

An experimental test of the role of predators in the maintenance of a genetically based polymorphism

R. CALSBEEK* & R. M. COX†

*Department of Biological Sciences, Dartmouth College, Hanover, NH, USA

†Department of Biology, University of Virginia, Charlottesville, VA, USA

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Abstract

Polymorphisms provide one of the most useful tools for understanding the maintenance of genetic and phenotypic variation in nature. We have previously described a genetically based polymorphism in dorsal patterning that is expressed by female brown anole lizards, *Anolis sagrei*, which occur in Bar, Diamond and intermediate Diamond–Bar morphs. Previous studies of island populations in The Bahamas support a role for selection in maintaining the polymorphism, but the agents responsible for this selection remain unclear. We tested two main hypotheses regarding the importance of predation as a selective agent that maintains the polymorphism within populations. First, we tested whether correlational selection favours different combinations of morph, locomotor performance and escape behaviour by measuring morph-specific natural selection on sprint speed, running endurance and the propensity of females to either ‘freeze’ or ‘run’ in response to attempted capture. Morphs did not differ in any of these traits, nor did correlational selection consistently favour any particular combinations of morph and anti-predator behaviour. Second, we experimentally excluded bird and snake predators from two entire island populations, allowed these predators access to two additional islands and then measured subsequent differences in natural selection on morphs in each population. Predators reduced the survival of Bar and Diamond females, but not of genetically intermediate Diamond–Bar females. These results provide limited evidence that predation may play a role in maintaining this polymorphism, although the functional traits that could account for differential susceptibility to predation remain unclear.

Introduction

Evolutionary theory makes the straightforward prediction that, all else being equal, alternative phenotypes that vary in fitness should not be able to coexist within a single population (Maynard Smith, 1982; Ryan *et al.*, 1992). Still, phenotypic and genetic variation is ubiquitous in nature. Understanding the factors that maintain this variation within populations has therefore become a central goal of evolutionary biology, and polymorphisms have proven to be invaluable tools for this purpose (Ryan *et al.*, 1992; Smith & Skulason, 1996;

Svensson *et al.*, 2005). Genetically based polymorphisms in body coloration or patterning are especially useful because they provide a source of visible phenotypic variation that is easily measured as an indicator of underlying genetic variation (Calsbeek *et al.*, 2010a; Cox & Calsbeek, 2011).

One of the general patterns to emerge from studies of genetically based polymorphisms is that alternative morphs may coexist within a population despite unequal fitness (Sinervo & Lively, 1996; Kerr *et al.*, 2002; Galeotti *et al.*, 2003; Mappes *et al.*, 2005). This coexistence can be facilitated through a variety of mechanisms, including temporal or spatial variation in the form of selection (Calsbeek *et al.*, 2002), negative frequency-dependent selection (Sinervo & Lively, 1996; Widemo, 1998; Alonzo & Warner, 2000), apostatic selection (Franks & Oxford, 2011; Holmer & Green,

Correspondence: Ryan Calsbeek, Department of Biological Sciences, Dartmouth College, Hanover, NH 03755, USA.
Tel: 603 646 9917; fax: 603 646 1345;
e-mail: ryan.calsbeek@dartmouth.edu

2011), correlational selection (Brodie, 1992; Lancaster *et al.*, 2010; Vanhoenacker *et al.*, 2010) and gene flow among demes in a metapopulation (King, 1987; King & Lawson, 1995). Although many of these processes can be inferred simply from the pattern of selection on morphs, a complete understanding of how and why polymorphism is maintained requires a causal understanding of the actual selective agents driving these patterns.

Experimental studies that manipulate proposed agents of selection and then measure changes in the selective outcome are rare and have been much better developed for plants than for animals (Pilson, 1996; Mauricio & Rausher, 1997; Juenger & Bergelson, 1998; Stinchcombe & Rausher, 2002; Strauss *et al.*, 2005; Smith & Rausher, 2008). Although similar studies in wild animal populations are less common, notable examples include experimental work demonstrating the role that predators play in shaping life-history evolution in guppies (Reznick *et al.*, 1990; Reznick *et al.*, 1997; Bronikowski *et al.*, 2002; Gordon *et al.*, 2009), the role of competition in shaping selection on morphology in stickleback fish (Rundle *et al.*, 2003; Bolnick, 2004) and the roles of both predation and competition in shaping selection on body size and limb morphology in lizards (Losos *et al.*, 2004, 2006; Calsbeek & Smith, 2008; Calsbeek & Cox, 2010). Here, we present results from observational studies of selection along with an experiment in which we manipulate the selective environment to directly assess whether predators act as agents of morph-specific selection on a sex-limited polymorphism in dorsal colour pattern.

We have previously described a polymorphism in which female brown anole lizards (*Anolis sagrei*) in The Bahamas exhibit one of three genetically determined dorsal colour patterns: Bar (B), Diamond (D) or Diamond-Bar (DB) (Calsbeek *et al.*, 2008). Dorsal patterns can be scored immediately upon hatching and are fixed throughout ontogeny. The DB pattern is phenotypically intermediate, and although breeding studies indicate that this polymorphism is controlled by multiple loci, the DB morph is also genetically intermediate in the sense that DB females are capable of producing offspring of all three morph types (Calsbeek *et al.*, 2010b). This polymorphism does not appear to be maintained by geographic variation in morph-specific selection among populations (Calsbeek *et al.*, 2010b) or by alternative reproductive strategies corresponding to dorsal pattern morphs (Cox & Calsbeek, 2011). However, comparisons of morph-specific viability among years suggest that the survival of the intermediate DB morph is typically high and increases along with its frequency in the population (Calsbeek *et al.*, 2010b). This could potentially maintain the polymorphism by a multilocus analogy to overdominance (i.e. heterosis or heterozygote advantage; Cox & Calsbeek, 2011), but the actual selective agents and functional mechanisms that could

be responsible for this type of selection are not clear. Another possibility is that B females are cryptic when perched on narrow branches (Schoener & Schoener, 1976), whereas D females are cryptic in leaf litter, such that predators maintain the polymorphism via disruptive selection against intermediate forms that are not cryptic on either background (Gray & McKinnon, 2006). Predators could also maintain the polymorphism through apostatic selection by developing a search image and disproportionately preying upon the most common morph (Gray & McKinnon, 2006), as suggested for other *Anolis* species with polymorphisms in dorsal pattern (Paemelaere *et al.*, 2011a). Any of these hypothesized mechanisms for the maintenance of polymorphism could be driven by morph-specific predation, but no study to date has tested the role of predators in this system.

Here, we build on previous experiments that investigated the role of predation in driving selection on the morphology of male lizards (Calsbeek & Cox, 2010) to test the hypothesis that alternative dorsal patterns expressed by female lizards are differentially susceptible to predator-based mortality. Predator-mediated selection is thought to maintain variation in dorsal patterning of water snakes (King, 1987), side-blotched lizards (Lancaster *et al.*, 2007), heliconius butterflies (Mallet & Gilbert, 1995), peppered moths (Majerus *et al.*, 2000) and stick insects (Nosil, 2004). In some cases, selection may act on combinations of dorsal patterning and anti-predator behaviours (Brodie, 1992; Lancaster *et al.*, 2010). For example, in garter snakes, striped morphs tend to flee in a straight line away from predators, whereas blotched morphs reverse direction during escape. These correlated patterns of behaviour and dorsal pattern create optical illusions that presumably disorient predators when attempting to catch snakes, and measures of survival reveal that correlational selection favours appropriate combinations of dorsal pattern and escape behaviour (Brodie, 1992).

We combine observational and experimental studies of natural selection to test the potential importance of predators in driving morph-specific selection and contributing to the maintenance of polymorphism. First, we use 4 years of observational data to test the hypothesis that natural selection favours different combinations of behaviour and/or running performance that could render morphs differentially susceptible to predators. We then perform a set of replicated experiments in which we either include or exclude bird and snake predators from entire island populations of lizards to directly test the hypothesis that morph-specific differences in survival are driven by predation. We predict that if predation is important in the maintenance of this polymorphism, then patterns of morph-specific survival should differ in the presence and absence of predators. We interpret our results in the light of the patterns expected for overdominance (selection under predation

favours the genetically intermediate DB morph), disruptive selection (selection under predation favours cryptic B and D morphs) and apostasis (selection under predation favours the rare B morph and acts against the common DB morph) as mechanisms for the maintenance of polymorphism.

Methods

Study system and general methods

The brown anole (*Anolis sagrei*, Polychrotidae) is a small (40–70 mm adult size), semi-arboreal lizard native to islands throughout the West Indies. We studied geographically distinct populations of *A. sagrei* located on separate offshore islands around the main island of Great Exuma in The Bahamas. The first population, located on Kidd Cay (23°30' N, 75°45' W), has been studied intensively since 2004, and details regarding its ecology and demography are available elsewhere (Calsbeek, 2007; Calsbeek & Bonneaud, 2008; Calsbeek & Smith, 2008). In this and other nearby populations on Great Exuma, the DB morph occurs at high frequencies (mean 42%, range 35–55%), the D morph occurs at intermediate frequencies (mean 35%, range 25–51%), and the B morph occurs at low frequencies (mean 23%, range 1–39%) (Calsbeek *et al.*, 2010a, b).

All observational studies of selection (i.e. no manipulation of predators), along with performance measures that are reported in this study, were made on Kidd Cay during 2005–2008. During 2008 and 2009, we also performed experimental manipulations of the predator guild on four other small islands just offshore of Great Exuma. These islands are all located within 2 km of each other, and are similar in many respects (e.g. vegetation structure, precipitation), but they differ in several other respects. For example, Kidd Cay (4200 m²) is about twice as large as the experimental islands (range: 800–2700 m²), is connected to the mainland by a narrow causeway, is the site of a small hotel and is naturally home to a greater diversity of potential competitors and predators of *Anolis* lizards. Although anoles on all islands are susceptible to predation by birds (e.g. mockingbirds, *Mimus polyglottos*, green herons, *Butorides striatus*), Kidd Cay also supports predatory reptiles (lizards, *Ameiva auberi*, and snakes, *Alsophis vudii* and *Epicrates striatus*) that are naturally absent from our experimental islands, as well as several potential competitors (*Anolis distichus*, *A. smaragdinus*) that are absent or extremely uncommon on the experimental islands. In addition, whereas all islands contain a mixture of native shrubs (e.g. sea grape, *Coccoloba uvifera*; buttonwood, *Conocarpus erectus*) that make up the majority of perching habitat used by anoles, Kidd Cay also supports larger tree species (e.g. Palm, *Pseudophoenix* spp., Australian pine, *Casuarina equisetifolia*). Although we draw inference about the role of predators in shaping

selection from Kidd Cay (hereafter, observational studies) as well as from our smaller experimental islands (hereafter, experimental manipulations), we never consider them together in the same analyses owing to these differences.

Observational studies of natural selection

During May of each year (2005–2008), we captured every visible female on Kidd Cay using a silk noose attached to the distal end of a fishing pole. Prior to capture, each female's escape behaviour in response to the approaching investigator was scored as either 'freeze' (female remained motionless or moved only slightly but made no attempt to flee) or 'run' (female attempted to escape by running at least four body lengths away from the pole). Human approach has been routinely used to score escape behaviour (Stankowich & Blumstein, 2005; Martin & Lopez, 2010), and we use this method under the assumption that attempted capture elicits a response comparable to that of a lizard attempting to escape a predator. Upon capture, we measured snout-vent length using a ruler (SVL, nearest 0.5 mm) and body mass using a Pesola spring scale (nearest 0.1 g). We scored dorsal morph (B, D, DB) and then permanently and uniquely marked each individual using either a series of elastomer dyes (Nauwelaerts *et al.*, 2000), injected subcutaneously into the underside of each limb, or by numeric toe-clipping. Following measurement of sprint speed and endurance, we applied a small spot of white paint to the hind limb of each animal to prevent immediate recapture and released each animal back to its spot of original capture within 8 h. At the conclusion of the breeding season (September), we recaptured all surviving lizards. Because we searched the site exhaustively, recapture success is a reliable estimate of survival and lizards not recaptured during our census were assumed to have died. We tested for morph-specific variation in survival using a generalized linear model (GLM) with a logit link function to account for the binomial distribution of survival data and including morph as a factor. We measured selection acting on escape behaviour (freeze or run) using a similar GLM including both morph and escape behaviour as factors. We measured correlational selection by testing for morph × behaviour interactions with respect to survival.

Running performance

All trials of running performance were conducted during the afternoon following initial capture. We measured endurance by running females to exhaustion (measured as the loss of righting response) on a motorized treadmill inclined at 20° and rotating at 0.4 km h⁻¹. We measured maximum sprint speed by chasing females up a dowel (2.5 cm diameter) that was



Fig. 1 (a) One of the experimental islands used as a predator exclusion treatment in this study. Insert (b) shows a close-up view of no-tangle bird netting used to exclude avian predators while leaving habitat accessible to anoles.

likewise inclined at 20°. We recorded sprinting trials using a digital video camera and then digitized and analysed video using MOTIONANALYSIS software (available from M. Chappell University of California, Riverside, CA, USA). At the time of this study, software could be downloaded at <http://warthog.ucr.edu>. Each female was sprinted three times, and we used the greatest speed achieved over any 10-cm interval in any of these three trials as the measure of maximal sprint speed for each animal. Additional information on these performance assays is available elsewhere (Calsbeek & Irschick, 2007; Calsbeek & Bonneauud, 2008; Calsbeek, 2009). We included size (SVL) as a covariate in our analyses of running performance. We tested for differences among morphs using ANOVA with each performance variable as the dependent variable and morph (B, D, DB) as the main effect.

Experimental manipulations of predation

During May of 2008 and 2009, we captured 600 females from a site near Georgetown, Great Exuma, and released 150 of these females to each of four small islands on which we manipulated the predation regime. Initial morph frequencies did not differ across experimental islands and were representative of the overall frequencies in the source population, where DB was common (mean \pm SD = 53.0 \pm 3.2%), D was intermediate (31.5 \pm 6.2%), and B was the least common morph (15.5 \pm 4.2%) in each replicate. Lizards were measured and marked as on Kidd Cay, except that we did not score antipredator behaviour or running performance. As part of a separate study testing for morph-specific reproductive strategies (Cox & Calsbeek, 2010), half of the females released onto each island received a surgical manipulation of reproductive investment (bilateral ovariectomy), whereas the second half received a sham surgery. For the present study, we report results from the full data set pooling females from both treatments and including surgical treatment as a factor in

our analyses. We also present results from each analysis using only the subset of data representing females that received a sham surgery. In all cases, our results were qualitatively identical regardless of whether we used the full data set or the subset of data from sham females, and we never detected a significant effect of surgical treatment.

During 2008, we established one 'no-predator' treatment in which all avian predators were excluded from an island by covering the entire island with No-Tangle bird-proof netting (Gardener's Supply Company, Burlington, VT, USA). Nets were secured to the island and stitched together using plastic cable-ties, such that the entire island was enshrouded in netting (Fig. 1) for the duration of the experiments (4 months). During 2009, we replicated the 'no-predator' treatment on a different island and we also established two 'predator' treatments on islands that were left exposed to natural levels of avian predation. We also added three adult male snakes (Bahamian racer, *Alsophis vudii*) to each of these islands. Racers are important predators of anoles on larger islands in The Bahamas, but they are naturally absent on many smaller cays, including those used in this experiment. As such, lizards in our two 'predator' replicates were subject to both bird and snake predation, whereas lizards in our two 'no-predator' replicates were not exposed to either type of predator.

We note that, because our 'no-predator' treatments were conducted in 2008 and 2009, whereas our 'predator' treatments were only conducted in 2009, predation treatment is partially confounded with year. This could complicate interpretation of predator effects if, for example, survival were to differ dramatically between years due to variation in environmental conditions. However, because our analyses are focused on morph-specific patterns of survival within each island, it seems less likely that any annual variation in environmental conditions would also act in such a way as to create morph-specific patterns of mortality. We also note that the addition of nets to our 'predator-free' islands may

have introduced minor artefacts due to netting (e.g. increased shade and altered thermal environment, increased habitat for arthropod prey) that are not related to our predator manipulations *per se*. We have previously added netting to the perimeter of some islands as a control for such effects (Calsbeek & Cox, 2010), but we elected not to do so in this experiment because preliminary studies in enclosed arenas indicated that snakes could become entangled in the netting (R. M. Cox, pers. obs.). Again, whereas any such artefacts of netting could potentially alter the patterns of overall survival, we have no reason to suspect this would lead to differences in morph-specific mortality between netted and unnetted islands.

As on Kidd Cay, we exhaustively surveyed each experimental island in the fall (September) and recaptured all visible survivors. On each individual island, we tested for significant survival differences among morphs using GLM with identity link functions. We also pooled individuals from all islands, classified them by morph and by predator treatment, then tested for this same effect using GLM with an identity link function. We tested for morph-specific effects by including a morph \times predator treatment interaction in this analysis. We also tested for significant predator effects on each morph separately. As noted above, all of these analyses were conducted both with the female's surgical treatment as a factor, and separately for sham females alone.

Results

Average survival to the fall census on Kidd Cay was 33% (range 27–45%) across all 4 years of this study. Average survival to the fall census on offshore cays was 34% (range 26–45%), similar to patterns on Kidd Cay. We did not measure significant morph-specific variation in the probability of survival (i.e. natural selection on morph) in any year or on any island (GLM for Kidd Cay all $\chi^2 < 2.32$, all $P > 0.32$ and for experimental islands all $\chi^2 < 5.41$, all $P > 0.06$).

Running performance and antipredator behaviour

Morphs did not differ in running endurance (ANOVA $F_{2,572} = 0.47$, $P = 0.62$; effect of SVL $F_{1,572} = 18.23$, $P < 0.0001$; effect of year $F_{3,572} = 510.20$, $P < 0.0001$; Fig. 2a) or sprint speed (ANOVA $F_{2,255} = 0.14$, $P = 0.87$; effect of SVL $F_{1,255} = 0.58$, $P = 0.45$; effect of year $F_{1,255} = 140.66$, $P < 0.001$; Fig. 2b). Significant year effects were due to higher running endurance in 2008 and faster sprint speeds in 2006 (the former likely attributable, at least in part, to subtle variation in the speed of rotation of our treadmill between years), but these differences were consistent among the morphs and no interaction terms were significant. Selection did not act differently among the morphs with regard to

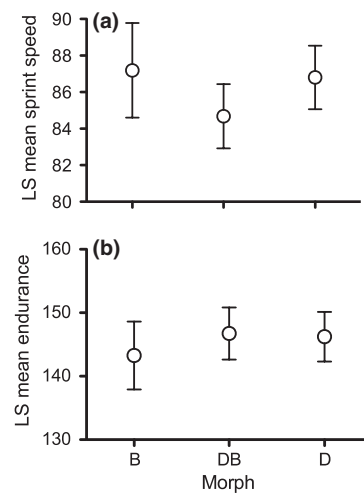


Fig. 2 Morphs did not differ in running endurance (top) or maximum sprint speed (bottom). Figures show least-square mean values (+SE) of trait scores after correcting for year effects and variation due to body size.

Table 1 Selection gradients ($\beta \pm 1$ SE) presented separately by year and individually by morph for females measured on Kidd Cay. Sprint speed was measured only during 2006 and 2007 (see methods). Only the 2007 selection gradient for sprint speed acting on DB females (shown in boldface) was significant ($P < 0.05$).

| Year | Morph | Beta sprint speed | Beta endurance |
|------|-------------------|---------------------|----------------|
| 2005 | B | N/A | 0.30 (0.56) |
| | DB | N/A | -0.07 (0.36) |
| | D | N/A | 0.36 (0.21) |
| | Overall selection | N/A | 0.20 (0.16) |
| 2006 | B | -0.04 (0.24) | 0.04 (0.21) |
| | DB | -0.19 (0.15) | 0.20 (0.18) |
| | D | -0.13 (0.25) | 0.03 (0.20) |
| | Overall selection | -0.13 (0.11) | 0.05 (0.11) |
| 2007 | B | 0.07 (0.25) | 0.13 (0.29) |
| | DB | -0.40 (0.19) | 0.11 (0.18) |
| | D | 0.16 (0.17) | 0.07 (0.21) |
| | Overall selection | -0.06 (0.11) | 0.16 (0.11) |
| 2008 | B | N/A | 0.41 (0.27) |
| | DB | N/A | 0.03 (0.15) |
| | D | N/A | 0.31 (0.20) |
| | Overall selection | | 0.20 (0.11) |

either endurance (GLM morph \times endurance: $\chi^2 = 0.09$, $P = 0.95$; covariate for SVL $P = 0.09$) or sprint speed (GLM morph \times sprint speed: $\chi^2 = 2.31$, $P = 0.31$; covariate for SVL $P = 0.01$). This overall lack of correlational selection was similar among years (data not shown in Table 1 because morph is a categorical variable, and hence, there is no correlational gradient, *sensu stricto*, for these analyses).

Morphs did not differ in their propensity to freeze or run in response to an approaching investigator (GLM $\chi^2 = 0.09$, $N = 571$, $P = 0.95$; effect of year $P = 0.95$;

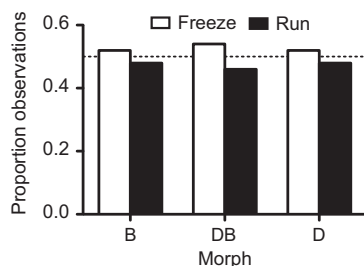


Fig. 3 Morphs did not differ in their propensity to either 'freeze' or 'run' in response to simulated predation (approach with a pole).

Fig 3). During 2005, selection tended to favour B females that ran and DB and D females that froze, although this correlational selection was not significant among morphs (morph \times behaviour: $\chi^2 = 4.43$, $P = 0.11$, Fig. 4a). During 2006, this pattern reversed, such that selection favoured B females that froze and DB and D females that ran (morph \times behaviour: $\chi^2 = 5.88$, $P = 0.052$, Fig. 4b). During 2007, there was a weak tendency for DB females to survive better when they ran ($\chi^2 = 3.89$, $P = 0.048$ within DB females), but correlational selection was not significant among morphs (morph \times behaviour: $\chi^2 = 2.38$, $P = 0.30$, Fig. 4c). Finally, during 2008, survival was not influenced by antipredator behaviour within any morph and correlational selection was nonsignificant among morphs (morph \times behaviour: $\chi^2 = 1.09$, $P = 0.58$, Fig. 4d). Interannual variation in correlational selection was marginally significant (morph \times behaviour \times year: $\chi^2 = 11.94$, $P = 0.06$), but there was no consistent overall pattern of correlational selection favouring particular combinations of morph and antipredator behaviour (morph \times behaviour: $\chi^2 = 1.69$, $P = 0.43$). Given the marginal significance of these results, it is worth noting that none of these tests would remain significant after a

correction for multiple comparisons made among the 4 years of data in this study.

Experimental manipulations of predation

When pooling individuals across predation replicates, survival was higher on islands where we excluded predators compared to islands where we introduced snakes and allowed access by birds (GLM $\chi^2 = 7.39$, $P = 0.007$; effect of surgical treatment $P = 0.18$; Fig. 5). This effect of predation treatment remained significant when we limited the analysis to females that received a sham surgery (GLM $\chi^2 = 5.03$, $P = 0.02$). Although survival was higher on each of the two 'no-predator' islands than on either of the 'predator' islands, we note that this significant effect of predation on survival is driven primarily by the high survival on one of the two 'no-predator' replicates (Fig. 5a). To account for this bias, we also used a nested analysis (island nested within treatment). The effect of predation treatment remained significant in this analysis (nested GLM $\chi^2 = 6.82$, $P = 0.009$) and also when we limited the analysis to females that received a sham surgery (nested GLM $\chi^2 = 4.15$, $P = 0.04$).

When pooling individuals across predation replicates, the effect of predation on survival varied significantly among morphs. This pattern was similar between replicates (Fig. 6a) and was statistically significant both in the full data set (GLM morph \times predation $\chi^2 = 8.86$, $P = 0.01$, effect of surgical treatment $P = 0.23$; Fig. 6b) and when we limited the analysis to sham females (morph \times predation $\chi^2 = 6.31$, $P = 0.04$). Analysing results separately within each morph revealed that this interaction arose because survival was significantly reduced in the presence of predators for B females ($\chi^2 = 6.01$, $P = 0.01$; effect of surgical treatment $P = 0.29$) and D females (D: $\chi^2 = 9.72$, $P = 0.001$; effect

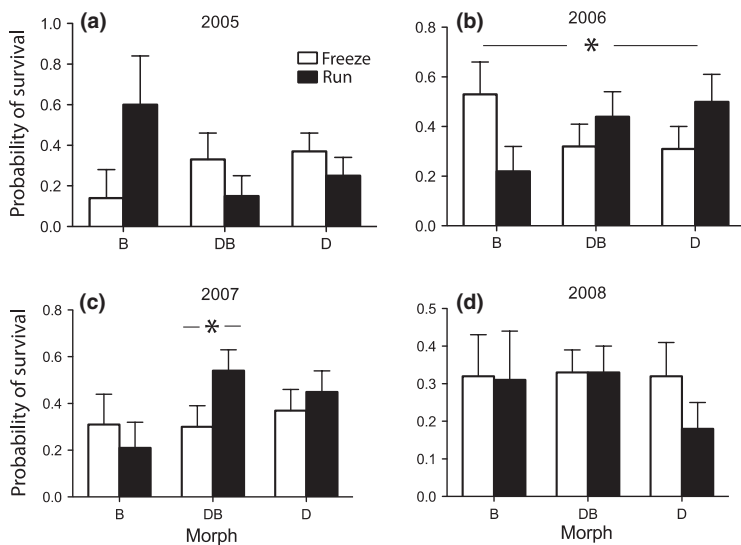


Fig. 4 Correlational selection acting on combinations of dorsal pattern morph and escape behaviour varied among the 4 years of our study (morph \times behaviour \times year; $P = 0.06$) but was only significant within 1 year (2006; morph \times behaviour: $P = 0.05$). Histogram bars shown mean values of survival (+SE). Asterisks above histogram bars indicate statistical significance ($P \leq 0.05$) of correlational selection across morphs (b) or differences in survival as a function of behaviour within individual morphs (c).

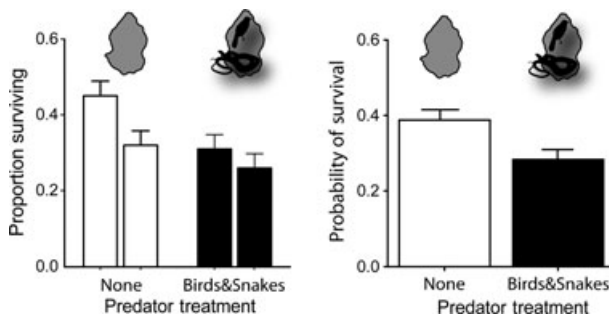


Fig. 5 Variation in survival as a function of predator treatment. Lizards on islands from which predators were experimentally excluded had significantly higher survival compared to lizards on islands exposed to bird and snake predation. Bars show mean values of survival (+SE), and illustrations above depict predation treatments. The left panel shows data from individual island replicates (a); the right panel shows data pooled across both island replicates of each treatment (b). Standard errors are shown using individuals as units of observation.

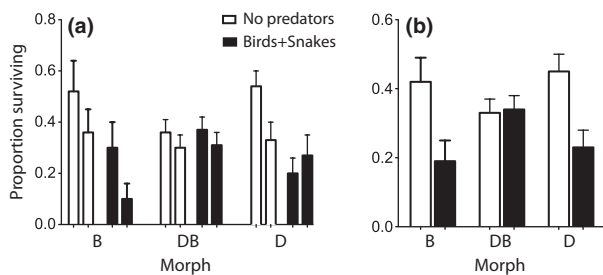


Fig. 6 Survival varied by morph and predation treatment. Data are means (+SE) plotted separately for each island replicate (a), and individuals pooled across both island replicates of each treatment (b). When pooling data across replicates of our study, DB females survived significantly better than did B or D females in the presence of predators. Morphs did not differ in survival on islands from which predators were excluded.

of surgical treatment $P = 0.32$), whereas DB females did not experience a significant reduction in viability in the presence of predators ($\chi^2 = 0.01$, $P = 0.91$; effect of surgical treatment $P = 0.72$; Fig. 6a). These morph-specific differences in predation effects remained significant when we limited the analysis to females that received a sham surgery (B: $\chi^2 = 3.86$, $P = 0.05$; D: $\chi^2 = 6.97$, $P = 0.008$; DB $\chi^2 = 0.07$, $P = 0.79$). Results were nearly identical when we nested island within predation treatment (nested GLM B: $\chi^2 = 4.64$, $P = 0.09$; D: $\chi^2 = 6.01$, $P = 0.006$; DB: $\chi^2 = 1.32$, $P = 0.52$), but the difference was less pronounced for B females when we included only females that received a sham surgery (B: $\chi^2 = 3.09$, $P = 0.21$; D: $\chi^2 = 11.68$, $P = 0.002$; DB $\chi^2 = 3.17$, $P = 0.20$).

Although patterns of morph-specific survival were generally similar across all islands within a given predation treatment, morph differences in survival were not

significant within individual island replicates of our predation treatments. In one replicate of our 'no-predator' treatment, we found a weak tendency for higher survival of B and D females relative to DB ($\chi^2 = 4.46$, $P = 0.10$), whereas survival of all morphs was essentially equivalent in the second replicate ($\chi^2 = 0.35$, $P = 0.84$). Survival of DB females tended to exceed that of B and D females in each replicate of our 'predator' treatment, but these effects were marginally significant in each case ($\chi^2 = 4.21$, $P = 0.12$ and $\chi^2 = 5.45$, $P = 0.07$). Again, results were qualitatively similar when we limited the analysis to females that received a sham surgery (results from the two 'no-predator' islands: $\chi^2 = 5.28$, $P = 0.07$; and $\chi^2 = 0.98$, $P = 0.61$, results from the two 'predator' islands: $\chi^2 = 4.88$, $P = 0.09$; and $\chi^2 = 2.60$, $P = 0.27$). Thus, the significant morph \times predation effects that we observed when pooling individuals across replicates are driven by a consistent tendency for DB to survive better than B and D in the presence of predators, combined with an inconsistent tendency for B and D to survive better than DB in the absence of predators (Fig. 6).

Discussion

The polymorphism in dorsal pattern expressed by *A. sagrei* females has many attributes that are consistent with a stable, genetically based polymorphism. The trait is heritable (Calsbeek *et al.*, 2008). It is found in nearly all populations thus far surveyed on the Great Bahamas Bank, and several lines of evidence suggest that it may be subject to ongoing natural selection (Calsbeek *et al.*, 2008, 2010b; Cox & Calsbeek, 2011). Despite these attributes, we still do not understand the specific causal mechanisms that maintain the polymorphism. The present study provides some limited evidence that predation may play a causal role in driving selection by favouring the phenotypically intermediate morph, which can produce daughters of all three morphotypes. Below, we first discuss each of the implications from our observational data and experimental results and then consider possible alternative means of maintaining variation in dorsal pattern in this system. Although dorsal patterns could represent selectively neutral phenotypic variation (Gould & Lewontin, 1979), we focus our discussion on adaptive processes that could maintain the polymorphism.

The role of behaviour and performance

Our study revealed no evidence that morphs occupy alternative fitness optima for locomotor performance. Morphs did not differ in sprint speed or endurance, nor did correlational selection consistently favour different combinations of morph and locomotor performance. Whereas some polymorphic species do show morph-specific variation in locomotor performance

(Sinervo *et al.*, 2000a; Lancaster *et al.*, 2007), others do not (Brodie, 1989). It is possible that the equipment and conditions used in our collection of performance data (e.g. a motorized treadmill and an enclosed sprint track under ambient field temperatures) were not sufficient to produce ecologically relevant measures of endurance and escape speed (Irschick *et al.*, 2005a,b). However, we have previously detected statistically significant and ecologically intuitive patterns of natural selection acting on variation in endurance and sprint speed measured with identical methods in male anoles (Calsbeek & Irschick, 2007). In contrast to males, the relatively small amount of morphological variation in females may constrain how variation in sprinting speed and running endurance contributes to variation in survival (Calsbeek, 2007), especially given the strong influence of body size and limb length on these performance traits (Losos *et al.*, 1994; Irschick *et al.*, 2005a,b). If this is the case, then the lack of a pattern in our data is perhaps not surprising, given that morphs do not differ substantially in body size or shape (Calsbeek *et al.*, 2008).

Correlational selection on escape behaviour and morph exhibited a marginally significant pattern of interannual variation (i.e. morph \times behaviour \times year interaction). However, morphs did not differ in their escape behaviour, nor did natural selection favour any consistent combinations of morph and escape behaviour when viewed across all 4 years of our study. This suggests that morphs may not be under selection to respond differently to an approaching predator, at least not with respect to the behaviours and stimuli used in this study. Such a scenario might be expected if, for example, some dorsal patterns are cryptic, whereas others are visually disorienting during escape. It is possible that the patterns running the length of the female dorsum are insufficiently cryptic or disorienting to have adaptive value in predator avoidance (Brodie, 1989, 1992). However, predators tend to develop specific search images for prey items and any pattern that breaks up that search image would likely provide at least some benefit (Mappes *et al.*, 2005). A more likely explanation for the lack of correlational selection is that the particular behaviours that we chose to measure in this study simply do not account for biologically important differences in predator avoidance.

A possible alternative explanation for the lack of evidence for correlational selection in our observational study is that selection varies through time, such that our 4-year study was insufficient to document important differences among morphs. This explanation might hold if, for example, correlational selection is itself frequency dependent and varies through time with changes in morph frequency (Calsbeek *et al.*, 2010b). This scenario has been postulated for side-blotched lizards, whose dorsal patterns are thought to be under the control of maternal effects, subject to frequency-dependent selection,

and vary in response to changes in the social environment (Lancaster *et al.*, 2007, 2010). However, in the absence of long-term measures of morph-specific selection extending beyond the 4-year period of our study, we conclude that selection does not consistently favour alternative fitness optima for antipredator behaviours or locomotor capacities in female *A. sagrei* morphs.

Experimental manipulations of the predator guild

Differences in overall survival between our two predator treatments suggest that our experimental manipulations were effective. Overall, lizards on islands from which we excluded predators survived better compared to lizards on islands that were exposed to predation by birds and snakes (Fig 4). One caveat to this result is that the high survival rate on one of our two 'no-predator' islands was responsible for a large part of this inferred predation effect (Fig. 5). If this particular island was more conducive to survival, or if variable environmental conditions led to higher survival in 2008, the year in which we established this replicate (all others were established in 2009), then the high levels of survival that we attribute to the absence of predators could also reflect island or year effects. However, it is more difficult to conceive of a scenario in which any such island or year effect could drive a pattern of morph-specific variation in survival in a manner such as we observed. Survival of B and D females increased significantly when we excluded predators, whereas DB females exhibited similar rates of survival irrespective of the presence or absence of predators, and this pattern was broadly consistent across all islands (Fig 6).

Apostasis is the process whereby predators disproportionately target common phenotypes over rare phenotypes, often by developing a search image for common colours or patterns, thereby generating negative frequency-dependent selection (Allen, 1988; Gray & McKinnon, 2006). Under this scenario, we would expect the uncommon B morph to have a survival advantage over the more common D and DB morphs in the presence of predators, but not in their absence. By contrast, we found that B females had the lowest overall survival in the presence of predators and experienced a large increase in survival when predators were removed, whereas the DB morph exhibited uniformly high survival in both the presence and absence of predators. Moreover, we have not detected any rare-morph advantage when comparing survival across island populations in which the frequencies of B and D naturally vary from rare to common (Calsbeek *et al.*, 2010a, b). This lack of support for apostatic selection agrees with a recent study of another anole expressing a sex-limited dorsal pattern polymorphism, in which neither long-term changes in morph frequencies nor within-generation survival rates provided evidence for frequency-dependent selection (Paemelaere *et al.*, 2011a).

Another possibility is that predator-mediated disruptive selection favours phenotypic extremes because they are each cryptic in different microhabitats or substrates. For example, the longitudinal stripe of the B morph has been suggested to provide a cryptic advantage to females perched on twigs (Schoener & Schoener, 1976), whereas the diamond pattern on D females might render them cryptic in leaf litter or other complex substrates. However, our experimental results are in direct contrast to the pattern expected under stabilizing selection, as the intermediate DB morph was the only phenotype to experience high survival in both the presence and the absence of predators. Despite the intuitive appeal of this hypothesis, examples of disruptive selection acting within populations to maintain colour polymorphisms are relatively scarce (Gray & McKinnon, 2006), and we find no evidence for this process in *A. sagrei*.

Our experimental results are most consistent with the hypothesis that predators give rise to a process analogous to overdominance (i.e. heterosis or heterozygote advantage) by favouring the genetically intermediate morph, with the caveat that this polymorphism is thought to be influenced by multiple loci (Calsbeek *et al.*, 2010a, b). Despite historical emphasis on overdominance as an explanation for the maintenance of genetic diversity, support for its role in the maintenance of colour polymorphism is limited (Gray & McKinnon, 2006). Moreover, the underlying mechanisms whereby predators could give rise to such an effect in *A. sagrei* are unclear. In particular, it is difficult to understand how the intermediate DB morph could have a survival advantage mediated by crypsis. However, we note that the intuitive alternative hypothesis that the B and D phenotypes are variously cryptic on narrow branches or complex substrates is based largely on indirect evidence from other populations (Schoener & Schoener, 1976) and our subjective assessment of crypsis. Whether any of these dorsal patterns are actually cryptic to bird or snake predators is unknown. It is also unclear whether the survival advantage of DB females is due to direct selection on the dorsal pattern or indirect selection on variation in correlated traits. For example, dorsal patterns could be in linkage disequilibrium with display rate or foraging behaviour, which are themselves subject to selection by predation (Stamps & Gon, 1983). In any case, a survival advantage for DB females when predation pressures are high could help to maintain the polymorphism in nature, because females with heterozygous DB genotypes can produce progeny with both B and D phenotypes (Calsbeek *et al.*, 2010b).

Although our results do not illustrate a direct mechanism by which predation pressures *per se* would maintain this polymorphism, it is possible that the selective consequences of the polymorphism vary either spatially or temporally. Selection might vary spatially if, for

example, the polymorphism has direct adaptive significance elsewhere in the geographic distribution of *Anolis*. Dorsal pattern polymorphisms similar to the one described here for *A. sagrei* have been documented among many species of anole throughout their range (Schoener & Schoener, 1976; Kohler & Obermeier, 1998; Townsend & Wilson, 2009; Paemelaere *et al.*, 2011b). Anole species in the West Indies are derived from mainland South and Central America (Poe, 2004), and these ancestral populations are thought to be more strongly influenced by predation compared to the anoles of the West Indies (Andrews, 1979). Thus, it may be that the dorsal polymorphism evolved under the influence of these predation pressures and is no longer relevant in populations that subsequently dispersed to the West Indies.

Given the limited evidence for predators in maintaining the polymorphism in these study populations, future studies of the *Anolis* polymorphism in The Bahamas should focus on alternative mechanisms for driving the patterns of frequency-dependent selection documented elsewhere (Calsbeek *et al.*, 2010b). Possible alternative agents of selection include differences in territorial defence among morphs (Shuster, 1989), differential susceptibility to harassment by males (Svensson *et al.*, 2005) or alternative reproductive strategies (Sinervo *et al.*, 2000b). Of these alternatives, we currently have data to address only the last, and several lines of descriptive and experimental data suggest that reproductive investment and its associated costs do not differ among morphs (Cox & Calsbeek, 2011).

Our study builds on the existing literature on selection estimates measured in the wild (Hoekstra *et al.*, 2001; Kingsolver *et al.*, 2001; Hereford *et al.*, 2004; Cox & Calsbeek, 2009; Siepielski *et al.*, 2009) by providing one of the rare field-based measures of a change in natural selection following experimental manipulations of a proposed agent of selection. Additional experimental manipulations of this sort will bring us closer to a full understanding of how natural selection operates in the wild. We suggest that, whereas correlative estimates of selection have contributed greatly to the way we understand evolutionary processes, experimental manipulations that measure changes in selection are too rarely performed and that future studies should aim to rectify this shortcoming.

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