

Testing the island effect in adaptive radiation: rates and patterns of morphological diversification in Caribbean and mainland *Anolis* lizards

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Many of the classic examples of adaptive radiation, including Caribbean *Anolis* lizards, are found on islands. However, *Anolis* also exhibits substantial species richness and ecomorphological disparity on mainland Central and South America. We compared patterns and rates of morphological evolution to investigate whether, in fact, island *Anolis* are exceptionally diverse relative to their mainland counterparts. Quite the contrary, we found that rates and extent of diversification were comparable—*Anolis* adaptive radiation is not an island phenomenon. However, mainland and Caribbean anoles occupy different parts of morphological space; in independent colonizations of both island and mainland habitats, island anoles have evolved shorter limbs and better-developed toe pads. These patterns suggest that the two areas are on different evolutionary trajectories. The ecological causes of these differences are unknown, but may relate to differences in predation or competition among mainland and island communities.

Keywords: macroevolution; island biogeography; evolutionary rate; convergence; disparity; community evolution

1. INTRODUCTION

Islands are famous as natural laboratories of evolution. One common phenomenon of island evolution is adaptive radiation, in which a colonizing species diversifies to produce a wide array of species adapted to many different ecological niches (e.g. Carlquist 1974). Famous examples include Darwin's finches of the Galápagos, which exhibit a diversity of morphologies that usually characterize different families of birds (Burns *et al.* 2002), and the remarkably diverse cichlid fish of the African Rift Lakes (lakes being the counterpart of islands for aquatic taxa; Fryer & Iles 1972; Kornfield & Smith 2000).

The standard explanation for such evolutionary diversity is that colonizing species find themselves in an ecologically depauperate land with few competitors and predators and many available resources (Simpson 1953; Schluter 2000). The result is evolutionary divergence as descendant species evolve to specialize on different portions of the available resource spectrum. A corollary of this view, which is no doubt correct in many cases, is that mainland taxa, constrained by the abundance of other sympatric species, should evolve in a slower and more limited fashion (Schluter 1988; Webb *et al.* 2002). Yet few studies have compared the extent of evolutionary diversification of closely related taxa that live in mainland and island settings (but see Schluter 1988, 2000; Bromham & Woolfit 2004).

Lizards of the genus *Anolis* provide an ideal opportunity to examine such ideas. The *Anolis* clade is substantially more phenotypically diverse than related clades (Losos & Miles 2002), and the evolutionary diversity of Caribbean anoles, in particular, has been intensively studied (Losos *in press*). On each island of the Greater Antilles, lineages have diversified to produce a suite of species adapted to use different habitats; notably, the same set of habitat specialists—termed 'ecomorphs' (Williams 1972)—has evolved on each island (Williams 1983; Losos *et al.* 1998, 2006). Caribbean anoles have become a widely cited example of adaptive radiation that is now commonly portrayed in introductory and advanced textbooks.

Less appreciated, however, is the fact that anoles have diversified to a great extent in Central America and northern South America. Indeed, more species occur on the mainland than on the Caribbean (197 versus 154; Nicholson *et al.* 2005). Moreover, a preliminary study that included 16 mainland species indicated that the extent of ecomorphological variety was comparable in anoles of the two areas (Irschick *et al.* 1997).

Here, we assemble a much larger dataset to compare the evolutionary radiation of anoles in the two areas. We focus on the extent to which radiation on the mainland has occurred in similar ways to that on islands. Specifically, we ask the following questions.

- (i) Does the overall extent of variation differ in the two areas?
- (ii) Have mainland and island evolutionary lineages evolved to occupy different parts of morphological space?

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2008.0686> or via <http://journals.royalsociety.org>.

- (iii) Have homoplasy and consequent lack of phylogenetic signal, so prominent in the Greater Antilles due to convergent evolution of ecomorphs, also occurred extensively in the mainland, or has evolution been more conservative in a continental setting?
- (iv) Have rates of morphological evolution differed in the two areas?

(a) Background on anole phylogeny

When possible, our analyses are conducted within the context of current understanding of anole evolutionary relationships (Poe 2004; Nicholson *et al.* 2005). In particular, the anole phylogeny can be broken into three groups (figure 1). The basal split in the phylogeny separates a group of predominantly mainland anoles (henceforth referred to as M1) from the rest of the clade (monophyly of this mainland clade is suggested, but not corroborated; an alternative possibility is that the Greater Antillean clade arose from within this mainland clade; Poe 2004; Nicholson *et al.* 2005). Because out-groups to anoles occur in Central and South America, the mainland is probably the ancestral site of origin for anoles (Nicholson *et al.* 2005; for further discussion, see Losos *in press*).

The sister group to mainland clade M1 is a clade containing most anole species. The most recent common ancestor of this group clearly occurred in the Caribbean, probably as the result of a colonization event (Nicholson *et al.* 2005). Nested well within this ancestrally Caribbean clade is a large clade of mainland anoles (which we refer to as M2), presumably the result of a back-colonization from the Greater Antilles to the mainland (Nicholson *et al.* 2005; figure 1). We refer to the paraphyletic remainder of this clade, which is found throughout the Caribbean, as C. Within the M1 clade, oceanic islands have been colonized twice, once by *Anolis agassizi* on Malpelo Island in the Pacific Ocean and once by the *roquet* series of anoles in the southern Lesser Antilles. Within M2, oceanic islands also have been colonized twice, once by *Anolis townsendi* on Cocos Island in the Pacific Ocean and once by the sister taxa (based on morphological data) *Anolis concolor* and *Anolis pinchoti* on the Caribbean islands of San Andrés and Providencia. Although several anole species occur on near-shore continental islands from the mainland, most such islands share recent connections with the mainland; we therefore restrict our comparisons with species on oceanic islands, most of which have long independent histories (e.g. Glor *et al.* 2005).

2. MATERIAL AND METHODS

(a) Study system and collection of data

For use in phylogenetic comparative studies, 35 mainland and 57 Caribbean species were examined. We also measured an additional 59 species that have not been included in molecular phylogenetic studies, although we do know to which of the three major lineages they belong (Poe 2004); we used this larger dataset to perform non-phylogenetic comparisons of mainland and island taxa (see the electronic supplementary material for species lists). Mainland species included in a recent phylogenetic study of the clade (Nicholson *et al.* 2005) were chosen based on the availability in museum collections without additional *a priori* criteria;

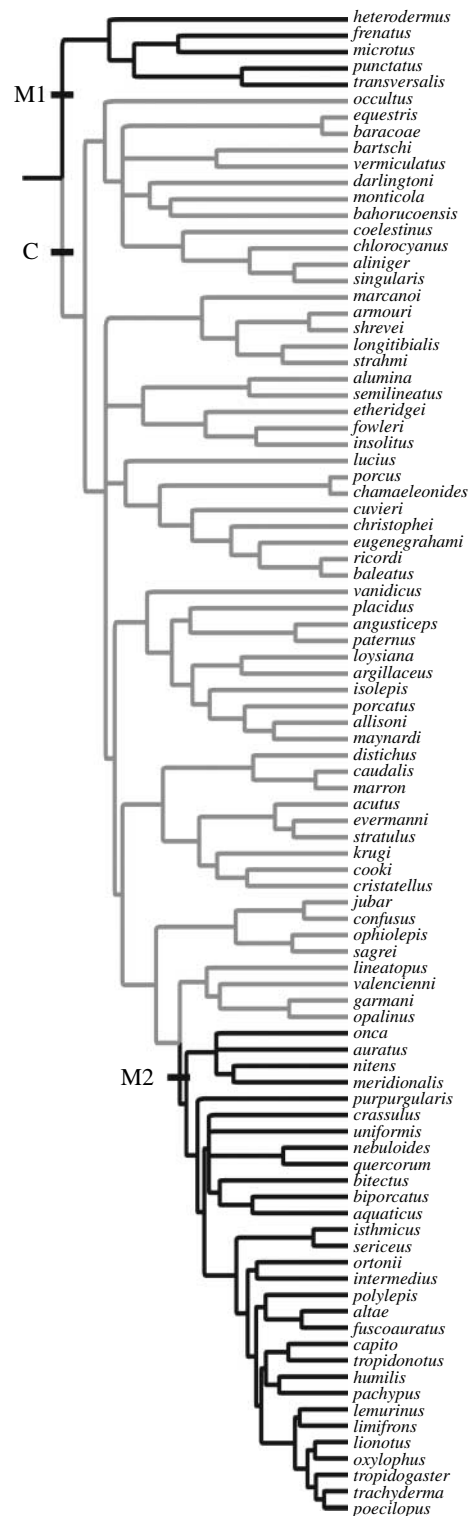


Figure 1. Phylogeny of *Anolis* depicting both mainland (black) and Caribbean (grey) radiations. M1 refers to a basal clade comprising northern South American and southern Central American species; C refers to the Caribbean anole radiation; M2 refers to a mainland radiation derived from within the Caribbean radiation and distributed throughout Central America and northern South America.

note that because few M1 species are included in Nicholson *et al.*'s (2005) study, that clade was represented by only five species in the phylogenetic analyses, but 27 species in the remaining analyses. Two sets of Caribbean species were compared. First, 15 species were randomly chosen by

selecting specimens from museum shelves without the consideration of ecomorph type. Subsequently, additional species were selected to supplement the species list with the criterion that each ecomorph was represented by at least one species on each island on which that ecomorph type occurs. We refer to these groups as the 'random' and 'full' Caribbean datasets. Except where noted below, statistical analyses for the two datasets were qualitatively identical and, consequently, are reported only for the full dataset.

As in the previous morphometric studies of anole ecomorphs (e.g. Losos *et al.* 1998; Beuttell & Losos 1999), only adult males were used for collection of the morphological data. Sample sizes for mainland species were 1–25 (mean = 7.4) and for Caribbean species 1–13 (mean = 4.1). For samples in which only a single individual was measured, the specimen measured was usually a paratype or holotype.

(i) Morphometric measurements

We measured a variety of morphological variables for which interspecific differentiation appears to have an adaptive basis; previous research has indicated that morphological differentiation in these characters is related to the use of different parts of the environment (Harmon *et al.* (2005) and references therein). Eighteen variables were measured, following Warheit *et al.* (1999) and Beuttell & Losos (1999). All measurements were taken with calipers except the number of lamellae, which was determined with the aid of a stereomicroscope. Variables were named according to their underlying skeletal elements, even though measurements were taken externally.

- *Snout–vent length (SVL)*. From the tip of the snout to the anterior margin of the cloaca.
- *Head length*. Distance from the occiput to the tip of the snout.
- *Head width*. Measured at the widest point of the head.
- *Head height*. Measured at the maximum depth of the head.
- *Humerus length*. Distance from the point at which the forelimb enters the body to the elbow.
- *Ulna length*. From the apex of the elbow to the centre of the wrist.
- *Metacarpus length*. From the ventral mid-point of the wrist to the base of finger IV.
- *Finger IV length*. From the tip of the claw on finger IV to the point of insertion in the hand.
- *Number of adhesive lamellae on finger IV (manus)*. Counted from the base of the digit to the end of the third phalanx.
- *Toe pad width (manus)*. Maximum width of adhesive pad on finger IV of the manus.
- *Pelvis width*. Body width at the pelvic girdle.
- *Pelvis height*. Body height at the pelvic girdle.
- *Femur length*. Distance from the insertion in the body wall to the knee.
- *Tibia length*. Distance from the knee to the centre of the ankle on the ventral surface.
- *Metatarsus length*. Distance from the ventral mid-point of the heel to the base of toe IV.
- *Toe IV length*. From the tip of the claw on toe IV to the insertion into the foot.
- *Number of adhesive lamellae on toe IV (pes)*. Counted from the base of the digit to the end of the fourth phalanx.
- *Toe pad width (pes)*. Maximum width of adhesive pad on toe IV of the pes.

(ii) Phylogenetic relationships of Anolis

The phylogeny used in this study is based on 1483 aligned positions of mitochondrial DNA (Nicholson *et al.* 2005; figure 1). Species that were not measured were excluded from the phylogeny.

(b) Testing for morphological differences among mainland and island species

First we calculated the mean value of each variable for each species. All variables were ln transformed to increase the homogeneity of variances. To remove the effect of body size, we calculated residuals by regressing each variable against SVL, using species means.

To calculate the position of species in a multivariate morphological space with reduced dimensionality, we conducted a principal components (PC) analysis on the correlation matrix of the size-corrected variables and the natural logarithm of SVL. PC scores from the first six PC axes, which explain 90.5 per cent of the variation in the dataset, were used for all analyses that follow.

We tested for differences among major groups of anoles in this morphological space using phylogenetic MANOVA. We compared the positions of three groups of lizards (Caribbean anoles and mainland clades M1 and M2; see below) by calculating Wilks' λ for differences in the position of the multivariate mean of these groups in the space defined by the first six PC axes. We then simulated 1000 random datasets on the tree under a multivariate Brownian motion model, using the evolutionary variance and covariance matrix estimated from independent contrasts (Revell *et al.* 2007). We compared the Wilks' λ statistic from the data to the null distribution generated from these simulations and calculated the probability value as the proportion of simulated statistics that were more extreme than the actual value. This analysis tests the hypothesis that the three groups are more different than would be expected from random differentiation given the phylogeny.

We also conducted a separate analysis on a larger morphological dataset, including 59 species not present in the phylogeny (total 151). We tested the difference among mainland- and island-dwelling anoles using MANOVA. To examine whether this difference (island–mainland) was distinct from the difference among the mainland M1 and M2 clades, we examined the canonical variate axis from the island–mainland MANOVA and performed an ANOVA on species scores along this axis, with M1 versus M2 as an effect. Further, to specifically examine how island and mainland anoles, and how M1 and M2 clades, differ in traits, we conducted an ANOVA on PC scores, first comparing mainland versus island species and then comparing M1 versus M2 species.

We then performed a discriminant function analysis (DFA) to assess how well mainland and island anoles could be classified based on morphology. In this study, we focused on the lineages within M1 and M2 that have colonized islands. We were specifically interested in whether these more recent island colonists exhibit island morphologies despite their mainland phylogenetic affinities.

(c) Testing for phylogenetic effect

We used both the *K*-statistic (Blomberg *et al.* 2003) and Mantel tests to investigate the relationship between morphological and phylogenetic similarities for each group of species. We calculated the *K*-statistic independently for each of the six PC axes, determining significance with 999 permutations (Blomberg *et al.* 2003), using code kindly provided by

S. Kembel. We then performed Mantel tests, which combine all six PC axes but are less powerful (L. Harmon & R. Glor 2008, unpublished manuscript). For each Mantel test, we first produced matrices representing the phylogenetic and morphological distances between all pairs of species. For the morphological matrix, we calculated the Euclidian distance between each pair of species in the space defined by the six first PCs. The phylogenetic distance matrix represents the patristic distance between each pair of species in the phylogeny from Nicholson *et al.* (2005). We then compared the phylogenetic and morphological distance matrices using a Mantel test with 9999 random permutations. In our dataset, mainland species were selected randomly with respect to habitat use, while Caribbean species in the full dataset were selected to represent a diversity of ecomorph categories. Because this might bias our statistical tests of the extent of convergence in this group, we also analysed the random set of 15 Caribbean species, in which species were selected without reference to their habitat use (electronic supplementary material). We carried out separate tests for all three datasets (mainland (including M1 + M2), full Caribbean and random Caribbean).

To examine whether the relationship between morphological and phylogenetic similarities differs between the Caribbean and mainland datasets, we used a randomization test. First, we randomly assigned all species in the dataset to either the Caribbean or the mainland, maintaining the empirical sample size for each. We then calculated the Mantel correlation (ρ) between phylogeny and morphology for mainland and island in each of these randomized datasets, and found the difference between the ρ -values for the mainland and island datasets. We compared the empirical difference in the Mantel ρ -statistic between these two groups with the distribution of differences from the randomized datasets. We conducted this analysis with both the full Caribbean and random datasets. In the latter case, for each permutation 15 randomly selected species were designated as Caribbean and the mainland sample size was maintained; the remaining species were discarded.

(d) Estimating phenotypic rates of evolution

We assessed whether anole lineages underwent different rates of morphological evolution in mainland versus Caribbean radiations. The *Anolis* phylogeny, pruned to the 92 Greater Antillean and mainland species for which morphological data were available, was divided, as previously detailed, into three groupings based on shared history and geography (M1, C and M2; figure 1). Rates of morphological evolution were calculated for the six PC axes of trait variation assuming a Brownian motion model of evolution (O'Meara *et al.* 2006). First, using a maximum-likelihood estimator, a single optimal rate was calculated for all taxa in the tree. Then, using the same technique, rates were calculated separately for each geographical subset (M1, C and M2). To test whether evolutionary rates differed substantially among subsets, we compared the likelihood of having separate rates of evolution for each group of anoles to the likelihood of having a single rate of evolution across all groups. These comparisons were made using both likelihood-ratio tests and an AIC_c model selection approach (the former consistently yielded more conservative results). Tests were performed on all pairwise combinations of the three groups of anoles to determine which geographical radiations evolved at substantially different rates from others. All analyses were executed using the software program BROWNIE (O'Meara *et al.* 2006). Rate comparisons were performed using the 'censored' rates test in BROWNIE,

and parametric bootstrapping (1000 replicates) was employed in the likelihood-ratio tests to account for bias introduced by the small sample size of taxon group M1. One M2 species, *Anolis onca*, is morphologically unusual (this species has 'lost' its toe pads; Nicholson *et al.* 2006) and has a large effect on the estimation of rates; for this reason, we recalculated evolutionary rates excluding this species.

3. RESULTS

(a) Testing for morphological differences among mainland and island species

In the PC analysis, the first six PC axes explained 90.5 per cent of the variation among species. The eigenvalues and loadings for these axes are reported in table 1.

In the phylogenetic comparison, the three groups of lizards (Caribbean, mainland M1 and mainland M2) differ significantly in their position in morphological space (figure 2a, Wilks' $\lambda=0.59$, phylogenetic simulation $p<0.001$). Significant differences also exist between the mainland and Caribbean taxa (Wilks' $\lambda=0.59$, phylogenetic simulation $p<0.001$) and the two mainland clades (Wilks' $\lambda=0.33$, phylogenetic simulation $p<0.001$).

For the larger non-phylogenetic dataset, we find similar results. From the MANOVA, we find a significant mainland-island effect (Wilks' $\lambda=0.64$, $F_{6,144}=13.44$, $p<0.001$). Along the single canonical variate axis derived from the MANOVA, the two mainland clades did not differ ($F_{1,91}=0.42$, $p=0.519$). Performing an ANOVA on each PC axis, we determined that mainland and island anoles were significantly different on PC I, PC II and PC V, while mainland clades M1 and M2 differed significantly on PC I, PC III and PC IV (table 2).

The DFA distinguished mainland and island anoles based on the morphological data in PC I–PC VI (Wilks' $\lambda=0.65$, $p<0.001$). Using a conservative cross-validation approach, the discriminant function predicted island and mainland affinities with 69.7 and 77.6 per cent accuracy, respectively. Of the four M1 taxa that have colonized islands (three members of the *roquet* series from the southern Lesser Antilles, as well as *A. agassizi*), two (*Anolis aeneus* and *Anolis blanquillanus*) were classified as island anoles by this DFA. The remaining two (*A. agassizi* and *Anolis richardi*) were classified as mainland anoles, but *A. agassizi* was intermediate in its discriminant function score, denoting similarity to island species (figure 3). Of the three M2 taxa that have colonized islands, one (*A. concolor*) was classified as an island anole, whereas the remaining two (*A. pinchoti* and *A. townsendi*) were classified as mainland anoles, although both had intermediate (island-like) scores within the M2 anoles (figure 3).

(b) Testing for phylogenetic effect

Values of Blomberg's K -statistic are similar for the full Caribbean and mainland groups. The expected value of the K -statistic is one under a Brownian motion model of phenotypic evolution, and both Caribbean and mainland anoles exhibit lower values than the neutral expectation; table 3. Using Mantel tests, phylogenetic and morphological distances are positively related for all groups of taxa, although this correlation is significant only for the full Caribbean dataset (mainland: $r=0.24$, $p=0.13$; full Caribbean dataset: $r=0.21$, $p<0.001$; random Caribbean subset: $r=0.17$, $p=0.07$). The strength of the

Table 1. Loadings, eigenvalues and variance explained from a principal components (PC) analysis on body size (SVL) and 17 additional morphological variables (all ln transformed and regressed to remove the effects of size).

variable	PC I	PC II	PC III	PC IV	PC V	PC VI
snout-vent length	0.00	0.00	0.00	1.00	0.00	0.00
head length	-0.26	0.57	0.45	0.00	0.51	-0.03
head width	0.36	0.52	0.67	0.00	0.08	-0.19
head height	0.27	0.41	0.70	0.00	0.36	0.07
humerus length	0.86	0.07	-0.14	0.00	0.00	-0.39
ulna length	0.90	0.11	-0.03	0.00	0.05	-0.31
metacarpal IV length	0.75	-0.06	0.12	0.00	-0.29	-0.30
finger IV length	0.87	0.09	-0.11	0.00	-0.03	0.11
femur length	0.89	-0.07	-0.30	0.00	0.20	0.12
tibia length	0.92	-0.09	-0.26	0.00	0.16	0.08
metatarsal IV length	0.91	-0.09	-0.19	0.00	0.16	0.13
toe IV length	0.88	0.03	-0.22	0.00	0.16	0.28
lamella number, finger IV	-0.17	0.86	-0.44	0.00	-0.08	-0.02
lamella width, finger IV	-0.10	0.94	-0.20	0.00	-0.11	-0.02
lamella number, toe IV	-0.12	0.83	-0.49	0.00	-0.02	0.07
lamella width, toe IV	-0.09	0.94	-0.23	0.00	-0.04	0.00
pelvic girdle width	0.52	0.34	0.46	0.00	-0.49	0.10
pelvic girdle height	0.53	0.20	0.56	0.00	-0.35	0.35
eigenvalue	7.0	4.2	2.5	1.0	1.0	0.6
per cent	38.9	23.1	13.8	5.6	5.5	3.6

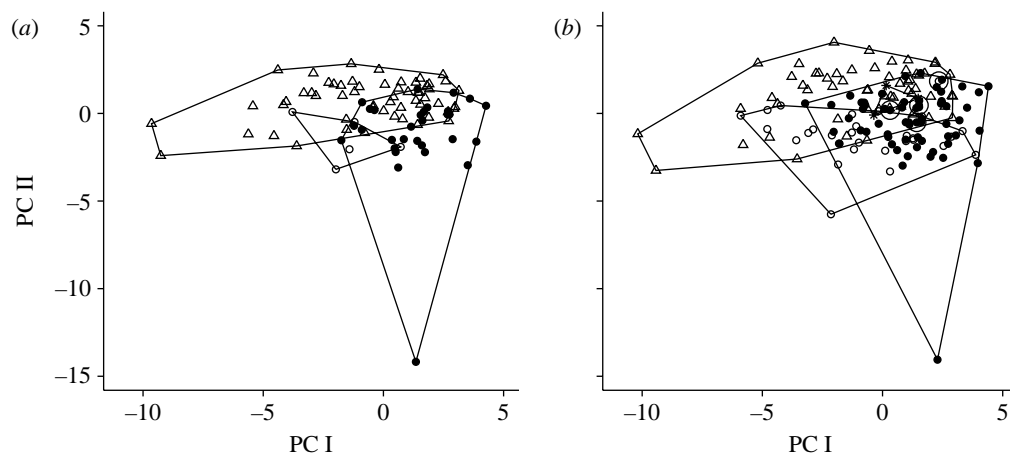


Figure 2. Position of species in morphological space. Statistical analyses were based on six PC axes, but for illustrative purposes, the first two PC axes are shown. The outlier with a low score on PC II is *A. onca*, which differs from other anoles in its absence of expanded toe pads. (a) Species included in the phylogeny (open circles, mainland M1; filled circles, mainland M2; triangles, Caribbean) and (b) expanded dataset including many species not represented in the phylogeny. The *roquet* species group, which colonized the southern Lesser Antilles from the mainland M1 clade, is indicated by asterisks, and *A. agassizi*, an M1 species that colonized the Pacific island of Malpelo, is indicated by an open circle within a circle. Each island colonist from the M2 lineage is indicated by a filled circle within a circle.

correlation is slightly greater among mainland than Caribbean species, but this difference was not statistically significant (randomization test, full dataset, $p=0.53$; random Caribbean subset, $p=0.30$).

(c) Estimating phenotypic rates of evolution

Caribbean anoles (C) experienced a higher rate of evolution than mainland radiation M1 for PC I, which is strongly correlated with limb and digit lengths (table 4). For PC II, which is loaded most strongly by pad width and lamella number on both fingers and toes, M2 exhibits a higher rate of evolution than either C or M1, a result that is less well supported, but nonetheless robust even when the unique taxon *A. onca* is excluded from the analysis (table S1). PC III, which exhibits a contrast between head and girdle

Table 2. Results of ANOVA on PC I-PC VI. (Two sets of analyses were conducted, one on all species using mainland or island affinity as an effect and the other only on mainland taxa using clade membership (M1 versus M2) as an effect. Significant results are in italics.)

	island-mainland		mainland-mainland	
	$F_{1,149}$	p	$F_{1,91}$	p
PC I	8.47	<i>0.004</i>	15.05	<i>< 0.001</i>
PC II	44.19	<i>< 0.001</i>	1.34	0.250
PC III	0.43	0.514	13.74	<i>< 0.001</i>
PC IV	0.92	0.340	60.21	<i>< 0.001</i>
PC V	9.81	<i>0.002</i>	0.39	0.532
PC VI	0.85	0.358	1.42	0.237

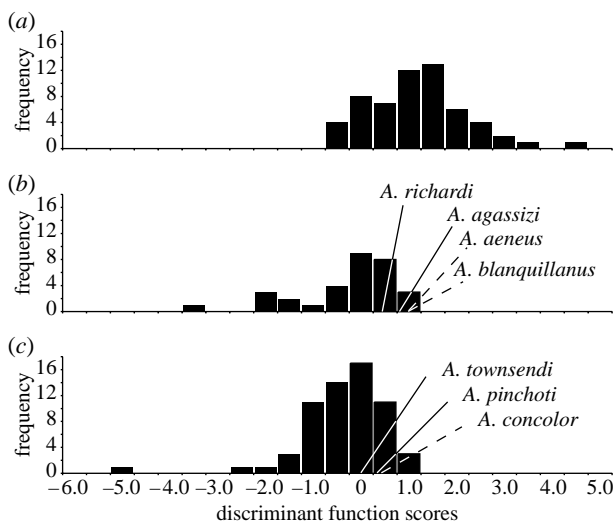


Figure 3. Results of a DFA classifying all measured species as 'mainland' versus 'island'. Distributions of discriminant function scores for the (a) Caribbean, (b) mainland M1 and (c) mainland M2 lineages of anoles are shown. Dashed lines indicate island-dwelling species from mainland lineages that were correctly classified as 'island'. Solid lines indicate island-dwelling species from mainland lineages that were misclassified as 'mainland'.

measurements versus finger and toe measurements, experienced a higher rate of evolution in the M2 clade, although this difference among groups appears to be entirely due to the presence of *A. onca* (table S1). PC IV, representing body size (using SVL as a proxy), and PC V (head length versus pelvis width) were not found to evolve at significantly different rates among groups. PC VI, which showed a contrast between pelvis height and forelimb measurements, was found to evolve more rapidly in C and M2 than in the M1 clade. In summary, C exhibits a higher rate than M1 on two PC axes, M2 is higher than M1 on two PC axes and M2 is greater than C on two PC axes.

4. DISCUSSION

(a) Ecological opportunity and adaptive radiation

Clearly, adaptive radiation is not an island phenomenon in *Anolis*. Our analyses reveal that mainland anoles exhibit many of the same features that are seen in the Caribbean anole radiation. Not only is the extent of ecomorphological divergence substantial in the mainland (M1 and M2 together are roughly as disparate as C; figure 2), but also the rates of evolutionary change in the mainland M2 clade have actually been as great as or greater than that in the Caribbean (table 4; see also Schluter 2000, ch. 7). Moreover, Greater Antillean anoles are renowned for the extent to which morphological similarity is uncoupled from phylogenetic relatedness (the result of repeated evolution of the same ecomorphs across islands; but see Poe 2005), but examination of the relationship between phylogenetic and morphological similarities indicates that homoplasy, evidenced by a relatively weak correlation between morphology and phylogeny, is just as prevalent in the mainland.

The classic explanation for adaptive radiation on islands is that an ancestral species, finding itself in a location with abundant ecological opportunity, diversifies widely. The recognition that mainland anoles are similarly diverse

presents a wrinkle, but one could always assume that, for some reason, just as much ecological opportunity was present in mainland locations as on the islands. Certainly it is true that even though the non-anole lizard fauna in the mainland neotropics is much more diverse than that on the Caribbean islands, nonetheless, very few mainland lizard species are similar to anoles in being arboreal, insectivorous and diurnal (Vitt & Zani 1996; Vitt *et al.* 1999; Duellman 2005). So, perhaps by good fortune, anoles have had similar levels of ecological opportunity available to them in both locations. An alternative possibility is that anole evolutionary diversification has not been prompted by pre-existing ecological opportunity but, rather, by some other factor. One possibility is that the evolution of the subdigital toe pad has served as a key innovation, providing anoles with the opportunity to interact with the environment in ways not possible by other taxa, and thus facilitating their evolutionary radiation (Losos *in press*). Distinguishing between these two possibilities will not be easy.

(b) Different patterns of anole radiation on islands and mainland

Although both mainland and Caribbean anoles have radiated extensively, several important differences exist between the patterns of radiation in these settings. First, Caribbean and mainland anoles have radiated over only partially overlapping portions of morphological space (figure 2; see also Velasco & Herrel 2007). A primary difference is in the toe pads, which correlate with PC II in our analyses; as a generality, many Caribbean anoles have better-developed toe pads than do any mainland species, a finding in agreement with the previous studies (Macrini *et al.* 2003; Velasco & Herrel 2007). Caribbean anoles have markedly more lamellae and wider toe pads than the early branching South American M1 clade (figure 2a,b). The M2 clade, however, not only contains members with fairly well-developed toe pads, as in the Caribbean, but also many taxa with thin toes and low lamella counts (a trend that is taken to the extreme in *A. onca*, which has lost expanded toe pad scales entirely). Most likely, the radiation into both of these areas of morphospace may account for the higher rate of toe pad evolution in M2 than in either other group.

By contrast, Caribbean anoles exhibit substantially greater variation in relative limb length (PC I) than do mainland anoles. This difference primarily results from some of the extremely short-legged twig anoles such as *Anolis occultus* and *Anolis sheplani*. Although some mainland anoles appear on morphological and ecological grounds to be twig anoles, such as *Anolis pentaprin* and members of the *Phenacosaurus* clade (Beuttell & Losos 1999; Losos *in press*), these species do not show the extreme reduction in limb length seen in some, but not all, Caribbean twig anoles (highly negative values for PC I in figure 2).

The morphology of members of the M2 clade is particularly notable given that this clade arose from within the Caribbean anoles; despite this heritage, in many traits, M2 shows more marked affinities with the other mainland clade (M1) than with its Caribbean progenitors. Both mainland groups contain many species with more poorly developed toe pads and longer legs than any Caribbean species, and neither achieve the maximal level of toe pad development seen in the Caribbean (figure 2b). Similarly, but in the reverse direction, mainland-island transitions have occurred twice within M1 (Poe *et al.* 2007), once in

Table 3. Phylogenetic signal (Blomberg's K -statistic) in Caribbean and mainland anoles, as well as a random subset of Caribbean anoles.

	full Caribbean		mainland		random Caribbean	
	K	p	K	p	K	p
PC I	0.53	0.001	0.47	0.040	0.58	0.170
PC II	0.45	0.004	0.39	0.087	0.96	0.270
PC III	0.34	0.001	0.40	0.013	0.71	0.230
PC IV	0.57	0.001	0.30	0.001	1.00	0.060
PC V	0.32	0.001	0.40	0.061	0.44	0.330
PC VI	0.43	0.002	0.38	0.470	1.17	0.080

Table 4. Comparison of rates of morphological evolution using PC axes. ('Single rate' is the best-fit phenotypic rate for the entire phylogeny. Subset rates inferred to be greater than the rates in at least one other geographical subset by AIC_c tests are in bold; those inferred to be significantly greater by likelihood-ratio tests appear in italics. Substantial rate differences are described in the far right column.)

character	% var. explained	M1 rate	C rate	M2 rate	single rate	comparisons
PC I	38.9	0.027	0.096	0.085	0.088	C > M1 ^a
PC II	23.1	0.020	0.021	0.135	0.058	M2 > M1 ^a ; M2 > C ^{b,c}
PC III	13.8	0.027	0.024	0.079	0.042	M2 > C ^{b,d}
PC IV	5.6	0.005	0.014	0.011	0.012	
PC V	5.5	0.024	0.014	0.018	0.016	
PC VI	3.6	0.001	0.013	0.013	0.012	C > M1 ^{a,d} ; M2 > M1 ^{a,d}

^a Moderate AIC_c support for multiple rates among group pairs.

^b Strong AIC_c support for multiple rates.

^c Support of multiple rates with $p < 0.001$.

^d That likelihood-ratio tests support multiple rates with $p < 0.05$.

A. agassizi (open circle contained within a circle in figure 2b), found on Malpelo Island, and once in the *roquet* species group of the southern Lesser Antilles (asterisks in figure 2b). In both cases, these species, although members of the M1 clade, have evolved towards a region more characteristic of Caribbean species (figure 3). Several members of M2 have also recolonized islands; all of these species (indicated by filled circles contained within a circle in figure 2b) also lie within the morphological space of Caribbean species, although our dataset does not allow the estimation of whether that represents a shift from their ancestral position, given that overlap exists between Caribbean and M2 species (figures 2b and 3). It appears, in summary, that mainland and Caribbean anoles occupy only partially overlapping space. Island species have evolved away from the morphologies of their mainland ancestors at least thrice (the Caribbean radiation, the *roquet* group, and *A. agassizi*; possibly more depending on the ancestral condition for Caribbean M2 species; figure 2b), and recolonization of the mainland by M2 was accompanied by a shift back towards the ancestral mainland portion of morphological space.

We note that the evolutionary changes that distinguish mainland and island anoles are distinct from differences among mainland subclades. Whereas mainland and island taxa differ in PC I, PC II and PC V, which largely represent limb length and toe pad characteristics, mainland clades M1 and M2 differ in a different suite of characters (PC I, PC III and PC IV; table 2). Therefore, although mainland clades M1 and M2 have evolved to resemble each other in many traits, these two lineages have exhibited divergent patterns of diversification in many others.

Despite the fact that mainland and island anoles occupy demonstrably different regions of morphological space, there is currently no clear evidence for consistent mainland–island differences in the rates of phenotypic evolution. For limb length traits that were the most variable traits measured (PC I in table 4), mainland M1 anoles show relatively low rates of evolution, but both Caribbean (C) and M2 mainland anoles exhibit high rates. A similar finding emerges from PC VI, which represents a contrast between pelvis height and forelimb length. These results indicate that the tempo of phenotypic evolution can be similar in both mainland and island settings, although the pattern of evolution is clearly different. The slower rates of evolution among M1 taxa remain more challenging to explain. If ecology is indeed a determinant of evolutionary rates, the observed difference in rates may indicate a difference in the ecological conditions of M1 diversification.

Why mainland and Caribbean anoles have radiated in different parts of morphological space is not clear. Previous studies have shown that the relationship between habitat use and morphology is fundamentally different between the two groups—anoles using the same habitat have evolved different morphological features in the two areas (Irschick et al. 1997; Macrini et al. 2003; Velasco & Herrel 2007).

The similarity in morphological diversity between the two mainland clades compared with the Caribbean radiation suggests that it is the environmental differences between the island and mainland that have driven corresponding morphological differences. Andrews (1979) suggested that a fundamental difference exists between mainland and Caribbean anole communities: in the

Caribbean, populations are food limited and regulated by intraspecific interactions, whereas on the mainland, populations are regulated by predators (see review in Losos *in press*). To the extent that this hypothesis is correct (keeping in mind that it is based on studies on relatively few species), then ecomorphological differences may reflect the different selective pressures facing mainland and island anoles: on the mainland, anoles may be selected to use the habitat in ways that avoid drawing the attention of predators and maximize the ability to escape them, whereas on the islands, selective factors related to competing with conspecifics for food and mating opportunities may be paramount (Irschick *et al.* 1997; Macrini *et al.* 2003).

An alternative possibility is that mainland anoles have been forced to use different habitats or behave differently as a result of interspecific competition. Many types of insectivores are much more diverse on the mainland, including non-anole lizards, birds, mammals and frogs. Although at first glance, the habitat use of anoles on the islands and the mainland is comparable (Castro-Herrera 1988; Irschick *et al.* 1997; Velasco & Herrel 2007), the presence of other insectivores, most likely birds, may have caused anoles to use the environment in different ways, and thus evolve different morphological features (for discussion, see Lister 1976*a,b*; Wright 1981; Moermond 1983; Waide & Reagan 1983; Wright *et al.* 1984). Further work is required to better understand the functional basis for the different ecomorphological relationships between mainland and island anoles, as well as the ecological selective forces driving these differences.

A starting point for further exploring the patterns we have described will be to better sample mainland anole faunas. Partly because they are rare, and partly because they have historically received little attention, many groups of mainland anoles are under-represented in museum collections and in phylogenetic analyses. This is particularly true not only for the M1 anoles (*Phenacosaurus* and *Dactyloa* clades), but also for many M2 anoles. Our non-phylogenetic analyses, which include many of these taxa, suggest that addition of more mainland anoles is unlikely to alter our conclusions about island-mainland differences. However, for the phylogenetic analyses, which employ a smaller dataset, it is possible that the addition of more M1 species will change our understanding of *Anolis* evolutionary rates. Currently, these analyses include only five of roughly 60 M1 species, and do not include taxa with poorly developed toe pads—an omission that may negatively bias rate estimates. The low estimates for M1 should accordingly be interpreted with caution. Increased attention to mainland anole systematics and ecology will continue to provide fruitful comparison to the well-known patterns observed in the Caribbean.

5. CONCLUSIONS

Adaptive radiation in *Anolis* lizards is much more complicated than traditionally recognized. Not just a phenomenon of island colonization and diversification, anole radiation has occurred independently in both the mainland and the Greater Antilles, producing a clade that is exceptionally disparate compared with other related lizard clades (Losos & Miles 2002). However, patterns of radiation differ in the two areas: repeated colonizations of Caribbean islands have led to consistent

morphological shifts (i.e. evolutionary diversification in areas of morphological space characterized by shorter limbs and better-developed toe pads) that are reversed in a back-colonization of the mainland. Rates of character evolution show yet another pattern, in which the most basal clade of mainland anoles exhibits consistently low rates, while Caribbean anoles and the mainland colonists exhibit higher rates. At present, we have more questions than answers. Detailed study of mainland anole evolution could present many new insights into the patterns and processes of adaptive radiation.

We thank the following institutions for allowing us to examine specimens in their care: Museum of Comparative Zoology, Harvard University; National Museum of Natural History, Smithsonian Institution; Natural History Museum and Biodiversity Center, University of Kansas; Department of Biology, University of Texas at Arlington; Florida Museum of Natural History; Sam Noble Oklahoma Museum of Natural History; Museu Paraense Emílio Goeldi; Museu de Zoologia da Universidade de São Paulo. G.P. was supported by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and a Short-Term Visitor Award by the Smithsonian Institution, D.L.M. was supported by an NSF GRFP fellowship and L.J.H. was supported by a UBC Biodiversity Centre post-doctoral fellowship. S. Kembel provided R code for the analysis of phylogenetic signal. The manuscript benefited from discussion with R. B. Langerhans and the comments of two anonymous reviewers.

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