mass, and body mass after oviposition were measured as previously described (Hatle et al. 2002).

Hemolymph samples (5 μL) were placed in 250 μL of buffer and frozen until analysis by an enzyme-linked immunosorbent assay for Vg (Borst et al. 2000). We estimated MHP titers as described by Hatle et al. (2001); Bradford's (1976) assay was used to measure total hemolymph proteins, and then Vg was subtracted from total protein. All the samples from a single individual were tested simultaneously to avoid possible effects of interassay variation on determination of the timing of the maximal titers. Further, the order in which individuals were analyzed was randomized to avoid possible effects due to variation over time. To determine the maximum titer of Vg for an individual, we simply compared all the Vg titers through development for that individual and identified the sample with the highest level of Vg. Hence, although there may not appear to be a distinct maximum among the means for each treatment group (e.g., Fig. 1), for each individual there was a distinct maximum level of Vg and age at maximum Vg. We identified MHP_{max} titers and ages at MHP_{max} by this same method.

Reproductive tactics were analyzed as described (Hatle et al. 2002). The protein data were analyzed using three two-way MANOVAs. We did not use phylogenetically based statistical methods (Felsenstein 1985; Garland and Adolph 1994; but see Ricklefs and Starck 1996; Reeve and Sherman 2002) because there are only three populations in the study. The first MANOVA tested for effects of diet, population, and the interaction of population and diet on age at Vg_{max} and time from Vg_{max} to oviposition. The second MANOVA tested for effects on age at MHP_{max} and time from MHP_{max} to oviposition. The third MANOVA tested for effects on Vg_{max} titer and MHP_{max} titer. When necessary, variables were log transformed to meet assumptions of normality and homogeneity of variances. For all analyses, we used SAS PROC GLM (SAS Institute 1989). We interpret significant MANOVA results on the basis of standardized canonical coefficients as described by Scheiner (2001).

In our experiment, a significant effect of diet would indicate plasticity in the timing of the maximal protein titers or in the titers themselves and therefore the developmental switch in protein regulation (hereafter, protein timing). A significant effect of population would indicate that populations differ in their protein timing or maximal titer (but not necessarily their plasticity), which could imply divergence of protein regulation

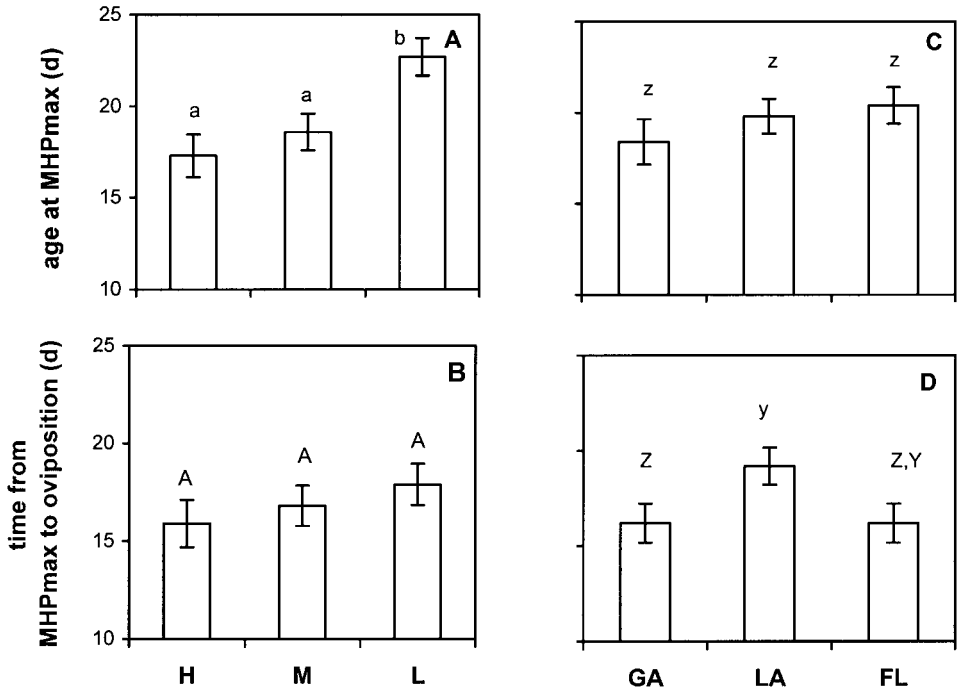

Figure 4. Diets differed in age at maximum titer of major hemolymph proteins (MHPs), but populations did not differ in age at maximum titer of MHPs. In contrast, diets did not differ in time from maximum titer of MHPs to oviposition, whereas populations did differ in time from maximum titer of MHPs to oviposition. Lubber grasshoppers populations each were raised from adult molt to first oviposition on three diet levels. For each variable, means for diets or means for populations that are associated with the same letter are not significantly different at experimentwise $\alpha = 0.05$. Error bars represent 1 SEM.

across populations. A significant interaction of diet and population would indicate that the plasticity of protein timing or titers differs among populations, suggesting population divergence in the level of plasticity.

Results

Reproductive Tactics

Trends in age at oviposition, clutch mass, and somatic storage retained after oviposition for the grasshoppers in this study were similar to those previously reported in the parallel study (Hatle et al. 2002). Age at oviposition was significantly affected by both diet ($F_{2,40} = 13.3$, $P < 0.0001$) and population ($F_{2,40} = 3.24$, $P = 0.0496$) but not the interaction of diet and population ($F_{4,40} = 1.36$, $P = 0.264$; Table 1). High-fed and medium-fed grasshoppers oviposited sooner than did low-fed grasshoppers, and GA grasshoppers laid sooner than did FL and LA grasshoppers. Clutch mass was significantly affected by diet ($F_{2,40} = 10.9$, $P = 0.0002$) but not by population ($F_{2,40} = 0.46$, $P = 0.635$) or interaction ($F_{4,40} = 0.44$, $P = 0.778$; Table 1). Finally, somatic storage was not affected by diet ($F_{2,40} = 0.45$, $P = 0.639$), population ($F_{2,40} = 0.67$, $P = 0.517$), or interaction ($F_{4,40} = 2.33$, $P = 0.72$). The lack of significant differences in clutch mass and somatic storage among populations was somewhat different from the results of our parallel study (Hatle et al. 2002), although trends were in the same directions. When the data from the two experiments were combined, the statistical conclusions were the same as those of Hatle et al. (2002). Most important for this article, the ages at oviposition in this study were significantly affected by diet and population but not by the interaction of diet and population.

Timing of Vg_{max} and MHP_{max}

Vitellogenin timing was significantly affected by both diet (Table 2; Fig. 1; Fig. 2*A*, 2*B*) and population (Table 2; Fig. 1; Fig. 2*C*, 2*D*). The interaction of population and diet was not significant (Table 2). Standardized canonical coefficients revealed that both diet and population effects were primarily due to variation in age at Vg_{max}. Low-fed grasshoppers reached Vg_{max} significantly later than did both high-fed (univariate $P < 0.0001$) and medium-fed ($P = 0.0004$) grasshoppers, and GA grasshoppers reached Vg_{max} significantly sooner than did both LA ($P < 0.0001$) and FL ($P = 0.0058$) grasshoppers.

Timing of MHPs was significantly affected by diet (Table 2; Fig. 3; Fig. 4*A*, 4*B*) and population (Table 2; Fig. 3; Fig. 4*C*, 4*D*). The interaction of population and diet was not significant (Table 2). Canonical coefficients revealed that the diet effect

Table 3: MANOVA results for maximum protein titers in lubber grasshoppers from three populations, each raised from adult molt to first oviposition on three diet levels

	Pillai's Trace	F	df	P	Standardized Canonical Coefficients	
					Vg_{max} Titer	MHP_{max} Titer
Diet	.4716	6.33	4, 82	.0002	−.196	1.411
Population	.4464	5.89	4, 82	.0003	−.129	1.414
Diet × population	.1862	1.05	8, 82	.4042		

Note. Developmental titers of both vitellogenin (Vg) and major hemolymph proteins (MHP) were measured in individuals. For significant main effects, standardized canonical coefficients are included.

was primarily due to the age at MHP_{max}, whereas the population effect (which was weaker) was primarily due to the time from MHP_{max} to oviposition. Low-fed grasshoppers reached MHP_{max} significantly later than did both high-fed ($P = 0.0012$) and medium-fed ($P = 0.0068$) grasshoppers. There was no population effect on the age at MHP_{max} (all $P > 0.20$). LA grasshoppers had a longer time from MHP_{max} to oviposition than did GA grasshoppers ($P = 0.0165$).

Maximal Protein Titers

Maximal protein titers were significantly affected by diet and population, but the interaction of population and diet was not significant (Tables 3, 4). Canonical coefficients revealed that both the diet and population effects were largely due to the MHP_{max} titer. Low-fed grasshoppers had a lower MHP_{max} titer than did both high-fed and medium-fed ($P \leq 0.0001$) grasshoppers. GA grasshoppers had a lower MHP_{max} titer than did both LA ($P < 0.0001$) and FL ($P = 0.0002$) grasshoppers.

Discussion

The mechanisms by which individuals within a species produce variable phenotypes are of interest because it is this variation on which natural selection can act. Two major sources of intraspecific variation are developmental plasticity and interpopulation variation. In this study, we examined simultaneous plasticity (due to diet levels) and interpopulation variation in the physiology of egg production that occurs in a grasshopper species. We found that both sources of variation significantly affected the developmental titers of both a reproductive protein (Vg) and storage proteins (MHPs; Figs. 2, 4), and this variation is associated with reproductive tactics. However, the interaction of plasticity and interpopulation variation (i.e., the genotype by environment interaction) did not significantly affect the developmental titers of these proteins.

Interpopulation Variation in Vg and MHP Timing

Our three study populations differed in the combination of three reproductive tactics: age at reproduction, clutch size, and somatic storage (Hatle et al. 2002). Here, we showed that these three populations differed in their timings of protein titers that Hatle et al. (2001) showed to be physiological measures of oocyte development. GA grasshoppers reached Vg_{max} earlier than did either FL or LA grasshoppers (Fig. 2). This indicated that the timing of the peak concentration of a protein specifically allocated to reproduction (i.e., Vg) differed among populations in a pattern that was concordant with variation in the timing of oviposition (Hatle et al. 2002).

This pattern of variation in reproductive timing contrasted with the phylogenetic relationships of these grasshoppers (Mutun 1999; see also "Introduction"). The observed patterns implied that the differences in reproductive physiologies might not have been merely correlates of the phylogenetic history of these populations. Taken together, these data suggested that the reproductive physiology of GA grasshoppers has diverged from

Table 4: Maximum titers of vitellogenin (Vg) and major hemolymph proteins (MHPs)

	Max. Titer of Vg (mg/mL)	Max. Titer of MHPs (mg/mL)
Diet:		
High	8.15 (6.54–10.16)^{a}	96.4 (91.1–102.0)^{z}
Medium	5.00 (4.15–6.05)^{a}	96.4 (91.9–101.1)^{z}
Low	6.48 (5.34–7.87)^{a}	69.8 (66.5–73.4)^{y}
Population:		
GA	6.61 (5.23–8.34)^{A}	69.7 (65.6–73.9)^{Z}
LA	8.10 (6.78–9.68)^{A}	98.2 (95.5–100.9)^{Y}
FL	4.95 (4.10–5.97)^{A}	94.8 (90.4–99.5)^{Y}

Note. GA = Georgia; LA = Louisiana; FL = Florida. Lubber grasshopper populations each were raised from adult molt to first oviposition on three diet levels. Because the data are backtransformed from log transformations, we present them as means and asymmetrical upper and lower bounds of SE intervals. For each variable, means for diets or means for populations that are associated with the same letter are not significantly different at experimentwise $\alpha = 0.05$. See "Results" for details.

that of FL and LA grasshoppers. This study contributed to the growing body of evidence that shows that insect populations can vary at the physiological level (e.g., Hoffman 1991; Rountree and Nijhout 1995*a*, 1995*b*; Krebs and Feder 1996; Peric-Mataruga et al. 1997; Berenbaum and Zangerl 1998; Hoffmann and Harshman 1999; Dahlhoff and Rank 2000; Patrick et al. 2002).

In contrast to age at Vg_{max}, age at MHP_{max} did not differ among populations (Fig. 4). This pattern existed despite differences in the timing of oviposition among populations (Hatle et al. 2002; Table 1) and despite the occurrence of MHP_{max} during the canalized phase of egg production (Hatle et al. 2001; Fig. 4). Hence, the age at maximum titer of a reproductive protein differed across populations, but the age at maximum titer of storage proteins did not differ across populations. Our data suggested that the physiological control of these functionally related proteins may be independent.

Time from MHP_{max} to oviposition was slightly but significantly greater in LA grasshopper than in GA grasshoppers (Fig. 4*D*), which was not predicted. We hypothesized (C. A. Jones and S. A. Juliano, unpublished data) that the beginning of vitellogenesis depends on attaining a threshold of MHP storage. This hypothesis postulates that once this threshold is met, MHP is converted to Vg and then transported into the growing oocytes; the time required for these physiological events is postulated to be part of the canalized phase of oogenesis (Olson et al. 2001; Tillman et al. 2001). One interpretation of our data is that LA grasshoppers have a higher threshold for initiating vitellogenesis than do GA grasshoppers, resulting in the greater ages at oviposition and higher MHP titers we observe in LA grasshoppers. Further, LA and GA grasshoppers have similar clutch masses (Hatle et al. 2002; Table 1), implying that the rates of conversion and transport of proteins into oocytes for LA grasshoppers may be lower than those of GA grasshoppers.

Interpopulation Variation in Maximum Titers

Maximum titers of MHP were lower in GA grasshoppers than in LA and FL grasshoppers (Table 4). Time-constrained animals would be expected to allocate relatively more resources to reproduction as opposed to storage (Rowe et al. 1994). Thus, GA grasshoppers may be better suited for a shorter active season in their younger age at Vg_{max} (Fig. 2), younger age at oviposition (Hatle et al. 2002; Table 1), lower levels of MHP (Table 4), and lower somatic storage (Hatle et al. 2002).

Lack of Population by Environment Interactions

Documenting significant interpopulation variation in the physiology underlying developmental plasticity is a first step toward addressing how mechanisms of plasticity evolve (Hodin 2000). Further, predictions of evolutionary lability in plasticity imply that there is intraspecific genetic variation of plasticity, which would be evident as a population (genotype) by environment interaction (West-Eberhard 1989, 2003; Hodin 2000; Hodin and Riddiford 2000). However, we found no significant population by environment interactions for any of the variables in this study or our parallel study (Hatle et al. 2002). This suggests that individuals from all three populations may have similar responses to the range of food availability used in these experiments (e.g., in all three populations, high-fed grasshoppers reach Vg_{max} earlier than low-fed grasshoppers reach Vg_{max}). Thus, we find no evidence that plasticity is adapted to local conditions for reproductive tactics (Hatle et al. 2002), protein timing (Figs. 2, 4), or protein levels (Table 4).

Although ages at both Vg_{max} and MHP_{max} varied among diets (Figs. 2*A*, 4*A*), times from Vg_{max} to oviposition and MHP_{max} to oviposition did not vary among diets (Figs. 2*B*, 4*B*). These results are consistent with a previous study for a single laboratory population of lubber grasshoppers from south Florida. This previous study demonstrated that both Vg_{max} and MHP_{max} occurred during the canalized phase of egg production (Hatle et al. 2001). Together, these studies strengthen the evidence that the range of plastic reproductive responses that are possible in lubber grasshoppers might be limited by physiological mechanisms associated with this canalized phase (Ricklefs and Wikelski 2002).

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