Ontogenetic Mechanisms Underlying a Geographic Size Cline in a Grasshopper, *Romalea microptera*

JASON E. JANNOT,[1,2] JESSICA BRINTON,[1] KEVIN KOCOT,[1] OLCAY AKMAN,[3] AND STEVEN A. JULIANO[1]

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ABSTRACT Geographic clines in body size have been described for many species, but relatively few investigations have tested hypotheses for the ontogenetic mechanisms maintaining geographic clines. We formalize and test the predictions for the role of ontogenetic mechanisms (e.g., hatching timing and size, juvenile developmental time, juvenile growth rate) in maintaining a longitudinal cline in adult body size of lubber grasshoppers [*Romalea microptera* (Beauvois)]. To obtain hatching timing and size, we collected eggs from wild females from several populations along the longitudinal gradient in 2 yr (2006 and 2007) and measured hatchling size and hatch date. To obtain juvenile developmental time and growth rates, we surveyed populations along the longitudinal gradient during 2 yr (2006 and 2007) and estimated developmental time and growth rates. We found the developmental time (hatching to fourth instar) and female growth rates increase from west to east along the cline. Patterns of hatching timing and hatching size were not consistent with the size cline. The size cline becomes evident in the third instar and is magnified in the fourth and fifth instars. Our data suggest that the size cline arises from some combination of clinal variation in developmental time and female growth rates; prolonged development and greater growth rates lead to larger mean size. Equally important, we found no evidence that differences in hatching time or size are ontogenetic causes of this cline. Our hypotheses for ontogenetic mechanisms producing an adult size cline should serve as a template for ecologists seeking to understand the ontogenetic basis of spatial variation in phenotypes.

KEY WORDS development, growth rate, hatching size, interpopulation variation, south Florida

Geographic clines in body size are frequently observed among populations of a single species and have proved to be excellent model systems for studying intraspecific phenotypic variation (Bergmann 1847, Ray 1960, Lindsey 1966, Endler 1977, Mousseau and Roff 1989, Brennan and Fairbairn 1995, Mousseau 1997, Gilchrist and Partridge 1999, Huey et al. 2000, Ashton and Feldman 2003, Berner et al. 2004, Blanckenhorn and Demont 2004, Schauble 2004, Meiri et al. 2005, Conover et al. 2006). Even though geographic clines in body size have been described for many species, relatively few investigations have tested hypotheses for the ontogenetic mechanisms maintaining geographic clines (Arnett and Gotelli 1999, Gockel et al. 2001, Merila and Crnokrak 2001, Palo et al. 2003, Sears and Angilletta 2004, Toju and Sota 2006). Geographic clines in adult body size provide a perfect opportunity to test alternative mechanistic hypotheses for the basis of size variation, including differences in initial size, developmental time, or growth rate (Sibly et al. 1997, Gilchrist and Partridge 1999, Huey et al. 2000).

In this study, we focus on the role of ontogenetic mechanisms in maintaining a geographic cline in adult body size. Interpopulation variation in three developmental mechanisms could produce variation in size at maturity among populations: size at hatching or birth, juvenile developmental time, or individual juvenile growth rate (Fig. 1). To simplify, we assume exponential growth in size. Variation in size at hatching could produce interpopulation differences in size at maturity even if juvenile developmental times and individual growth rates are equal among populations (Fig. 1a). If size at hatching is the only mechanism operating (i.e., Fig. 1a), then the difference in size between populations will increase over time (Stearns 1992 pp. 174–175). Interpopulation differences in juvenile developmental times also could produce interpopulation variation in adult size, independent of hatching size and individual growth rate (Fig. 1b). Populations with longer juvenile developmental times spend more time growing and therefore may attain a larger size at maturity relative to populations of individuals with relatively shorter developmental times, even if initial size and growth rates are equal (Fig. 1b). Interpopulation variation in developmental time could arise in at least two nonmutually exclusive ways: 1) all populations hatch at the same time but vary in total developmental time or 2) populations vary in

[1] BEES Section, School of Biological Sciences, Illinois State University, Normal, IL 61790-4120.
[2] Corresponding author, e-mail: jjannot@ilstu.edu.
[3] Department of Mathematics, Illinois State University, Normal, IL 61790-4520.

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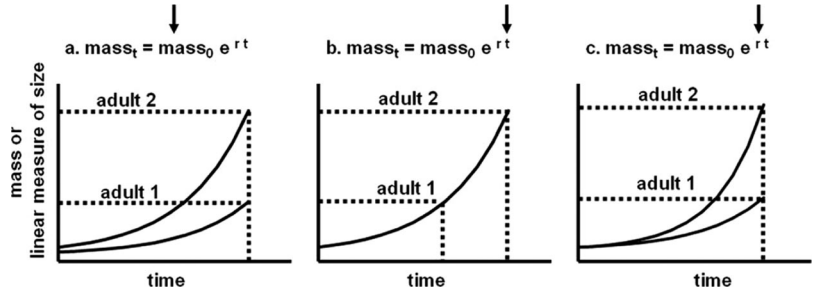

Fig. 1. Three ontogenetic mechanisms which could underlie geographic clines in adult body size: (a) hatching size: small initial differences become large differences in final size because of exponential growth (growth rates do not differ); (b) developmental time: prolonged development results in larger final size; and (c) growth rate: greater rate of increase results in greater final size. Arrows point to the parameter that differs in each figure. Dotted lines intersect at the size-time point where each individual matures to the adult stage (adult 1, adult 2). $Mass_0$, mass at hatching; r, exponential rate of growth in size; t, total developmental time from hatching to adult.

hatching times but have a fixed season suitable for development. Assuming fixed growth rates and season length, animals hatching early in the season should have longer developmental times than animals hatching later in the season. Finally, different individual juvenile growth rates could produce interpopulation differences in size at maturity, independently of hatching size or developmental time. Populations that have higher individual growth rates attain greater individual mass (or linear dimensions) at a given time than do populations with lower individual growth rates, even if hatching size and developmental times are the same among populations (Fig. 1c).

Mean adult body size for populations of the lubber grasshopper *Romalea microptera* (Beauvois) (Orthoptera: Acrididae) exhibits a longitudinal cline, increasing from west to east in south Florida (Huizenga et al., 2008; see Supplemental Material). This longitudinal cline might represent local adaptation because lubbers are flightless, with low mobility and limited gene flow among populations. In addition, across south Florida, local populations of *R. microptera* exist in habitats that differ greatly in soil, vegetation composition and structure, hydroperiod, and proximity to water (Rehn and Grant 1961, Capinera et al. 1999). Therefore, habitat-specific selection on life history variables such as hatching timing, hatchling size, developmental rate, and growth rate could produce differences among populations in these ontogenetic traits.

Our goal in this study was to identify the ontogenetic mechanisms responsible for maintaining this size cline. In this article, we do not attempt to identify the selective agents and evolutionary processes (e.g., adaptation versus plasticity) producing the cline but instead seek to identify the proximate mechanisms underlying the cline. To test for the role of ontogenetic mechanisms in maintaining this body size cline, we sampled lubber populations in south Florida in 2006 and 2007. Because of the west (small) to east (large) adult size cline (Huizenga et al., 2008; Supporting Information), we predicted that, relative to eastern populations, western populations would have some combination of later hatching times, smaller hatchlings, shorter developmental periods, and lower individual growth rates. We also test for the ontogenetic timing of inter-population size differences by examining when during development (i.e., what instar) the size cline becomes evident.

Materials and Methods

Study Species and Populations. Lubber grasshoppers are univoltine. In south Florida, lubber nymphs typically begin hatching in February. Nymphal growth and development proceed through the spring and adults begin to show up in late May and are present through August (Rehn and Grant 1961, Capinera et al. 1999). Lubbers have five juvenile instars (see Supplemental Material online: Jannot_etal_Size_Cline_Grasshopper_Suppl_Info.pdf). Linear measures of the hard exoskeleton are fixed for the duration of each instar and the adult stage. Hence, interpopulation variation in linear measures of adult size results from one or more of the developmental mechanisms described above.

In total, 12 *R. microptera* populations were chosen from a longitudinal transect in subtropical south Florida (Fig. 2). Sites were chosen based on accessibility and previous surveys, indicating relatively high densities of lubbers (AA, FX, TL, SVS; Huizenga et al. 2008). Driving distance between the most distant sites (DANHOUSE to CASINO) is ≈125 km.

Field Studies. To obtain information about interpopulation variation in hatching time and size, we measured the timing of hatching and sizes of hatchlings from eggs laid by females collected in June and July 2006 (AA, TL, SVS) and in July 2007 (AA, FX, TL BURNS, SVS, CASINO). Females were allowed to oviposit once in individual 946-ml plastic cups filled 50% with white, moistened sand (N = clutches that hatched 2006: AA = 4, TL = 12, SVS = 4; 2007: DANHOUSE = 3, AA = 13, FX = 2, TL = 24, BURNS = 2, SVS = 25, CASINO = 7). Egg cups were transported from the field to Illinois State University, kept moist, stored on a laboratory benchtop (≈25–30°C), and monitored daily for hatching. The timing of hatching was quantified as the number of days between ovipo-

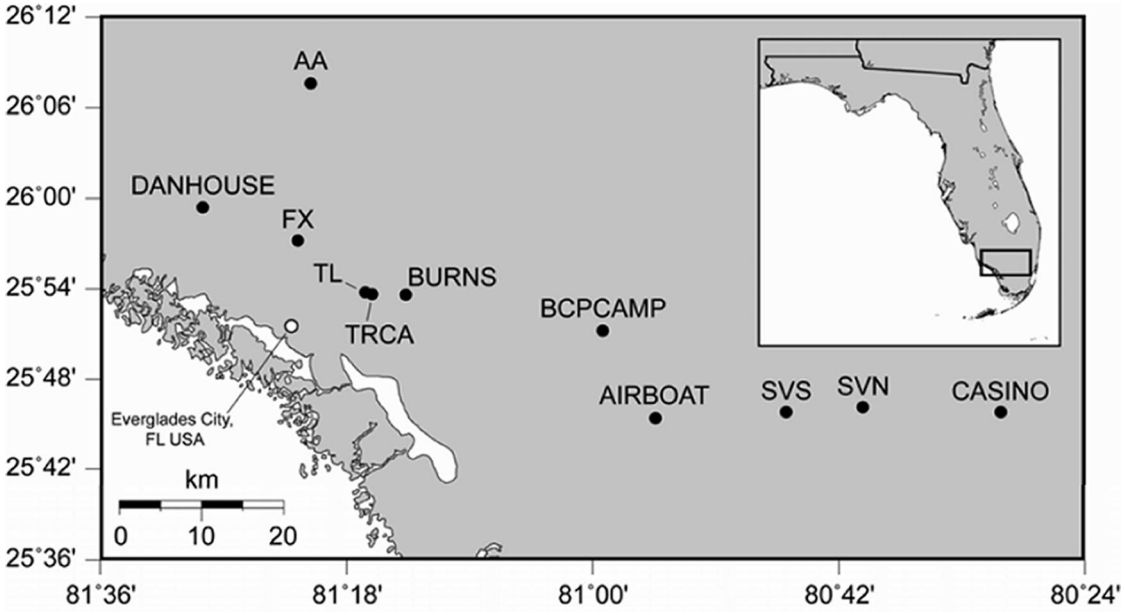

Fig. 2. Twelve field sites located in south Florida (see black box in inset). From DANHOUSE to CASINO is ≈125 km (driving distance).

sition and the hatching of the first individual. Twenty-four hours after hatching, individuals were digitally photographed to obtain linear dimensions and fresh mass determined to the nearest 0.001 mg for 10 hatchlings per clutch.

We quantified interpopulation variation in growth rate and developmental time from six (2006: AA, FX, TL, TRCA, SVS, SVN) or 10 (2007: DANHOUSE, AA, FX, TL, BURNS, BCPCAMP, AIRBOAT, SVS, SVN, CASINO; Fig. 2) populations. In 2006, five of six populations were surveyed every 1–2 d from 24 March to the 4 April and approximately weekly from 10 May to 30 June. The sixth population (SVS) was only sampled during the weekly surveys (May–June). At each location, twenty plots (2 by 5 m) were arrayed linearly. On each sampling date, all individuals were collected from 15 randomly chosen plots/site. We could not sample all 20 plots each time because the number of animals obtained was too large to process in 24 h. Animals from different plots were placed in separate containers and transported to the field laboratory for data collection. After data collection (see below), animals were fed Romaine lettuce ad libitum; held overnight at the field laboratory; and the following day, returned to the center of the plot from which they were collected. In 2007, we omitted plot sampling, and instead sampled 10 populations each week during March and April 2007 by searching 0.5 h per site and collecting all *R. microptera* observed.

In both years, digital photographs (Cannon PowerShot A610) were used to measure pronotum length (millimeters) and femur length (millimeters) by converting the number of pixels to millimeters by using a ruler placed in each photograph (Adobe Photoshop version 6.0, Adobe Systems, Mountain View, CA). In addition, to pronotum and femur lengths, we recorded sex, mass (Jennings JPrecision Digital Pocket 0.002 g scale), and the number of individuals per instar.

Statistical Analyses. To test for a cline in hatching times, we used analysis of variance (ANOVA) (PROC GLM, SAS Institute, Cary, NC), with number of days from oviposition to hatching as the dependent variable and site as the independent variable. To test for a cline in linear hatchling size, we conducted a multivariate analysis of covariance (MANCOVA) (SAS PROC GLM), with mean hatchling pronotum and femur lengths (averaged among offspring from a single clutch) as the dependent variables, location as the independent, and the dam's pronotum and femur lengths as the covariates. To test for a cline in hatchling mass, we used an ANOVA, with the mean hatchling fresh mass (milligrams; averaged among offspring from a single clutch) as the dependent and location as the independent variables. In all three analyses, the 2 yr were run separately because days to hatching and hatchling size (linear and mass) were significantly different between the 2 yr within sites (ANOVAs $P \leq 0.001$).

To obtain information on developmental progress over time at each site, we applied the Kiritani–Nakasuji–Manly Method (KNM; Manly 1990) to the cumulative frequency counts of instars at each site-date to estimate the duration from hatching to the fourth instar. The KNM method assumes: survival rate does not change over time; sampling starts at or before the beginning of the first stage (instar 1) and continues until all individuals leave the last stage (through stage change or death); and losses from the population via emigration are negligible (Manly 1990). We tested the constant survival rate assumption by performing an

Table 1. Mean (least squares, ± 1SE) hatchling pronotum and femur lengths (millimeters), mass (milligrams), and time to hatching (days from oviposition to first hatchling) for three (2006) or seven (2007) populations of lubber grasshoppers in south Florida (see text for methods)

Yr	Site	Pronotum length (mm, ±1 SE)	Femur length (mm, ±1 SE)	Mass (mg, ±1 SE)	Time to hatching (d ±1 SE)
2006	AA	2.94 (0.16)	5.17 (0.26)	7.89 (0.29)	183.4 (5.0)
2006	TL	2.92 (0.15)	5.23 (0.23)	7.51 (0.22)	180.3 (5.7)
2006	SVS	2.50 (0.19)	4.67 (0.30	7.03 (0.51)	197.5 (5.0)
2007	DHP	2.64 (0.12)	5.19 (0.17)	5.40 (0.40)	212.3 (14.1)
2007	FX	2.76 (0.16)	5.17 (0.22)	N.A.	210.5 (17.3)
2007	AA	2.39 (0.06)	4.74 (0.08)	5.46 (0.22)	260.2 (7.1)
2007	TL	2.20 (0.04)	4.46 (0.06)	5.38 (0.20)	273.2 (5.0)
2007	BURNS	2.55 (0.15)	4.88 (0.20)	4.86 (0.44)	207.0 (17.3)
2007	SVS	2.47 (0.04)	4.74 (0.06)	5.24 (0.14)	N.A.
2007	CAS	2.62 (0.08)	5.08 (0.11)	5.98 (0.29)	206.7 (9.3)

N.A., data not available.

analysis of covariance (ANCOVA) on the natural log-transformed total count frequency of instars 2–5 (2006) or 2–4 (2007) as a function of time (Julian day) and location (PROC GLM in SAS). A significant negative linear relationship would indicate that survival rate is constant over time (Manly 1990). Count frequency declined linearly with time for both sexes in both years (all $P < 0.001$), indicating that nymphal survival rate is constant over time. We met the sampling assumption because sampling began as first instars appeared and continued until most populations were in the fifth instar (2007) or adult stage (2006). Emigration seems to be negligible because these grasshoppers are flightless and rarely disperse more than ≈100 m (J.E.J., unpublished data).

We estimated average individual growth rate as the mean $\log_{10}$ mass fourth instar/mean duration from hatching to fourth instar for females from both years and from males in 2007. This approach assumes no interpopulation differences in hatchling size (see below). Mean duration from hatching to fourth instar was estimated by KNM (above). We only obtained growth rate from males in 2006 from a single population; therefore, we conducted two separate analyses: female growth rate as a function of year, degrees longitude and interaction; and male growth rate in 2007 as a function of degrees longitude.

To test for when during ontogeny the size cline becomes apparent, we used one ANCOVA for each instar (PROC GLM in SAS) with the mean pronotum length of each instar (instars 2–5) as the dependent variable, sex as the independent variable, and degrees longitude east as the covariate. Degrees longitude east was quantified as the differences between the longitudes of each of the sites and that of our westernmost site (DANHOUSE, Fig. 2). To test for a cline in developmental time, we used ANCOVA to examine how the mean time from hatching to fourth instar (dependent variable), was influenced by sex, year (independent variables), and degrees longitude east (covariate). To test for a cline in growth rates ($\log_{10}$ mass/developmental duration), we used ANCOVA with growth rate as the dependent variable, sex and year as the independent variables, and degrees longitude east as the covariate. For each of these analyses, we tested for class variable (sex and year) interactions with degrees east in each case.

For all analyses, residual and normal quantile plots were inspected to check for homogeneity of variances and normality. All analyses were conducted with SAS version 9.1 on Windows XP platform (SAS Institute).

Results

Adult Lubber Grasshopper Size Cline. Consistent with the pattern documented by Huizenga et al. (2008), the average size of adult grasshoppers increases from west to east along our longitudinal transect in both years (see Supplemental Material).

Hatching Time and Size. There was no significant difference in hatching times among the three populations tested in 2006 (ANOVA $F_{2,8} = 3.16$, $P = 0.10$; Table 1). There was a significant difference in hatching times among the 2007 populations; however, the hatching times did not show the pattern predicted if hatching times contributed to the west-to-east cline in adult size. Hatch was not later in the west, rather hatching times were greatest at the central sites (ANOVA $F_{6,65} = 12.97$, $P < 0.0001$; Table 1). Mean pronotum and femur lengths of hatchlings did not differ among the three field sites in 2006 (MANCOVA with female pronotum and femur lengths as covariates, site effect: $F_{4,12} = 0.59$, $P = 0.79$; Table 1). Pronotum and femur lengths of hatchlings did significantly differ among the seven field sites in 2007; however, as with hatching times, the differences in pronotum and femur lengths did not predict the west-east cline in adult size (MANCOVA with female pronotum and femur lengths as covariate, site effect: $F_{12,132} = 3.66$, $P < 0.0001$; Table 1). Hatchling mass did not differ among sites in 2006 (ANOVA $F_{2,10} = 1.20$, $P = 0.35$; Table 1) or in 2007 (ANOVA $F_{5,42} = 1.42$, $P = 0.23$; Table 1).

Instar Size. There is an ontogenetic component to the size cline. Whereas there is no consistent interpopulation variation in the size of first (see above) or second instars (Table 2; female pronotum length adjusted mean ± 1 SE = 4.15 ± 0.04 mm, male adjusted mean ± 1 SE = 4.21 ± 0.04 mm), the sizes of both third and fourth instars increased significantly from west to

Table 2. ANCOVA results for pronotum length of each nymphal instar as a function of sex, degrees longitude, and the sex × degrees interaction

Instar	Source	df	MS	*F*	*P*	Intercept	Slope
II	Sex	1,324	0.0035	1.32	0.25		
	Degrees	1,324	0.0025	0.94	0.33		
III	Sex	1,382	0.00819669	5.3	0.02		
	Degrees	1,382	0.0333	21.5	<0.0001	6.19 (1.01)	1.09 (1.02)
IV	Sex	1,186	0.0518	50.45	<0.0001		
	Degrees	1,186	0.02429894	23.65	<0.0001	9.56 (1.01)	1.10 (1.02)
V	Sex	1,186	0.0389	19.32	<0.0001		
	Degrees	1,186	0.00003	0.01	0.91	♀15.45 (1.03)	♀1.07 (1.07)
	Sex × degrees	1,186	0.0095	4.72	0.03	♂13.67 (1.02)	♂0.93 (1.05)

Intercepts (1 SE) and slopes (1 SE) are for the regression of pronotum length on degrees longitude (instars III and IV) or pronotum length on degrees by sex (instar V).

east (Fig. 3a and b; Table 2). Pronotum length of third and fourth instars also exhibited female-biased sexual dimorphism (Table 2; mean ± 1 SE, third instars: females = 6.50 ± 0.04 mm, males = 6.36 ± 0.04 mm; fourth instars: females = 10.66 ± 0.08 mm, males = 9.87 ± 0.08 mm), but no sex–longitude interaction (Table 2). The size of fifth instars yielded a significant sex-by-degrees longitude interaction. Both female and male fifth instar pronotum length increased from west to east; however, female pronotum length increased faster than male pronotum length along the cline (Fig. 3c and d; Table 2).

Developmental Time. Estimated time from hatching to fourth instar increased from west to east, but this relationship does not depend on sex, year, or interactions (Fig. 4; ANCOVA degrees longitude east: $F_{1,20} = 15.79$, $P = 0.001$; sex: $F_{1,20} = 0.00$, $P = 0.96$; year: $F_{1,20} = 0.04$, $P = 0.84$; all interactions $P \geq 0.50$). The west-to-east increase in the average first-fourth instar duration remains significant even when the site with the longest estimated duration (=SVN ≈54 d; see Fig. 4) is removed (ANCOVA degrees longitude east: $F_{1,17} = 10.02$, $P = 0.01$; sex: $F_{1,17} = 1.04$, $P = 0.32$; year: $F_{1,17} = 2.03$, $P = 0.17$; all interactions $P \geq 0.50$).

Growth Rate. Female growth rate increased from west to east (degrees longitude $F_{1,8} = 10.99$, $P = 0.01$; Fig. 5) but year ($F_{1,8} = 0.70$, $P = 0.43$) and the year–longitude interaction ($F_{1,8} = 0.00$; $P = 0.98$) were not significant. Male growth rate in 2007 did not vary among sites (degrees longitude: $F_{1,5} = 0.03$, $P = 0.88$; Fig. 5).

Discussion

Our study is among the first to test alternative hypotheses for ontogenetic mechanisms that could result

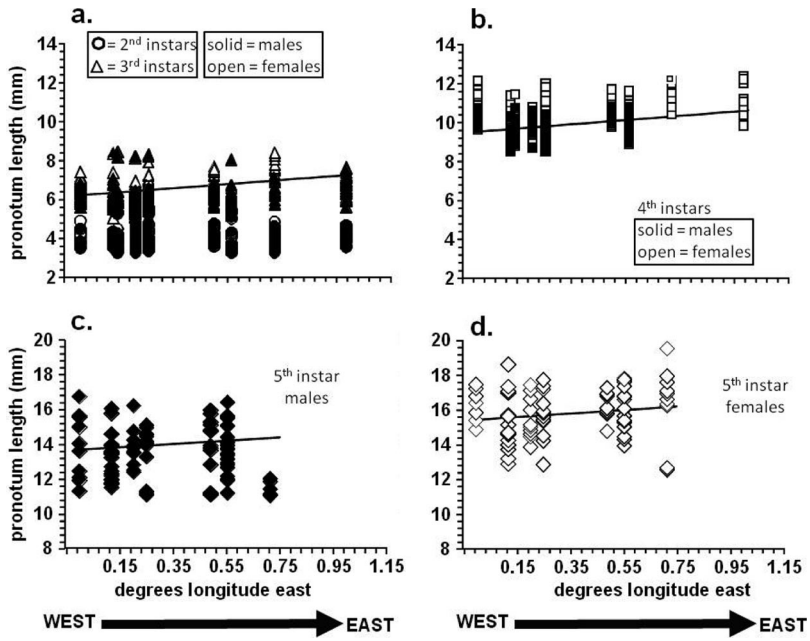

Fig. 3. Pronotum length as a function of degrees longitude east for (a) male (solid symbols) and female (open symbols) instars 2 (circles) and 3 (triangles), line is for instar 3 sexes combined; (b) male (solid symbols) and female (open symbols) instar 4, line for sexes combined; (c) male instar 5; and (d) females instar 5 (data from 2007 only).

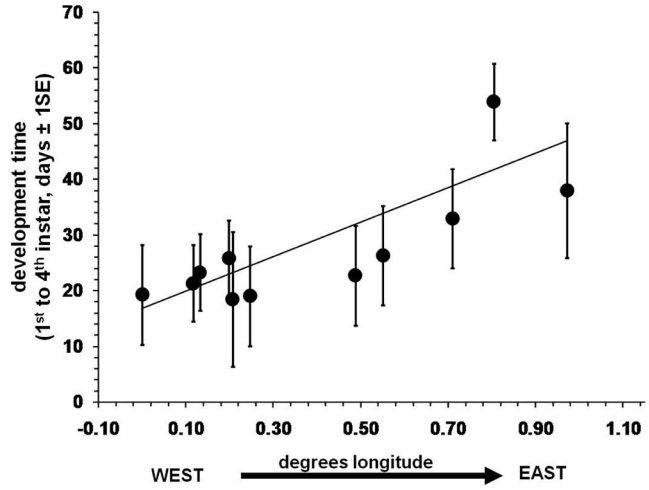

Fig. 4. Duration (days ± 1SE) of from first to fourth instar along the longitudinal gradient from west to east. Points represent means across years and sexes (intercept ± 1 SE = 16.86 ± 4.77, slope ± 1 SE = 30.95 ± 7.79).

in a geographic cline in adult size. The longitudinal size cline occurs in the third instar and is amplified in the fourth and fifth instars. We have demonstrated that the cline is associated with a combination of clinal variation in the developmental time from first to fourth instar and female (but not male) growth rates. Equally important, we found no evidence that differences in hatching time or size are associated with this cline. Our hypotheses for ontogenetic mechanisms producing an adult size cline should serve as a template for biogeographers and ecologists seeking to understand the ontogenetic basis of spatial variation in phenotypes, which can in turn indicate the sources and targets of selection acting on phenotypes to produce clinal variation. Below, we discuss the potential ecological mechanisms that could produce spatial variation in each ontogenetic mechanism.

Ontogenetic Mechanism: Hatching Time and Size. Hatching time and size either did not vary among grasshopper populations (2006) or varied in patterns that would not contribute to the adult size cline (2007). Therefore, hatching time and size are unlikely to be the root cause of the adult size cline. Unlike developmental time and growth rate, variation in hatching size per se (as opposed to egg size) has rarely been tested as a proximate mechanism underlying geographic size clines (but see Sinervo and Huey 1990, Ims 1997, Telfer and Hassall 1999. Under investigation

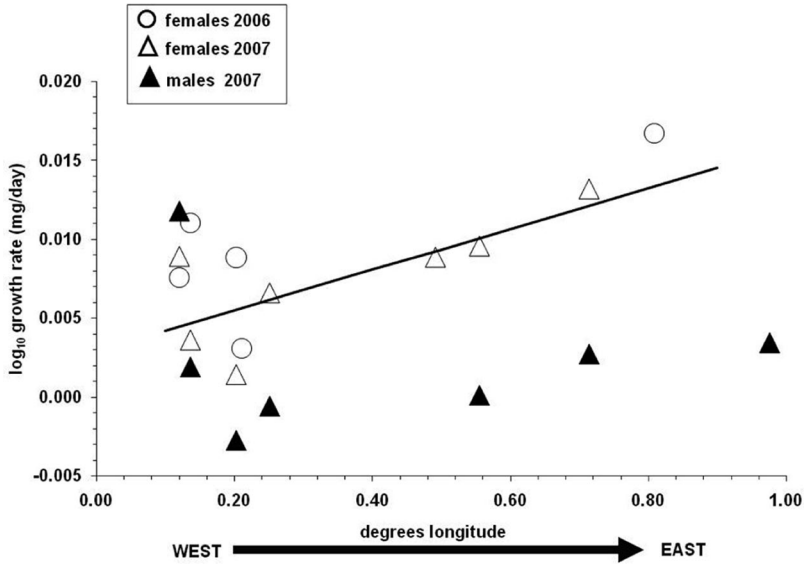

Fig. 5. Female (open symbols) and male (solid) growth rate (log_{10} mass fourth instar/duration instars 1–4) along the longitudinal gradient from west to east. Line represents female data from both years combined (intercept ± 1 SE = 0.0029 ± 0.0023, slope ± 1 SE = 0.013 ± 0.006).

of hatching size as a mechanism could be because of neglect or the "file drawer problem"—underreporting nonsignificant results (Csada and James 1996). Of the few published studies that explicitly tested hatching size as a ontogenetic mechanism, two demonstrated hatching size as a plausible mechanism for interpopulation size variation (Sinervo and Huey 1990, Ims 1997). Our study joins the one other study (Wright et al. 2004) in finding no evidence that interpopulation differences in hatching size are associated with clinal variation in adult size. Another study on grasshoppers actually demonstrates a negative correlation between hatching size and adult body size (Telfer and Hassall 1999).

Ontogenetic Mechanism: Developmental Time. Time from hatching to fourth instar increases from west to east, paralleling observed cline in adult body size; suggesting that larger size is attained via a longer juvenile growth period during these instars. As with growth rates (see below), interpopulation differences in developmental time could be a result of plasticity or local adaptation. Differences in grasshopper developmental time are often a response to seasonal time constraints (Sibly and Monk 1987, Berner et al. 2004). Longer developmental times in grasshoppers can be correlated with an additional developmental stage (Grant et al. 1993, Telfer and Hassall 1999, Berner and Blanckenhorn 2006), although we found no evidence for this in our analysis (see Supplemental Material). Differences in developmental time might be proximally induced by differences in temperature or photoperiod (Grant et al. 1993, Telfer and Hassall 1999, Berner and Blanckenhorn 2006), but for our populations, photoperiod differences among our field sites seem unlikely because of limited latitudinal variation among our sites (Fig. 2). Eastern populations have both greater growth rate and longer developmental time compared with western populations. The combination of prolonged development and greater growth seems to be inconsistent with the hypothesis that current differences among sites in food quantity or quality are the only environmental inputs causing these differences. Low food typically produces both prolonged development and reduced growth in many insects, including *R. microptera* (Flanagin et al. 2000). Thus, we would expect prolonged development to be associated with low growth rate and small size if current differences in nutrition were the proximate cause of the observed cline in size.

Ontogenetic Mechanism: Growth Rate. Individual growth rate of females increases from west to east and therefore might contribute to the observed adult size cline. Interpopulation variation in individual growth rates has been demonstrated for other grasshopper species (Telfer and Hassall 1999, Fielding and Defoliart 2007). However, our results are in contrast with at least one other study that demonstrated that the individual growth rates of high altitude grasshoppers did not vary among populations (Berner and Blanckenhorn 2006).

Observed differences in growth rates among our south Florida populations could arise via effects of environmental differences in ecological time (plasticity) or evolutionary time (local adaptation), and we cannot distinguish between plasticity and local adaptation at this time. There are, however, two environmental factors that could affect growth rate in ecological and evolutionary time and merit future investigation: nutritional conditions and enemies. Growth rates could vary because of varying nutritional conditions, such as low quality or quantity of food or lack of specific nutrients in the diet. Results from other grasshoppers suggest that plant chemistry and poor nutritional content can reduce individual growth rates (Asshoff and Hättenschwiler 2005, Berner et al. 2005, Hahn 2005) but that generalist feeders such as *R. microptera* show behavioral and physiological flexibility that can compensate for environmental nutritional constraints (Raubenheimer and Simpson 2003, Berner et al. 2005, Fielding and Defoliart 2007). Surveys of plant species composition and density at our field sites (AA, FX, TL, TRCA, BL, SVS, SVN; Fig. 2) suggest that plant diversity, host plant density, and the species of plants consumed by adult lubbers differ among sites (K.K., unpublished data). Whether identity or quality of food plants differentially influences juvenile growth rates of grasshoppers at different sites remains to be tested. Both intra- and interspecific competition can reduce individual growth rates of phytophagous insects (Kaplan and Denno 2007), although the evidence for intra- and interspecific competition in grasshoppers and other phytophagous insects is equivocal (Evans 1992, Ritchie and Tilman 1992, Belovsky and Slade 1995, Chase 1996, Liu et al. 2007) and models show that effect of competition on grasshopper life history traits is influenced by the spatial distribution of resources (Fielding 2004).

Prey growth rates can be influenced by predation, either as a plastic response during development or as an evolved response over longer time scales (Benard 2004). In either case, predators tend to reduce foraging behavior, and therefore, growth rates, of prey (Benard 2004). Nymphs and adults of *R. microptera* are chemically defended against most vertebrate predators (Whitman et al. 1991) so that interpopulation differences in vertebrate predation pressure are unlikely to cause growth rate variation. Invertebrate predators (e.g., spiders, assassin bugs, carabid beetles) have been observed preying on nymphs at our field sites (J.E.J., unpublished data). In another grasshopper, risk of spider predation reduced growth rates of individuals by 3–5% (Danner and Joern 2003). Thus, invertebrate predation pressure could contribute to lower growth rates of individuals in western relative to eastern populations if there is spatial variation in the distribution of invertebrate predators.

In conclusion, understanding the ontogenetic mechanisms producing clines can be useful because it can narrow the field of tenable hypotheses about ultimate mechanisms producing phenotypic variation. We have formalized predictions about the ontogenetic mechanisms that could produce clinal variation in adult body size and tested those predictions for our model system. We find that differences in growth rate

and developmental time are the most likely ontogenetic mechanisms producing body size variation along this cline. Understanding the origins of geographic patterns of size variation will only be possible by connecting the pattern with the underlying ontogenetic mechanisms.

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