

# PROBING THE ADAPTIVE LANDSCAPE USING EXPERIMENTAL ISLANDS: DENSITY-DEPENDENT NATURAL SELECTION ON LIZARD BODY SIZE

Ryan Calsbeek<sup>1,2,3</sup> and Thomas B. Smith<sup>3,4</sup>

<sup>1</sup>Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755

<sup>2</sup>E-mail ryan.calsbeek@dartmouth.edu

<sup>3</sup>Center for Tropical Research, Institute of the Environment, University of California, Los Angeles, California 90095

<sup>4</sup>Department of Ecology and Evolutionary Biology, University of California, Los Angeles, California 90095

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*Anolis* lizards in the Greater Antilles are thought to have diversified through natural selection on body size and shape, presumably due to interspecific competition and variation in locomotor performance. Here we measure natural selection on body size over three years and across seven replicate populations of the brown anole, *A. sagrei*. We experimentally manipulated an important component of the environment (population density) on several small islands to test the role of density in driving natural selection. Results indicate that the strength of natural selection was proportional to population density ( $r^2 = 0.81$ ), and favored larger body sizes at higher density, presumably owing to the enhanced competitive ability afforded by large size. Changes in the distribution of body size by selective releases of lizards to islands show that this effect did not arise by pure density dependence, since smaller individuals were disproportionately selected against at higher densities. We measured significant broad sense heritability for body size in the laboratory ( $h^2 = 0.55$ ) indicating that selection in the wild could have an evolutionary response. Our results suggest an important effect of population density on natural selection in *Anolis* lizards.

**KEY WORDS:** *Anolis* lizard, density dependence, island, natural selection, selection gradient.

Density-dependent natural selection has a longstanding and important role in ecology. First developed in the context of island biogeography (MacArthur and Wilson 1967), the theory of density dependence has traditionally been invoked to explain patterns of population regulation (e.g., r- vs. K selection; MacArthur and Wilson 1967; Sinervo et al. 2000), the evolution of behavior (e.g., competitive ability, resource holding potential [RHP]; Fretwell and Lucas 1969; Maynard-Smith and Brown 1986); the role of competition in structuring populations (Roughgarden 1971; Rummel and Roughgarden 1985; Svensson and Sinervo 2000); and in creating diversity (Rosenzweig 1978; Pimm 1979; Bolnick 2004).

Density-dependent selection may be particularly important in adaptive radiations (Schluter 2000), in which competition is believed to be a driving force in diversification (e.g., character displacement; Dayan and Simberloff 2005; Grant and Grant 2006) and may, under the right conditions, lead to speciation (Schluter 2000, p. 124; Gavrillets 2004, p. 17). Recent evidence suggests that the adaptive radiation of the highly colorful lake Victoria cichlid fishes may have been driven, at least in part, by male–male competition for nest sites. Competition is intense between similarly colored males, promoting frequency and density-dependent selection in which males of dissimilar color have a fitness advantage. Subsequent female choice may have led to reproductive

isolation and speciation (Seehausen and Schluter 2004). Competition in differing habitats has also been considered to be important to the *Anolis* lizard radiation in the Greater Antilles (Pacala and Roughgarden 1982; Losos 1994), a hypothesis that motivates the present study. Here we examine both the strength of selection and the role that competition (i.e., density) plays as a causative agent of natural selection using island populations of the brown anole, *A. sagrei*.

Early measures of density-dependent selection were indirect (Solbrig 1971; Smouse 1976; Butlin et al. 1984), and were rooted in the use of correlations between ecological variables and phenotype frequency as evidence for selection. However, without an experimental approach, it may be impossible to resolve the origin of these correlations (Moreno et al. 1997). Lande and Arnold's (1983) paper revolutionized the use of regression techniques for estimating the strength and form of selection. The statistical approach, now in common practice, is to estimate the strength of selection ( $s$ ) by measuring the covariance between fitness and a trait or traits of interest:

$$s = \text{COV}(W, z),$$

where  $W$  represents fitness and  $z$  the phenotype. It follows that the strength of directional selection (the selection gradient) can be estimated from the regression of relative fitness on standardized traits (Lande and Arnold 1983; Brodie et al. 1995). Although the approach is useful for measuring the strength and form of natural selection, understanding the causality that underlies selection is equally important but far more challenging. Correlations between fitness and traits alone are not sufficient to demonstrate causality (Endler 1986; Mitchell-Olds and Shaw 1987; Wade and Kalisz 1990). For example, identifying the underlying forces that drive selection may require an environmental context when ecology influences fitness. Thus, a more rigorous approach to understanding how selection operates requires the use of experiments that manipulate relevant environmental variables (Mitchell-Olds and Shaw 1987; Wade and Kalisz 1990; Anholt 1991; Krupa and Sih 1993; Hopper et al. 1996; Svensson and Sinervo 2000; Le Galliard et al. 2005; Anderson and Johnson 2006; Grant and Grant 2006).

The adaptive radiation of anoles comprises nearly 150 species, which generally fall into one of six eco-morphological species groups called "ecomorphs" (Williams 1983). Ecomorphs are characterized by variation in body size and limb morphology that is correlated with habitat use (Losos 1990; Losos et al. 1994; Irschick and Losos 1998). Individuals with long limbs tend to reside on broad perches, and long limbs enhance sprint speed on flat surfaces (Irschick and Losos 1998). Shorter limbed lizards are more often found on narrow perches in which shorter limbs reduce running speed, but enhance agility relative to longer limbs. Although variation in limb length influences locomotor perfor-

mance, variation in body size is likely to influence competitive ability (Schoener and Schoener 1982; Schoener 1983). It is this variation in competitive ability that forms the basis of study in the present manuscript.

Body size is an excellent predictor of competitive ability in wild lizard populations (Stamps and Krishnan 1994; Olsson and Shine 2000; Calsbeek and Sinervo 2002) and as a consequence should be under density-dependent selection. To investigate the strength of natural selection on body size in island-lizard populations, we experimentally manipulated population density on a group of small study islands. We present data from a combination of natural and experimental populations of *A. sagrei*, in which we manipulated population densities of lizards through selective release of wild caught lizards to small islands. Anoles are highly territorial (Tokarz 1987; Qualls and Jaeger 1991; Stamps and Krishnan 1995, 1998; Paterson 2002) and we predicted that our manipulations of density would influence the intensity of competition among lizards. Specifically, we predicted that the intensity of selection would increase at high density, and that larger lizards would have a competitive advantage over smaller lizards. Our study contributes to the body of experimental tests of density dependence performed in the wild (Mitchell-Olds and Shaw 1987; Wade and Kalisz 1990; Anholt 1991; Krupa and Sih 1993; Dudley and Schmitt 1996; Hopper et al. 1996; Svensson and Sinervo 2000; Bolnick 2004; Le Galliard et al. 2005; Anderson and Johnson 2006) and illustrates the potential role that intraspecific competition may play in influencing natural selection in the wild.

## Material and Methods

### GENERAL METHODS AND STUDY ISLANDS

*Anolis sagrei* is a small (40–70 mm snout-vent length; SVL) semi-arboreal lizard with a broad tropical and subtropical distribution. It is the most common anole in the Bahamas and one of the species in the Greater Antilles adaptive radiation. Most lizards in our study population (~96%) mature and die in a single year. We studied wild populations of *A. sagrei* from 2003 to 2005 on islands in the Bahamas. All but one of our estimates of selection were performed on small islands where we captured and tracked the fate of every individual lizard in each population. On the large island of Great Exuma, we caught every individual lizard on a study plot that was bound on three sides by water, and on the fourth side by a busy highway. We captured all lizards during each spring (May–June) from 2003 to 2005. On capture all lizards were weighed (g), measured (SVL and hindlimb length [mm]), and assigned a unique four-color combination of elastomer markings, which we injected into the underside of the hind- and forelimbs. Color tags serve as permanent individual identification and are preferable to toe-clipping because clipping the specialized toe pads of *Anolis* lizards negatively impacts locomotor performance (Bloch and Irschick

**Table 1.** Summary of experimental design showing the density treatment on each island studied during 2003–2005, and whether selection was measured on one or both sexes. FBC, Flamingo bay cay; NC, Nightmare cay; KC, Kidd cay; GE, Great Exuma; NL, natural low density; EL, experimental low density; EM, experimental medium density; EH, experimental high density; M, male; F, female. In years when we only studied selection on males, female lizards were not manipulated and their potential effects on selection therefore remained constant.

Year	Island	Treatment	Sex measured
2003	GE	NL	M
2003	KC	NL	M, F
2004	KC	EL	M
2004	FBC	EM	M
2005	KC	NL	M, F
2005	FBC	EH	M, F
2005	NC	EM	M

2005). On natural plots, lizards were released to their original point of capture. On experimental plots (see below), lizards were transplanted to an adjacent study island and released randomly with respect to body size.

We performed replicated studies of natural selection on separate study islands near Great Exuma, Bahamas. Study sites were carefully matched by habitat type. These islands have previously proved suitable for sustaining large numbers of *A. sagrei* (Losos et al. 2001), and are similar in both their general ecological properties and their proximity to the main island of Great Exuma (~500 m offshore). During 2003 we studied selection on the main island of Great Exuma (“Exuma”), and on an isolated near-shore island (“Kidd cay”). During 2004 we again studied selection on Kidd cay but replaced the Exuma site with a second offshore cay (“Flamingo bay cay”) because our Exuma site was being cleared

for hotel construction. During 2005, we added a third offshore cay to our sample (“Nightmare cay”). We report estimates of natural selection on male lizards from these seven selection studies. In addition, we measured selection on female lizards in three of these studies (Kidd cay during 2003 and 2005 and Flamingo bay cay during 2005).

We used a combination of natural and experimental approaches in studying selection at the different sites (Table 1). In all cases, lizards that were experimentally introduced to our study cays were captured from adjacent populations on the main island of Great Exuma. We captured and measured a total of 1128 lizards during the course of this study (Table 2). Because most (> 95%) *A. sagrei* in our study populations live for only a single year (Calsbeek et al. 2006), each year we studied selection on a new cohort of subadult lizards.

#### EXPERIMENTAL MANIPULATIONS OF THE ENVIRONMENT

We calculated lizard densities at each plot on the basis of the number of lizards captured and released per square meter of vegetated area on each study island. We measured vegetated area on Kidd cay from an available government survey map, and on remaining sites by running line transects across the island, and calculating both island areas and the extent of vegetation. Plots varied in size (Table 2) from the relatively large Kidd cay (~1604 m<sup>2</sup>) to the relatively small Flamingo bay cay (~431 m<sup>2</sup>). By incorporating a range of lizard population sizes on the experimental islands (range 97–170; Table 2), we studied a set of low, medium, and high-density lizard populations. Average densities were 0.134 ± 0.02 lizards/m<sup>2</sup> on low-density islands (range 0.113–0.148), 0.256 ± 0.04 lizards/m<sup>2</sup> on medium-density islands (range 0.225–0.286), and 0.462 lizards/m<sup>2</sup> on the high-density island. All densities should be considered biologically relevant on

**Table 2.** The strength of selection increased across 10 replicates from 2003 to 2005. \**P* < 0.05, \*\**P* < 0.005, \*\*\**P* < 0.0005. FBC, Flamingo bay cay; NC, Nightmare cay; KC, Kidd cay; GE, Great Exuma. Units for lizard density are #lizards/ m<sup>2</sup>. β and γ are the linear and nonlinear selection coefficients, respectively, and were calculated from separate multiple regressions that included the hind limb residuals (from limb length regressed on body size).

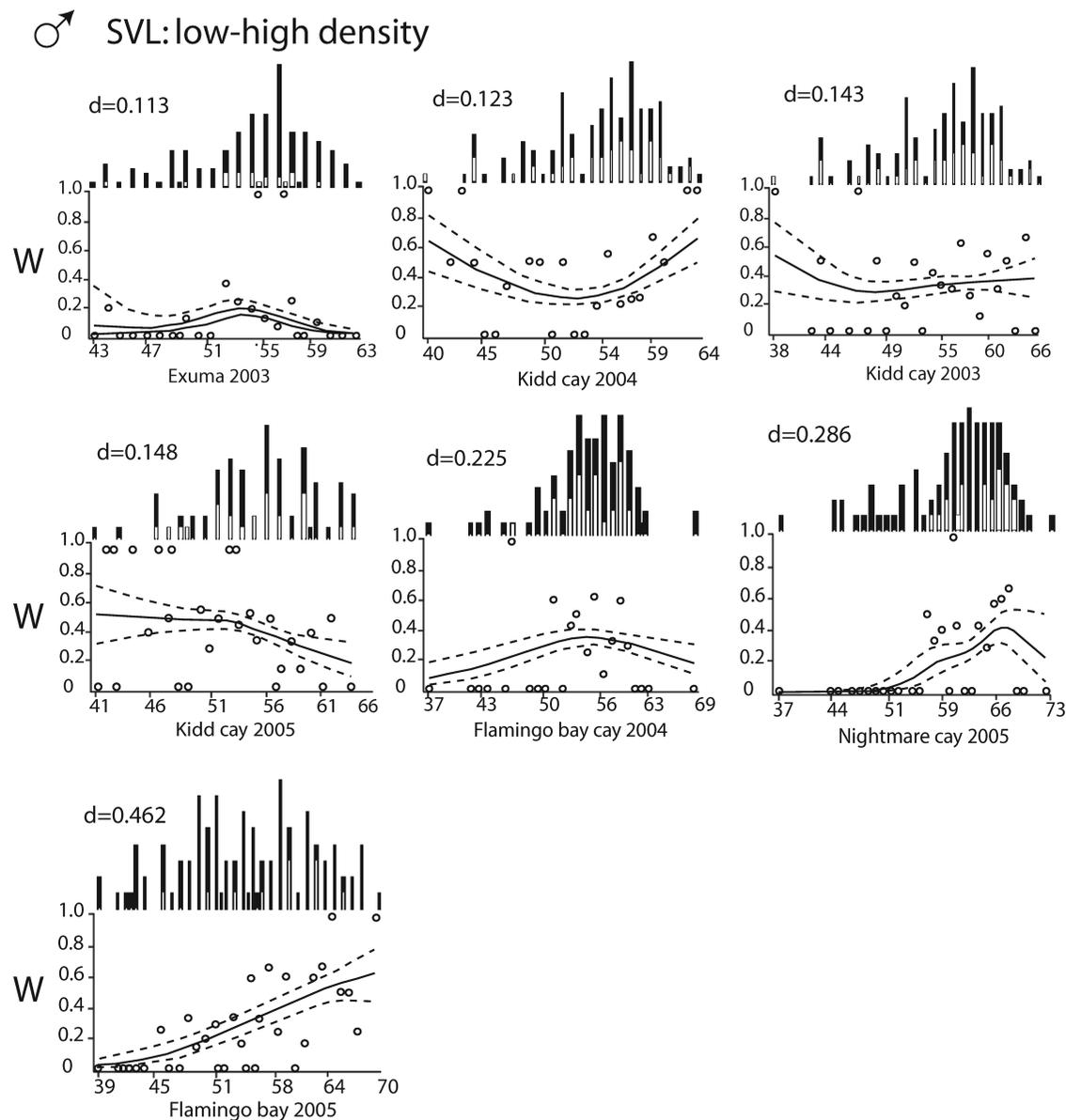
Year	Island	Area (m <sup>2</sup> )	N	Sex	Density	Survival (%)	β	SE	γ	SE
2003	KC	1604	99	F	0.143	35.40%	0.13	0.13	0.03	0.09
2003	GE	1500	170	M	0.113	11.80%	-0.03	0.21	-0.328*	0.17
2003	KC	1604	130	M	0.143	33.10%	0.02	0.19	0.11	0.10
2004	KC	1604	97	M	0.123	32.00%	-0.02	0.09	0.19*	0.08
2004	FBC	431	97	M	0.225	29.90%	0.09	0.15	-0.138*	0.09
2005	KC	1604	127	F	0.148	33.10%	0.12	0.15	-0.17	0.11
2005	KC	1604	111	M	0.148	40.50%	-0.17	0.12	-0.09	0.18
2005	NC	518	98	M	0.286	27.60%	0.54***	0.21	-0.03	0.20
2005	FBC	431	91	F	0.462	33.00%	0.85***	0.24	0.18	0.21
2005	FBC	431	108	M	0.462	34.30%	0.56***	0.19	0.03	0.13

the basis of the naturally occurring densities reported here, and elsewhere (Losos and Spiller 1999). In controlling the release of lizards to our experimental islands, our primary intention was to manipulate population densities. As a by-product of this experiment, we also altered the distribution of body sizes on experimental islands. The general effect of our body size manipulation was to shift the variance in the phenotypic distribution from low to high in alternate years and on different plots. For example, on Flamingo bay cay between 2004 and 2005, we experimentally increased the variance in male body size from 27.7 to 54.6, and

from 2003 to 2004 we experimentally decreased the variance in male body size on Kidd cay from 33.5 to 23.1 (Fig. 1).

**FITNESS ESTIMATES**

We estimate fitness as survival over the four-month period from initial capture in late May and early June, to our population censuses conducted during late September and early October. This time frame encompasses survival to sexual maturity and the end of the first breeding season. Censuses can be considered exhaustive because we recaptured all uniquely tagged (color marks) lizards



**Figure 1.** Fitness functions for male body size on low- to high-density plots with frequency distributions of body size shown above each spline. “W” represents fitness and data points show the probability of survival against the independent variable snout-vent length. Fitness functions show the best-fit cubic spline (solid line) with 95% confidence intervals (hatched lines) generated from 500 bootstrap replicates (Schluter 1988). Dark histogram bars show the phenotypic distribution before selection, light bars the distribution following selection. Lizard densities ( $d = \text{lizards}/\text{m}^2$ ) are given for each replicate to the left of the frequency distribution.

from each of our study islands. Lizards that were not recovered during our censuses were considered dead; a reasonable assumption given that emigration from islands is likely to be an extremely rare event except during hurricanes (Calsbeek and Smith 2003), none of which affected our study islands during the three years of this study. Although the majority of surviving lizards were captured within the first two days at each plot, censuses continued for two to three weeks, or until three consecutive days of searching turned up no new marked lizards.

We measured the strength of linear (i.e., directional,  $\beta$ ) and quadratic (either stabilizing or disruptive,  $\gamma$ ) selection in each population from separate multiple regressions of survival to fall against lizard body size (SVL) and the residuals of hind limb length (removing the effects of body size). We calculated relative fitness (standardized by the population mean) separately for each sex. All trait distributions were standardized to mean zero and unit standard deviation. The regression of relative fitness on standardized snout-vent length with limb length included in the model estimates the strength of selection (selection gradient) in each population (Lande and Arnold 1983). The strength of selection was estimated from the slope of the regression line using standard parametric statistics. Because the dependent variable "survival" was binomially distributed (live-1, die-0), we report significance values of each selection gradient from a logistic regression that accounts for binomial error variance (Janzen and Stern 1998). In our initial analyses, we also tested for an effect of mean body size, and variance in body size on survival. We visualized the forms of selection separately for each population using the cubic spline (Schluter 1988). Because a subset of islands were measured in multiple years, and because selection gradients were in some cases measured for both sexes from the same island, our results may be influenced by some degree of nonindependence. To account for this, and to further validate the above results, we performed a complementary analysis using the entire data set (all populations/years; > 1000 lizards). We tested the dependent variable "survival" against the independent variables: sex, body size, year, island area, density, and higher-order interaction terms.

To estimate the relationship between population density and fitness, we regressed our individual estimates of the selection gradient against population density. Each estimate of the selection gradient has an associated error, and we therefore estimated the relationship between selection and density using both reduced major axis regression and a randomization test. We performed our randomization test by bootstrapping the individual regressions of survival and body size from each study population. We performed 1000 bootstrap replicates of  $\beta$  for each population, randomly chose one estimate per population, and regressed these estimates against population density. We repeated this procedure 10,000 times and then calculated the median value of  $\beta$ , as well as the 5% and 95% confidence intervals from the regressions.

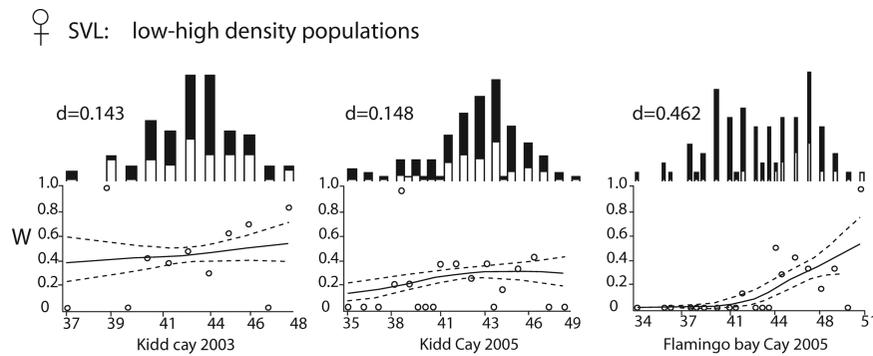
## HERITABILITY OF BODY SIZE

During 2004 we captured 50 gravid females from an adjacent population approximately 2 km from our study sites on the main island of Great Exuma and brought them into the laboratory to lay eggs. Females were housed in separate 10-gallon terraria and were provided with full spectrum lighting (12L:12D), and ad libitum food (*Achaeta* crickets) and water. Each female laid eggs in a potted plant, and eggs were left unmolested in the pots to incubate. Because females were housed individually, maternity of all offspring was known with certainty. Progeny were raised to adult body size in terraria apart from parents, initially fed a diet of baby crickets and *Drosophila*, and after two weeks, were fed adult crickets. At sexual maturity, F1 female progeny were allowed to mate with F1 males (no mating within sib groups). The F2 progeny were raised in the same conditions described earlier. We measured the broad sense heritability in body size that arises between dams and progeny as twice the slope of the regression between dams and progeny. Although this estimate may be confounded by maternal effects (Falconer and MacKay 1996), we report it here because most dams mated in the field prior to capture, and paternity could only be scored for a subset of F1 progeny. This analysis included the regression of both F2 progeny on F1 dams and F1 progeny on field caught dams. We present the least-squares regression of the family mean SVL on dam SVL based on  $N = 141$  progeny from 33 different dams (mean family size =  $3.8 \pm 1.8$  progeny).

## Results

### SELECTION ON INDIVIDUAL STUDY ISLANDS

Univariate fitness functions for all study islands and all years illustrate variation in the strength and form of selection that was related to density (Fig. 1, Fig. 2). Although the nature of selection varied, mean percent survival to fall was similar across most study plots (31%, SE = 0.02), and was not correlated with density (spearman  $\rho = -0.33$ ,  $P = 0.29$ ; rates of survival for individual study islands are given in Table 2). During 2003 we measured selection on two naturally occurring low-density island populations: one population on Kidd cay and a second population on Great Exuma. On Great Exuma, disturbance by construction surveyors and clearing of underbrush probably increased mortality and resulted in low survival (12%; Table 2) at this site. Body size was under weak and nonsignificant directional selection (Figs. 1 and 3) on low-density Kidd cay for both males and females ( $\beta = 0.02$ , logistic regression Wald  $\chi^2 = 0.03$ ,  $P = 0.85$ , and  $\beta = 0.13$ ,  $\chi^2 = 0.88$ ,  $P = 0.34$  for males and females, respectively, covariate for limb length, both  $P > 0.24$ ). On low-density Exuma, directional selection was nonsignificant ( $\beta = -0.03$ , Wald  $\chi^2 = 0.06$ ,  $P = 0.87$ ; covariate for limb length  $P = 0.87$ ), whereas stabilizing selection tended to favor males of intermediate size ( $\gamma_{1,1} = -0.328$ , Wald  $\chi^2 = 4.42$ ,  $P = 0.03$ ; Fig. 1).



**Figure 2.** As in Figure 1, but splines are for female body size on low- and high-density plots.

During 2004 we experimentally replaced all of the male lizards on Kidd cay but left the natural density unchanged. We again detected weak and nonsignificant directional selection on males (Fig. 1, Table 2), but we also measured significant disruptive selection on male size ( $\gamma_{1,1} = 0.19$ ; Wald  $\chi^2 = 5.67$ ,  $P < 0.01$ ; covariate for limb length  $P = 0.52$ ). On medium-density Flamingo bay cay, selection was stabilizing and tended to favor males of slightly larger than average size (Fig. 1) ( $\gamma_{1,1} = -0.138$ ;  $\chi^2 = 5.94$ ,  $P < 0.01$ ). During 2005 we measured selection on the naturally occurring low-density population on Kidd cay, and on experimentally manipulated populations on medium-density Nightmare cay and high-density Flamingo bay cay. Selection on Kidd cay was stabilizing during 2005 in males and females (no effect of sex,  $\gamma_{1,1} = -0.18$ ,  $\chi^2 = 5.85$ ,  $P < 0.01$ ; covariate for limb length  $P = 0.59$ ). By contrast, selection was strong ( $\beta > 0.5$ ; Kingsolver et al. 2001) and directional on the medium-density island favoring larger male body size on Nightmare cay ( $\beta = 0.535$ , Wald  $\chi^2 = 9.39$ ,  $P = 0.009$ ; covariate for limb length  $P = 0.9$ ) (Fig. 1), and on the high-density island favoring larger male and female body sizes on Flamingo bay cay ( $\beta = 0.557$ , Wald  $\chi^2 = 15.11$ ,  $P < 0.0001$  and  $\beta = 0.846$ , Wald  $\chi^2 = 8.16$ ,  $P = 0.004$  for males and females, respectively; both covariates for limb length  $P > 0.50$ ; Figs. 1 and 2).

Results were significant when we tested for an effect of mean body size, and variance in body size on survival (Table 3). More-

**Table 3.** Analysis of variance of the strength of selection ( $\beta$ ) acting on density and body size (linear and quadratic terms) shows that density-dependent selection was not “pure” in that not all populations and years were affected by density in the same way.

Source for $\beta$	df	Mean square	F	P
Density	1	0.093	2.93	0.147
Body size	1	0.416	13.19	0.015
Density <sup>2</sup>	1	0.196	6.21	0.055
(Body size) <sup>2</sup>	1	0.434	13.76	0.014
Density $\times$ body size	1	0.362	11.48	0.019
Residual	5	0.032		

over, a pooled analysis of all populations across all years, which partially accounts for nonindependence of study islands measured in multiple years, supported the above results and indicated that the strength of selection varied by body size (density  $\times$  body size;  $F_{1,1123} = 12.70$ ,  $P < 0.0005$ ; Table 4).

**DENSITY AND THE SELECTION DIFFERENTIAL**

We calculated changes in the mean and variance in lizard body size following each episode of natural selection. The change in mean body size after selection is the selection differential (Lande and Arnold 1983) and was positively correlated with density ( $r_{\text{density}} = 0.775$ ,  $F_{1,8} = 12.02$ ,  $P < 0.0085$ ). The change in variance was also correlated with the strength of selection, but the sign of this relationship depended on whether selection was linear or quadratic. Variance in body size decreased with increasing linear selection pressure ( $r_{\beta} = 0.801$ ,  $F_{1,8} = 14.37$ ,  $P < 0.005$ ; see Fig. 1), and not surprisingly, increased with increasing quadratic selection pressure ( $r_{\gamma} = 0.588$ ,  $F_{1,8} = 4.29$ ,  $P = 0.07$ ). This latter result is the logical outcome of the fact that stabilizing selection decreases trait variance and has a negative coefficient ( $-\gamma$ ), whereas disruptive selection increases trait variance and has a positive coefficient ( $+\gamma$ ).

**Table 4.** Analysis of variance illustrating that variation in body size and population density among study islands explains survival differences among individuals. There was no effect of sex, and no higher-order interaction terms were significant in the model.

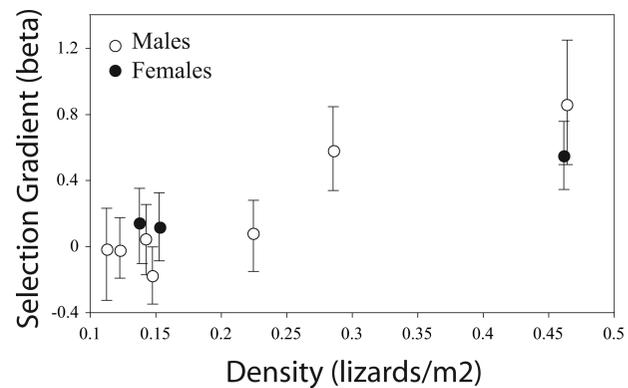
Source for W	df	Mean square	F	P
Body size	1	1.52	7.95	0.0049
Density	1	3.86	20.18	0.0001
Year	2	2.87	7.50	0.0006
Island area	1	0.95	4.95	0.02
Density $\times$ body size	1	2.42	12.70	0.0004
Density $\times$ year	2	3.81	9.96	0.0001
Residual	1115	0.191		

Finally, we measured significant broad sense heritability of body size in the laboratory ( $h^2 = 0.55 \pm 0.13$ ;  $F_{1,32} = 4.47$ ,  $P < 0.04$ ). We did not detect any significant effect of year or progeny sex in this analysis. For comparison, we also report the coefficient of additive genetic variation  $CV_A = 2.79$  for body size. Houle (1992) has shown that, in some cases, high residual variation in fitness-related traits makes  $CV_A$  a more useful measure of evolutionary potential than is heritability.

We approximated the adaptive peak for body size in each population using the Mitchell-Olds and Shaw method (Mitchell-Olds and Shaw 1987). We excluded the two cases of strong directional selection on Flamingo bay cay in 2005 because the optimum was found to be outside the range of phenotypes measured in the study. We then regressed the value of the adaptive optimum for body size against the local density in each study population. We found a significant positive relationship between density and optimum body size, after controlling for sex differences ( $F_{2,5} = 2.87$ ,  $P < 0.03$ , effect of sex  $F_{2,7} = -2.54$ ,  $P = 0.05$ ). Thus, the strength of directional selection increased with increasing density, and so too did the optimum lizard body size. This suggests that body size and density together are important in determining the strength of selection.

#### DENSITY EFFECTS ON THE STRENGTH OF SELECTION

Each replicate island provides a point estimate of the strength of selection on lizard body size. We regressed these measures of selection ( $\beta$  or  $\gamma$ ) against the density of lizards on each island to estimate the importance of density-dependent natural selection. The strength of directional selection increased with increasing lizard density and this relationship was highly significant ( $r^2 = 0.81 \pm 0.19$ ,  $F_{1,8} = 34.31$ ,  $P < 0.0004$ ; Fig. 3). There was no significant relationship for nonlinear terms ( $\gamma$ ), and there was no significant effect of year or study island apart from those derived from our density treatment (both  $P = 0.24$ ). In addition, there was no difference between the small cays (Nightmare and Flamingo bays) and the larger Kidd cay in the relationship between  $\beta$  and density ( $F_{2,6} = 2.5$ ,  $P = 0.16$  for null hypothesis that slopes and intercepts were equal; Neter and Wasserman 1990, pp. 160–167). Reduced major axis regression further supported the relationship between the strength of selection and density (delete-one jackknife  $r^2 = 0.841 \pm 0.10$ ), as did the bootstrapped estimates of  $\beta$  (median value of  $\beta = 2.24$ , 95% CI = 1.54–2.93; Fig. 3). We detected a significant interaction effect between the mean body size and population density on each island (density  $\times$  mean SVL  $F_{1,4} = 7.77$ ,  $P < 0.04$ ), indicating that smaller lizards were disproportionately selected against at high density compared with low density. This result suggests that density-dependent regulation in our study was not “pure,” that is, selection is likely to be partially influenced by frequency dependence (Fryxell et al. 1999) on the basis of the



**Figure 3.** The strength of selection ( $\beta$ ) increased with increasing density. The panel shows results for males (open circles) and females (filled circles) together. Male and female points from the same location are slightly separated along the x-axis for ease of visualization. Points show median values of  $\beta$  with 5% and 95% confidence intervals that were generated from 10,000 bootstrapped replicates of the data. The regression of  $\beta$  on density was highly significant and explained more than 80% of the variation ( $P < 0.0002$ ).

result that the change in density did not affect all populations and years equally.

#### Discussion

Studies of natural selection on traits important to fitness are seldom replicated either spatially or temporally (Merila et al. 2001; McAdam and Boutin 2003; Svensson et al. 2005), and often consider important ecological factors such as density to be noise (but see below). Here, we have used a combination of natural and experimentally induced variation in lizard densities over multiple years to reveal the environment's role as an agent of natural selection. Laboratory studies, particularly with *Drosophila* (Mueller and Ayala 1981; Shakarad et al. 2005), have provided a large literature on density-dependent selection, and a broad range of studies from nature, including studies of fish (Bolnick 2004), plants (Dudley and Schmitt 1996), mimicry systems (Anderson and Johnson 2006), insects (Anholt 1991; Krupa and Sih 1993; Hopper et al. 1996), and other lizards (Svensson and Sinervo 2000; Le Galliard et al. 2005), have experimentally manipulated density in the wild to demonstrate its importance to selection. More rare are studies that use experimental variation in both the phenotype and the environment to study natural selection (Svensson and Sinervo 2000). Although difficult to perform in the wild, such “double level” experiments (Svensson and Sinervo 2000) that vary both phenotype and environment provide additional information for understanding the mechanisms that lead to natural selection. Future manipulations of offspring size (Svensson and Sinervo 2000)

could provide additional statistical power to additional tests of density-dependent selection in anoles.

The strength of directional selection on lizard body size increased with increasing population density in our study. Although many point estimates of the selection gradient were individually nonsignificant (see Table 2), the central result of our study shows that density effects explained over 80% of the variation in the strength of selection (Fig. 3). Although large body size was clearly adaptive at high density, lower densities (e.g., 0.113–0.148 lizards/m<sup>2</sup>) tended to favor individuals of more intermediate size. During 2004 we measured significant disruptive selection in the low-density population on Kidd cay, indicating that under some conditions, selection may include an optimum for smaller male body size. This result is almost certainly due, at least in part, to the slightly larger size of Kidd cay compared to the other offshore islands. By virtue of its larger size, Kidd cay contains more adult trees, which provide broad diameter perching surfaces to the lizards. Previous work (Losos 1990; Losos et al. 1994; Irschick and Losos 1998) has shown that the diversification of anoles in the Greater Antilles is driven in part by variation in habitat use; performance differences favor larger and relatively longer-limbed lizards on broad perching surfaces, and smaller, relatively shorter-limbed lizards on narrow perching surfaces (Losos and Sinervo 1989). Habitat differences cannot explain the entire pattern; however, as larger and longer-limbed lizards are consistently favored on offshore cays (Losos et al. 2004, and this study) where there is also a substantial amount of narrow perch-diameter vegetation available to lizards. Moreover, selection was stabilizing on Kidd cay during 2005. Rather, some interaction between population density and habitat use may make competitive effects more important at high density, and variation in locomotor performance more important at low density. As such, selection in low-density populations might be expected to show greater interannual variation with changes in habitat structure, although competitive effects would maintain consistency in the form of selection at high density. Finally, it is important to note that any selection on other traits or moments correlated with body size may have indirectly influenced selection on body size in this study (Lande and Arnold 1983; Brodie et al. 1995). The role of habitat use in driving selection on morphology remains under current investigation in our laboratory.

One caveat to consider here is that we were unable to manipulate density *within* islands. This problem arose because small islands like Nightmare cay are simply so small (e.g., ~500 m<sup>2</sup>) that low-density populations would contain too few individuals to provide robust estimates of the selection gradient (Brodie et al. 1995; Kingsolver et al. 2001). Thus, an alternative explanation that might partially explain the patterns of selection found in our study is that an island effect other than variation in density drove differences in selection. Although we cannot rule out this possibil-

ity, density was the only significant effect in a multiple regression of  $\beta$  versus both density and island area. Moreover, including island identity as a random factor in our analyses did not change our results qualitatively, and neither the slopes nor intercepts differed between the larger and smaller islands in the relationship between  $\beta$  and density. We therefore believe that population density remains the most likely explanation for the patterns observed.

Traits that have a heritable basis should respond to selection in proportion to the strength of selection and the heritability of the trait. There are a large number of studies demonstrating the heritability of body size in a variety of taxa (Campton 1992; Keightley and Hill 1992; Merila and Fry 1998; Ahnesjo and Forsman 2003) and our own broad sense estimates indicate significant heritability of snout-vent length in *Anolis* lizards. Thus, the selection reported here should have an evolutionary response.

### COMPETITION AND NATURAL SELECTION

Interspecific competition has been implicated in the diversification of anoles because competition plays an important role in setting up selection based on differences in habitat use. Our results suggest that intraspecific competition also has an important influence on selection in these lizards. Although we did not directly measure agonistic interactions in the context of our density manipulations, it is reasonable to assume that competitive interactions should increase at high density (Brockelman 1975). Anecdotal observations in high-density populations suggest that the frequency and/or intensity of male–male interactions increased at high population density (personal observation, D. Spiller pers. comm.). Moreover, body size is an important predictor of competitive ability in these (Tokarz 1985) and other lizards (Stamps and Krishnan 1995; Olsson and Shine 2000; Calsbeek and Sinervo 2002) further supporting a role for competition in our results. Interestingly, patterns of selection at high density were identical in males and females, indicating that competitive interactions among females may be selectively important as they are in males. Female–female interactions have received considerably less attention than male–male interactions (but see Comendant et al. 2003) and likely have an underappreciated significance in the population dynamics of this and other taxa.

During 2003 Great Exuma had the lowest lizard density of any of our study sites and the magnitude of the selection gradient ( $\gamma_{1,1} = -0.328$ ) was one of the highest in our study. At the other density extreme, the directional selection gradient measured during 2005 in our high-density population on Flamingo bay cay ( $\beta = 0.846$ ) is one of the strongest selection coefficients reported from the wild (Kingsolver et al. 2001). Thus, although the strength of directional selection clearly increased with density in our study, the effect may be subtly more complicated than the linear relationship depicted in Figure 4. Our results suggest that the form of selection (e.g., directional, stabilizing, disruptive) may likewise

vary within populations, especially at low density. Curvature in the fitness functions (Schluter 1988) was inconsistent at low density, and selection took both stabilizing and disruptive forms in different years. However, our quantitative estimates reveal that trait variance increased following selection at low density, but decreased at high density. This role of density in shaping the adaptive landscape is not consistent with that predicted by classical ecological theory, which posits that high population density and competition, and consequent negative frequency-dependent selection could drive disruptive selection (Kondrashov and Kondrashov 1999). This idea has received little empirical support, however (but see Bolnick 2004), and clearly more work in this area is warranted.

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