EVOLUTION

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

Oriol Lapiedra,[1]* Thomas W. Schoener,[2] Manuel Leal,[3] Jonathan B. Losos,[1]† Jason J. Kolbe[4]†

Biologists have long debated the role of behavior in evolution, yet understanding of 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 on eight small islands either with or without *Leiocephalus carinatus*, a major ground predator. We found that 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, 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 toward 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). 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 interindividual variation in behavior (9–11) has revealed that behavior often varies consistently among individuals within a population (12). These studies have also suggested that this variation has fitness consequences (13–16). These results set the stage to investigate the hypothesis that natural selection on interindividual variation in behavior could drive different ecological and evolutionary trajectories for populations under distinct selective regimes (7, 8, 17–20). Assessing a hypothesis like this one 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, we measured the rapidity of individuals to explore new and potentially dangerous

environments and the time individuals spend on the ground and are 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 trade-off (27, 28). *Anolis sagrei* individuals more willing to explore new environments should survive better in the absence of terrestrial predators (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 273 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 S2).
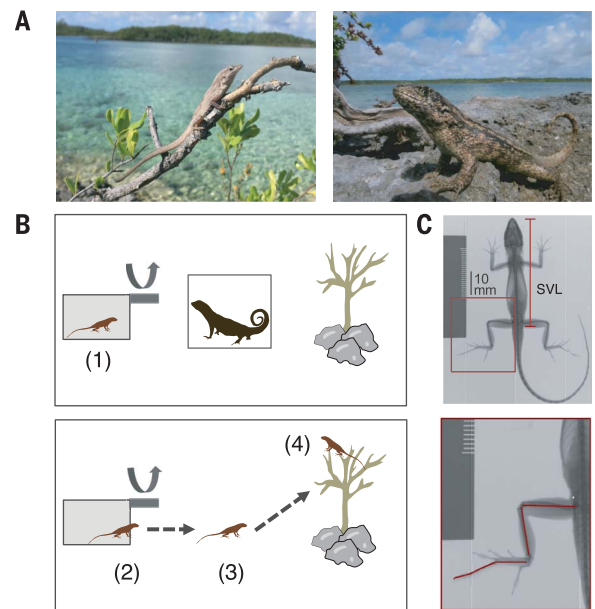
Before translocation, we used outdoor laboratory behavioral assays [following (25, 26)] to

Fig. 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), an *A. sagrei* individual was gently placed into a wooden refuge inside a butterfly cage. During a 3-min habituation period, we placed a clear plastic cage that contained a live adult *L. carinatus* between the refuge and a natural perch. Then, we remotely opened the door of the refuge and the *A. sagrei* individual was able to see the predator for 5 min (1). At the end of this period, we closed the door of the refuge and removed the plastic container with the *L. carinatus* from the experimental cage. After another 5-min 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 when the lizard either climbed the perch or hid underneath the rocks. See (26) for details. (**C**) Example of an x-ray image from which we measured the morphological traits in this study (i.e., SVL and hindlimb length).

[1]Museum of Comparative Zoology and Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA. [2]Department of Evolution and Ecology, University of California, Davis, CA 95616, USA. [3]Division of Biological Sciences, University of Missouri, Columbia, MO, USA. [4]Department of Biological Sciences, University of Rhode Island, Kingston, RI, USA.
*Corresponding author. Email: olapiedragonzalez@fas.harvard.edu
†These authors contributed equally to this work.

characterize interindividual variation in two behaviors known to consistently vary among individuals (25) (table S1). After being exposed to the presence of an individual *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 by conducting vegetation transects [following (26, 32)]. One 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 recaptured lizards on each of the experimental islands and identified surviving adult lizards from their individually unique subcutaneous tags.

On the basis of recapture data, we found that survival was lower on predator islands than on 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 more than twice as high on predator islands (33.9 cm) than on 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) (figs. S8 and S9), we hypothesized that natural selection on interindividual 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—a pattern not observed on predator islands (Fig. 2A). On predator islands, females that spent less time exposed on the ground had a greater chance of survival (Fig. 2B). 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).
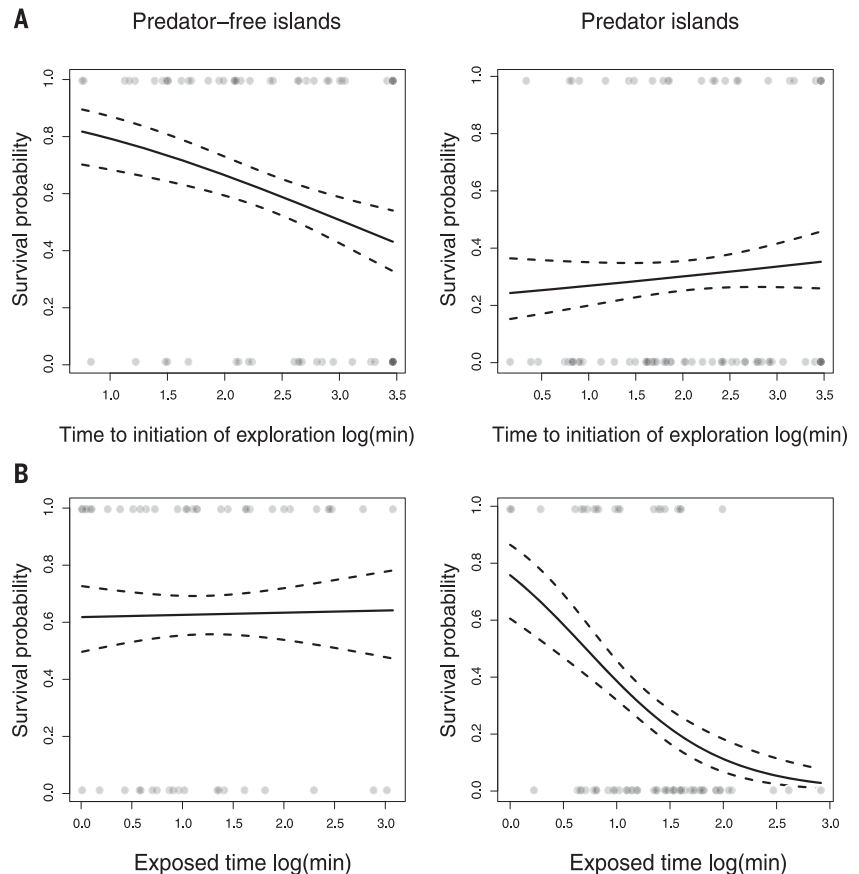
That we only found significant selection on time spent on the ground on predator islands for females, but not males, suggests a greater effect from predatory lizards on females than

on males. In support of this possibility, female mortality was higher on predator islands than on predator-free ones ($\chi^2 = 9.7$, $P = 0.002$), whereas for males there was no difference ($\chi^2 = 2.9$, $P = 0.086$; Fig. 3A). In addition, on predator islands, use of the ground was also lower in females than in males (11.9% versus 22.9%, respectively; $\chi^2 = 41.9$, $P < 0.001$; 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, 4 months after experimental translocation, females were in poorer body condition on predator islands than on predator-free islands ($P < 0.001$) (fig. S3), 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 whether 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

Fig. 2. Association between individual variation in behavior and survival of *A. sagrei* females after the 4-month experimental period. (**A** and **B**) Time to initiation of exploration in a new environment (A) and time exposed on the ground (B) are represented separately for predator-free versus predator islands. Solid lines represent the fitted model logistic regression; dashed lines denote 95% confidence intervals. Dots represent individual values for both survivors (dots at top of each panel) and nonsurvivors (dots at bottom of each panel); darker dots are indicative of several individuals having similar values. See table S4 for a representation of results pooling both sexes together.
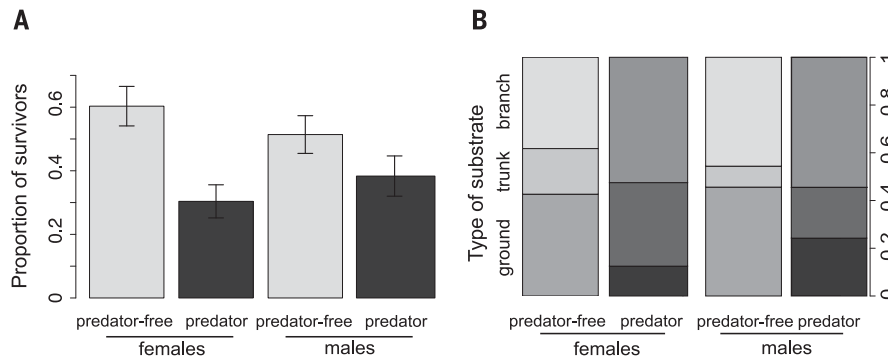
Fig. 3. Comparison of survival frequencies and habitat use between sexes and experimental treatments. (**A**) The proportion of females surviving was higher on predator-free islands than on predator islands, but this difference was marginally nonsignificant for males. Error bars indicate SEM. (**B**) Both sexes used the ground less on predator islands, but this difference was greater for females than for males.

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

		Estimate	SE	z	P value
		Predator-free islands (n = 63)			
	(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)			
	(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

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 and fig. S6). This is consistent with our observation that females used the ground more often on predator-free islands than on predator islands (Fig. 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 on predator islands, smaller females survived better than larger females ($P = 0.013$; 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 with, and independent of, 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 [snout-vent length (SVL)] accounted for 9.8%. These findings suggest that although both behavior and morphology can simultaneously contribute to survival, their importance is context-dependent and varies under different selective regimes.

Although behavior largely defines how animals interact with the environment, the evolutionary consequences of interindividual variation in behavior remain largely unknown (*7, 8*). Our replicated field study provides evidence that natural selection operates differently on interindividual 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 interindividual variation in behavior as a relevant phenotypic dimension in studies of adaptation (*7, 8, 35*). Moreover, we found 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 can be generalized across species remains to be determined. 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.

REFERENCES AND NOTES

1. E. Mayr, *Animal Species and Evolution* (Harvard Univ. Press, 1963).
2. C. M. Bogert, *Evolution* **3**, 195–211 (1949).
3. R. B. Huey, P. E. Hertz, B. Sinervo, *Am. Nat.* **161**, 357–366 (2003).
4. D. Sol, D. G. Stirling, L. Lefebvre, *Evolution* **59**, 2669–2677 (2005).
5. O. Lapiedra, D. Sol, S. Carranza, J. M. Beaulieu, *Proc. R. Soc. B* **280**, 20122893 (2013).
6. M. M. Muñoz, J. B. Losos, *Am. Nat.* **191**, E15–E26 (2018).
7. S. R. X. Dall, S. C. Griffith, *Front. Ecol. Evol.* **2**, 1–7 (2014).
8. M. Wolf, F. J. Weissing, *Trends Ecol. Evol.* **27**, 452–461 (2012).
9. S. R. X. Dall, A. I. Houston, J. M. McNamara, *Ecol. Lett.* **7**, 734–739 (2004).
10. A. Sih, A. Bell, J. C. Johnson, *Trends Ecol. Evol.* **19**, 372–378 (2004).
11. D. Réale, S. M. Reader, D. Sol, P. T. McDougall, N. J. Dingemanse, *Biol. Rev. Camb. Philos. Soc.* **82**, 291–318 (2007).
12. A. M. Bell, S. J. Hankison, K. L. Laskowski, *Anim. Behav.* **77**, 771–783 (2009).
13. N. J. Dingemanse, C. Both, P. J. Drent, J. M. Tinbergen, *Proc. R. Soc. B* **271**, 847–852 (2004).
14. J. N. Pruitt, J. J. Stachowicz, A. Sih, *Am. Nat.* **179**, 217–227 (2012).
15. C. D. Santos *et al.*, *Sci. Rep.* **5**, 15490 (2015).
16. N. G. Ballew, G. G. Mittelbach, K. T. Scribner, *Am. Nat.* **189**, 396–406 (2017).
17. M. Wolf, G. S. van Doorn, O. Leimar, F. J. Weissing, *Nature* **447**, 581–584 (2007).
18. N. J. Dingemanse, M. Wolf, *Philos. Trans. R. Soc. B* **365**, 3947–3958 (2010).
19. D. Réale, N. J. Dingemanse, A. J. N. Kazem, J. Wright, *Philos. Trans. R. Soc. B* **365**, 3937–3946 (2010).
20. S. R. X. Dall, A. M. Bell, D. I. Bolnick, F. L. W. Ratnieks, *Ecol. Lett.* **15**, 1189–1198 (2012).
21. J. A. Endler, *Natural Selection in the Wild* (Princeton Univ. Press, 1986).
22. J. B. Losos, T. W. Schoener, D. A. Spiller, *Nature* **432**, 505–508 (2004).
23. J. B. Losos, *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles* (Univ. of California Press, 2009).
24. T. W. Schoener, D. A. Spiller, J. B. Losos, *Nature* **412**, 183–186 (2001).
25. O. Lapiedra, Z. Chejanovski, J. J. Kolbe, *Glob. Change Biol.* **23**, 592–603 (2016).
26. See supplementary materials.
27. P. A. Bednekoff, S. L. Lima, *Proc. R. Soc. B* **271**, 1491–1496 (2004).
28. D. S. Wilson, A. B. Clark, K. Coleman, T. Dearstyne, *Trends Ecol. Evol.* **9**, 442–446 (1994).
29. M. Drakeley, O. Lapiedra, J. J. Kolbe, *PLOS ONE* **10**, e0138016 (2015).

3 of 4

30. M. López-Darias, T. W. Schoener, D. A. Spiller, J. B. Losos, *Ecology* **93**, 2512–2518 (2012).
31. D. S. Steinberg *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **111**, 9187–9192 (2014).
32. J. J. Kolbe, M. Leal, T. W. Schoener, D. A. Spiller, J. B. Losos, *Science* **335**, 1086–1089 (2012).
33. T. W. Schoener, *Ecology* **49**, 704–726 (1968).
34. J. S. Wyles, J. G. Kunkel, A. C. Wilson, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4394–4397 (1983).
35. A. Sih, M. C. O. Ferrari, D. J. Harris, *Evol. Appl.* **4**, 367–387 (2011).

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/360/6392/1017/suppl/DC1
Materials and Methods
Figs. S1 to S9
Tables S1 to S5
References (*36–42*)

Science

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

Oriol Lapiedra, Thomas W. Schoener, Manuel Leal, Jonathan B. Losos and Jason J. Kolbe

Predation favors the unadventurous
Selection is likely to shape behavior by acting on behavioral differences between individuals. Testing this idea has been challenging. Lapiedra *et al.* took advantage of a chain of small islands in the Caribbean colonized by anole lizards. A series of repeated behavioral selection experiments were set up in which brown anole populations were established with and without predators. On predator-free islands, animals that were more exploratory were favored, whereas when predators were present, less adventurous animals survived better. Selection for behavior occurred simultaneously with morphological selection but was predominant when predators were present.
Science, this issue p. 1017

ARTICLE TOOLS	http://science.sciencemag.org/content/360/6392/1017
SUPPLEMENTARY MATERIALS	http://science.sciencemag.org/content/suppl/2018/05/30/360.6392.1017.DC1
REFERENCES	This article cites 37 articles, 9 of which you can access for free http://science.sciencemag.org/content/360/6392/1017#BIBL
PERMISSIONS	http://www.sciencemag.org/help/reprints-and-permissions

Use of this article is subject to the Terms of Service