

The evolution of locomotor morphology, performance, and anti-predator behaviour among populations of *Leiocephalus* lizards from the Dominican Republic

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Most ecomorphological studies use a comparative approach to examine adaptation by studying variation among species. A question of considerable interest is whether ecomorphological patterns observed among species also exist at the population level. We studied variation in morphology, performance, and behaviour in four populations of *Leiocephalus personatus* and two populations of *Leiocephalus barahonensis* in the Dominican Republic. We combined these data with measurements of predation intensity and habitat structure to test for convergence at the population level. We predicted that predation intensity would be higher in open habitats and that lizards in these habitats would have traits conferring higher predator evasion capacity (increased wariness, faster sprint speeds, and longer limbs). Principal components analysis suggests that sites tend to differ with respect to the abundance and spacing of low-lying vegetation (i.e. percentage of shrub cover and distance to nearest vegetation), but we did not detect any striking differences among sites in tail-break frequencies or attacks on clay lizard models. Consistent with predictions we find that in open habitats, lizards tend to have longer limbs, faster sprint speeds (relative to body size), and longer approach distances. These patterns corroborate findings in other ground-dwelling lizard species and indicate that they have evolved at least twice among populations of *Leiocephalus* lizards. The results of this study also suggest that these traits have evolved rapidly despite recent or ongoing gene flow. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, **93**, 445–456.

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INTRODUCTION

A major aim of evolutionary biology is to explain geographical variation in phenotypic traits. Geographic variation in environmental conditions provides an arena within which adaptation can occur. Alternative environments across geographical space can result in different organismal traits conferring high fitness in different areas (Endler, 1977; Schluter, 2000). This geographical pattern may result in a mosaic of locally-adapted populations. Although conclusively documenting adaptation requires satisfaction of a number of criteria (Baum & Larson, 1991;

Rose & Lauder, 1996; Schluter, 2000), a repeated pattern of trait variation in similar ecological contexts within and among species provides strong evidence for adaptation (Losos, 1990; Harvey & Pagel, 1991; Robinson & Wilson, 1994; Schluter, 2000; Langerhans & DeWitt, 2004).

A common ecomorphological pattern found among lizards is the association between habitat use, body shape, and whole animal performance (Losos, 1990; Vitt *et al.*, 1997; Melville & Swain, 2000; Herrel, Meyers & Vanhooydonck, 2002; Elstrott & Irschick, 2004). Several previous studies examined ecomorphological relationships within species (Van Damme, Aerts & Vanhooydonck, 1997, 1998; Vitt *et al.*, 1997; Herrel, Meyers & Vanhooydonck, 2001; Vanhooydonck, Van Damme & Aerts, 2002;

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Irschick *et al.*, 2005); however, only one studied more than two populations (Herrel *et al.*, 2001) and only one other utilized phylogenetic information (Vitt *et al.*, 1997). Ecomorphological patterns observed among species provide predictions for patterns of variation expected among populations within species. Among terrestrial snow skinks (*Niveoscincus*) and Galapagos lava lizards (*Microlophus*), the degree of 'habitat openness' is correlated with limb length variation, predator avoidance behaviour, and locomotor performance (Snell *et al.*, 1988; Melville & Swain, 2000, 2003; Miles *et al.*, 2001). Open habitats (i.e. habitats with relatively little vegetation) should favour the evolution of traits that increase predator avoidance capacity (i.e. longer hindlimbs, increased wariness, and increased sprinting abilities). Nonetheless, these studies generally do not quantify predation intensity in each habitat.

Caribbean curly tailed lizards (*Leiocephalus*) are an excellent system to examine adaptation to alternative environments because the genus is widespread, exists in a variety of habitat types, and phylogeographical hypotheses are available (Schwartz & Henderson, 1991). All members of the genus are ground-dwelling, although some species and populations utilize arboreal perches to a degree (M. E. Gifford and A. Herrel, pers. observ.). On Hispaniola, these lizards inhabit a variety of habitats ranging from open savanna and sandy beaches to dry tropical thorn-forests with a dense, cluttered understory.

Sparsely-vegetated habitats may constitute a different predator environment than densely-vegetated habitats (Van Damme *et al.*, 1998). Therefore, in the present study, we initially test for differences in predation intensity between habitat types using clay lizard models and the frequencies of lizards with broken tails. Furthermore, we test for patterns of ecomorphological variation among populations of *Leiocephalus* lizards found in densely- and sparsely-vegetated habitats. We predict that lizards found in sparsely vegetated habitats will have longer limbs, sprint faster, and exhibit different predator avoidance behaviours (e.g. larger approach distances; Bulova, 1994) than those found in densely vegetated habitats. These comparisons are made between two populations from each of three independent mitochondrial DNA (mtDNA) lineages to test for repeated patterns of ecomorphological variation. Included in this study are four populations of *Leiocephalus personatus* and two populations of *Leiocephalus barahonensis*.

MATERIAL AND METHODS

POPULATIONS SAMPLED

The six populations used in this study were chosen based on preliminary observation of lizard-habitat

associations and guided by mtDNA phylogeographical results (M. E. Gifford, unpubl. data). In each of three lineages, two populations were chosen that differed in apparent habitat cover based on preliminary anecdotal observations. Each pair included one population from a coastal (i.e. beach) site and the other from an inland site. Inland sites tend to contain a larger diversity and density of vegetation than coastal sites. Hereafter, the populations are denoted as follows. For *L. personatus*, comparison 1 (COMP1) represents a contrast between the inland Monte Cristi population (MC) and coastal Sosua population (S), and comparison 2 (COMP2) represents a contrast between the coastal Las Terrenas population (LT) and the inland Santo Domingo population (SD). For *L. barahonensis*, comparison 3 (COMP3) represents a contrast between the coastal La Cienaga population (LC) and the inland population from Parque Nacional Jaragua (PNJ). All field work was conducted during July and August 2006.

DNA SEQUENCING AND ANALYSIS

Mitochondrial DNA (ND2) was amplified and sequenced from one individual for each population. A single individual was used because preliminary phylogeographical results indicated a lack of haplotype sharing among lineages (M. E. Gifford, unpubl. data.). ND2 was amplified using published (L4437; Gifford *et al.*, 2004) and species-specific polymerase chain reaction (PCR) primers (*L. personatus*, TYR.r1-5'-GTAGGCTGTAGTCCTATTTACTG-3'; *L. barahonensis*, ASN.r8-5'-TGTTGTGGGATCGAGGCCACCTA-3') under amplification conditions previously described by Gifford *et al.* (2004). PCR products were cycle-sequenced using Big Dye Terminator Ready-Reaction Kits (Perkin-Elmer) and visualized on an ABI 3130 automated sequencer. The absence of indel polymorphisms and premature stop codons resulted in unambiguous manual alignment of DNA sequences. GenBank accession numbers are provided in the Appendix.

MODELTEST 3.7 (Posada & Crandall, 1998) was used to determine an appropriate model of sequence evolution using the Aikake Information Criterion. Phylogenetic relationships among populations were estimated in PAUP* (Swofford, 1999) using maximum likelihood with the model of sequence evolution chosen by MODELTEST. We assessed node support using nonparametric bootstrapping (1000 pseudoreplicates; Felsenstein, 1985). All nodes received high bootstrap support (i.e. > 95%).

MEASUREMENTS OF HABITAT USE

Habitat data were recorded by starting at a randomly chosen location in the study site and moving back and

forth across the site to ensure complete coverage. Only animals that were spotted without being disturbed were included so that habitat associations could be confidently measured. To assess vegetative associations, we surveyed the study site for lizards and, for each individual observed, we recorded its sex and five measures of vegetative cover. For each animal, we recorded the following characteristics in a 2-m diameter circle around the animal: (1) percentage of shrub cover; (2) percentage of herbaceous cover; (3) percentage of canopy cover; (4) percentage of litter cover (fallen branches, leaves, and other vegetative debris); and (5) the distance to the nearest vegetation that could provide refuge measured to the nearest centimeter. All vegetative cover measurements, except canopy cover, were taken at ground level. Measures of percentage cover were based on visual estimates by the same two researchers (M. E. Gifford and D. L. Mahler). These methods are similar to those used by Melville & Swain (2000, 2003) and Schulte *et al.* (2004), and were chosen to maintain consistency across studies.

MEASUREMENTS OF PREDATION INTENSITY

Because, in open habitats, lizards may be more vulnerable to predation, one may expect to find differences reflected in indices used to estimate predation pressure. Two methods were used to quantify predation pressure in each population: tail-break frequency and the frequency of attack on clay model lizards. Because *Leiocephalus* lizards use a tail-curling behaviour in predator encounters (Schwartz & Henderson, 1991; Cooper, 2001), and because tail colour often contrasts with dorsal coloration, the frequency of tail breaks may represent a measure of predation pressure (but see also Schoener, 1979; Schoener & Schoener, 1980). The second method used clay lizard replicas cast from molds of preserved specimens. An alcohol-preserved specimen of each sex was first blotted dry with paper towels and then coated with a silicone-based molding product commonly used in the culinary industry (Silicone Plastique; Culinart, Inc.). The resulting molds were coated with a releasing agent (Universal Mold Release, Smooth-On, Inc.), and filled with a urethane plastic material (Smooth-Cast 325, Smooth-On, Inc.). After curing for approximately 20 min, an exact replica of the lizard specimen was produced. Each replica was coated with approximately 2 mm of modelling clay and painted with acrylic paint to visually match digital photographs of the species of interest. We deployed 40 models at each site for COMP1 and 20 models at each site for COMP3. We did not deploy models for COMP2 because one of the sites was located in a park (see Results). Models were left undisturbed in the field for

24 h. We are confident that our models were adequate representations of the species in nature as some field-recovered models had lizard-tooth impressions, most likely from conspecifics.

MORPHOLOGICAL VARIABLES

Morphological measurements taken from each individual included: snout-vent length (SVL), length of the femur (FEM), tibia (TIB), metatarsus on the hindlimb (METH), longest toe on the hindlimb (TOEH), humerus (HUM), radius (RAD), metacarpus on the forelimb (MECF), and longest toe on the forelimb (TOEF). Total length of the hindlimb (HL) and forelimb (FL) were represented by the sum of the hind- and forelimb elements, respectively. All morphological measurements were taken on live specimens by one of the authors (A.H.) using Mitutoyo digital calipers (± 0.01 mm).

MEASUREMENTS OF MAXIMUM SPRINT SPEED

All lizards used for the measurement of sprint speed were those used for morphological measurements. We recorded tail condition (i.e. intact, broken, or regenerated), for all animals, and gravidity (i.e. gravid or not), for females. Both of these variables are suggested to be correlated with decreased sprinting ability in some lizards (tail autotomy: Ballinger, Nietfeldt & Krupa, 1979; Punzo, 1982, but see Daniels, 1983; Brown, Taylor & Gist, 1995; gravidity: Sinervo, Hedges & Adolph, 1991), however, none showed significant effects in the present study (tail condition, $F_{2,205} = 1.118$, $P = 0.329$; gravidity, $F_{1,104} = 1.708$, $P = 0.194$). Therefore, we included performance measures from all animals in the analyses. We conducted performance trials at field-ambient temperatures of 29–33 °C, which is slightly lower than body temperatures measured in the field (32–37 °C; *L. personatus*: M. E. Gifford, unpubl. data; *L. barahonensis*: Micco *et al.*, 1997). The thermal sensitivity of sprint speed in these populations is unknown, so we recorded all measurements at comparable lizard body temperatures. To measure maximum sprint speeds we used a 2-m long electronic racetrack equipped with infrared photocells spaced every 25 cm. The track surface consisted of Styrofoam boards covered with cork to provide traction. We induced lizards to run by chasing them down the length of the track. Each individual was raced three times with at least 30 min of recovery between trials. Trials were scored as either 'good' or 'poor'; only 'good' trials were used to estimate maximum sprint speed. For each lizard, the fastest 25-cm interval among trials was recorded as the maximum sprint speed for that individual. Studies of other lizard species using a similar methodology

suggest that these measurements are highly repeatable (Huey & Dunham, 1987).

MEASUREMENTS OF ESCAPE BEHAVIOUR

For escape behaviour, the same person (M. E. Gifford) conducted all trials and attempted to wear similarly coloured clothing each day. When a lizard was spotted, it was approached, at a slow constant pace, until it fled. Only lizards that were undisturbed upon first sight were included. The distance between the person and the lizard before flight was measured, to the nearest cm, and termed the 'approach' distance. The distance the lizard fled before stopping was measured as the 'flight' distance. Means and standard errors for all habitat, morphological, performance, and behavioural measurements are included in Table 1.

DATA ANALYSIS

All statistical analyses were conducted using JMP IN, version 5.1 (SAS Institute) on transformed variables (\log_{10} for morphological and behavioural variables and distance to nearest vegetation; arcsine for habitat variables represented by proportions). The non-independence of data points imposed by the evolutionary relationships among populations violates the assumptions of traditional statistical procedures (Felsenstein, 1985). To control for this non-independence, we only made statistical comparisons between sister-populations.

All morphological measurements were correlated with body size (SVL) so statistical analyses were conducted on 'size-free' variables represented by the residuals from regressions of each \log_{10} -transformed variable on \log_{10} -SVL. Principal components analysis (PCA) on the correlation matrices was used to reduce both the morphological (size adjusted) and habitat (not size adjusted) variables to smaller sets of composite variables that describe the underlying structure of each data set. Only PC axes with eigenvalues greater than 1.0 were included in the following analyses (Jackson, 1993). We tested for differences in morphology (residuals), habitat use, and behaviour between sister-populations using two-way analysis of variance (ANOVA) with population ($N = 2$) and sex ($N = 2$) as fixed effects. Because maximum sprint speed increases with body size (SVL) among lizard species (Garland & Losos, 1994), we conducted analysis of covariance (ANCOVA) to test for differences in log-transformed sprint speed between populations while controlling for body size (log-transformed SVL). Differences in tail-break frequencies and clay model attacks between sister-populations were tested using chi-square or Fisher's exact tests.

RESULTS

PHYLOGENETIC ANALYSIS

Maximum likelihood produced a single tree with high bootstrap support (all nodes > 95%, Fig. 1). Consistent with preliminary results, we recovered three lineages each containing two populations. Figure 1 illustrates the qualitative differences in habitat structure within each lineage.

HABITAT USE

Table 1 provides summary statistics for habitat, behavioural, morphological, and performance variables. The PCA on habitat variables yielded three PC axes with eigenvalues greater than 1, which together explain 79.22% of the total variation. PC1 accounted for 33.15% of the variation and showed high positive loadings for percent shrub and high negative loadings for the distance to nearest vegetation (Table 2). The percentage of canopy and litter cover loaded positively on PC2 (25.91%, Table 2) and the percentage of herbaceous cover loaded positively on PC3 (20.16%, Table 2).

PREDATION INTENSITY

The proportion of lizards with broken or regenerated tails did not differ in any comparison (COMP1, MC = 60.5%, S = 61.5%; $\chi^2 = 0.01$, d.f. = 1,82, $P = 0.92$; COMP2, LT = 58.3%, S = 58.1%; $\chi^2 = 0.00$, d.f. = 1,79, $P = 0.98$; COMP3, LC = 65.2%, PNJ = 63.6%; $\chi^2 = 0.007$, d.f. = 1,56, $P = 0.90$), sexes, or sexes within populations. The proportion of clay models with evidence of bird attacks differed significantly in COMP1 (MC = 32.5%, S = 5.3%; $\chi^2 = 10.25$, d.f. = 1,78, $P = 0.001$) but did not differ significantly in COMP3 (PNJ = 5%, LC = 15.8%, $\chi^2 = 1.258$, d.f. = 1,39, $P = 0.28$). We did not deploy clay lizard models for COMP2 because the SD population is located in a public park that receives a high level of human traffic.

ANTIPREDATOR BEHAVIOUR

Escape behaviour showed significant population and interaction effects. All population pairs differed significantly in approach distances (COMP1, $N_{MC} = 13$, $N_S = 16$, $F_{1,29} = 31.0$, $P < 0.001$; COMP2, $N_{LT} = 18$, $N_{SD} = 20$, $F_{1,38} = 28.32$, $P < 0.001$; COMP3, $N_{LC} = 19$, $N_{PNJ} = 21$, $F_{1,40} = 22.05$, $P < 0.001$). Thus, lizards at coastal sites (S, LT, and LC) were significantly warier than lizards at inland sites (MC, SD, and PNJ). There was also a significant population by sex interaction effect in COMP3 ($F_{1,35} = 6.23$, $P < 0.05$), indicating that the magnitude of the effect of population and sex differed in each population. By contrast, flight distances only differed significantly in COMP1

Table 1. Morphometric, performance, habitat, and behavioural data for each population

Morphology/performance	<i>Leiocephalus personatus</i>					<i>Leiocephalus barahonensis</i>						
	N	MC	N	S	N	LT	N	SD	N	LC	N	PNJ
Snout-vent length	15	63.7 ± 1.40	22	65.3 ± 0.86	18	66.3 ± 0.92	24	63.3 ± 1.40	8	65.5 ± 1.99	16	73.7 ± 1.11
Femur	28	51.4 ± 0.77	17	52.3 ± 1.29	18	52.5 ± 1.03	19	53.7 ± 1.17	13	54.9 ± 0.85	17	59.4 ± 0.47
		11.3 ± 0.22		12.5 ± 0.19		13.5 ± 0.23		12.1 ± 0.32		13.6 ± 0.40		15.4 ± 0.31
Tibia		9.0 ± 0.22		9.4 ± 0.24		9.4 ± 0.17		9.4 ± 0.22		11.0 ± 0.22		11.5 ± 0.12
		13.4 ± 0.23		14.9 ± 0.19		14.8 ± 0.20		14.1 ± 0.31		15.9 ± 0.38		17.6 ± 0.24
Metatarsus		10.8 ± 0.23		11.7 ± 0.26		11.3 ± 0.18		11.5 ± 0.20		12.9 ± 0.22		13.9 ± 0.11
		8.4 ± 0.13		9.1 ± 0.15		9.3 ± 0.15		8.6 ± 0.16		9.7 ± 0.31		10.7 ± 0.12
Toe (hindlimb)		6.5 ± 0.14		7.1 ± 0.13		7.3 ± 0.07		7.1 ± 0.09		8.2 ± 0.11		8.6 ± 0.09
		11.6 ± 0.15		13.0 ± 0.16		12.7 ± 0.18		12.4 ± 0.22		14.7 ± 0.30		14.9 ± 0.16
Hindlimb		9.2 ± 0.13		10.4 ± 0.20		10.1 ± 0.15		10.6 ± 0.13		11.9 ± 0.14		12.3 ± 0.13
		44.7 ± 0.57		49.5 ± 0.57		50.3 ± 0.69		47.1 ± 0.95		53.9 ± 1.30		58.7 ± 0.70
Humerus		35.5 ± 0.68		38.5 ± 0.75		38.1 ± 0.50		38.6 ± 0.58		44.1 ± 0.58		46.2 ± 0.35
		9.3 ± 0.17		10.0 ± 0.12		10.4 ± 0.15		9.6 ± 0.23		10.9 ± 0.25		12.2 ± 0.16
Radius		7.5 ± 0.11		7.9 ± 0.17		7.9 ± 0.11		7.9 ± 0.14		8.8 ± 0.17		9.5 ± 0.12
		8.4 ± 0.14		9.0 ± 0.12		8.9 ± 0.13		8.4 ± 0.20		9.6 ± 0.25		10.3 ± 0.16
Metacarpus		6.6 ± 0.13		6.9 ± 0.16		6.8 ± 0.09		6.9 ± 0.12		7.7 ± 0.11		8.1 ± 0.07
		3.0 ± 0.08		3.1 ± 0.06		3.2 ± 0.06		3.1 ± 0.05		3.2 ± 0.11		3.6 ± 0.06
Toe (forelimb)		2.5 ± 0.05		2.5 ± 0.07		2.6 ± 0.04		2.7 ± 0.05		2.7 ± 0.08		3.0 ± 0.06
		5.2 ± 0.09		5.6 ± 0.07		5.5 ± 0.09		5.3 ± 0.10		6.3 ± 0.08		6.4 ± 0.09
Forelimb		4.2 ± 0.06		4.6 ± 0.09		4.5 ± 0.06		4.7 ± 0.07		5.4 ± 0.09		5.4 ± 0.07
		25.8 ± 0.39		27.8 ± 0.30		28.0 ± 0.39		26.4 ± 0.54		29.9 ± 0.64		32.6 ± 0.37
Sprint speed (cm*s ⁻¹)	13	20.7 ± 0.31	22	21.9 ± 0.39	16	21.7 ± 0.25	24	22.3 ± 0.33	8	24.6 ± 0.34	16	26.0 ± 0.24
	25	199.8 ± 8.92	16	229.5 ± 7.49	18	209.9 ± 7.18	17	216.6 ± 8.92	13	205.9 ± 11.71	16	209.7 ± 5.73
Habitat		152.8 ± 4.87		213.9 ± 9.95		159.0 ± 5.00		193.9 ± 11.8		192.7 ± 8.85		180.6 ± 8.74
% shrub cover	10	26.5 ± 6.10	11	7.5 ± 4.24	10	1.5 ± 0.50	10	0.0 ± 0.00	12	1.7 ± 0.94	11	16.1 ± 4.19
% herbaceous cover	11	40.5 ± 3.33	12	9.6 ± 4.01	10	0.6 ± 0.50	11	0.0 ± 0.00	13	3.1 ± 1.75	10	17 ± 4.29
% canopy cover		7.1 ± 2.32		11 ± 3.80		16.9 ± 8.93		17 ± 4.16		15.8 ± 5.22		31.4 ± 7.87
% litter cover		15 ± 3.09		15.1 ± 5.15		15.8 ± 9.64		16.4 ± 2.44		9.8 ± 3.61		29.7 ± 8.03
Distance to nearest vegetation (cm)		22.8 ± 7.04		3.6 ± 3.63		22.2 ± 8.71		30.5 ± 6.34		5 ± 2.61		49.1 ± 4.76
Escape behaviour		19.1 ± 5.91		0.4 ± 0.42		31 ± 9.77		38.2 ± 7.78		10.4 ± 6.16		31 ± 6.90
Approach distance (cm)	5	17 ± 6.33	8	10.1 ± 3.46	8	22.3 ± 7.70	10	19.2 ± 4.24	10	9.1 ± 2.29	9	16.8 ± 4.78
	7	23.4 ± 6.34	9	5.9 ± 1.42	9	38.7 ± 7.23	11	22.3 ± 4.34	12	23.6 ± 5.36	9	12 ± 3.35
		56.5 ± 9.26		186.5 ± 44.44		143.3 ± 35.26		302.5 ± 38.27		141.7 ± 25.06		79.54 ± 15.47
		67.1 ± 8.57		310.4 ± 103.16		114.7 ± 22.84		258.8 ± 54.76		179.6 ± 41.07		76 ± 26.81
Predation intensity		135.8 ± 35.61	8	481.3 ± 94.42	8	324.5 ± 56.91	10	182 ± 25.38	10	767 ± 104.58	9	242.8 ± 26.75
Tail-break frequency	28	143 ± 25.88	9	402.8 ± 51.94	9	366.1 ± 28.57	11	115.1 ± 21.88	12	399.2 ± 51.43	9	274.4 ± 41.42
Clay models	40	97 ± 32.86	40	162.5 ± 66.27	18	100.3 ± 35.42	19	73.8 ± 27.28	20	99 ± 35.32	20	85.6 ± 23.22
		33.9 ± 6.760		137.5 ± 30.98		93.6 ± 24.21		80.3 ± 24.12		46.8 ± 9.2		45.6 ± 10.78
	15	0.60	22	0.68	18	0.50	24	0.54	8	0.63	16	0.69
	28	0.61	17	0.53	18	0.67	19	0.63	15	0.67	17	0.59
	40	0.325	40	0.053					20	0.158	20	0.05

For each variable, the top row includes data for males and the bottom row includes data for females. Sample sizes are given in the first entry for each data set. Table entries are means ± standard error. All measurements in mm.

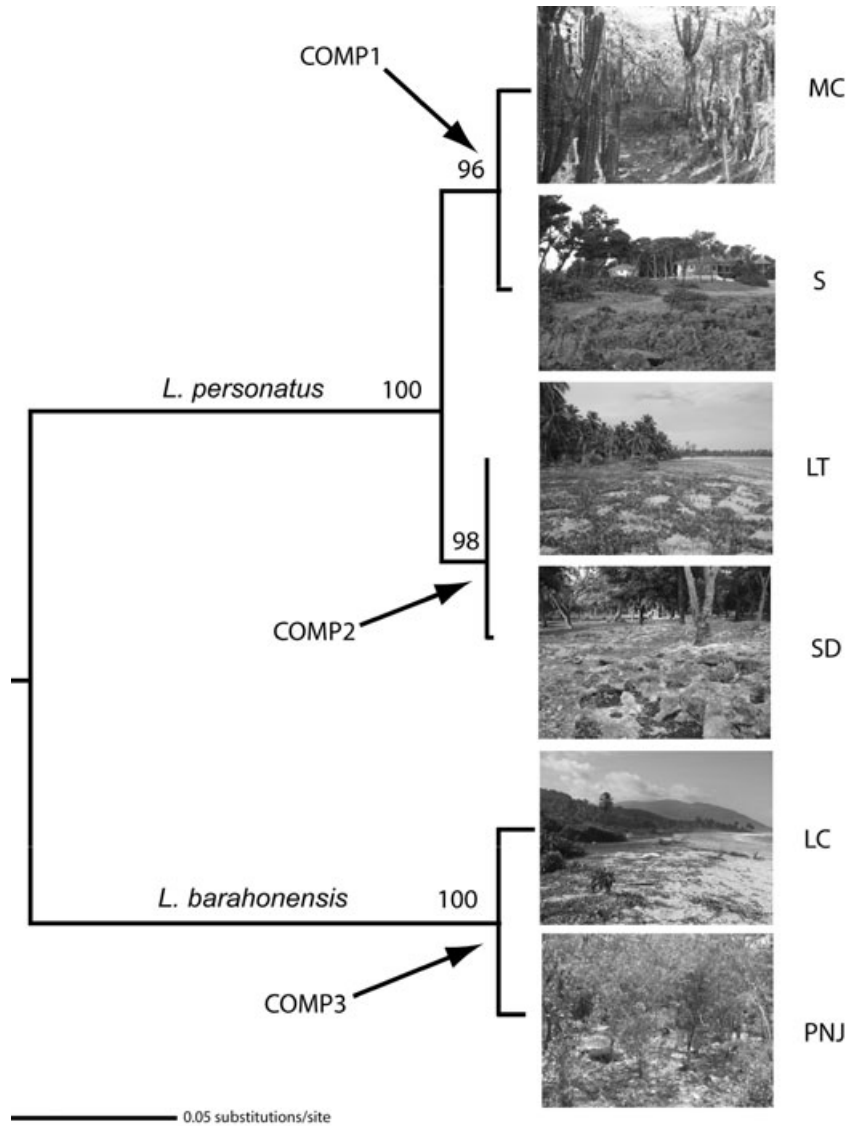


Figure 1. Phylogram of the relationships among *Leiocephalus* populations produced using maximum likelihood and the TVM + I model of sequence evolution. Numbers above branches represent nonparametric bootstrap values calculated using 1000 pseudoreplicates. Photographs indicate the degree of vegetative cover at each site. COMP1: MC, Monte Cristi (inland); S, Sosua (coastal); COMP2: LT, Las Terrenas (coastal); SD, Santo Domingo (inland); COMP3: LC, La Cienaga (coastal); PNJ, Parque Nacional Jaragua (inland).

($F_{1,29} = 8.65$, $P < 0.01$), indicating that lizards on the coast (S) fled a further distance than those at the inland site (MC). Sexes did not differ in either approach or flight distances in any comparison.

MORPHOLOGY

Body size (SVL) did not differ significantly between population pairs in *Leiocephalus personatus* (COMP1, males, $N_{MC} = 15$, $N_S = 22$, $F_{1,37} = 1.277$, $P = 0.266$, females, $N_{MC} = 28$, $N_S = 17$, $F_{1,45} = 0.286$, $P = 0.595$; COMP2, males, $N_{LT} = 18$, $N_{SD} = 24$, $F_{1,42} = 3.043$,

$P = 0.09$, females, $N_{LT} = 18$, $N_{SD} = 19$, $F_{1,37} = 0.449$, $P = 0.507$), but SVL of both sexes of *L. barahonensis* differed between populations (COMP3, males, $N_{LC} = 8$, $N_{PNJ} = 16$, $F_{1,24} = 14.986$, $P = 0.0008$, females, $N_{LC} = 15$, $N_{PNJ} = 17$, $F_{1,32} = 20.299$, $P < 0.0001$). In the latter, lizards at the inland site (PNJ) are larger than lizards on the coast (LC). The PCA on size adjusted morphological data resulted in a single axis with an eigenvalue greater than 1 explaining 69.7% of the variation (Table 3). All but one limb element (the metacarpus) loaded strongly and positively on this axis, therefore positive PC1 scores indicate longer limb elements.

Table 2. Eigenvalues, loadings, and percentage of variance explained for principal component axes retained from analysis of habitat variables

Variable	PC1	PC2	PC3
Eigenvalue	1.658	1.296	1.008
Shrub cover	0.749	-0.270	-0.436
Herbaceous cover	0.503	0.131	0.753
Canopy cover	0.278	0.798	0.151
Litter cover	-0.112	0.754	-0.447
Distance of nearest vegetation	-0.868	0.002	0.167
% variance explained	33.150	25.910	20.160

Substantial loadings are in bold. For statistical details, see text.

Table 3. Eigenvalues, loadings, and the percentage of variance explained for the principal component axes retained from analysis of size-adjusted morphological variables

Variable	PC1	PC2
Eigenvalue	6.969	0.889
Femur	0.807	-0.105
Tibia	0.889	-0.169
Metatarsus	0.836	-0.054
Toe (hindlimb)	0.878	-0.031
Hindlimb	0.975	-0.096
Humerus	0.799	-0.016
Radius	0.832	-0.157
Metacarpus	0.428	0.890
Toe (forelimb)	0.823	0.049
Forelimb	0.958	0.129
% variance explained	69.690	8.900

Substantial loadings are in shown in bold.

ANALYSIS OF HABITAT USE AND MORPHOLOGY

ANOVA results for habitat and morphological data are presented in Table 4. A comparison of habitat data between populations indicates significant population and interaction effects. PC1 differed significantly in COMP1 and COMP3. The habitat at the inland sites (MC and PNJ) contained a higher percentage of shrub cover and a shorter distance to the nearest vegetation than the habitat at coastal sites (S and LC, Fig. 2A). PC1 did not differ in COMP2. PC2 only differed significantly and showed a significant population by sex interaction effect in COMP3. Variation on this axis suggests that the inland site contains a higher percentage of canopy and litter cover than the coastal site. Habitat PC3 only differed significantly in COMP1; the inland site was characterized by less

Table 4. Results of analysis of variance (ANOVA) comparing populations within lineages for the principal component axes from habitat and morphological analyses

	Population	Sex	Population × Sex
Habitat			
COMP1			
PC1	21.81***	0.72	0.24
PC2	3.69	0.24	0.10
PC3	27.13***	0.018	1.02
COMP2			
PC1	2.86	1.08	0.33
PC2	0.28	3.57	0.68
PC3	1.45	0.59	0.11
COMP3			
PC1	22.25***	0.07	0.08
PC2	8.56**	0.03	7.40**
PC3	2.87	1.70	0.69
Morphology			
COMP1			
PC1	36.63***	0.91	0.13
COMP2			
PC1	0.71	2.62	0.15
COMP3			
PC1	13.74***	2.31	0.49

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table entries represent F -values from two-way ANOVAs with population ($N = 2$) and sex ($N = 2$) as fixed effects.

herbaceous cover than the coastal site. The only significant interaction effect (population by sex) was for PC2, suggesting that males at the coastal site tended to occupy areas with less canopy and litter cover than females whereas at the inland site males tended to occupy habitats with more canopy and litter cover than females.

For morphology, PC1 differed significantly in COMP1 and COMP3. Thus, lizards at inland sites have significantly shorter limb elements than lizards at coastal sites (Fig. 2B). PC1 did not differ significantly in COMP2. A phylogenetic effect is evident in the morphology data such that *Leiocephalus barahonensis* tend to have longer limbs than *L. personatus*. Despite this interspecific variation, the pattern of variation observed within species is consistent (i.e. lizards in coastal habitats tend to have longer limbs than those in inland habitats; Fig. 2).

SPRINT SPEED PERFORMANCE

ANCOVA results comparing \log_{10} -transformed sprint speed and \log_{10} -transformed SVL for each pair of populations showed no significant differences in slopes (COMP1, $F_{1,77} = 0.31$, $P = 0.587$; COMP2, $F_{1,75} = 2.74$, $P = 0.102$; COMP3, $F_{1,53} = 0.36$, $P = 0.55$),

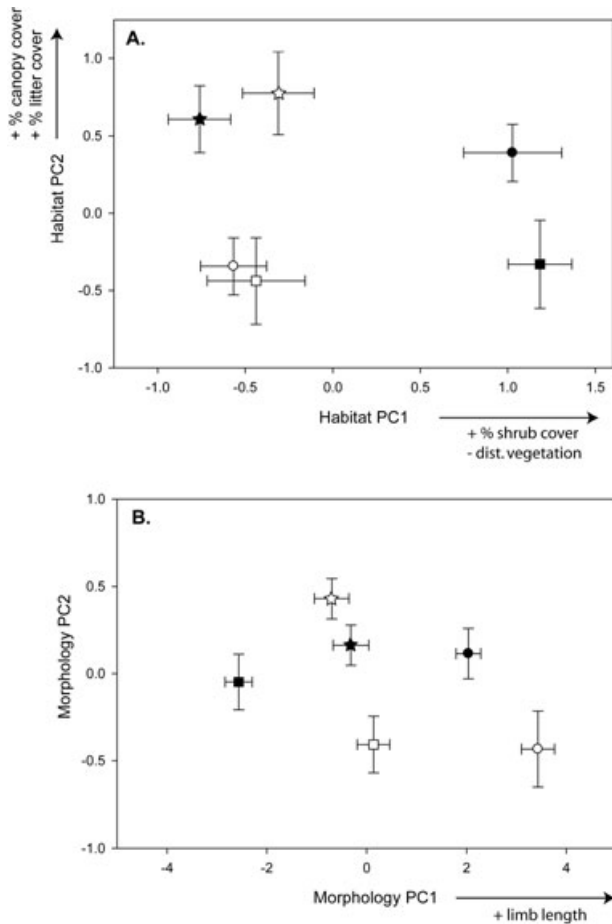


Figure 2. Bivariate plots of the principal components analysis for habitat (A) and morphology (B) data. PC2 in (B) (y-axis) represents metacarpus length (MEC) but was not included in statistical analyses because it had an eigenvalue less than 1 (nonsignificant based on the Broken Stick method). Symbols represent means \pm standard error. Boxes represent COMP1 (S-MC, Sosua-Monte Cristi), stars represent COMP2 (SD-LT, Santo Domingo-Las Terrenas), and circles represent COMP3 (LC-PNJ, La Cienaga-Parque Nacional Jaragua). Filled symbols indicate inland sites and open symbols coastal sites.

but showed significant differences in intercepts in COMP1 ($F_{1,77} = 37.18$, $P < 0.0001$) and COMP2 ($F_{1,75} = 6.71$, $P = 0.012$). There was no significant difference in intercept in COMP3 ($F_{1,53} = 0.93$, $P = 0.34$), although the intercept for the coastal population was higher than that for the inland population. Consequently, for a given body size, lizards from the coastal populations S and LC sprint faster (for their body size) than lizards from the inland populations MC and PNJ, respectively; the latter not being significant (Fig. 3A, C). By contrast, in COMP2, lizards in the inland population (SD) sprint faster than lizards from the coastal population (LT) (Fig. 3B).

DISCUSSION

Evolutionary biologists have long been interested in the adaptive nature of phenotypic diversity. To study this, researchers generally compare species that exist under putatively different selective pressures. This approach has been particularly effective when similar patterns of phenotypic variation are associated with similar patterns of selective pressures across species (Losos & Miles, 1994). With respect to lizards, the 'openness' of a habitat (i.e. the degree of vegetative cover experienced by a species) has been suggested to impose different selective pressures related to mortality. In particular, lizards with certain trait combinations may be more vulnerable to predation in open habitats and hence those with traits conferring increased predator avoidance capacity should be favoured (Bauwens *et al.*, 1995). Because natural selection is thought to act most strongly on whole animal performance capacity, adaptation to open environments was predicted to involve increased sprint speeds (Garland & Losos, 1994; Melville & Swain, 2000).

Although few studies have explicitly examined this hypothesis (i.e. 'habitat openness') in lizards, those that have were focused at the species level (Pianka, 1969; Jaksic & Núñez, 1979; Jaksic, Núñez & Ojeda, 1980; Melville & Swain, 2000; Schulte *et al.*, 2004). However, to our knowledge, no studies have examined this hypothesis in multiple closely-related populations. Our study provides evidence that the amount of vegetative cover in a lizard's habitat influences the evolution of traits associated with predator evasion capacity at the *population level*.

HABITAT USE AND PREDATION

In *Leiocephalus* lizards from the Dominican Republic, habitat use differed among coastal and inland sites within three phylogenetically independent comparisons, although not significantly in the Las Terrenas-Santo Domingo comparison (COMP2). Each comparison consisted of a pair of habitats that differed with respect to the abundance of low lying vegetative cover (i.e. percentage of shrub cover) and the proximity of the nearest vegetation that could serve as refuge from an approaching predator. Areas with a low percentage of vegetative cover and a low proximity to refuges constitute 'open' habitats. It is generally suggested that, in more open habitats, predation intensity may be greater than in closed habitats. Overall, the data presented in this study do not support this assumption. We found that predation intensity, indexed by tail-break frequencies, did not differ between any pair of populations. This may suggest that predation intensity is invariant across

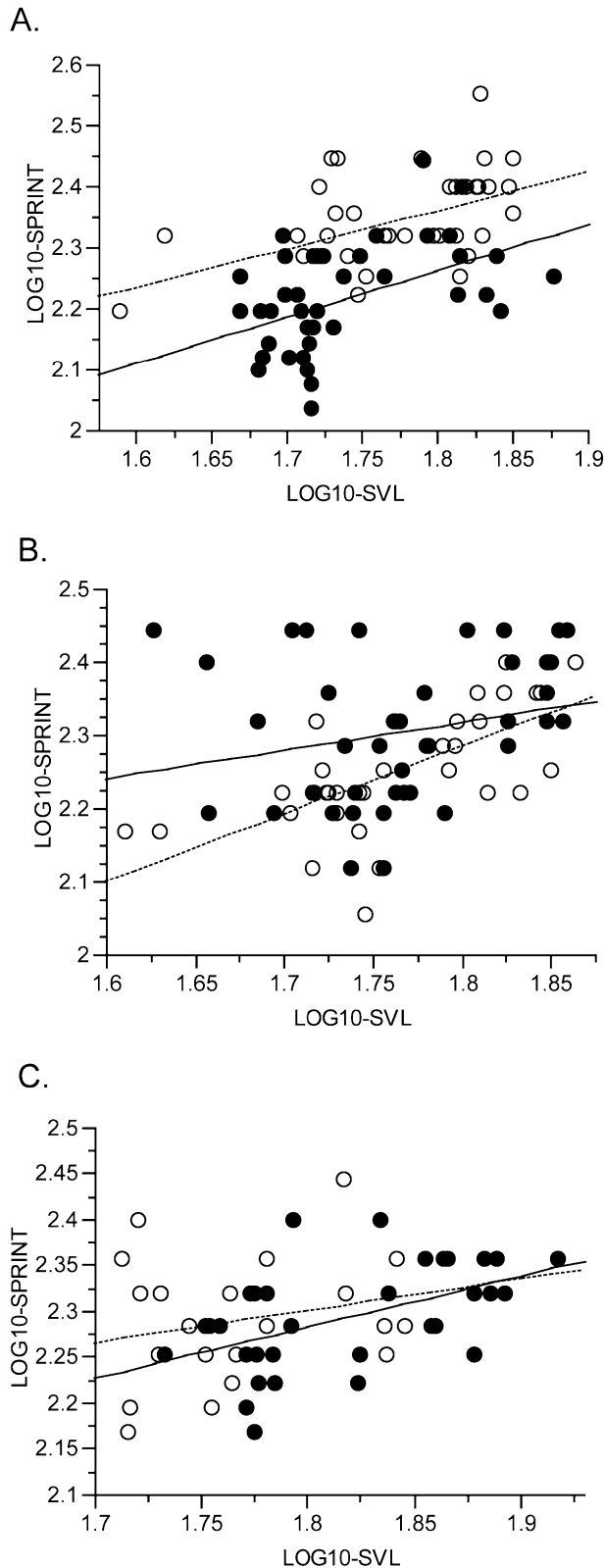


Figure 3. Bivariate plots of \log_{10} -transformed sprint speed ($\text{LOG}_{10}\text{-SPRINT}$) against \log_{10} -transformed snout-vent length ($\text{LOG}_{10}\text{-SVL}$) for each comparison: (A) COMP1, (B) COMP2, (C) COMP3. In each plot, open circles represent coastal populations and filled circles represent inland populations. Dashed and solid lines represent ordinary least-squares regressions for coastal and inland populations, respectively.

sites, or that other causes of injury (e.g. agonistic interactions) are frequent in these populations (Schoener, 1979). Furthermore, predation intensity could differ between sites but the faster animals in open habitats may escape predation attempts more readily, and hence not exhibit a higher frequency of tail breaks. Data from clay lizard replicas offer mixed results with respect to predation intensity. In the case of COMP1, model data suggest that lizards in the inland, closed habitat (MC) actually experience higher predation pressure. Conversely, in COMP3, there is no significant difference in predation pressure between populations, although a larger proportion of models were attacked in the coastal, open habitat (15.8% at LC versus 5% at PNJ). One likely reason for this discrepancy is that, in COMP1, the inland site contains a large number of human commensals (domestic chickens) that are absent from all other sites. It appeared that chickens did account for a number of recorded predation events, evidenced by 'peck marks' on clay models rather than triangular bite marks, characteristic of predatory avian attacks (Brodie, 1993). In summary, these results suggest that there is no striking difference in predation pressure between open and cluttered habitats. Even if predation occurs at similar ambient levels in all habitats, it may have a different selective effect on locomotion and behaviour in open versus cluttered habitats.

ASSOCIATION OF BEHAVIOUR, MORPHOLOGY, AND PERFORMANCE WITH HABITAT

Consistent with interspecific studies and predictions of local adaptation, in two of three phylogenetically independent population comparisons, lizards in more open habitats have longer limbs, run at faster relative sprint speeds, and have longer approach distances. These two sets of populations represent two different species, suggesting that the trait correlations have evolved independently in each. In COMP2 (LT-SD), lizards differ neither in habitat use nor limb morphology but do differ in escape behaviour. Hence, all coastal populations exhibit longer approach distances.

Although some studies suggest faster lizards allow closer approach of a putative predator (i.e. human or

bird model; Vanhooydonck & Van Damme, 2003), lizards in the present study tended to show larger approach distances in open habitats relative to closed habitats (i.e. faster lizards had larger approach distances); a pattern consistent with that observed in two species of North American sand lizards (Bulova, 1994). For lizards that rely on fleeing as a predator avoidance mechanism, this strategy may provide extra time for effective escape, which would be beneficial in open habitats where lizards are usually a greater distance from refuge sites.

TRAIT LIABILITY AND THE RATE OF MORPHOLOGICAL CHANGE

The finding that closely-related populations in two species of *Leiocephalus* lizards differ in a manner consistent with expectations of local adaptation suggests that these traits have evolved at a rate exceeding genetic divergence. Vitt *et al.* (1997) reached a similar conclusion in their study of two populations of *Tropidurus* lizards. They found patterns of morphological differentiation were associated with a divergence in habitat occupation among two populations of *Tropidurus hispidus* in Brazil that differed by less than 2% sequence divergence. Although we cannot confidently estimate divergence times among populations with the few molecular data presented here, it is clear that trait variation has evolved very rapidly despite recent or ongoing gene flow (approximately 1% average uncorrected sequence divergence between haplotypes of sister populations). Further phylogeographical analyses are needed to better address this question.

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REFERENCES

- Ballinger RE, Nietfeldt JW, Krupa JJ. 1979.** An experimental analysis of the tail in attaining high speed in *Cnemidophorus sexlineatus* (Reptilia; Squamata; Lacertilia). *Herpetologica* **35**: 114–115.
- Baum DA, Larson A. 1991.** Adaptation reviewed: a phylogenetic methodology for studying character macroevolution. *Systematic Zoology* **40**: 1–18.
- Bauwens D, Garland T Jr, Castilla AM, Van Damme R. 1995.** Evolution of sprint speed in lacertid lizards: morphological, physiological, and behavioral covariation. *Evolution* **49**: 848–863.
- Brodie EDIII. 1993.** Differential avoidance of coral snake banded patterns by free-ranging avian predators in Costa Rica. *Evolution* **47**: 227–235.
- Brown RM, Taylor DH, Gist DH. 1995.** The effect of caudal autotomy on locomotor performance of wall lizards (*Podarcis muralis*). *Journal of Herpetology* **29**: 98–105.
- Bulova SJ. 1994.** Ecological correlates of population and individual variation in antipredator behavior of two species of desert lizards. *Copeia* **1994**: 980–992.
- Cooper WE Jr. 2001.** Multiple roles of tail display by the curly-tailed lizard *Leiocephalus carinatus*: pursuit deterrent and deflective roles of a social signal. *Ethology* **107**: 1137–1149.
- Daniels CB. 1983.** Running: an escape strategy enhanced by autotomy. *Herpetologica* **39**: 162–165.
- Elstrott J, Irschick DJ. 2004.** Evolutionary correlations among morphology, habitat use and clinging performance in Caribbean *Anolis* lizards. *Biological Journal of the Linnean Society* **83**: 389–398.
- Endler JA. 1977.** *Geographyraphic variation, speciation, and clines*. Princeton, NJ: Princeton University Press.
- Felsenstein J. 1985.** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.
- Garland T Jr, Losos JB. 1994.** Ecological morphology of locomotor performance in squamate reptiles. In: Wainwright PC, Reilly SM, eds. *Ecological morphology, integrative organismal biology*. Chicago, IL: The University of Chicago Press, 240–302.
- Gifford ME, Powell R, Larson A, Gutberlet RL Jr. 2004.** Population structure of a phenotypically variable teiid lizard (*Ameiva chrysolema*) from Hispaniola: the influence of a geologically complex island. *Molecular Phylogenetics and Evolution* **32**: 735–748.
- Harvey PH, Pagel MD. 1991.** *The comparative method in evolutionary biology*. Oxford: Oxford University Press.
- Herrel A, Meyers JJ, Vanhooydonck B. 2001.** Correlations between habitat use and body shape in a phrynosomatid lizard (*Urosaurus ornatus*): a population-level analysis. *Biological Journal of the Linnean Society* **74**: 305–314.
- Herrel A, Meyers JJ, Vanhooydonck B. 2002.** Relations between microhabitat use and limb shape in phrynosomatid lizards. *Biological Journal of the Linnean Society* **77**: 149–163.
- Huey RB, Dunham AE. 1987.** Repeatability of locomotor

- performance in natural populations of the lizard *Sceloporus merriami*. *Evolution* **41**: 1116–1120.
- Irschick DJ, Carlisle E, Elstrott J, Ramons M, Buckley C, Vanhooydonck B, Meyers JJ, Herrel A. 2005.** A comparison of habitat use, morphology, clinging performance, and escape behavior among two divergent green anole lizard (*Anolis carolinensis*) populations. *Biological Journal of the Linnean Society* **85**: 223–234.
- Jackson DA. 1993.** Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. *Ecology* **74**: 2204–2214.
- Jaksic FM, Núñez H. 1979.** Escaping behavior and morphological correlates in two *Liolaemus* species of central Chile (Lacertilia: Iguanidae). *Oecologia* **42**: 119–122.
- Jaksic FM, Núñez H, Ojeda FP. 1980.** Body proportions, microhabitat selection, and adaptive radiation of *Liolaemus* lizards in central Chile. *Oecologia* **45**: 178–181.
- Langerhans RB, DeWitt TJ. 2004.** Shared and unique features of evolutionary diversification. *American Naturalist* **164**: 335–349.
- Losos JB. 1990.** Ecomorphology, performance capability, and scaling of West Indian *Anolis* lizards: an evolutionary analysis. *Ecological Monographs* **60**: 369–388.
- Losos JB, Miles DB. 1994.** Adaptation, constraint, and the comparative method: phylogenetic issues and methods. In: Wainwright PC, Reilly SM, eds. *Ecological morphology, integrative organismal biology*. Chicago, IL: The University of Chicago Press, 60–98.
- Melville J, Swain R. 2000.** Evolutionary relationships between morphology, performance, and habitat openness in the lizard genus *Niveoscincus* (Scincidae: Lygosominae). *Biological Journal of the Linnean Society* **70**: 667–683.
- Melville J, Swain R. 2003.** Evolutionary correlations between escape behaviour and performance ability in eight species of snow skinks (*Niveoscincus*: Lygosominae) from Tasmania. *Journal of Zoology, London* **261**: 79–89.
- Micco SM, Lahey GJ, Sosa RA, Powell R, Censky EJ, Parmerlee JS Jr. 1997.** Natural history of *Leiocephalus barahonensis* (Tropiduridae) on the Península de Barahona, Hispaniola: an examination of two populations. *Herpetological Natural History* **5**: 147–156.
- Miles DB, Snell HL, Snell HM. 2001.** Intrapopulation variation in endurance of Galapagos lava lizards (*Microplophus albemarlensis*): evidence for an interaction between natural and sexual selection. *Evolutionary Ecology Research* **3**: 795–804.
- Pianka ER. 1969.** Sympatry of desert lizards (*Ctenotus*) in Western Australia. *Ecology* **50**: 1012–1030.
- Posada D, Crandall KA. 1998.** ModelTest: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.
- Punzo F. 1982.** Tail autotomy and running speed in the lizards *Cophosaurus texanus* and *Uma notata*. *Journal of Herpetology* **16**: 329–331.
- Robinson BW, Wilson DS. 1994.** Character release and displacement in fishes: a neglected literature. *American Naturalist* **144**: 596–627.
- Rose MR, Lauder GV. 1996.** *Adaptation*. San Diego, CA: Academic Press.
- Schluter D. 2000.** *The ecology of adaptive radiation*. New York, NY: Oxford University Press.
- Schoener TW. 1979.** Inferring the properties of predation and other injury-producing agents from injury frequencies. *Ecology* **60**: 1110–1115.
- Schoener TW, Schoener A. 1980.** Ecological and demographic correlates of injury rates in some Bahamian *Anolis* lizards. *Copeia* **4**: 839–850.
- Schulte JA II, Losos JB, Cruz FB, Núñez H. 2004.** The relationship between morphology, escape behaviour and microhabitat occupation in the lizard clade *Liolaemus* (Iguanidae: Tropidurinae*: Liolaemini). *Journal of Evolutionary Biology* **17**: 408–420.
- Schwartz A, Henderson RW. 1991.** *Amphibians and reptiles of the west indies: descriptions, distributions, and natural history*. Gainesville, FL: University of Florida Press.
- Sinervo B, Hedges R, Adolph SC. 1991.** Decreased sprint speed as a cost of reproduction in the lizard *Sceloporus occidentalis*: variation among populations. *Journal of Experimental Biology* **155**: 323–336.
- Snell HL, Jennings RD, Snell HM, Harcourt S. 1988.** Intrapopulation variation in predator-avoidance performance of Galápagos lava lizards: the interaction of sexual and natural selection. *Evolutionary Ecology* **2**: 353–369.
- Swofford DL. 1999.** *PAUP*: phylogenetic analysis using parsimony (* and other methods)*, Version 4.0. Sunderland, MA: Sinauer.
- Van Damme R, Aerts P, Vanhooydonck B. 1997.** No trade-off between sprinting and climbing in two populations of the lizard *Podarcis hispanica* (Reptilia: Lacertidae). *Biological Journal of the Linnean Society* **60**: 493–503.
- Van Damme R, Aerts P, Vanhooydonck B. 1998.** Variation in morphology, gait characteristics, and speed of locomotion in two populations of lizards. *Biological Journal of the Linnean Society* **63**: 409–427.
- Vanhooydonck B, Van Damme R. 2003.** Relationships between locomotor performance, microhabitat use and anti-predator behaviour in lacertid lizards. *Functional Ecology* **17**: 160–169.
- Vanhooydonck B, Van Damme R, Aerts P. 2002.** Variation in speed, gait characteristics and microhabitat use in lacertid lizards. *Journal of Experimental Biology* **205**: 1037–1046.
- Vitt LJ, Caldwell JP, Zani PA, Titus TA. 1997.** The role of habitat shift in the evolution of lizard morphology: evidence from tropical tropidurids. *Proceedings of the National Academy of Sciences of the United States of America* **98**: 3828–3832.

APPENDIX

GenBank accession numbers for samples used in the present study.

Sample and population affiliation	GenBank accession number
MEG104 – Monte Cristi	EF591769
MEG153 – Sosua	EF591770
MEG252 – Las Terrenas	EF591771
MEG308 – Santo Domingo	EF591772
MEG520 – La Cienaga	EF591773
MEG24 – Parque Nacional Jaragua	EF591774

MEG, field tag numbers for samples collected by the lead author.