

# DETERMINISM IN THE DIVERSIFICATION OF HISPANIOLAN TRUNK-GROUND ANOLES (*ANOLIS CYBOTES* SPECIES COMPLEX)

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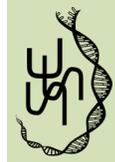
The evolutionary processes that produce adaptive radiations are enigmatic. They can only be studied after the fact, once a radiation has occurred and been recognized, rather than while the processes are ongoing. One way to connect pattern to process is to study the processes driving divergence today among populations of species that belong to an adaptive radiation, and compare the results to patterns observed at a deeper, macroevolutionary level. We tested whether evolution is a deterministic process with similar outcomes during different stages of the adaptive radiation of *Anolis* lizards. Using a clade of terrestrial–scansorial lizards in the genus *Anolis*, we inferred the adaptive basis of spatial variation among contemporary populations and tested whether axes of phenotypic differentiation among them mirror known axes of diversification at deeper levels of the anole radiation. Nonparallel change associated with genetic divergence explains the vast majority of geographic variation. However, we found phenotypic variation to be adaptive as confirmed by convergence in populations occurring in similar habitats in different mountain ranges. Morphological diversification among populations recurs deterministically along two axes of diversification previously identified in the anole radiation, but the characters involved differ from those involved in adaptation at higher levels of anole phylogeny.

**KEY WORDS:** Adaptive radiation, contingency, landscape genetics, recurrence, spatial convergence, speciation.

Does evolution repeat itself time and time again, or do the contingencies of history and differences in environmental setting across space and through time act to thwart such repeatability, pushing each evolutionary instance along its own unique course (e.g., Gould 1989, 2002; Conway Morris 2003)? At the macroevolutionary level, this question has been investigated by examining distinct lineages that have evolved in similar circumstances to see if similar patterns of diversification result. Recent years have seen increasing numbers of such cases of convergent patterns of evolutionary diversification among distinct clades, hinting at a deterministic aspect to evolution (Schluter and McPhail 1993;

Grant and Grant 1995; Schluter 1996; Losos et al. 1998; Danley and Kocher 2001; Young et al. 2009; Losos 2010, but see Grant and Grant 2002).

Investigating the evolutionary processes that produce adaptive radiations is particularly difficult because adaptive radiations are by definition recognized after the radiation has occurred. One way to connect pattern to process is to study the processes driving divergence today among populations of species that belong to an adaptive radiation and then to compare the results to patterns observed at deeper phylogenetic levels. If processes scale seamlessly from the microevolutionary to the macroevolutionary



level, then it may be possible to link studies of geographic differentiation occurring within species today to macroevolutionary phenomena (Mandelbrot 1982; Green 1991; Khatri et al. 2009). However, several phylogenetic studies have suggested that different “stages” of adaptive radiation occur such that the forms of selection and direction of diversification differ during different phases of a radiation (reviewed in Streebman and Danley 2003). This hypothesis would predict that the processes driving divergence today may bear little resemblance to those which occurred during earlier phases of adaptive radiation. Indeed, one widely held view is that ecological opportunity—an abundance of available resources little used by competitors—is often the impetus for radiation to occur (Schluter 2000; Yoder et al. 2010), but one would expect that such opportunity would erode as a radiation runs its course, such that very different selective conditions may characterize early and late phases of radiation (Losos 2010; Mahler et al. 2010). The stages hypothesis, however, has never been tested by comparing patterns of contemporary geographic variation to macroevolutionary diversification within an adaptive radiation (but see Moreno et al. 1997).

Such a comparison would entail two parts. First, integral to adaptive radiation is the hypothesis that species have adapted to use different parts of the environment. If geographic variation recurrently mirrors macroevolutionary patterns, then one would expect that geographic variation also would have an adaptive basis, leading to patterns of spatial convergence of populations occupying similar ecotones across a landscape (cf. Gavrillets 2004). Such convergent evolution among populations has been found, for example, in land snails (Ozgo and Kinnison 2008) and lizards (Stenson et al. 2002; Kaliontzopoulou et al. 2010; Thorpe et al. 2010).

Second, we predict more specifically the form that adaptation should take at different evolutionary scales. If micro- and macroevolution scale seamlessly, then selection should be driving differentiation in the same way at both levels, perhaps only differing in magnitude of differentiation. Alternatively, the stages hypothesis predicts that geographic variation should be different from patterns evident in earlier stages of adaptive radiation.

The evolutionary diversification of Caribbean *Anolis* lizards has become a textbook case of replicated adaptive radiation, with similar sets of ecological specialists, termed ecomorphs, evolving independently on each of the four Greater Antillean islands (Williams 1972; Losos et al. 1998; Losos 2009). Although dispersal among islands occurred early in anole radiation, the majority of species arose through within-island diversification, in part the result of divergence into different ecomorphs, but most frequently as diversification within same-ecomorph clades (Losos et al. 1998). With few exceptions, each ecomorph type has arisen only once on an island, but after arising, ecomorphs have often proliferated, producing clades of many species in a single eco-

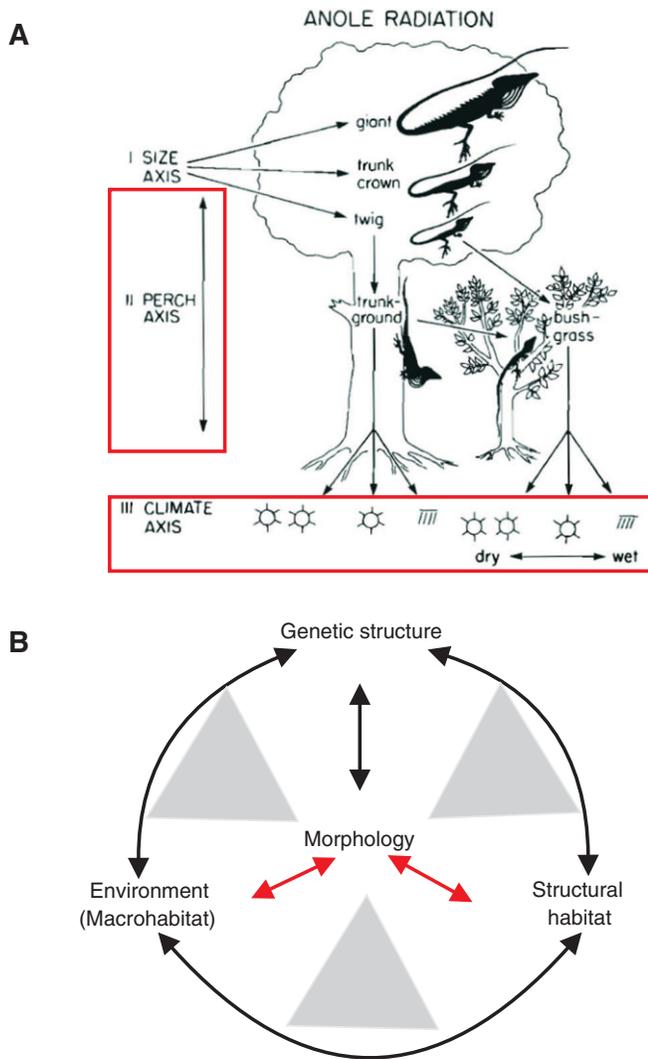
morph category (Losos 2009). Consequently, understanding the factors involved in within-ecomorph intra-island diversification is important in understanding the major part of species diversity of the anole radiation.

Intraspecific diversification within islands has been studied in some Lesser Antillean anole species, which are renowned for the extent of their geographic variability (Lazell 1972), but which have not diverged into multiple species. Recent work has suggested that patterns of diversification in morphology among those populations are driven by environmental variation (Thorpe et al. 2008, 2010; reviewed in Losos 2009). In contrast, although geographic variation exists in Greater Antillean anole species (as evident by the many subspecies described in the 1960s and 1970s; Schwartz and Henderson 1991), relatively little research has been devoted to its adaptive basis (but see Underwood and Williams 1959; Arnold 1980; Glor et al. 2003, 2004; Thorpe et al. 2008, 2010; Glor and Warren 2010), much less to investigating whether patterns of adaptive diversification are recurrent at different scales in this model case of adaptive radiation.

Although the anole ecomorphs are adapted to different aspects of the structural habitat (e.g., perch height and diameter; Fig. 1A), Williams (1972) suggested that most intra-ecomorph diversification reflected adaptation to different macrohabitats (i.e., different climate/vegetation associations; Fig. 1A). This is seen clearly in the clade of trunk-ground anoles, members of the *Anolis cybotes* clade (commonly referred to as “cybotoids”), on the Greater Antillean island of Hispaniola, which is a lineage that diversified in situ into eight trunk-ground species. Although the most widespread species, *A. cybotes*, can be found across Hispaniola, inhabiting mesic to semi-xeric macrohabitats, other cybotoid species have small geographic distributions and are restricted to specific macrohabitats. Differences in macrohabitat type among these species correlate with variation along some of the same morphometric axes that distinguish the ecomorphs (Glor et al. 2003).

Within *A. cybotes* itself, a high level of geographically structured genetic differentiation in mitochondrial DNA has been found, the most distantly related clades approximating the degree of genetic differentiation that is typical among distinct species of reptiles (Glor et al. 2003). *Anolis cybotes*, including the taxa *Anolis shrevei* and *Anolis armouri* which are paraphyletically nested within it, is composed of populations that are similar in appearance and cannot be distinguished from the color of their dewlaps. In this article, we use populations of *A. cybotes*, *A. armouri*, and *A. shrevei* (in the following referred to as the “*Anolis cybotes* species complex”) as a model group to test the following two hypotheses of deterministic evolution within adaptive radiations.

- (1) *Does diversification occur recurrently on the same axes during different stages of adaptive radiation?* First, we test



**Figure 1.** (A) Proposed axes of diversification among and within ecomorphs (Williams 1972). Axes tested for *Anolis cybotes* species complex (trunk-ground ecomorph) diversification, outlined in red. Vertical—structural habitat use axis, horizontal—environment/macrohabitat axis. (B) Flowchart outlining workflow to test for environment and structural habitat effects on morphological diversification (red arrows) while controlling for effects of genetic relatedness (black arrows) and joint effects (gray triangles).

whether patterns of morphological diversification in populations of the widespread *A. cybotes* species complex parallel the macrohabitat divergence seen among the clade containing all cybotoid species, or whether they parallel the patterns of structural habitat adaptation that characterize divergence among the ecomorphs (Fig. 1A). Alternatively, differences among populations might simply reflect the amount of time they have been evolving separately from a common ancestor, in which case we expect the morphological and ecological similarity of populations to reflect their evolutionary relat-

edness. Of course, these factors need not be independent (Fig. 1B). For example, lizards in different macrohabitats might also occupy different parts of the structural habitat, or populations that inhabit more distinctive climates may also be more genetically differentiated. We therefore partition the amount of morphological variation explained by each of three factors—genetic differentiation, structural habitat use, and macrohabitat differentiation—independently and jointly.

- (2) *Does convergence occur among populations experiencing similar environments?* Just as convergence occurs at the species level among species radiating on different islands, intraspecific evolution may be deterministic over the physical landscape by promoting spatial convergence in patterns of contemporary adaptation among populations. One particularly strong cause of climatic variation on the geographic scale of an island is elevation. The geography of the Dominican Republic, with several parallel mountain chains that are separated by vast stretches of lowland, provides a particularly strong test both of the specific hypothesis of adaptation to different environmental conditions that occur along an elevational gradient and the general question of whether the same patterns of evolution arise repeatedly in different populations experiencing similar environmental contexts.

## Methods

### SAMPLING

Populations of *A. cybotes* are mostly allopatric and are paraphyletic with respect to the species *A. armouri* and *A. shrevei* that form two separate clades within the *A. cybotes* phylogeny (Glor et al. 2003; Alföldi et al. 2011). *Anolis armouri* and *A. shrevei* occur in the highlands of the Cordillera Central and Sierra Baoruco respectively, whereas populations of *A. cybotes* are distributed throughout Hispaniola. The monophyly of this group (*A. cybotes*, *A. armouri*, and *A. shrevei*) with respect to the other species in the cybotoid species clade is supported with high bootstrap values (Glor et al. 2003; Alföldi et al. 2011). Consequently, we regard populations of *A. cybotes*, *A. armouri*, and *A. shrevei* as belonging to a single species complex (the *A. cybotes* species complex) and analyze them jointly. We also refrain from analyses of the (albeit currently recognized) species of uncertain taxonomic status, *A. breslini* and *A. haetianus*, until further material and data are available. The *A. cybotes* species complex inhabits a wide range of semi-xeric to mesic habitats from sea level to high altitudes (Glor et al. 2003).

On three field trips in October 2009, April 2010, and June 2010, habitat use data, georeferenced distribution data (obtained with a Garmin 60CSx GPS, WGS84, decimal degrees), tissue

samples, and specimens of the *A. cybotes* species complex were collected from across the Dominican Republic, including all major mountain ranges and intermediate lowlands. Major mountain ranges are the Sierra Septentrional, Sierra Baoruco, Sierra de Neyba, and the Cordillera Central with peaks up to 3087 m elevation, which are separated by vast stretches of lowlands. Smaller montane areas whose separation from the major mountain chains is not as obvious are the Eastern flanks of the Sierra Baoruco near Polo Monte, the Sierra Martín García, and the Sierra de Ocoa, each with peaks up to 1000 m elevation. Sampling localities span elevations from -22 m below sea level in the Valle de Neyba to ~3000 m on Pico Duarte (Cordillera Central). We attempted to sample the most important montane ecotones, as well as previously identified clade boundaries (Glor et al. 2003), and boundaries between major macrohabitats (e.g., coastal mangrove, xeric), and we attempted to generate high sampling coverage of the Dominican Republic overall. Because of the continuous distribution of the *A. cybotes* species complex over the studied landscape and the associated arbitrariness in defining populations, an individual-based sampling scheme was chosen (Lemmon and Lemmon 2008; Bloomquist et al. 2010; Gaggiotti 2010).

Structural habitat used data were collected for 551 adult males, and 224 of these were taken as whole animal specimens for morphological analysis. The preserved specimens were deposited in the herpetological collection of the Museum of Comparative Zoology (MCZ), Harvard University and subsequently analyzed for further morphological characters. Locality information and museum accession numbers can be found in Table S1. We obtained 511 tissue samples, including muscle tissues from the 224 whole animal specimens preserved in 90% EtOH and 287 additional tail tip samples collected from specimens that were subsequently released on site.

### MOLECULAR GENETIC DATA

A fragment of the mitochondrial ND2 gene was amplified from the 511 tissue samples to compare these specimens to the study of Glor et al. (2003). DNA extraction was performed using standard salt-extraction protocols (Bruford et al. 1992). Thermocycling reactions followed protocols described in Glor et al. (2003), with primers modified to align optimally to *A. cybotes* (forward L4437-cyb 5'-AAG CTA TTG GGC CCA TAC C-3'; reverse H5730-cyb 5'-AGC GAA TRG AAG RCC GCT GG-3'). The successfully amplified products were purified using exonuclease I and shrimp alkaline phosphatase or antarctic phosphatase according to the manufacturer's instruction (New England Biolabs, Ipswich, MA, USA). Purified polymerase chain reaction templates were sequenced on both strands using dye-labeled dideoxy terminator cycle sequencing on an ABI 3130 automated DNA sequencer. Fragments were edited and aligned manually to produce a final alignment of 1202 bp length in total. Novel sequences

were submitted to GenBank (KC981250–KC981646). Not all tissue samples amplified successfully, and further ND2 sequences were added to the alignment from GenBank (obtained from Glor et al. 2003; Table S1).

### MORPHOLOGICAL DATA

We obtained data on a range of morphological features from both live and preserved specimens. *In vivo*, we measured the following morphological characteristics: simple body and head dimensions were measured using digital calipers to the nearest 0.01 mm (snout-vent-length [SVL], head length [HL], head width [HW], head height [HH]). Lizard live weight was determined using a digital scale (Ohaus PS 251, Parsippany, NJ, USA). We obtained the following morphological data for each of the 224 preserved museum specimens: 13 toepad and hindfoot characteristics were measured, most of them on the right fourth toe (T4) and on the foot using digital flatbed scanners with a resolution of 2400–3200 dpi, and analyzed with the software ImageJ (NIH, Bethesda, MD). Some of these characters were meristic, including number of lamellae on the toepad (NLP), number of scales between the pad to knee (from first scale after toe pad lamellae to last scale before flexor of knee, NPK), number of scales knee to sole (NKP), number of scales connecting scale rows of the third toe (T3) and T4 at their base (N34), number of scales across the width of the foot starting at the base of the second toe (T2D), number of scales across the width of the foot starting at the base of T3 (T3D), number of scales on T4 until break on sole (T4B), and number of scales on T3 until break on palm (T3B). Other characters were quantitative: pad area (PA), pad length (PL), pad width (PW), fourth toe length (T4L), and foot length (FOL). If the right foot was damaged, the left foot was measured. All foot measurements were taken twice and the mean was used to minimize measurement error. Flatbed scans were also used to measure seven claw characteristics on T4 of the hindfoot, modified after Zani (2000; Fig. 1 therein): claw base width (CLC), sine and cosine of claw radians (CLD, CLE), claw height (CLF), claw phalanx length (CPL), claw tip angle ( $\theta$ ), and claw curvature (CC). Specimens also were X-rayed using an INSPEX 20i digital X-ray system (compiled by Kodex, Inc.) with a resolution of 3.94 lp/mm (density dependent). Skeletal quantitative traits were subsequently analyzed using the software tpsDig2 (©Sunysb.edu). We measured 26 quantitative skeletal traits from these X-ray images, focusing on ecologically significant characters (Losos 2009; Mahler et al. 2010, for abbreviations and computation of skull measurements see Supplementary Information S11): hindlimb phalanx length (HLP), hindlimb metatarsal length (HLMT), tibia length (T), fibula length (F), femur length (FE), pelvis width (PW), metacarpal length (MT), ulna length (U), radius length (R), humerus length (H), shoulder width (SW), length of jaw from tip to opening in-lever (OI), length of jaw from closing in-lever to jaw tip (CLI), whole head (WH), out-lever

(O), snout plus eye (SE), eye length (REL), frontal parietal to snout (FPS), braincase width (BW), head width across retroarticulars (HWR), head width across jugals (HWJ), head width quadrates (HWQ), snout width at front of eye (SFE), lower jaw length (RLJL), distance from quadrate to symphysis (RQS), distance from orbita to symphysis (ROS). All X-ray measurements were obtained and averaged to minimize measurement error. Variables were tested for normal distribution using Kolmogorov–Smirnov tests in STATISTICA (©StatSoft, Tulsa, OK); the hypothesis of normal distribution was not rejected for any variable, and all were log-transformed before computing residuals to SVL for size correction of all variables. To reduce dimensionality of the data, tables of multiple variables that were obtained by different measurement methods were each subjected to principal component analyses (PCAs). PCAs were conducted separately on the flatbed scan and X-ray data, yielding eight (toepads, foot, and claw, TPC1–8) and five (X-rays, XPC1–5) PC axes with eigenvalues > 1.0 (Supplementary Tables S2–S5). Analyses were conducted on these PC scores, along with size-corrected head and body mass measurements.

#### STRUCTURAL HABITAT USE DATA

Perch height (PHL), perch diameter (PD), and distance of the lizard to the nearest perch (DNP), variables known to be important in anole ecology, were collected for each observed lizard. Data were only collected for lizards that were seen before they were disturbed, and never for lizards that were first seen moving. Distance to nearest perch (DNP), a measure of the structural properties of the microhabitat (Pounds 1988), was measured as the closest distance between the spot where the lizard perched (taken mid-body) and the nearest perch structure to which it could jump and that could support it. We considered potential nearest perches as small as 2 mm, the smallest diameter on which lizards were observed. If the nearest perch was greater than 99 cm in diameter (e.g., a rock face of several meters in diameter), we used a value of 99 for its diameter because perches of greater diameter are essentially flat to the lizard and thus functionally equivalent. A PCA was performed on these variables to reduce dimensionality, yielding two PCs with eigenvalues > 1.0 (HPC1, HPC2; Supplementary Tables S6–S7). These variables constituted the habitat use data set for subsequent analyses. In addition, we recorded the categorical variables perch type (plant, rock, log) and orientation of the lizard on the perch (quasi-horizontal [0–30°] or quasi-vertical [31–90°]).

#### DATA MODELING FOR LANDSCAPE ANALYSES

We performed spatial statistics by regression of data extracted from maps using General Linear Model (GLM), canonical correlation, and redundancy analyses. Recent papers (e.g., Legendre

and Fortin 2010) recommend such table-based ordination analyses instead of matrix-based tests.

Genetic distances were determined by computing a matrix of uncorrected *p*-distances between the sequenced specimens in MEGA (©2003–2008 version 4.1). To transform this matrix into table format, we extracted principal components of the neighborhood matrix (PCNMs; Borcard and Legendre 2002; Borcard et al. 2004) in R (package *vegan*; Oksanen et al. 2011). Principal components of the neighborhood matrix with negative eigenvectors were discarded, yielding 12 PCNMs. Subsequently, 10 meaningful PCNM axes were selected using stepwise-forward analysis of covariance (ANOVA) in R (function *ordiR2step*; Oksanen et al. 2011) discarding PCNMs 1 and 5 (Table S8).

As can be seen in Table S1, not all data were recorded for the same individuals, and sometimes, single measurements were missing (e.g., if the scales on toe pads were not countable). To perform spatial statistics on the maximal set of cases, each variable from the three data sets (morphology, habitat use, and genetic distance) was interpolated separately over the spatial extent of the Dominican Republic using the simple Kriging algorithm in ArcGis (©ESRI, version 9.3), which interpolates unknown values from surrounding known values via a covariance function. In this way, we also corrected for the nonrandom scheme of sampling of the original data points that might otherwise have led to biases in model estimation. Depending on the hypothesis tested, we continued with data extracted from random points over these maps. The more conservative approach would have been to prune from the data set cases with missing data instead of interpolating, thereby discarding many of the original spatial data points. To investigate whether analyses using interpolated data differ from analyses using the original data set, we conducted a GLM regression on this reduced data set (102 observations) with subsequent test of spatial autocorrelation of the residuals using Moran's *I*. These analyses, presented in the Supporting Information, indicate that the results are qualitatively unchanged.

We used interpolated environmental data in the form of bioclimatic layers from the WORLDCLIM database (bio1–19; Hijmans et al. 2005), and elevation from the GTOPO30 W100N40 digital elevation model (US Geological Survey).

#### DATA EXTRACTION AND REGRESSION

For regression analyses, values for all variables (morphology, habitat use, genetic distance, and environment) were extracted from the Kriging interpolated maps for 568 points generated from a random distribution (equaling the number of georeferenced GPS samples in the original data set). Repeating the analyses with three more random samples of 568 points did not show different results, so we refrain from reporting those here. The extracted data for environment (bio1–19 plus elevation) were subjected

to a PCA (Tables S9, S10), yielding four PCs with eigenvalues  $> 1.0$  (EnvPC1–4), where elevation significantly contributed to EnvPC1. A GLM was then used in STATISTICA for hypothesis testing. A potential error for Kriging-interpolated data is that the interpolation will be computed over the total extent of the surface including areas from which there are no data. If this absence of data points results from missing sampling, the procedure is valid. However, data interpolation over areas that are not inhabited by the species group (e.g., due to unsuitable climate) will introduce errors when comparing the interpolated data to potential explanatory variables (such as climate). We corrected for potential errors introduced by interpolation of species-absent areas by computing a MAXENT model of species distribution (Phillips et al. 2004, 2006) using our own sampling points for the *A. cybotes* species complex as well as distribution data for the whole island of Hispaniola of 2367 additional specimens deposited at the MCZ. The obtained values for the occurrence probability of the *A. cybotes* species complex at each of the 568 random points as computed by MAXENT were added to the analysis data set and used as weighting variable for GLM in STATISTICA.

To test our first hypothesis, that patterns of morphological differentiation in the *A. cybotes* species complex parallel those seen at higher phylogenetic levels, we applied variance partitioning using R (package *vegan*, function *varpart*). The *varpart* function partitions the variation of a response table with respect to the explanatory tables using redundancy analysis ordination (*rda*; Oksanen et al. 2011) without requiring that collinearity in the explanatory tables be removed first (Oksanen et al. 2011). Tables were generated from each data set and used as input files. For the morphological data set, the morphological PCs (TPC1–8, XPC1–5) were transformed into a data table together with body condition and the caliper head measurements. Similarly, the habitat use PCs (HPC1 and HPC2) composed of a data table, as did the environmental PCs (EnvPC1–4) and the genetic distances (genPC 2–4, 6–10).

To test hypothesis 2, that populations in similar environments converge morphologically, an additional test data set was prepared. We generated a second data set for all variables containing extracted map data for 2000 randomly generated points to obtain a more accurate representation of geospatial characteristics than obtained by the 568-point sampling data set, approximating the total number of raster cells in the layers. To test replicated, directional evolution across similar elevational ecotones, we extracted map data for the Kriging interpolated maps of morphology, environment, and habitat use for lowlands (LOW) and each montane ecotone (Cordillera Central [CC], Sierra Baoruco [SB], Sierra de Neyba [SN], eastern flanks of Sierra Baoruco near Polo Monte [PM], Sierra Martín García [SMG], and Sierra de Ocoa [SO]). These mountain chain areas were manually delimited in ArcGIS

using the digital elevation model. Elevations  $> 1000$  m were furthermore classified as “highlands.” Highlands in the Sierra Septentrional are isolated from each other as well as restricted to a very narrow spatial extent so that no map values could be extracted for them.

## HYPOTHESIS TESTING

- (1) We tested whether areas that harbor genetically similar lizards differ in environmental parameters using occurrence probability-weighted (values derived from MAXENT) regression. We tested whether a relationship exists between morphology and habitat use using occurrence-probability weighted canonical correlation analyses. To partition the relative and joint effects of environment, habitat use, and genetic differentiation, we performed an occurrence-probability weighted regression analysis on interpolated and noninterpolated data. To further infer the relative contribution of each significantly contributing factor, the variance explained by significant predictor tables was subsequently partitioned. Significance of these fractions was further tested using ANOVA in *rda*. Using the same approach as for the whole model, variance partitioning was then applied for each morphological variable separately.
- (2) To infer spatially convergent ecomorphological evolution over similar environmental gradients, a canonical correlation between habitat use and morphological variables (“ecomorphological,” in one set) and environment (in the other set) was performed on the 2000 point data set, and ecomorphological values for each mountain chain and for lowlands were plotted. Differences of ecomorphological canonical roots (CRs) between all montane ecotones and the other, nonmontane sampling points were determined with Mann–Whitney *U* tests. Ecomorphological values were then extracted for the lowlands and for each montane ecotone, and separately tested for differences using Kruskal–Wallis ANOVA. To test whether all morphological variables are changing over elevational gradients to the same extent, we added variance-partitioning analyses using single morphological variables to identify variables whose variance is better explained by the environment than by phylogenetic relatedness. Elevational differences between the categorical variables perch type and orientation of the lizard on the perch were tested using Kruskal–Wallis ANOVA.

We performed power analyses for all tests and added them to the test result tables. For correlations, confidence intervals were estimated for correlation coefficients using Fisher’s refined *Z*.

**Table 1.** Multivariate tests of significance (Wilks' test) for occurrence-probability based regression of environmental PCs on genetic distance principal components of the neighborhood matrix. Because of large sample size, measures of power are given: partial  $\eta^2$  (the proportion of total variance attributable to each factor), noncentrality (effect size according to sample size),  $\alpha$  (probability of type II error,  $1-\beta$ ).

	<i>W</i>	<i>F</i>	<i>P</i>	Partial $\eta^2$	Noncentrality	$\alpha$
Intercept	0.000	233732830	<0.001	1.000	2.337328E+09	1.000
EnvPC1	0.584	12	<0.001	0.416	1.184566E+02	1.000
EnvPC2	0.450	20	<0.001	0.550	2.027542E+02	1.000
EnvPC3	0.130	111	<0.001	0.870	1.113074E+03	1.000
EnvPC4	0.442	21	<0.001	0.558	2.093181E+02	1.000

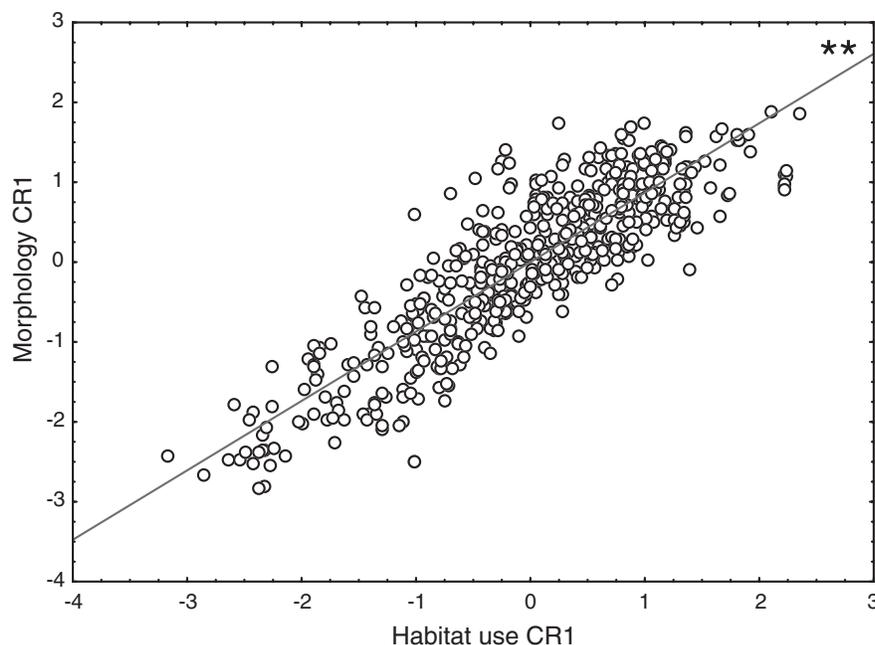
## Results

### (1) DOES DIVERSIFICATION OCCUR RECURRENTLY ON THE SAME AXES DURING DIFFERENT STAGES OF ADAPTIVE RADIATION?

First, we tested whether degree of genetic similarity and degree of environmental similarity are significantly related—areas harboring more closely related populations tend to be more similar in their environmental niches (macrohabitat; Table 1). Canonical correlation analysis extracted two CRs from each variable set (habitat use and morphology), explaining in total 100% of the variance from the habitat use data set (probably due to the limited number of variables) and 26.6% of variance from the morphology data set. The two data sets were highly correlated (Fig. 2; Canonical  $R = 0.87$ ,  $\chi^2 = 1438.3$ ,  $P < 0.0001$ ; Tables S11–S13, Fig. S1). Structural habitat use was strongly correlated with body mass and shape characteristics: the habitat use CR1 was strongly influenced by perch height and distance to nearest perch (HPC1),

and morphological CR1 received high weights from body mass, snout width at front of eye (XPC5), and characters measured on the hindfoot—an indicator of relative foot width measured by scales across the foot from second and third toe (TPC4), and claw phalanx length (TPC6). In the second CRs, perch diameter (HPC2) was correlated with the head dimensions (XPC2, XPC4), scale row length on T3 and T4 (TPC5), and claw angle and curvature (TPC7).

A species distribution model for the *A. cybotes* species complex was computed using MAXENT (training AUC = 0.852; Fig. S2). Occurrence probability from this model was used as a weighting variable in multivariate regression. However, exclusion of the MAXENT weighting variable from the model did not change the results (not shown). Multiple regression analysis on Kriging interpolated maps showed that not only habitat use, but all potential explanatory variables, significantly contribute to morphological variation over the landscape (Table 2). These



**Figure 2.** Ecomorphological canonical correlation between morphology and habitat use of the *Anolis cybotes* species complex throughout the Dominican Republic ( $r = 0.8691$ ,  $P < 0.0001$ , Fisher's  $Z$  refined confidence interval estimation  $0.8472 < r < 0.8877$ ).

**Table 2.** Univariate results of occurrence-probability weighted multiple regression showing significant effects (Wilks' test,  $W$ ) of all explanatory variables on morphological variance. Because of large sample size, measures of power are given: partial  $\eta^2$  (the proportion of total variance attributable to each factor), noncentrality (effect size according to sample size),  $\alpha$  (probability of type II error,  $1\beta$ ).

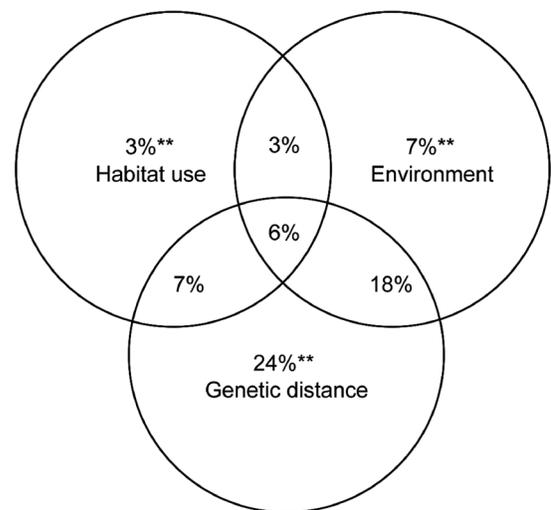
	$W$	$F$	$P$	Partial $\eta^2$	Noncentrality	$\alpha$
Intercept	0.236	27.545	<0.001	0.770	491.434	1.000
HPC1	0.515	8.020	<0.001	0.542	173.677	1.000
HPC2	0.617	5.293	<0.001	0.399	97.793	1.000
genPC2	0.306	19.338	<0.001	0.725	386.847	1.000
genPC3	0.473	9.510	<0.001	0.475	133.143	1.000
genPC4	0.414	12.066	<0.001	0.646	268.267	1.000
genPC6	0.428	11.415	<0.001	0.681	314.336	1.000
genPC7	0.375	14.214	<0.001	0.623	242.450	1.000
genPC8	0.551	6.952	<0.001	0.436	113.585	1.000
genPC9	0.624	5.140	<0.001	0.380	90.126	1.000
genPC10	0.295	20.396	<0.001	0.710	359.315	1.000
genPC11	0.711	3.472	<0.011	0.193	35.061	0.970
genPC12	0.427	11.432	<0.001	0.575	198.500	1.000
EnvPC1	0.248	25.870	<0.001	0.741	420.869	1.000
EnvPC2	0.431	11.243	<0.001	0.563	189.225	1.000
EnvPC3	0.357	15.387	<0.001	0.651	274.407	1.000
EnvPC4	0.485	9.072	<0.001	0.494	143.254	1.000

results are consistent with those from multiple regression analysis on a reduced set of 102 observed points, in which a Moran's  $I$  test showed no evidence of spatial autocorrelation, meaning they can be treated as independent observations (Tables S14, S15). Therefore, variance partitioning was necessary to find the best predictor and discern joint from independent variation. Using *varpart* in R, the model with habitat use, environment, and genetic distance as three explanatory tables explained 69% of total variance in morphology as the response table, with all of them being significant ( $P < 0.001$ ; Fig. 3). Among the explanatory tables, the largest part of morphological variation explained by our model was attributed to genetic distance (24%). Seven percent of the morphological variance was jointly explained by genetic differentiation and differences in structural habitat use, and 6% by the joint covariation of all three variables. Environmental differences explained 7% of the variation, difference in structural habitat use 3%, and the covariation between them another 6%. Also, 18% of total morphological variance was explained by the joint effect of genetic differentiation and environmental variation.

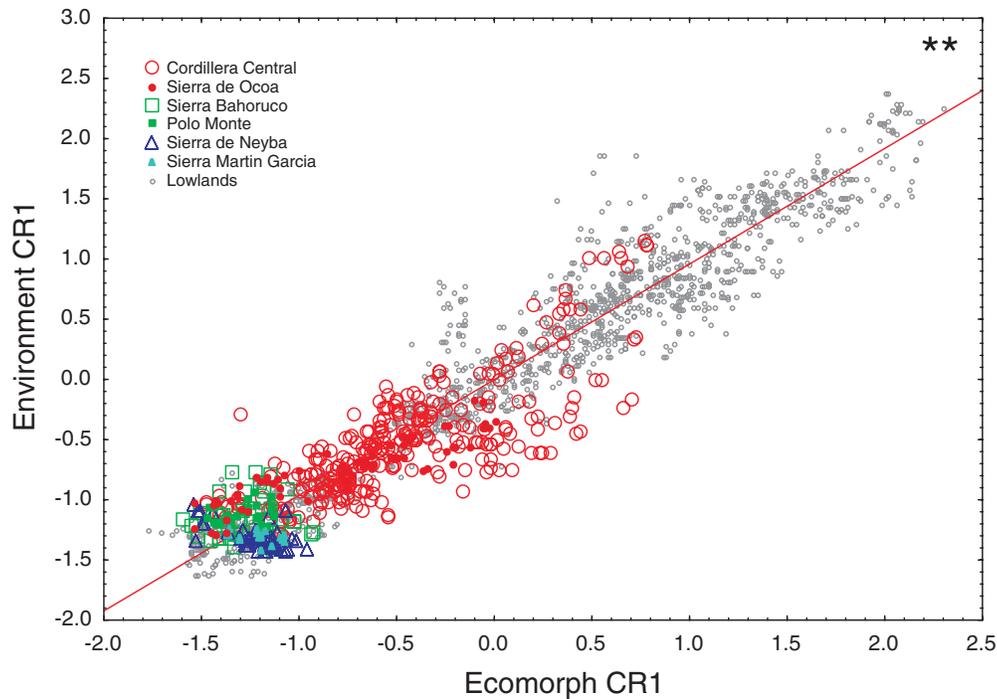
## (2) DOES CONVERGENCE OCCUR AMONG POPULATIONS EXPERIENCING SIMILAR ENVIRONMENTS?

The canonical correlation analysis between ecomorphological (morphology and habitat use) and environmental (elevation and climate) variables extracted four CRs from each variable set, explaining 100% of the variance in environment and 44.4% of ecomorphological variance. The canonical correlation between

these sets was significant (canonical  $R = 0.96$ ,  $\chi^2 = 11066.0$ ,  $P < 0.0001$ ; Tables S16–S18). Figure 4 shows the correlation between ecomorphological CR1 and environmental CR1, with the data points obtained from different mountain chains depicted by different symbols. Environmental CR1 receives high weights from precipitation variables (EnvPC2). Similar ecomorphological val-



**Figure 3.** Variance partitioning of whole model (69% of total variance explained \*\*significance of fractions  $P < 0.0001$ ). Values in circles represent partitions of variance in lizard morphology explained by the predictors structural habitat use, environment, and genetic distance separately. Areas of overlap between circles show shared variance between predictors.



**Figure 4.** Significant ecomorph–environment canonical correlation ( $r = 0.9608$ ,  $P < 0.0001$ , Fisher’s  $Z$  refined confidence interval estimation  $0.09573 < r > 0.9640$ ). Concentration of montane ecotone data points on lower left side of the correlation indicates replicated directional evolution.

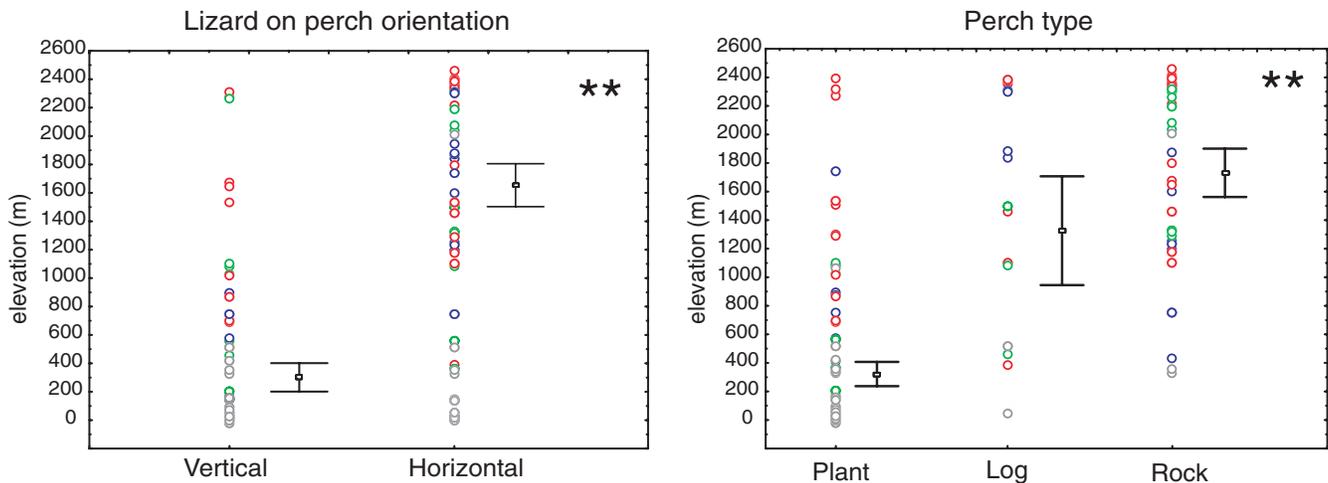
ues obtained from different mountain chains and among distantly related clades subsequently demonstrates the presence of similar ecomorphological shifts along similar environmental gradients (the montane ecotone). This morphological convergence was less evident in the correlations between the other CRs (Fig. S3). As an alternative means of looking for an association between ecomorphological trait variation and environment, we separated localities into montane and nonmontane, with 1000 m being the cut-off. Mann-Whitney  $U$  tests showed that all environmental CRs differed between montane and nonmontane sampling points (Table 3). Ecomorphological CR1 received high weights from both habitat use and morphology variables. Variables with high weights were perch height and distance to nearest perch (HPC1), head width (HW), length of scale rows on T3 and T4 (TPC5), head width at jugals (XPC3), closing in lever, head width at quadrates (XPC4), and snout width at front of eye (XPC5), showing that these quantitative traits are being jointly shaped by environmen-

tal variation. Using the same approach as for the whole model, variance partitioning was applied for each morphological variable separately. This revealed that variation in XPC4 and XPC5 (but not of XPC3, HW, and TPC5) is explained better by environmental variation than by genetic similarity (Table S17; as an example; Fig. S4 shows Kriging-interpolated maps of XPC4 and XPC5 and their partitioned variance).

In summary, environmental variation promotes correlated variation in perch height, head width, and some characteristics of the toepad, which is partially independent from genetic associations. In fact, if perch type and orientation of the lizard on the perch are plotted against elevation, it becomes obvious that the *A. cybotes* species complex shows a shift in perch type with elevation (KW-H = 112.71,  $P < 0.0001$ ; Fig. 5) and equally in the physical orientation of the lizard on the perch (KW-H = 98.13,  $P < 0.0001$ ; Fig. 5). Specifically, at higher elevations, lizards inhabit low perches such as rocks and logs, sitting on them horizontally,

**Table 3.** Mann–Whitney  $U$  test for differences of ecomorphology canonical roots 1–4 between highlands (elevation >1000 m, HI) and lowlands (elevation <1000 m, LO). Right three columns show results of power analysis of parametric  $U$  test equivalent ( $t$ -test).

Ecomorph canonical root	$U$	$Z$	$P$ -level	Sigma	Critical value of $t$	$\alpha$
CR1	122426.0	11.362	<0.001	1.000	1.961	1.000
CR2	106543.0	13.221	<0.001	1.000	1.961	1.000
CR3	180182.0	−4.602	<0.001	1.000	1.961	0.951
CR4	77591.0	−16.610	<0.001	1.000	1.961	1.000



**Figure 5.** Changes in occupied perch type and orientation of lizards on the perch with elevation. Double asterisks indicate significant differences between groups ( $P < 0.0001$ , tested with Kruskal–Wallis analysis of covariance). Data points are shown as gray circles—lowlands, red circles—Cordillera Central, green circles—Sierra Baoruco, blue circles—Sierra de Neyba and mean  $\pm$  95% confidence interval plots.

whereas plants are the most frequently used perch type at lower elevations, with the lizards occupying a more vertical position along the main perch axis. The Kruskal–Wallis tests showed significant differences ( $P < 0.0001$  for all tests) in ecomorphological canonical root 1 (CR1) scores between lowland and montane sites for geographically separated mountain chains and associated lowlands in the Dominican Republic (Cordillera Central, KW-H = 133.15; Sierra Baoruco, KW-H = 78.25; Sierra de Neyba, KW-H = 90.97; Polo Monte or eastern flank of Sierra Baoruco PM, KW-H = 47.75; Sierra Martin Garcia SMG, KW-H = 30.58; and Sierra de Ocoa SO, KW-H = 66.69; Fig. 6), and lowlands (LOW; Fig. 6), showing the recurrent nature of the morphological evolutionary response to elevational gradients.

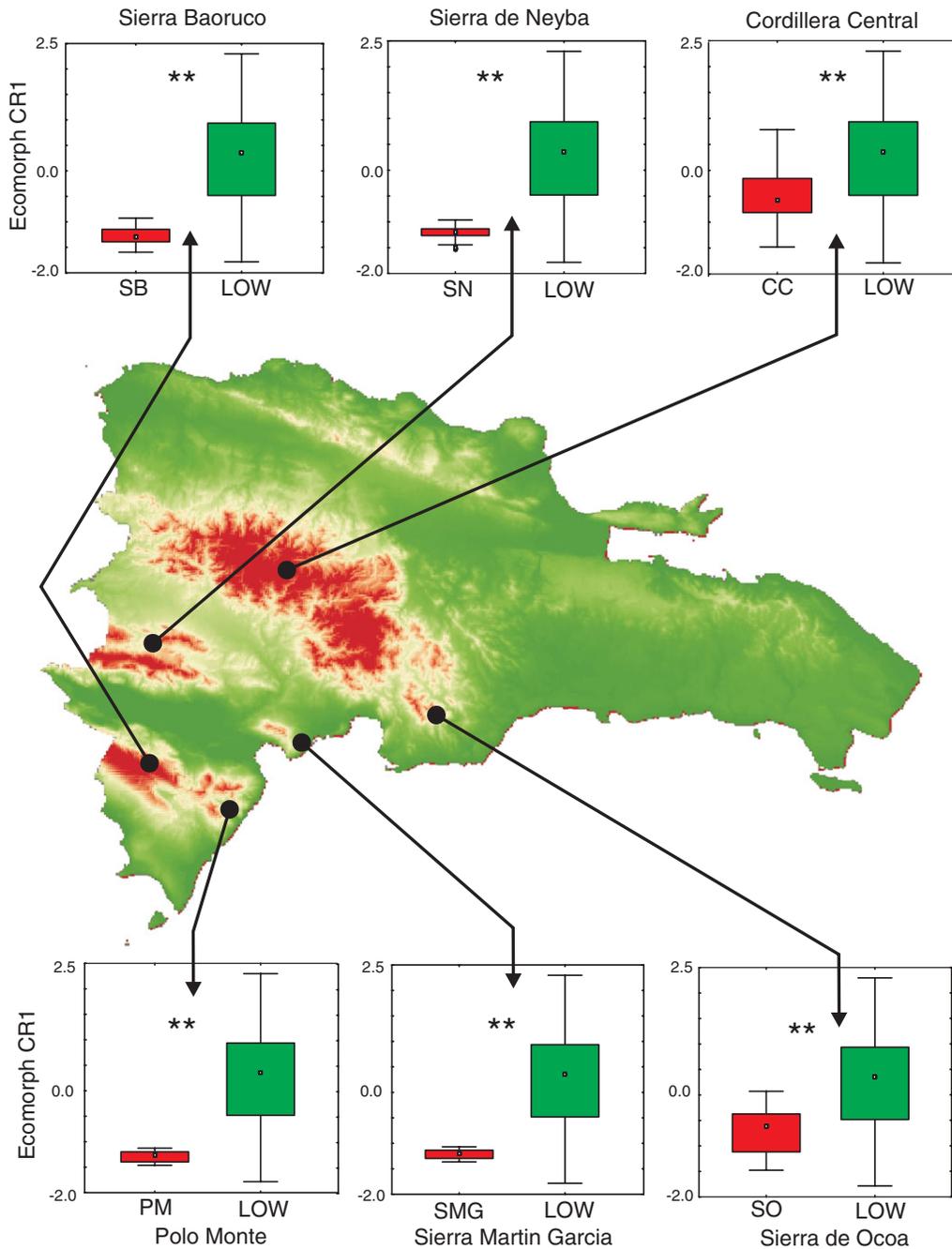
## Discussion

Evolutionary diversification of Caribbean *Anolis* lizards has become a textbook example of adaptive radiation, in this case replicated four times across the islands of the Greater Antilles. Two features of these radiations are important here. First, across islands, the same set of habitat types, termed ecomorphs, has evolved convergently on each island. To a large extent, the features that are convergent among members of the same ecomorph and divergent between ecomorphs represent adaptations to use different parts of the structural habitat (e.g., the diameter and spacing of the supports on which they move). Second, within an island radiation, once each ecomorph type has evolved, many clades diversify, producing a suite of species, all members of the same ecomorph class, but adapted to use different macrohabitats that vary in climate and vegetation structure.

An ongoing debate in evolutionary biology concerns the correspondence between micro- and macroevolutionary change. Can the small-scale changes that occur within and among populations be scaled up to the major changes that distinguish species and higher taxa? An alternative possibility, particularly in the context of adaptive radiation, is that conditions change during the course of a clade's history such that one would not expect evolutionary patterns that manifest among populations and species today to mirror those that occurred in the early stages of an adaptive radiation.

We chose to examine the replicability of patterns of anole diversification among populations of the *A. cybotes* species complex in the Dominican Republic. This group is particularly suited for comparison to the anole radiation with large for two reasons. First, populations of the complex occur in a wide variety of habitats and thus have the potential to diversify both in terms of structural and macrohabitat. Second, the rugged topography of the Dominican Republic, featuring a number of different mountain chains, provides the possibility for divergence along elevational transects if populations are, indeed, adapting to different environments, combined with the possibility of convergence across landscape as lineages independently adapt to similar circumstances.

Our results reveal that patterns of differentiation in this complex parallel those seen in the Caribbean anole radiations. Between 13% and 44% of morphological variation can be explained by divergence in macrohabitat and structural habitat use, paralleling patterns of diversification in the anole radiations. Across mountain ranges, phenotypic divergence along elevational gradients has occurred in parallel, indicating a pattern of convergent divergence analogous to that seen among the Greater Antillean radiations.



**Figure 6.** Box plots showing significant differences (\*\* $P < 0.0001$ ) in ecomorphological canonical root 1 scores between montane ecotones of geographically separated mountain chains in the Dominican Republic (Cordillera Central CC, Sierra Baoruco SB, Sierra de Neyba SN, upper plot row), and lowlands (LOW). Additional recognized mountains that are more or less separated from these are shown in the lower plot row (Polo Monte or eastern flank of Sierra Baoruco PM, Sierra Martin Garcia SMG, and Sierra de Ocoa SO). Montane regions in the Sierra Septentrional are not cohesive enough to permit testing at this scale. Significant differences between mountain chains and lowlands indicate replicated adaptive evolutionary responses under the same selective pressure, and the uniformly lower canonical scores for mountains than for highlands show equidirectionality of the response.

**DOES DIVERSIFICATION OCCUR RECURRENTLY ON THE SAME AXES DURING DIFFERENT STAGES OF ADAPTIVE RADIATION?**

We found significant evidence for adaptive divergence in some morphological characters among *A. cybotes* populations in

response to both variation in structural habitat and macrohabitat use; however, the best predictor of morphological differentiation is the genetic differentiation between populations. Previous work has established the existence of numerous, highly differentiated mitochondrial clades within the *A. cybotes* complex (Glor et al.

2003). Our results confirm the existence of these genetic groups and show that these clades are substantially differentiated morphologically as well, including differences in several limb, toe, and scale characters. In fact, genetic differentiation explains 24% of the morphological differentiation among populations. Whether such differences are the result of genetic drift or idiosyncratic adaptation to particular selective conditions facing each clade is unknown, but this variation does not parallel patterns of adaptation seen at higher levels of anole radiation. However, genetically differentiated clades often occur in different environments, such that macrohabitat and genetic differentiation are confounded, and 18% of the morphological variation is jointly predicted by differences in macrohabitat and genetics. Another 7% of morphological variation partitions to genetically differentiated populations that differ in structural habitat, and 6% more correlates with joint differentiation in genetics, macrohabitat, and structural habitat use.

Nonetheless, morphological variation was significantly related to environmental features independent of genetic differentiation among populations. Seven percent of variation was solely attributable to variation in macrohabitat, 3% to variation in structural habitat use, and another 3% to joint effects of macrohabitat and structural habitat use. These results support the hypothesis that at least some differentiation at the population level in the *A. cybotes* complex parallels that seen at higher levels of anole adaptive radiation. However, one could look at these results in two ways.

A more conservative outlook would argue that only 13% of variation can be solely explained by one or both environmental factors, and thus the majority of variation has occurred in ways unlike that seen at higher levels of radiation. However, that view discounts the 31% of the morphological variation that is jointly explained by genetic differentiation and one or the other, or both, of the environmental factors. Because of the confounding with genetic differentiation, it is not possible to determine whether the environment has been directly responsible for this variation, but potentially as much as 44% of morphological variation may be a result of the same factors that drove higher levels of divergence among anoles. The data at hand cannot differentiate these viewpoints.

Regardless, one result seems clear: macrohabitat explains a greater share of morphological differentiation than structural habitat use. This result is not surprising. Given that all members of the *A. cybotes* complex are trunk-ground anoles, they generally use similar structural habitats. By contrast, occurring from habitats as different as montane forest and semidesert, populations experience a great diversity of macrohabitats, and thus have had substantial opportunity to adapt divergently to these macrohabitats. In this respect, it is not surprising that differentiation within the *A. cybotes* complex is more similar to that seen among species

within the same ecomorph clade than to divergent adaptation among different ecomorphs.

### SPATIAL CONVERGENCE IN ECOMORPHOLOGICAL DIVERSIFICATION

In the Dominican Republic, environmental features (that have been found to predict genetic and morphological structure in the *A. cybotes* species complex) vary across the landscape. The second test for deterministic evolution was whether ecomorphological divergence across similar environmental gradients—mountainous ecotones on spatially separated mountain chains—occurred in parallel. We found significant similarity between environmental and ecomorphological features: environmental parameters (especially precipitation) and ecomorphology for all mountain ranges are significantly correlated. Montane and lowland areas significantly differed in their ecomorphological values for the *A. cybotes* species complex, showing recurrent ecomorphological shifts over similar, spatially separated environmental gradients. Variables that showed such patterns of convergent evolution were related to perch height, vegetation structure, several head width characters, scale characteristics of the foot, and snout width. In a repeated variance-partitioning analysis, we found that head width and snout width covary with elevational clines independently from genetic association.

These elevational effects on anole morphology and habitat use are clearly apparent in the use of specific perch structures. At lower elevations, lizards of the *A. cybotes* species complex usually perch vertically on plant structures (tree trunks), whereas at higher elevations, lizards perch horizontally on logs and rocks even though trees are available (upland pine forests are composed of *Pinus occidentalis* alone or mixed with other broadleaf trees until 2100 m elevation; World Wildlife Fund 2009). These patterns occur in many different genetic populations (i.e., being taxonomically regarded as different clades of *A. cybotes* or as *A. armouri* or *A. shrevei*). It needs to be kept in mind however that although the observed perch and associated morphology shift was significant, the portion of morphology that varied with the populations adapted to different macrohabitat was relatively larger. The replicated nature of these patterns over three major and three minor mountain chains suggests an adaptive basis for morphological change (Losos 2011).

Many lizards are known to exhibit such interspecific (e.g., Bickel and Losos 2002; Herrel et al. 2002) or intraspecific (Kaliontzopoulou et al. 2010; Hopkins and Tolley 2011) morphological variation corresponding to habitat changes. In our example, habitat-related morphological variation was restricted to characteristics of the foot and head dimensions. Such intraspecific ecomorphological variation in head dimensions has also been found in *Podarcis bocagei* lizards (Kaliontzopoulou et al. 2010), where populations inhabiting walls (saxicolous populations) and

mountain habitats (where both rocks and the ground were used) showed flattened skulls as compared to populations inhabiting sand dunes, a difference similar to that seen between montane and lower-elevation populations of the *A. cybotes* species complex. As of now, the exact nature of the changes in selective pressure in high elevations is still unclear—future studies might reveal whether the primary agent of selection acting on phenotypes is on physiology (Hertz and Huey 1981), reproduction (Huey 1977), behavior, or on something else.

#### PARALLELS TO DIVERSIFICATION IN OTHER ANOLE CLADES

The morphological changes among populations of the *A. cybotes* species complex in response to changing environmental conditions parallel morphological evolution observed among the other clades of cybotoid anoles, where diversification was also found to be related to macrohabitat divergence (Glor et al. 2003). Furthermore, the findings on spatial morphological convergence in the *A. cybotes* species complex bear similarities to patterns of intraspecific divergence in anoles of the Lesser Antilles; similar patterns are seen in populations of *A. roquet* on Martinique and *A. ocellatus* on Dominica, although they involve different morphological characters. Along a montane gradient, *A. roquet* diverges morphologically and clade independently in terms of body measurements, dewlap hues, scalation, and pattern characteristics (Thorpe et al. 2010), whereas in a similar situation in Dominica, *A. ocellatus* diverges in the number of scales spanning the body was negatively correlated with rainfall, but not with elevation, and vegetation was correlated with body patterns (Malhotra and Thorpe 1991). Adaptive divergence along the environmental (macrohabitat/climate) axis, as proposed by Williams (1972), therefore seems to be an important process in intra-island intraspecific and intra-ecomorph clade diversification.

#### MORPHOLOGICAL CHARACTERS INVOLVED IN DIVERGENCE AND CONVERGENCE

Because macrohabitat variation appears to a stronger determinant of morphological differentiation in the *A. cybotes* complex, it is not surprising that the morphological characters that vary among populations do not correspond to the morphological characters that are most commonly found to vary among ecomorphs, which differ in structural habitat and not in macrohabitat. The primary morphological characters that vary among ecomorphs, such as relative limb length and size of the toepad, were not associated with habitat use in the *A. cybotes* species complex. Within ecomorph clades, species differ in macrohabitat, but no research to date has examined which phenotypic characters—if any—differ among closely related species differing in macrohabitat, although aspects of scalation may be one likely candidate (Losos 2009). Among populations of the *cybotes* complex, variation in body

mass (stocky in highlands versus slender in lowlands) and characteristics of the claws (phalanx length, tip angle and curvature) were responsible for significant phenotype–macrohabitat correlation. Relative body mass is known to vary among ecomorphs (trunk-ground anoles being stocky and, at the other extreme, twig anoles being slender), but further study needs to be directed toward examining variation in claw morphology among ecomorphs. More importantly, future research should investigate whether these characteristics differ among closely related species that occupy different macrohabitats.

Zani (2000) examined clinging performance and foot morphology in an array of different lizards and found toe pad variation to be related to better clinging performance on smooth surfaces. In contrast, claw morphology was found to be related to clinging performance on rough substrates. In the *A. cybotes* species complex, higher elevation populations have higher claws, larger tip angles, and longer and more curved claws, suggesting that they have better clinging performance on rough substrates—which seems odd given the fact that habitat use shifts from primary occurrence on vertical tree trunks at low elevations to using the grounds, rocks, and horizontal logs at high elevations. However, claw morphology has not been studied in relation to other functional demands such as escape performance and requires further attention.

### Conclusions

We found evidence for deterministic evolution in the *A. cybotes* species complex across space and through time; the genetic and morphological diversification of populations occurs repeatedly along axes of diversification previously identified in the anole radiation, but different sets of characters are involved in population divergence than in speciation. Regarding the relative contribution of these axes, variation in macrohabitat exerted a greater effect than variation in structural microhabitat. Our observations of spatial convergence across the landscape and genetically independent habitat-associated morphological variation support the conclusion that phenotypic variation among populations of the *A. cybotes* species complex is adaptive. The scale of these patterns—morphological differences among populations being substantially less than among the ecomorph classes—probably reflects both the much smaller variation in habitat use among populations than among ecomorphs, as well as competitive constraints stemming from competition with other sympatric anole species precluding occupation of, and thus adaptation to, a larger range of structural habitats.

More generally, our results show both similarities and differences between microevolutionary and macroevolutionary diversification. At both levels, taxa appear to diversify in response to variation in the same broad environmental factors. Nonetheless, the exact details of how the diversification occurs vary

among phylogenetic levels. Extensive divergence in both morphology and genetics among populations of widespread species is common among anoles on all four islands of the Greater Antilles. The next step in addressing these questions involves looking at other species to see if their population divergence has occurred in similar ways.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

### Supplementary Information S11.

**Figure S1.** Significant canonical correlation of habitat use and morphology for second canonical roots (CRs;  $r = 0.8447$ ,  $P < 0.0001$ , Fisher's  $Z$  refined confidence interval estimation  $0.8191 < r > 0.8665$ ).

**Figure S2.** Logistic MAXENT model of species distribution for the *Anolis cybotes* species complex on Hispaniola (Dominican Republic outlined in black); black points—own sampling points and sampling points obtained from Glor et al. (2003), gray points—additional data points from specimens deposited in the Museum of Comparative Zoology, Harvard University. Red shades on map denote low occurrence probability; green shades denote high occurrence probability.

**Figure S3.** Significant canonical correlations between the ecomorph-environment canonical roots CR2–CR4.

**Figure S4.** Example for morphological characters whose variance is best explained by the environment.

**Table S1.** Localities, coordinates, GenBank accession numbers, museum voucher numbers, and list of recorded parameters of studied specimens of the *Anolis cybotes* species complex.

**Table S2.** Eigenvalues and explained variance for principal component analysis of variables obtained by flatbed scans of anole feet (residuals to SVL).

**Table S3.** Factor loadings for Principal Component Analysis of variables obtained by scanning anole feet (residuals to SVL).

**Table S4.** Eigenvalues and explained variance for Principal Component Analysis of morphological variables obtained from X-ray images of the skeleton (residuals to SVL).

**Table S5.** Factor loadings for Principal Component Analysis of morphological variables obtained by analyzing X-ray images of the skeleton (residuals to SVL).

**Table S6.** Eigenvalues and explained variance for principal component analysis of habitat use variables.

**Table S7.** Factor loadings for principal component analysis of habitat use variables.

**Table S8.** Forward-stepwise selection of the genetic principal components of the neighborhood matrix using analysis of covariance in *rda* (R: vegan).

**Table S9.** Eigenvalues and explained variance for principal component analysis of environmental variables (elevation, and 19 bioclimatic variables extracted from WORLDCLIM).

**Table S10.** Factor loadings for principal component analysis of environmental variables (elevation, and 19 bioclimatic variables extracted from WORLDCLIM).

**Table S11.** Results of occurrence-probability weighted canonical correlation analysis between ecological and morphological variable sets.

**Table S12.** Canonical weights for habitat use variable set. Marked are weights  $> 0.4$ .

**Table S13.** Canonical weights for morphology variable set. Marked are weights  $> 0.4$ .

**Table S14.** Results of occurrence-probability weighted multiple regression of data set pruned to cases with no missing values.

**Table S15.** Overall model results for each dependent variable, displaying the overall fit of all parameters in the model.

**Table S16.** Canonical correlation of “Ecomorph” data set with environment data set obtained from 2000 random points.

**Table S17.** Canonical weights for “Ecomorph” variable set.

**Table S18.** Canonical weights for environment variable set.

**Table S19.** Results of variance partitioning (R: vegan, *varpart*) using single morphological variables as dependent.