

Intraspecific variation in *Anolis sagrei* mirrors the adaptive radiation of Greater Antillean anoles

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Anolis lizards of the Greater Antilles represent one of the classic examples of vertebrate adaptive radiation. The same morphological types ('ecomorphs') have evolved repeatedly in response to similar ecological pressures on different islands. We tested whether patterns of within species diversification were congruent with between species patterns and the processes leading to the adaptive radiation of Greater Antillean anoles by measuring variation in performance-related morphological characters in the brown anole, *Anolis sagrei*. We measured morphological and genetic variation in two different habitat types on each of five islands in the Bahamas. We estimated population structure and rates of gene flow within and among islands using eight microsatellite markers. Intraspecific variation in performance-related morphological characters was similar to the pattern of interspecific variation that characterizes the adaptive radiation of this group in the Greater Antilles. For example, limb length was correlated with perch diameter within *A. sagrei* as has also been shown among species of anole. Morphological divergence in traits has occurred despite relatively high levels of gene flow both within and among islands. These results are discussed in the context of the divergence-with-gene-flow model of speciation. The results provide important intraspecific evidence that the diversification of anoles has been shaped by natural selection and show how ecologically-based selection pressures explain diversification at both the population and species levels. © 2007 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2007, 90, 189–199.

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INTRODUCTION

Despite controversy concerning the mechanisms of evolutionary change below and above the species levels, few studies have attempted to relate patterns of intraspecific and interspecific differentiation, and to explore how concordance in such patterns can be informative in understanding the nature of diversification (Emerson & Arnold, 1989; Emerson, 1991; Moreno, Barbosa & Carrascal, 1997). Here, we attempt to show that the processes that drive diversification within populations are the same mechanisms that may eventually lead to morphological divergence among species. We examine the influence of gene flow and

natural selection on population level diversification. In particular, we examine the extent to which natural selection can drive divergence in populations that are connected by gene flow. Although controversial, because such a process requires selection to overcome the homogenizing effects of recombination (Felsenstein, 1981), a growing number of empirical (Pashley, 1988; Carroll, Dingle & Klassen, 1997; Lu & Bernatchez, 1999; Rundle *et al.*, 2000; Smith, Schneider & Holder, 2001; Nosil, Crespi & Sandoval, 2002; Ogden & Thorpe, 2002; Coyne & Orr, 2004) and theoretical (Doebeli & Dieckmann, 2003; Gavrillets, Li & Vose, 2000; Gavrillets, 2004) studies suggest that divergence by natural selection is possible, and perhaps probable, even in the face of moderate levels of gene flow (Endler, 1977).

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Adaptive radiations are useful systems in which to investigate and contrast the mechanisms that drive intra- and interspecific diversification (Schluter, 2000). An adaptive radiation occurs when ecologically-based selection pressures cause rapid speciation within a group (Grant, 1968; Fryer, 1996; Givnish, 1998; Albertson *et al.*, 1999; Barrier *et al.*, 1999; Barrier, Robichaux & Purugganan, 2001). A spectacular example of vertebrate adaptive radiations is found in *Anolis* lizards on islands in the Caribbean, where more than 140 species have evolved from a common ancestor (Jackman *et al.*, 1999). These species are often characterized by divergence in body size and limb dimensions that are well correlated with habitat use (Losos, 1990a; Irschick *et al.*, 1997; Losos *et al.*, 2001). Different species can be grouped into morphological classes, termed 'ecomorphs' (Williams, 1972, 1983), which are named for the distinct habitats in which they tend to occur (e.g. grass bush, trunk ground, trunk, trunk crown, twig, crown). Abundant evidence from previous studies suggests that competition (Schoener, 1975; Lister, 1976; Losos, Irschick & Schoener, 1994) and natural selection on locomotor performance (Losos, 1990b; Losos, Warheit & Schoener, 1997) may have driven the diversification of anoles. For example, species that occupy habitats characterized by broad perch diameters (e.g. tree trunks) tend to have relatively longer limbs because long limbs increase maximum sprint speed (Losos, 1990a). By contrast, species that perch on narrower surfaces (e.g. twigs) tend to have relatively shorter limbs (Losos & Sinervo, 1989) owing to the enhanced agility of short limbs on narrow perches. These correlations between morphology and habitat use are considered to be adaptive and are thought to have played an influential role in the adaptive radiation of these lizards (Losos *et al.*, 2001).

Based on phylogenetic evidence, similar ecomorphs appear to have arisen repeatedly on different islands (Losos *et al.*, 1997, 1998), suggesting that parallel patterns of selection, and not multiple colonization events, are primarily responsible for the distribution of ecomorphs on islands (Jackman *et al.*, 1999). The repeated evolution of ecomorphs on different islands indicates the importance of ecology in driving diversification in this group; the inference being that ecologically-based natural selection creates predictable and repeatable patterns of morphological variation.

In a previous study, Losos *et al.* (1994) predicted that patterns of morphological variation within *Anolis* species would parallel the correlations of morphology and habitat use seen among species. They measured performance-related morphology (e.g. limb length and body size) and tested for correlations with habitat use on different islands in the Bahamas. Losos *et al.* (1994) concluded that morphological divergence had

occurred as a result of shifts in habitat use on different islands; however, the degree of differentiation was less than that predicted based on the observed differences in habitat. The authors suggested that the timing of colonization of the islands in the Pliocene had provided sufficient time for diversification and that some mechanism was constraining adaptive evolution of the lizards. One possibility considered by the authors was that the homogenizing influence of gene flow (Slatkin, 1985; Futuyma, 1987) may have slowed the evolutionary process. Losos *et al.* (1994) did not present data on gene flow among Bahamian islands, but predicted that levels of gene flow would be very low and that, whatever the rates of gene flow, they were likely insufficient to have prevented divergence among lizard populations. Here, we present a formal test of their hypothesis.

There were two main objectives in this study: (1) to examine whether intraspecific morphological divergence in anoles is similar to the interspecific divergence among ecomorphs (Losos *et al.*, 1994) and (2) to determine whether gene flow between islands may constrain this divergence. The prediction that intra- and interspecific patterns of morphological divergence should be concordant is based on the assumption that species divergence is ultimately the result of local natural selection on habitat use (Fig. 1). We predict that if the processes leading to intraspecific divergence are the same processes leading to interspecific divergence, then intraspecific variation in morphological traits should mirror interspecific differences in habitat use (Losos *et al.*, 1997). Furthermore, if gene flow is a con-

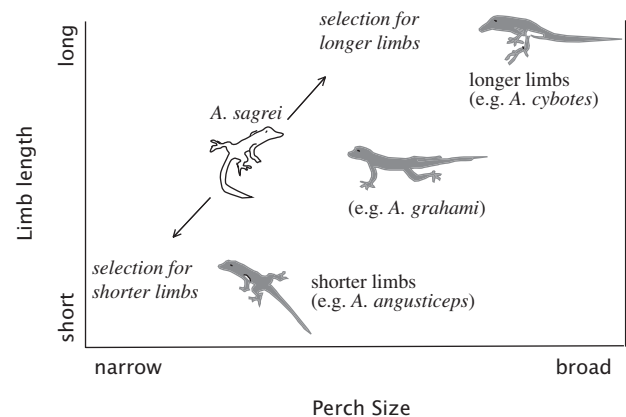


Figure 1. If natural selection drives the adaptive radiation of anoles, then selection on morphology that is related to habitat use in *Anolis sagrei* should drive morphological changes that correspond to differences observed among ecomorphs. Thus, we predicted that patterns of morphological variation within *A. sagrei* should be parallel to the variation among ecomorphs. Shaded lizards depict species level variation in ecomorphs compared to *A. sagrei* (left).

straining force in this system, then we predict that rates of gene flow among islands should slow adaptive divergence and the formation of genetic structure among islands.

MATERIAL AND METHODS

STUDY ORGANISM, FIELD WORK, AND GENERAL METHODS

The study took place on five islands on the Great Bahama bank (Fig. 2) during June to July 2002 and May to June 2004. During 2002, we worked sites on: Cat Island (24°27'N, 75°30.5'W), Andros (24°25'N, 77°57.5'W), Eleuthera (25°9'N, 76°13.5'W), Great Exuma (23°31'N, 75°49.5'W), and South Bimini (25°43'N, 79°15.5'W). During 2004, we focused exclusively on the Great Exuma population. The brown anole, *Anolis sagrei*, is the most common anole in the Bahamas. It is a member of the trunk-ground ecomorph, but like all ecomorphs, may be found in a variety of habitat types (Williams, 1983; Losos, 1990b). It is a short-lived lizard that matures and dies in 1–3 years (Schoener & Schoener, 1978, 1982).

On each island, during 2002, we sampled at least ten lizards from each of three to five locations along a linear transect (Fig. 2) whose end points were approximately 20 km apart (except on South Bimini where the island's small size limited the length of our transect to ~6 km). We captured 45–57 lizards from

each island (mean = 51) and recorded sex, mass, and snout–vent length (SVL) for all individuals (213 males and 38 females in total). Analyses of data from 2002 indicated a positive trend in correlations between body size measures (e.g. mass and SVL) and habitat use, but these correlations were weak (see Results). To further investigate the relationship between habitat use and body size traits, we repeated our measures of morphology and habitat use during 2004, focusing on a single population (Georgetown, Great Exuma). We captured 289 males from Great Exuma and recorded perch diameter as in 2002. We also measured perch height during 2004. In both years, we measured hind-limb and forelimb lengths from the point of insertion into the abdomen to the femoral-tibial and humero-radio-ulnar joints. All of these measurements were made blind with respect to habitat use. These traits have previously been linked to habitat use and are important for performance (Losos, 1990a; Irschick & Losos, 1998). We removed a 2-mm piece of tissue from the tip of the tail as a source of DNA for molecular analyses (see below). All lizards were temporarily marked with a small spot of white paint on their hind leg to prevent recapture of the same individuals.

We recorded the perch diameter of each lizard at its location just prior to capture. Lizards that were moving when first sighted, or lizards perched on the ends of leaves were not scored for perch diameter (*sensu* Rand, 1964; 1967; Schoener, 1975). Perch diameter was estimated by visual comparison against a small

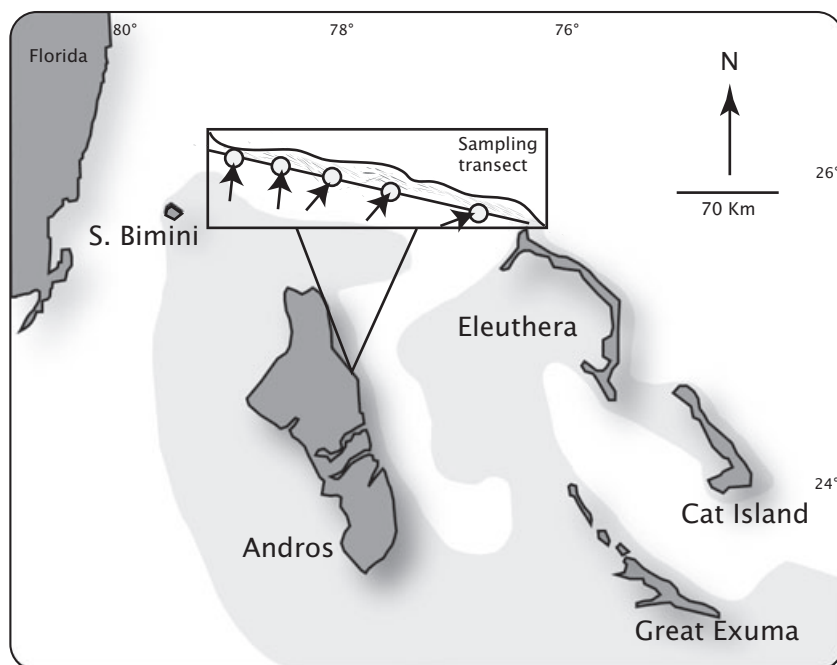


Figure 2. Map of the study islands in the Bahamas, including a magnified view of one study transect. Arrows indicate collecting sites along the transect. The lightly shaded area indicates the Great Bahama Bank.

ruler or with dial calipers. Perch height (in 2004 only) was estimated with a tape measure. For each island, we grouped habitats with perch diameters greater than 150 mm into a categorical variable called 'mature' habitat. Habitats with perch diameters less than 150 mm were grouped into a categorical variable called 'scrub' habitat. Both habitat types occur together on all islands and form a mosaic of overlapping broad and narrow perches.

INTERSPECIFIC MORPHOLOGICAL VARIATION

Data on interspecific morphology and habitat use were taken from Losos (1990a), except for perch height and diameter data for *Anolis occultus*, which were provided by J. Losos directly. To compare variation among species with the intraspecific data that we collected in the field, we regressed the mean values of hind and forelimb lengths against SVL for 13 species of *Anolis* given in Losos (1990a; table 1). We excluded the two giant species of anole in Losos (1990a; table 1) (*Anolis cuvieri* and *Anolis garmani*) because their SVL were more than two-fold greater than the mean value for the rest of the species in the comparison and were outliers in our analysis.

STATISTICAL ANALYSIS

Residual analyses have recently been criticized as being inappropriate for use in analyses of variance (Darlington & Smulders, 2001; Garcia-Berthou, 2001). However, for our work to be comparable to past studies (Losos, 1990a), differences in residual body proportions in mature and scrub habitats are presented. To more appropriately control for body size variation, our statistical analyses include nested analysis of covariance (ANCOVA), with SVL as a covariate to control for variation due to body size. Unless otherwise noted, all of our data were approximately normally distributed and met the criteria for parametric statistics. All of our statistical tests were two-tailed.

ANALYSIS OF GENETIC DATA

Microsatellite markers were cloned from a single *A. sagrei* captured from one of our study populations, and were developed using standard cloning procedures (Bardelbeden *et al.*, 2004). We extracted genomic DNA from tail tissue by overnight incubation at 55 °C in 500 µL of 5% Chelex (Biorad) and 2 µL of proteinase K solution (at 20 mg/mL) followed by centrifugation and 1 : 10 dilution of the extract. Loci were amplified from the genomic template via the polymerase chain reaction, and we assessed length polymorphism among individuals on an automated DNA sequencer (ABI 3700) using fluorescent labelling of one of the primers in each pair.

We tested assumptions of independence among loci and for Hardy–Weinberg equilibrium using the linkage disequilibrium and Hardy–Weinberg options in the software package GENEPOP, version 3.2a (Raymond & Rousset, 1994, 1995). We also used GENEPOP to estimate F_{ST} values between habitat types and among islands. Pairwise F_{ST} and significance values were estimated from 1000 bootstrap replicates using ARLEQUIN, version 1.1 (Schneider, Roessli & Excoffier, 2000). We calculated heterozygosity values (observed, average and total) using FSTAT (Goudet, 1995). Finally, we tested for a relationship between morphological divergence and gene flow by comparing two matrices: \ln (morphological distance) and $F_{ST}/(1 - F_{ST})$ (Rousset, 1997). We assessed the significance of this correlation using a Mantel test with 9999 randomizations in the R package (Casgrain, Legendre & Valdour, 2001).

We further examined population structure among islands using the model based clustering analysis implemented in the program STRUCTURE (Pritchard, Stephens & Donnelly, 2000). STRUCTURE uses the distribution of allele frequencies from multilocus genotypes to assess the probability that an individual comes from one of K populations. We tested two models of population differentiation: the no admixture model which assumes that each individual is derived uniquely from one of the K populations, and the admixture model which allows individuals to have ancestry in two populations (e.g. parents from separate populations). The results from the two models were nearly identical and only the results from the admixture model are reported because it may be more biologically realistic. We tested a range of K -values from one to five, with a burn in period of 10 000 and 100 000 replications. STRUCTURE estimates the posterior probability $\ln(\text{Pr}[X/K])$ to determine the most likely clustering of populations (Pritchard *et al.*, 2000).

RESULTS

INTRASPECIFIC BODY SIZE AND HABITAT USE IN *A. SAGREI*

Correlations between body size and perch diameter were strong for mass but not SVL during 2002, but trends in both cases suggested that larger males tended to occupy broader diameter perches (nested regressions: mass $r^2 = 0.34$, $P < 0.01$; effect of island, $P < 0.001$; SVL $r^2 = 0.29$, $P < 0.2$; effect of island, $P < 0.001$). During 2004, both correlations with perch diameter were significant in a test using our much larger sample size from the single island Great Exuma (mass $r^2 = 0.038$, $P < 0.001$; SVL $r^2 = 0.027$, $P < 0.006$) and mass was also correlated with perch height

($r^2 = 0.02$, $P < 0.01$). Thus, we include SVL as a covariate in the following analyses to correct for the influence of body size on correlations between limb length and habitat use.

PERFORMANCE-RELATED MORPHOLOGICAL CHARACTERS AND HABITAT USE

We found strong support for our prediction that intraspecific morphological variation in performance-related characters would vary with habitat use. Figure 3 illustrates the following comparisons and illustrates the morphological divergence measured between different habitat types on the same islands. During 2002, lizards captured on broad diameter perches had relatively longer hind limbs (ANCOVA: $F_{1,239} = 10.73$, $P < 0.001$) and forelimbs (ANCOVA: $F_{1,239} = 8.63$, $P < 0.004$) than did lizards captured on narrow perches after correcting for body size, sex, and island effects (Table 1; for residuals comparison, see Fig. 4A, B). The results were also significant when we calculated mean values of limb length in both habitat types on all five islands, and used these ten comparisons in an ancova controlling for body size (Hindlimb, nested ANCOVA: $F_{1,7} = 5.1$, $P < 0.05$; SVL, $P < 0.005$; Forelimb, nested ANCOVA: $F_{1,7} = 6.3$, $P < 0.04$; SVL, $P < 0.008$). Finally, correlations were significant ($P < 0.04$) and nearly significant ($P < 0.07$) for hindlimb (but not forelimb) length with perch diameter and perch height, respectively, within the Great Exuma population during 2004 (Fig. 5). This is the first demonstration that limb length and habitat use are correlated within populations of *Anolis* lizards.

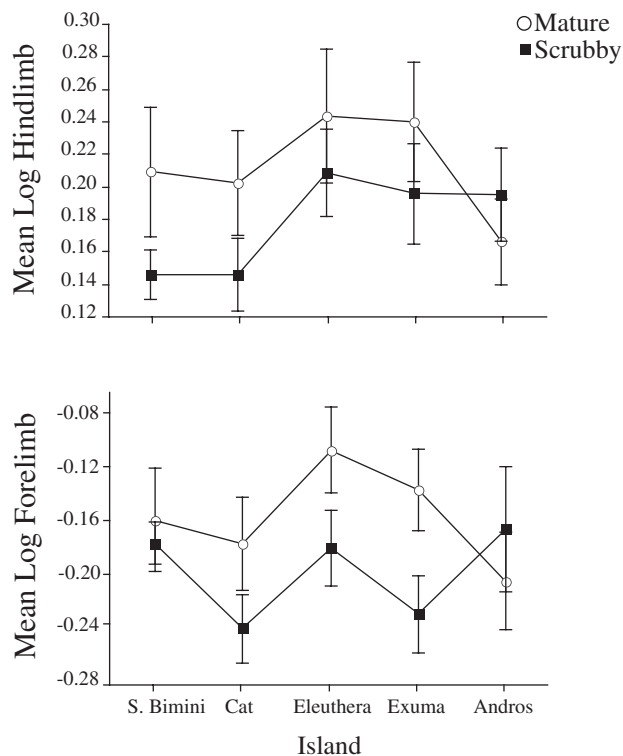


Figure 3. Values of hind and forelimb lengths on five study islands illustrate morphological divergence between habitat types within island populations. Open circles, represent mature vegetation; closed squares, scrubby vegetation. Data points are mean \pm standard error values calculated in each habitat type. For statistical comparisons, see Table 1.

Table 1. Analysis of covariance table showing variation in hindlimb and forelimb lengths as a function of island, sex, snout-vent length (SVL), perch size, and the nonsignificant perch size \times island interaction

| | d.f. | Mean square | <i>F</i> | <i>P</i> |
|----------------------------|------|-------------|----------|----------|
| Hindlimb | | | | |
| Island | 4 | 0.03 | 3.345 | 0.0109 |
| Sex | 1 | 0.116 | 12.954 | 0.0004 |
| SVL | 1 | 2.643 | 295.969 | 0.0001 |
| Perch size | 1 | 0.096 | 10.735 | 0.0012 |
| Perch size \times island | 4 | 0.012 | 1.312 | 0.2663 |
| Residual | 239 | 0.009 | | |
| Forelimb | | | | |
| Island | 4 | 0.008 | 1.054 | 0.379 |
| Sex | 1 | 0.057 | 7.68 | 0.006 |
| SVL | 1 | 0.997 | 134.819 | 0.0001 |
| Perch size | 1 | 0.064 | 8.634 | 0.0036 |
| Perch size \times island | 4 | 0.007 | 0.946 | 0.4381 |
| Residual | 239 | 0.007 | | |

Both hind and forelimb lengths were larger in broad diameter perches compared with narrow diameter perches. d.f., degrees of freedom.

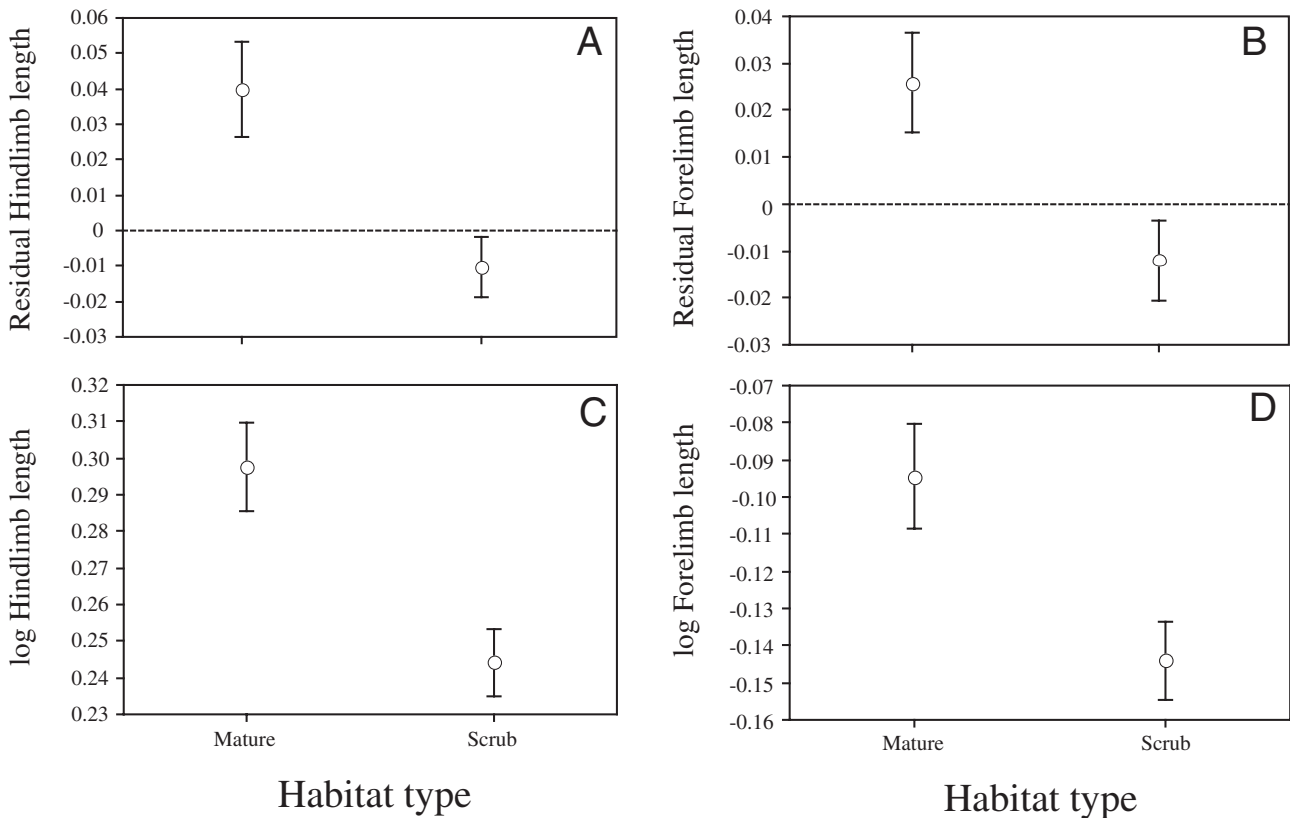


Figure 4. Differences in limb length as a function of perch diameters during 2002. Residuals from the regression of log-transformed hindlimb lengths (A) and forelimb lengths (B) on snout–vent length (SVL) were significantly larger for male lizards in mature habitats (diameter >15 cm) perches compared with male lizards in scrub habitats (< 15 cm). The pattern was also significant for both log-transformed hindlimb (C) and forelimb (D) lengths in a nested analysis of covariance, using SVL as a covariate to control for variation due to body size. Histogram bars in (A) and (B) represent the mean \pm standard error.

Similarly, interspecific variation in limb length was correlated with the mean perch diameter used among species of anole (Fig. 6). Mean interspecific forelimb length was significantly correlated with mean perch diameter (ANCOVA: $F_{1,10} = 7.83$, $P < 0.01$; SVL, $P < 0.0003$) and mean interspecific hind limb length was nearly significantly correlated with mean perch diameter (ANCOVA: $F_{1,10} = 3.43$, $P = 0.09$; SVL, $P < 0.005$). Neither trait was significantly correlated with perch height in the interspecific level, although, for additional multivariate comparisons that are significantly correlated with habitat use, see Losos (1990a).

MICROSATELLITE ANALYSES

We detected significant linkage disequilibrium ($P < 0.05$) between two of nine microsatellite loci. One of these was excluded and we performed all of our analyses with the eight remaining loci (Table 2). All other markers may be considered independent. Some

Table 2. Microsatellite data for five study populations showing mean allele size (A), the number of alleles (N_A), observed heterozygosity (H_o), mean heterozygosity (H_s) and total heterozygosity (H_t) for each locus

| Locus | A | N_A | H_o | H_s | H_t |
|-------|---------|-------|-------|-------|-------|
| p61 | 277.24 | 10 | 0.414 | 0.837 | 0.892 |
| p63 | 102.11 | 9 | 0.373 | 0.721 | 0.888 |
| p91 | 93.622 | 12 | 0.681 | 0.874 | 0.884 |
| p94 | 228.932 | 10 | 0.536 | 0.795 | 0.908 |
| p95 | 88.152 | 9 | 0.419 | 0.632 | 0.734 |
| p77 | 118.69 | 14 | 0.618 | 0.883 | 0.902 |
| p76 | 124.008 | 16 | 0.48 | 0.892 | 0.913 |
| p38 | 85.6275 | 10 | 0.748 | 0.905 | 0.907 |

of the eight remaining loci were significantly out of Hardy–Weinberg equilibrium in some of the island populations. However, none of the island populations were out of equilibrium at all loci, nor was any locus

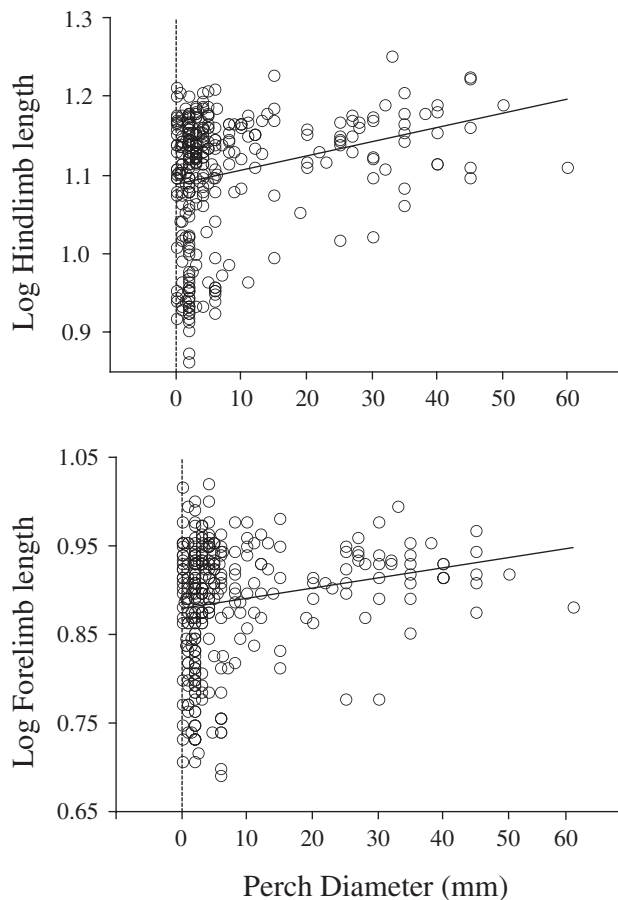


Figure 5. Correlations between limb length and perch diameter on Great Exuma during 2004 indicate that performance-related morphology with *Anolis sagrei* is congruent with patterns of morphological diversity measured among species of anole.

out of equilibrium in all island populations. Therefore, we conclude that our results are not adversely affected by the presence of null alleles.

We detected no significant intra-island population structure between habitats, indicating that high levels of gene flow between habitat types prevent genetic differentiation between mature and scrub habitats within islands (Table 3). However, we did detect population structure between populations on some pairs of islands. All pairwise population comparisons of F_{ST} were significantly different from zero except for the comparison between Andros and Great Exuma (Table 4). Overall, rates of gene flow between islands are relatively high (average $Nm \sim 7$), and, as demonstrated previously (Calsbeek & Smith, 2003), are highest between Andros and Great Exuma. Gene flow between these two islands is sufficiently high to prevent differentiation (Table 4). Morphological divergence decreases with increasing levels of gene

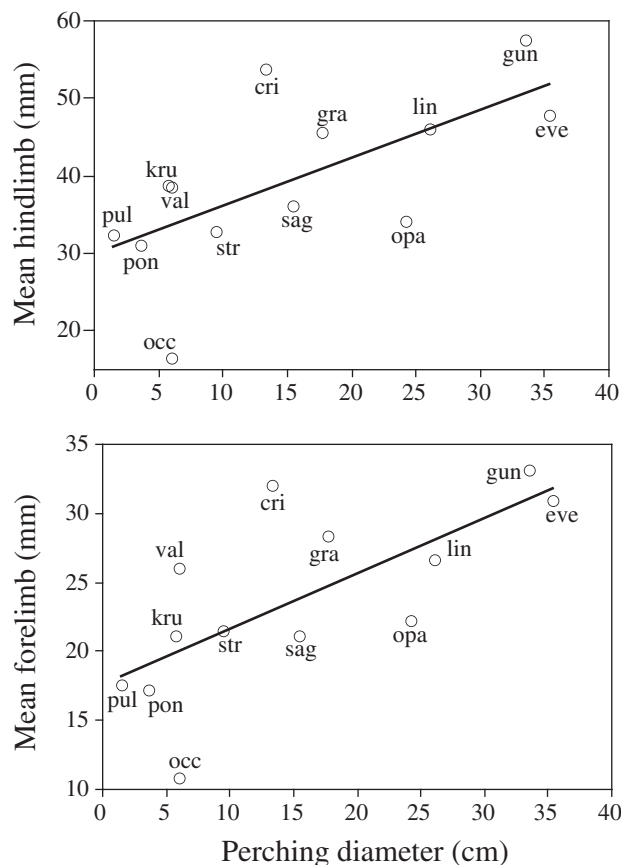


Figure 6. Interspecific relationships between hindlimb and forelimb lengths and habitat use. Data are adapted from Losos (1990a) and J. Losos (pers. comm.). *Anolis* species: cri, *crisatellus*; eve, *evermanni*; gun, *gundlachi*; kru, *krugi*; occ, *occultus*; pon, *poncensis*; pul, *pulchellus*; str, *stratulus*; gra, *grahami*; lin, *lineatopus*; opa, *opalinus*; sag, *sagrei*; val, *valencienni*.

Table 3. Pairwise F_{ST} values between habitat types on each island. We found no evidence for differentiation within islands as a function of habitat type

| Habitat comparison | F_{st} | P (mature vs. scrub) |
|--------------------|----------|------------------------|
| South Andros | 0.0093 | 0.47 |
| Bimini | -0.0091 | 0.84 |
| Cat Island | -0.001 | 0.91 |
| Eleuthera | 0.0318 | 0.18 |
| Great Exuma | 0.002 | 0.522 |

flow among islands (Mantel's test, $P < 0.02$; Calsbeek & Smith (2003).

The results from our analyses using STRUCTURE corroborate the F_{ST} approach above (Table 5). The clustering method strongly supported division into $K = 4$ populations [$\text{Pr}(K) = 1.0$]. None of the other esti-

Table 4. Pairwise F_{ST} values between study islands

| | South Andros | Bimini | Cat Island | Eleuthera |
|-------------|--------------|--------|------------|-----------|
| Bimini | 0.07* | – | – | – |
| Cat Island | 0.07* | 0.13* | – | – |
| Eleuthera | 0.10* | 0.12* | 0.14* | – |
| Great Exuma | 0.008 (NS) | 0.10* | 0.07* | 0.10* |

*Significant difference ($P < 0.05$).

All island populations were significantly differentiated from one another except South Andros and Great Exuma (highlighted), which have the highest numbers of migrants between islands (reported in Calsbeek & Smith, 2003).

Table 5. Results from the clustering analysis implemented in the software package STRUCTURE

| No. of populations (K) | $\ln \text{Pr}(X/K)$ | P |
|----------------------------|----------------------|-----|
| 1 | –4713 | 0.0 |
| 2 | –4349 | 0.0 |
| 3 | –4344 | 0.0 |
| 4 | –4283 | 1.0 |
| 5 | –4347 | 0.0 |

The results group the five island populations into four effective gene pools and suggest that high levels of gene flow between Andros and Great Exuma are responsible for grouping those islands into a single population.

mates for K resulted in a relevant estimate of population grouping. Clustering into four populations is most likely the result of high levels of gene flow between Great Exuma and Andros islands, effectively creating a single population, whereas South Bimini, Cat Island, and Eleuthera form three distinct populations.

DISCUSSION

Adaptive radiations provide unique opportunities to study patterns of diversity at different taxonomic levels. Here, we show that studying adaptive divergence at the population level may aid in understanding the processes that lead to adaptive radiations. Specifically, we have shown that intraspecific morphological divergence in characters related to performance (e.g. body size and limb length) are similar to the interspecific patterns of divergence exhibited by ecomorphs in the adaptive radiation of anoles (Losos *et al.*, 1994). Among ecomorphs (Fig. 6), species that perch on large diameter surfaces (e.g. tree trunks) tend to be larger and have longer hind and fore limbs compared with species that perch on smaller diameter surfaces (e.g. twigs) (Williams, 1972, 1983; Losos, 1990a; Losos &

Irschick, 1996; Irschick & Losos, 1998). Similarly, intraspecific hindlimb and forelimb lengths were significantly greater for *A. sagrei* captured on broad perches compared with individuals captured on narrower perches, even after correcting for body size. These morphological correlations with habitat use are similar to the findings of Losos *et al.* (1994) in *A. sagrei*. However, the added genetic information in the present study indicates that this morphological differentiation occurs despite high levels of gene flow between habitats on the same islands and despite moderate gene flow among populations on different islands. Moreover, the correlations that we report from the island population on Great Exuma are the first to demonstrate that limb length and habitat use are correlated within, as well as between, populations.

Together, these intraspecific results support assertions by Losos *et al.* (1994) that ecologically-based selection pressures drive morphological divergence among populations of *Anolis*. In addition, our results provide evidence that gene flow among habitats can be an important force (Table 3). Despite high levels of gene flow between habitats, strong natural selection apparently generates population-level adaptive divergence that is similar to the interspecific differentiation characterizing the adaptive radiation. We also measured moderate levels of gene flow occurring among islands. We assume here that contemporary gene flow is similar in magnitude and direction to historical patterns that have acted on anoles throughout their diversification in the Caribbean. This supports our assertion that the mechanisms of diversification acting within populations are the same as those that have given rise to species level diversity, and is a salient result because it demonstrates that studies of intraspecific variation can be useful for better understanding the processes that lead to diversification.

A recent laboratory study of *A. sagrei* demonstrated that a portion of the variance in limb length associated with habitat use arises due to phenotypic plasticity (Losos *et al.*, 2000). Juvenile lizards that were raised in the laboratory and provided with broad perching surfaces grew significantly longer hindlimbs and forelimbs compared with juvenile lizards that were provided with only narrow perching surfaces. Some of the variation in limb length observed in the wild may therefore be due to plastic changes in morphology (Losos *et al.*, 2000). However, the difference in limb length resulting from differences in perch use in the laboratory was very small, and less than half of the difference in limb length shown in the wild in the present study (mean difference in residual limb length from the laboratory ~0.025 vs. 0.061 in the wild). We have also shown that gene flow has homogenizing effects on morphological divergence between populations (Calsbeek & Smith, 2003); this result would not

be expected if variation in traits were purely plastic with no genetic basis (Smith *et al.*, 1997; Hendry, Taylor & McPhail, 2002). Thus, our results suggest that, although plasticity may contribute to differences in morphology in these lizards, some of the variation reported in the present study (and in the laboratory study cited above) also arises from genetic variation at loci that influence limb length. Breeding studies are currently underway in our laboratory to better resolve this issue.

DIVERGENCE WITH GENE FLOW SPECIATION

Despite the longstanding controversy surrounding the importance of allopatry to the speciation process (Mayr, 1942, 1963), there is a growing appreciation for cases of population level divergence in the face of ongoing gene flow (Lu & Bernatchez, 1999; Schneider *et al.*, 1999; Smith *et al.*, 2001; Ogden & Thorpe, 2002). We have shown that morphological divergence within islands and population divergence between islands has occurred despite ongoing gene flow among populations of *A. sagrei*. Elsewhere (Calsbeek & Smith, 2003), we demonstrate that patterns of gene flow between islands are best explained by over-water dispersal of adult lizards following island inundation by hurricanes. Rates of gene flow via ocean currents in the Bahamas are moderate to high (Nm range = 3.66–19.65; Calsbeek & Smith, 2003) and should place an upper limit on rates of morphological divergence (Slatkin, 1987; Barton & Hewitt, 1989). Indeed, the results from our analysis using the clustering algorithm implemented in STRUCTURE suggest that gene flow between island populations on Great Exuma and Andros are sufficiently high ($Nm = 19.65$; Calsbeek and Smith, 2003) to group them together as a single population. Nevertheless, even very high rates of gene flow between contiguous habitats within islands do not appear to prevent morphological divergence in characters important to performance (e.g. limb length), and large Nm values may delay but not prevent speciation (Gavrilets, 2002). One exception to this pattern may be the case presented on Andros island. There, the relationship between habitat type and limb length was reversed compared with all other islands. Although this difference was not significant on Andros, the difference could potentially reflect local maladaptation arising from relaxed selection pressure and/or high levels of gene flow to Andros from the other islands.

The present study provides further support for the hypothesis that natural selection can overcome the homogenizing influence of gene flow (Ehrlich & Raven, 1970; Rice & Hostert, 1993). Lizard populations on different islands tend to be morphologically divergent between habitats but morphologically similar within

habitats. Our results are similar to those of a recent study of anoles in the Lesser Antilles (Stenson, Malhotra & Thorpe, 2002), which revealed large morphological disparity among 33 populations of *Anolis oculatus* despite high rates of nuclear gene flow between many of these populations. The authors report a strong correlation between F_{st} and geographical distance, and suggest that the morphological divergence that they observe is due to strong natural selection. In addition, another study of *A. oculatus* (Ogden & Thorpe, 2002) demonstrated how the divergence with gene flow model of speciation might operate in anoles. Ogden & Thorpe (2002) found that morphological divergence was particularly pronounced across sharp habitat transitions and was correlated with reduced levels of gene flow. Similarly, reduced gene flow among populations of the Tenerife lizard, *Gallotia galloti*, is correlated with divergence in ultraviolet characteristics (Thorpe & Richard, 2001). Although the latter two studies suggest the nascent beginnings of reproductive isolation linked with morphological divergence, both studies report divergence despite ongoing gene flow between populations. Although other mechanisms of diversification may also be present, our results suggest that the divergence with gene flow model is likely to be an important process in the diversification of Greater Antillean anoles.

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