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Density-dependent polyphenism and geographic variation in size among two populations of lubber grasshoppers (*Romalea microptera*)

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Abstract. 1. Density-dependent phase polyphenism occurs when changes in density during the juvenile stages result in a developmental shift from one phenotype to another. Density-dependent phase polyphenism is common among locusts (Orthoptera: Acrididae).

2. Previously, we demonstrated a longitudinal geographic cline in adult body size (western populations = small adults; eastern populations = large adults) in the eastern lubber grasshopper (*Romalea microptera*) in south Florida. As lubbers are confamilial with locusts, we hypothesised that the longitudinal size cline was partly due to density-dependent phase polyphenism.

3. We tested the effect of density, population, and density × population interaction on life-history traits (pronotum length, mass, cumulative development time, growth rate) of, and proportion surviving to, each of the five instars and the adult stage in a 2 × 3 factorial laboratory experiment with two lubber populations, each reared from hatchling to adult at three different densities.

4. The effect of density on life history and survival was independent of the effects of population on life history and survival. Higher densities led to larger adult sizes (pronotum, mass) and lower survivorship. The western population had smaller adult masses, fewer cumulative days to the adult stage, and higher survivorship than the eastern population.

5. Our data suggest that lubber grasshoppers exhibit density-dependent phase polyphenism initiated by the physical presence of conspecifics. However, the plastic response of adult size to density observed in the laboratory is not consistent with the relationship between phenotypes and adult density in the field. Genetic differences between populations observed in the laboratory could contribute to size and life-history differences among lubber populations in the field.

Key words. Acrididae, development, growth, life history, size, survival.

Introduction

Much of life-history theory is directed at understanding how and why life-history variation arises and evolves (Roff, 1992; Stearns, 1992; Abrams *et al*., 1996). One source of life-history variation is phenotypic plasticity. Most models predict that animals should exhibit phenotypic plasticity in response to variable environments–that is, their physiology, morphology, and behaviour should change in response to environmental conditions. Phenotypic plasticity is particularly common during the early stages of development, when tissues and organs are in the process of differentiation (West-Eberhard, 1989; Schlichting & Pigliucci, 1998; Pigliucci, 2001).

Both abiotic and biotic factors in the environment can select for different forms of phenotypic plasticity. One biotic

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variable that can induce phenotypic plasticity is population density (Gilbert, 2001). For example, plasticity in response to crowding occurs in many insects, including species in the orders Coleoptera, Hemiptera, Homoptera, Lepidoptera, and Orthoptera, and results in the phenomenon of density-dependent phase polyphenism (Applebaum & Heifetz, 1999; Gilbert, 2001; West-Eberhard, 2003; Braendle *et al*., 2006; Simpson & Sword, 2008). Density-dependent phase polyphenism occurs when differences in density during the juvenile stages produces a developmental shift from one phenotype to another (Peters & Barbosa, 1977; Applebaum & Heifetz, 1999; Gilbert, 2001). Density-dependent behavioural polyphenism can also occur in adult locusts (Simpson & Sword, 2009).

The best studied examples of density-dependent phase polyphenism occur among locusts and grasshoppers (Orthoptera: Acrididae). In locusts and other grasshoppers, high densities of nymphs lead to increased physical contact with conspecifics, which in turn, induces polyphenism in physiology, development, morphology, reproduction, and behaviour of the nymphs and adults. High juvenile densities lead to the *gregarious* form characterised by: large hatchlings, high metabolism, greater feeding rate, few instars, short juvenile development period, high juvenile growth rates, a tendency to aggregate and small adults, relative to the *solitarious* form (Kennedy, 1961; Uvarov, 1966; West-Eberhard, 2003; Simpson & Sword, 2008). Not only are these traits expressed in the grasshopper family Acrididae (e.g. *Locusta* and *Schistocerca* species) (Kennedy, 1961; Simpson & Sword, 2008), but similar traits are also expressed in response to density in other orthopteran species (West-Eberhard, 2003). Mosaic individuals with both *solitarious* and *gregarious* traits are frequently observed because: (1) each trait in the complex is independent; (2) the response to density is a graded response; (3) the response is mediated by environmental variables independent of density (e.g. temperature, humidity); and (4) the effects can be transmitted maternally and are not fully expressed for several generations. Therefore, depending on conditions, the traits expressed by any individual are usually some combination of the two extreme morphs (Kennedy, 1961; Uvarov, 1966; Pener & Yerushalmi, 1998; West-Eberhard, 2003; Lester *et al*., 2005; Simpson & Sword, 2008).

Eastern lubber grasshoppers (*Romalea microptera*, Orthoptera: Acrididae) are univoltine, flightless, gregarious grasshoppers found throughout the southeastern United States. Lubber populations in south Florida exhibit a geographic cline in adult body size and densities from west (small adults, high adult densities) to east (large adults, low adult densities) (Huizenga *et al*., 2008; Jannot *et al*., 2009). Eastern lubber grasshoppers are considered to be relatively gregarious (Whitman, 1988, 1990; Hatle *et al*., 2002b). We hypothesised that the observed geographic cline in body size is due, in part, to density-dependent phase polyphenism, as is observed in other species of Acrididae (see above). We test this hypothesis by examining the life-history effects of density on two populations of lubber grasshoppers from south Florida. Field studies of *R. microptera* suggest that adult densities are highest in the west relative to the east (J. E. Jannot, unpublished data). Current knowledge of phase-dependent life-history differences in traits such as development time, growth rate, and size-at-maturity in insects is based on results that vary considerably among and within species (Kennedy, 1956, 1961; Uvarov, 1966; Antoniou & Robinson, 1974; Peters & Barbosa, 1977; Heifetz & Applebaum, 1995; Pener & Yerushalmi, 1998; Applebaum & Heifetz, 1999; Bouaichi & Simpson, 2003; Maeno & Tanaka, 2008). We predicted that at high rearing densities in the nymphal stages, lubbers would have short development times, high growth rates, and small adult sizes, compared to low densities (Peters & Barbosa, 1977; Applebaum & Heifetz, 1999). Alternatively, if the geographic cline is a result of local adaptation and therefore, not a product of plasticity, then we predict that individuals from a western site (small adult size) should exhibit shorter development times, higher growth rates, and smaller adult sizes than individuals from an eastern site (large adult size). These patterns would be independent of rearing density. We tested these predictions in a laboratory experiment using the offspring of wild-caught females from two south Florida lubber populations. We also measured the proportion surviving to each stage to determine the effects of density on mortality. We predicted that high densities should have negative effects on individuals; therefore, the proportion surviving to each stage should decrease as densities increase.

Methods

Adult females were obtained from two geographically distinct populations of *R. microptera* in south Florida (western site = located at 26.12°N, 81.34°W; eastern site = located at 25.76°N, 80.77°W; driving distance between sites = 88.84 km; see Jannot *et al*. 2009 for map). The two populations differ in average adult body size with adult females from the western population ~30% shorter pronotum length than females from the eastern population (Huizenga *et al*., 2008; Jannot *et al*., 2009). Populations also differ in density with adult densities at the western site higher than at the eastern site (J. Jannot, unpublished data). Females from both populations were placed on individual egg cups (~946 ml plastic cups with ~12 cm sand at ~5–10% moisture) and fed romaine lettuce *ad libitum* until oviposition. Each female was allowed to oviposit only once. Egg cups were covered and placed in an incubator (LD 14:10 h; thermal cycle 32°C:28°C). The hatchlings from these eggs were reared singly in individual plastic containers (~946 ml plastic cups thermal and light cycles as above, with *ad libitum* romaine lettuce) to the adult stage and propagated in the laboratory. Within each population, males and females were randomly paired, allowed to copulate, and then females were placed on egg cups until oviposition. Each female oviposited only once.

To minimise parental effects, hatchlings from the laboratory-reared generation (i.e. first generation reared in the same laboratory environment) were used in the density experiment. As grasshoppers hatched, individuals from separate clutches of the same population were combined into a single container (to mix clutches) and then randomly assigned to one of three population densities: two, four, or eight individuals per cage, resulting in nymphal densities (m^{-2}) of 14, 27, or 55, respectively. Prior to cage assignment, we measured the pronotum

length (mm) and mass (mg) of each first instar (= hatchling). Each hatchling was digitally photographed to measure pronotum length using a scale in the photograph. The experiment was a 3 density × 2 population factorial, with four replicates of each density-population combination. Individuals were only exposed to other individuals from the same population (i.e. western and eastern were not mixed). Groups were raised in identical plastic storage bins (~0.26 m × 0.30 m × 0.49 m; bottom area = 0.147 m^2) with a wire mesh top to allow for airflow. To provide ample vertical climbing space, two 6 mm wooden dowels were glued diagonally, from bottom to top, in opposite directions inside the cage. The inside walls of each cage were scoured with sandpaper to provide footholds for climbing. Heat lamps were placed above the cages so that each cage provided similar thermoregulatory opportunities. Once a day, each replicate cage received a mixture of romaine lettuce and dried oats in the following manner: 300% × average fifth instar mass × number of animals per container (2 animals = 0.9 g oats + 9.0 g lettuce per day; 4 animals = 1.8 g oats + 18.0 g lettuce per day; 8 animals = 3.6 g oats + 36.0 g lettuce per day). This level of feeding was maintained for the entire experiment and there was no sign that food levels ever became limiting, because excess food remained in the cages at the end of each day, even after some animals moulted to the adult stage. Every time an individual moulted, it was weighed (nearest 0.01 mg) and measured for pronotum length (digital calipers for instars II–V and adults). Each individual was marked with a non-toxic, white paint marker so that moulted and non-moulted individuals within a single cage could be distinguished from each other. The experiment ended when all the individuals in a cage moulted to the adult stage.

During the experiment, we obtained measures of size at each moulting (pronotum length, mass), number of cumulative days to the moult (i.e. from start of experiment to the moult to instar x). As individuals were not followed, we calculated the mean size and the mean cumulative days to the moult for each instar for all individuals in a single container. Instars were determined based on previous studies of animals in the field (Jannot *et al*., 2009). Growth rate for each instar, for each container, was determined as: ($\log_{10}$ $mass_{x+1}$ − $\log_{10}$ $mass_x$)/days from x to $x + 1$. Growth rate to the adult stage was calculated for each container as: ($\log_{10}$ $mass_{adult}$ − $\log_{10}$ $mass_{1st\ instar}$)/days from hatching to adult moult. We calculated the proportion surviving for each instar, for each container, as the number of animals alive at instar x/number of animals initially placed into the container (i.e. either 2, 4, or 8).

Box–Cox transformation (SAS© proc transformation) indicated that none of the size variables (pronotum length, mass) needed to be transformed. However, Box–Cox indicated that cumulative time to each stage needed to be $\log_{10}$ transformed and proportion surviving to each stage needed to be transformed by squaring.

We used five separate MANOVAs (SAS© proc GLM), one for each instar (II to adult) to determine the effect of density, population, and the density × population interaction (independent variables) on size-at (pronotum length, mass), cumulative time to ($\log_{10}$ transformed), growth rate of, and proportion surviving to (square transformed) instar x. Means for each container were used in the analyses. All analyses were conducted with SAS© v. 9.1 on Windows XP platform (Copyright © 2002–2003 SAS Institute Inc., Cary, NC, U.S.A.).

Results

For all traits, there were no significant density-by-population interactions (Table 1; Figs 1 and 2; Supporting Information Figures S1–S5). Therefore, we present only our analyses of the main effects of density and population.

Density effects

The size, life history, and survival of all five stages were affected by density (Table 1; Figs 1 and 2; Supporting Information Figures S1–S5). Pronotum length (stages III to A) made

Table 1. MANOVA results for pronotum length, cumulative time to the stage, mass, growth rate, and proportion surviving for each stage (instars II–V and A = adult) as a function of density, population, and the density-by-population interaction.

Stage	Source	Pillali's trace	*F*	d.f.	*P*
II	Density	1.05	3.51	10, 28	0.01
	Population	0.32	1.25	5, 13	0.34
	Density × population	0.71	1.54	10, 28	0.18
III	Density	0.93	2.45	10, 28	0.03
	Population	0.54	3.01	5, 13	0.05
	Density × population	0.69	1.49	10, 28	0.20
IV	Density	1.08	3.29	10, 28	0.006
	Population	0.75	7.85	5, 13	0.001
	Density × population	0.78	1.77	10, 28	0.11
V	Density	1.00	2.80	10, 28	0.02
	Population	0.46	2.22	5, 13	0.11
	Density × population	0.59	1.16	10, 28	0.35
A	Density	1.07	3.24	10, 28	0.01
	Population	0.73	6.89	5, 13	0.002
	Density × population	0.76	1.71	10, 28	0.13

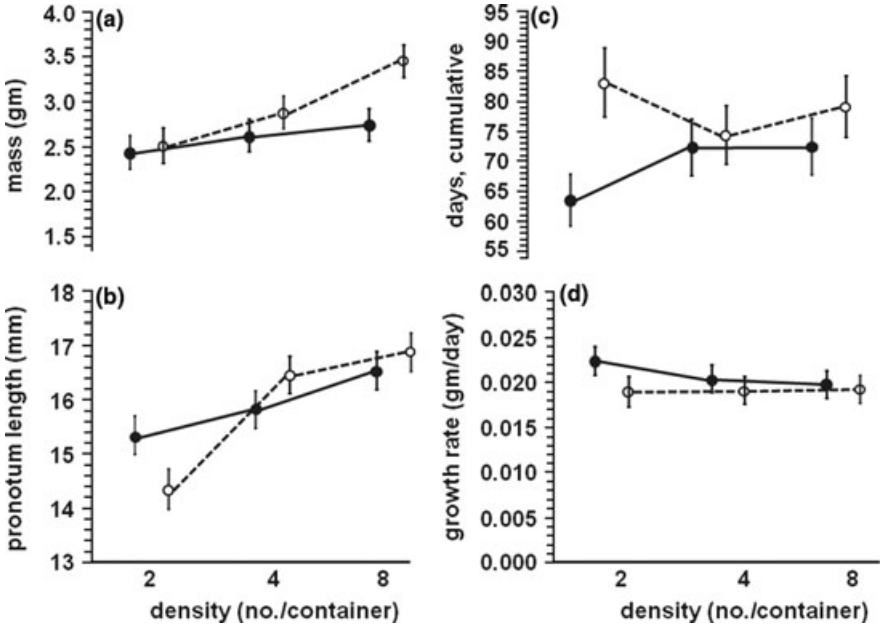

Fig. 1. Adult (least-square means ± 1 SE) (a) mass, (b) pronotum length, (c) cumulative days to the adult stage, and (d) growth rate as a function of density (number of animals per container) for each population (●, western population; ○, eastern population). MANOVA indicated that density × population interactions were not significant.

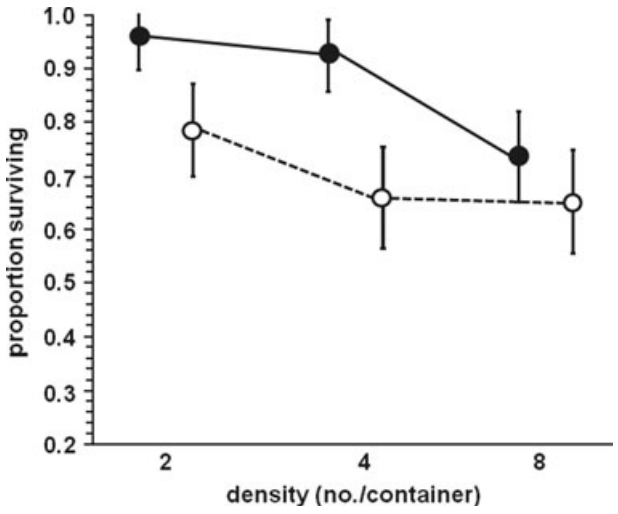

Fig. 2. Adult proportion surviving (least-square means ± 1 SE) as a function of density (number of animals per container) for each population (●, western population; ○, eastern population). MANOVA indicated that density × population interactions were not significant.

the largest contribution to the density effect and was negatively correlated with proportion surviving (SCC1, Table 2). Cumulative days to the adult stage and adult growth rate also made a major contribution to the density effect on adults, independent of adult pronotum length (SSC2, Table 2). In the adult stage, as density increased, mass, pronotum length, and cumulative days increased (Fig. 1a–c), growth rate was unaffected (Fig. 1d), and proportion surviving declined (Fig. 2). These patterns were generally consistent throughout development (Supporting Information Figures S1–S5).

Population effects

The size, life history and survival of instars III and IV and the adult stage differed between populations (Table 1; Figs 1 and 2; Supporting Information Figures S1–S5). Pronotum length accounted for a large portion of the variation among populations in instars III and IV and was negatively correlated with mass during these stages (SCC1, Table 2). In the adult stage, cumulative days and growth rate accounted for a large portion of the variation among populations and were negatively correlated with proportion surviving (SCC1, Table 2). Proportion surviving was lower (Fig. 2) and development time (cumulative days) was longer (Fig. 1c) in the eastern population compared to the western population. Adult mass was greater in the eastern population (Fig. 1a), whereas adult pronotum length and growth rate did not differ between the two populations (Fig. 1b,d). Throughout juvenile development, individuals from the western population had longer pronotums than individuals from the eastern population (Supporting Information Figure S2); however, because eastern had a longer development time than western (Fig. 1c), the two populations ended up with similar adult pronotum lengths (Fig. 1b). The population difference in adult mass appeared mainly in the fifth instars (Supporting Information Figure S1d), but was likely due to differences in development time (Fig. 1c) as growth rate did not differ between populations during development (Fig. 1d; Supporting Information Figure S4). The western population had a higher proportion surviving throughout development relative to the eastern population (Fig. 2; Supporting Information Figure S5).

Discussion

Our data provide evidence that density-dependent phase polyphenism could contribute to body size variation in lubber

Table 2. Standardised canonical coefficients (SCC) for each stage (instars II–V, A = adults) for the MANOVAs in Table 1.

		SCC1					SCC2				
Stage	Source	Pronotum	Mass	Days	Growth rate	Proportion surviving	Pronotum	Mass	Days	Growth rate	Proportion surviving
II	Density	0.35	0.99	0.55	0.34	−0.86	0.85	0.62	−0.10	−0.32	0.66
	Population	0.83	0.31	−0.21	0.29	0.32	–	–	–	–	–
	Density × population	0.40	1.34	−0.31	−0.07	−0.36	0.63	0.22	1.03	0.37	−0.25
III	Density	1.74	−0.23	0.15	−1.07	−0.57	−1.12	1.44	0.76	0.90	−0.23
	Population	1.75	−0.72	0.55	1.25	−0.04	–	–	–	–	–
	Density × population	−1.86	0.68	0.12	1.28	0.89	−0.40	0.77	1.16	0.50	−0.24
IV	Density	1.58	0.09	0.85	0.83	−0.10	−1.95	1.66	0.95	2.30	−0.59
	Population	1.98	−1.62	−0.28	−0.54	0.78	–	–	–	–	–
	Density × population	1.07	−0.44	0.39	1.35	0.43	−0.14	0.19	−0.92	−0.35	0.68
V	Density	1.25	1.02	0.87	0.28	−0.43	−1.44	1.32	0.02	0.94	−0.41
	Population	−0.52	1.37	0.71	0.80	−0.57	–	–	–	–	–
	Density × population	1.31	0.30	−0.06	−0.43	−0.07	−0.18	1.17	0.24	1.33	0.55
A	Density	2.18	−0.26	0.46	−0.34	−0.41	−1.06	1.35	3.00	2.96	−0.94
	Population	0.44	1.16	2.94	2.50	−1.05	–	–	–	–	–
	Density × population	−0.96	0.38	3.82	3.49	−0.06	−0.49	1.50	−0.39	−0.09	0.27

grasshoppers. However, phase polyphenism does not appear to explain the size gradient observed among our south Florida populations, because the direction of the plastic response in size does not match the pattern of adult density variation in nature. Therefore, it seems unlikely that density-dependent phase polyphenism contributes to the size cline among our south Florida populations. At high densities, we predicted short development times, high growth rates, and small adult sizes, compared to low densities. Surprisingly, we found the opposite pattern: at high densities, development times were slightly longer leading to larger sizes at maturity, whereas growth rate was not consistently associated with size at maturity. As we predicted, we found that individuals from the western population exhibited shorter development times and smaller adult masses than individuals from the eastern population. These results are consistent with our field studies, which also demonstrated shorter development times and lower growth rates in western relative to eastern populations (Jannot *et al*., 2009). They suggest that the differences observed in the field are features of the populations, rather than products of different field environments. Our data suggest that mass-at and time-to maturity are related to both density and population independently, suggesting both plasticity and genetics could influence adult lubber sizes. However, cumulative juvenile development time appears to be the result of fixed differences among populations and likely results in the adult body size cline we observe at our field sites.

Nonetheless, we did observe density-dependent phase polyphenism in response to nymphal densities during our experiment. One alternative to the density-dependent phase polyphenism hypothesis for the results in this experiment is 'selective death' of small individuals at high densities. The selective death hypothesis postulates that small individuals at high densities have a higher mortality rate than small individuals at low densities and large individuals at high densities. Proportion surviving declined with increasing densities, suggesting that selective death could be important. However, several other lines of evidence indicate that selective death is not a likely explanation for the observed size–density pattern. First, food was never limiting–at the end of each day there was always ample food left over within every cage, therefore starvation of small individuals due to interference competition seems unlikely. Second, selective death at high densities predicts two very specific, but opposite, statistical patterns. (1) The coefficient of variation for adult size within cages should be large at low densities and become smaller in high density, because small animals at high density would die, increasing the mean and shrinking the variance in size in the high density containers. There was no significant difference in the coefficients of variation for adult pronotum length or mass among density treatments (MANOVA $f_{4,34} = 0.71,\ P = 0.59$). (2) There should be *no difference* in size among the largest individuals in each replicate cage for each treatment. Again, we find no support for this pattern, because the size of the largest individuals within cages increased significantly with density ($F_{4,34} = 3.07$, $P = 0.03$; Supporting Information Table S1). A final important piece of evidence is that development time, not growth rate or survival, was important for establishing the observed density effect. This suggests that the observed differences among densities are a true developmental shift from solitary to gregarious form, as opposed to a change in growth rate or survival. All the data taken as a whole indicate that the pattern of size, growth, and development that we observed in the experiment is likely density-dependent phase polyphenism.

The evolutionary history of density-dependent polyphenism in grasshoppers and other orthopterans supports our claim. Current evidence suggests that phase polyphenism has evolved multiple times and predicts that the physiological basis for phase change was present relatively early in the evolution of locusts (Song, 2005, 2008). Evidence from non-grasshopper orthopterans (for a summary, see p. 300 in West-Eberhard, 2003) suggests that some elements of density-dependent phase

polyphenism might have been in place even before evolutionary divergence of grasshoppers from other orthopterans. If the phylogenetic hypothesis is correct and the origin of phase polyphenism occurred early in the evolutionary history of Orthoptera, then it is no surprise that we observed elements of density-dependent plasticity in the lubber grasshoppers, which are close relatives of locusts.

Density-dependent phase change in locusts is thought to be an adaptation for coping with environmental conditions experienced under high population densities. For example, migratory behaviour increases under food limitation (Ellis, 1953), which is more likely at high densities and is sometimes associated with gregariousness (but not always; see Simpson & Sword, 2009). Interestingly, we have observed large migrations of lubber grasshoppers nymphs, similar to those observed in locusts (Uvarov, 1977; Simpson & Sword, 2008), at our western field site (Supporting Information Video S1) and migrations of lubber grasshopper nymphs have been recorded at other locations in Florida in the past (Watson & Bratley, 1939, 1940; Watson, 1941). While not definitive evidence for density-dependent gregarisation of lubber nymphs in the field, these migrations do suggest that environmental conditions such as local food limitation might occur and could lead to high densities of lubber nymphs in the field. For locusts, the interaction between population density and spatial distribution of food determines the proportion of gregarious animals in the population. As population density increases and host plants become more spatially clumped, chemical, visual, and mechanical contact between grasshoppers increases (Collett *et al*., 1998; Despland *et al*., 2000), presumably altering endocrine regulation so that animals switch from the solitary to the gregarious form (Breuer *et al*., 2003), leading to a higher proportion of gregarious animals in the population (Collett *et al*., 1998; Despland *et al*., 2000). Food was never limiting in our study, suggesting that one of the cues for the developmental switch is the physical presence of competitors (chemical, visual, and mechanical cues) which is consistent with cues used by other insects (Roessingh *et al*., 1998; Rogers *et al*., 2003; Lester *et al*., 2005; Lihoreau & Rivault, 2008). We currently do not have information on the role of food limitation, host-plant spatial distribution, or endocrine regulation in producing a phase change in lubbers. Other adaptive hypotheses predict that phase polyphenism in response to population density reduces the risks of predation (Simpson *et al*., 2005) or disease (Wilson *et al*., 2002). The role of predators and parasites in producing phase polyphenism in lubbers remains to be investigated. Comparing density-dependent phase change in lubbers to locusts will provide a clearer understanding of the ecological and evolutionary significance of density-dependent phase polyphenism.

While density and other environmental conditions might play a significant role in determining phase expression, our data suggest that genetic differences between the two populations are important for determining survival and life-history outcomes and likely results in the size cline observed at our field sites. Results of a recent study suggest that phase change is initiated by density conditions (plastic), but its expression can be genetically modified for different levels among different populations (Chapuis *et al*., 2008). However, in our laboratory study, phase change induced by changes in density were independent of population differences in size and life-history traits. For example, adult pronotum length exhibits a plastic response to density with little or no genetic component, whereas development time has a large genetic component with little or no plastic response to density. Thus, both plastic and genetic components are likely to play important, but independent, roles in the adult phenotypes of lubber grasshoppers. However, it is likely that only genetic differences among populations (e.g. development time) determine the size cline observed in the wild.

In conclusion, our data provide evidence that (1) lubber grasshoppers exhibit density-dependent phase polyphenism initiated by the physical presence of conspecifics; (2) phase polyphenism in response to density is not a likely explanation for observed inter-population geographic variation in adult size; and (3) genetic differences between populations contribute to life-history variation of lubbers which results in a cline in adult size among our south Florida populations. Lubber grasshoppers have proved to be a useful model organism for studying physiology, development, anatomy, life history, and ecology (Hatle *et al*., 2001, 2002a,b, 2004; Juliano *et al*., 2004; Mutun & Borst, 2004; Fei *et al*., 2005; Li *et al*., 2005; Mefferd *et al*., 2005; Vincent, 2006; Homeny & Juliano, 2007; Jannot *et al*., 2009). Our data suggests that researchers should coordinate efforts to use lubbers as a model for density-dependent phase polyphenism and provide a much-needed comparison with locusts.

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