Research

Geographic variation in body size, sexual size dimorphism and fitness components of a seed beetle: local adaptation versus phenotypic plasticity

R. Craig Stillwell and Charles W. Fox

R. C. Stillwell (rcstill@email.arizona.edu), Dept of Ecology and Evolutionary Biology, Univ. of Arizona, BioSciences West 310, Tucson, AZ 85721-0088, USA. – C. W. Fox, Dept of Entomology, Univ. of Kentucky, S225 Agricultural Science Center North, Lexington, KY 40546-0091, USA.

Variation in body size, growth and life history traits of ectotherms along latitudinal and altitudinal clines is generally assumed to represent adaptation to local environmental conditions, especially adaptation to temperature. However, the degree to which variation along these clines is due to adaptation vs plasticity remains poorly understood. In addition, geographic patterns often differ between females and males – e.g. sexual dimorphism varies along latitudinal clines, but the extent to which these sex differences are due to genetic differences between sexes vs sex differences in plasticity is poorly understood. We use common garden experiments (beetles reared at 24, 30 and 36°C) to quantify the relative contribution of genetically-based differentiation among populations vs phenotypic plasticity to variation in body size and other traits among six populations of the seed-feeding beetle *Stator limbatus* collected from various altitudes in Arizona, USA. We found that temperature induces substantial plasticity in survivorship, body size and female lifetime fecundity, indicating that developmental temperature significantly affects growth and life history traits of *S. limbatus*. We also detected genetic differences among populations for body size and fecundity, and genetic differences among populations in thermal reaction norms, but the altitude of origin (and hence mean temperature) does not appear to explain these genetic differences. This and other recent studies suggest that temperature is not the major environmental factor that generates geographic variation in traits of this species. In addition, though there was no overall difference in plasticity of body size between males and females (when averaged across populations), we did find that the degree to which dimorphism changed with temperature varied among populations. Consequently, future studies should be extremely cautious when using only a few study populations to examine environmental effects on sexual dimorphism.

Growth and life history traits of animals often vary geographically. For example, body size of many animals, both endotherms and ectotherms, frequently varies with latitude and altitude (Bergmann 1847, Partridge and Coyne 1997, Blanckenhorn et al. 2006, Stillwell et al. 2007a). However, the underlying factors producing these broad-scale patterns are still poorly understood. Latitudinal and altitudinal gradients in temperature are thought to generate many observed altitudinal and latitudinal clines. Organisms can respond to variation in temperature through the direct effect of temperature on phenotypes (phenotypic plasticity), or through long-term adaptation to temperature (and thus evolution of either the mean phenotype or the thermal reaction norm). Disentangling the degree to which phenotypic variation is due to genetic adaptation vs plasticity is central to understanding how temperature generates geographic variation and clines in animals (Angilletta and Dunham 2003, Stillwell and Fox 2005, Karl and Fischer 2008).

Studies of geographic variation in body size and other traits demonstrate that much of this variation is commonly genetically based. For example, animals often show clines in body size and egg size (Chown and Klok 2003, Ashton 2004, Blanckenhorn and Demont 2004, Karl et al. 2008) that persist after rearing in a common garden experiment (Partridge and Coyne 1997, Gilchrist and Partridge 1999, Armbruster et al. 2001), indicating that they are genetically-based. Latitudinal clines are also congruent across continents for some cosmopolitan species (Coyne and Beecham 1987, Capy et al. 1993, Imasheva et al. 1994, James et al. 1995, Van't Land et al. 1995) and evolve relatively fast upon introduction to new continents (Huey et al. 2000), suggesting that natural selection on these traits likely varies substantially with latitude. Temperature is commonly proposed to be the environmental variable generating variation in selection along the cline. Laboratory studies of fruit flies support this hypothesis. For example, body size evolves directly in response to temperature (in laboratory natural selection experiments) in directions consistent with latitudinal clines that are observed in nature (Anderson 1966, 1973, Cavicchi et al. 1985, 1989, Partridge et al. 1994). However, other studies have failed to detect evidence

of temperature-mediated selection consistent with observed clinal variation (Stillwell et al. 2007a, 2008).

Plastic responses of body size and other traits to developmental temperature are also common, and frequently parallel geographic patterns in traits. For example, survivorship, development time, body size and egg size tend to increase with decreasing developmental temperature (Atkinson 1994, Fox and Czesak 2000, Angilletta and Dunham 2003, Angilletta et al. 2004, Kozlowski et al. 2004, Kingsolver and Huey 2008), while growth rate and fecundity tend to decrease with decreasing temperature (Atkinson and Sibly 1997, Ernsting and Isaaks 2000, Stillwell and Fox 2005). Because evolution at different latitudes/temperatures creates patterns that are nearly identical to those created by development at different temperatures, it can be difficult to distinguish genetic adaptation vs phenotypic plasticity in natural populations. Furthermore, populations can even vary in the degree or direction of plasticity they exhibit in response to temperature as a result of adaptation to different thermal environments, generating variation among populations in thermal reaction norms (Morin et al. 1999, Gilchrist and Huey 2004, Stillwell and Fox 2005, Kingsolver et al. 2007).

Though many studies focus on how temperature generates variation in overall body size, most studies ignore sexual size dimorphism and differences in plasticity between the sexes. Most organisms exhibit some degree of sexual size dimorphism both among species and among populations within species (Teder and Tammaru 2005, Blanckenhorn et al. 2006). For example, recent studies have shown that geographic variation in size can differ between males and females, creating latitudinal and altitudinal clines in sexual size dimorphism (Blanckenhorn et al. 2006, Bidau and Martí 2007, Howes and Lougheed 2007, Stillwell et al. 2007a). This suggests that temperature or some other climatic/ecological factor that varies with latitude or altitude is differentially affecting selection on the sexes. Likewise, recent studies have shown that males and females can respond differently to developmental temperature (differences in plasticity of size between the sexes), creating variation in size dimorphism (differential-plasticity hypothesis; Fairbairn 2005). Both adaptive and proximate hypotheses have been proposed to explain sex differences in plasticity of body size (Fairbairn 2005, Teder and Tammaru 2005, Stillwell and Fox 2007), but how the sexes achieve such diverging growth trajectories even though they share the same genome remains unknown (Badyaev 2002, Rhen 2007). Although recent studies have found that the sexes often do differ in plasticity of body size (Fairbairn 2005, Fernández-Montraveta and Moya-Laraño 2007, Stillwell and Fox 2007; but see Delph and Bell 2008), few studies have specifically explored whether sex differences in plasticity vary among populations.

Here we use the seed-feeding beetle *Stator limbatus* (Coleoptera: Chrysomelidae: Bruchinae) as a model system to examine the relative contribution of adaptation vs temperature-mediated plasticity in generating geographic variation in body size and sexual size dimorphism. Body size of *S. limbatus* varies geographically, increasing with latitude and thus following Bergmann's rule (Stillwell et al. 2007a). Similarly, body size of *S. limbatus* decreases with increasing developmental temperature following the temperature-size

rule observed in most ectotherms (Stillwell and Fox 2005, Kingsolver and Huey 2008). Plastic responses to temperature vary among populations, and one study comparing only two populations suggested that populations are adapted to different temperatures; e.g. the lower altitude population performed better (grew larger and matured faster) than the high altitude population when reared at higher temperature, whereas the higher altitude population performed better when reared at low temperature (i.e. they exhibited population differences in thermal reaction norms consistent with predictions based on adaptation to temperature) (Stillwell and Fox 2005). Sexual size dimorphism also varies geographically in *S. limbatus*. Males are generally larger than females, a pattern that is opposite to the general female-biased dimorphism observed in most species of insects (Fairbairn 1997, Esperk et al. 2007, Székely 2007), but the magnitude of this dimorphism decreases with increasing latitude (Stillwell et al. 2007a). In a related species of seed beetle, *Callosobruchus maculatus*, temperature-induced plasticity of body size differs between the sexes, creating temperature-induced variation in size dimorphism (Stillwell and Fox 2007). However, no previous study has explored whether size dimorphism varies with temperature for *S. limbatus*.

Using a common garden experiment at three temperatures we compare thermal reaction norms for six populations from Arizona, USA, spanning 810 m in altitude and ∼ 6°C in mean annual temperature, to (a) quantify the relative contribution of genetically-based differentiation vs plasticity in generating variation in body size, and (b) test whether norms of reaction vary among populations. Also, we test the differential-plasticity hypothesis to see if geographic variation in sexual size dimorphism can be created by sex differences in response to temperature.

Methods

Natural history of *Stator limbatus*

Stator limbatus is a generalist seed parasite of legumes in the dry tropical forests of South and Central America and in the deserts of Mexico and the southwestern United States (Johnson and Kingsolver 1976, Johnson et al. 1989, Nilsson and Johnson 1993). *S. limbatus* has been collected from >70 species of primarily mimosoid or caesalpinioid legumes throughout its wide geographic range, but only one or a few hosts are encountered in most locations. For example, *S. limbatus* primarily uses *Acacia greggii* (Fabaceae: Mimosoideae), *Parkinsonia florida* (Fabaceae: Caesalpinioideae; previously *Cercidium floridum*) and *P. microphylla* (Fabaceae: Caesalpinioideae) as hosts in central Arizona, USA.

The life cycle of *S. limbatus* revolves around seeds. Females oviposit directly onto host seeds inside of fruits that have either dehisced or been damaged by other organisms (e.g. mice, other bruchine seed beetles such as *Mimosestes* spp., etc.). Eggs hatch and larvae burrow into the seed directly underneath the egg. Larval growth and pupation take place entirely within a single seed; larvae cannot move among seeds. This allows us to control larval density and eliminate larval interactions (including competition), which

Table 1. Populations of *Stator limbatus* collected from various locations throughout Arizona, USA. Average annual temperatures were obtained from weather stations located nearest to each collection locality (National Climatic Data Center, Asheville, NC, USA). Female and male mass (mean ± 1 SEM) for each population was calculated by averaging across all temperature treatments.

Population	Host	Latitude/ Longitude	Female mass (mg)	Male mass (mg)	Altitude (m)	Annual mean temperature (°C)	Location
Wenden	*Acacia greggii*	33°81′N/ 113°56′W	3.09 ± 0.04	3.20 ± 0.04	562	21.5	La Paz County, Arizona, USA
Apache	*Acacia greggii*	33°53′N/ 111°3′W	3.20 ± 0.04	3.36 ± 0.04	641	21.9	Maricopa County, Arizona, USA
Tucson	*Parkinsonia florida*	32°40′N/ 110°96′W	3.25 ± 0.04	3.40 ± 0.04	787	19.6	Pima County, Arizona, USA
Verde	*Acacia greggii*	34°56′N/ 111°8′W	3.13 ± 0.05	3.33 ± 0.05	945	16.3	Yavapai County, Arizona, USA
Kingman	*Acacia greggii*	35°40′N/ 113°75′W	3.14 ± 0.04	3.32 ± 0.04	1088	16.4	Mohave County, Arizona, USA
Oracle	*Acacia greggii*	32°61′N/ 110°77′W	3.16 ± 0.04	3.40 ± 0.04	1372	16.7	Pinal Country, Arizona, USA

affects body size (Amarillo-Suárez 2006). Upon emergence from the seed, adults mate and females begin to lay eggs within ∼ 24–48 h in the laboratory.

Stator limbatus, like many species of seed beetles that have evolved to use dry seeds in dry climates, is facultatively aphagous. They need only the resources inside of a single seed to complete development and reproduce (i.e. they are capital breeders). Additional food and water are not necessary. Adult feeding can increase the lifespan of adult seed beetles, but adult feeding has only a small positive effect on female fecundity (Fox 1993, Fox and Dingle 1994, Tatar and Carey 1995).

Study populations

We used six *S. limbatus* populations collected from different geographic localities in Arizona, USA. These populations spanned 562 to 1372 m in altitude and 16–22°C in mean annual temperature (Table 1). Mature fruits were collected from several trees at each location and were shipped back to the lab where seeds bearing eggs were placed individually in 35-mm petri dishes. More than 100 emerging adult beetles of each population were used to initiate each colony. All colonies were maintained on *A. greggii* seeds at ∼ 100 families for one generation (at 28°C, 15:9 L:D) prior to the experiment. Larvae were reared at a density of 1 larva per seed, at 28°C, 15:9 L:D. Egg-to-adult survivorship is >90% on seeds of *A. greggii* (Fox et al. 1994), minimizing the influence of natural selection (including adaptation to the laboratory).

Experimental protocol

Overview of the experiment

We performed a common garden experiment at three temperatures (24, 30 and 36°C) to examine temperature-induced plasticity, genetic differences among populations and genetic (population level) variation in temperature-induced plasticity for egg-to-adult survivorship, body size, sexual size dimorphism and female lifetime fecundity. Full-sib families of each population were raised for two generations at each of three temperatures (n = 21 to 52 for each temperature × population combination; total = 685 full-sib families) to ensure that we removed any environmentally-based parental effects. Data were collected on the second generation only. The temperatures we used are within the range of temperatures normally encountered in the field (within central and southern Arizona, daily temperatures range from 14°C to 39°C during late summer and early fall when beetles are most active; <http://www.ncdc.noaa.gov>; National Climatic Data Center, Asheville, NC, USA).

Both generations were raised in 35-mm petri dishes, on seeds of *A. greggii*, inside temperature controlled Percival growth chambers. The developing larvae were rotated daily through growth chambers to minimize the effects of spatial variation in temperature.

Details

To initiate the experiment, we randomly paired emerging colony males and females within each population and placed the mated pairs in 35-mm petri dishes with ∼ 10 *A. greggii* seeds. ∼ Three seeds per female were scraped to one egg per seed to eliminate larval competition. These eggs and resulting offspring were generation A. Seeds bearing eggs from each full-sib family were randomly and evenly divided (split-brood design) across the three temperature treatments to ensure that each genotype was present at all temperatures. Offspring were raised to adult individually in 35-mm petri dishes.

Emerging beetles from generation A were collected every 2–3 days. Virgin males and females were randomly paired within each temperature-by-population combination and placed in 35-mm petri dishes containing 10 seeds of *A. greggii*. Dishes were checked every 2–3 days for the presence of eggs. These eggs and resulting offspring are generation B, and are the individuals on which we collected the data presented here. Seeds bearing eggs were scraped to one egg per seed. This was repeated every 2–3 days until females had laid eggs on ∼ 8–10 seeds, at which point the adults were discarded. Offspring were raised to adult individually in 35-mm petri dishes.

Emerging beetles from generation B were collected daily and weighed on an electronic balance to the nearest 0.1 mg. To quantify fecundity, four individuals (randomly selected at the egg stage by marking their dish) from each family were paired with individuals of the opposite sex from the

same temperature-by-population combination. These pairs were placed in 60-mm petri dishes with 40 *A. greggii* seeds and allowed to lay eggs until death.

Overall, 5218 offspring from 685 full-sib families were raised to adult and weighed. Lifetime fecundity data were scored for 1106 female offspring from 588 full-sib families.

Analyses

Sexual size dimorphism (SSD) was estimated for each full-sib family of each population at each temperature using the Lovich and Gibbons (1992) index, in which SSD = (size of the larger sex/size of the smaller sex) − 1, made positive when females are the larger sex and negative when males are the larger sex. This index has the best statistical properties of all dimorphism indices that have been proposed (Lovich and Gibbons 1992, Smith 1999). We used this index for analyses of sexual dimorphism, instead of testing for sex-by-environment interactions in ANOVA, because interactions in ANOVA are biased by scale effects and would thus be misleading in the presence of large temperature effects on body size (Dobson and Wigginton 1996, Blanckenhorn et al. 2006).

Statistical analyses were done with SAS 9.1 using ANOVA (type III sums of squares). We used family means as our lowest level of independence. All traits except egg-to-adult survivorship were approximately normally distributed and had equal variances among temperatures (including the sexual size dimorphism index). Egg-to-adult survivorship (mean per family) was arcsine-square root transformed to meet as best as possible the assumptions of ANOVA. For our analysis we used mixed model ANOVA, in which temperature and sex were treated as fixed main effects and population (and all interactions involving population) was treated as a random effect. ANOVAs were first performed using the full model with all possible interaction terms present; the non-significant three-way term (population-by-temperature-by-sex) was dropped from the final model in the analysis of body size. If the population effect was significant in the full analysis of variance, we performed a second analysis (analysis of covariance with altitude as a covariate), using population means as our lowest level of independence, to test whether traits varied with altitude. The analysis examining population effects and the analysis exploring the altitude effects are two independent analyses and thus do not confound population and altitude effects.

The main focus of our study is on the interactions involving temperature. However, interactions between factors in an ANOVA measure changes in the linear difference between treatment means and are thus dependent on scale (Stanton and Thiede 2005, Stillwell et al. 2007b). When one variable (e.g. temperature) has a large effect on the overall means, linear differences do not correspond to proportional changes; e.g. a 10% difference in mean size at low temperature is a smaller proportion of total body size than the same difference in size at higher temperature. Because temperature had a large effect on two traits in our study, body size and female lifetime fecundity (p < 0.0001 for both traits), we performed our analysis as a two-step process. First, we examined main effects (effects of temperature, population and, where relevant, sex) using

Table 2. Analysis of variance (type III sums of squares) for the effects of rearing temperature and population on egg-to-adult survivorship, body mass, sexual size dimorphism and female lifetime fecundity of the seed beetle *Stator limbatus*. †Interactions for body mass and fecundity are from analyses on relative trait values (individual value/ mean for each temperature treatment).

	DF	F	p
Egg-to-adult survivorship			
Temperature	2	4.01	0.05
Population	5	1.35	0.32
Temperature × Population	10	2.57	0.005
Error	695		
Body mass			
Temperature	2	465	<0.0001
Population	5	4.51	0.04
Sex	1	89.0	0.0002
Temperature × Population †	10	2.12	0.02
Temperature × Sex †	2	1.30	0.27
Population × Sex †	5	1.17	0.32
Error	1344		
Sexual size dimorphism			
Temperature	2	1.26	0.32
Population	5	0.46	0.80
Temperature × Population	10	2.77	0.002
Error	650		
Lifetime fecundity			
Temperature	2	167	<0.0001
Population	5	3.44	0.05
Temperature × Population †	10	3.41	0.0002
Error	570		

ANOVA on family means. Then, to test for interactions between temperature and population, we created relative trait values following Stanton and Thiede (2005); we divided each individual trait value by the overall mean (averaged across populations and sexes) within each temperature treatment, removing the large effect of temperature (Stanton and Thiede 2005, Stillwell et al. 2007b). This method is preferable to using log transformation to remove scale effects because log transformation can make biological interpretation difficult (Grissom 2000, Stanton and Thiede 2005).

Results

Population, altitude and temperature main effects

Because temperature can strongly influence growth and life history traits of ectothermic organisms, we expected temperature to affect all traits in this study. We observed this for most traits (Table 2). Overall, there was a marginal effect of temperature on egg-to-adult survivorship; survivorship was greatest at low (24°C) and intermediate temperature (30°C; pairwise comparison (linear contrast) between 24°C and 30°C: $F_{1,695} = 0.62$, p = 0.43) and lowest at the highest temperature (36°C; temperature effect: p = 0.05; Fig. 1). Body size of both males and females increased with decreasing temperature (p < 0.0001; Fig. 2) consistent with the general pattern in ectothermic animals (Atkinson 1994). Males were substantially larger than females (sex effect: p = 0.0002; Fig. 3), as is typical in this species (Fox et al. 2007), but averaged across all populations there was no effect of temperature on sexual size dimorphism (p = 0.32; Fig. 2, 3) (but see interaction effects, below).
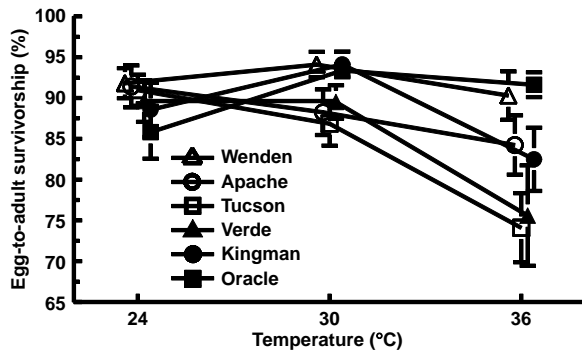
Figure 1. Egg-to-adult survivorship of populations of *Stator limbatus* raised at three different temperatures (24, 30, 36°C). Standard errors (± 1 SE) are included, but are smaller than the symbols for some experimental treatments.

Lifetime fecundity of females decreased monotonically with increasing rearing temperature (p < 0.0001; Fig. 4). The temperature effect on fecundity was not entirely due to the decrease in female body size with increasing temperature; although large females laid more eggs than small females (ANCOVA: $F_{1,568} = 219$, p < 0.0001), and females mated to large males laid more eggs (ANCOVA: $F_{1,568} = 131$, p < 0.0001), the effect of rearing temperature on fecundity remained highly significant after removing the effects of male and female body size (ANCOVA: $F_{2,31} = 198$, p < 0.0001). Interestingly, when removing the effect of size (both females and males), females laid more eggs when reared at intermediate temperature (least square

means for 24°C = 26.2 eggs; 30°C = 31.1 eggs; 36°C = 23.2 eggs).

We observed no differences among the six study populations for egg-to-adult survivorship or sexual size dimorphism (Table 2), but there were differences among the populations in body size and fecundity (body size: p = 0.04; Fig. 2; fecundity: p = 0.05; Fig. 4). There was no effect of a population's collection altitude on most traits (ANCOVA: $F \leq 2.89$, $p \geq 0.11$ for all traits excluding body size). However, collection altitude significantly affected body size (ANCOVA: $F_{1,26} = 7.34$, p = 0.01), though, when analyzed separately for the two sexes this relationship was significant only for male size ($F_{1,12} = 6.62$, p = 0.02; males from the highest elevation were largest) and not for female size ($F_{1,12} = 1.27$, p = 0.28) (Table 1). This difference in significance between males and females in the relationship between altitude and body size did not translate into a detectable relationship between altitude and sexual dimorphism ($F_{1,12} = 2.89$, p = 0.11).

Population-by-temperature and altitude-by-temperature interactions

The response to temperature differed considerably among populations for all traits (p ≤ 0.02 for all traits; Table 2), indicating genetic differentiation among populations in thermal reaction norms. However, there was no genetic differentiation in thermal reaction norms among altitudes for mortality, body size or fecundity (altitude-by-temperature interaction: $F \leq 1.60$, $p \geq 0.22$ for all three traits), indicting the populations did not respond to the predicted directions based on their origin of altitude (i.e. the highest altitude populations were not the largest at low temperature while simultaneously the smallest at high temperature).

Although there was no overall variation in the degree of sexual size dimorphism among populations (averaged across populations; see above), the populations responded very differently to temperature (p = 0.002). The most dramatic example of this was exhibited by the Wenden population; Wenden was the most dimorphic population when reared at 30°C, but was the least dimorphic when reared at 24°C and 36°C (Fig. 3). This different response to temperature was because the degree of dimorphism changed with temperature in some populations (Wenden: $F_{2,110} = 5.32$, p = 0.006; Tucson: $F_{2,123} = 3.00$, p = 0.05; Oracle: $F_{2,133} = 5.84$, p = 0.004) while others showed no change with temperature (Apache: $F_{2,114} = 1.59$, p = 0.21; Verde: $F_{2,67} = 1.07$, p = 0.35; Kingman: $F_{2,103} = 0.17$, p = 0.85). The variation among populations in the temperature effect on dimorphism was also due to a large population effect at high temperature ($F_{5,193} = 2.94$, p = 0.01; $F \leq 1.83$, $p \geq 0.11$ for 24°C and 30°C); Oracle exhibited the largest degree of dimorphism while Tucson exhibited the smallest (Fig. 3). Though there was no average change in dimorphism with altitude, there was a nearly significant altitude-by-temperature interaction ($F_{1,12} = 3.71$, p = 0.06); the lower altitude populations were less dimorphic at 24°C and more dimorphic at 30°C, whereas the higher altitude populations were less dimorphic at 30°C and more dimorphic at 36°C (Fig. 3).
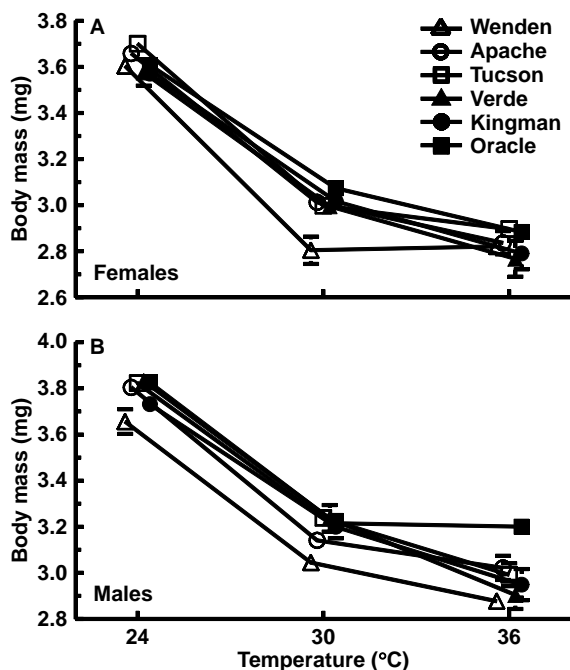

Figure 2. Adult body mass of females (A) and males (B) of populations of *Stator limbatus* raised at three different temperatures (24, 30, 36°C). Standard errors (± 1 SE) are included, but are smaller than the symbols for some experimental treatments.
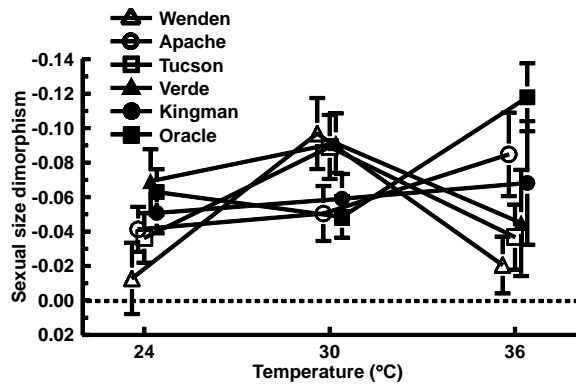
Figure 3. Sexual size dimorphism of populations of *Stator limbatus* raised at three different temperatures (24, 30, 36°C). Sexual dimorphism was estimated for each full-sib family of each population at each temperature as (mean size of the larger sex (mg)/ mean size of the smaller sex (mg)) − 1, made negative when males were the larger sex and positive when females were the larger sex. Note that all estimates were negative (the dashed line indicates no dimorphism) indicating males were always larger than females. Standard errors (±1 SE) are included, but are smaller than the symbols for some experimental treatments.

Discussion

Organisms can respond to variation in temperature through the direct effect of temperature on phenotypes (phenotypic plasticity) or through evolution of mean size and/or the thermal reaction norm. We observed, as expected, substantial temperature-induced plasticity for most of the traits we examined in *Stator limbatus* (egg-to-adult survivorship, body size and fecundity). In addition, we detected genetic differences among populations for body size and female lifetime fecundity, and we detected variation among populations in thermal reaction norms for all traits. There was little evidence for altitudinal clines in most traits for these beetles, but body size did vary (slightly but significantly) with altitude for males (beetles were larger-bodied when collected at higher elevations) but not females. In addition, the shape of the thermal reaction norms did not correspond to predictions based on adaptation to temperature – we predicted that higher altitude populations would mature larger at low temperature (compared to lower

altitude populations) and lower altitude populations to mature larger at high temperature, but this was not observed. We also found that the sex difference in plasticity of body size varied substantially among populations; the degree to which temperature affected dimorphism varied considerably among our study populations.

Adaptation to temperature in *S. limbatus*

Much of the evidence for adaptation to temperature comes from studies that sample across a range of latitudes and altitudes. In this study we collected six populations of *S. limbatus* from an 810 m range in altitude (6°C range in mean annual temperature; Table 1). Despite this small range in altitude and temperature, we detected genetic differences among the six populations for body size; beetles collected from the lowest and highest altitudes were the smallest and largest, respectively. However, when examining the sexes separately, this altitudinal cline was significant only in males and even then the effect was small. Our results suggest that some environmental variable along the altitudinal cline affects selection on body size, but that the effect is small. However, our study cannot test whether temperature variation along the cline generates the selection that produced the observed pattern in body size. It is more likely that other variables contribute to selection producing geographic variation in body size. For example, the latitudinal cline in body size of *S. limbatus* is best explained by clinal variation in host plant seed size, moisture and seasonality, and the latitudinal cline in sexual dimorphism in *S. limbatus* is best explained by variation in moisture (Stillwell et al. 2007a). These same variables (e.g. moisture) could likewise change with altitude, but studies exploring the impact of these other variables on selection and plasticity are sorely lacking.

Fecundity varied among populations but not in a manner predicted from a latitudinal cline in selection on body size and egg size; our two highest altitude populations, Oracle and Kingman, had the highest and lowest mean fecundity, respectively. This pattern is difficult to interpret. Ectotherms have been shown to evolve larger eggs in cooler environments (Armbruster et al. 2001), which may be affected by tradeoffs with fecundity (Smith and Fretwell 1974). However, we did not measure egg size in this study. Furthermore, one of our study populations (Tucson) was collected from a different host plant than the other populations, which possibly confounds our interpretation of population differences because populations of *S. limbatus* are known to evolve egg size differences due to adaptation to their host plants (Fox and Mousseau 1996, Fox et al. 2001). However, deleting this population from our analysis does not produce a cleaner relationship between fecundity and altitude. Further research is needed to determine whether fecundity or egg size in *S. limbatus* can evolve due to climate.

Population differentiation in thermal reaction norms is expected to evolve when organisms experience temporal and spatial variation in temperature. Recent studies have shown population differentiation in thermal reaction norms for body size (Morin et al. 1999, Gilchrist and Huey 2004, Stillwell and Fox 2005, Kingsolver et al. 2007) and other

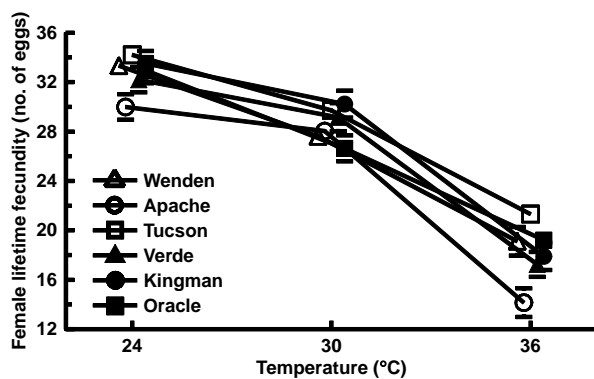Figure 4. Female lifetime fecundity of populations of *Stator limbatus* raised at three different temperatures (24, 30, 36°C). Standard errors (±1 SE) are included, but are smaller than the symbols for some experimental treatments.

traits (Norry et al. 2001, Bochdanovits and de Jong 2003). For example, in the cabbage white butterfly, *Pieris rapae*, body size increases with increasing rearing temperature in a population from North Carolina, USA, whereas body size decreases with increasing temperature in a population from Washington, USA (Kingsolver et al. 2007). These reaction norms evolve different slopes because the North Carolina population experiences strong selection for body size under warm conditions, whereas the Washington population experiences strong selection for size under cooler conditions. Similarly, in a previous study on *S. limbatus* (Stillwell and Fox 2005) we found that a higher altitude population matured sooner and was larger when raised at low temperature, while the lower altitude population matured sooner and was larger when raised at high temperature, suggesting these populations have adapted to their native climates. Results of this study do not support that adaptive interpretation of our previous *S. limbatus* results. Although we found population differentiation in thermal reaction norms (i.e. population-by-temperature interactions) for body size and other traits in this current study, the populations did not respond in the predicted directions based on their native climates (i.e. we did not detect altitude-by-temperature interactions) in contrast to our previous study (Stillwell and Fox 2005, which compared only two populations). The inconsistent results between our studies suggest that the number of populations included in a study can have major implications for understanding the evolution of thermal reaction norms. As a result, future studies should be cautious when making conclusions about the evolution of thermal reaction norms from results with only one or only a couple populations of a species.

Why did our six study populations not exhibit reaction norms consistent with predictions based on adaptation to the temperatures experienced at their collection altitude? Two possible explanations are: (1) the temperatures we used in our study are too high. The temperatures we chose were based on the temperatures beetles are likely to experience in the field during the time when beetles are most likely to be active in Arizona, USA (Methods). However, beetles are likely stressed and unable to develop through the hottest periods of the day, and may even be stressed at the average temperatures experienced at a site, especially at low altitudes. Had we used fluctuating temperatures that include periods of lower stress, our results might better reflect experiences which occur in nature (Kingsolver et al. 2007). (2) Even considering the caveat of whether our high non-fluctuating temperatures represent stressful conditions for beetles, our results likely suggest that, although temperature may partially contribute to clines in body size, it is not the major climatic variable driving the evolution of clines in these beetles. A previous study (Stillwell et al. 2007a) found that the latitudinal cline in body size of *S. limbatus* is best explained by clinal variation in host plant seed size, moisture and seasonality (beetle body size increases with increasing host seed size, decreasing moisture and increasing seasonality). This and other recent studies on *S. limbatus* (Moya-Laraño et al. 2007, Stillwell et al. 2007a, 2008) are consistent in that none support the hypothesis that temperature is the major environmental factor that generates the clinal variation in selection that produces clines in body size and other traits of this species.

Rather, other environmental variables such as seed size, moisture and seasonality are as likely responsible for creating geographic variation in selection that explains geographic variation in growth and life history traits of *S. limbatus*.

Intraspecific variation in sexual size dimorphism in *S. limbatus*

Probably the most intriguing result of our study is that temperature affects sexual dimorphism in some of our *S. limbatus* populations, but that the magnitude and direction of the effect varies substantially among populations. Most studies on sexual size dimorphism assume that the magnitude of dimorphism is fixed within a species. However, recent studies have shown that the magnitude of sexual size dimorphism can vary within species (Teder and Tammaru 2005, Blanckenhorn et al. 2006, 2007). This intraspecific variation in dimorphism could be due to genetic differences in mean sizes of males and females among populations, probably due to geographic variation in the degree of sexual selection on males (Fairbairn 2005). Alternatively, this variation could be due to a sex difference in plasticity of body size (differential-plasticity hypothesis; Fairbairn 2005). Several recent studies have demonstrated that the magnitude of dimorphism within populations can vary considerably with environmental conditions due to a sex difference in plasticity of body size (Blanckenhorn 1997, Morin et al. 1999, Fischer and Fiedler 2000, 2001, Fairbairn 2005, Teder and Tammaru 2005, Bonduriansky 2007, Fernández-Montraveta and Moya-Laraño 2007, Gianoli et al. 2007, Mikolajewski et al. 2007, Stillwell and Fox 2007, Karl and Fischer 2008), supporting the differential-plasticity hypothesis. However, few studies have explored whether sex differences in plasticity of body size vary among populations within a species.

In *S. limbatus*, males are the larger sex, but the degree of this dimorphism decreases with increasing latitude (Stillwell et al. 2007a). Our study of latitudinal clines in *S. limbatus* used field-collected specimens, so it is not clear whether the variation among populations was genetic or due to environmental conditions (plasticity). In the current study, we found no overall variation in sexual dimorphism among populations, and no main effect of temperature on sexual dimorphism. However, we found a highly significant temperature-by-population interaction for sexual dimorphism because the magnitude of size dimorphism changed with temperature in some populations but not in others. This suggests that the degree to which males and females differ in their sensitivity to rearing conditions varies among populations of *S. limbatus*. Prior studies have shown that populations within a species are consistent in either supporting or rejecting the differential-plasticity hypothesis (Blanckenhorn 1997, Morin et al. 1999, Fairbairn 2005, Stillwell and Fox 2007, Delph and Bell 2008), whereas our study shows that some populations within a species support the differential-plasticity hypothesis while others do not. Variation among populations in the degree of sex differences in plasticity of size has major implications for studies testing the differential-plasticity hypothesis; conclusions drawn from experiments on only a single population, or

only a single sex, could be misleading. To be able to generalize from laboratory plasticity studies, researchers need to consider multiple populations of a species.

Why do some populations or species support the differential-plasticity hypothesis while others do not? Fairbairn (2005) suggested that variation in sexual size dimorphism among populations is due to canalization of traits closely associated with fitness, which will differ between sexes; if body size is more important to fitness in one sex then body size is expected to be developmentally canalized against environmental perturbation in that particular sex. In *S. limbatus*, body size has large effects on fitness of both males and females. Selection on male size is mediated through contributions of nuptial gifts and male effects on female receptivity, and estimates of selection intensities on male size are of similar magnitude to estimates on female size (Fox et al. 2007). However, the relative amount of selection on female vs. male size changes with host species and likely other environmental variables (Fox and Czesak 2006), including temperature (Moya-Laraño et al. 2007). The relationship between size and fitness may thus vary among populations such that which sex is developmentally canalized, and whether both sexes are developmentally canalized, could vary among populations, such that plasticity would vary among populations in a manner observed in this study. Thus, whether sex differences in plasticity can be explained by the adaptive canalization hypothesis requires knowing details on how natural and sexual selection is operating on males vs. females within each population. We know little about the sources of selection on body size in our six study populations. Consequently, it is unclear whether developmental canalization can account for the sex differences in plasticity we found in some populations of *S. limbatus* in this study.

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