Contributed Paper

The Contribution of Habitat Loss to Changes in Body Size, Allometry, and Bilateral Asymmetry in Two *Eleutherodactylus* Frogs from Puerto Rico

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Abstract: *Amphibian populations have been declining worldwide and the exact mechanisms underlying these changes are not well understood. We examined environmentally induced phenotypic changes that may reflect ongoing stresses on individuals and therefore their ability to persist in increasingly changing landscapes. Specifically, we evaluated the contribution of habitat loss on the size, allometry, and levels of fluctuating asymmetry of* Eleutherodactylus antillensis *and* E. coqui, *2 common species that are endemic to Puerto Rico. We x-rayed frogs collected at 9 sites that differed in the amount of forest cover and measured their snout-vent, radio-ulna, femur, and tibio-fibula lengths.* E. antillensis *and* E. coqui *were smaller in the highly disturbed (≤20% forest cover) than in the intermediately (20–70% forest cover) and little-disturbed (≥70% forest cover) landscapes. In* E. antillensis *but not in* E. coqui, *the slope and intercept of the curves relating snout-vent length with the length of the 3 bones differed with degree of forest cover, suggesting an effect of habitat loss on body shape. In* E. antillensis *and* E. coqui, *differences between right and left sides corresponded to true fluctuating asymmetry; however, only the radio-ulna length of* E. coqui *showed a trend toward an increase in fluctuating asymmetry with habitat loss. Because body size scales with a variety of physiological, life history, and ecological traits, conservation programs aimed at monitoring morphological changes in amphibians may help in understanding the mechanisms that contribute to their persistence in changing environments.*

Keywords: allometry, amphibians, body size, fluctuating asymmetry, Greater Antilles, habitat loss, phenotypic variation, Puerto Rico

La Contribución de la Pérdida de Hábitat a Cambios en el Tamaño Corporal, Alometría y Asimetría Bilateral en Dos Ranas *Eleutherodactylus* de Puerto Rico

Resumen: *Las poblaciones de anfibios han declinado mundialmente y los mecanismos subyacentes en estos cambios no son bien conocidos. Examinamos cambios fenotípicos inducidos ambientalmente que pueden reflejar el estrés continuo sobre individuos y por lo tanto su habilidad para persistir en paisajes en cambio constante. Específicamente, evaluamos la contribución de la pérdida de hábitat sobre el tamaño, la alometría y los niveles de asimetría fluctuante de* Eleutherodactylus antillensis *y* E. coqui, *dos especies comunes endémicas a Puerto Rico. Tomamos radiografías a ranas recolectadas en 9 sitios que difieren en la cantidad de cobertura forestal y medimos sus longitudes hocico-cloaca, radio-ulna, fémur y tibia-fíbula.* E. antillensis *y* E. coqui *fueron más pequeños en los paisajes muy perturbados (≤20% de cobertura forestal) que en los paisajes con perturbación intermedia (20–70% de cobertura forestal) y que en los poco perturbados (≥70% de cobertura forestal). En* E. antillensis *pero no en* E. coqui, *la pendiente y la intercepción de las curvas que relacionan la longitud hocico-cloaca con la longitud de los 3 huesos difirió con el grado de cobertura forestal, lo que sugiere un efecto de la pérdida de hábitat sobre la forma del cuerpo. En* E. antillensis *y* E. coqui, *las diferencias entre los flancos derecho e izquierdo correspondieron a una asimetría fluctuante real; sin embargo, sólo la longitud*

del radio-ulna de E. coqui *mostró una tendencia hacia el incremento de la asimetría fluctuante con la pérdida de hábitat. Debido a que el tamaño corporal incrementa con una variedad de características fisiológicas, de historia de vida y ecológicas, los programas de conservación enfocados a monitorear los cambios morfológicos de anfibios pueden ayudar a entender los mecanismos que contribuyen a su persistencia en ambientes cambiantes.*

Palabras Clave: alometría, anfibios, Antillas Mayores, asimetría fluctuante, pérdida de hábitat, Puerto Rico, tamaño corporal, variación fenotípica

Introduction

A recent worldwide assessment of amphibian populations provided unequivocal evidence that this group of vertebrates is declining rapidly (Stuart et al. 2004). Nearly 8% of the known species of amphibians are listed as threatened, and the conservation status of most species has changed since earlier assessments (Baillie et al. 2004). The underlying environmental causes of these rapid changes include habitat loss, overexploitation, and unknown causes (Stuart et al. 2004; Mendelson et al. 2006). Yet the exact mechanisms underlying changes in amphibian populations remain little understood. One way to investigate the mechanisms behind these changes is to focus on phenotypic changes that can affect the performance of individuals and therefore their ability to persist in increasingly variable environments.

Phenotypic changes in amphibians may be adaptive or induced environmentally. Body size and the overall shape or allometry of amphibians varies in predictable ways along environmental gradients, which reflects to some extent an adaptive response to extreme climatic conditions, shortages of food, and predation (e.g., Lee 1993; Cummins & Swan 1995; Morrison et al. 2004; Phillips et al. 2006). Likewise, the occurrence of deformities (Loeffler et al. 2001; Blaustein & Johnson 2003) and subtle differences in the allometry (McCollum & Leimberger 1997) and symmetry of bilateral traits (Chubinishvili 1998; Wright & Zamudio 2002; Alford et al. 2007) may be induced during the development of amphibians in response to human-induced environmental stresses. The fitness and survival costs associated with these subtle morphological changes may affect amphibian populations on a long-term basis.

The contribution of habitat loss, in particular forest fragmentation, to phenotypic changes in amphibians is poorly known. Habitat loss is a complex phenomenon that may affect amphibian populations directly through elimination of suitable habitats required for breeding and foraging (Tocher et al. 1997), modification of microclimatic conditions that influence physiological processes (Marsh & Pearman 1997), and disruption of habitat connectivity that affects dispersal (Funk et al. 2005). It may indirectly affect amphibians by changing the abundance of predators, parasites, pathogens, and prey in a habitat

(Lips 1999). For a handful of species, body size is smaller in disturbed than undisturbed habitats (Mazerolle 2001; Neckel-Oliveira & Gascon 2006) and trait asymmetry may follow an opposite trend (Wright & Zamudio 2002; Lauck 2006). The species that exhibit these trends have a tadpole stage. It remains to be seen whether forest loss and fragmentation induces phenotypic changes in amphibians with direct development (no tadpoles), which represent a considerable fraction of species in tropical assemblages.

Unlike other researchers, we focused on 2 subtropical species of frogs with direct development, and we asked whether habitat loss influenced their body size, allometry, and trait symmetry. The 2 species, *Eleutherodactylus antillensis* and *E. coqui*, are endemic to Puerto Rico and widely distributed across the island (Joglar 1998; Rivero 1998).

Methods

Study Area

This study was conducted in the northern karst region of Puerto Rico, which represents 13% of the island's total area (Fig. 1) and is characterized by extensive secondary forests, agricultural fields and pastures, and expanding urban areas (Lugo et al. 2001). This area has a complex topography in which small hills are interspersed among small valleys that formed after the collapse of sinkholes. This region has a mean total annual rainfall and temperature of 1400 mm and 16° C, respectively, and is classified as subtropical moist forest (Ewel & Whitmore 1973).

Sampling

We used geological maps (Monroe 1976; Rodríguez-Martínez 1995), aerial photographs, and ground truthing to select our study sites (Table 1; Fig. 1b). In each of 3 areas representing major limestone formations, we selected 3 sites that differed in terms of the amount of forest cover: (1) low levels of disturbance (≥70% forest cover), (2) intermediate levels of disturbance (20–70% forest cover), and (3) high levels of disturbance (forest cover ≤20%) (Table 1; Fig. 1c). The percentage of forest cover at each study site was estimated over a 600 × 600 m
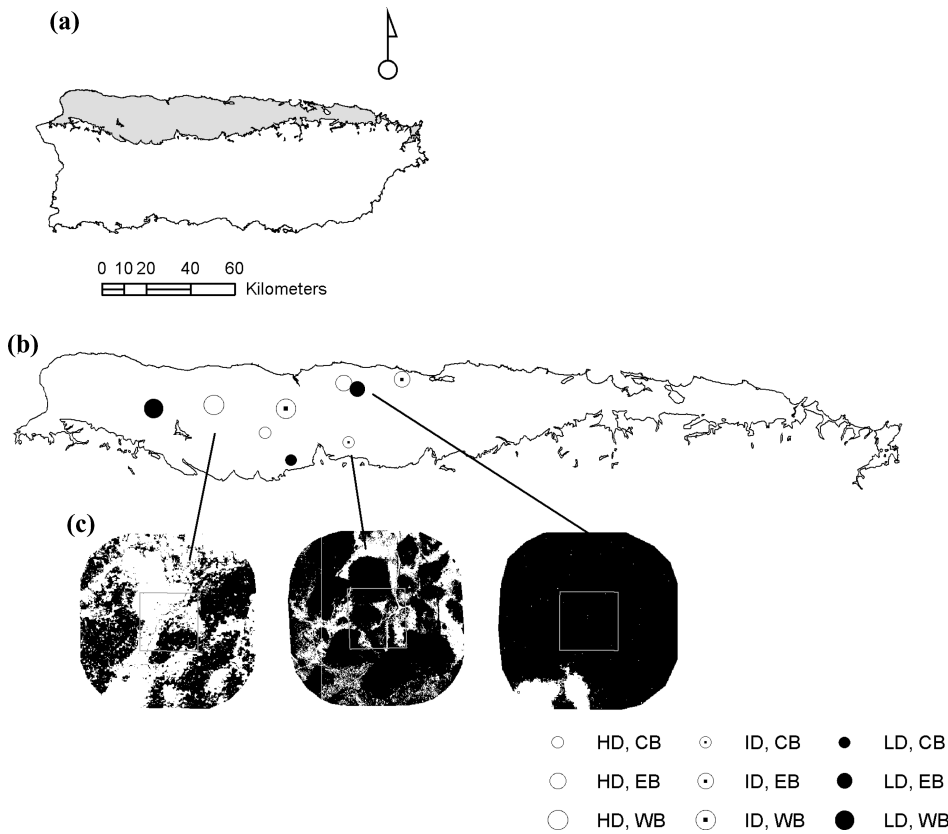
(a)

(b)

(c)

Figure 1. The island of Puerto Rico showing (a) the northern karst region in gray and (b) the 9 study sites classified according to their degree of disturbance (LD, low disturbance; ID, intermediate disturbance; HD, high disturbance) and geological substrate (WB, western block; CB, central block; EB, eastern block). (c) Degree of disturbance expressed as percent forest cover (black) versus other land uses (white) within a 600 × 600 m area centered on each plot.

area centered on the 4-ha study plots where frogs were collected. To quantify the amount of forest cover, we ran a supervised classification on high-resolution IKONOS (2-m resolution) images taken in 2002 and supplemented with color aerial photographs (1-m resolution) taken between 2001 and 2002 (Centro de Recaudaciones Municipales de Puerto Rico-CRIM). After the initial classification, the images were reclassified to produce binary images

Table 1. Characteristics of study sites in the northern karst region of Puerto Rico indicating their underlying geology (blocking factor) and degree of disturbance (treatment).

Block[a]	Treatment, site	Municipality	Latitude	Longitude	Maximum elevation (m)	Percent forest	E. antillensis (n[b], U/F/M)	E. coqui (n[b], U/F/M)
WB	high, Abra Honda	Camuy	18°27′39″N	66°51′26″W	175	19.70	11	9
							0/1/11	0/0/9
	intermediate, Mata de Plátano	Arecibo	18°25′30″N	66°43′39″W	150	69.40	15	10
							1/2/12	1/2/7
	low, Guajataca	Isabela	18°′25′00″N	67°57′30″W	200	81.76	11	11
							0/1/10	2/5/4
EB	high, Factor	Arecibo	18°27′30″N	66°37′30″W	30	17.85	13	10
							0/0/13	3/0/7
	intermediate, La Esperanza	Manati	18°26′43″N	66°31′62″W	60	40.06	11	13
							0/4/7	0/2/12
	low, Cambalache	Arecibo	18°27′40″N	66°33′53″W	70	94.25	13	11
							0/2/11	0/0/11
CB	high, Esperanza	Arecibo	18°21′29″N	66°47′37″W	200	13.08	10	13
							0/1/9	2/4/7
	intermediate, Sabana Hoyos	Arecibo	18°22′31″N	66°38′31″W	200	60.67	10	14
							2/0/8	1/1/12
	low, Río Abajo	Utuado	18°19′27″N	66°43′41″W	250	85.44	13	18
							1/0/12	1/5/12

[a] Abbreviations: WR, western block underlaid by the Aymamon limestone; CB, central block underlaid by the Aymamon formation and other deposits; EB, eastern block underlaid by the Montebello limestone.

[b] Under each species is the number of individuals measured (n), including the number of males (M) and females (F). The sex of some individuals could not be determined (U).

representing 2 cover classes: tree cover and other land uses, the latter including agricultural fields, pastures, and urban areas (Fig. 1c). Hereafter, we refer to the above cover classes as landscape types.

At each site we established a 4-ha plot that was subdivided into five 200-m-long transects. Sampling of frogs took place between 19:00 and 03:00 on 2 consecutive nights following a predefined route along all 5 transects from October 2002 through January 2003 and in May 2003. Once we collected the minimum number of frogs per site (approximately 12 individuals/species) we stopped sampling. Each captured individual was weighed to the nearest 0.01 g with a balance (Ohaus Scout TM II, Pine Brook, New Jersey) and placed in a plastic bag for transport to a nearby station, where the field measurements were completed. Captured animals were euthanized by putting them in ice water. Individuals were taped to Petri dishes with limbs in a uniform orientation and injected with and submerged in 70% alcohol for fixation (Meteyer et al. 2000). All collections and animal-use protocols were approved by the Puerto Rican Department of Natural Resources (permit 031C038) and the Institutional Animal Care and Use Committee from the University of Puerto Rico at Rio Piedras (permit 204-2003).

We obtained radiographs of the specimens with a Faxitron Specimen Radiography System (model MX-20; Faxitron X-Ray Corporation, Wheeling, Illinois) at the U.S. Geological Survey National Wildlife Health Center (Madison, Wisconsin). All specimens were oriented ventral dorsally with the fore and hind limbs clearly exhibited and exposed to 18 kV for 120 seconds with 0.3 mA of continuous current (Meteyer et al. 2000). On each radiograph plate, we placed a lead ruler to establish a standard scale, which we used later for calibration purposes. The radiographs were scanned at 600 × 600 dpi and saved as digital bitmap images to collect morphological measurements to the nearest 0.01 mm with Scion Image Beta 4.0.2 (Scion Corporation, Frederick, Maryland). Radio-ulnas were measured from the most axial point of the ulna to the olecranon, femurs from the lateral condyle to the femur head, and the tibio-fibulas from the most proximal point to the most axial point of each bone. The right and left side of each bone were measured twice (J.D.A.) with each starting side chosen at random.

E. antillensis and *E. coqui* are dimorphic species; males are smaller than females (Joglar 1998; Rivero 1998). Because the calling behavior of males may facilitate their detection and capture, it was likely that our samples were biased toward males. We examined the gonads of each frog to establish the exact proportion of males, females, and juveniles (Table 1) in the sample.

Data Analyses

We thoroughly examined the data for outliers resulting from human error before conducting the 3 sets of sta-

tistical analyses because outliers can have a tremendous effect in morphological studies (Bailey & Byrness 1990; Palmer & Strobeck 2003). In the first set of analyses, we evaluated the effect of landscape type on snout-vent length (SVL) and its allometric relationship with the 3 bones measured. We used a complete randomized-block-design analysis of variance (ANOVA) to evaluate differences in size across the 3 landscape types. Limestone formation was included as a blocking factor, and landscape type with 3 levels (low, intermediate, and high disturbance) was the factor of interest. We used blocking to control for external sources of variation (i.e., geology, elevation, vegetation, and climate are similar within blocks; Table 1) (Manly 1992). To evaluate changes in the allometry of frogs across the 3 landscape types we used analysis of covariance (ANCOVA), with SVL as the covariate, landscape type as the factor of interest, and length of the 3 bones as the dependent variables.

In the second set of analyses, we examined whether the differences between right and left sides of each bone corresponded to fluctuating asymmetry (Palmer & Strobeck 2003). We used a mixed-model ANOVA in which individual and side of each bone with 2 repeated measurements each were included as a random and fixed factor, respectively (Palmer & Strobeck 2003). Significance of the side term (i.e., H_o: $R_i = L_i$, where R_i and L_i are the lengths of the right and left sides of the ith bone) suggests directional asymmetry (Palmer & Strobeck 2003), whereas the individual × side interaction term suggests presence of fluctuating asymmetry or antisymmetry. We also estimated measurement error (ME) as $MS_E/MS_{SI} × 100$, where MS_E and MS_{SI} are the error and side × individual mean squares, respectively (Palmer & Strobeck 2003). The traits for which the interaction term was significant were evaluated to test whether the signed differences (R_i–L_i) followed a normal distribution with mean zero. A normal distribution is indicative of fluctuating asymmetry (FA) and thus developmental instability (Van Valen 1962; Polak 2003). We used a Kolmogorov–Smirnov test to compare the observed distribution against a normal distribution with mean zero. We visually inspected the probability density functions of the signed differences of each bone to further characterize departures from a normal distribution.

The third set of analyses tested differences in FA among landscape types. We used a complete randomized block design ANOVA to examine differences in the unsigned differences ($|R_i$–$L_i,|$) of FA. This generates a truncated distribution in which the mean and standard deviation of the data are highly correlated (Palmer & Strobeck 2003). Therefore, the evaluation of unsigned FA means through ANOVA is equivalent to Levene's test for homogeneity of variances (Palmer & Strobeck 2003).

We ran the first and second set of analyses including all individuals and then removed females; this did not alter results, so we present analyses based on all individuals.

All statistical analyses were conducted with S-Plus 8.0 for Windows (Insightful Corporation, Seattle, Washington), whereas analyses of the images were conducted with Erdas Imagine 8.7 (Leica Geosystems, St. Gallen, Switzerland).

Results

The size of *E. antillensis* as reflected by the SVL differed among treatments ($F = 8.98$, df = 2, $p = 0.0001$; Fig. 2). SVL was smaller in the highly disturbed landscapes than in the intermediate and little-disturbed landscapes (Tukey's multiple comparisons, $p < 0.050$; Fig. 2). The SVL also differed in *E. coqui* ($F = 13.51$, df = 2, $p < 0.0001$; Fig. 2). Frogs were smaller in the highly and intermediately disturbed landscapes than in the little-disturbed landscapes (Tukey's multiple comparisons, $p < 0.050$).

The relationship between SVL and length of each bone was linear for the 2 species across the 3 landscape types (common slope was significantly different from zero for the 3 traits and 2 species, $p < 0.001$; Fig. 3). In *E. antillensis* the slopes relating SVL with the radio-ulna ($p = 0.04$), femur ($p = 0.001$), and tibio-fibula ($p = 0.003$) differed among the 3 landscapes (Fig. 3a–c). Once the highly disturbed landscape was removed from the analyses, the intermediate and little-disturbed landscapes were indistinguishable, and we could not detect any landscape
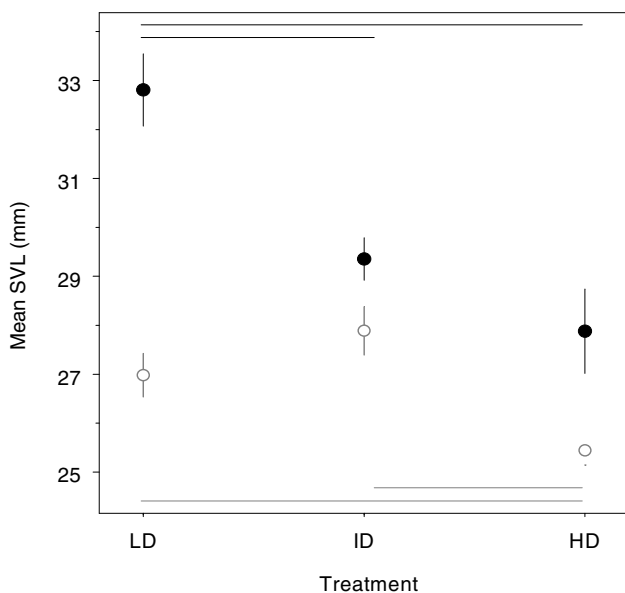

Figure 3. Allometric relationships between snout-vent length (SVL), radio-ulna (RU), femur (FE), and tibio-fibula (TF) lengths for (a-c) E. antillensis *and (d-f)* E. coqui *in the low (black), intermediate (blue), and high (fuchsia) disturbance landscapes (D, disturbance). Numbers in the key represent the y intercept and slope, respectively.*

effect on the allometry of this species. In *E. coqui* slopes were similar across the 3 treatments and results of the ANCOVA failed to show an effect of landscape type on the relationship between SVL and length of the 3 bones ($p > 0.25$; Fig. 3d–f).

In *E. antillensis* and *E. coqui* differences between lengths of right and left sides seemed to represent true asymmetry as indicated by the significant individual × side interactions ($p < 0.0001$) for each bone (Table 2). Measurement error in all but one case (RU in *E. coqui*) was <10%. Distribution of the signed differences between right and left sides ($R-L$) did not differ from a normal distribution with mean zero for the 3 bones in *E. antillensis* and the femur and tibio-fibula in *E. coqui*, which indicates true FA (Fig. 4a–c, e–f). On the other hand, distribution of the signed differences between right and left for the radio-ulna in *E. coqui* was leptokurtic

Figure 2. Snout-vent length (SVL) of E. antillensis *(white circles) and* E. coqui *(black circles) in the 3 landscape types (LD, low disturbance; ID, intermediate disturbance; HD, high disturbance). Points and bars represent mean and 1 SE, respectively. The horizontal lines join treatment pairs that were not significantly different.*
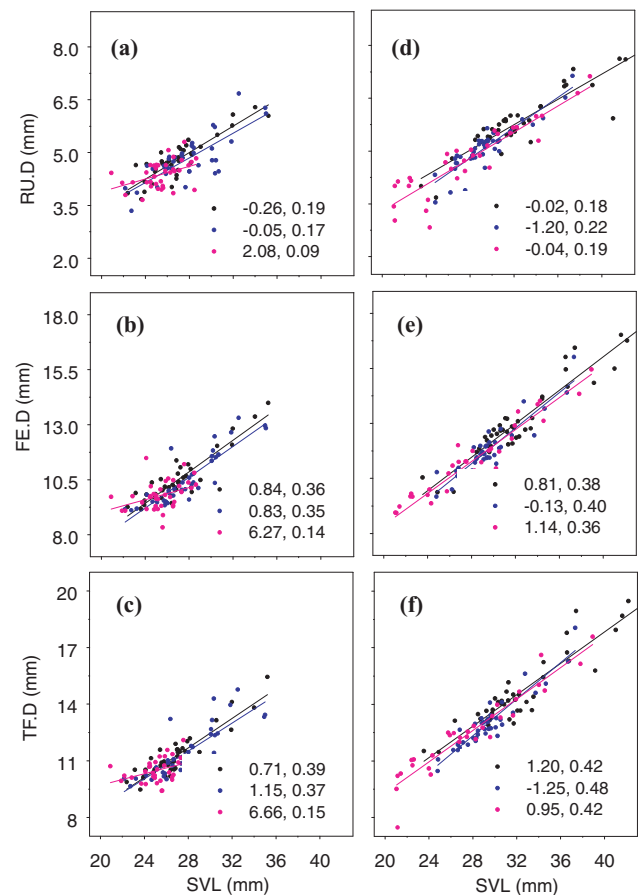
Table 2. Results of the analysis of variance (ANOVA) for testing the within-side variation relative to bone measurement error (individual × interaction).[a]

Species	Bone[b]	ME[c] (%)	df[d]	F[e]
E. antillensis	RU	5.7	111	17.3
	FE	2.1	111	47.2
	TF	8.0	111	11.5
E. coqui	RU	21.0	110	4.8
	FE	3.9	110	25.3
	TF	9.8	110	9.8

[a]*All* p < 0.0001.
[b]*Abbreviations: RU, radio-ulna; FE, femur; and TF, tibio-fibula.*
[c]*Measurement error expressed as* $MS_E/MS_{sI} \times 100$, *where* MS_E *and* MS_{sI} *are the error and side × individual mean squares of the mixed model ANOVA, respectively.*
[d]*Degrees of freedom for the individual × side interaction term.*
[e]*The* F *statistic for the individual × side interaction term.*

(Fig. 4d); however, we considered it FA following recommendations in Palmer and Strobeck (2003). Fluctuating asymmetry and SVL were not correlated; therefore, there was no evidence of a size effect on FA in *E. antillensis* or *E. coqui*.

Levels of fluctuating asymmetry in *E. antillensis and E. coqui* did not vary among landscape types (Fig. 4g-h, k-l).

Nevertheless, in *E. coqui* there was a trend suggestive of an increase in FA for the radio-ulna in the highly modified habitat ($F = 2.82$, df = 2, $p = 0.06$; Fig. 4j).

Discussion

In the northern karst region of Puerto Rico, *E. antillensis* exhibited smaller sizes in the highly disturbed landscapes than in the intermediately and little-disturbed landscapes, whereas in *E. coqui*, smaller sizes were found in the highly and intermediately disturbed landscapes than in the little-disturbed landscapes. Moreover, in *E. antillensis* but not in *E. coqui*, variation in the slope and intercept of the curves relating SVL with bone length suggests an effect of habitat loss on the allometry of this species. Evidence for an increase of fluctuating asymmetry in highly disturbed habitats was weak, with only one bone in *E. coqui* showing a trend in this direction. Our results suggest that habitat loss can contribute to phenotypic changes in these 2 species of frogs. Yet because no comparable data exist for these or other species with direct development, we could not determine conclusively the significance of these changes.

E. antillensis and *E. coqui* are the 2 most widely distributed species in Puerto Rico and are sympatric
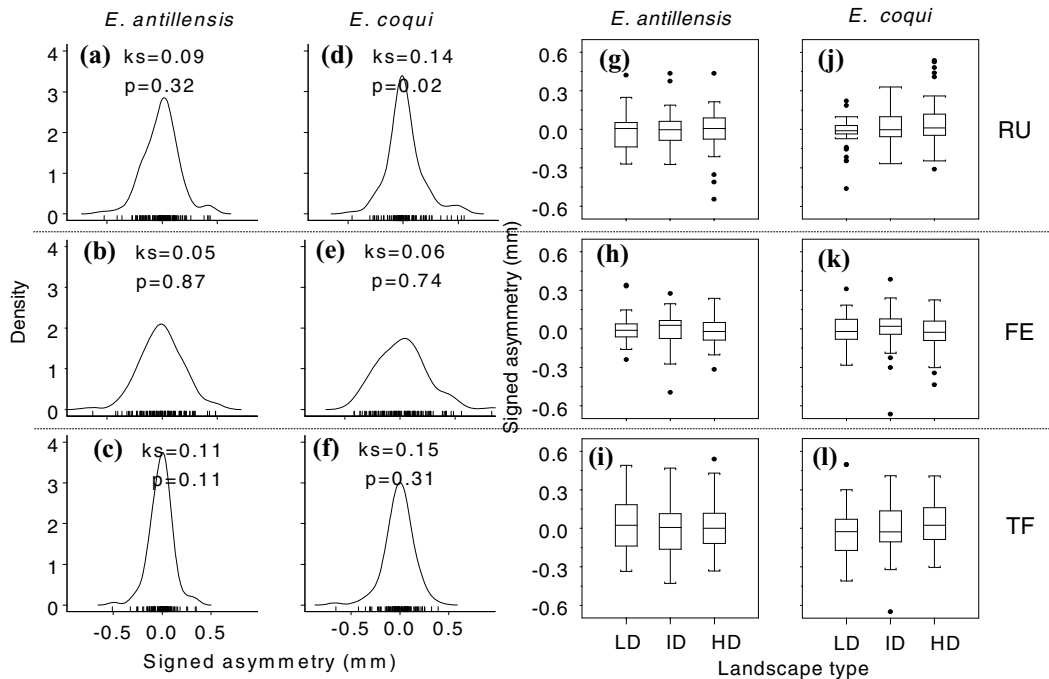

Figure 4. (a–f) The results of Kolmogorov–Smirnov tests (KS) for departures from a normal distribution (μ = 0, SD = data) in E. antillensis *and* E. coqui *are shown for each of the 3 bones examined. Probability-density functions for the signed differences between right and left sides of bones. (g–l) Box plots of the signed differences between right and left sides of radio-ulna (RU), femur (FE), and tibio-fibula (TF) of* E. antillensis *and* E. coqui *in the 3 landscape types (LD, low disturbance; ID, intermediate disturbance; and HD, high disturbance) (FA, fluctuating asymmetry).*

throughout most of their range, yet *E. antillensis* is less abundant than *E. coqui* at middle elevations and is absent from the highest peaks of the island. In *E. coqui*, and most likely *E. antillensis*, changes in microclimatic conditions, predator density, and food availability may explain the observed phenotypic variability across the 3 landscape types we evaluated. Whether these changes are adaptive, associated with age differences, or induced during development needs further evaluation.

The natural morphological variation reported for *E. coqui* across elevation and humidity gradients in Puerto Rico indicates there is the potential for adaptive local responses, as has been shown for frogs elsewhere (Berven 1982; Ashton 2002). *E. coqui* exhibits a 2-fold increase in size from low (male and female average SVL is 28.5 mm and 35.4 mm, respectively) to high (46.2 mm vs. 60.5) elevations (Beuchat et al. 1984; Joglar 1998; Rivero 1998; Fogarty & Vilella 2002). In addition, *E. coqui* exhibits a clinal variation in size along a west–east gradient (Fogarty & Vilella 2002). Yet in the context of our study, we think adaptive changes to variable conditions are an unlikely explanation for our results given our sampling design.

A second explanation for the observed morphological differences among habitats could be the result of age differences among individuals. For example, frogs from highly or intermediately disturbed landscapes may be individuals that recently dispersed to open habitats from nearby forests. Another possible explanation is that large individuals are less likely to escape predation in highly disturbed landscapes; therefore, there are more small individuals in these habitats. Two lines of evidence fail to support this explanation. First, *E. antillensis* and *E. coqui* are sympatric throughout most of their range. At a lowland site in northern Puerto Rico, *E. antillensis* was more abundant than *E. coqui* in early-successional habitats dominated by herbaceous vegetation, whereas the opposite was true in late-successional habitats dominated by arboreal vegetation (N. Ríos-López, unpublished data). *E. antillensis* is smaller than *E. coqui* and has a slightly different allometry, as indicated by the slopes relating SVL with bone length (this study, Fig. 3), which suggests that predation may not necessarily account for a bias distribution of small versus large individuals in open habitats. Second, if calling behavior is a sign of reproductive status, then our samples contained a large proportion of reproductive males. Therefore age may not explain size differences among habitats. Rather, developmental shifts in response to forest loss may explain the presence of small calling males in disturbed habitats, a possibility that we discuss later.

Several lines of evidence indicate that phenotypic differences observed in *E. coqui* and *E. antillensis* may be induced environmentally during development. First, as in our study, Woolbright (1991) found that males and females of *E. coqui* attain smaller sizes in forest gaps

created by hurricanes and treefalls. These habitats are highly ephemeral and unpredictable, and age was discarded as potential explanation of the observed results. Second, the allometry of *E. coqui* may change depending on the time at which eggs hatch, and in turn this may be affected by the presence of predators as happens with other species (Warkentin 1995, 2000). Buckley et al. (2005) induced early hatching of *E. coqui* eggs under laboratory conditions to simulate the presence of predators and found that froglets from eggs that hatched early have relatively smaller hind limbs than froglets in the control group. Lastly, in a separate study in which we used a double-staining procedure to differentiate ossified and cartilaginous bone components in *E. antillensis* and *E. coqui*, we observed asymmetrical patterns of bone development, including skeletal abnormalities and missing elements in manus and pes and possible heterochronic shifts in skeletal development in response to habitat loss (C.R., unpublished data).

Altogether our data suggest that habitat loss can influence the developmental trajectories of *E. antillensis* and *E. coqui*, as it has for species with a tadpole stage. In fact, the presence and abundance of predators (LaFiandra & Babbitt 2004; Moore et al. 2004), number of competitors (Relyea 2004), pollutants (Gallant & Teather 2001; McCoy & Harris 2003; Soderman et al. 2007), pathogens (Blaustein & Johnson 2003; Johnson et al. 2003; Parris & Cornelius 2004), water and food availability (Boorse & Denver 2003; Thumm & Mahony 2006), and habitat fragmentation (Mazerolle 2001; Neckel-Oliveira & Gascon 2006; Lauck 2006) can induce phenotypic changes in species with indirect development. Yet the long-term fitness consequences of these changes are not well understood.

Habitat loss may also influence levels of FA, a phenotypic measure of the degree to which individuals can withstand environmental and genetic stresses during development (Polak 2003). Yet studies in natural populations of amphibians and reptiles are inconclusive. Results of one set of studies shows higher levels of FA in disturbed than in undisturbed habitats (Sarre 1996; Wright & Zamudio 2002; Lauck 2006), whereas we and Davis (1997, 2004) found little evidence that supports an increase of FA with habitat loss. Only *E. coqui*, and only one bone, showed a trend toward an increase in FA in the highly disturbed landscape. The lack of a strong effect of habitat loss on FA in our study may be the result of 3 nonmutually exclusive causes. First, our small samples sizes may have translated into limited statistical power. Although our sample sizes (approximately 36 individuals/treatment/species) were in the range (9–92 individuals) of those reported by other researchers examining FA in amphibians (Chubinishvili 1998; Bosch & Marquez 2000; Gallant & Teather 2001; Wright & Zamudio 2002; McCoy & Harris 2003; Parris & Cornelius 2004; Soderman

et al. 2007), they were still below the number (40 or 50) that is thought to detect the subtle differences between right and left sides that characterize fluctuating asymmetry (Palmer & Strobeck 2003). Second, there may have been no landscape-type effect on FA, which may indicate that habitat loss at the level we studied does not increase developmental instability.

Finally, the variability in FA at the scale of our study may have been masked by islandwide increased levels of FA. It is possible that superimposed on the stresses associated with habitat loss are those associated with the insular character of Puerto Rico, which may limit dispersal and increase vulnerability of amphibians to large-scale natural disturbances such as hurricanes and invasive exotic species. Insular populations exhibit higher levels of FA than mainland populations (Soulé 1967), and levels of FA vary in response to distance from the mainland and island size (e.g., Soulé 1967; Sarre & Dearn 1991; Juste et al. 2001). Nevertheless, differences among studies in terms of the traits measured (metric vs. meristic) and methods used to analyze the data (e.g., evaluation of measurement error, examination of type of asymmetry) limits further conclusions.

We propose that studies examining phenotypic variation of amphibians across habitats affected to different degrees by human activities might particularly help in the understanding of their global population declines. Yet we do not recommend focusing on trait asymmetry alone to monitor amphibians (Alford et al. 1999, 2007), or any other species (Leary et al. 1985; Clarke 1995; Leung et al. 2003). Trait asymmetry is not only difficult to estimate (Wright & Zamudio 2002; McCoy & Harris 2003; Delgado-Acevedo 2005) but it also reflects only one aspect of development. This, together with the idiosyncratic response of species, may lead to the conclusion that populations are healthy. Instead, conservation monitoring programs should consider measurement of traditional morphological traits (e.g., SVL, body mass, limb length) and degree of trait asymmetry (Berggren 2005). An increase in the variance of trait size and trait asymmetry (Gallant & Teather 2001) may be accompanied by other phenotypic changes and may therefore reflect more profound developmental changes in organisms. Because body size scales with a variety of physiological, life history, and ecological traits (Duellman & Trueb 1986), monitoring morphological changes in amphibians may help in understanding mechanisms that contribute to their persistence in changing environments.

Acknowledgments

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