

# A comparison of habitat use, morphology, clinging performance and escape behaviour among two divergent green anole lizard (*Anolis carolinensis*) populations

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We measured available and actual habitat use, morphology, escape behaviour and clinging ability in a large sample ( $N = 242$ ) of green anoles, *Anolis carolinensis*, in a habitat consisting primarily of segregated dense clumps of broad leaves, *Aspidistra elatior* (Tulane University campus, LA) to compare against similar data collected previously from a more typical habitat c. 30 km away, consisting of continuous strands of bushes and trees (Good Hope Field, St. Charles Parish, LA). At Tulane the anoles perched primarily on the broad, smooth leaves of broad leaves, whereas in Good Hope Field (GHF) they predominantly perched on branches and tree trunks. The two populations differed significantly in morphology. In Tulane, the anoles tended to have shorter distal hindlimb elements, longer forelimb elements, and were more 'slender' than those at GHF. A comparison of escape behaviour showed population and sex differences. In both populations, females had significantly longer approach distances (i.e. were more 'wary') than males. These distances were, in addition, significantly longer at GHF than at Tulane for both sexes; this may be due to the potentially higher diversity and abundance of predators at GHF, although habituation to humans may also play a role. Anoles at Tulane had significantly larger toepads and higher clinging abilities than those at GHF. The enhanced clinging abilities of anoles at Tulane may have arisen due to their propensity to use smooth leaves as their primary substrate. Overall, our data reveal substantial ecological, behavioural, morphological, and functional differences among populations, some of which may be adaptive. © 2005 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2005, 85, 223–234.

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## INTRODUCTION

A central tenet of evolutionary biology is that environmental differences among local populations often result in profound adaptive differences in morphology, behaviour, and performance capacity (e.g. Endler, 1977; Thompson, 1990; Edwards & Kot, 1995; Kopf *et al.*, 1996; Herrel *et al.*, 2005). There are numerous examples of this phenomenon. Plant populations occu-

pying soils containing differing amounts of heavy metal have evolved adaptive differences in heavy metal tolerance (e.g. Antonovics *et al.*, 1971). Similarly, guppy populations occupying streams in which they are subject to differing levels of predation intensity have rapidly evolved life-history differences (e.g. Reznick *et al.*, 1990, 1997). In some cases, adaptive changes may be plastic (e.g. *Anolis* lizards, Losos *et al.*, 1997, 2000); in others, morphological or behavioural differences are mediated by genetic differences (Travis, 1994). Regardless, the notion that local popu-

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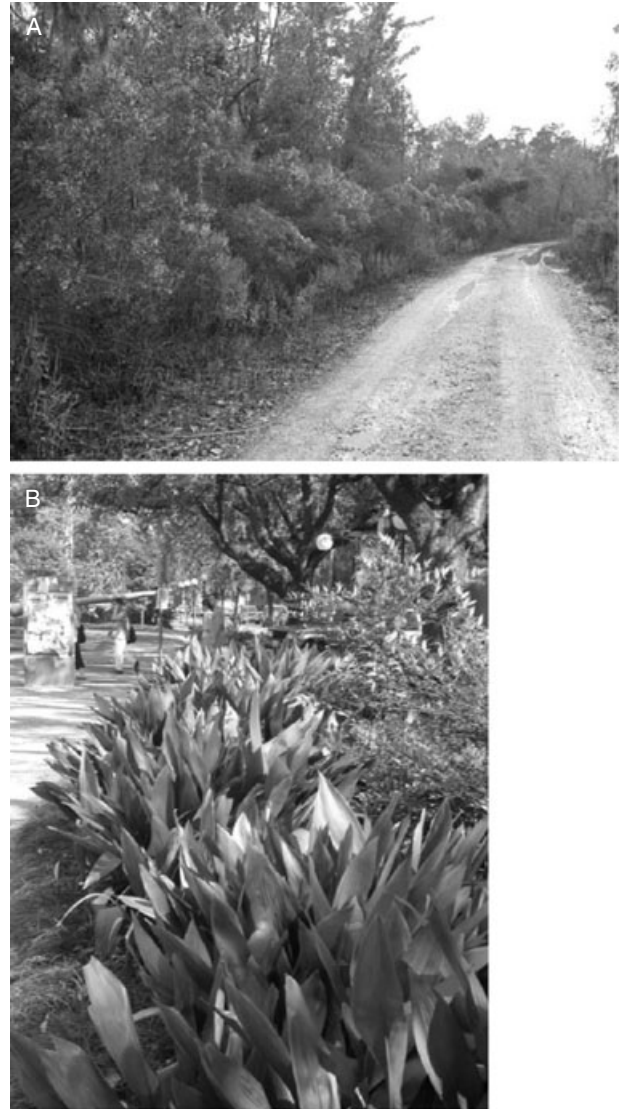
lations will adapt to environmental variation is a central one in evolutionary biology, particularly for ideas regarding speciation (see Otte & Endler, 1989 and references therein).

Most intraspecific studies have examined differences among populations in morphology, life-history or behaviour. In contrast, fewer have looked at whole-organism performance capacity, such as maximum sprinting, endurance or even evaporative water loss (see Snell *et al.*, 1988; Dmi'el *et al.*, 1997; Perry *et al.*, 1999). As discussed by previous authors, natural selection is most likely to act directly on 'higher-level' performance capacities, such as sprinting ability, as opposed to 'lower-level' traits, such as morphology (Arnold, 1983; Garland & Losos, 1994; Irschick & Garland, 2001).

Consider an example of two populations of terrestrial lizards of the same species, one of which occurs in a habitat with a larger number of predators. If predators in both environments primarily capture these lizards by running after them, then one might predict that the population with more predators would evolve both longer hindlimbs and higher sprint speeds to avoid them (e.g. Snell *et al.*, 1988; O'Steen *et al.*, 1990). However, only a few studies have actually documented such differences, and even when they have, the underlying ecological cause of such differences is not always apparent.

In the companion paper (see Irschick *et al.*, 2005, this issue), we have examined differences in morphology, performance (jumping capacity and clinging ability), and habitat use among different age/sex classes (adult males, adult females, and juveniles) within a lowland population of *Anolis carolinensis* (Good Hope Field, henceforth GHF) in St. Charles Parish, south-eastern Louisiana. In this paper, we extend this approach by comparing many of these same characteristics between populations that are highly divergent in overall habitat structure.

To briefly review (see Irschick *et al.*, 2005 for details), one anole population (GHF) occurs in a lowland freshwater swamp that is relatively undisturbed by people, and contains complex vegetation, such as large cypress trees and bushes (Fig. 1A). The second population (Tulane) occurs *c.* 30 km away, on the campus of Tulane University (Orleans Parish, LA). The Tulane habitat (Fig. 1B) is characterized by simpler vegetation, in large part due to the urban and artificial nature of the setting. It is dominated by dense clumps of palmetto (*Aspidistra elatior*) with relatively short (< 2 m) leaves and few large trees or bushes, and has been present for a relatively long period (> 25 years). Green anoles have similarly been present for at least 25 years (R. Thomas, pers. comm.).



**Figure 1.** Representative images of habitats at (A) Good Hope Field (GHF) and (B) Tulane campus (containing large numbers of *Aspidistra elatior* leaves). Note the marked difference in both vegetation type and habitat openness.

Given the ability of *Anolis* lizards to rapidly evolve in morphology and behaviour when encountering a novel habitat (Losos *et al.*, 1997), one would reasonably expect that both populations have had ample opportunity to diverge and that habitat differences would profoundly affect morphology and performance. Of course, any comparison between closely related populations must be tempered by the realization that differences can be plastic (Losos *et al.*, 2000).

These habitat differences between GHF and the Tulane campus could profoundly affect morphology and performance. One aspect of performance that is

important for arboreal lizards is clinging ability (Irschick *et al.*, 1996; Zani, 2000, 2001; Elstrott & Irschick, 2004). All *Anolis* lizards possess toepads that adhere to both rough and smooth surfaces. No studies have examined the effects of surface smoothness on clinging ability (but see Zani, 2000, 2001 for more details on studies relating surface texture to clinging), but a priori one might predict that populations that perch on relatively smooth substrates (e.g. palmetto leaves) should have enhanced clinging abilities relative to those that perch on rough substrates (e.g. bark). Because anoles at Tulane may occupy relatively smooth palmetto leaves, we thus predict that anoles on the Tulane campus should have enhanced clinging abilities for enabling effective movement on smooth surfaces.

Previous studies have shown that lizard species often differ among populations in their escape behaviour (Bulova, 1994; Martin & Lopez, 1995; Cooper, 1997; Lailvaux *et al.*, 2003; Whiting *et al.*, 2003). In some cases this can be linked to differences in habitat structure (e.g. visibility), and the presumed threat of predation. Because GHF and Tulane appear to differ dramatically in habitat structure, one might also expect lizards in the two habitats to differ in escape behaviour, although we cannot make predictions for how this behaviour might differ.

One way of quantifying escape behaviour is to measure approach distances (= distance between a threat and the lizard as the lizard begins to run). This method assumes that a human threat mimics a natural threat at some level (Bulova, 1994). Because the Tulane population naturally has a larger number of people surrounding the anole habitat, it is possible that the lizards may be more habituated to humans. As structural habitat use (e.g. preferred perch diameter) may vary among these two populations, morphological characteristics such as limb dimensions may differ as well, given the strong relationship among anole species between structural habitat use and hindlimb length (see Losos *et al.*, 2000 for a discussion of the plastic effects of surface diameter on limb dimensions).

We tested these ideas by measuring external morphology, structural habitat use, clinging ability, and escape behaviour in adult male, adult female, and juvenile green anoles (*A. carolinensis*) on the Tulane campus to compare against the data already collected for the GHF population (Irschick *et al.*, 2005). We addressed the following specific questions: (1) How do the random (i.e. available) and actual patterns of habitat use differ between GHF and Tulane? (2) Do external morphology, clinging ability and escape behaviour differ between populations? (3) Is it possible to relate differences in morphology, performance, and escape behaviour to differences in structural habitat for these two populations?

## MATERIAL AND METHODS

### HABITAT SAMPLED

Irschick *et al.* (2005) presented details on the GHF population and these details are only briefly reviewed here. Lizards ( $N = 242$ ) in Tulane were sampled along a 380 m linear transect, a road on which vegetation was present on either side (Fig. 1B). This is analogous to the 755 m linear transect for the GHF population, which was also a road with narrow strips of vegetation on either side (Fig. 1A). Thus, lizards in both populations occupy narrow strips (GHF), or clumps (Tulane) of vegetation on both sides of a barrow road. The habitat structure at Tulane consists of clumps of vegetation 2–10 m apart. The habitat is artificially maintained (e.g. regular watering via a sprinkler system, occasional removal of dying plants), although lizards are generally undisturbed (Irschick, pers. observ.). Thus, lizards are largely isolated in different clumps of vegetation. Sampling was performed in vegetation on both sides of the transect.

The GHF transect consists of a rarely used dirt access road bordered on either side by relatively thin, but continuous, strips of vegetation 3–4 m wide, and surrounded by an open-water swamp that effectively blocks lateral movements of the lizards relative to the transect. The vegetation is a mixture of low-lying shrubs and grass interspersed with larger trees and bushes.

### MEASUREMENTS OF RANDOM AND ACTUAL HABITAT USE

All fieldwork at Tulane University took place between 1 March and 15 May 2003. The availability of the structural habitat for *A. carolinensis* was quantified by measuring the availability of perches at 0.5, 1 and 2 m (relatively few lizards perched above 2 m, see Results) at regular intervals along the transect. 2-m-long rods were placed horizontally and perpendicularly to the transect, at heights of 0.5, 1 and 2 m, with the centre-point located approximately 250 cm away from the road (and thus located around the middle of the strip of vegetation on either side). We defined a perch as any surface in between two nodes. Thus, for each perch, we measured perch diameter, perch length, the distance to the nearest perch ( $D_{np}$ , taken from the middle of each perch), and the diameter of that closest perch ( $PD_{np}$ ). The transect was sampled every 5 m on both sides of the transect (left and right sides), resulting in a total of 156 sample points.

To determine the actual habitat use of anoles in the habitat, we walked along the transect daily when lizards were active (09.00 to 17.00 h), and captured any lizard sighted. We carefully scanned all areas to eliminate the potential bias of searching for lizards where

they were likely to be most common. We recorded the following variables for each lizard upon capture: substrate type (e.g. tree trunk, branch), perch height, diameter, and length,  $D_{np}$  and  $PD_{np}$ . The position of each lizard was marked using small coloured flags, and after the appropriate measurements were taken, lizards were returned to their original point of capture (typically within 24 h). We attempted to sample evenly by walking the entire transect each day, except when there was torrential rain or when ambient temperatures were unusually low (i.e. less than about 25 °C).

#### MEASUREMENTS OF ESCAPE BEHAVIOUR

We collected escape behaviour data in the field for adult male, female and juvenile *A. carolinensis* from September to November 2002 at Tulane University and GHF. The same person (EC) conducted all escape trials, and attempted to wear similarly coloured clothing each day. Once a lizard was sighted, one person, acting as a threat, approached it with an outstretched arm at a constant pace until it fled. The distance between the fingertip of the person and the lizard at the moment of flight was the 'approach' distance. The distance the lizard travelled before stopping was measured as the 'flight' distance. The 'final' distance was calculated by adding the two distances together.

#### *Clinging ability*

We collected large samples ( $N = 242$ ) of three age/sex classes (adult males, adult females, juveniles) from Tulane during normal activity periods for clinging and jumping trials either by hand or a noose attached to the end of a pole. Lizards were placed in plastic bags and transported to Irschick's laboratory at Tulane University. Lizards were always released within 48 h at the point of capture.

Clinging ability was measured by inducing lizards to adhere to an acetate sheet attached to the surface of the force plate with tape (using the same methods as Elstrott & Irschick, 2004). We only included those trials in which lizards exerted a maximum effort by extending both forelimbs and placing their toepads flush on the sheet. Because the substrate was smooth, our measure of clinging ability did not include the claws. Each lizard participated in a session consisting of three trials, with approximately 1 h of rest between each one.

During a trial, the lizard was removed from the incubator and placed with its front feet on the sheet (see below). The lizard was then repeatedly dragged horizontally at a constant speed (i.e. not jerked) across the force plate for 30 s. We estimated the pulling speed to be approximately 5 cm/s, and only one investigator (DI) conducted these trials to ensure consistency.

Slight differences in the velocity of dragging do not affect force output, but rapid acceleration can potentially affect it (Autumn, unpubl. data). The lizard was returned to the incubator, and the top five performances recorded for that trial. Lizards were allowed to rest between different trials for at least 2–3 h. All individuals were placed inside an incubator at 32 °C (similar to the preferred field temperature) for at least 1 h prior to performance measurement.

#### MORPHOLOGICAL VARIABLES

The following morphological variables were measured for each individual: mass, snout–vent length (SVL), and the length of the tail, humerus, radius, metatarsus of the forelimb, longest toe of the forelimb, femur, tibia, metatarsus of the forelimb and longest toe of the hindlimb. All morphological measurements (excluding mass and toepad area) were taken using Mitutoyo digital calipers ( $\pm 0.01$  mm). The images of both sets of forelimb toepads were digitized using an HP Scanjet 5370C and saved as JPEG files. Their combined areas were calculated using the program TpsDig. Mass was recorded by placing lizards inside a small cup on a Denver instruments M-220 electronic balance (accuracy to the nearest 0.01 g).

#### DATA ANALYSIS

Following similar protocols to those described in the companion paper (Irschick *et al.*, 2005) we divided the data into three groups. Juveniles were below 40 mm SVL and displayed no obvious sexual traits (e.g. enlarged dewlap). Adult females were greater than 40 mm SVL; they exhibited a narrow tail base and a reduced dewlap area. Adult males were greater than 45 mm SVL; they exhibited enlarged tail bases and dewlap areas.

We compared random vs. actual patterns of habitat use by employing Kolmogorov–Smirnov tests. We pooled the 0.5, 1.0 and 2.0 m random sample for Tulane because we wanted to represent the entire spectrum of available perches for anoles (nearly all of which perched between 0.5 and 2.0 m, see Table 1). We conducted the following comparisons: (1) within Tulane (actual vs. random); (2) within GHF (actual vs. random); (3) Tulane-GHF (actual vs. actual), and (4) Tulane-GHF (random vs. random).

For GHF anoles, we only conducted random habitat samples at 1 and 2 m, but because the 0.5 and 1 m Tulane random samples were statistically indistinguishable for all variables (KS tests, all  $P$ -values  $> 0.20$ ), the two sets of measurements are largely equivalent. These sets of comparisons thus test both within- and among-population differences in whether the habitat use of anoles reflects random habitat

**Table 1.** Descriptive statistics for random and actual habitat measures for three different sex and age classes of *Anolis carolinensis* for Tulane (see companion paper for similar values for GHF). Values are means  $\pm$  1 SE, with median values in parentheses. *Abbreviations:* PH, perch height; PD, perch diameter; PL, perch length;  $D_{np}$ , distance to nearest perch;  $PD_{np}$ , diameter of nearest perch

	PH	PD	PL	$D_{np}$	$PD_{np}$
RANDOM ( $N = 379$ perches)	–	$13.3 \pm 1.4$ (5)	$60.0 \pm 4.4$ (38)	$19.1 \pm 2.3$ (6)	$8.3 \pm 0.8$ (4)
ACTUAL					
Juveniles ( $N = 75$ )	$70.2 \pm 3.2$ (66.0)	$7.8 \pm 0.4$ (8.0)	$51.0 \pm 3.5$ (45.0)	$10.2 \pm 1.7$ (7.0)	$7.4 \pm 0.3$ (7.0)
Females ( $N = 70$ )	$61.8 \pm 3.0$ (60.5)	$8.3 \pm 0.9$ (9.0)	$51.8 \pm 4.1$ (46.0)	$8.0 \pm 0.8$ (6.0)	$7.0 \pm 0.4$ (8.0)
Males ( $N = 88$ )	$69.2 \pm 3.1$ (67.5)	$10.2 \pm 1.3$ (9.0)	$60.7 \pm 4.7$ (46.0)	$13.0 \pm 2.4$ (8.0)	$8.9 \pm 1.5$ (8.0)

**Table 2.**  $D_{max}$  values from Kolmogorov–Smirnov tests comparing random and actual habitat distributions for Tulane and GHF. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Abbreviations per Table 1

Comparison	Kind of comparison	PD	PL	$D_{np}$	$PD_{np}$
d.f.		20	22	17	22
Within Tulane	Actual-Random	0.40**	0.51***	0.53***	0.39**
Within GHF	Actual-Random	0.74***	0.71***	0.37*	0.39**
Tulane-GHF	Actual-Actual	0.66***	0.43***	0.53***	0.80***
Tulane-GHF	Random-Random	0.40**	0.23	0.40*	0.50***

patterns. We did not divide the Tulane and GHF data sets into different age/sex classes because we were primarily interested in global patterns of habitat use. We did not use a correction for multiple comparisons but have provided an estimate of  $P$ -values for the KS tests to enable the reader to assess the magnitude of statistical differences, and thus the biological significance.

We tested for differences in morphology (residuals) and habitat use (non-size-adjusted) between the two populations by first conducting principal components analyses (PCA, rotated using varimax rotation) on log-transformed variables for the pooled data sets, and then conducting univariate ANOVAs on the resulting PCs using population type ( $N = 2$ ) and age/sex ( $N = 3$ ) as the two factors. We only included those PCs with eigenvalues greater than 1.0 (Jackson, 1993).

We compared escape behaviours (approach, flight, and final distance) both among populations ( $N = 2$ ) and among males and females within each population ( $N = 2$ ) using two-way ANOVAs with population and sex being fixed factors (not enough juveniles could be examined to compare across populations). Because clinging ability and toepad area both increase with size among anoles (Irschick *et al.*, 1996), we conducted analysis of covariance (ANCOVA) to compare log-transformed toepad area and clinging ability ( $y$ -axis) vs. log-transformed SVL ( $x$ -axis) using population type as the relevant category in separate regressions.

## RESULTS

### HABITAT USE

We sampled a total of 242 green anoles on the Tulane campus (see Table 1 for sample sizes for adult males, adult females, and juveniles). Consistent with anecdotal observations, anoles in the Tulane population perched almost exclusively on large palmetto (*Aspidistra elatior*) leaves (71%), with a smaller number perching on branches (3%), tree trunks (4%), signs/fence posts (7%), and other surfaces (15%). This contrasted markedly with the GHF population, where they primarily perched on branches (68%) and tree trunks (18%), with a smaller number using leaves or stems of grass (8%), and other surfaces (6%).

Within Tulane and GHF, actual habitat usage distributions were always significantly different from random (Table 1). Thus, actual habitat use in both populations is not simply a reflection of the underlying distribution of ecological variables. Similarly, Tulane and GHF differ at a highly significant level when comparing actual distributions for all ecological variables (Table 2). With the exception of perch length, the random distributions in both populations also differed significantly for all variables (Table 2). Thus, Tulane and GHF differ dramatically both in their underlying available habitat and actual habitat use (see analyses

for testing directionality of effects below). Figure 2 provides an example of random and actual distributions for both populations for perch diameter. Figure 3 shows the actual distributions for perch height, showing a propensity for Tulane anoles to perch at lower heights than GHF anoles (KS test,  $D_{\max} = 0.62$ , 27 d.f.,  $P < 0.001$ ).

The PCA for the habitat variables (pooling Tulane and GHF) yielded five PCs, although only PCs 1 (2.71) and 2 (1.08) had eigenvalues greater than 1.0, together explaining *c.* 40.5% of the variation. We used only these two PCs in further analyses (Table 3). PC 1 explained 19.9% of the total variation, and exhibited high positive loadings for the diameter of the nearest perch (Table 3). Only distance to nearest perch had a high (and positive loading) with PC 2.

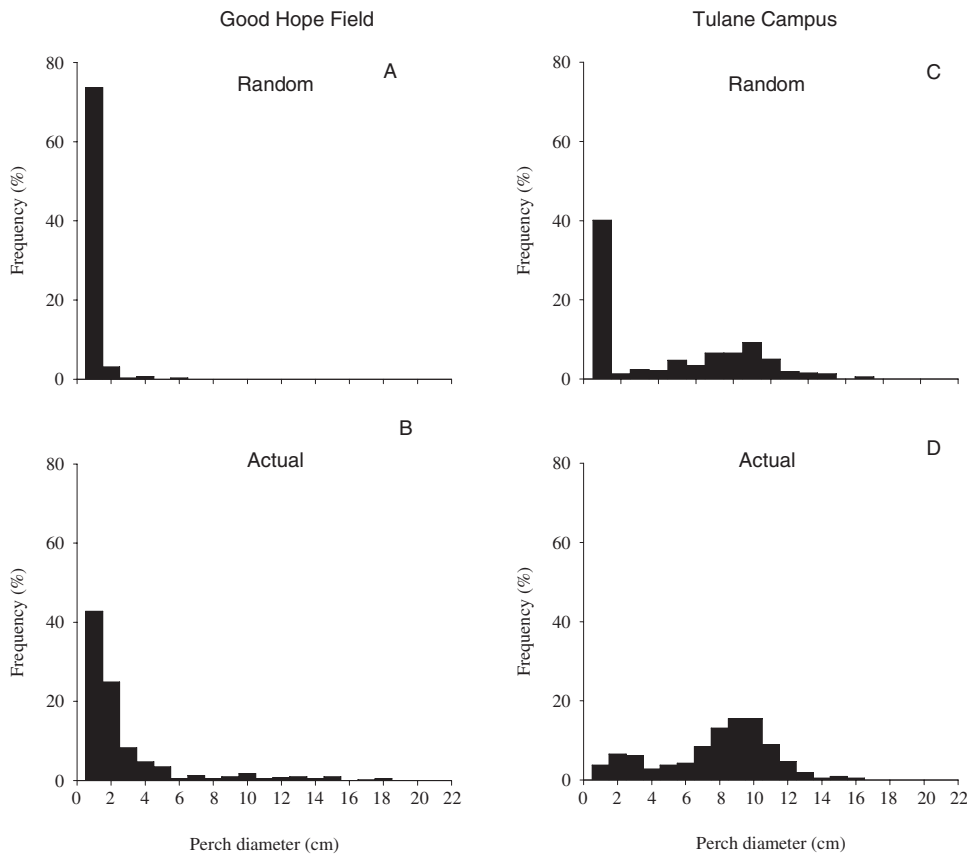
#### ESCAPE BEHAVIOUR

Escape behaviour exhibited significant sex and population effects. For both populations, females had

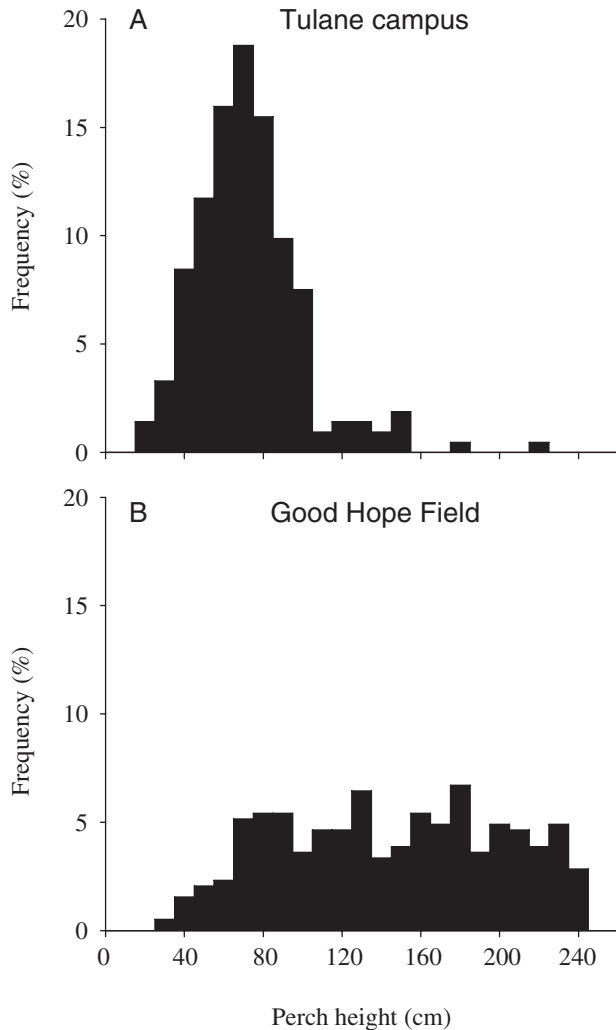
longer approach distances (and were thus more wary), than males ( $F_{1,73} = 9.9$ ,  $P < 0.01$ ), and approach distances were substantially larger in GHF than in Tulane ( $F_{1,73} = 28.2$ ,  $P < 0.001$ , Fig. 4). Thus, females are generally more 'wary' than males, and anoles of both sexes are more wary in the GHF population com-

**Table 3.** Loadings from a PCA for five habitat variables pooling GHF and Tulane anole populations. Substantial loadings are in bold. Abbreviations per Table 1

	PC 1	PC 2
PD	0.327	0.184
PH	-0.252	0.021
PL	0.122	0.260
$D_{np}$	0.103	<b>0.956</b>
$PD_{np}$	<b>0.894</b>	0.120
% variance explained	19.9	20.6



**Figure 2.** Frequency distributions of random (A, C), and actual (B, D) perch diameters for GHF (A, B) and Tulane (C, D). Sample sizes: GHF random ( $N = 211$ ), Tulane random ( $N = 379$ ), GHF actual ( $N = 403$ ), Tulane actual ( $N = 242$ ). Each actual distribution pools males, females, and juveniles

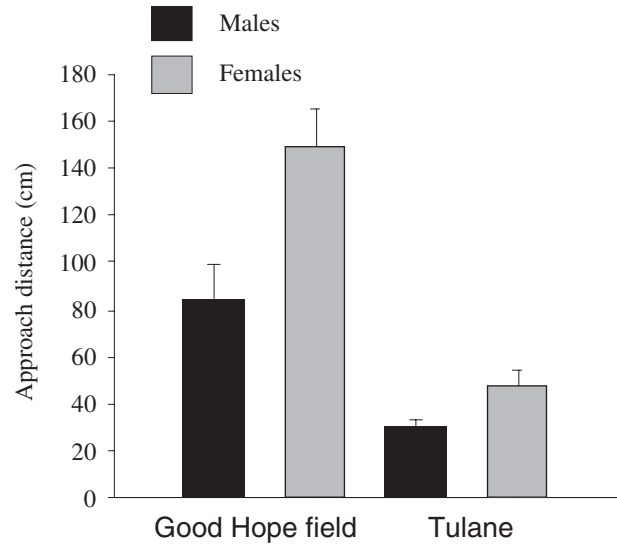


**Figure 3.** Frequency distributions of perch heights used by anoles at (A) Tulane and (B) GHF. Each distribution pools males, females, and juveniles.

pared to that at Tulane. By contrast, flight distances did not vary either among sexes ( $F_{1,67} = 0.002$ ,  $P > 0.25$ ), or among populations ( $F_{1,67} = 0.04$ ,  $P > 0.50$ ) (Fig. 4). Anoles also showed a population ( $F_{1,67} = 20.8$ ,  $P < 0.001$ ) and sex effect ( $F_{1,67} = 6.5$ ,  $P < 0.05$ ) for final distances.

#### MORPHOLOGY

Table 4 provides summary statistics for morphological variables for Tulane anoles. Only PCs 1–3 (together explaining 33.7% of the variation) had eigenvalues greater than 1.0 (2.54, 1.46 and 1.25, respectively), so we included only these PCs in further analyses. The length of the metatarsus of the hindlimb had a high and positive loading with PC 1, the length of the



**Figure 4.** Mean (+ 1 SE) approach distances for adult males and females from both GHF and Tulane.

radius with PC 2 (Table 5), and mass with PC 3 (Table 5).

#### UNIVARIATE TESTS COMPARING MORPHOLOGY AND HABITAT USE

For habitat, only PC 1 differed significantly between populations, while PC 2 showed a significant age/sex effect (Table 6). For morphology, all three PCs differed significantly between populations, with PCs 2 and 3 showing significant age/sex effects (Table 6) and PC 1 showing a significant interactive effect of population and age/sex (Table 6). Figure 5 shows a representative plot of PC 1 (morphology) vs. PC 1 (habitat) for all Tulane and GHF anoles.

In terms of morphology, the anoles of GHF tended to have larger values for PCs 1 and 3, but smaller values for PC 2. Inspection of Table 5 reveals that they exhibited longer lengths of the metatarsus element of the hindlimb, shorter lengths of the radius of the forelimb, and higher values of mass (i.e. they have longer hindlimbs, shorter forelimbs, and are more stocky) compared to those of Tulane. The lone significant interactive effect (population by age/sex) was in PC 1, reflecting the tendency of the Tulane juveniles to have shorter hindlimbs. Thus, relative limb dimensions seem to reverse their rank order within age/sex classes across different populations.

In terms of habitat, Tulane had larger values of PC 1 compared to GHF, as they occurred in habitats with greater diameters of nearby perches ( $PD_{np}$ ). This is consistent with qualitative observations, as Tulane anoles occur on densely packed clumps of broad leaves.

**Table 4.** Mean ( $\pm 1$  SE) morphological measures for three different sex and age classes of Tulane *Anolis carolinensis*. All measurements (except for mass, g) in mm

Variable	Juveniles	Adult females	Adult males
SVL	40.2 $\pm$ 0.41	51.0 $\pm$ 0.50	59.9 $\pm$ 0.90
Mass	1.3 $\pm$ 0.03	2.7 $\pm$ 0.09	4.3 $\pm$ 0.20
Femur	7.6 $\pm$ 0.09	9.2 $\pm$ 0.11	11.1 $\pm$ 0.17
Tibia	7.8 $\pm$ 0.10	9.5 $\pm$ 0.09	11.4 $\pm$ 0.17
Metatarsus hindlimb	4.4 $\pm$ 0.09	5.5 $\pm$ 0.09	6.6 $\pm$ 0.12
Longest toe hindlimb	5.5 $\pm$ 0.07	6.4 $\pm$ 0.07	7.8 $\pm$ 0.11
Humerus	5.5 $\pm$ 0.07	6.7 $\pm$ 0.07	7.8 $\pm$ 0.12
Ulna	5.1 $\pm$ 0.07	6.3 $\pm$ 0.08	7.5 $\pm$ 0.11
Metatarsus forelimb	1.9 $\pm$ 0.05	2.1 $\pm$ 0.04	2.6 $\pm$ 0.05
Longest toe forelimb	3.0 $\pm$ 0.05	3.5 $\pm$ 0.05	4.3 $\pm$ 0.07

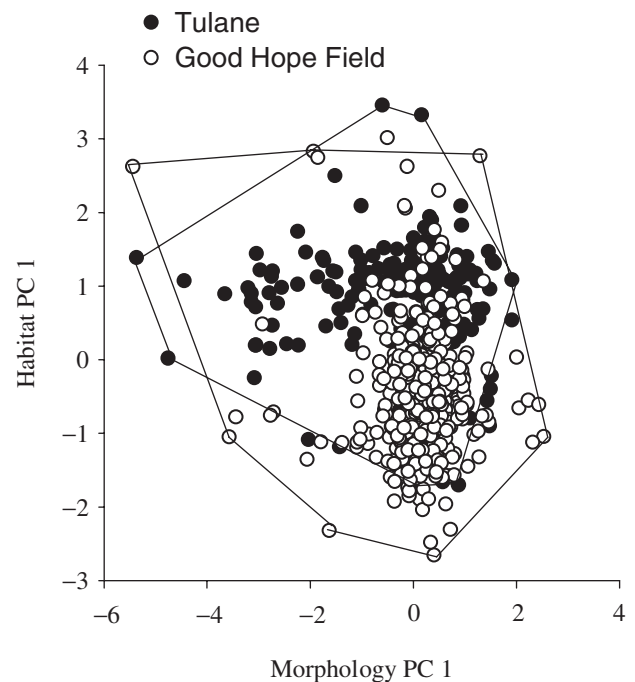
**Table 5.** Loadings from a PCA for nine size-adjusted morphological variables pooling GHF and Tulane anole populations. Substantial loadings are in bold. All measurements (except for Mass, g) in mm

Variable	PC 1	PC 2	PC 3
Mass	-0.005	0.030	<b>0.973</b>
Femur	0.138	0.030	0.118
Tibia	0.210	0.179	0.025
Metatarsus hindlimb	<b>0.954</b>	-0.027	-0.006
Longest toe hindlimb	0.019	0.104	0.097
Humerus	0.184	-0.182	0.140
Ulna	-0.030	<b>0.965</b>	0.137
Metatarsus forelimb	0.034	0.027	0.093
Longest toe forelimb	0.037	0.027	0.094
% variance explained	11.23	11.25	11.20

#### CLINGING ABILITY

Toepad area increased significantly with size within both the Tulane (slope = 1.80,  $y$ -int = -1.96,  $r^2 = 0.84$ ,  $P < 0.001$ ), and GHF (slope = 1.87,  $y$ -int = -2.17,  $r^2 = 0.84$ ,  $P < 0.001$ ) populations. The populations did not differ significantly in the slope of the line relating log-transformed toepad area vs. log-transformed SVL (ANCOVA, slopes test,  $F_{1,579} = 0.977$ ,  $P > 0.25$ ), but the Tulane population had a significantly higher  $y$ -intercept (ANCOVA,  $y$ -intercepts test,  $F_{1,580} = 132.2$ ,  $P < 0.001$ ). Thus, for their size, Tulane anoles have significantly larger toepads.

The ANCOVA comparing log-transformed clinging ability (dependent variable) vs. log-transformed SVL (independent variable) for the two populations showed no significant differences in the slope ( $F_{1,268} = 0.79$ ,  $P > 0.25$ ), but showed that the Tulane anoles have a significantly higher  $y$ -intercept ( $F_{1,269} = 20.1$ ,

**Figure 5.** Scatterplot of PC 1 (morphology) vs. PC 1 (habitat use) for all anoles (males, females, and juveniles) from GHF and Tulane. Each point represents an individual lizard.

$P < 0.001$ ) and thus, for a given body size, tend to cling somewhat more strongly.

#### DISCUSSION

Our comparison of habitat use, morphology and performance between two populations of *A. carolinensis* lizards revealed several key findings:

1. The two populations differ dramatically in both available and actual habitat use. The Tulane habi-



**Table 6.** Results from univariate ANOVAs comparing GHF and Tulane anole populations for the two principal components derived from both habitat and morphology analyses. Values are  $F$ -values from two way ANOVAs using population ( $N = 2$ ) and sex/age class ( $N = 3$ ) as the factors in a two-way ANOVA. \* $P < 0.05$ ; \*\* $P < 0.01$ , \*\*\* $P < 0.001$

	Population	Sex/age class	Pop*sex/age
d.f.	1595	2595	2,595
HABITAT			
PC 1	240.06***	1.21	0.13
PC 2	0.04	3.00*	1.77
MORPHOLOGY			
PC 1	22.58***	0.27	3.60*
PC 2	137.42***	7.10**	1.14
PC 3	29.91***	12.90***	0.50

tat is composed of segregated clumps of broad leaves that are fairly close to the ground, whereas the GHF habitat is composed of continuous arrays of dense bushes and trees, offering a wider range of perch heights, but with typically narrower diameters. Consistent with this observation, the anoles in Tulane perch on leaves far more frequently (71%) than those in GHF (8% perch on leaves, 68% on branches or tree trunks). The perch heights (based on KS tests) and the average diameter of nearby perches (based on ANOVAs) also differ significantly.

2. Tulane anoles tend to have relatively shorter hindlimb elements, longer forelimb elements, and are significantly more 'slender'.
3. Consistent with a priori predictions, Tulane anoles have significantly larger toepads and greater clinging ability.
4. GHF anoles had significantly longer approach distances for both males and females. A surprising finding was a significant sex effect, with females being more wary than males in both populations.

A key tenet of ecological theory is that sexes or ontogenetic classes will often use different microhabitats to reduce competition, particularly when a resource linked with habitat use (e.g. food) is limiting (Schoener, 1968; Werner & Gillam, 1984). This hypothesis has generally found support in previous studies of Caribbean anole species (Schoener, 1968; Schoener & Schoener, 1971a, b; Perry, 1996; Irschick *et al.*, 2000; Herrel *et al.*, 2005), where the general trend is for males to perch higher than females, which in turn perch higher than juveniles. Furthermore, males tend to use broader perches than either females or juveniles (Schoener, 1968; Schoener & Schoener, 1971a, b; Irschick *et al.*, 2000).

Our comparison of two mainland anole populations is only partially consistent with this trend. First, different age and sex classes do not appear to segregate the habitat based on a perch height axis in either population (Table 1 of this paper, see Table 1 of companion paper; also see Jenssen & Nunez, 1998 for a similar result). Second, while some niche segregation exists for perch diameter, the rank order of intraspecific usage differs between the Tulane and GHF populations. At Tulane, the classes follow a pattern similar to that of the Caribbean anoles (males occupy the broadest perches, followed by females, and then juveniles), whereas at GHF, females (mean = 5.4 cm) occupy broader perches than males (mean = 4.0 cm; see Jenssen & Nunez, 1998). Thus, in both populations, the total amount of intraspecific niche segregation seems less than for Caribbean anoles, and there is not a consistent trend in terms of which the classes occupy particular niches.

One factor that could affect the degree of intraspecific habitat segregation when comparing Caribbean and North American mainland anole populations is the abundance and availability of insect prey. Previous studies have shown that food is limiting on Caribbean islands (Andrews, 1979; Roughgarden, 1995), in part because of high anole densities and relatively low insect diversity and abundance. While anole densities in the two Louisiana populations are also extremely high (Irschick, pers. observ.), overall insect diversity and abundance appear to be much higher than for most Caribbean islands (Irschick, pers. observ.; A. Herrel, unpubl. data). If true, intraspecific competition for food might be higher for the Caribbean anoles than for the populations examined here. Further field studies may shed light on this.

On a cautionary note, the two populations were compared during different seasons (Fall 2002 for GHF, Spring 2003 for Tulane), and it is therefore possible

that some of the documented ecological differences could reflect seasonal rather than population-level differences. Green anoles do change their foraging patterns between the growing season and winter (Jenssen *et al.*, 1995), although there are insufficient data to demonstrate conclusively a seasonal effect on preferred perch height and diameter (but see Jenssen & Nunez, 1998). Work on Puerto Rican anoles (Lister, 1981) has shown relatively little ecological change across the seasons. Further study is also needed to address the importance of seasonality in habitat use.

As predicted, both males and females had longer approach distances in GHF than in Tulane. There are two possible and interrelated factors that may explain this difference. First, the abundance and diversity of predators may be higher in the swamp of GHF than in the urban habitat of Tulane. Our observations are consistent with this, as we frequently observed large numbers of snake, bird, and invertebrate predators of anoles in GHF, which we have not observed in Tulane. Demonstrating interpopulational differences in predation pressure is challenging. However, planting clay models mimicking green anoles (e.g. Brodie, 1993) or examining survival rates in predator-proof enclosures might shed light on this issue. A second factor is that humans were much more common on the Tulane campus compared to GHF; Tulane anoles may therefore not perceive an approaching human as a potential threat.

A surprising finding was that females were apparently more 'wary' than males in both populations, as evidenced by their significantly longer approach distances. Previous studies have shown that juvenile, female, and male lizards can sometimes differ in wariness and, more generally, in strategies for escaping predators (Bauwens & Thoen, 1981; Martin & Lopez, 1995; Irschick, 2000; Lailvaux *et al.*, 2003; Whiting *et al.*, 2003). For example, Bauwens & Thoen (1981) showed that gravid female *Lacerta vivipara* lizards used crypsis, rather than flight, to elude potential predators as a potential consequence of their gravid (and hence somewhat performance-impaired) condition. However, the majority of green anole females examined in both habitats were not gravid during escape trials, so this factor would not seem to provide sufficient explanation for the observed sexual difference. In contrast to our findings, adult male *Platysaurus wilhelmi* took refuge significantly earlier than did adult females, and also fled over shorter distances when approached by a human, suggesting that females rely on crypsis as an escape strategy (Lailvaux *et al.*, 2003; Whiting *et al.*, 2003).

One possible explanatory factor is that because green anole males tend to perch conspicuously (Stamps, 1977), it is possible that they are more accustomed to being threatened by predators. In combina-

tion with their higher overall maximum sprint speeds (see e.g. Macrini & Irschick, 1998, who examined *A. lineatopus*), this may make them less willing to flee from a favourable perch. For green anoles, both maximum bite force (A. Herrel, unpubl. data) and maximum jumping ability (see Irschick *et al.*, 2005; horizontal distance jumped) increase significantly with size. Females are therefore at a disadvantage relative to males both in terms of effectively fleeing predators, and also perhaps at defending themselves against a predator by biting once captured (Leal & Rodriguez-Robles, 1995). They may thus be 'compensating' for their relatively poor performance overall by being more wary (Martin & Lopez, 1995; Irschick & Losos, 1998; Irschick, 2000; Herrel *et al.*, 2005).

We showed that the two populations differed significantly in distal limb dimensions. Interestingly, our data show that Tulane anoles have significantly shorter hindlimb elements, but longer forelimb elements. The adaptive significance of this is unclear. Previous discussions of limb length have focused on the strong relationship between preferred perch diameter and relative hindlimb length (Losos & Sinervo, 1989). However, our data reveal the opposite trend, as the average perch diameters used by the Tulane anoles were generally greater than those used by the GHF anoles (cf Table 1 here and in the companion paper).

An unresolved issue in our study is whether the documented differences in morphology are simply plastic changes in response to environmental heterogeneity. Indeed, studies of the brown anole *A. sagrei* showed that animals raised on narrow perches have significantly shorter hindlimbs than those raised on broad ones, suggesting that interpopulational differences in limb morphology could be plastic (Losos *et al.*, 2000). Based on this finding, one might predict that the Tulane anoles would generally exhibit longer limb dimensions because of their proclivity to spend time on broad leaves. However, our data reveal the opposite. This suggests that either limb dimensions change with perch diameter in a different manner for *A. carolinensis* than for *A. sagrei*, or that the documented differences between the Tulane and GHF populations may be genetically based, and are not plastic. Further experiments are needed to resolve this. Another possibility might be that Tulane juvenile anoles use narrow leaves when very small, then shift to broader leaves when older. However, inspection of our habitat data (Table 1) shows that juveniles also use relatively broad perches (mean = 7.8 cm).

Our data also show a significant difference in relative toepad area and relative clinging ability among populations. As predicted, the Tulane population, perching on the smooth surfaces of leaves, had significantly larger toepad areas and higher clinging ability than the GHF population, which primarily perched on

rough (e.g. bark) surfaces. However, as with differences in limb length, whether these differences have arisen as a plastic or genetically fixed response is unclear. Raising juveniles from both populations on both types of surface may shed further light on this (see Losos *et al.*, 2000).

In conclusion, we have documented significant ecological, behavioural, and functional differences between two adjacent yet highly divergent anole populations. We suggest that some of these differences may be adaptive, but further work examining both the basis of habitat segregation (e.g. insect availability and use) and whether the documented differences are genetic or plastic, would be useful.

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