GEOGRAPHIC VARIATION, FREQUENCY-DEPENDENT SELECTION, AND THE MAINTENANCE OF A FEMALE-LIMITED POLYMORPHISM

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A central problem in evolutionary biology is to understand how spatial and temporal variation in selection maintain genetic variation within and among populations. Brown anole lizards (*Anolis sagrei*) exhibit a dorsal pattern polymorphism that is expressed only in females, which occur in "diamond," "bar," and intermediate "diamond-bar" morphs. To understand the inheritance of this polymorphism, we conducted a captive breeding study that refuted several single-locus models and supported a two-locus mode of inheritance. To describe geographic variation in morph frequencies, we surveyed 13 populations from two major islands in The Bahamas. Morph frequencies differed substantially between major islands but were highly congruent within each island. Finally, we measured viability selection on each island to test two hypotheses regarding the maintenance of the polymorphism: (1) that spatial variation in selection maintains variation in morph frequencies between islands, and (2) that temporal variation in selection across years maintains variation within islands. Although bar females had relatively lower survival where they were rare, our data do not otherwise suggest that selection varies spatially between islands. However, diamond-bar females were subject to positive frequency-dependent selection across years, and the relative fitness of bar and diamond females alternated across years. We propose that this polymorphism is maintained by temporal variation in selection coupled with the sheltering of alleles via a two-locus inheritance pattern and sex-limited expression.

KEY WORDS: Drift, genetic variation, island, lizard, polymorphism, selection.

One of the most long-standing debates in evolutionary biology concerns the relative importance of selection pressures operating at different temporal (Eldredge et al. 2005) and spatial scales (Thompson 1999; Svensson and Sinervo 2004). Understanding spatial and temporal variation in selection is important because variation in selection pressures can influence levels of genetic diversity within populations (Gillespie and Turelli 1989), the forms of genetic variation between populations (e.g., local co-adapted gene complexes and linkage disequilibria; Wade and Goodnight 1998), the maintenance of clines (Endler 1977), and the dynamics of a species' range (Case and Taper 2000). As such, the debate has influenced much of our modern concep-

tion of evolutionary biology, including perspectives concerning the relative importance of selection in large panmictic populations (Fisher 1930) versus interconnected demes (Wright 1982), and the importance of rectifying rapid evolutionary change (Huey et al. 2000; Gilchrist et al. 2001) with the long periods of stasis observed in the fossil record (Eldredge et al. 2005).

Selection can vary, whether spatially or temporally, in a variety of ways. For example, selection can differ in magnitude, direction, or form (Kingsolver et al. 2001; Hereford et al. 2004; Svensson and Sinervo 2004). Measured over short and long time scales, or small and large spatial scales, these estimates may bear little resemblance to one another (Grant and Grant 2002). Thus,

there is increasing appreciation of our need for additional studies of selection measured in more than a single population or in a single year (Siepelski et al., in press), to better understand how the temporal and geographic mosaics of selection influence patterns of biological diversity (Thompson 1989, 1996; Gomulkiewicz et al. 2000). This growing appreciation for the variability of selection pressures has done much to improve our understanding of how genetic variation can be maintained, even in the face of erosive forces such as strong selection and genetic drift (Hubby and Lewontin 1966; Lewontin and Hubby 1966; Kimura 1983).

Our goal in this article is to determine whether spatial and temporal variation in selection contribute to the maintenance of phenotypic and genetic diversity within and among populations of brown anole lizards, *Anolis sagrei*. These lizards present an excellent opportunity to simultaneously track phenotypic and genetic diversity because females express a genetically based polymorphism (Calsbeek et al. 2008). Females on the Great Bahamas bank exhibit one of three alternative dorsal patterns: (1) a dorsum with well defined and interconnected polygons called "Diamond" (hereafter D), (2) a dorsum with a longitudinal stripe, called "Bar" (hereafter B), and (3) a highly variable intermediate phenotype called "Diamond-bar" (hereafter DB) (Fig. 1). Elsewhere in The Bahamas (e.g., the Little Bahamas bank), females are also polymorphic (Schoener and Schoener 1976). Although B morphs occur on both the Great and Little Bahamas banks, D and DB morphs are not observed on the little Bahamas bank. Here, we present new data from a captive breeding study designed to elucidate the mode of inheritance underlying the polymorphism. We then explore geographic variation in the frequency of female morphs and show that morph frequencies are highly divergent between two major island groups on the Great Bahamas bank (B is rare on Great Exuma, D is rare on Eleuthera). Finally, we present four years of mark–recapture data to estimate viability selection on each island and test two related hypotheses concerning the maintenance of this variation within and between island populations.

First, we test the hypothesis that geographic variation in morph frequency "between" island populations is maintained by spatial variation in natural selection. This hypothesis predicts that selection should favor the common morph in a given population, and that fixation of a single morph is prevented by gene flow from adjacent populations. Thus, if variation in morph frequencies between islands is due to differences in selection, we predict that B females, which are common on Eleuthera and rare on Great Exuma, should be favored on Eleuthera and disfavored on Great Exuma. By contrast, D females, which are common on Great Exuma and rare on Eleuthera, should be favored on Great Exuma and disfavored on Eleuthera. A recent study demonstrated that alternative female morphs may be subject to different forms of natural selection on Great Exuma (Calsbeek et al. 2008), but that
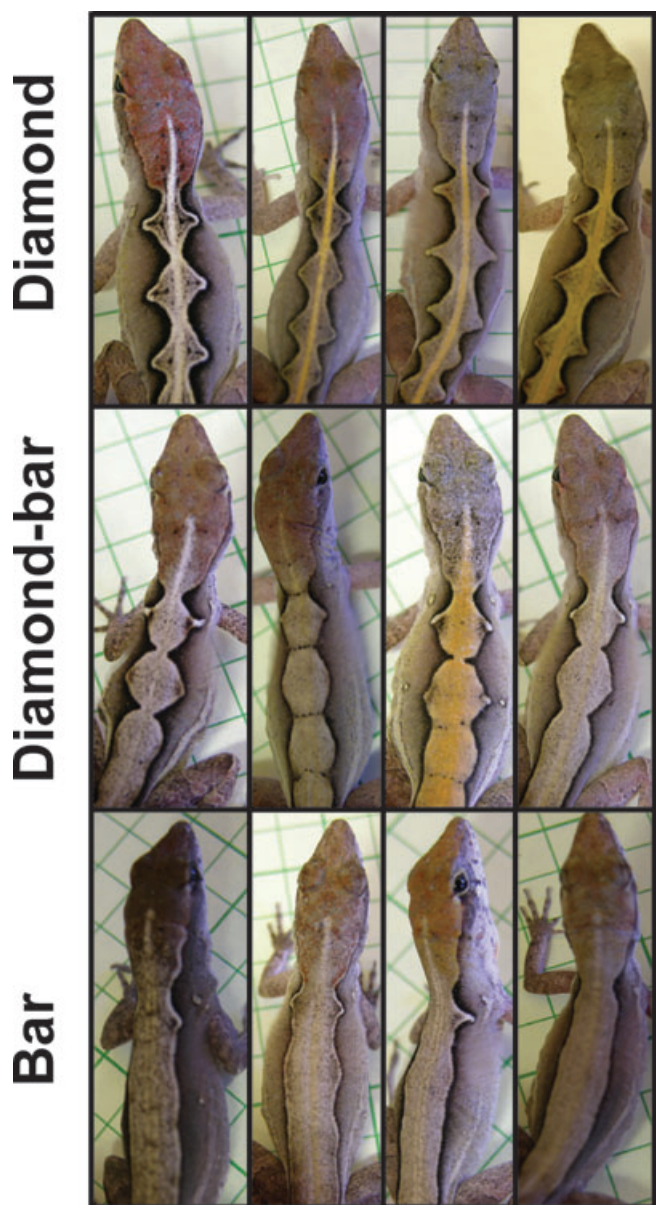

Figure 1. Each row illustrates representative variation in female dorsal patterning. Morphs were diagnosed based on the presence of an uninterrupted diamond pattern running the length of the dorsum (D; top row), the presence of a solid longitudinal bar (B; bottom row), or misshapen diamonds or irregular stripes (DB; center row).

study did not quantify geographic variation in selection. Moreover, different island populations in The Bahamas are connected by gene flow that occurs via overwater dispersal (Calsbeek and Smith 2003), suggesting that the polymorphism could persist via gene flow even in the face of strong local selection against the rare morph.

Second, we test whether polymorphism is maintained "within" populations by frequency-dependent natural selection.

Negative frequency-dependent selection (i.e., rare morph advantage) is generally considered one of the primary means of preserving genetic variation within populations (Trotter and Spencer 2008). Many polymorphisms are associated with alternative fitness optima corresponding to differential resource specialization (Schluter 1996; Nosil et al. 2002) or alternative mating tactics (Gross 1985; Sinervo and Lively 1996; Alonzo and Warner 1999), and these alternative fitness optima often lead to negative frequency-dependence. Unlike the first hypothesis concerning geographic variation in morph-specific selection, this hypothesis predicts that the fitness of a morph is related to its frequency in the population, rather than its local environment per se. To test this hypothesis, we estimate morph-specific correlations between frequency and fitness for each population and each year of our study. If the dorsal pattern polymorphism persists due to negative frequency-dependent selection, we predict a negative correlation between frequency and fitness for one or all morphs.

Methods

The brown anole (*A. sagrei*) is a small, semi-arboreal lizard that is distributed throughout the West Indies. This species is sexually dimorphic in body size, morphology, and coloration, including the dorsal pattern polymorphism that is the focus of this study, and which is expressed only in females. We studied *A. sagrei* on and around the islands of Great Exuma and Eleuthera in The Bahamas. Most (70–90%) females at these study sites mature and die during a single reproductive season. For all studies, we captured females by hand, or by using a silk noose attached to the end of a fishing pole. Individual females were scored as D based on the presence of an uninterrupted diamond pattern and as B based on the presence of a solid longitudinal bar. Females with misshapen diamonds or irregular stripes were scored as DB morphs (see Fig. 1 for examples of typical variation in morphs).

BREEDING STUDY

During 2007, we captured adult females from a remote study site near Georgetown, Great Exuma and transported them back to the laboratory to lay eggs. Females mated naturally in the wild prior to capture. Because this species can store sperm for several months (Calsbeek et al. 2007), we were able to collect multiple eggs from most females (range: 0–16). Females were housed individually in 10-gallon (50 × 25 × 30 cm) terraria, and were provided with a potted plant to use as a nesting site. Each terrarium was exposed to full spectrum 5% UVB lighting and had a 40-watt incandescent lamp as a heat source. Lamps were set to a 12:12 photoperiod. Females were watered daily and fed ad libitum crickets (*Achaeta domestica*), which were dusted periodically with a vitamin and mineral supplement (Fluker's Repta-Vitamin, Port Allen, LA). Hatchlings were removed from

tanks at first sighting, sexed, weighed on a digital balance (nearest 0.01 g.), measured for snout-vent length (SVL; nearest 0.5 mm), and toe-clipped for permanent identification. Female progeny were scored for dorsal pattern morph, which is visible at hatching and remains fixed throughout ontogeny. Progeny from field-caught dams were raised to maturity and were then used to sample a second generation during 2008. Each virgin female was individually housed with two potential sires, and progeny were collected and processed using the same protocols as described for 2007.

To elucidate the mode of inheritance of the polymorphism, we compared the ratios of progeny morphs against the expected ratios from a series of one- and two-locus Mendelian models, each of which account for three phenotypic classes. We began with simple one-locus models and moved to progressively more complex two-locus models. In each case, we asked whether the model under consideration could produce both the observed patterns of offspring morph ratios and the biogeographic patterns of morph distribution observed in the wild (see below). We used the female morph frequencies observed in the laboratory to estimate allele frequencies for use in each model. We then made the simplifying assumption that morph alleles in sires were at Hardy–Weinberg equilibrium in the same frequencies as observed in females. We made this assumption because the sex-limited nature of the polymorphism made it impossible to score male genotypes a priori. We then calculated expected ratios of morphs in progeny given the putative genotypes of dams and the expected allele frequencies in males. We used chi-square tests to compare observed morph frequencies against the expected ratios from one- and two-locus Mendelian models. Analyses were conducted separately for each morph class and significant departures from expected ratios were taken as evidence refuting a given model of inheritance.

GEOGRAPHIC VARIATION IN MORPH FREQUENCY

To describe geographic variation in morph frequency, we surveyed 13 populations arrayed at two different spatial scales. To assess large-scale geographic variation in morph frequency between Eleuthera and Great Exuma (ca. 150 km apart), we surveyed four separate sites on each major island. We also surveyed five populations on small offshore islands within 3 km of Great Exuma (Fig. 2). The inclusion of multiple populations on each major island and on offshore islands near Great Exuma allowed us to assess small-scale geographic variation in morph frequency within Eleuthera and Great Exuma. For each survey, we captured 50–125 females (mean = 93) and recorded the dorsal pattern of each. All females were weighed with a Pesola spring scale (nearest 0.1 g) and measured for SVL with a metal ruler (nearest 0.5 mm). We also sampled a small (2 mm) piece of tail tissue from each individual for future genetic studies. To prevent double counting, we temporarily marked each individual with a small spot of white paint and then released each animal at its exact spot of capture. We
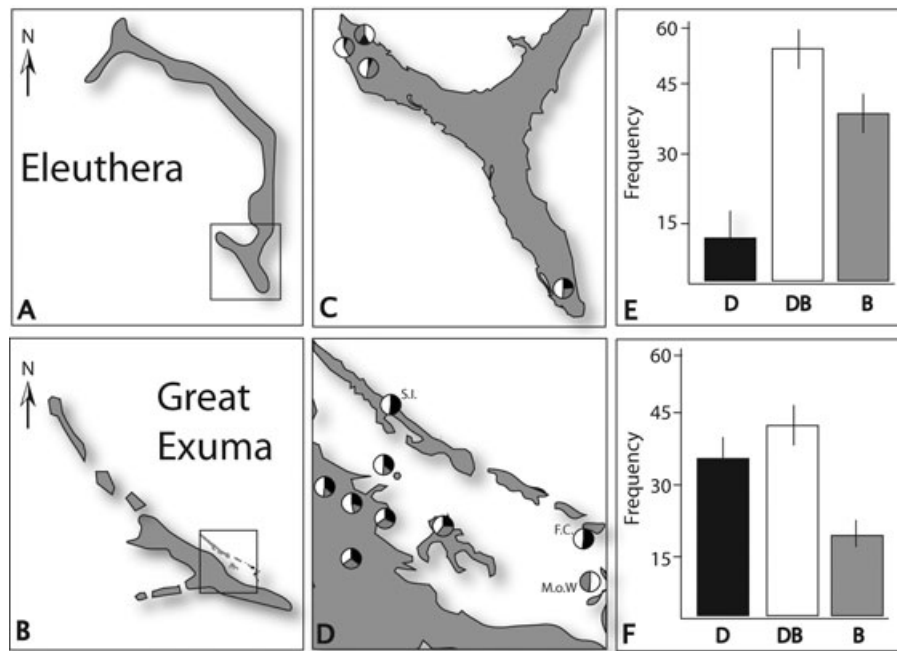
Figure 2. Maps show whole island views of our two principle study islands. (A) Eleuthera, and (B) Great Exuma, with outlined areas showing greater detail in panels (C) and (D). Pie charts show the relative frequency of the three female morphs at each study site. In panel D, initials designate the three barrier islands that lie to the east of Great Exuma, the only sites on which one morph (D or B) was found to be nearly absent. These are Stocking Island (S.I.), Fowl Cay (F.C.), and Man o' War cay (M.o.W.). Panels (E) and (F) show the mean frequency (+SE) of each morph averaged across all study sites on each island group.

calculated the frequency of each morph in each individual population and then tested for large-scale geographic differences in morph frequency using analysis of variance (ANOVA) with major island (Eleuthera or Great Exuma) as the main effect. These analyses were conducted separately for each morph. We also tested for an overall difference in the distribution of morphs using a contingency chi-square.

SELECTION STUDIES

We studied the viability of female morphs over the course of the breeding season at one site on each of our two primary study islands. We studied natural populations of lizards on Kidd Cay, near Georgetown, Great Exuma (23°31′N, 75°49.5′W), and on a peninsula near The Island School on the north side of Cape Eleuthera (24°50′N, 76°19′W). Kidd Cay is a medium-sized (ca. 1500 m^2) island that is connected to the main island of Great Exuma by a paved causeway. This site is dominated by large trees (e.g., palms *Pseudophoenix* spp, and Australian Pine *Casuarina equisetifolia*) and smaller scrub (e.g., sea-grape *Coccoloba uvifera* and buttonwood *Conocarpus erectus*). We studied selection at this site over four consecutive breeding seasons (2005–2008). We also studied selection at our Eleuthera site for two seasons (2007–2008). This site is similar in size to the Great Exuma site, and is dominated by *C. equisetifolia* and scrub vegetation. We established boundaries for this study site based on water on three

sides and a road that served as a probable barrier to dispersal off the peninsula.

At each site, we attempted to capture every female during each spring (mid-May on Exuma, early June on Eleuthera). All females were scored for dorsal pattern morph, weighed with a Pesola spring scale (nearest 0.1 g), and measured with a metal ruler (nearest mm). Females were permanently marked with a unique combination of colored elastomer tags that we injected into the ventral side of each limb. To prevent unnecessary recapture, we marked each female with a small spot of temporary white paint on the hind limb. Four months after the initial capture period (i.e., September–October), we returned to each study site and performed an exhaustive census of all surviving lizards. Censuses continued for two weeks, or until three consecutive days of searching turned up no new lizards. Lizards not recaptured during our census were considered to have died. This is a reasonable assumption given the extremely low frequency of dispersal away from established home ranges (Calsbeek 2009) and the fact that our study sites were bounded primarily by water and roads, which serve as barriers to dispersal. Primary sources of mortality on these islands include both predation (primarily by snakes, *Alsophis vudii*, and birds, particularly mockingbirds, *Mimus* spp.*,* and green herons*, Butorides virescens*) and competition (Calsbeek and Smith 2007). Females lay a single-egg clutch at approximately one-week intervals throughout the breeding season,

thus variation in fecundity should be closely linked to variation in survival.

We estimated relative fitness in each study population by scaling the mean survival of each morph class by the mean probability of survival for all females in that population. Because survival has a binomial distribution (live/die), we estimated significance values for tests of geographic variation in morph frequency using generalized linear models (GLM) with a logit link function. To test for spatial variation in selection, we used GLM with survival as the dependent variable, major island (Eleuthera or Great Exuma) and morph (D, B, DB) as main effects, and then tested for a significant morph $\times$ island interaction. To test for frequency-dependent selection, we performed a regression of the probability of survival against initial frequency for each morph. To do this, we treated each year of data as an independent observation and combined estimates from Eleuthera and Great Exuma, including island as a factor. We examined differences in the nature of frequency-dependent selection (i.e., positive, negative) among morphs by combining data on relative fitness of all three morphs and testing for a morph $\times$ frequency interaction, with each morph's contribution to the model weighted by the number of observations. We

also ran this model using data only from Great Exuma (the island for which we had four years of data) to verify that effects were due to morph frequencies and not differences among islands per se.

Results

BREEDING STUDY

We raised a total of 263 progeny (125 daughters) from 69 dams during 2007 and another 220 progeny (100 daughters) from 63 dams during 2008. We rejected the one-locus model of morph inheritance based on significant deviations from expected progeny ratios for all morph classes (Table 1). Indeed, statistical significance was inestimable for the B and D progeny classes on account of zero values in the chi-square denominator (the prediction of a one-locus model is that neither B nor D dams should produce progeny of the alternative morph). However, two alternative models each provided a reasonable (although imperfect) fit to the prediction that the polymorphism is controlled by two loci, each with two alleles. The first of these models predicts that D (D) is dominant to B (d) at the first locus, and that dominant epistasis

Table 1. Left columns show Progeny ratios of female morphs produced by dams of the three alternative dorsal morphs in the laboratory (observed). (A) Field captured dams that had mated prior to capture. (B) F1 dams raised in the laboratory and bred to each of two alternative sires. (C) Data from both years combined. Right columns (D–F) show expected ratios used to test each of three hypothetical models for patterns of inheritance. In addition to testing the pooled dataset, we also tested each year separately. Results were all similar and for brevity, all comparisons are made against (C). Although none of the models provided a perfect fit, we found that morph ratios in progeny, along with the biogeographic patterns of morph distribution on islands (see text) were highly consistent with a two-locus model and generally supported the recessive epistasis model (F).

A.		Observed Daughters			D. Expected Single codominant locus				
		B	DB	D	B	DB	D	χ^2	P
2007	B	11	13	2	18	25	0	NA	0
Dam	DB	3	36	10	18	50	29	31.85	<0.00001
	D	4	18	26	0	35	47	NA	0

B.					E. Two loci; dominant epistasis				
		B	DB	D	B	DB	D		
2008	B	10	6	1	9	5	12	21.29	0.0001
Dam	DB	3	32	15	3	34	11	0.67	NS
	D	3	12	18	6	10	32	4.88	0.01

C.					F. Two loci; recessive epistasis				
		B	DB	D	B	DB	D		
Both	B	21	19	3	18	16	9	4.61	NS
Dam	DB	6	69	25	14	59	26	6.41	0.05
	D	7	30	45	9	25	49	1.88	NS

at the second locus (*A_*) overrides production of either "pure" phenotype, resulting in the DB phenotype. Although DB dams produced progeny in ratios that closely matched the predicted values, we observed significant deviations from the expected ratios of progeny morphs in D and B females within in each year separately, as well as in the combined dataset from both years (Table 1). An alternative two-locus model assuming recessive epistasis produced a better fit to the data (Table 1). A two-locus model with recessive epistasis again assumes that the diamond allele (*D*) is dominant at the first locus, but that two copies of the recessive allele at a second locus override the expression of B or DB and produces the D phenotype. The only significant deviation from the expected ratios in this model came from one morph class in the pooled dataset, in which DB dams slightly overproduced B daughters ($\chi^2 = 6.41$, $P < 0.05$; Table 1).

GEOGRAPHIC SURVEYS

Morphs differed in size (SVL), and this variation was similar across both major islands. On Exuma, B females were significantly smaller than D and DB females (mean $SVL_B = 40.22$ mm, $SVL_D = 41.59$ mm, $SVL_{DB} = 41.65$ mm; ANOVA $F_{2,871} = 7.46$, $P = 0.0002$). On Eleuthera, D females were significantly larger than both B and DB females (mean $SVL_D = 41.98$, $SVL_B = 40.93$ mm, $SVL_{DB} = 40.79$ mm; ANOVA $F_{2,532} = 4.32$, $P = 0.01$). All differences remained significant in post-hoc tests.

Survey data revealed large and statistically significant geographic differences in morph frequency between Great Exuma and Eleuthera (Contingency $\chi^2 = 55.70$, df = 2, $P < 0.0001$; Fig. 2). The B morph was rare (range: 1–39%) in all populations sampled on Great Exuma and on all but one of its near-shore islands. The one exception to this pattern occurred on Man o' War Cay, to the east of Great Exuma (Fig. 2), where we recorded a B frequency of 50%. In contrast to populations on Great Exuma, B was common (range: 27–47%) at all sites surveyed on Eleuthera. Mean frequency of B differed significantly between Great Exuma (23%) and Eleuthera (39%, ANOVA $F_{1,14} = 4.57$, $P = 0.05$). The D morph was common on Great Exuma (range: 25–51%) and on all but one of its near-shore islands (2% on Man o' War Cay), but it was rare on Eleuthera (range: 7–24%). Mean frequency of D differed significantly between Great Exuma (34%) and Eleuthera (14%, ANOVA $F_{1,14} = 9.49$, $P = 0.008$). The DB morph was common in all populations (range: 35–59%) and mean DB frequency did not differ between Great Exuma (42%) and Eleuthera (46%, ANOVA $F_{1,14} = 0.89$, $P = 0.36$).

SELECTION ON THE MORPHS

We began our study of selection on female morphs during 2005, one year after we had experimentally replaced all of the males in our study population as part of a separate study of natural selection (see Calsbeek and Smith 2007). We note this here because the
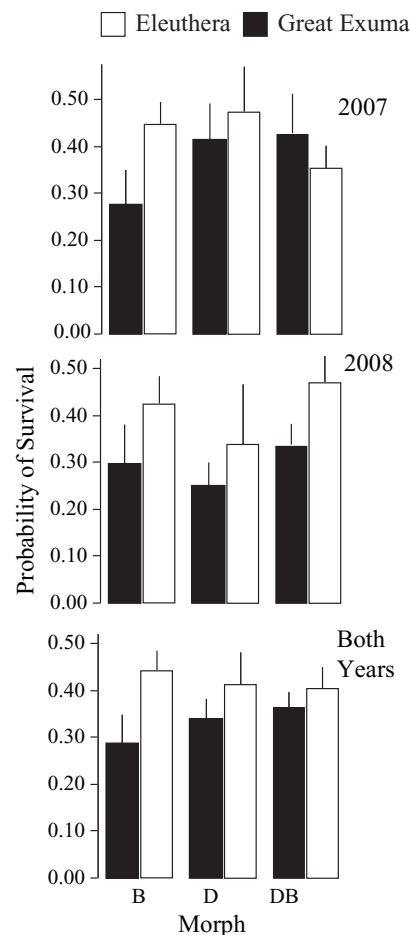

Figure 3. Survival probabilities +(1 SE) of the morphs on each major study island during the two years of comparison (2007 and 2008), and for both years combined (bottom panel). Although overall survival was consistently higher on Eleuthera compared with Great Exuma, there were no consistent differences in morph-specific survival either within or between islands.

2004 population manipulation is likely to have shifted the study population's morph frequencies out of equilibrium at the beginning of the present study. We did not detect any morph-specific selection as a function of body size in any year of our study. During 2005 and 2006, we measured variation in survival only on our Great Exuma site. Morphs did not differ in their probability of survival during 2005 (GLM $\chi^2 = 1.81$, df = 2, $P = 0.41$) or 2006 (GLM $\chi^2 = 0.33$ df = 2, $P = 0.85$). During 2007 and 2008, we expanded our study to include measures of selection on our Eleuthera site. Consistent with data from the first two years of study, we detected no morph-specific variation in survival within either site during 2007 (Great Exuma: GLM $\chi^2 = 2.31$, df = 2, $P = 0.31$; Eleuthera: GLM $\chi^2 = 1.74$ df = 2, $P = 0.42$) or 2008 (Great Exuma: GLM $\chi^2 = 2.31$, df = 2, $P = 0.55$; Eleuthera: GLM $\chi^2 = 0.82$ df = 2, $P = 0.67$) (Fig. 3).

We measured significant differences in overall viability between lizards from Great Exuma and Eleuthera. Survival rates
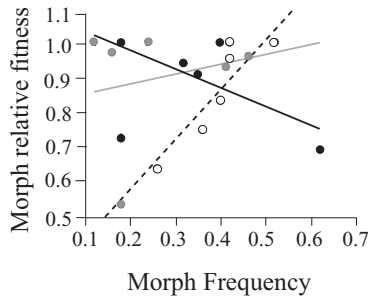
Figure 4. Four years of survival data reveal that morph fitness was partially frequency-dependent. Fitness of DB increased with increasing frequency (positive frequency-dependence). However, D fitness tended toward negative frequency-dependence, and B fitness was independent of frequency over the course of our study.

Figure 5. Despite the frequency-dependent fitness interactions among morphs, morph frequencies did not show consistent cyclic dynamics among years (data for Great Exuma only). Instead, DB females steadily rose to a frequency near 50% over the course of our study, a pattern consistent with positive frequency-dependence of DB fitness.

were higher on Eleuthera compared to Great Exuma during 2008 (GLM $\chi^2 = 3.83$, df = 1, $P = 0.05$) but not 2007 (GLM $\chi^2 = 0.61$, df = 1, $P = 0.44$). Differences in survival remained significant when we pooled data from 2007 and 2008 and included "year" as a random factor (ANOVA $F_{1,584.2} = 3.99$, $P = 0.04$). This difference was largely driven by the significantly higher survival of B females on Eleuthera compared to Great Exuma (0.44 vs. 0.28; GLM $\chi^2 = 4.43$, df = 1, $P = 0.03$) (Fig. 3).

Finally, we estimated survival probabilities for morphs as a function of local frequency in each population and for each year (Fig. 4). We measured a significant interaction effect, indicating that frequency-dependent natural selection differed among morphs (GLM for morph frequency × survival $\chi^2 = 8.24$, df = 2, $P = 0.02$). This interaction reflects the fact that DB females were subject to positive frequency-dependent selection (GLM $\chi^2 = 5.50$, df = 2, $P = 0.04$, effect of island $P = 0.63$), whereas D females were under negative frequency-dependent selection ($\chi^2 = 4.20$, df = 1, $P = 0.04$ effect of island $P = 0.19$). There was no relationship between the frequency of B females and their probability of survival ($\chi^2 = 0.06$, df = 1, $P = 0.81$, effect of island, $P = 0.92$). Results were similar when we considered only data from Great Exuma to avoid confounding local variation between islands with frequency-dependence per se. On Great Exuma, DB females were subject to positive frequency-dependent selection ($\chi^2 = 4.23$, df = 1, $P = 0.04$), whereas D females were under negative frequency-dependent selection ($\chi^2 = 4.20$, df = 1, $P = 0.04$) and the frequency of B females was unrelated to their probability of survival ($\chi^2 = 0.04$, df = 1, $P = 0.85$). The interaction effect remained statistically significant in the restricted dataset with only four observations per morph (GLM: morph frequency × survival $\chi^2 = 7.52$, df = 2, $P = 0.03$).

Although fitness of morphs differed as a function of their frequencies, this inferred frequency-dependent selection did not appear to drive annual cycles in morph frequency (Fig. 5). Instead, DB frequency rose consistently over the four years of study
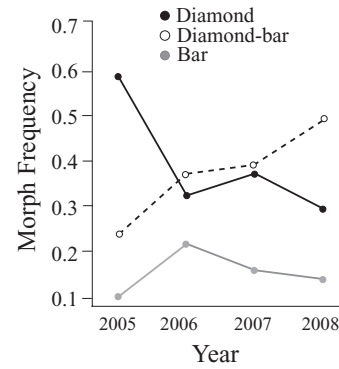
on Great Exuma. This pattern is consistent with the predicted evolutionary increase of DB in response to the observed positive frequency-dependence of DB fitness. By contrast, B and D frequencies appeared to oscillate out of phase in three of the four years of our study, although the pattern was not statistically significant (test of independence in morph abundance [$\chi^2 = 10.72$ df = 6, $P = 0.1$]).

Discussion

Our study adds to the growing body of work capitalizing on discrete polymorphisms to understand the maintenance of genetic variation in nature (Sinervo and Lively 1996; Kerr et al. 2002; Svensson et al. 2005). We have shown that *Anolis* lizards on the Great Bahamas Bank exhibit a dorsal pattern polymorphism that is expressed only by females. Frequencies of the three alternative morphs were largely consistent among sub-populations sampled at relatively small spatial scales (i.e., on and around Great Exuma and on Eleuthera) but were highly divergent between the two main study islands. Whereas the frequency of the DB morph was similar on Eleuthera and Great Exuma, D females were relatively common on Great Exuma and uncommon on Eleuthera, and the reverse was true for B females. What explains this geographic variation in dorsal pattern? Our results do not suggest a central role for spatial variation in selection in maintaining variation in morph frequencies between populations. However, our data do provide preliminary evidence that mixed forms of frequency-dependence can maintain variation in the polymorphism within populations.

Although survival was generally higher on Eleuthera than on Great Exuma, there were no consistent differences in morph-specific viability either within or between islands. The one exception to this general result was the modestly higher survival of B females on Eleuthera during 2007. Although this result is

consistent with the prevalence of B females on Eleuthera, it provides only weak evidence for interisland differences in morph-specific viability. On the whole, our data do not strongly support the hypothesis that observed differences in morph frequencies between Great Exuma and Eleuthera reflect underlying differences in morph-specific viability selection. This result differs from other studies that have shown geographic mosaics in selection over both large (Gosden and Svensson 2008) and small spatial scales (Blanckenhorn et al. 1999; Jann et al. 2000) that can contribute to the maintenance of phenotypic and genetic variation.

Our selection data do provide preliminary support for the hypothesis that morph fitness is frequency-dependent, although not in the typical form of negative frequency-dependence observed in other studies (Sinervo and Lively 1996; Kerr et al. 2002; Svensson et al. 2005). Whereas fitness of D females decreased slightly with increasing frequency (i.e., negative frequency-dependence or a "rare-Diamond" advantage), fitness of DB females increased with increasing frequency (positive frequency-dependence). Fitness of B females was independent of their frequency, at least over the relatively short time course of this study (four total years of selection data). This last result may stem from the fact that B frequency remained very low in all years on Great Exuma, making it difficult to accurately measure selection as a function of changes in B frequency. Although positive frequency-dependent selection is often predicted to lead to fixation of a single strategy, the putative genotypes that characterize DB females (see below) can reconstitute B or D female morphs (or both), even at high DB frequency. This facet of the genetic basis of the polymorphism may also explain why, despite frequency-dependence of morph fitness, we observe no cyclic variation in morph frequency among years (Sinervo and Lively 1996; Kerr et al. 2002; Svensson et al. 2005).

Although suggestive as a means of contributing to the maintenance of variation, we still do not understand the causes of frequency-dependent selection. Previous studies have shown that frequency-dependent selection can arise from a variety of causes. Examples of negative frequency-dependence include the balance between territorial and sneaker strategies (Shuster and Wade 1992), optimal out-crossing in plants (Eckert and Barrett 1992; Eckert et al. 1996), and cryptic strategies for avoiding sexual conflict (Svensson et al. 2005). Positive frequency-dependence is often measured in predator–prey dynamics (Mappes et al. 2005) and is also known to interact with negatively frequency-dependent forms (Endler and Mappes 2004) reviewed in (Sinervo and Calsbeek 2006). It would be edifying to know the process that creates frequency-dependence in this system.

A previous study (Calsbeek et al. 2008) showed that the dorsal pattern polymorphism in *A. sagrei* has a genetic basis, but the available data in that study could not address the mechanism of inheritance. Here, we have greatly expanded on that study by collecting two generations of progeny in the laboratory. Observed morph ratios in progeny of DB females closely match those predicted by a two-locus mode of inheritance (Table 1). Although our data are most consistent with the influence of recessive-epistasis at the second locus (Table 1), this conclusion is based on the simplifying assumption that male genotypes are in Hardy–Weinberg equilibrium. Our inability to score male genotypes severely constrains our ability to test additional models of inheritance or to rule out alternative explanations for the observed deviations from the models tested here. For example, under the dominant-epistatic model, neither D nor B females produced progeny in their predicted ratios. Instead, D and B females each under-produced progeny of the opposite morph in both years of our breeding study. This pattern might be explained by assortative mating if females could somehow assess the morph-specific alleles carried by males. Morph-biased assortative mating has recently been demonstrated in Gouldian finches, *Erythrura gouldiae*, in which females that mate with sires of opposite head color produce less-fit offspring (Pryke and Griffith 2009). Until we can accurately assess male genotypes, our conclusions regarding the exact mode of inheritance, including the possibility that morphs represent a more continuous form of variation, must remain tentative.

Although we cannot specify the exact two-locus model, the biogeography of the polymorphism on several islands to the east of Great Exuma provided further corroborative support for our proposal that the polymorphism is controlled by at least two loci. Bar females were exceedingly rare on Stocking Island ($<1\%$) and Fowl Cay (6%), where D and DB females both reached frequencies of near 50%. By contrast, B and DB morphs each accounted for ca. 50% of the females on Man o' War Cay, where we captured only one D female (2%). The most parsimonious explanation for the loss of either D or B morphs despite persistence of DB females is a two-locus mode of inheritance. For example, under the recessive epistasis model, this pattern could be explained by the loss of the recessive *bar* allele *(d)* on Stocking Island and Fowl Cay and the loss of the recessive allele at the second locus *(a)* on Man o' War Cay. Loss of the *bar* allele implies that females on Stocking Island and Fowl Cay are homozygous for the dominant *diamond* (*D*) allele. Diamond and DB females would then differ only by the presence or absence of a dominant allele at the epistatic locus. Similarly, loss of the recessive allele at the epistatic locus on Man o' War Cay would imply that all females are homozygous for the dominant *A* but may have either the dominant or recessive alleles at the morph locus. By contrast, no single-locus model for this system can explain the maintenance of two morphs despite absence of the third, and at the same time account for the observed production of all three dorsal morphs by individual dams in the laboratory.

Fixation of alternative alleles on these three islands may have occurred via selection, genetic drift, or a founder effect in which different islands were colonized by females homozygous for alternative morph alleles. Selection favoring alternative morphs on different islands seems an unlikely explanation given the close proximity and similar environmental conditions on all three of these islands (Fig. 2). Either remaining scenario is biologically plausible and differentiating between them is beyond the scope of the present study. However, we observed all three morphs on several smaller islands in our study, even though stochastic loss of alleles should, at least theoretically, be more likely on these smaller islands. This implies that either insufficient time has passed to lose alleles by drift, or that over-water dispersal among islands (Calsbeek and Smith 2003) replaces these lost alleles at frequent intervals on the smaller cays.

Gene flow between major islands (e.g., Eleuthera and Great Exuma) has been shown to follow the flow of ocean currents (Calsbeek and Smith 2003). Currents flow east to west in The Bahamas and also among many cays near Great Exuma. Importantly, the three islands that are fixed for one of the alternative morph alleles all lay on the eastern-most range of the Exuma island chain (Fig. 2). These islands should therefore be the least likely to receive input from neighboring populations, whereas the smaller cays on which we observed all three morphs could receive input from a variety of adjacent sites. A similar process might likewise help to maintain the polymorphism at larger spatial scales (e.g., among islands on the Great Bahamas Bank). For example, gene flow between island and mainland populations of garter snakes plays an important role in the maintenance of another dorsal polymorphism (King and Lawson 1995; Lawson and King 1996). Future studies will be aimed at understanding the potential importance of dispersal and gene flow in the maintenance of morphs across multiple spatial scales.

Finally, although expression of the polymorphism is entirely sex-limited to females, males almost certainly still carry alleles for the trait. For analytical convenience, we assumed allele frequencies in males to be in Hardy–Weinberg equilibrium. However, any indirect selection acting on correlated traits in males would alter allele frequencies passed on to female progeny. This, in turn, could have confounded our estimates of morph frequency and morph-specific fitness variation because the frequencies and fitness of alleles may differ in males and females (Calsbeek and Bonneaud 2008). If males carry a biased sample of alleles, this could also explain deviations from expected ratios of progeny morphotypes predicted by our proposed genetic mechanism.

Our study provides a working hypothesis for how the female limited polymorphism in *A. sagrei* is maintained in island populations. We hypothesize that, so long as some DB females have heterozygous genotypes, positive frequency-dependent selection acting on DB females will preserve both the *bar* and *diamond* al-

leles, as well as both dominant and recessive alleles at the epistatic locus. This process is analogous to heterozygote advantage and is predicted to limit the average frequency of DB females to ca. 50% (as the frequency of DB females increases, so too will the probability of producing B and D daughters). The predicted average frequency (∼50%) for DB females is highly congruent with those observed in our geographic surveys (see Results). We further propose that males, which never express the polymorphism, shelter *bar* and *diamond* alleles from selection and contribute to the maintenance of the polymorphism. Although sheltering alleles in males and DB females can preserve the polymorphism within populations, we did not find strong evidence to suggest that differing selection pressures between islands underlie the differences in morph frequencies between Eleuthera and Great Exuma. Future studies that incorporate molecular estimates of gene flow within and between islands are needed to better understand the potential importance of connectivity among islands in maintaining variation.

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