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SELECTION DOES NOT FAVOR LARGER BODY SIZE AT LOWER TEMPERATURE IN A SEED-FEEDING BEETLE

R. Craig Stillwell,[1,2] Jordi Moya-Laraño,[3,4] and Charles W. Fox[5,6]

[1]*Department of Ecology and Evolutionary Biology, University of Arizona, BioSciences West 310, Tucson, Arizona 85721*

[2]*E-mail: rcstill@email.arizona.edu*

[3]*Departamento de Ecología Fucional y Evolutiva, Estación Experimental de Zonas Áridas, Consejo Superior de Investigaciones Científicas, Almería, Spain*

[4]*E-mail: jordi@eeza.csic.es*

[5]*Department of Entomology, University of Kentucky, S225 Agricultural Science Center North, Lexington, Kentucky 40546*

[6]*E-mail: fox@uky.edu*

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Body size of many animals increases with increasing latitude, a phenomenon known as Bergmann's rule (Bergmann clines). Latitudinal gradients in mean temperature are frequently assumed to be the underlying cause of this pattern because temperature covaries systematically with latitude, but whether and how temperature mediates selection on body size is unclear. To test the hypothesis that the "relative" advantage of being larger is greatest at cooler temperatures we compare the fitness of replicate lines of the seed beetle, *Stator limbatus*, for which body size was manipulated via artificial selection ("Large," "Control," and "Small" lines), when raised at low (22°C) and high (34°C) temperatures. Large-bodied beetles (Large lines) took the longest to develop but had the highest lifetime fecundity, and highest fitness (r_C), at both low and high temperatures. However, the relative difference between the Large and Small lines did not change with temperature (replicate 2) or was greatest at high temperature (replicate 1), contrary to the prediction that the fitness advantage of being large relative to being small will decline with increasing temperature. Our results are consistent with two previous studies of this seed beetle, but inconsistent with prior studies that suggest that temperature-mediated selection on body size is a major contributor to the production of Bergmann clines. We conclude that other environmental and ecological variables that covary with latitude are more likely to produce the gradient in natural selection responsible for generating Bergmann clines.

KEY WORDS: **Bergmann's rule, body size, latitudinal cline, seed beetles, temperature.**

A commonly observed pattern in nature is that body size of animals increases with increasing latitude, a pattern known as Bergmann's rule (Bergmann clines; Bergmann 1847; Stillwell et al. 2007a). Bergmann clines were first observed in endothermic animals (Bergmann 1847), but they have since been found in numerous ectotherms (Blanckenhorn et al. 2006; Stillwell et al. 2007a). Bergmann clines are known to be genetically based; populations collected from different geographic regions and reared in common environmental conditions generally retain their differences in size (Partridge and Coyne 1997; Gilchrist and Partridge 1999). These clines are often repeatable across continents in the same 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 rapidly following colonization of new continents (Huey et al. 2000). They are thus likely produced by natural selection rather than drift, but the sources of selection remain poorly understood (Stillwell et al. 2007a).

Although many ecological and environmental variables covary with latitude and could generate Bergmann clines, average temperature is frequently assumed to be the environmental variable mediating selection and thus producing these body size clines in animals (Stillwell et al. 2007a). However, it is unclear how, and if, temperature mediates selection on body size. Surface area-to-volume ratios decrease with increasing body size such that larger animals lose heat more slowly in colder environments. The effect of size on surface area-to-volume ratios is thus commonly invoked as the likely explanation for clines in endotherms (Bergmann 1847; Ashton et al. 2000; Ashton 2002), but comparative studies shed doubt on this conclusion (Ashton et al. 2000; Ashton 2002). Also, the surface area-to-volume ratio hypothesis is not a compelling explanation for the observed clines in ectotherms (Blanckenhorn and Demont 2004; Blanckenhorn et al. 2006; Stillwell et al. 2007a). We thus have no satisfactory explanation for how, or even if, temperature mediates selection on body size, especially for ectotherms.

Nevertheless, that latitudinal clines in mean temperature at least influence the evolution of body size is indicated by two observations—altitudinal clines frequently mirror latitudinal clines (Partridge and Coyne 1997; but see Karan et al. 2000) and body size evolves in *Drosophila* thermal selection experiments (natural selection experiments in which experimental populations are allowed to adapt to different temperature regimes; Anderson 1966, 1973; Cavicchi et al. 1985, 1989; Partridge et al. 1994a), generally in the directions consistent with latitudinal clines observed in nature. Egg size, development time, and growth rate, all of which are genetically correlated with body size, also evolve in these *Drosophila* thermal selection experiments (Partridge et al. 1994b; Azevedo et al. 1996; Griffiths et al. 2005). Latitudinal and altitudinal clines in body size could thus evolve either due to temperature-mediated variation in selection on body size along the cline, or due to temperature-mediated variation in selection on traits genetically correlated with size (Cavicchi et al. 1989; Bochdanovits and de Jong 2003a,b).

To evaluate how temperature affects selection on body size, body size has to be manipulated and the fitness consequences of variation in size must be quantified across a range of temperatures. There are two general ways to manipulate body size: through phenotypic manipulations or through evolutionary (selection) experiments. Most ectotherms mature at larger body sizes when reared at lower temperatures (Atkinson 1994), providing an easy means of manipulating body size. However, rearing temperature simultaneously affects a large suite of physiological, morphological, and life-history traits (Stillwell and Fox 2005; Stillwell et al. 2007b), making it nearly impossible to disentangle the effects of size on fitness from the complex effects of larval experience/environment on fitness. Using lines that are laboratory adapted to low versus high temperature or populations from different geographic localities is likewise problematic because these lines/populations are likely to differ in many traits uncorrelated with size, but which evolve in response to temperature, and which affect responses to temperature.

One way to disentangle the effects of selection on body size from the effects of other traits that influence responses to temperature is to directly manipulate body size by artificial selection and measure the fitness consequences of large versus small animals at low versus high temperature (McCabe and Partridge 1997; Reeve et al. 2000). Other traits inevitably evolve in response to selection on size, but these are traits genetically correlated to size and thus directly relevant to the evolution of body size and selection on body size. Studies of *Drosophila melanogaster* that compare flies selected for large versus small size suggest that the balance between natural selection for early maturation (and thus small size) versus natural selection for increased fecundity/fertility/longevity (and thus large size) differs between low and high temperature because the survival and fecundity advantages of being larger are greater at low temperature (McCabe and Partridge 1997; Reeve et al. 2000; for a review of selection on size see Blanckenhorn 2000, 2005). This is consistent with the hypothesis that temperature-mediated variation in selection favors larger individuals in cooler climates, and thus at higher latitudes and altitudes, producing Bergmann clines. However, similar studies that compare yellow dung flies selected for large versus small size have given inconsistent results (Reim et al. 2006; Teuschl et al. 2007). Moreover, the only two studies (McCabe and Partridge 1997; Reeve et al. 2000) that have used artificial selection to evaluate the relative fitness advantages of large versus small size at low versus high temperature, and that have supported the hypothesis that temperature-mediated variation in selection generates latitudinal clines, have been conducted on a single set of selection lines of a single species, *D. melanogaster*. Furthermore, recent studies have demonstrated that body size of *Drosophila* does not evolve in response to temperature when larval competition, which is affected by temperature, is controlled (Santos et al. 2004, 2005, 2006), casting doubt on the hypothesis that temperature-mediated variation in selection on body size creates Bergmann clines.

Here we use the seed beetle, *Stator limbatus* (Coleoptera: Chyrsomelidae: Bruchinae), as a model system to test the hypothesis that selection on body size changes with temperature. *S. limbatus* is a generalist seed-feeding beetle that is widely distributed from northern South America to the southwestern United States. Body size of *S. limbatus* (average mass $\sim$ 3-4 mg) varies substantially among populations, and increases with latitude,

following Bergmann's rule (Stillwell et al. 2007a). The variation in body size is substantial (the largest populations are >50% larger in mass than the smallest populations) and is at least partially genetically based (variation in size among populations persists after many generations of laboratory rearing; Amarillo-Suárez 2006; Amarillo-Suárez and Fox 2006). The cline is most likely produced by natural selection, with selection favoring larger beetles at higher latitudes (Stillwell et al. 2007a). Using artificially selected lines of *S. limbatus*, Moya-Laraño et al. (2007) showed that smaller males have an advantage during scramble competition for mates because smaller males takeoff more quickly and thus reach females more quickly than do larger males. In addition, they found that selection favoring smaller males was greatest at cooler temperatures, opposite to the prediction based on Bergmann clines. However, scramble competition is only one component of total selection in nature, and only occurs in males. We thus need to examine other components of fitness, and selection in females, before we can generalize effects of temperature on overall fitness variation.

In this study we test the hypothesis that natural selection on body size varies with temperature, and thus whether temperature could potentially mediate clinal variation in selection. We do this by quantifying the relative fitness consequences of variation in body size of female *S. limbatus* at low versus high temperature. We compare replicate lines of beetles for which body size was manipulated via artificial selection. Specifically, we imposed directional selection on female body size for nine generations to create replicate large-, medium-, and small-bodied populations of beetles. We then examined egg-to-adult survivorship, egg-to-adult development time, adult body mass, lifetime fecundity, and a composite measure of fitness of the selection lines at two test temperatures to evaluate the relative advantage of being larger versus smaller at low versus high temperature.

Materials and Methods

NATURAL HISTORY OF *STATOR LIMBATUS*

Stator limbatus (Horn) 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). Although only a few hosts are encountered in most locations, *S. limbatus* has been collected from > 70 species of primarily mimosoid or caesalpinioid legumes throughout its wide geographic range. Most hosts are native (~50 spp.), although many are aliens (>20 spp.; Morse and Farrell 2005a,b).

The life cycle of *S. limbatus* revolves around seeds. Females oviposit directly onto host seeds inside fruits that have either dehisced or been damaged by other organisms (e.g., mice, other bruchine 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) that have been problematic for some studies of *Drosophila* (Santos et al. 2004). Upon emergence from the seed, adults mate and females begin to lay eggs within ~ 24–48 h in the laboratory. Average adult lifespan for mated beetles is approximately 18 days at 22°C and 5 days at 34°C (Stillwell et al., unpubl. data).

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 a single seed to complete development and reproduce (i.e., they are capital breeders). Additional food and water are thus 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 POPULATION

We initiated our selection experiment using a population of *S. limbatus* collected from Oracle, Arizona. Beetles were collected along Hwy 77 and adjacent roads in Oracle, Pinal County, Arizona, USA (32.61° N 110.77° W; 1372 m above sea level) in August 2002 from mature fruits of several *Acacia greggii* trees. Fruits were shipped back to the laboratory and seeds bearing eggs were placed individually in 35-mm petri dishes. More than 200 emerging adult beetles were used to establish a laboratory colony. This colony was maintained for two generations on *A. greggii* seeds at ~ 100 families each generation, prior to initiation of artificial selection. 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) during the artificial selection experiment.

SELECTION LINES

We imposed artificial selection for nine generations. All beetles were raised in laboratory growth chambers at 28°C (15:9 h, light:dark) at a density of 1 larva per seed. Selection was then relaxed for five or six generations (replicates 1 and 2, respectively) prior to this experiment. At the start of this experiment Large line females were 16% larger than Control line females and Small line females were 32% smaller than Control line females. The realized heritability (h^2) for body mass (averaged across lines and replicates) was 0.45 (Moya-Laraño et al. 2007).

Details on the creation of the selection lines are described elsewhere (Moya-Laraño et al. 2007). In brief, we imposed selection for large (Large lines) and small (Small lines) adult females in each of two separate replicates ("replicate 1" and "replicate 2"). These lines were paired with two replicates of unselected control

lines (Control lines). Each replicate was initiated with 125 randomly mated pairs of beetles. Two eggs chosen from each of the 125 pairs were used to initiate the Control line. Offspring from the 25 largest females (largest 20%) were used to create each Large line, and offspring from the 25 smallest females (smallest 20%) were used to create each Small line. Thus, within replicates, the Large, Control, and Small lines were created with the same set of females.

To maintain the selection lines, we reared 10 offspring to adult per family per line per generation. This produced up to 250 adult offspring, and thus 125 female offspring (minus those that died during development, which averaged $\sim$ 5% per generation). All females were weighed within 12 h of adult emergence, then paired with a randomly chosen male (selection was imposed only on female size), and allowed to lay eggs on 10–12 *A. greggii* seeds. We selected the 25 largest (Large lines) or 25 smallest (Small lines) females, from which we raised 10 offspring to produce the next generation.

To maintain the Control lines, two randomly chosen offspring per family were reared to adult, randomly mated, and allowed to lay eggs. This was repeated each generation unless the number of families created dropped below 100 (due to larval mortality and occasional pairs failing to lay eggs) after which, for one generation, we reared three offspring per family.

EXPERIMENTAL DESIGN

Overview of the experiment

To examine the relative advantage of large versus small female body size at low versus high temperature, we measured egg-to-adult survivorship, egg-to-adult development time, body mass and female lifetime fecundity of offspring for each selection line when reared at 22°C (15:9, L:D) and 34°C (15:9, L:D) (3 lines $\times$ 2 replicates $\times$ 2 rearing temperatures $=$ 12 treatment combinations). These temperatures 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). To remove environmentally based parental effects, we raised all lines in the experimental treatments for two generations and collected data only on the second generation. To ensure that we tested similar genotypes of beetles (within lines) in each temperature treatment, we used a split-brood design to create generation A of the experiment. Offspring from each full-sibling family of each replicate and selection line were randomly and equally divided among the two temperature treatments. These offspring were raised to adult and allowed to lay eggs. Larvae hatching from these eggs, which are generation B, were reared in the same temperature treatment as their parents. We measured fitness characters on Generation B.

All larvae were reared in 35-mm petri dishes, on seeds of *A. greggii*, inside temperature-controlled Percival reach-in growth chambers. Developing larvae were rotated daily through the chamber to control for spatial variation within growth chambers.

Details

To initiate generation A of the experiment, we randomly divided eggs from each family (of each replicate of each line of the selection experiment) between the two temperature treatments. Three eggs per family were assigned to each treatment from the Large and Control lines and four eggs per family were assigned to each treatment from the Small lines. We raised more offspring from Small lines because Small lines suffered from greater mortality. Offspring were reared to adult at one egg per seed, one seed per dish (so that all emerging beetles were virgin). Emerging offspring were collected once daily and mated with a random individual of the opposite sex within the same replicate-line-temperature combination. Mated beetles were confined in 35-mm petri dishes with either 10 *A. greggii* (Large and Control lines) or 12 *A. greggii* seeds (Small lines). Dishes were checked twice daily for eggs until a female had laid eggs on all of the seeds or until the female failed to oviposit for 48 h. Eggs were reared to adult at one egg per seed (excess eggs were scraped off) and one seed per dish.

We checked for emerging adult beetles twice daily. All adults were weighed on an electronic balance to the nearest 0.1 mg within $\sim$12 h of emergence. We measured fitness characters on approximately 60% of the offspring per family (randomly selected at the egg stage). The remaining 40% were used in a different study. Females were isolated for 24 h to mature eggs before we allowed them to mate. Each female was then paired with a virgin male from the same replicate-selection line-temperature combination. Mated pairs were placed in 60-mm petri dishes containing 50 *Albizia julibrissin* seeds and allowed to lay eggs until death to estimate female lifetime fecundity. We used *A. julibrissin* to estimate fecundity because seeds are available in bulk from horticultural seed suppliers and are much less expensive than *A. greggii* seeds. No beetles were reared on *A. julibrissin* seeds during the experiment; we only used these seeds to measure adult fecundity.

In addition to the fitness components that we measured, we created a composite measure of fitness (r_C). r_C was calculated for each family as $\ln(R_0)/\tau$, where R_0 is the lifetime reproductive success (proportion of offspring surviving to adult in the family $\times$ average fecundity of females in the family) and τ is the mean generation time for individuals in that same family (Fairbairn 2006). Generation time was estimated as egg-to-adult development time plus the time between emergence and the average age at which a female laid her middle egg. We calculated the time between emergence and the average age at which females laid their middle egg for 22°C and 34°C using data from previous studies (Stillwell and

Fox 2005; C.W. Fox, unpubl. data). Generation time was included in our measure of fitness because *S. limbatus* are multivoltine with overlapping generations. Estimating fitness with generation time is thus necessary for the results to be biologically meaningful. However, calculating fitness as only lifetime reproductive success (R_0, calculation of which does not include generation time) produces similar results (Table 1).

In total, 10,528 eggs from 1190 full-sibling families were raised to adult. Nine thousand and three of these individuals emerged and were weighed. Female lifetime fecundity was recorded for 2263 females.

STATISTICAL ANALYSES

All data were analyzed with analysis of variance (ANOVA) using SAS 9.1 (SAS Institute, Cary, North Carolina, USA). We used family means for all analyses, and considered the selection lines our lowest level of independence (with replicates nested within lines, as in McCabe and Partridge 1997; Reeve et al. 2000). All data except egg-to-adult survivorship and egg-to-adult development time were approximately normally distributed and had equal variances between temperature treatments. They were thus not transformed prior to analysis. Egg-to-adult development time was log-transformed before analysis to stabilize the variances between temperatures. Egg-to-adult survivorship (calculated as the proportion of surviving offspring for each family) was arcsine-square root transformed to meet as best as possible the assumptions of ANOVA.

The main focus of our study is on interactions between rearing temperature and selection line. However, interactions between factors in an ANOVA measure change in the linear difference between treatment means and are thus dependent on scale (Stanton and Thiede 2005; Stillwell et al. 2007b). We are interested in how rearing temperature affects relative differences between treatments, for which ANOVA can be misleading; for example, a difference in fecundity of three eggs between selection lines has different meaning at high than at low temperature because the overall average fecundity changes with rearing temperature. Consequently, we performed our analysis as a two-step process. First, we examined main effects (effects of temperature, selection line, and replicates) using ANOVA on absolute data. Then, to test for interactions between temperature and selection lines we created relative trait values following Stanton and Thiede (2005); we divided each individual trait value by the overall mean (averaged across lines and replicates) within each temperature treatment, removing the large effect of temperature and standardizing variances between temperatures (Stanton and Thiede 2005; Stillwell et al. 2007b). This test for significant interactions is thus a test of whether the fitness of large beetles relative to small beetles changes with

Table 1. Analysis of variance (Type III sums of squares) for the effects of rearing temperature, selection line and replicate on egg-to-adult survivorship, egg-to-adult development time, adult body mass, female lifetime fecundity and two composite measures of fitness (r_C and R_0) of the seed beetle *Stator limbatus.* ANOVAs were performed using the full model with all possible interaction terms present. All interactions are from analyses on relative trait values (individual value/mean for each temperature treatment). Egg-to-adult development time data were log-transformed for the analysis of main effects, but relative trait values were created from nontransformed data for the analysis of interactions.

	df	*F*	*P*
Egg-to-adult survivorship			
Temperature	1	3.56	0.06
Replicate (Line)[2]	3	0.61	0.61
Line	2	13.7	0.03
Temperature × Line	2	0.48	0.66
Temperature × Replicate (Line)[3]	3	2.21	0.09
Error[1]	1178		
Egg-to-adult development time			
Temperature	1	104039	<0.0001
Replicate (Line)[2]	3	3.24	0.02
Line	2	11.4	0.04
Temperature × Line	2	0.01	0.99
Temperature × Replicate (Line)[3]	3	29.8	<0.0001
Error[1]	1114		
Adult body mass			
Temperature	1	1495	<0.0001
Replicate (Line)[2]	3	9.32	<0.0001
Line	2	87.6	0.002
Temperature × Line	2	0.58	0.61
Temperature × Replicate (Line)[3]	3	3.12	0.03
Error[1]	1120		
Female lifetime fecundity			
Temperature	1	1244	<0.0001
Replicate (Line)[2]	3	47.3	<0.0001
Line	2	4.54	0.13
Temperature × Line	2	0.66	0.58
Temperature × Replicate (Line)[3]	3	3.59	0.01
Error[1]	991		
Fitness (r_C)			
Temperature	1	136	<0.0001
Replicate (Line)[2]	3	2.13	0.09
Line	2	17.8	0.02
Temperature × Line	2	5.22	0.11
Temperature × Replicate (Line)[3]	3	0.87	0.46
Error[1]	1168		
Fitness (R_0)			
Temperature	1	461	<0.0001
Replicate (Line)[2]	3	15.2	<0.0001
Line	2	4.95	0.11
Temperature × Line	2	0.61	0.60
Temperature × Replicate (Line)[3]	3	3.97	0.008
Error[1]	1170		

[1]Error term for Temperature, Replicate (Line) and Temperature × Replicate (Line).
[2]Error term for Line.
[3]Error term for Temperature × Line.

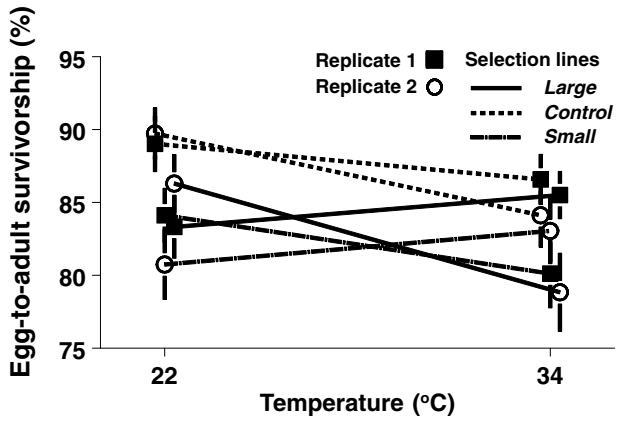

Figure 1. Egg-to-adult survivorship of the Large, Control, and Small artificial selection lines of *Stator limbatus* reared and tested at two different temperatures (22 and 34°C). Pairwise comparisons (linear contrast) for Large vs. Control: $F_{1,1178} = 8.97$, $P = 0.003$; Large vs. Small: $F_{1,1178} = 0.90$, $P = 0.34$; Control vs. Small: $F_{1,1178} = 15.1$, $P = 0.0001$.

temperature. Log-transformation can also be used to alleviate the scaling effect of temperature. However, log transformation can impair biological insight at the expense of meeting the statistical assumptions (Grissom 2000; Stanton and Thiede 2005).

Results

MAIN EFFECT OF SELECTION LINE

As expected, the selection lines differed considerably in body mass and other traits (large line effects for egg-to-adult survivorship, egg-to-adult development time, adult body mass, female lifetime fecundity, and fitness (r_C); Table 1). Beetles in the Control lines had 4.6 and 6.5% higher egg-to-adult survivorship than either the Large or Small selection lines ($P < 0.01$ for both pairwise comparisons; see figure legends for all pairwise comparisons) although the Large and Small selection lines did not differ in survivorship from one another ($P > 0.05$; Fig. 1). This suggests that disturbing beetles from their natural variation in size only partially affected survivorship, and that the selection lines experienced at most a small loss of fitness due to loss of genetic variation during artificial selection. On average, beetles of the Large and Small selection lines took 0.8 and 0.5 days longer (2.5 and 1.6%), respectively, to develop than beetles from the Control lines ($P < 0.0001$ for both pairwise comparisons; Fig. 2A). Beetles in the Large selection lines, as expected, were 28% larger than beetles in Small lines and 16% larger than beetles in the Control lines ($P < 0.0001$ for all pairwise comparisons; Fig. 2B). Beetles in the Large lines also laid 55% more eggs than beetles in the Small lines, and 22% more eggs than beetles in the Control lines

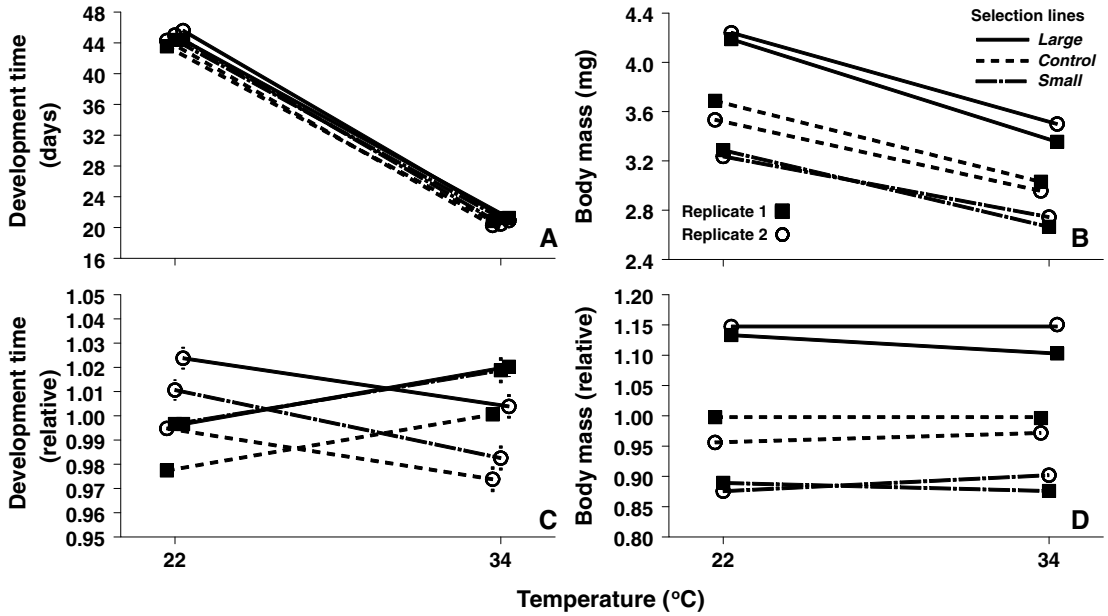

Figure 2. Egg-to-adult development time (A) and adult body mass (B) of the Large, Control, and Small artificial selection lines of female *Stator limbatus* reared and tested at two different temperatures (22 and 34°C). Relative development time (C) and relative body mass (D) are the means after removing the large temperature effect (individual trait/mean trait for each temperature treatment, following Stanton and Thiede (2005) and Stillwell et al. (2007b)). Egg-to-adult development time data were also collected for males, but we present data for females only because our focus is on female traits (results for males were qualitatively similar). Standard error bars are included, but are smaller than the symbols for some experimental treatments. Pairwise comparisons of development time (linear contrast) for Large vs. Control: $F_{1,1114} = 71.7$, $P < 0.0001$; Large vs. Small: $F_{1,1114} = 8.77$, $P = 0.003$; Control vs. Small: $F_{1,1114} = 27.9$, $P < 0.0001$. Pairwise comparisons of body mass (linear contrast) for Large vs. Control: $F_{1,1120} = 652$, $P < 0.0001$; Large vs. Small: $F_{1,1120} = 1589$, $P < 0.0001$; Control vs. Small: $F_{1,1120} = 236$, $P < 0.0001$.

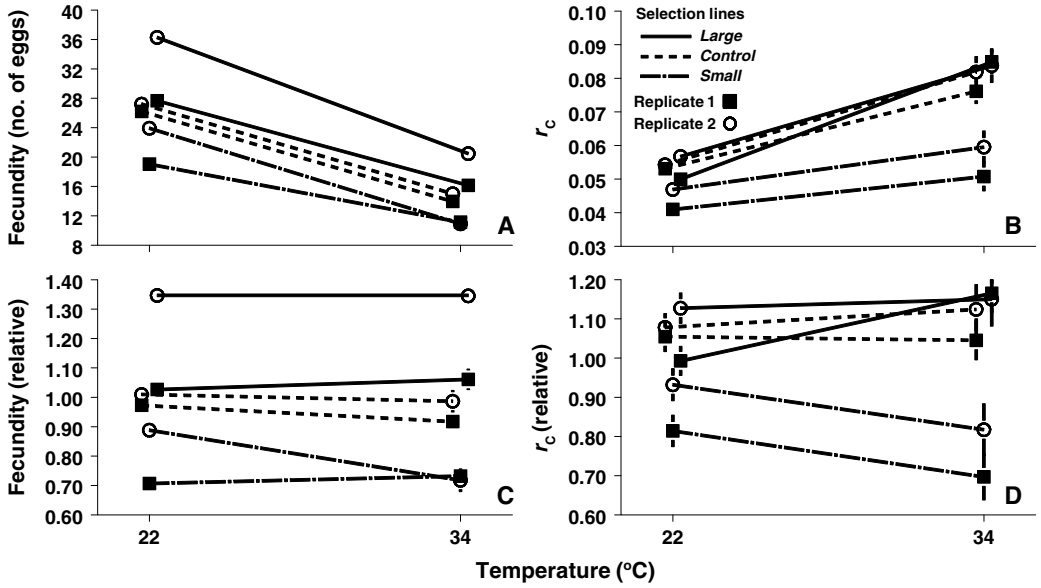

Figure 3. Female lifetime fecundity (A) and fitness (r_C; B) of the Large, Control, and Small artificial selection lines of female *Stator limbatus* reared and tested at two different temperatures (22 and 34°C). Relative fecundity (C) and relative r_C (D) are the means after removing the large temperature effect (individual trait/mean trait for each temperature treatment, following Stanton and Thiede (2005) and Stillwell et al. (2007b)). r_C was calculated for each family as $\ln(R_0)/\tau$, where R_0 is the lifetime reproductive success (proportion of offspring surviving × fecundity) and τ is the mean generation time for each family (Fairbairn 2006). Standard error bars are included, but are smaller than the symbols for some experimental treatments. Pairwise comparisons of fecundity (linear contrast) for Large vs. Control: $F_{1,991} = 126$, $P < 0.0001$; Large vs. Small: $F_{1,991} = 427$, $P < 0.0001$; Control vs. Small: $F_{1,991} = 104$, $P < 0.0001$. Pairwise comparisons of r_C (linear contrast) for Large vs. Control: $F_{1,1168} = 1.16$, $P = 0.28$; Large vs. Small: $F_{1,1168} = 64.9$, $P < 0.0001$; Control vs. Small: $F_{1,1168} = 50.0$, $P < 0.0001$.

($P < 0.001$ for all pairwise comparisons; Fig. 3A), even though the line effect was not significant ($P = 0.13$). However, the effect size was large ($F_{2,3} = 4.54$), suggesting that the small number of replicates (2 replicates of each line) resulted in low statistical power; when the replicates were analyzed separately the line difference in fecundity was highly significant for both replicates (replicate 1: $F_{2,535} = 71.2$, $P < 0.0001$; replicate 2: $F_{2,456} = 158$, $P < 0.0001$). The differences between Large and Small lines in survivorship, development time, and fecundity translated into large differences in overall fitness—Large line beetles had 39% higher fitness (r_C) than Small line beetles ($P < 0.0001$), but Large line beetles did not differ from Control line beetles ($P = 0.28$; Fig. 3B).

MAIN EFFECT OF TEMPERATURE

Because the temperatures experienced during immature development and during adult female oviposition can dramatically affect growth and life-history traits of ectotherms (Atkinson 1994; Stillwell and Fox 2005; Stillwell et al. 2007b), we expected temperature to affect all traits in this study. This was observed for all traits (Table 1). In general, beetles had 3% higher survivorship ($P = 0.06$; Fig. 1) when they were reared at 22°C. They also took 24 days longer to develop ($P < 0.0001$; Fig. 2A) and were 21% larger when reared at 22°C ($P < 0.0001$; Fig. 2B).

Females laid on average 83% more eggs ($P < 0.0001$) when they were reared at 22°C (Fig. 3A). The large temperature effect on fecundity led to females having much higher reproductive success (R_0) when reared at 22°C. However, because of the large effect of temperature on generation time (generation time was much shorter at 34°C vs. 22°C) beetles had 45% higher fitness (r_C) when reared at 34°C ($P < 0.0001$; Fig. 3B).

TEMPERATURE-BY-SELECTION LINE INTERACTIONS

Although we expected Large line beetles to have higher absolute fitness than Small line beetles at all temperatures (due to the fecundity advantage of being large) we also expected the magnitude of this advantage to change with temperature. Specifically, we predicted that the relative fitness of large versus small beetles would change with temperature, with large beetles having greater relative fitness at lower temperature. This was not observed for most traits examined. We detected no significant interactions between rearing temperature and selection line for egg-to-adult survivorship, egg-to-adult development time, body mass or fecundity ($P > 0.50$ for all traits; Table 1; Figs. 1–3). We also did not detect a significant interaction between rearing temperature and selection line for relative fitness (r_C: $P = 0.11$) or relative reproductive success (R_0: $P = 0.60$), inconsistent with the prediction that variation in temperature is the major mediator of variation in selection

along the latitudinal cline. However, the effect size of the interaction was large for r_C ($F_{2,3} = 5.22$) although it was not large for R_0 ($F_{2,3} = 0.61$). To ensure that our lack of significance was not an artifact of a small number of experimental lines, we examined the interaction effect on r_C separately for each replicate. We found that the fitness (r_C) of the body size lines did not change with temperature for replicate 2 (nonsignificant temperature-by-line interaction for r_C: $F_{2,534} = 1.33$, $P = 0.26$) but did change with temperature for replicate 1 (significant temperature-by-line interaction for r_C: $F_{2,634} = 4.62$, $P = 0.01$). In replicate 1, the fitness of small beetles (Small relative to Large lines) decreased with temperature, and the fitness of large beetles (Large relative to Small lines) increased with temperature—the relative difference in fitness between the Large and the Small lines was greater at 34°C (67%) than at 22°C (22%)—opposite to the predicted direction based on the hypothesis that larger beetles have a fitness advantage at lower temperatures (Fig. 3D).

Discussion

Temperature is generally considered the most likely environmental variable mediating natural selection along latitudinal and altitudinal gradients and creating the observed latitudinal and altitudinal clines in body size. Here, using artificially selected lines of the seed beetle *S. limbatus*, we directly tested the hypothesis that the advantage of being larger-bodied (relative to smaller-bodied) is greater at low than at high temperature. We found that the fitness of large-bodied beetles (beetles artificially selected for large size), relative to small-bodied beetles (those selected for small size), did not change with temperature (replicate 2) or was greatest at higher temperature (replicate 1; Fig. 3D). Both of these results are contrary to our prediction that the fitness advantages of being larger will increase with decreasing temperature. Our results are thus inconsistent with a main prediction of the hypothesis that temperature-mediated variation in natural selection on body size creates Bergmann clines.

Evidence supporting the hypothesis that temperature-mediated variation in selection on body size produces Bergmann clines in ectothermic animals comes from extensive studies on the fruit fly, *Drosophila melanogaster*. In *D. melanogaster*, body size increases with latitude, following Bergmann's rule (Coyne and Beecham 1987; Capy et al. 1993; Imasheva et al. 1994; James et al. 1995; Van't Land et al. 1995). In laboratory natural selection experiments, in which flies were maintained at, and thus allowed to adapt to, low vs. high temperature, flies evolve to be larger at low temperature and smaller at high temperature (Anderson 1966, 1973; Cavicchi et al. 1985, 1989; Partridge et al. 1994a). However, these studies did not control for larval densities, which increase at higher temperatures (Santos et al. 2004). Recent work has demonstrated that body size can evolve in response to variation in larval density (Tucić et al. 1997; but see Santos et al. 1997). It is not clear whether temperature or larval density mediated the rapid evolution of body size in these experiments, nor is it even clear that body size itself was the target of selection (Bochdanovits and de Jong 2003a; Santos et al. 2004). Similar studies on *D. subobscura*, in which larval density was controlled, have shown that body size does not evolve when flies are cultured for several years at low or high temperatures (Santos et al. 2004, 2005, 2006).

However, recent work has more convincingly demonstrated that temperature can mediate the fitness consequences of variation in body size. McCabe and Partridge (1997) and Reeve et al. (2000) used an artificial selection experiment to create large and small *D. melanogaster* and then tested the fitness of both males and females of these selection lines at low vs. high temperature. McCabe and Partridge (1997) showed that the large-line females lived considerably longer and produced more offspring, at the lowest temperature, relative to small-line females. Also, Reeve et al. (2000) demonstrated that the fitness of large-line males was greatest at the lowest temperature. Both results indicate that the advantage of being large (relative to small) is greater in cooler than in warmer environments, such that variation in temperature with latitude should favor the evolution of larger flies at higher latitudes, as observed in Bergmann clines.

Our results are inconsistent with these *Drosophila* studies. Body size of *S. limbatus* increases with increasing latitude (Stillwell et al. 2007a), as in many *Drosophila* species, leading to the same prediction as in *Drosophila*—that the magnitude of selection favoring large beetles (relative to small beetles) should be greatest at cooler temperatures if temperature is the variable generating the cline. We found that, for both of the estimates of fitness used in this study (r_C and R_0), larger females had higher fitness than smaller females at all temperatures. However, in contrast to the prediction that the magnitude of selection for large beetles should be greatest at cooler temperatures, we found that the relative advantage of being large did not change with temperature (r_C in replicate 2) or was greatest at the warmer experimental temperature (r_C in replicate 1). This result matches that from a study on the fitness of male *S. limbatus* during scramble competition. Using these same artificially selected lines of *S. limbatus* (plus another set of similar lines), Moya-Laraño et al. (2007) found that selection favoring small males is greatest at cooler temperatures. Both of these experimental studies of *S. limbatus* are consistent with the biogeographic study in which we found that, although body size co-varies with latitude in *S. limbatus*, the observed cline is not best explained by a gradient in average temperature along the cline (Stillwell et al. 2007a). Instead, the latitudinal cline is best explained by clinal variation in three other environmental factors: host plant seed size, moisture, and seasonality (beetle body size increases with increasing host seed size,

decreasing moisture and increasing seasonality). The results of all of these studies with *S. limbatus* are thus consistent in that none supports the hypothesis that temperature-mediated selection on body size is likely the explanation for the latitudinal cline in body size observed for this beetle. Instead, other sources of selection must co-vary with latitude and be responsible for mediating selection along the cline. The environmental variables most likely to mediate selection along the cline are seed size, moisture, and seasonality (Stillwell et al. 2007a).

Although our results here were inconsistent with our initial prediction, we did find that relative fitness of large versus small beetles changed with temperature for replicate 1, but opposite to the predicted direction. This could be a consequence of temperature mediation of desiccation rates (McCabe and Partridge 1997). If large beetles are more resistant to desiccation than small beetles, because large size reduces the surface-to-volume ratio and increases absolute water content (Chown and Gaston 1999), then larger beetles will be favored at higher temperatures. Observations with *Drosophila* are consistent with this. Desiccation resistance in flies often increases with increasing latitude (Hoffmann and Harshman 1999), covarying with body size (Van Herrewege and David 1997). Also, large body size evolves swiftly in response to low versus high relative humidity (in laboratory natural selection experiments), with larger size evolving at lower humidity (Kennington et al. 2003). This suggests that clinal variation in moisture could drive the evolution of clines in size, consistent with the results of our biogeographic study in *S. limbatus* (Stillwell et al. 2007a).

Our estimates of fitness in this study reflect only a subset of the total contributors to fitness in nature. Although a previous study found similar results for male flight and scramble competition, many other components of the life cycle in which beetles experience selection have not yet been considered. For example, females must locate hosts that are often distributed patchily, and their larvae must often tolerate high levels of larval competition inside seeds. Selection occurring during these (and other) stages of the life cycle may be mediated by temperature and thus contribute to the evolution of body size along the cline. Furthermore, the inclusion of other temperatures into our experiment could have produced different results. The temperatures we used in this study were selected because they are within the normal range of temperatures that *S. limbatus* typically encounter in the field (see Materials and Methods). However, reaction norms in response to temperature are often complex (Stillwell and Fox 2005; Stillwell et al. 2007b) such that the observed line differences could vary with the range of temperatures included, and could be influenced by variation in temperature within treatments (e.g., fluctuating vs. constant temperatures). Thus, although our results so far are inconsistent with the prediction that temperature-mediated selection on body size is responsible for generating the observed latitudinal clines in body size, the difference in results between our studies and studies of *D. melanogaster*, and the inconsistency among the various *Drosophila* studies, indicate that much work is needed before we develop an understanding of how and whether temperature mediates selection on body size. Equally important, future studies should consider the potential importance of other environmental variables, especially seasonality and water availability, which vary geographically and clinally.

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