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### Hind-Limb Length Plasticity in *Anolis carolinensis*

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**ABSTRACT.**—The positive relationship between hind-limb length and perch diameter is well established in *Anolis* lizards, both among populations of some species and among species. Interspecific comparisons indicate that longer legs confer an advantage for increased running speed on broad substrates, whereas shorter limbs provide greater maneuverability on narrow surfaces. In this light, phenotypic plasticity for hind-limb length previously detected in *Anolis sagrei* may be adaptive because hatchlings exposed to only broad substrates developed relatively longer hind limbs for their body size compared to hatchlings exposed to only narrow substrates. We tested the generality of hind-limb length plasticity in *Anolis* by conducting a hatchling growth experiment on *Anolis carolinensis*, a distant relative of *A. sagrei* and a different type of habitat specialist. Similar to *A. sagrei*, *A. carolinensis* grown in cages with different sized perches showed hind-limb length plasticity, but the magnitude of difference between treatments and sexes was less for *A. carolinensis* than for *A. sagrei*. This finding suggests either hind-limb plasticity is widespread within the genus *Anolis* or that it has evolved independently at least twice.

A recent study revealed hind-limb length plasticity in the lizard *Anolis sagrei* (Losos et al., 2000). Hatchling lizards reared with only broad perches had significantly longer hind limbs for a given body size than those raised with only narrow perches; this was the first study to show this type of plasticity in a vertebrate. Furthermore, this plasticity mirrored the correlation

between hind-limb length and perch diameter found among natural (Losos et al., 1994) and experimental (Losos et al., 1997) populations of this species. That is, *A. sagrei* shows a positive relationship between the mean perch diameter and the mean relative hind limb length among populations. This same relationship, greatly magnified, also exists among species in the *Anolis* radiation (Williams, 1983; Losos, 1990). The functional significance of the relationship between hind-limb length and perch diameter is clear: species with relatively longer legs run faster on broad surfaces, whereas those with relatively shorter legs are better

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suiting for maneuvering on narrow substrates (Losos and Sinervo, 1989; Losos and Irschick, 1996; Irschick and Losos, 1998; Spezzano and Jayne, 2004). Although intraspecific studies of the functional relationship between hind-limb length and perch diameter are lacking, similar biomechanical factors likely apply within species as well.

Although hind-limb plasticity has implications for adaptive differentiation within a species, its importance in the broader context of the *Anolis* radiation is unclear. In particular, *A. sagrei* has perhaps the greatest breadth of habitat use among all anole species (e.g., Schoener, 1968; Losos et al. 1994; Mattingly and Jayne, 2004); if plasticity were to have arisen in a single species as an adaptation to variable habitat use, *A. sagrei* would be the most likely candidate. Alternatively, such plasticity could characterize all anoles or even a more inclusive clade (cf. Erickson, 1997). Determining whether plasticity in hind-limb length is a unique property of *A. sagrei*, as opposed to being widespread within the genus, is an important first step toward assessing its importance in the adaptive radiation of *Anolis* lizards.

To examine the generality of phenotypic plasticity in hind-limb length within the *Anolis* radiation, we conducted hatchling growth experiments on *Anolis carolinensis*. Distantly related to *A. sagrei* (Nicholson et al., 2005), *A. carolinensis* is a trunk-crown habitat specialist, whereas *A. sagrei* is a trunk-ground habitat specialist (Williams, 1983). Yet, similar to *A. sagrei*, a positive relationship between perch diameter and relative hind-limb length exists among populations of *A. carolinensis* in the Bahamas (Losos et al., 1994). Thus, phenotypic plasticity may be important in hind-limb differentiation among populations of *A. carolinensis*. This experiment was designed to provide insight into the potential phylogenetic distribution of hind-limb plasticity as well as into its occurrence in other habitat specialists.

#### MATERIALS AND METHODS

We conducted hatchling growth experiments from August 2002 through March 2003 and August 2003 through April 2004. Each year, 112 hatchling lizards were obtained from a commercial dealer in Laplace, Louisiana. Lizards were placed in plastic cages  $28.5 \times 17.5 \times 21$  cm, misted twice daily, and fed wingless fruit flies and hatchling crickets dusted with mineral supplements every two or three days. The bottoms of the cages were covered with traction sand and ZooMed Reptisun 5.0 UVB fluorescent bulbs provided lighting above each row of cages. Animal room conditions were maintained at 26°C, 70% relative humidity, and on a light:dark cycle of 13:11 h. The only perches available to lizards were either two narrow bamboo dowels 1 cm in diameter and 30 cm long ("narrow" treatment) or one piece of wood 23 cm long  $\times$  9 cm wide  $\times$  4 cm thick ("broad" treatment) leaned against the walls of the cages. In each year, 56 cages were used, with each cage initially containing two lizards. Lizards were haphazardly assigned to cages with the requirement that lizards placed in the same cage were matched for size, usually  $< 2$  mm difference in snout-vent length (SVL), and then randomly assigned to a treatment. Cages were arranged on a metal rack with 14 per shelf in two rows and alternating treatments.

At the beginning of the experiment, prior to assigning lizards to treatments, we measured SVL and hind-

limb length (HL) to the nearest 0.5 mm using a ruler. The same person (JJK) measured all lizards in this study. HL was measured as the distance from the insertion of the limb into the body wall to the distal tip of the claw on metatarsal IV. One individual per cage was toe-clipped for future identification. At the end of each experiment, SVL and HL were measured and sex determined.

To reject the possibility that differences in starting conditions or survival influenced HL at the end of the experiment, we tested for differences in initial HL between treatments, sex, year, survival, and the interaction between treatment and survival using analysis of covariance (ANCOVA) with initial SVL as a covariate. To make the results of this study directly comparable to the previous study of *A. sagrei* (Losos et al., 2000), we determined whether the experimental treatment affected hind-limb growth in two ways. First, we tested for a difference in HL at the end of the experiment using ANCOVA with final SVL as the covariate and treatment, sex, and year as the factors. Second, we determined the difference in relative hind-limb growth (RHG) between the treatments. RHG was measured as  $(\text{final HL} - \text{initial HL}) / (\text{final SVL} - \text{initial SVL})$  for all lizards surviving for the entire experiment. We evaluated RHG using a factorial three-way analysis of variance (ANOVA) that included treatment, sex, and year as factors. SVL, HL, and RHG were ln-transformed prior to analyses. *A priori* predictions for larger HL and RHG in broad treatment lizards and males were tested with one-tailed *P*-values, all other tests were two-tailed (Losos et al., 2000). If both lizards in a cage survived the entire experiment, then the mean values for SVL, HL, and RHG were calculated and used in subsequent analyses.

#### RESULTS

Survival was 86% (96/112) and 55% (62/112) in the first and second years, respectively. At the start of experiments, ANCOVA showed no difference in HL while controlling for SVL between treatments ( $F_{1,101} = 0.01$ ,  $P = 0.935$ ), sexes ( $F_{1,101} = 0.71$ ,  $P = 0.401$ ), survival ( $F_{1,101} = 0.19$ ,  $P = 0.666$ ), or the interaction between treatment and survival ( $F_{1,101} = 0.21$ ,  $P = 0.644$ ; Table 1); slopes were homogeneous for all factors (all  $P > 0.30$ ). There was, however, a significant difference between years ( $F_{1,101} = 14.07$ ,  $P = 0.0003$ ): relative hind-limb lengths at the start of the experiments were slightly longer for hatchlings in 2002 as compared to 2003.

At the end of the experiments, SVL growth did not differ between treatments ( $F_{1,150} = 1.17$ ,  $P = 0.281$ ), and no interactions among treatment, sex, and year were significant (all  $P > 0.05$ ); but lizards grew more in 2002 than in 2003 ( $F_{1,150} = 97.64$ ,  $P < 0.0001$ ), and males grew more than females ( $F_{1,150} = 11.27$ ,  $P = 0.001$ ; Table 1). Analyzing cage means for final HL revealed lizards in the broad treatment ( $F_{1,65} = 7.21$ ,  $P = 0.005$ , one-tailed) and males ( $F_{1,65} = 22.14$ ,  $P < 0.0001$ , one-tailed) had longer hind limbs for a given body size (Fig. 1), whereas the effect of year was almost significant ( $F_{1,65} = 3.50$ ,  $P = 0.068$ ). Slopes were homogeneous for all factors (all  $P > 0.15$ ) in this ANCOVA. Using cage means for RHG, the three-way ANOVA indicated that hind-limb growth rate was higher for broad treatment lizards ( $F_{1,96} = 2.89$ ,  $P = 0.046$ , one-tailed) and males

TABLE 1. Initial and final snout-vent lengths (SVL), hind-limb lengths (HL), and relative hind-limb growth (RHG) for *Anolis carolinensis* by year, sex, and treatment. Values are mean  $\pm$  SE (range, sample size). SVL and HL values (mm) are from individuals and RHG is based on cage means. Sample sizes are 209 for initial values (15 individuals were not sexed), 158 for final values (survivors only), and 104 for RHG (cage means).

Year	Sex	Treatment	Initial SVL	Initial HL	Final SVL	Final HL	RHG
2002	Male	Broad	27.1 $\pm$ 0.22 (25.0–29.5, 25)	17.2 $\pm$ 0.22 (15.0–19.5, 25)	41.9 $\pm$ 0.66 (34.5–47.0, 24)	28.0 $\pm$ 0.55 (22.0–32.0, 24)	0.722 $\pm$ 0.014 (0.636–0.867, 15)
		Narrow	27.0 $\pm$ 0.37 (24.0–30.5, 23)	17.1 $\pm$ 0.25 (15.5–19.5, 23)	41.8 $\pm$ 0.66 (36.5–47.5, 20)	27.5 $\pm$ 0.43 (24.5–31.5, 20)	0.717 $\pm$ 0.019 (0.608–0.840, 15)
	Female	Broad	26.3 $\pm$ 0.22 (24.5–28.0, 27)	16.7 $\pm$ 0.18 (15.0–19.0, 27)	38.7 $\pm$ 0.42 (34.0–44.5, 25)	25.3 $\pm$ 0.32 (22.5–29.0, 25)	0.687 $\pm$ 0.012 (0.633–0.769, 13)
		Narrow	26.8 $\pm$ 0.18 (24.5–28.5, 28)	16.9 $\pm$ 0.17 (15.5–19.5, 28)	38.7 $\pm$ 0.38 (35.5–43.5, 27)	24.7 $\pm$ 0.29 (22.0–29.0, 27)	0.648 $\pm$ 0.019 (0.476–0.746, 13)
2003	Male	Broad	27.0 $\pm$ 0.26 (25.0–29.0, 18)	16.8 $\pm$ 0.17 (15.5–18.5, 18)	36.2 $\pm$ 0.85 (32.0–39.5, 9)	23.5 $\pm$ 0.66 (20.5–26.0, 9)	0.719 $\pm$ 0.018 (0.688–0.789, 5)
		Narrow	26.9 $\pm$ 0.27 (24.5–31.0, 22)	16.7 $\pm$ 0.18 (15.0–18.0, 22)	37.3 $\pm$ 0.63 (32.0–40.0, 11)	24.0 $\pm$ 0.45 (20.5–26.0, 11)	0.704 $\pm$ 0.030 (0.636–0.870, 7)
	Female	Broad	27.0 $\pm$ 0.20 (24.0–29.0, 36)	16.6 $\pm$ 0.14 (14.5–18.5, 36)	36.3 $\pm$ 0.3 (33.0–39.0, 20)	23.2 $\pm$ 0.24 (21.5–25.0, 20)	0.732 $\pm$ 0.029 (0.556–1.083, 19)
		Narrow	27.0 $\pm$ 0.26 (24.5–29.5, 30)	16.8 $\pm$ 0.17 (15.0–18.5, 30)	36.1 $\pm$ 0.40 (32.0–40.0, 22)	23.0 $\pm$ 0.30 (20.0–26.0, 22)	0.683 $\pm$ 0.018 (0.560–0.813, 17)

( $F_{1,96} = 3.40$ ,  $P = 0.034$ , one-tailed; Fig. 2), but there was no effect of year ( $F_{1,96} = 0.81$ ,  $P = 0.371$ ) or any interactions (all  $P > 0.15$ ; Table 1).

DISCUSSION

This study demonstrates that hind-limb plasticity is not a unique property of *A. sagrei*. Rather, it also exists in *A. carolinensis*, a species adapted to a different habitat than its distant relative, *A. sagrei*. Uncorrected sequence divergence of 23.7% for the mitochondrial gene ND2 suggests these species diverged roughly 18 million years ago (Macey et al., 1998; Nicholson et al., 2005). Thus, either hind-limb plasticity arose independently multiple times in *Anolis* or the common ancestor of *A. carolinensis* and *A. sagrei* had hind-limb plasticity. The latter explanation suggests widespread hind-limb plasticity within the genus.

The pattern of hind-limb plasticity in *A. carolinensis* mirrors the positive relationship between hind-limb length and perch diameter observed among natural populations of this species (Losos et al., 1994). The 1 cm and 9 cm perch diameters used in this experiment are similar to perch diameters used by juvenile and subadult *A. carolinensis* in the field (range 0.3–10.8 cm; Schoener, 1968). Thus, the stimulus used to elicit the plastic response in hind-limb length in this experiment is biologically relevant to field conditions. The question still remains whether differences among populations are strictly genetically based, mediated through the environment (i.e., phenotypic plasticity), or a combination of both.

A parallel situation occurs in *A. sagrei*: a correlation exists among populations between hind-limb length

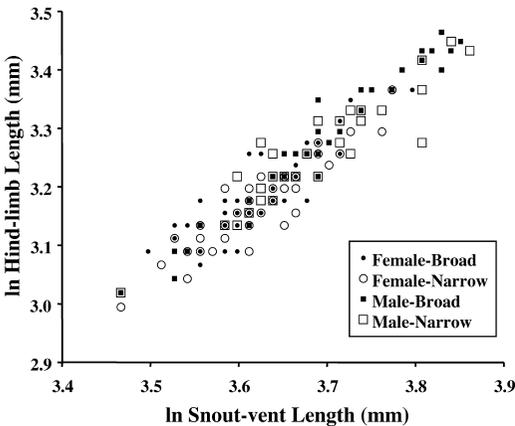


FIG. 1. The relationship between hind-limb length and snout-vent length for lizards in the two treatments at the end of the experiment. Data from the two years were pooled.

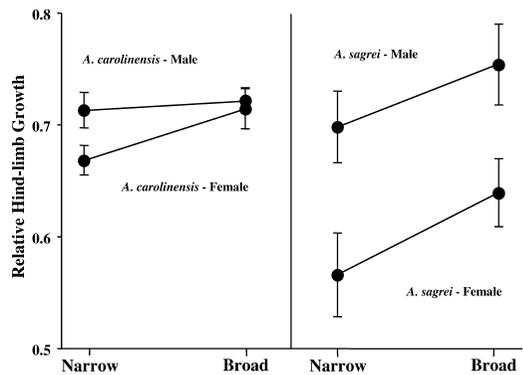


FIG. 2. Relative hind-limb growth (RHG) reaction norms for both sexes of *Anolis carolinensis* and *Anolis sagrei* using cage means. Values are means and error bars denote  $\pm 1$  SE. Data for *A. sagrei* are from Losos et al. (2000), but here raw RHG values are presented instead of ln-transformed values. See text for differences between the experiments that may complicate direct comparisons between the two species.

and habitat use (Losos et al., 1994), and laboratory studies reveal that hind-limb length is a plastic trait (Losos et al., 2000). Recent studies show natural selection on hind-limb length in Bahamian *A. sagrei* (Losos et al., 2004) and a high heritability for hind-limb length in a Florida *A. sagrei* population (J. Kolbe, L. Revell, and J. Losos, unpubl. data). Thus, natural selection on hind-limb length occurs, and a quantitative genetic basis for hind-limb variation exists in natural populations, which suggests that genetically based differences in hind-limb length might rapidly evolve among populations of *A. sagrei*. Similar studies in *A. carolinensis* may help clarify the cause of population differences in this species.

This study documents a second species of *Anolis* with phenotypic plasticity in hind-limb length. Broad substrates during hatchling development result in lizards with relatively longer hind limbs for both *A. sagrei* (Losos et al., 2000) and *A. carolinensis* (this study). Despite this similarity, several interesting differences in the pattern of hind-limb growth exist between the two species. Although there was a significant difference in hind-limb growth between males and females for both *A. carolinensis* and *A. sagrei*, the magnitude of the difference was much greater for *A. sagrei* (Fig. 2). Furthermore, the difference between male and female *A. carolinensis* was greater in the narrow treatment, whereas differences between male and female *A. sagrei* were similar in both treatments (Fig. 2). Thus, female *A. carolinensis* and both sexes of *A. sagrei* had similar, and much steeper, reaction norm slopes than male *A. carolinensis*.

Several caveats exist, however, to direct comparisons of reaction norms between these two species. First, some conditions varied between experiments with *A. carolinensis* and *A. sagrei*, including cage size, number of perches, and slight differences in perch diameter. Second, experiments with *A. carolinensis* lasted 2–3 months longer than those with *A. sagrei*. Thus, the magnitudes on RHG are not standardized for time. Finally, the two species grew over different size ranges. *Anolis carolinensis* grew on average from 26.9–38.7 mm SVL, whereas *A. sagrei* grew from 33.0–42.2 mm SVL. These differences may confound interpretations because the shape of the hind-limb growth curve likely varies at different times during development and among species (T. Sanger, pers. comm.).

Although it would be difficult, if not impossible, to fully reconstruct the role of phenotypic plasticity in the adaptive radiation of *Anolis*, the primary importance of interspecific competition in driving the evolutionary radiation of *Anolis* provides a good focal point for future work (reviewed in Losos, 1994). Many comparative (Jenssen, 1973; Schoener, 1975) and experimental (Pacala and Roughgarden, 1982; Rummel and Roughgarden, 1985) studies show anoles shift their structural habitat use in the presence or absence of congeners. These shifts likely lead to changes in perch diameter that could provide the stimulus for a plastic response in hind-limb length. Designing seminatural experiments using likely competitors and realistic perch characteristics will provide good tests of whether phenotypic plasticity in hind-limb length could have played a role in the *Anolis* radiation. Future studies should also standardize experimental conditions, duration, and growth range of lizards to facilitate direct comparisons

of reaction norms among species as well as include more divergent habitat specialists, such as twig anoles.

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