

Experimentally assessing the relative importance of predation and competition as agents of selection

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Field experiments that measure natural selection in response to manipulations of the selective regime are extremely rare¹, even in systems where the ecological basis of adaptation has been studied extensively. The adaptive radiation of Caribbean *Anolis* lizards has been studied for decades^{2–5}, leading to precise predictions about the influence of alternative agents of selection in the wild. Here we present experimental evidence for the relative importance of two putative agents of selection in shaping the adaptive landscape for a classic island radiation. We manipulated whole-island populations of the brown anole lizard, *Anolis sagrei*, to measure the relative importance of predation versus competition as agents of natural selection. We excluded or included bird and snake predators across six islands that ranged from low to high population densities of lizards, then measured subsequent differences in behaviour and natural selection in each population. Predators altered the lizards' perching behaviour and increased mortality, but predation treatments did not alter selection on phenotypic traits. By contrast, experimentally increasing population density dramatically increased the strength of viability selection favouring large body size, long relative limb length and high running stamina. Our results from *A. sagrei* are consistent with the hypothesis⁶ that intraspecific competition is more important than predation in shaping the selective landscape for traits central to the adaptive radiation of *Anolis* ecomorphs.

Natural selection is often measured in wild animal populations, but only rarely are changes in selection quantified in response to experimental manipulations of the putative ecological agents of selection^{7,8}. As a result, our current view of selection in the wild is based almost entirely on correlations⁹. This represents a major impediment to understanding the ecological basis of the most important force of evolution¹⁰. Here, we simultaneously manipulate predation and competition to disentangle the causal agents of natural selection using an experimental framework in the wild.

Lizards in the genus *Anolis* comprise one of the most well-studied vertebrate radiations on the planet, with nearly 400 species distributed throughout the Caribbean and the mainland Americas^{5,11,12}. In the Greater Antilles, patterns of *Anolis* diversification are correlated with differences in habitat use, and species are grouped into 'ecomorph' classes based on associations between morphology and the habitat in which species are most often found³. *Anolis* ecomorphs have evolved independently on separate islands^{5,12}, suggesting consistent patterns of selection on key traits such as body size and relative limb length. These traits are subject to selection because they are ecologically relevant to locomotor performance and fitness in different habitats. For example, relative limb length differentially influences speed and agility on broad- and narrow-diameter vegetation, and this probably contributes to the efficiency of prey capture and territory defence¹³. Larger body size is particularly favoured when access to resources is determined by fighting ability¹⁴, and variation in body size may also relate

to trophic and thermal resource partitioning^{15,16} and susceptibility to predators^{17,18}. Thus, the relations between morphology, performance and fitness are well understood, and can be linked to ecological factors such as predation and competition, providing a foundation for experimental tests of natural selection in wild populations¹⁹.

One long-standing prediction about the selective agents acting on anoles is that the adaptive landscape of mainland anoles is primarily shaped by predation, whereas that of island populations in the West Indies is driven by competition^{5,6}. This hypothesis is motivated by the observation that mainland anoles generally occur at lower density, have greater food abundance, earlier maturation, reduced sexual dimorphism and lower adult survivorship than island anoles^{5,6}. All of these differences are predicted by classic island biogeography theory for populations that are differentially affected by predation and competition²⁰. Recent studies have experimentally demonstrated that both predation¹⁷ and competition²¹ can influence selection in wild anole populations, yet no study has simultaneously examined their relative importance in determining the strength and form of selection.

We tested the relative significance of predation and competition by manipulating replicate island populations of the brown anole, *A. sagrei*, in The Bahamas. We selected small, offshore study islands based on their similarity in vegetation structure, such that variation in island area (range 800–2,300 m²) would generate a natural range of population densities (0.09–0.30 lizards per square metre)²² when seeded with lizards ($n = 40$ –80 males, 150 females per island). During spring (May), we captured and uniquely marked lizards from a single population on the nearby main island of Great Exuma and then released them to our experimental islands. Females were included in estimates of total lizard density on each island, but our analyses of selection focused on adult males (see Methods). For each male, we measured body size (snout–vent length to the nearest 1.0 mm), relative hindlimb length (to the nearest 0.5 mm) and stamina (running endurance on a treadmill, in seconds) following standard methods¹⁹. Four months later, at the end of the breeding season (September), we conducted population censuses to recapture survivors and measure the strength and form of viability selection on these traits^{21,23}. Because our study islands are small and preclude dispersal, we can reliably catch nearly all (approximately 95%)²¹ surviving lizards, resulting in robust estimates of natural selection.

We conducted three predator treatments (no predators, bird predators only, bird and snake predators) with two island replicates of each treatment. Islands in the 'no predators' treatment were enshrouded with No-Tangle bird-proof netting to exclude avian predators. Islands in the 'bird predators' treatment received an equal amount of netting around their perimeters to control for the structural addition of nets, but were left open to avian predators. Islands in the 'bird and snake predators' treatment were seeded with predatory snakes (*Alsophis vudii*, Bahamian racer), such that lizards were exposed to both avian and terrestrial predators. Racers are naturally

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absent from our small experimental islands, although they are abundant on most major islands in The Bahamas, where we have repeatedly observed them hunting and capturing adult male anoles. To manipulate simultaneously the strength of intraspecific competition, we distributed our three predation treatments across a range of low (mean 0.14 lizards per square metre) to high (mean 0.26 lizards per square metre) population densities. In total, we introduced 1,329 individual lizards to our experimental islands. Finally, we monitored an unmanipulated island as a natural reference population. Power analyses (see Supplementary Information) indicated a high probability of detecting important treatment effects (mean power 0.96, range 0.72–0.99).

Variation in survival on experimental islands (range 0.20–0.70; mean 0.40) was similar to that of the natural reference population measured over six years (range 0.26–0.48; mean 0.37)¹⁹. Patterns of survival and perching behaviour confirmed the efficacy of our predation manipulations and revealed particularly strong effects of snake predators. Survival was lowest when lizards were exposed to both bird and snake predators and did not differ between other predation treatments (generalized linear model (GLM) with logit link function: $\chi^2 = 6.54$; $n = 6$; $P < 0.03$; Fig. 1). Male lizards on islands exposed to both bird and snake predators were also recaptured higher in the canopy than were lizards in the two other predation treatments (GLM: $\chi^2 = 12.67$; $n = 6$; $P < 0.005$; Fig. 1). This predator-induced change in perching behaviour is consistent with another recent study of the same species involving a different terrestrial predator¹⁷. However, despite the clear impact of terrestrial predators on survival and perching behaviour, the strength and form of selection on morphological and performance traits did not differ across our predation treatments (Fig. 2). Thus, terrestrial predators increased lizard mortality and altered habitat use, but these changes did not alter patterns of phenotypic selection.

In contrast to predation treatments, our density manipulations provided strong evidence for a central role of competition in shaping the adaptive landscape. Viability selection was stronger at high density than low density for body size (GLM: $\chi^2 = 3.35$; $n = 7$; $P = 0.06$), stamina (GLM: $\chi^2 = 5.36$; $n = 7$; $P = 0.02$) and residual hindlimb

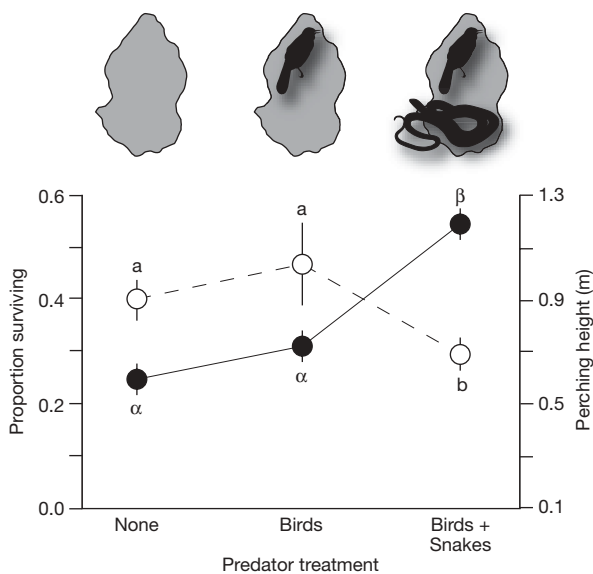


Figure 1 | Differences in survival (open circles) and perching behaviour (filled circles) as a function of predator treatment. Survival was lowest on experimental islands exposed both to bird and snake predators. Lizards on these six islands were also perched significantly higher in the canopy when recaptured. Letters indicate significant post-hoc differences among treatment groups for survival (a, b) and perch height (α , β). Error bars show variance (± 1 s.e.m.) in survival across experimental replicates and variance (± 1 s.e.m.) in perching behaviour across individual lizards. Illustrations depict experimental treatments.

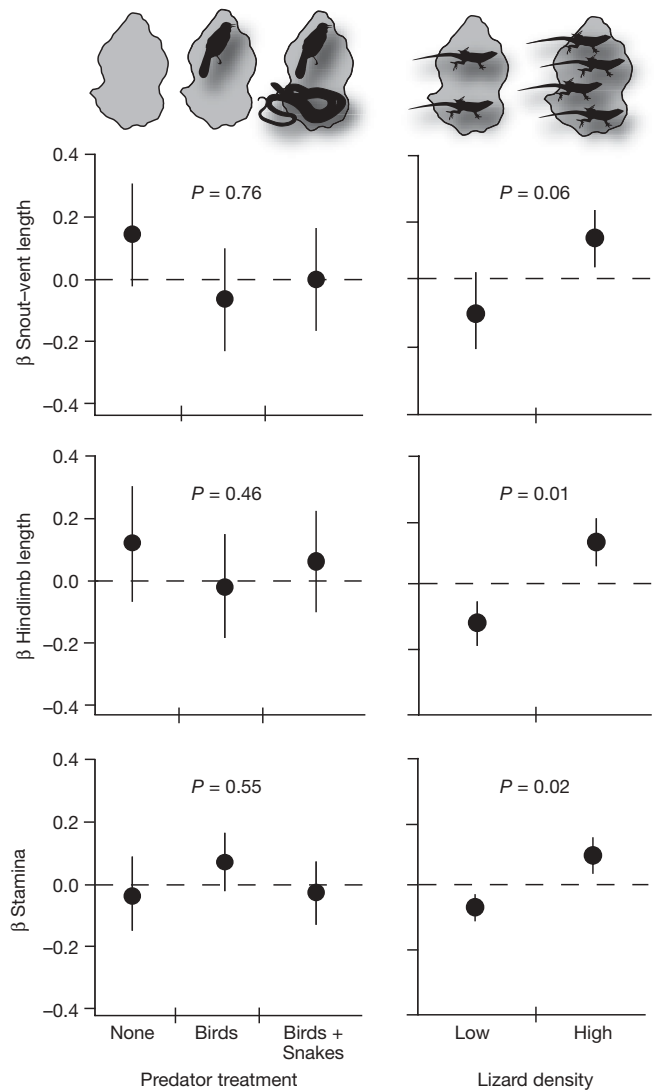


Figure 2 | Left panels show mean values (± 1 s.e.m.) of selection gradients measured in two replicates across each of three predation treatments. Right panels show mean values (± 1 s.e.m.) of selection gradients measured at low and high population densities (Supplementary Fig. 2 shows regressions of gradients against continuous variation in density). High and low densities were greater or less than 0.2 lizards per square metre, respectively (ref. 28). Differentials were measured within each island and then corrected for year effects. Illustrations above each panel depict treatments.

length (GLM: $\chi^2 = 6.25$; $n = 7$; $P = 0.01$) (Fig. 2). These results are consistent with a previous study of this species in which population density was manipulated across seven populations and 3 years²¹. As in that study, the present data suggest that high population density alters the strength and form of viability selection acting on anole phenotypes. Large body size provides a competitive advantage in many reptiles²⁴, and traits such as limb length and stamina are also presumably important in competitive situations, given their central roles in prey capture and territory defence. Thus, increased competition leads to strong selection on these traits, whereas reduced competition relaxes this selection pressure and may favour lower trait values (Fig. 2).

To test the relative importance of predation and competition in our study, we compared linear models that included terms for competition alone, predation alone and for both competition and predation together. We used Akaike's information criterion with a second-order correction (AIC_c)²⁵ to assess the best-fit model. Comparison of AIC_c scores revealed that the model including a term

Table 1 | Comparison of AIC_c scores for alternative models to explain variation in natural selection arising from competition (density), predation or both competition and predation

Trait	Agent of selection	Sum of squared errors	Number of parameters	AIC _c	ΔAIC _c
Snout-vent length	Competition	0.13	3	-14.46	0
	Predation	0.26	4	4.95	19.41
	Competition + predation	0.10	5	40.26	54.66
Relative hindlimb length	Competition	0.17	3	-12.45	0
	Predation	0.19	4	2.75	15.20
	Competition + predation	0.07	5	37.76	50.21
Stamina	Competition	0.03	3	-24.17	0
	Predation	0.06	4	-5.32	18.85
	Competition + predation	0.03	5	31.10	55.27

AIC_c scores for each trait were calculated from separate models, and we report the sum of squared errors and number of parameters from each model. ΔAIC_c values highlight that models with competition as the sole factor were preferred in all of our selection estimates.

for competition, but not predation, provided the best explanation for variation in selection on all phenotypic traits (Table 1).

Our results strongly implicate competition as the central driver of viability selection on island populations of *A. sagrei*. Predation could still contribute to variation in the form of selection, but its effects are clearly weak relative to those of competition. Larger numbers of experimental replicates may be required to demonstrate any significant effects of predators¹⁷. However, the logistical constraint of greater experimental replication is often smaller sample sizes within replicates¹⁷, which can vitiate the estimates of selection themselves. Measuring the influence of predation on natural selection may also depend on the timescales over which selection is measured, especially if behavioural shifts in response to predation change the selective landscape through time²⁶. Consistent with previous work²⁶, our manipulations of predators resulted in differences in perching behaviour: lizards exposed to snake predation were recaptured significantly higher off the ground than lizards from other predator treatments. If this behavioural shift were permanent, one might predict an eventual shift towards selection favouring relatively shorter limbs to facilitate arboreal locomotion²⁶.

Although we have shown that viability selection on adult anoles is more strongly influenced by competition than by predation, we note that a full consideration of lifetime fitness requires estimates of viability selection on juveniles and sexual and fecundity selection on adults²⁷. Finally, we note that interpretations of any experimental study should consider the extent to which an experiment mimics natural variation. Our experimental densities were similar to natural variation²⁸ and produced large effects on selection. By removing predators from entire islands, we probably generated variation in predation that was below natural levels, yet we detected no effect on selection. All else being equal, this strengthens our interpretation that competition is relatively more important than predation in driving viability selection on body size, relative limb length and stamina in brown anoles. Body size and relative limb length are of particular interest because they are key morphological features in the adaptive radiation of *Anolis* ecomorphs in the Greater Antilles³.

We have provided the first experimental evidence for the relative importance of two putative agents of selection in shaping the adaptive landscape for a classic island radiation. Our results support the hypothesis that lizard populations in the West Indies are primarily influenced by competition rather than predation. The second half of the hypothesis, that predation rather than competition drives selection in mainland anoles, would be a next logical pursuit. Tests of this hypothesis will be made more difficult by the very facets of population biology that led to the predictions, namely lower population density and longer lifespan. More generally, we emphasize that additional experimental manipulations of natural selection in any study system will prove invaluable for our continued understanding of the processes that shape adaptive landscapes in natural populations¹.

METHODS SUMMARY

Selection analyses. Lizards were captured from the main island of Great Exuma, Bahamas (23° 29' N, 75° 45' W) and transplanted to small offshore islands. Each

lizard was given a permanent and unique marking by clipping one toe from each foot. We never clipped the fourth toe (which may be especially important for locomotion) and survival rates of toe-clipped lizards in this study did not differ from our previous studies using injectable coloured tags²⁸. We measured stamina by running lizards to exhaustion (measured as the loss of righting response) on a treadmill (0.4 km h⁻¹)²⁹. We released approximately 40 males to each island during 2008 and 80 males during 2009. In both years we also released approximately 150 females per island, which we consider here as part of the manipulation of lizard densities. However, we excluded females from our selection analyses because patterns of selection on females differ dramatically from those on males³⁰, and because these females were surgically manipulated as part of a separate study of reproductive investment. During 2008, we established one 'no predators' treatment and one 'bird predators' treatment, and monitored one unmanipulated reference island with a natural predation regime (150 males and 232 females). During 2009, we replicated our 'no predators' and 'bird predators' treatments on different islands to avoid confounding island effects with predation effects. We also added three adult racers to each of two additional islands, thus creating 'bird and snake predator' treatments (Supplementary Table 1). Surviving lizards were removed from each study island during our autumn censuses such that, each year, we began with a new experimental population of lizards.

During our recapture censuses, we estimated perch height for all lizards that were not in motion when first spotted. Recapture success is a reliable measure of survival on experimental islands^{19,21,23}. Experiments were initiated in May and recapture censuses took place at the end of September. This time frame encompasses most of the breeding season. We quantified selection by regressing relative survival (0 or 1 divided by mean survival rate) on trait values standardized to the population mean in unit variance. Significance ($P < 0.05$) was determined by logistic regression and treatment differences were inferred from significant treatment × trait interactions.

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