

Common Garden and Natural Selection Experiments Support Ecotypic Differentiation in the Dominican Anole (*Anolis oculatus*)

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ABSTRACT: The theory behind ecotypic differentiation and ecological speciation assumes a predominant role for natural selection working on characteristics with genetic variance, but experimental support for these assumptions is limited. Lesser Antillean anoles show marked ecotypic variation within islands and the potential for ecological speciation. Common garden rearing experiments on the Dominican anole (*Anolis oculatus*) suggest that the characters showing geographic variation have genetic variance and are not primarily determined by environmental plasticity. Replicated natural selection experiments using large-scale enclosures show that translocated montane samples experience significant (multivariate) directional selection in both wet and dry seasons in both males and females. The targets of selection appear to be spread among the various character systems. An experiment on 12 geographically segregated populations along a coastal xeric-montane rainforest gradient (four replicate enclosures) clearly showed that the magnitude of the directional selection intensity is positively related to the position along this gradient. The results of the common garden and natural selection experiments support the interpretation that the geographic differentiation is primarily driven by natural selection and are compatible with the potential for ecological speciation in this system.

Keywords: anoles, natural selection, common garden experiments.

Phenotypic geographic variation within a species can be due to natural selection for current ecological conditions

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or may reflect historical processes such as drift over time. When phenotypic and environmental factors co-vary geographically, this may suggest the former (Endler 1986), but such a correlation could be due to environmental plasticity (reaction norm) influencing the character variation (Thompson 1991; Losos et al. 2000) rather than natural selection. Hence, both ecological differentiation within species and ecological models of speciation based on empirical observations of phenotypic patterns (Smith et al. 1997) make two assumptions: first, the phenotypic variation is the product of genetic control (rather than direct environmental plasticity) and second, current natural selection (rather than a historical process) is the cause of the pattern. Few such studies offer experimental evidence to support either (let alone both) of these assumptions even though manipulative experiments may give valuable information on the processes and causes of evolution in a wide range of organisms from plants (Dudley 1996), insects (Bennington and McGraw 1996; McPeck 1997; Stoks et al. 1999), fish (Endler 1980; Warner and Schultz 1992; Schluter 1994, 2003), and terrestrial vertebrates (Malhotra and Thorpe 1991; Sinervo and DeNardo 1996; Losos et al. 1997).

Study System

Anoles are a useful study system because the relationship between morphology and niche at the interspecific (ecomorph) level is well established in Greater Antillean anoles (Williams 1983; Losos 2004) and there is also some indication of a link between morphological and ecological variation at the intraspecific level in these islands (Lister 1976; Jackman et al. 2002; Glor et al. 2003; Leal and Fleishman 2004). However, it is work on geographic variation in Lesser Antillean anoles that has attempted to rigorously test the link between phenotype and habitat at the intraspecific level (Malhotra and Thorpe 1997a, 1997b; Thorpe 2002) and has shown the occurrence of ecotypes (Malhotra and Thorpe 2000; Thorpe and Stenson 2003). Lesser An-

tillean anoles are particularly useful because there is substantial geographic variation within these small islands. This could be a consequence of selection due to distinct habitat zonation or a consequence of historical processes. Although either (or both) could be associated with the pattern of variation, there is overwhelming indirect evidence of the importance of natural selection in shaping the phenotype irrespective of relatively deep phylogenetic lineages within islands (Malhotra and Thorpe 2000; Thorpe 2002; Thorpe and Stenson 2003). This evidence comes from covariation between facets of the phenotype (scalation, body dimensions, color pattern, and hue) and ecological zonation within these islands and parallel patterns of apparent adaptation among independent lineages within and among islands.

The Dominican anole *Anolis oculatus* is typical of anoles on high-elevation Lesser Antillean islands in that it shows marked geographic variation in a wide range of features (scalation, color and pattern, and body dimensions; Malhotra and Thorpe 1997a, 1997b, 2000). As with other Lesser Antillean anoles, studies suggest a predominant role for natural selection. Outside of the recently volcanically disturbed southwest of Dominica, morphological features track physical and biotic zonation. Although character complexes from the different character sets may relate to complexes of biotic and physical variables (Malhotra 1992), parallel trends suggest that body dimensions tend to relate primarily to altitude, color and pattern to vegetation, and scalation to humidity/rainfall (Malhotra and Thorpe 1997a, 1997b, 2000; Thorpe et al. 2004). The details of the mechanisms are unknown, but it is possible to speculate. Physical factors are clearly important, and scale size may be related to susceptibility to desiccation such that larger scales (the inverse of scale number) with more sculptured surface area may be found in wetter areas. The association between vegetation and color/pattern is compatible with a male anole's requirement to balance being visible enough to be seen by conspecifics (to maintain territory and attract a mate) and being sufficiently cryptic to avoid predation (from snakes and birds). Hence, in light open xeric Caribbean woodland, a straw-colored dorsum with large black blotches in the male may be optimal, while in the dense littoral woodland, a darker orange/brown dorsum with white spots and a high tail crest may be optimal (Malhotra and Thorpe 2000). The morphological features for markers in such studies are chosen, explicitly or implicitly, with the assumption that their patterns of variation are not predominantly a result of environmental plasticity. Features such as weight, condition, or growth rate, which are more likely to be subject to plasticity, are generally avoided.

In addition to this indirect evidence, a preliminary experiment using large-scale field enclosures was used to

study selection intensity and the role of natural selection in producing these patterns (Malhotra and Thorpe 1991; Thorpe and Malhotra 1992). A study of four unreplicated ecotypes indicated significant selection intensity acting on montane translocates after a short time and a significant relationship between the magnitude of the selection intensity and the extent of ecological difference between the source locality and experimental site.

This article reports on the further experimental work that was undertaken to extend this, add replication, and investigate phenotypic plasticity. This work focused on one of the most prominent patterns of geographic variation in the Dominican anole, that is, the contrast between anoles from the north Caribbean xeric woodland and those from the montane rainforest. There are pronounced differences in the environment along this spectrum that can be illustrated by comparing two sites of focal interest: Cabrits from the xeric woodland and Syndicate from the montane rainforest (fig. 1). The xeric woodland site is just above sea level and has a mean temperature of 27°C, a strongly seasonal rainfall (five dry months) of 1.87 meters per annum (mpa), an open light woodland with a canopy up to 18 m, and generally no epiphytes. The montane rainforest site at 547 m has an aseasonal (no dry months) rainfall of 5.62 mpa and a mean temperature of 23°C, and it is dominated by large rainforest trees (>40-m closed canopy) festooned with epiphytes. This environmental difference is matched by marked visual and quantitative differences between anoles at the extremes of this spectrum (Malhotra 1992; Malhotra and Thorpe 1997a, 1997b, 2000). For example, xeric anoles can be small, with a straw-colored dorsum and numerous dorsal scales (extreme male means of 60 mm snout-vent length [SVL], 3% cyan in the dorsal hue, and 204 dorsal scale rows) while montane anoles can be large and greenish with few scales (extreme male means of 90 mm SVL, 47% cyan in the dorsal hue, and 135 dorsal scale rows).

To test the role of natural selection in determining this observed pattern of morphological geographic variation, a series of experiments were carried out. First, even though both genetic variance and environmental plasticity are likely to contribute to within- and among-group variation in natural systems, it is important to establish the occurrence and relative importance of genetic variance in any hypothesis involving natural selection. Consequently, a common garden experiment was carried out to evaluate this over the habitat gradient. Second, to establish the action and intensity of directional natural selection and its geographic facet, a series of replicated translocation experiments were carried out using large-scale enclosures. The first tested for selection intensity in an extreme habitat contrast in both seasons using both sexes, and the second

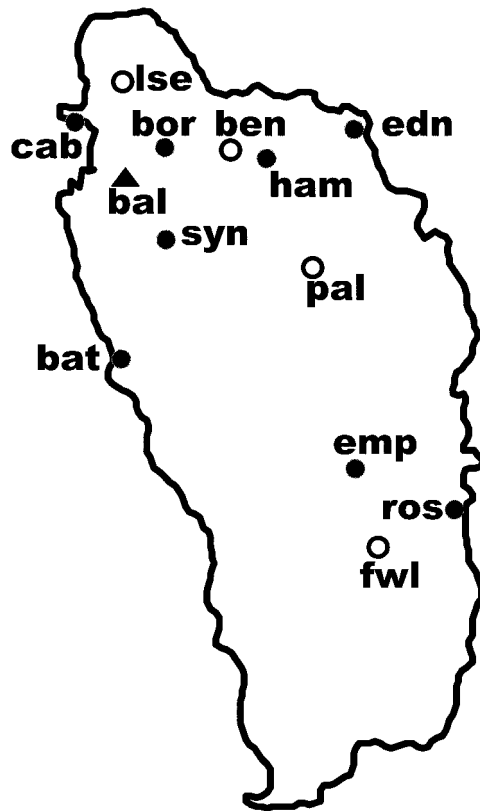


Figure 1: Map of Dominica showing sample and study sites. The common garden experiment was run at Balvine (*bal*; triangle) based on nine sites with an adequate number of reared offspring (filled symbols). Both natural selection experiments were run at Cabrits (*cab*). Experiment 1 was based on montane translocates from Syndicate (*syn*) and controls from Cabrits. Experiment 2 was based on all sites marked with circular symbols. Remaining abbreviations are as follows: *ben* = Bense Ridge, *bat* = Batali Estate, *bor* = Bornes, *edn* = Eden, *emp* = Emerald Pool, *fwl* = Fresh Water Lake, *ham* = Hampstead Ridge, *lse* = La Source Estate, *pal* = Palmiste Ridge, and *ros* = Rosalie.

tested the relationship between selection intensity and degree of ecological difference along the habitat gradient.

Methods

Common Garden Experiment

We ran a common garden experiment, where individuals from different localities are hatched and raised under the same natural conditions in the field (Garland and Adolph 1991), to determine whether and to what extent the geographic pattern has a genetic component. Gravid females were sampled from 12 localities across Dominica (fig. 1). The samples were chosen to represent a wet-montane-rainforest to xeric-north-coastal-Caribbean environmental graduation (montane-xeric gradient) associated with

strong phenotypic differentiation (Malhotra and Thorpe 2000). The resulting offspring (one per adult) were reared in a common garden situated in transitional woodland between northern Caribbean xeric woodland and montane forest types (Balvine; fig. 1). Gravid females from source populations were kept in an individual enclosure until the eggs were laid. On hatching, the offspring were toe clipped for identification and immediately released into the common garden enclosure. This took 1 month to complete. The enclosure was 200 × 210 × 70 cm, timber framed, and enclosed with UV-resistant 3-mm-gauge plastic mesh, which permitted the passage of invertebrate prey. The enclosure contained vegetation that provided a complex arboreal environment for the anoles identical to natural low vegetation structural complexity outside the enclosure. Ripe fruit was placed in the enclosure weekly to attract additional invertebrate prey. All surviving common garden anoles were captured and remeasured after they had been in the enclosure for 6 months, when the majority of anoles had attained or were close to mean adult body mass (capable of reproduction).

It was possible to record four scalation and six body dimension characters (app. A in the online edition of the *American Naturalist*) from the young adult females reared in the common garden (an approximate sample size of about 20–30 per locality was planned). The common garden-reared (CGR) specimens were compared with natural-reared (NR) specimens independently collected from the original locality where they would have developed under natural conditions. The actual mothers were not used to represent the NR specimens. This design does not control for those maternal effects determined before gestation, for example, maternal size, or those that operate in precapture gestation, but it does attempt to control for effects determined during postcapture gestation and postoviposition.

If the geographic patterns in the phenotypic characters of specimens sampled directly from the original localities (NR specimens) are due to plasticity rather than natural selection acting on characters with some genetic variance, then the predictions are as follows. First, there should be no significant interlocality difference in the morphological characteristics of CGR specimens. This was tested by comparing the morphological characteristics of CGR specimens among localities with a one-way ANOVA (scalation) or ANCOVA (body dimensions regressed against the standard size measure of SVL). Second, there should be significant differences between the phenotypic characteristics of CGR and NR specimens. This is tested by comparing the morphological characteristics of CGR and NR specimens with a two-way ANOVA (scalation) or ANCOVA (log body dimensions regressed against log SVL). One factor is geographic locality, and the other is CGR versus NR.

As well as a significant difference in the CGR versus NR factor, phenotypic plasticity may result in those localities most ecologically different from the common garden site having more morphological difference between CGR and NR specimens. This should show as a significant interaction between the two factors. Third, the montane-xeric gradient geographic variation in the NR specimens will not be correlated with the pattern of geographic variation from the CGR specimens. A canonical variate analysis (CVA) summarizes among-group differences while taking into account information redundancy in the characters (within-group covariance) and is one of the best methods for summarizing patterns of population differentiation (Thorpe 1976). A CVA was run on all groups, both NR and CGR specimens (to maximize sample size and degrees of freedom in the pooled within-group covariance matrix) and all characters (body dimensions were first size adjusted by pooled within-group regression as in ANCOVA). The simple pattern of geographic variation is summarized by the first variate. The correlation between the group centroids for NR specimens and CGR specimens tests the similarity of the patterns. Fourth, the multivariate divergence between phenotypes of CGR and NR specimens from a given locality (measured by Mahalanobis's D) should be correlated with the multivariate environmental divergence measured by the Euclidean distance between the source locality and common garden site. Hence, this is tested by the product-moment correlation between morphological and environmental divergence. The environmental divergence was measured across vegetation type (ranked as in natural selection experiment 2; Beard 1948) and the physical variables (Lang 1967) altitude, humidity, and mean annual temperature as derived and described by Malhotra and Thorpe (2000) and given in appendix A.

All four tests can suggest whether there is a genetic component determining the pattern of variation. However, tests 3 and 4 can also give an insight into the relative importance of plasticity and genetic control in determining the overall (multivariate) pattern of geographic variation.

Natural Selection Experiments: General

Two sets of field translocation experiments were run, primarily to test for directional selection intensity. Experiment 1 tested intensity and targets of selection (Brodie et al. 1995) on montane and xeric (control) ecotypes during the wet and dry seasons for both sexes. If the geographic variation along an environmental gradient is due to natural selection, the prediction is that there will be an association between the directional selection intensity and the position along the environmental gradient. This is tested by experiment 2.

Four lizard-proof field enclosures (approximately

11,000 m³ each) in the Caribbean xeric woodland at the Cabrits site (fig. 1, *cab*) were assiduously emptied of lizards over a 2-week period and then stocked with specimens that were individually numbered by clipping up to two toe tips, distal to the pad. This information was combined with information on sex and morphology to ensure correct identification of an individual. Hence, only a limited number of toes were clipped, and only a very minute amount of tissue was removed. Any marking system may be intrusive, and recent evidence suggests that removing the fleshy toes of anurans, particularly a large number of toes, impacts survival (McCarthy and Parris 2004). However, what little evidence there is for lizards (Borges-Landaez and Shine 2003) suggests that toe clipping does not impact locomotory performance. Moreover, although toe clipping could impact survival in lizards, we are not aware of evidence that it does so or that it influences differential selection in our type of experimental design, where both controls and montane specimens are similarly marked and selection intensity rather than survival rates are compared. The alternative of passive integrated transponder tagging can be more intrusive, especially for small animals, and tags can migrate in lizards and result in loss of the tag (Germano and Williams 1993) and possible damage to the lizard. Enclosure materials and design are given by Malhotra and Thorpe (1993); they represent as natural a set of conditions as is compatible with restraining the anoles. The physical and vegetation conditions are entirely natural, and predators such as birds had free access, although snakes were necessarily excluded by the design. The enclosures were comparable (but not identical) in size, and the body size of specimens differs among localities (montane anoles are larger) and sexes (males are larger), so the four enclosures were stocked to the same biomass per unit area (i.e., that of the original anole population; Malhotra and Thorpe 1991). Control samples from Cabrits (fig. 1, *cab*) were subjected to the same procedures as translocated samples (including translocation from an adjacent area), and all were regularly subjected to an intense census to record individual survival (probability of recapture was assumed to be 1.0). Before being released into the enclosure, lizards were photographed, and a series of characters, selected because they show geographic variation (Malhotra and Thorpe 1997a, 1997b, 2000), were recorded from the color (pattern), scallation, and body proportions (app. A). Sexes have to be treated separately in all analyses because of widespread sexual dimorphism in the morphological characters (Malhotra 1992).

The aim of this study was to investigate primarily selection intensity rather than the specific targets of selection (Brodie et al. 1995), but both require an estimate of fitness (lifetime reproductive success). In most circumstances, this

cannot be directly measured but is estimated by variables such as clutch size, survival, or condition. Male anoles are polygamous, and females have a clutch size of 1. Hence, reproductive success (let alone lifetime reproductive success) is not readily assessed in anoles. Using condition, such as SVL^3/wt , has disadvantages in anoles because it can be unduly influenced by the intake of food items that may be large in relation to anole body size and because in females it is influenced by whether they are gravid. Hence, survival over time is the best estimate of fitness in enclosure experiments on anoles and was used to investigate both selection intensity and selection targets.

Selection intensity (Brodie et al. 1995) was estimated as the extent of multivariate morphological difference, Mahalanobis's D , between survivors and nonsurvivors obtained from a 10-step discriminant function analysis and was measured from the start of the experiment to a given census period and between sequential census periods. The data for accumulated census periods are not (and are not treated as) independent but give an insight into the chronology of the process (app. B in the online edition of the *American Naturalist*). Hence, for selection intensity, fitness was estimated as survival (1) or nonsurvival (0) of an individual between a pair of census periods. Mahalanobis's distance between survivor and nonsurvivor group means is used because it takes into account the within-group covariance, which allows for information redundancy (character interdependence) and effectively standardizes the data (Thorpe 1976; Manly 1986). Not only does this give a measure of the degree of difference between group means, but it also allows a test of the null hypothesis of no difference between means (i.e., it tests for significant selection intensity), with the degrees of freedom of the approximate F statistic being determined by the number of characters (numerator) and number of individuals within groups minus the number of characters and groups (denominator; Dixon 1992). Hence, Mahalanobis's distance and discriminant function analysis can be used to give the extent and significance of selection intensity taken across several characters simultaneously (Malhotra and Thorpe 1991). The targets of selection were estimated by standardized partial regression of characters against fitness of an individual (Brodie et al. 1995), estimated as the time of survival of an individual across the census periods taken as an ordinal series with five states (0 for no survival at census 1, 0.25 for survival to census 1, 0.50 for survival to census 2, 0.75 for survival to census 3, and 1.00 for survival to census 4).

Natural Selection Experiment 1

This experiment was based on replicated comparison of montane and control xeric forms across seasons. Using

natural sex ratios, two enclosures were stocked with montane ecotypes (fig. 1, *syn*) while two were stocked with local anoles (fig. 1, *cab*) as controls. One experiment was run in the wet season (September 1995–January 1996), and another started anew in the dry season (January–July 1996). The enclosures were randomly assigned to control or montane types for the first experiment, and the allocation was transposed for the second experiment. The four enclosures were stocked to comparable biomass with an equal sex ratio, giving an average of 86 control and 45 montane specimens per enclosure per season. Enclosures censuses began at 14, 42, 68, and 96 days after stocking. If the morphological distance (Mahalanobis's D) between survivors and nonsurvivors (for a given sex, ecotype, season, and census) was low and nonsignificant, there was no evidence for directional selection, but if it was large and significant, this was taken as direct evidence for the differential-survival component of directional selection. The pronounced sexual dimorphism throughout the character system is presumably (or at the very least possibly) maintained by selection. Consequently, the intensity of selection could vary between the sexes. This, together with the effect of season and ecotype, is tested by a three-way ANOVA (factors were ecotype, season, and sex) run on the selection intensities (D) for replicates. This tested whether one ecotype experienced significantly higher selection intensity than the other ecotype, whether one season has significantly higher selection intensity than the other season, whether one sex experienced significantly higher selection intensity than the other sex, and whether there was any interaction between these three factors. Hence, a sample for a given ecotype of a given sex from a given season was comprised of the two D values for the pair of replicate enclosures.

Natural Selection Experiment 2

The most prominent ecological gradient in Dominica (and other high-elevation Lesser Antillean islands) is the contrast between the Caribbean coastal xeric woodland and the montane rainforest, and this is associated with geographic divergence in the phenotype. This gradient shows physical and vegetation differences that were assessed multivariately (Malhotra and Thorpe 1991, 2000). Twelve localities were selected to represent this gradient and were chosen from xeric woodland (Cabrits, Batali) through littoral/transitional/lower montane rainforest (examples are, respectively, Eden/Bornes/Hampstead Ridge) to upper montane rainforest (e.g., Syndicate, Bense Ridge) close to the transition to elfin woodland (e.g., Fresh Water Lake). To test for a correlation between selection intensity on morphology and degree of environmental difference experienced by the translocated samples, an increase in the

Table 1: ANOVA and ANCOVA results of the common garden experiment

| Character | Test 1 | | Test 2 | | | |
|-------------------------|----------|----------|----------|----------|----------------------|----------------------|
| | <i>F</i> | <i>P</i> | <i>F</i> | <i>P</i> | <i>F_I</i> | <i>P_I</i> |
| ANCOVA: | | | | | | |
| Upper leg length | 9.5 | .00 | .9 | .35 | 1.21 | .30 |
| Lower leg length | 17.9 | .00 | .6 | .44 | 1.28 | .25 |
| Toe length | 7.4 | .00 | 2.3 | .13 | 1.53 | .15 |
| Toe width | 6.4 | .00 | .6 | .42 | .76 | .64 |
| Head width | 4.8 | .00 | .5 | .49 | 2.30 | .02 |
| Head length | 4.7 | .00 | .3 | .57 | 1.92 | .06 |
| ANOVA: | | | | | | |
| Circum-trunk scales | 2.6 | .01 | .2 | .70 | .14 | 1.00 |
| Lamellae scales | 2.5 | .01 | .1 | .78 | 1.24 | .27 |
| Scale tubercularization | 6.0 | .00 | .7 | .42 | .41 | .91 |
| Ventral scales | 22.2 | .00 | .4 | .51 | .76 | .64 |

Note: For test 1, $df = 8, 254$. For test 2, $df = 1, 254$. Test 1 is for inter-locality variation in common garden-reared specimens. Test 2 is for a difference between common garden- and natural-reared specimens and whether the extent of any difference depends on geographic origin (*I*).

number of sample sites was required while maintaining an adequate sample per site in the four replicate enclosures. This was achieved by using only females; they are smaller than males and allow a larger sample size for the appropriate biomass. In this experiment, 362 females from the 12 localities were mixed within the four replicate enclosures (maintaining a comparable biomass per enclosure), giving an average of 90 females per enclosure and approximately equal numbers per locality type. The censuses were begun 48, 56, 84, and 112 days after stocking.

Results

Common Garden Experiment

Insufficient offspring were reared from three of the localities, but four scalation and six body dimension characters were recorded from the remaining nine localities (fig. 1) with the following samples sizes in alphabetic order of locality: 28, 29, 20, 20, 20, 31, 31, 30, 20. If the geographic patterns in the phenotypic characters of NR specimens are due to plasticity rather than natural selection acting on characters with some genetic variance, then there are several testable predictions. However, these predictions are not borne out. First, there should be no significant interlocality difference in the morphological characteristics of CGR specimens. However, all characters showed significant differences among localities (table 1, test 1). Second, there should be significant differences between the phenotypic characteristics of CGR and NR specimens when taking into account locality of origin and no interaction. No morphological characters showed significant

differences between CGR and NR specimens (table 1, test 2). However, in test 2, one character (head width) showed a significant interaction between locality and the CGR versus NR factor, suggesting some phenotypic plasticity. Third, the xeric-montane gradient of geographic variation in the NR specimens will not be correlated with the pattern of geographic variation from the CGR specimens. However, the pattern of divergence among geographic localities for CGR specimens is a similar xeric-montane gradient as found in the NR specimens, with CV1 centroids highly correlated at $r = 0.76$, $P < .001$. Fourth, the multivariate divergence between phenotypes of CGR and NR specimens at the same locality should be correlated with the multivariate environmental divergence between the source locality and the site of the common garden (Malhotra and Thorpe 2000). This is tested by product-moment correlation between the morphological and environmental divergence. However, there was no such correlation ($r = 0.001$, $P = .88$).

All four tests suggest that there is a genetic component to the pattern of geographic variation. Moreover, apart from the significant interaction effect for head width in test 2, the results across all characters in tests 1 and 2 are consistent. This consistency, together with the results from tests 3 and 4, suggests that the pattern of geographic variation in the characters considered is predominantly the result of genetic control rather than plasticity.

Natural Selection Experiment 1

The intensity of selection (D) was higher (fig. 2; table 2) in montane translocates than equivalent controls for both

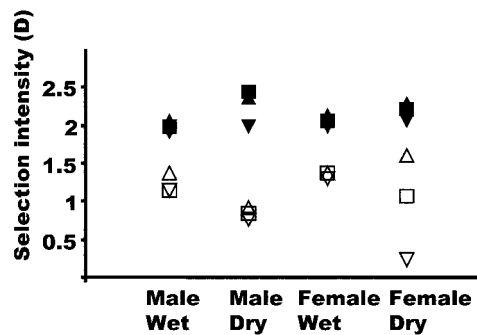


Figure 2: Natural selection experiment 1. Graphical comparison of the selection intensity acting on montane translocates from Syndicate (filled symbols) and controls from Cabrits (open symbols) for both wet and dry seasons and both males and females (census 3, 68 days). Selection intensity (Mahalanobis's D) pooled across replicates is indicated by a square (these are all significant for the montane samples but not significant for any control samples; table 2) and replicate values by a triangle. Replicate values are also significantly higher in montane samples than equivalent controls (ANOVA; table 2).

Table 2: Selection intensity (D) in census 3 of natural selection experiment 1 for each of the two replicate enclosures (replicates 1, 2)

| Ecotype, season, and sex | Replicate 1 (D) | Replicate 2 (D) | Pooled D |
|--------------------------|---------------------|---------------------|------------|
| Xeric: | | | |
| Dry: | | | |
| Female | 1.561 | .227 | 1.087 |
| Male | .935 | .780 | .858 |
| Wet: | | | |
| Female | 1.442 | 1.356 | 1.422 |
| Male | 1.346 | 1.178 | 1.117 |
| Montane: | | | |
| Dry: | | | |
| Female: | 1.989 | 2.237 | 2.133* |
| Male: | 2.341 | 1.941 | 2.427* |
| Wet: | | | |
| Female | 1.986 | 1.827 | 1.892* |
| Male | 1.900 | 2.017 | 1.942* |

Note: Selection intensity data was pooled (not averaged) across replicates 1 and 2, with significance for the latter given by an asterisk. ANOVA $F = 26.2, 0.1, \text{ and } 0.0$ for ecotype, season, and sex, respectively. ANOVA $P < .001, .49, \text{ and } .90$ for ecotype, season, and sex, respectively.

* $P < .05$.

sexes and for both wet and dry season experiments except for just two instances at an early stage (census 2) of the experiment (app. B). These higher D values for translocate montane samples show a significant difference between survivor and nonsurvivor morphology (significant selection intensities except for males in census 4 of the wet season experiment; app. B), whereas the selection intensities for controls are not significant. Also, the three-way ANOVA run on the replicates (fig. 2; table 2) shows a significant difference between the measured selection intensity of montane and control ecotypes but no significant difference between the seasons and sexes or for any interaction between these factors. Analysis of the selection intensity between sequential census periods indicates that selection appears to act throughout the duration of the experiment with a slight tendency to increase with time. There is no tendency for selection to impinge at just one phase of the experiment at the expense of others (app. B). While selection coefficients (indicating directional changes in traits that are targets of or are linked to targets of selection) were not the focus of this study, they indicate that the selection experienced by the translocated montane forms was spread across all character systems, that is, size, shape, hue, pattern, and scalation (app. C in the online edition of the *American Naturalist*).

Natural Selection Experiment 2

There was a high and significant correlation between ecological distance and selection intensity within each repli-

cate enclosure, with no significant difference between replicate slopes (i.e., the relationship between ecology and selection was significant and the same in each of the four enclosures). The pooled within-replicate regression (at census 4) had an intercept of 0.272, slope $b = 0.508$, $r = 0.829$, $P < .001$ (fig. 3). Once again, the effect of selection was progressive through the duration of the experiment, with this pooled within-replicate correlation increasing from census 1 to census 4 as $r = 0.036$, $r = 0.484$, $r = 0.648$, to $r = 0.829$, respectively.

Discussion

This article reports one of the very few replicated translocation experiments on natural selection in terrestrial vertebrates that are also supported by a common garden study. There are inherent difficulties involved with common garden experiments when natural selection rather than plasticity is largely responsible for the divergence because not all ecotypes may be adapted well enough to the site of the common garden to thrive. This can result in lower sample sizes, fewer localities, and a reduced character set. This was a problem in this study, but in spite of this, it was possible to obtain results for nine of the 12 localities. These results are not in accord with a predominant role for phenotypic plasticity but are compatible with characters having genetic variance on which natural selection can act. Hence, the null hypothesis that these patterns of geographic variation found in the Dominican anole have no genetic basis can be rejected. Moreover, it appears that the observed pattern of geographic variation in the char-

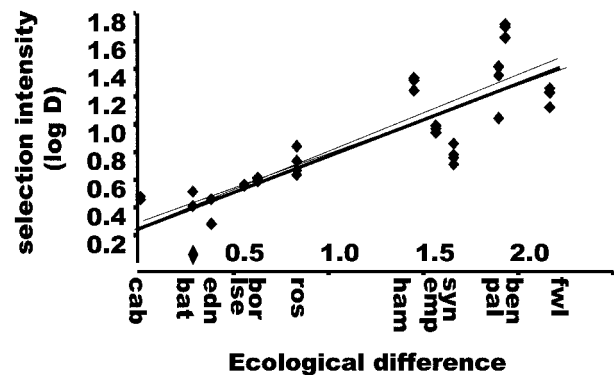


Figure 3: Natural selection experiment 2. The selection intensity of four replicate (enclosure) samples from each of 12 localities plotted against the ecological distance between the source locality and experimental site. Sample localities (below ordinate; codes in fig. 1) represent an ecological gradient from xeric coastal Caribbean habitats at the origin to progressively more montane wetter habitats. The pooled within-replicate slope is the thick line, with the four slopes for the individual replicate enclosures partly obscured by coincidence with it (*thin lines*).

acters under consideration is predominantly a result of genetic mechanisms, such as natural selection. This is not intended to suggest that there is little or no phenotypic plasticity but that it is not predominant in determining the coherent pattern of wet-dry geographic variation among the localities investigated in the characters investigated. Plasticity may influence within-locality variation and even among-locality variation in a haphazard or random manner. Moreover, other characters, for example, those measuring aspects of weight, condition, or growth rate, may well be predominantly plastic.

The original preliminary field experiment in 1991 demonstrated that for both sexes, montane ecotypes were rapidly subject to significant directional selection. However, the design did not allow for synchronous replication (Malhotra and Thorpe 1991) or for the effect of seasonality to be tested. This preliminary experiment, together with the pair of experiments (1995, 1996) on montane ecotypes and xeric woodland controls reported here, supplies chronological replicates over three periods. This chronological series shows that both sexes are rapidly subject to significant directional selection. Moreover, this is supported by the synchronous replicates of the latter two experiments. Hence, these experiments provide repeatable evidence of the differential survival component of directional natural selection acting on spatially differentiated populations of this Lesser Antillean anole. This selection effect occurs in both sexes in both the wet and dry seasons. For the translocated montane anoles, the selection intensity tends to be higher in the dry season than the wet season for both sexes and all census periods. However, this is not significant with the limited number of replicates used. Nevertheless, this trend is intuitive because the dry season might be expected to provide an even more hostile environment than the wet season with even stronger selection pressures for those specimens translocated from the humid montane rain forest.

While the pattern of spatial/ecotypic differences in selection intensity may be consistent over time, the specific magnitude of the selection intensity may vary. There are "wet" dry seasons and "dry" wet seasons, as well as occasional extreme conditions. For example, the beginning of the 1995 wet season experiment was marked by the passage of three hurricanes (Iris, Luis, Marilyn) in rapid succession. These stripped foliage from the trees, increasing light levels and altering humidity after the initial trauma of the hurricane. This particular climatic regime and consequent selection pressures may not be repeated exactly. Nevertheless, the occurrence of directional selection intensity under this experimental procedure appears to be robust.

Anoles on high-elevation Lesser Antillean islands with ecological zonation show strong geographic patterns of

phenotypic variation correlated with this ecological zonation generally irrespective of the phylogeographic lineages (Malhotra and Thorpe 2000; Thorpe 2002; Thorpe and Stenson 2003). The Dominican anole is, in this respect, typical of lizards in the Lesser Antilles and some other archipelagos (Brown et al. 1991; Thorpe and Malhotra 1996; Gübitz et al. 2000; Thorpe and Richard 2001). Experiment 2 provides clear evidence of the magnitude of the intensity of selection being strongly and positively dependent on the extent of ecological difference between the geographic source location and the site of the experiment. This is repeatable because each of the four replicate enclosures gives effectively the same result with significant correlations between ecological difference and selection intensity for each enclosure and no significant difference in regression intercepts or slopes between replicate enclosures. Consequently, the experiments reported here are compatible with the interpretation that these geographic patterns are due to natural selection. Although considerable caution is required when identifying targets of selection, it appears that the targets of selection come from all of the character systems (color pattern, hue, size, shape, and scalation) studied rather than just one, as appears to be the case in several studies of temporal variation (Boag and Grant 1981; Fleischer and Johnston 1982; Price et al. 1984; Endler 1986). Once again, this is compatible with the observation that in anoles, all of these character systems may show patterns of geographic variation that covary with ecological conditions on these islands (Malhotra and Thorpe 2000).

Such patterns of geographic variation and ecological speciation (Smith et al. 1997; Schneider et al. 1999; Ogden and Thorpe 2002; Tautz 2003) assume a central role for natural selection working on characters with genetic variance, but studies showing natural selection in action are still infrequent, particularly for terrestrial vertebrates (but see Endler 1986; Malhotra and Thorpe 1991; Schluter 1994, 2003; Losos et al. 1997). The reason for the paucity of such studies is that they pose several logistic difficulties. These difficulties include but are not limited to maintaining discrete experimental units under natural conditions, obtaining experimental units that are large enough to contain a sufficient number of specimens at natural density (MacNally 1997) to yield statistically significant results but small enough to regularly subject to a census, and finding a selection regime that is sufficiently intense to yield significant results within the time span of a fundable project. Nevertheless, manipulative field experiments, whether or not they address the geographic facet of natural selection by translocation, can give valuable insights into the evolutionary processes acting at the population level (Endler 1980; Malhotra and Thorpe 1991; Schluter 1994; Sinervo and DeNardo 1996; Losos et al. 1997; Janzen et al. 2000;

Svensson and Sinervo 2000), particularly when supported by common garden rearing experiments that rule out a primary role for phenotypic plasticity.

Lesser Antillean anoles have been model organisms for investigating both microevolutionary processes (Thorpe et al. 2004) and speciation (Ogden and Thorpe 2002; Losos 2004). The series of experiments reported here suggest genetic variance and directly demonstrate directional selection. Hence, they strongly support the hypothesis that the patterns of intraspecific geographic variation within islands are predominantly a result of natural selection for current ecological conditions and are also compatible with the potential for ecological (Ogden and Thorpe 2002) and adaptive (Tautz 2003) speciation in this system.

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Appendix A from R. S. Thorpe et al., “Common Garden and Natural Selection Experiments Support Ecotypic Differentiation in the Dominican Anole (*Anolis oculatus*)”
(Am. Nat., vol. 165, no. 4, p. 495)

Characters and Variables

Characters recorded in the common garden experiment are in table 1. They are self-explanatory except “scale tubercularization.” The white lateral scales can become enlarged and develop into raised tubercles, and six ordinal states encompass this range of tubercularization.

Characters recorded in natural selection experiments were as follows: snout-vent length (SVL); head length and width; length and width of the fourth toe of the hind foot; upper and lower leg length; the number of scale rows at midbody; the number of ventral scales; the number of lamellae beneath the fourth toe of the hind foot; the degree of tubercularization of lateral white scales; the number of small, medium, and large white lateral spots; width of lateral black patches in males; dorsal hue; and hue of the brightly colored skin surrounding the eye (hue recorded as three characters: percent cyan, yellow, and magenta). Size-dependent characters were adjusted by regression against SVL (using pooled within-group regression coefficients).

Kodachrome 64 photographic transparencies taken under standardized conditions were used for recording color hue and pattern characteristics. Hues were measured as percent yellow, magenta, and cyan using either printers' color charts (experiment 1) or Adobe Photoshop on digitized transparencies (experiment 2). These hues were recorded as three separate variables because previous analysis (Malhotra 1992) has shown that these elements of hue can show independent patterns of geographic variation. It was essential to record a large number of specimens very rapidly to minimize individual handling time and the translocation period across the experiment. Spectrometry as that of Thorpe (2002) was therefore unsuitable, but subsequent comprehensive spectrometry on the Dominican anole following the procedure by Thorpe (2002) indicates that the method used to characterize hue in these experiments was appropriate (A. Wootton, unpublished data). Environmental data are from Malhotra (1992) and Malhotra and Thorpe (2000). The altitude (m), temperature (°C), and humidity (%) for each site (fig. 1) are as follows: Cabrits: 75, 26.8, 61.3; Batali Estate: 10, 28.2, 66.3; Rosalie: 36, 25.4, 82.5; Eden: 18, 26.1, 76.1; La Source Estate: 150, 28.7, 71.4; Bornes: 247, 26.0, 70.3; Hampstead Ridge: 154, 26.6, 77.2; Bense Ridge: 532, 21.9, 88.9; Palmiste Ridge: 280, 24.8, 80.7; Emerald Pool: 393, 22.3, 78.2; Syndicate: 547, 23.3, 83.0; Fresh Water Lake: 756, 21.4, 89.6.

Appendix B from R. S. Thorpe et al., “Common Garden and Natural Selection Experiments Support Ecotypic Differentiation in the Dominican Anole (*Anolis oculatus*)”
(Am. Nat., vol. 165, no. 4, p. 495)

Selection Intensity over Time: Natural Selection Experiment 1

Selection intensity (SI) was recorded at census periods (0–4) and expressed as both accumulated selection intensity (0–1, 0–2, 0–3, 0–4) and sequential selection intensity (0–1, 1–2, 2–3, 3–4).

Accumulated SI. At the start of the experiment, there will be no selection because the survivor group will have all individuals and the nonsurvivor group none. Then, as selection acts take effect and the sample size of the nonsurvivor group increases, selection intensity may both increase and become significant. However, as the experiment progresses, the sample size of the survivor group may decrease to the point where significance is lost, even though the SI may be very large. Consequently, figure B1 gives the accumulated SI at censuses 2, 3, and 4 even though accumulated SIs are not statistically independent.

Sequential SI. Figure B2 graphs directional selection intensity (as log Mahalanobis’s D) between sequential periods and is plotted for both ecotypes (xeric controls and montane) and for both seasons (dry season and wet season) averaged across sexes. Overall selection appears to be acting across the duration of the experiments with a slight tendency for intensity to increase as the experiment progresses in time, which is significant for the montane ecotype (rank correlation, $r = 0.70$, $P < .005$) but not the xeric ecotype control ($r = 0.43$, $P > .05$).

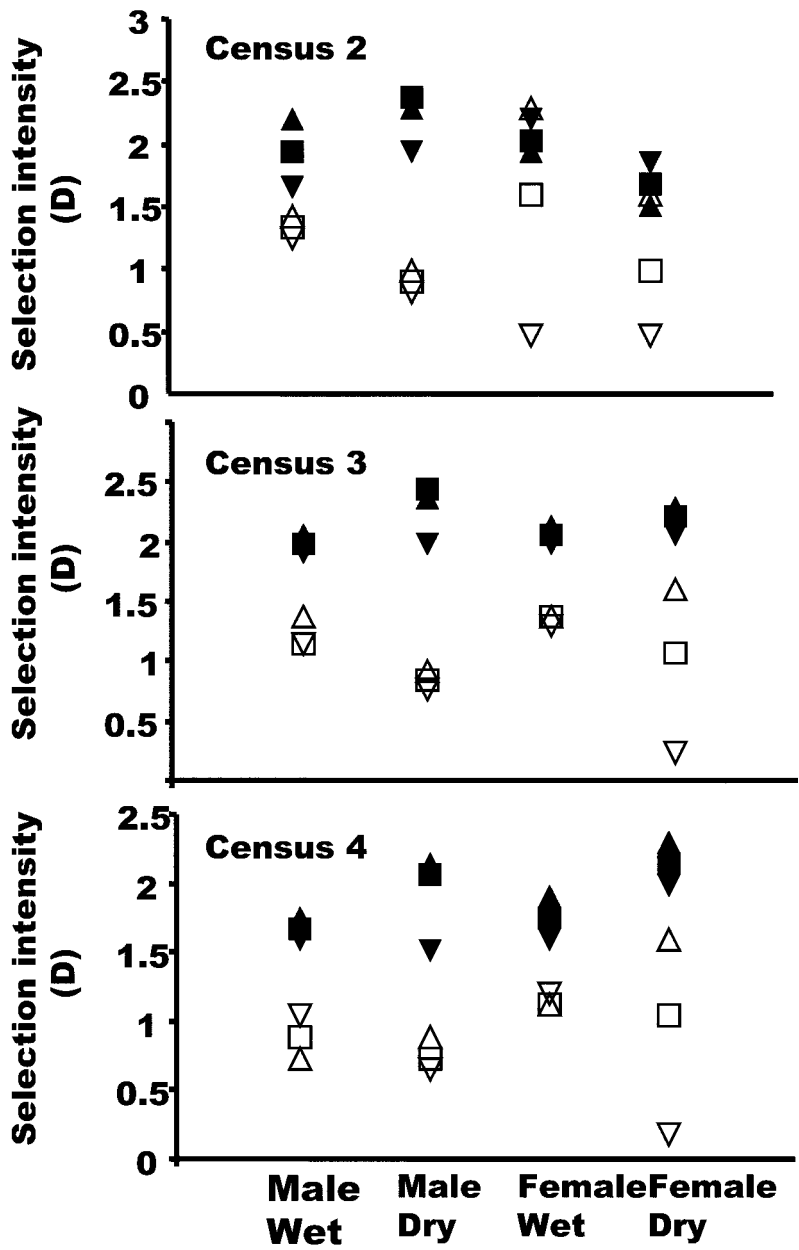


Figure B1: Natural selection experiment 1. Accumulated selection intensity at censuses 2, 3, and 4 (symbols as in fig. 2).

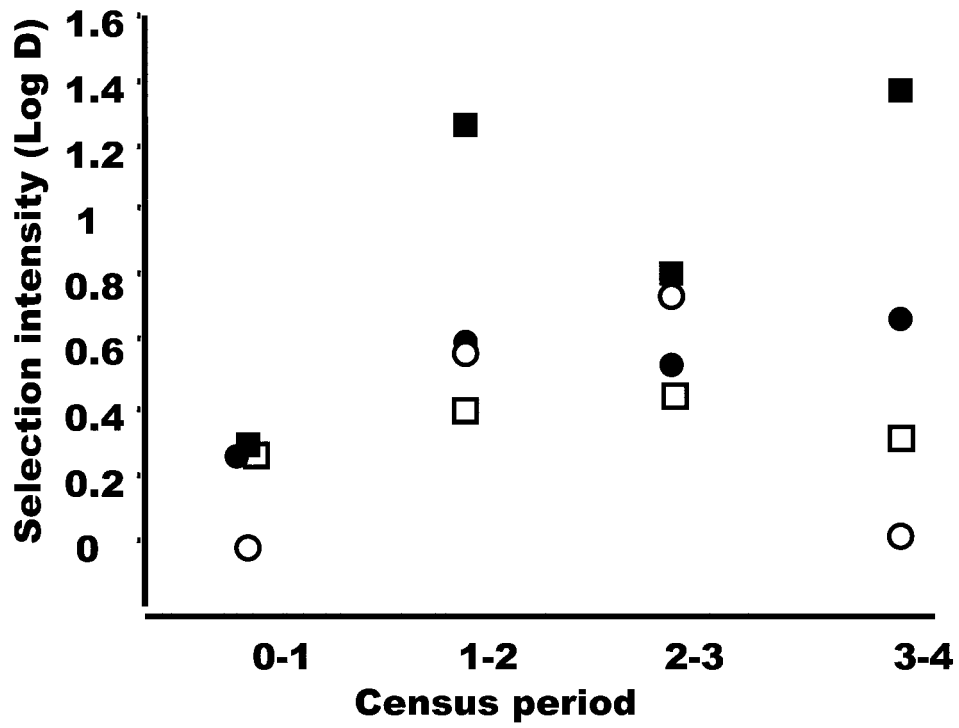


Figure B2: Natural selection experiment 1. Selection intensity between sequential periods for xeric controls (*open symbols*) and montane ecotypes (*filled symbols*) for dry season (*squares*) and wet season (*circles*).

Appendix C from R. S. Thorpe et al., “Common Garden and Natural Selection Experiments Support Ecotypic Differentiation in the Dominican Anole (*Anolis oculatus*)”
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Selection Coefficients for Translocated Montane Ecotype

Standardized partial regressions of characters (independent variables) against fitness (dependent variable) give selection coefficients or gradients (fitness is measured as survival over time: 0 for no survival, 0.25 for survival to census 1, 0.50 for survival to census 2, 0.75 for survival to census 3, and 1.00 for survival to census 4). The table of selection coefficients (table C1) indicates that the targets of selection are spread through the character systems. The characters are as in table 1 and appendix A. Selection targets can also be studied using logistic regression, but the focus of this study was on selection intensity rather than on selection targets.

Table C1
 Selection coefficients

| Character | Male | | Female | |
|-----------------------|--------|--------|--------|--------|
| | Wet | Dry | Wet | Dry |
| SVL | .379* | -.022 | -.069 | .450* |
| Upper leg length | -.130 | .421* | .328* | -.223 |
| Lower leg length | .171 | .230 | -.227 | .203 |
| Toe length | .075 | -.064 | .001 | .024 |
| Toe width | .396* | -.646* | .189 | .148 |
| Head width | .021 | .153 | -.045 | .240 |
| Head length | -.230 | -.231 | -.095 | -.545* |
| Circum-trunk scales | -.156 | .561* | -.113 | .195 |
| Lamellae | -.101 | .057 | -.078 | .024 |
| Scale tubercle | -.203 | -.188 | .263 | .048 |
| Ventral scales | .132 | .307 | .210 | -.163 |
| Eye yellow | -.010 | .151 | .134 | -.042 |
| Eye magenta | .272* | .105 | -.375* | .171 |
| Eye cyan | .105 | -.339* | -.077 | -.154 |
| Dorsal yellow | .393* | -.023 | -.042 | -.057 |
| Dorsal magenta | -.312* | .459* | .060 | .380* |
| Dorsal cyan | -.224 | -.032 | .050 | -.116 |
| Black blotch size | -.066 | -.594* | - | - |
| Anterior white mark | -.368* | -.035 | -.081 | -.010 |
| Midbody white marks | .133 | .259 | .347* | .232 |
| Posterior white marks | .076 | -.016 | -.129 | -.295 |

Note: SVL = snout-vent length.

* Significance of the partial regression coefficient at $P < .05$.