

Research article

## Variation in scale numbers is consistent with ecologically based natural selection acting within and between lizard species

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**Abstract.** Recent studies have demonstrated that changes in scale number are correlated with ecological variables such as precipitation, and this suggests that scale number may be under selection to maintain water balance in reptiles. Here, we present new evidence that variation in scale numbers within and among species of *Anolis* lizards is under ecologically based natural selection. We measured scalation of the brown anole, *Anolis sagrei*, in two habitat types on each of five islands in the Bahamas. We also measured scalation for 12 species of anole representing six different ecomorphs from the Greater Antilles. Within populations of *A. sagrei*, scale numbers increased with increasing precipitation and with decreasing temperature in open arid habitats. Variation measured among species of *Anolis* from the Greater Antilles showed similar patterns with temperature, precipitation, and elevation. Independent contrasts using scale count data indicated that variation in scale number was congruent within and between species, even after accounting for the influence of phylogeny. We measured natural selection (survival to maturity) on scale number in *A. sagrei* over two different habitat types in the Bahamas. Patterns of natural selection were congruent with the correlational results described. Finally, results from a breeding experiment in the laboratory provide preliminary evidence that variation in scale number is heritable, and suggests a mechanism for generating these correlations. Our results provide new evidence that the diversification of anoles has been shaped by natural selection and that ecologically based selection pressures help explain diversification at both the population and species levels.

**Key words:** adaptive radiation, independent contrasts, lizard, micro-climate, natural selection, scalation, selection

### Introduction

A growing number of studies (Horton, 1972; Soulé and Kerfoot, 1972; Lister, 1976a; Thorpe and Baez, 1987, 1993; Malhotra and Thorpe, 1997a, b) suggest that natural selection operates on scale number because of the importance of

scale number and size in reptilian water balance. The keratinized scales of reptiles serve as a protective shield against injury, and were a key adaptation for amniotic vertebrates that invaded land (Alibardi, 2003). In addition to protection against injury, scales may also serve thermoregulatory functions (Ruben and Jones, 2000), and are likely to influence water balance (Alibardi, 2003). The importance of scalation to water balance arises because the size and number of scales impact the surface area of skin that is exposed to the atmosphere. Fewer larger scales are generally keeled and overlapping (Soulé and Kerfoot, 1972), reducing skin exposure and hence the amount of evaporative water loss. Smaller, more granular scales increase the exposed skin surface area and increase rates of dehydration (Dmi'el, 2001; Neilson, 2002). While the skin between these protective scales is also keratinized, scalation likely influences local water flux. Thus, reptiles living in arid habitats often have large and overlapping scales, while lizards in more humid conditions have smaller scales with more exposed skin area, though there are exceptions to this general pattern (e.g. the small granular scales of desert geckos). These patterns appear to be generally consistent both within (Kerfoot, 1968) and between (Soulé and Kerfoot, 1972) species.

More than five decades of study have produced only a handful of studies demonstrating correlations between scale number and microclimatic variables important for water balance. In one of the earliest such studies, Hellmich (1951) demonstrated that South American lizards in the genus *Liolaemus* have greater numbers of smaller scales at lower temperatures and higher humidities across their range. Consistent changes in scalation have now been reported to be correlated with changes in a host of ecological variables [e.g. latitude and elevation (Thorpe and Baez, 1987), temperature (Soulé and Kerfoot, 1972), degree of habitat openness (Lister, 1976a) and rainfall (Malhotra and Thorpe, 1997a)]. In nearly all of these studies, scale numbers change with microclimate in the predicted direction: increasing scale numbers with increasing precipitation, decreasing temperature, increasing elevation etc... [but see (Horton, 1972; Sanders *et al.*, 2004) for exceptions]. Thus, mounting evidence suggests that scalation is probably not a neutral character but may be subject to natural selection. Here we test these ideas further by assessing congruence in the relationship between ecology and scalation both within and between species of *Anolis* lizards, and by measuring natural selection and heritability of scalation in *A. sagrei*.

*Anolis* lizards in the Caribbean have undergone an extraordinary adaptive radiation in which more than 140 species have diverged (Jackman *et al.*, 1999) and exhibit morphological variation that is associated with habitat use (Losos, 1990; Irschick *et al.*, 1997; Losos *et al.*, 2001). Morphological classes of anole are grouped into 'ecomorphs' (Williams, 1972, 1983) according to the habitats in which they most often occur (e.g. grass-bush, trunk-ground, trunk, trunk-crown, twig, crown). Previous work suggests that competition (Schoener, 1975; Lister,

1976b; Losos *et al.*, 1994) and natural selection on locomotor performance (Losos, 1990; Losos *et al.*, 1997) are key factors in the adaptive radiation of anoles. For example, species that occupy habitats characterized by broad perch diameters (e.g. tree trunks) tend to have long limbs relative to their body length because long limbs enhance sprint speed (Losos, 1990). By contrast, species that perch on narrower surfaces (e.g. twigs) tend to have shorter limbs (Losos and Sinervo, 1989) and greater agility on narrow perches. The relationships between morphology and habitat use are likely adaptive (Losos *et al.*, 2001) and have probably played an influential role in the diversification of these lizards. Furthermore, phylogenetic evidence suggests that similar ecomorphs have evolved repeatedly on different islands (Losos *et al.*, 1997, 1998), and that congruent patterns of selection, rather than repeated colonization events, explain the distribution of ecomorphs on islands (Jackman *et al.*, 1999). The repeated evolution of similar ecomorphs on different islands underscores the importance of ecologically based selection pressures in driving the diversification of anoles. To date however, only continuous morphological characters such as limb length and body size have been shown to be involved in the adaptive radiation of Greater Antillean anoles (Losos *et al.*, 1994, 2001). Possible correlations with habitat characteristics and the importance of selection on meristic traits such as scale number in the group remain unclear.

Here we test whether intraspecific variation in scale number is similar to the interspecific divergence measured among ecomorphs. The hypothesis that patterns of divergence should be congruent both within and between species follows from the assumption that species divergence is ultimately the result of divergent natural selection on intraspecific variation (Losos *et al.*, 1994). We predict that intraspecific variation in scalation should be correlated with differences in habitat use and microclimate, that these patterns should be congruent with interspecific variation among ecomorphs, and that natural selection acting within populations is the ultimate driving force behind these relationships.

We measured variation in scale numbers using the most common ecomorph in the Bahamas, the trunk-ground anole, *Anolis sagrei*. Although the inference of selection on body proportions (e.g. limb lengths) has been supported by numerous studies (Losos and Sinervo, 1989; Losos, 1990; Losos *et al.*, 1994; Irschick and Losos, 1998), the importance of selection on meristic traits (e.g. scale counts) in the adaptive radiation of anoles has been comparatively understudied. While scalation has not been implicated in the radiation of anoles, previous studies with *Anolis oculatus* (Malhotra and Thorpe, 1997a, b) and *Anolis sagrei* (Lister, 1976a) have suggested that changes in scalation may be related to microgeographic variation in climate. To test for the role of selection on meristic traits in the adaptive radiation of anoles, we measured intraspecific variation in scale numbers and survival in our study populations, and

measured interspecific variation among ecomorphs using museum collections. Based on previous studies (Horton, 1972; Soulé and Kerfoot, 1972; Malhotra and Thorpe, 1994, 1997a, b), and the importance of scalation to water balance, we predicted that variation in scalation should be related to differences in precipitation, elevation and temperature. Finally, we measured the heritability of scale numbers in a laboratory based breeding study.

## Methods

### *Study organism, field work and general methods*

This study took place on five islands on the Great Bahama bank (Fig. 1) during June and July 2002 and on small cays offshore of Great Exuma, Bahamas from May to October 2004. Islands and the locations of study sites were: Cat Island 24°27'N 75°30.5'W, South 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. The brown anole, *Anolis sagrei*, is extremely common in the Bahamas. It is a member of the trunk-ground ecomorph, but like all ecomorphs, it may be found in a variety of habitat types (Losos, 1990; Williams, 1983) including mature and scrubby vegetation. *Anolis sagrei* is short-lived and typically matures and dies in one to three years in the wild (Schoener and Schoener, 1978, 1982).

On each of our study islands during 2002, we captured at least 10 lizards from each of 3–5 locations along a linear transect whose end points were approximately 20 km apart (except on South Bimini where the island's small size constrained the length of our transect to ~6 km) (Fig. 1). We captured 45–57 lizards from each island (mean = 51) and recorded sex, mass, and snout-vent-length (SVL) for all individuals ( $N=213$  males and 38 females total). We counted the numbers of sub-ocular and labial scales. Sub-ocular and labial scales by themselves account for a relatively small fraction of the surface-area of *A. sagrei*, and probably contribute little to overall water regulation. However, these measures are correlated with other scale counts (e.g. numbers of ventral scale rows based on  $N=11$  captive males counted with the aid of a dissecting scope: sub-ocular scales  $r=0.58$ ,  $p=0.06$ ; labial scales  $r=0.65$ ,  $p<0.02$ ) and our measurements should therefore be useful estimates of differences in overall scalation among individual lizards. Our measures of scalation did not differ by sex based on data from a separate study (ANOVA  $F_{1,255}=2.77$ ,  $p=0.1$  and  $F_{1,255}=1.57$ ,  $p=0.2$  for sub-ocular and labial scales, respectively) and we pooled data from both sexes in the following analyses. All lizards were temporarily marked with a small spot of white paint on their hind leg to prevent recapture of the same individuals.

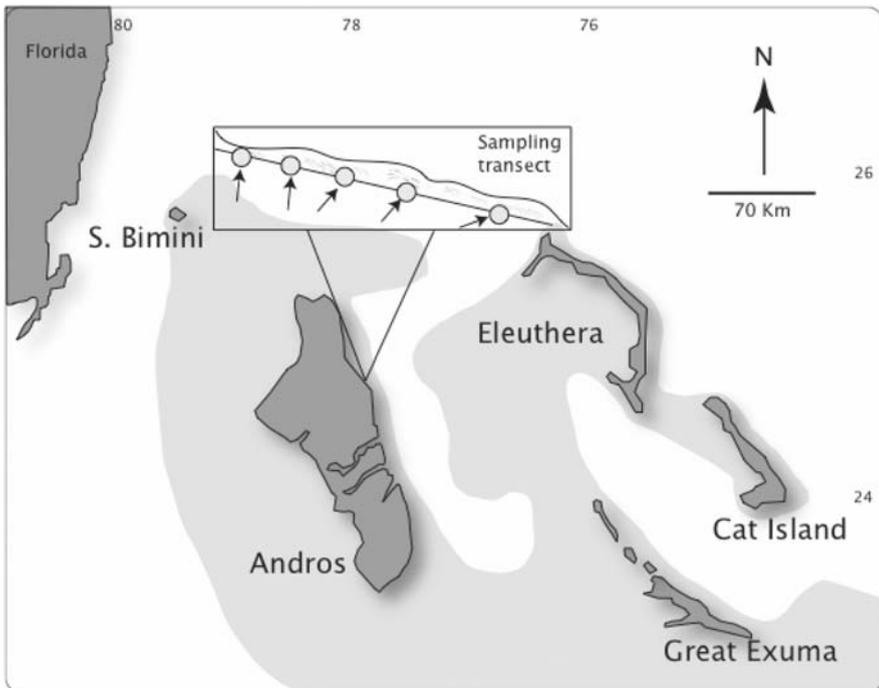


Figure 1. Map of our study islands in the Bahamas including a magnified view of one study transect. Arrows indicate collecting sites along the transect. The lightly shaded area indicates the Great Bahama Bank.

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 (following Rand, 1964, 1967; Schoener, 1975). Perch diameter was estimated by visual comparison against a small ruler. For each island, we grouped habitats whose perch diameters exceeded 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 in coastal areas, but the vegetation becomes predominantly scrubby as one moves inland (e.g. very few large diameter trees and more low lying brush vegetation).

#### *Museum specimens, interspecific comparisons, and phylogeny*

We measured 12 species representing the six Greater Antilles ecomorphs, available from the Museum of Vertebrate Zoology (MVZ) at the University of California, Berkeley (Table 1). As no museum specimens were available from Bahamian islands, we report results from specimens from the islands of the Dominican Republic, Haiti, and Puerto Rico. We recorded sex and

Table 1. Ecomorph data collected from museum samples. Sample sizes ( $N$ ) are accurate for all measurements except that two individuals in the *A. occultus* group could not be scored for sub-ocular scales, and one of these could not be scored for labial scales because of damage to these two specimens. Perching height and diameter data are from Losos (1990) and J. Losos (unpublished data)

Species	Ecomorph	$N$	Mean sub-oculars (SD)	Mean labials (SD)	Mean SVL (SD)	Perching height (m)	Perching diameter (cm)
<i>Bahoriucoensis</i>	Bush	14	4.93 (1.27)	7.43 (0.85)	42.79 (1.76)	1.50	2.68
<i>Cooki</i>	Trunk-ground	11	8.55 (1.44)	8.55 (0.69)	54.36 (6.06)	1.49	7.75
<i>Cybotes</i>	Trunk-ground	6	8.67 (1.37)	8.17 (0.75)	54.67 (8.36)	1.43	24.52
<i>Brevirostris</i>	Trunk	2	10 (1.41)	7.5 (0.71)	45 (1.41)	1.03	21.07
<i>Distichus</i>	Trunk	8	8.88 (2.70)	8 (0.76)	49.5 (6.48)	2.57	18.33
<i>Insolitus</i>	Twig	2	22.5 (6.36)	14 (1.41)	42.5 (2.12)	3.36	0.36
<i>Occultus</i>	Twig	6	12.83 (4.67)	14 (3.58)	37.56 (1.88)	2.30	6.00
<i>Alinger</i>	Trunk-crown	2	12 (0)	10 (1.41)	55.5 (0.71)	4.63	18.58
<i>Chlorocyanus</i>	Trunk-crown	1	10 (0)	11 (0)	51 (0)	3.57	10.62
<i>Coelestinus</i>	Trunk-crown	9	8.33 (2.12)	10.111 (0.93)	66.44 (10.35)	2.53	20.92
<i>Singularis</i>	Trunk-crown	1	11 (0)	11 (0)	40 (0)	3.00	8.00
<i>Cuveri</i>	Crown	6	11.33 (1.63)	12.67 (0.82)	115 (18.54)	3.25	8.50

snout-vent-length for each species in the same manner reported above for *A. sagrei*. Numbers of sub-ocular and labial scales were counted with the aid of a dissecting microscope. We then tested for correlations between mean elevation, mean January precipitation, and mean annual temperature and sub-ocular and labial scale counts. We used January precipitation data because these data represent conditions during the middle of the dry season, when selection is likely to be most intense. Mean temperature, elevation and precipitation for the interspecific analyses were calculated across each species range using the 30-year WORLDCLIM database (30-s. resolution), Version 1.1 (<http://biogeo.berkeley.edu/worldclim/worldclim.htm>) (Hijmans *et al.*, 2004) and ArcView 8.3. Intraspecific analyses used data collected across each island using the same WORLDCLIM dataset. We used raw scale counts rather than residual values corrected for snout-vent-length because there was no correlation between scale number and body size and no *a priori* reason to believe that scale number should change as a function of growth.

Phylogenetic history may contribute to the similarity among closely related species, and morphological divergence among ecomorphs may depend in part upon historical relationships. To correct for non-independence of morphological divergence among closely related taxa, we applied the method of independent contrasts (Felsenstein, 1985; Richman and Price, 1992) to interspecific data on ecomorphs. We calculated independent contrasts using the program CAIC (Purvis and Rambaut, 1995) with the criterion of equal branch lengths. A complete phylogeny for *Anolis* lizards is not currently available (Jackman *et al.*, 1997, 1999) and we substituted four species from our analyses for closely related species on the existing phylogeny (J. Losos unpublished data, J. Losos pers. comm. see legend for Fig. 3). We computed independent contrasts for temperature data and numbers of sub-ocular and labial scales among the 12 species of anole (Table 1). Independent contrasts computed for temperature data and scalation measures met the criteria for analysis using standard parametric regression according to the methods for verification recommended by Garland *et al.* (1992). All regressions performed with contrast data were forced through the origin (Garland *et al.*, 1992).

#### *Measuring selection on scalation*

During 2004, we performed a large-scale mark recapture study of natural selection on small off-shore islands (cays) representing two habitat types in the Bahamas. We captured and counted the scales of 231 sub-adult male *Anolis sagrei* from the island of Great Exuma. All lizards were given a unique combination of colored elastomer tags that we injected sub-dermally into each limb using a 25-gauge needle (information at <http://www.nmt-inc.com>). Each

colored tag was injected as a liquid that polymerized within 2 h and remained visible to the naked eye. Tags serve as permanent identification in the wild, allowing us to track the fate of every individual over the course of the study. Males were transplanted to cays that naturally support *A. sagrei* but which had been recently denuded of all male lizards (but not female lizards) as part of a larger study of natural selection on lizard morphology (Calsbeek and Smith, in prep.). Thus, interactions among males released to experimental islands were not influenced by prior residency on territories. Of the males measured and marked in this study, we released 74 to each of two cays containing only scrubby habitat (hereafter scrubby cays), and we released 83 to a cay containing both mature and scrubby vegetation (Kidd cay). Each of the scrubby cays lies several hundred meters offshore of the main island of Great Exuma, while Kidd cay is connected to the main island by a narrow causeway that is ~300 m long. In addition to differences in available habitat types, scrubby cays experience higher winds (Calsbeek, personal observation) and higher temperatures (Calsbeek, unpublished data) than Kidd cay. We returned to the cays 4 months later and recaptured all individuals on the island. We chose this time frame because it represents the nominal time to reach maturity (Schoener and Schoener, 1982) and the end of the first breeding season. All lizards not captured during this census period were considered to have died.

#### *Heritability of scale number*

We captured 50 sexually mature female *A. sagrei* from Great Exuma and brought them into the laboratory. Each female was housed in a separate 10-gallon terrarium and was provided with full spectrum lighting, and *ad libitum* food (*Achaeta* crickets) and water. Females were allowed to copulate in the laboratory with up to two males as part of a different study on sperm competition, but had also copulated with males in the field prior to capture. We genotyped all progeny, dams, and candidate sires using a library of eight microsatellite markers, and assigned paternity using the software package CERVUS. Maternity of all offspring was known with certainty, as females were housed separately. Simulations in CERVUS indicated a total paternity exclusionary power of 0.993. Of the 107 sexually mature progeny obtained in the laboratory, we could assign paternity (95% confidence) to 25 sons and 22 daughters from 10 different sires. We point out that some error in our paternity estimates could potentially bias our heritability estimates. To avoid the potentially confounding influence of maternal effects that can arise when including dams in regression analyses, we estimated heritability of scale number using weighted least squares regression (with number of progeny as weights) of sires on sons.

### *Statistical analyses*

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. While interspecific comparisons were corrected for phylogeny, we tested for spatial autocorrelation in intraspecific comparisons using Mantel's tests. No spatial autocorrelation was detected. We tested associations between scalation and ecological variables using ANCOVA comparing the relationships of scale numbers with temperature and precipitation between scrub and mature habitat types. We then performed separate linear regressions for each habitat type, of scale numbers against temperature and precipitation. We report values for selection gradients calculated from standard parametric statistics (Lande and Arnold, 1983) and we verified the significance of selection using logistic regression of the dependent variable survival (live/die) on the independent variable scale number. Owing to small numbers of surviving lizards on scrubby cays, we pooled these two islands after finding no difference in the form of selection between islands. We visualized the form of selection using non-parametric cubic-spline regression (Schluter and Nychka, 1994).

## **Results**

### *Scalation, ecology, and ecomorphs*

We found support for our first hypothesis that intraspecific patterns of scalation would vary with changes in temperature and precipitation. Within scrub habitat, mean numbers of both sub-ocular and labial scales were negatively correlated with temperature (log sub-oculars  $F_{1,4} = 18.09$ ,  $r^2 = 0.86$   $p < 0.05$ ; log labials:  $F_{1,4} = 13.12$ ,  $r^2 = 0.82$ ,  $p < 0.03$ ; Fig. 2a) and positively correlated with precipitation (log sub-oculars  $F_{1,4} = 14.16$ ,  $r^2 = 0.83$   $p < 0.03$ ; labials:  $F_{1,4} = 76.82$ ,  $r^2 = 0.96$ ,  $p < 0.003$ ; Fig. 2b) but none of these relationships were significant in mature habitat (log sub-oculars  $F_{1,4} = 0.114$ ,  $p = \text{n.s.}$ ; log labials:  $F_{1,4} = 1.875$ ,  $p = \text{n.s.}$ ; Fig. 2c,d). In an analysis of covariance, there was also a significant difference in the relationship between mean number of sub-ocular scales and ecological variables in the two habitat types (ANCOVA habitat type  $\times$  precipitation  $F_{1,6} = 11.52$ ,  $p < 0.01$ ; ANCOVA habitat type  $\times$  temperature  $F_{1,6} = 10.07$ ,  $p < 0.02$ ) though the difference was not significant for labial scales ( $F_{1,6} = 0.63$ ,  $p = \text{n.s.}$ ;  $F_{1,6} = 0.14$ ,  $p = \text{n.s.}$  for precipitation and temperature, respectively).

Our second hypothesis, that patterns of interspecific variation should be congruent with intraspecific variation, was also supported by our data. Analysis of museum specimens revealed similar correlations between sub-ocular and labial scale numbers and the same ecological variables related to climate (Fig. 3).

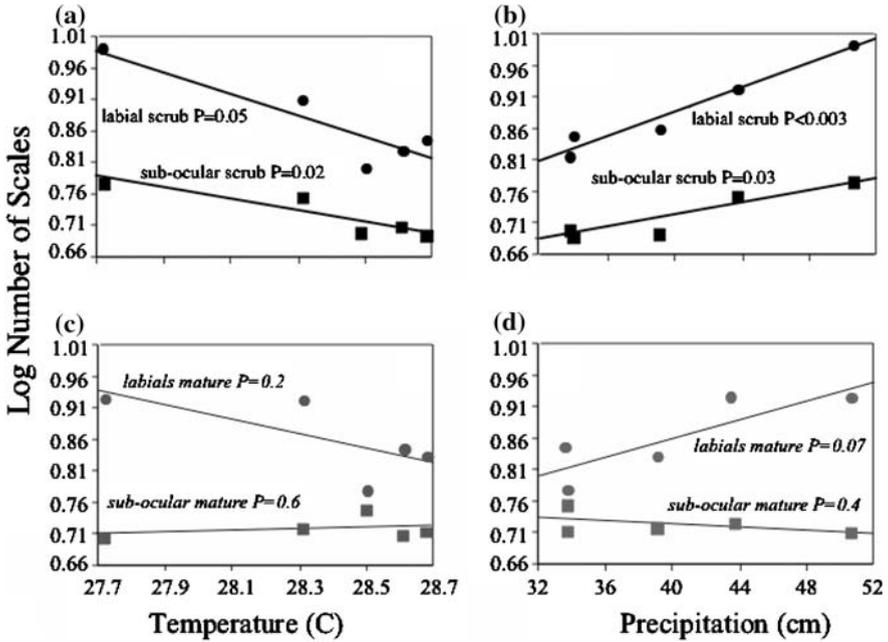


Figure 2. Numbers of sub-ocular (squares) and labial scales (circles) varied significantly with temperature (panel a) and precipitation (panel b) in scrub habitat but not in mature forest (panels c and d). Scale numbers were also significantly correlated with elevation (data not shown). Thus, the hotter and drier conditions in scrub habitats are associated with lower numbers of scales while this pattern is not significant in mature forest.

Numbers of sub-ocular scales were inversely correlated with the median temperature experienced by ecomorph populations ( $F_{1,10} = 19.16$ ,  $r^2 = 0.62$ ,  $p < 0.001$ ), as were numbers of labial scales ( $F_{1,10} = 19.16$ ,  $r^2 = 0.62$ ,  $p < 0.001$ ). Numbers of labial scales were also negatively correlated with precipitation ( $F_{1,10} = 14.88$ ,  $r^2 = 0.60$ ,  $p < 0.003$ ) and sub-ocular scales showed the same pattern though the regression was not significant ( $F_{1,10} = 1.91$ ,  $r^2 = 0.16$ ,  $p = \text{n.s.}$ ). Both measures of scalation were also positively correlated with elevation ( $F_{1,10} = 20.53$ ,  $r^2 = 0.67$ ,  $p < 0.002$  and  $F_{1,10} = 5.04$ ,  $r^2 = 0.27$ ,  $p < 0.04$  for sub-ocular and labial scales, respectively). We did not measure elevation for the intraspecific data because islands in the Bahamas are uniform in elevation. Species correlations with temperature and elevation remained highly significant after applying the method of independent contrasts ( $p < 0.01$  for both scale counts; Fig. 3), though in the interest of brevity, only temperature data are illustrated in the figure.

*Selection and heritability scalation*

We recaptured 59 of the 231 lizards (ca. 25% survival) released to the experimental cays (33 from scrubby cays and 26 from Kidd Cay). We found evidence

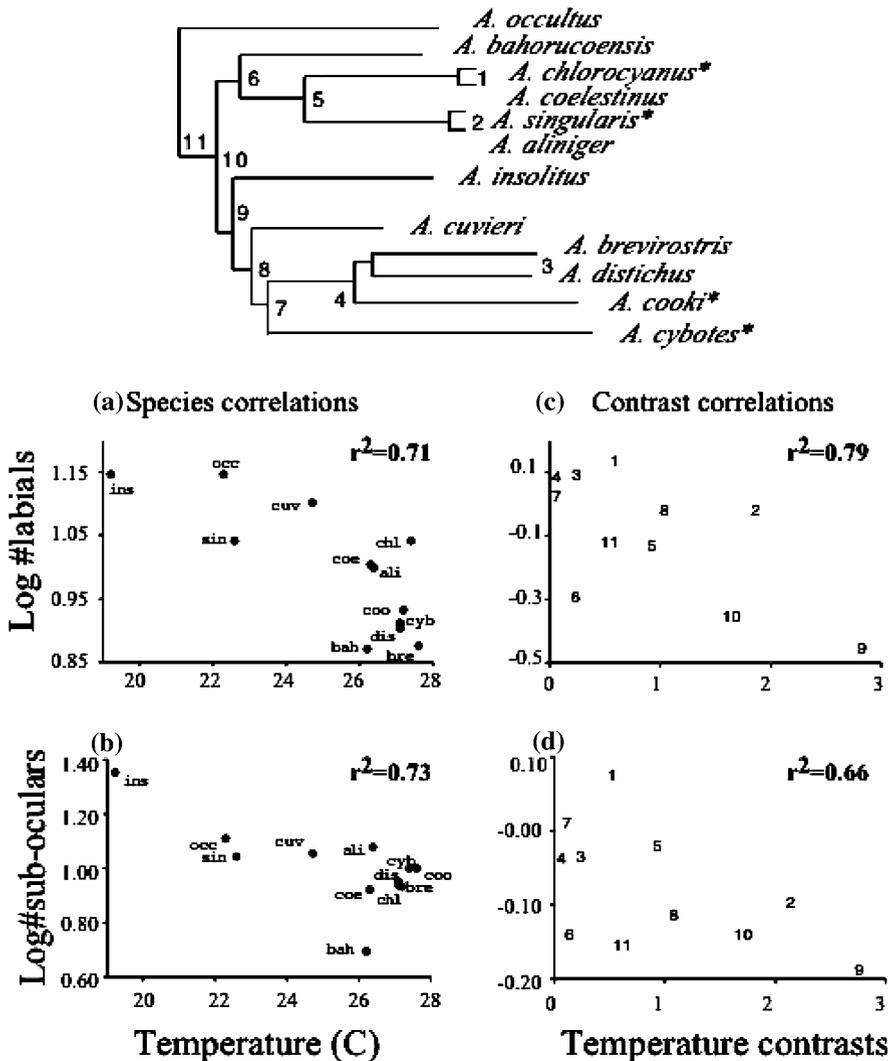


Figure 3. Top panel shows the phylogeny for some *Anolis* lizards (modified and redrawn from (Jackman *et al.*, 1999)). Asterisks denote species for which no published phylogenetic data are available and which were substituted for closely related species (based on unpublished phylogenetic information, J. Losos pers. comm.). The following species were substituted, based on their close similarity, for those missing from the phylogeny: *crisatellus* for *cooki*, *marcanoi* for *cybotes*, and *chlorocyanus* and *singularis* for *aliniger* and *coelestinus*. Panels a–d show raw and contrast data for (log) numbers of sub-ocular and labial scales for the different species of ecomorphs versus temperature (see methods). Three-letter abbreviations correspond to species in the phylogeny. Numbers refer to corresponding nodes at which independent contrasts were computed based on the phylogeny above. Correlations were still highly significant ( $p < 0.001$  and  $0.01$  for labial and sub-ocular scales, respectively) following independent contrasts (panels c and d).

for non-linear natural selection (weakly stabilizing) tending to favor fewer numbers of labial scales on scrubby cays ( $\gamma_{1,1} = -0.037$ ,  $SE = 0.018$ ; logistic regression  $p < 0.002$ , Table 2 and Fig. 4). We did not detect any significant natural selection on sub-ocular scales, nor did we measure significant selection on either of the two scale traits on Kidd cay. Selection pressures will only result in an evolutionary response if traits under selection have a heritable basis. Sire-son regression of 26 sons from 9 different sires (weighted by progeny number) provide evidence that scale numbers are indeed heritable ( $h^2 = 0.76 \pm 0.18$ ,  $p < 0.02$ ).

Table 2. (A) Natural selection on labial scale number was significant on scrubby cays ( $***p = 0.002$ ) but not on Kidd cay (B). Selection was non-linear (weakly stabilizing, see Fig. 4) and tended to favor individuals with fewer scales living on scrubby cays as reflected by the significant quadratic selection term. There was no evidence for selection on orbital scales on scrubby cays, nor was there any selection on either trait on Kidd cay

	Gradient	Std. error
(A) Serubby cays		
Labial scales	$\beta = 0.028$	0.042
Labial scales <sup>2</sup>	$\gamma = -0.037$	0.018***
Orbital scales	$\beta = -0.106$	0.322
(B) Kidd cay		
Labial scales	$\beta = -0.002$	0.077
Labial scales <sup>2</sup>	$\gamma = -0.016$	-0.024
Orbital scales	$\beta = 0.095$	0.56

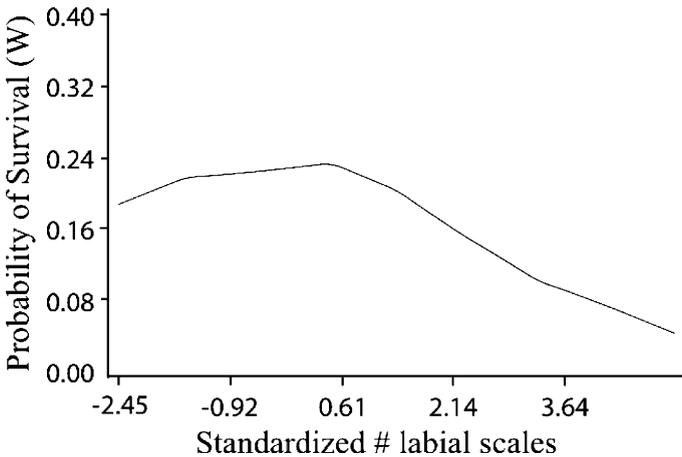


Figure 4. Cubic-spline regression showing survival to maturity as a function of labial scale number on scrubby cays in the Bahamas. Selection was weakly stabilizing and generally favored individuals with fewer numbers of scales. Significance of the selection gradient was assessed using logistic regression. Traits distributions were standardized to have a mean of zero and standard deviation of one prior to analysis.

## Discussion

The adaptive radiation of anoles provides a useful framework for studying the parallel nature of selection at different taxonomic levels. By examining morphological variation at both the intra- and inter-specific level, we have shown that studying adaptive divergence in scalation at the population level may aid in understanding the processes that give rise to adaptive radiations. Specifically, we provide evidence (1) that intraspecific divergence in scalation manifests itself across temperature and precipitation gradients in the Bahamas, (2) that this variation is similar to the interspecific patterns of divergence exhibited by ecomorphs in the adaptive radiation of anoles, and (3) that correlations with microclimate may be driven by ecologically based selection pressures on heritable traits (Malhotra and Thorpe, 1991). Elsewhere (Calsbeek *et al.* submitted), we have demonstrated similarly congruent patterns of selection acting on performance related morphological characters (e.g. limb length), and that this divergence occurs despite relatively high levels of gene flow among populations.

Our results provide an example of the power of congruent forms of natural selection acting on different populations to cause parallel forms of divergence. Our data suggest that ecologically based natural selection acting within populations, and the resulting patterns of geographic variation between climate and scalation, are also evident across species of *Anolis* lizards. If this is correct, then intraspecific variation should be generally useful for better understanding the processes that lead to speciation. Thus, we provide a potential example of how intraspecific variation in some characters provide a population level context for studying an adaptive radiation, since ecologically based selection appears to have acted similarly on variation both within and among species.

### *Variation and selection on scalation*

Similar to traits associated with locomotor performance (Calsbeek *et al.* submitted), intraspecific variation in scale number was correlated with habitat use. However, these patterns are likely to be related to differences in microclimate in mature and scrub habitats, rather than the functional significance of perch diameter to lizards of different limb length (see introduction). Numbers of both sub-ocular and labial scales increased with increasing precipitation and decreased with increasing temperature in scrub habitat but not in mature habitat. Lizards in scrub habitat experience greater exposure to sun and wind, resulting in hotter and drier conditions compared with the more protected areas in mature habitats (e.g. under the canopy of an adult tree). Thus, lizards in scrub habitats are probably under more intense selection pressure to maintain proper water balance and are thus selected to have fewer scales to prevent water loss. This suggests that selection may act on scalation based on

differences in microclimate favoring fewer numbers of scales in hot and/or dry conditions. The lack of significant correlation between scale numbers and micro-climate in mature habitats may result from the small number of populations reported in this study, however relaxed selection pressures in cooler conditions could also explain the result. If this is the case, then divergence between habitats appears to have occurred despite ongoing gene flow (Calsbeek and Smith, 2003; Calsbeek *et al.* submitted) among islands and between habitat types within islands.

Results from our natural selection study indicate that selection tended to favor individuals with fewer numbers of scales on islands with generally hotter and drier conditions (Fig. 4). Selection was relatively weak as reflected by the small values of the selection gradient (Table 2), and this may explain why we were only able to measure selection in one of the habitat types and on one of the scalation traits. There have been several indirect estimates of selection on scale number in reptiles (Lindell *et al.*, 1993; Forsman *et al.*, 1994; Olsson *et al.*, 1996). Nevertheless, to our knowledge this represents only the second study to report direct measures of selection acting on scale number in reptiles (Malhotra and Thorpe, 1991) and despite mixed results, suggests that scale number is indeed an adaptive trait. Moreover, we provide one of the first ever estimates of the heritability of scale number, a results that suggests selection on scale number can lead to evolutionary change.

The patterns reported here are consistent with those in a previous study of scalation in *Anolis sagrei* (Lister, 1976a). Consequently, we suggest that scalation may represent a general adaptation to prevent water loss in arid habitats (Soulé and Kerfoot, 1972), since fewer numbers of scales in hotter and drier conditions would decrease the amount of scale surface area exposed to the atmosphere (Horton, 1972; Malhotra and Thorpe, 1997a). However, as noted by Malhotra and Thorpe (1997a), the adaptive interpretation of these results may depend on whether the trait of interest is the scale itself, or the exposed skin between scales. Some have suggested that the opposite pattern of scalation with microclimate could also be adaptive (Hertz, 1980; Malhotra and Thorpe, 1997a), because smaller scales increase the surface area of interstitial skin and hence the amount of evaporative water loss.

Previous studies of scalation have either been conducted across broad climatic gradients (Malhotra and Thorpe, 1997a) or geographic ranges (Horton, 1972; Taylor and Buschman, 1993; Sanders *et al.*, 2004). By contrast, the correlational patterns observed in our study populations occur across extremely small changes in latitude (less than 2°; see Fig. 1). Nevertheless, small changes in latitude are correlated with large differences in annual rainfall among islands. Moreover, interspecific comparisons among ecomorphs indicate that shifts in scalation occur across broad changes in microclimate as well. Scale numbers increased with increasing elevation and decreased with

increasing temperature among population locations of ecomorphs. These correlations further support the hypothesis that *Anolis* lizards are under selection to have fewer scales at higher temperatures (and/or lower elevations) where conditions may be generally drier.

Species correlations remained significant following application of the independent contrasts method. This indicates that relationships among sister taxa are not solely responsible for generating the correlations in our data (i.e. closely related species are not morphologically more similar than distantly related species) and supports an adaptive explanation for our results. The lack of phylogenetic influence on morphological variation is not surprising given a recent study of anoles on Cuba (Losos *et al.*, 2003) that showed that the degree of overlap in habitat use was not related to phylogenetic similarity. Moreover, under an adaptive radiation model of speciation individual species may be considered independent without performing contrasts (Richman and Price, 1992; Price, 1997; Harvey and Rambaut, 2000). The argument for species level independence is predicated on the assumption that radiating lineages do not evolve in small, incremental steps through time (e.g. a random walk). Rather, the bulk of evolutionary change occurs around speciation events (a punctuated model of evolution). Harvey and Rambaut (2000) have shown that in some cases, despite an influence of phylogeny, parametric linear regressions using species values may be considered an appropriate estimate of the correlation between morphology and ecology (Price, 1997). However, because this issue remains a subject of ongoing debate we have followed Schluter's (2000) recommendation and have presented results from both analyses.

The congruence between scale numbers and ecological variables both within and among species indicate that morphological traits other than limb length and body size may play important roles in diversification within the *Anolis* group. Parallel patterns of scalation within and between species in our data indicate that the relevance of ecologically based selection on scalation may have been relatively under-appreciated in previous studies of the adaptive radiation of anoles. Future studies should attempt to elucidate the causal mechanisms involved in the divergence of both continuous and meristic traits in these and other lizards. In particular, additional direct estimates of the strength and forms of selection on scale number (and/or size) are needed in the wild, as are larger scale studies of the heritability of these traits.

There are several alternative explanations for the observed pattern of scalation. Meristic characters may exhibit a plastic response to differences in the environment (e.g. vertebral number in amphibians (Jockusch, 1997)) or may be under the influence of maternal effects, (though this has been shown not to be the case for scalation in garter snakes (Arnold and Peterson, 2002)). Scale numbers could also be pleiotropically linked to some other trait or traits that are under ecologically based selection (Fox, 1975; Lande and Arnold, 1983).

Some portion of the variation in scalation may also arise due to phenotypic plasticity as has been shown for some quantitative traits in *Anolis* (Losos *et al.*, 2000), though our breeding study in the laboratory indicates that a large proportion of the variation arises from additive genetic effects ( $h^2 = 0.76 \pm 0.18$  see above). Finally, body scales certainly serve as a form of physical protection from injury and/or infection (Alibardi, 2003) and there may be differences in likelihood of injury in different habitat types that could lead to alternative forms of selection on scale number or size. Further investigation of heritability and the role of selection on scale number (Thorpe *et al.*, 2005) is warranted, and these studies are currently underway in our laboratory. Importantly, based on results here, heritable variation in reptilian scale numbers is clearly under the influence of natural selection.

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