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Predator-driven natural selection on risk-taking behavior in anole lizards

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Title: Predator-driven natural selection on risk-taking behavior in anole lizards

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One sentence summary: "Natural selection on behavior"

Abstract

Biologists have long debated behavior's role in evolution, yet understanding its role as a driver of adaptation is hampered by the scarcity of experimental studies of natural selection on behavior in nature. After showing that individual *Anolis sagrei* lizards vary consistently in risk-taking behaviors, we experimentally established populations onto eight small islands either with, or without, *Leiocephalus carinatus*, a major ground predator. Selection predictably favors different risk-taking behaviors under different treatments: exploratory behavior is favored in the absence of predators whereas avoidance of the ground is favored in their presence. On predator islands, the selection on behavior is stronger than selection on morphology, whereas the opposite holds on islands without predators. Our field experiment demonstrates that selection can shape behavioral traits, paving the way to adaptation to varying environmental contexts.

Understanding the role of behavior in adaptation of animals to new environmental circumstances remains a major challenge in biology. Research has long addressed the debate about whether behavior spurs or impedes evolution (*1–3*) on phenotypic dimensions such as morphology (*4*, *5*) or physiology (*6*). In order to unravel the process by which behavior shapes adaptation, we must examine how natural selection operates among individuals in a population (*7*, *8*). Recent growth in the study of inter-individual variation in behavior (*9–11*) has revealed that behavior often varies consistently among individuals within a population (*12*), and recent studies have also suggested this variation has fitness consequences (*13–16*). These observations set the stage to investigate the hypothesis that natural selection on inter-individual variation in behavior could drive different ecological and evolutionary trajectories for populations under different selective regimes (*7*, *8*, *17–20*). Assessing a hypothesis like this under natural conditions requires controlled experiments in which natural selection is quantified under contrasting selective regimes generated by manipulating well-known selective pressures (*21*). Here we used small Caribbean islands as replicates to test directly whether and how natural selection operates on lizards with different behaviors and morphologies under different selective regimes.

We conducted this experiment on a well-studied predator-prey system involving the small lizard *Anolis sagrei* – commonly found on or near the ground (*22*, *23*) – and its ground-dwelling predator, the larger lizard *Leiocephalus carinatus* (*24*) (Fig. 1A). We focused on individual variation in two behaviors of *A. sagrei* (Fig. 1B) that are consistently repeatable across time and in different contexts within individuals of this species (*25*, see repeatability scores from this study in Table S1). Specifically, the rapidity of individuals to explore new and potentially dangerous environments and the time individuals spend on the ground and thereby potentially exposed to ground-dwelling predators (*26*). The ecological relevance of these risk-taking behaviors in *A. sagrei* is illustrated by a simple cost-benefit tradeoff (*27*, *28*). *A. sagrei* individuals more willing to explore new environments should survive better in the absence of significant predation pressures (*17*) because they are more likely to obtain resources. In contrast, *A. sagrei* individuals that spend more time exposed on the ground are more vulnerable to ground predators as compared with individuals that spend less time exposed on the ground (*22*, *25*, *29*). Previous studies have reported differences in habitat use and modulation of social signals in *A. sagrei* populations in the presence or absence of *L. carinatus* (*30*, *31*), leading us to hypothesize that variation in risk-taking behavior might be adaptive.

To experimentally examine natural selection on these risk-taking behaviors under natural conditions, we translocated 274 adult *A. sagrei* individuals onto eight small islands in the Bahamas (Fig. S2). Lizards were captured from source islands in the study area that generally have higher vegetation and host more complex biological communities (*32*) than our experimental islands, which have scrubbier, shorter vegetation and do not support resident populations of any known lizard predator (see Table S2A).

Before translocation, we used outdoor laboratory behavioral assays following (*25*, see details in *26*) to characterize inter-individual variation in two behaviors known to consistently vary among individuals (*25*, see also Table S1). After being exposed to the presence of a *L. carinatus* (position 1 in Fig. 1B), 'time to initiation of exploration in a new environment' was defined as the amount of time until the lizard started exploring the experimental cage by poking its head out of the wooden refuge (position 2 in Fig. 1B). 'Time exposed on the ground' corresponded to the interval of time during which the lizard was out of the refuge (position 3 in Fig. 1B) until it climbed on the perch or hid underneath the rocks (position 4 in Fig. 1B). Each lizard was X-rayed (Fig. 1C) and individually tagged before translocation onto experimental islands. We randomly assigned individuals to islands. Each island received lizards in proportion to its vegetated area, which was determined conducting vegetation transects following (*32)(see details in 26*). A week later, we added *L. carinatus* on four randomly selected islands, while the other four islands remained as predator-free controls. Four months later, we re-captured lizards on each of the experimental islands and identified surviving adult lizards from their individually unique sub-cutaneous tags.

Based on re-capture data, we found that survival was lower on predator islands as compared to predator-free islands (mixed-effects model including island ID as a random factor and modeled following the Binomial Distribution; $p < 0.001$; Fig. S3A). We also observed that *A. sagrei* from predator islands used the ground less frequently (16.9 % of observations) than those from predator-free islands (41.4 % of observations), and mean perch height was over twice as high on predator islands (33.9 cm) compared to predator-free islands (14.4 cm) ($t = -4.9$, $df = 102.5$, $p < 0.001$; Fig. S3B).

Because *A. sagrei* is a sexually dimorphic species in which males and females differ in both morphology and behavior (*23*, *33; see also Fig. S8-S10*), we hypothesized that natural selection on inter-individual variation in behavior could operate differently between sexes under different environmental conditions. On predator-free islands, natural selection favored females that took less time to initiation of exploration in the experimental trials conducted before release (Fig. 2), a pattern not observed on predator islands (Fig. 2). On predator islands, females that spent less time exposed on the ground had a greater chance of survival (Fig. 2). Behavior was not a significant predictor of survival for males (Fig. S3A). Whether or not *A. sagrei* were initially captured from islands with *L. carinatus* present did not significantly affect their chances of survival during the experiment (Table S3).

That we only found significant selection on time spent on the ground on predator islands for females, but not males, suggests a greater effect from predatory lizards on females compared to males. In support of this possibility, female mortality was higher on predator islands as compared to predator-free ones ($X^2 = 9.7$, $p = 0.002$), whereas for males there was no difference ($X^2 = 2.9$, $p = 0.086$; Fig. 3A). In addition, on predator islands, use of the ground was also lower in females than in males (11.9 % vs. 22.9 % respectively; $X^2 = 41.9$, $p < 0.001$; Fig. 3B). Because *A. sagrei* feeds primarily on the ground (*23*), the observed patterns of ground use suggest that females could be having more difficulties obtaining food resources on the predator islands. Indeed, four months after experimental translocation, females were in poorer body condition on predator islands than on predator-free islands ($p < 0.001$), a pattern not observed in males ($p = 0.68$) (Fig. S3). Together, these results suggest that differences in habitat use between sexes influence natural selection on behavioral traits.

A long-standing debate in evolutionary biology concerns the association between behavioral and morphological evolution (*1, 2, 34*). Our study design allowed us to investigate whether selection on morphological traits occurs simultaneously with selection on behavioral traits and to assess if selection on both phenotypic dimensions was correlated. Specifically, we tested a well-established morphological pattern in *Anolis* lizards: that the use of the ground or other broad surfaces favors longer limbs, which provide greater sprinting abilities (reviewed in (*23*)). We found that females with longer hindlimbs relative to their body size survived better than shorter-limbed individuals on predator-free islands ($p = 0.002$; Table 1; Fig. S6). This is consistent with our observation that females used the ground more often on predator-free islands than on predator islands (Figure 3b). On predator islands, relative hindlimb length did not affect survival ($p = 0.26$; Fig. S6). We did not find selection on the relative hindlimb length for males ($p > 0.80$) in either experimental treatment. In addition, we found that smaller females survived better on predator islands than larger individuals ($p = 0.013$; Table 1). Finally, selection on behavior and morphology was not correlated. For females from predator-free islands, selection for longer hindlimbs was independent of selection for increased exploratory behavior (shown by the lack of a significant interaction term in mixed models shown in Table 1). On predator islands, selection for smaller females was also independent of selection favoring individuals that spent less time exposed on the ground (Table 1). Overall, these results indicate that natural selection on behavior can occur simultaneously and independently with selection on morphology.

Given that selection operated on both morphology and behavior, we asked which of these factors explained a higher proportion of the variation in mortality in females (no significant factors were detected in males). An analysis of the proportion of variation in mortality explained by behavior versus morphology (*26*) revealed that, on predator-free islands, selection on hindlimb length explained 19.1% of the variation in mortality, whereas selection for more exploratory females accounted for 13.9%. Conversely, on predator islands, the proportion of variance in mortality explained by time exposed on the ground was 22.5%, whereas body size (SVL) accounted for 9.8%. These findings suggest that although both behavior and morphology can simultaneously contribute to adaptation, their importance is context dependent, varying under different selective regimes.

Although behavior largely defines how animals interact with the environment, the evolutionary consequences of inter-individual variation in behavior remain largely unknown (*7*, *8*). Our replicated field study provides evidence that natural selection operates differently on inter-individual variation in behavior under different, experimentally manipulated selective pressures. Moreover, our results indicate that differences in habitat use between sexes likely influence the strength of natural selection on behavioral traits. By showing that selection can simultaneously and independently operate on behavior and morphology, we demonstrate that rapid environmental changes can shape different phenotypic dimensions at the same time; the evolutionary outcome of such selection will depend on the genetic basis of these traits and the extent to which they are correlated. Our results thus underscore the need to explicitly integrate inter-individual variation in behavior as a relevant phenotypic dimension in studies of adaptation (*7*, *8*, *35*). In fact, we show that under increased predation pressure, behavior is a more important factor explaining survival than the morphological traits that have been the subject of previous investigation (*22*)—the extent to which these results will be general across species remains to be discovered. Our results demonstrate that consistent behavioral variation among individuals can be an important focus of selection when populations experience novel environmental conditions, an increasingly common situation in the current context of global change.

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Figure legends and Tables:

Figure 1 | Assessment of risk-taking behavior and morphological characterization of *A. sagrei* individuals. A, *Anolis sagrei* (left) and *Leiocephalus carinatus* (right) photographed on the experimental islands. **B,** Experimental assessment of behavioral traits (*26*). Following (*25*), *A. sagrei* were gently placed into a wooden refuge inside a butterfly cage. During a three-minute habituation period, we placed a clear plastic cage that contained a live adult curly-tailed lizard between the refuge and a natural perch. Then, we remotely opened de door of the refuge and the *A. sagrei* was able to see the predator for five minutes (1). At the end of this period, we closed the door of the refuge and removed the plastic container with the curly-tailed lizard from the experimental cage. After another five-minute habituation period we again opened the refuge cover and measured the 'time to initiation of exploration in a new environment' (2) -defined as the time interval between the time we opened the refuge cover and the time when the lizard started exploring the experimental cage by poking its head out of the refuge. We defined 'time exposed on the ground' as the interval between the 'exposed time start' (3), defined as the time when the experimental lizard went out of the refuge (i.e. all its body, excluding the tail), and the 'exposed time end' (4), the time the lizard either climbed the perch or hid underneath the rocks. Further details are provided in the Methods section. **C,** Example of an X-ray image from which we measured the morphological traits in this study (i.e. SVL and hindlimb length).

Figure 2 | Effects of time to initiation of exploration in a **new environment (A) and time exposed on the ground (B) for the survival of female *A. sagrei* on predator-free vs. predator islands.** Solid lines represent the fitted model logistic regression and dashed lines represent the 95% confidence intervals. Results pooling both sexes can be found in Table S4.

Figure 3 | Comparison of survival frequencies and habitat use between sexes and experimental treatments. A, The proportion of females surviving was higher on predator-free islands as compared with predator islands, but this difference was marginally non-significant for males. Error bars indicate +/- (SEM). **B,** Both sexes used the ground less on predator islands, but this difference was greater for females than for males.

Females					
Predator-free islands (n = 63)					
		Estimate	SE	z	p-value
	(Intercept)	2.82	1.05	2.7	0.007
Random effects					
	Island	0.18	0.423	0.43	0.669
Fixed effects					
	Time to initiation of exploration	-1.03	0.4	-2.55	0.011
	Relative hindlimb length	48.7	15.78	3.08	0.002
Predator islands (n = 68)					
		Estimate	SE	z	p-value
	(Intercept)	14.68	5.55	2.65	0.008
Random effects					
	Island	0	0	0	1
Fixed effects					
	Exposed time on ground	-1.27	0.61	-2.1	0.035
	Body size (SVL)	-0.34	0.14	-2.48	0.013

Table 1 | Best Mixed-effects models describing female survival on the experimental islands.

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Supplementary Materials

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Materials and Methods

Figures S1-S10

Tables S1-S5

References (36-43)