

# Brain Organization and Habitat Complexity in *Anolis* Lizards

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## Key Words

Brain evolution · Comparative neuroanatomy · Habitat complexity · Reptile

## Abstract

Across vertebrates, there is a broad correlation between neuroanatomy and the type of habitat preferred by a species. In general, species occupying habitats classified as more structurally complex have relatively larger brains and exaggerated structures related to navigating and exploiting those habitats. We empirically measured the structural habitat complexity of six species of Puerto Rican *Anolis* lizards, which have traditionally been classified as occupying three distinct habitat types. We also measured the volume of the whole brain as well as six structures putatively related to exploiting complex habitats in these species. We found a significant interspecific variation in structural habitat complexity, including a substantial variation between those belonging to the same ecomorph category. Despite this, we found no evidence to support the hypothesis that interspecific differences in habitat preferences, particularly differences in the relative structural complexity of those habitats, can favor a divergence in neuroanatomy. However, our findings indicate that, at a finer scale, species preferences for structural habitats vary greatly between *Anolis* species belonging to

the same ecomorph category. This variation might contribute to the community structure of anoles by allowing multiple sympatric species of the same ecomorph category to occupy what, at a coarse scale, appears to be the same structural niche. We propose that, in the case of arboreal species, differences in the complexity of arboreal habitats alone may not be sufficient to favor divergent brain evolution.

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## Introduction

There is a rich literature in the fields of animal behavior, ecology, and evolution evaluating the myriad effects of habitat complexity on species ecology. For example, it has been shown that interspecific differences in behavioral traits such as locomotion [Moermond, 1979a; Mattingly and Jayne, 2004], foraging behavior [Moermond, 1979b; Bezemer et al., 2010], territoriality [Stamps, 1977; Jensen et al., 2005], and aggression [Danley, 2011; Kobler et al., 2011] can be accounted for by interspecific differences in habitat preference, particularly the degree of structural habitat complexity. At the proximal level, the above differences might reflect interspecific differences in neuroanatomy, which provides the physical substrate through which selection can shape behavioral differenc-

es [Bell and Szabo, 1986; Jacobs et al., 1990; Baron et al., 1996; Striedter, 2005; Raby and Clayton, 2010].

The relationship between the size of the brain, including its constituent regions, and habitat complexity has been studied across a wide range of taxonomic groups. For example, in the seabird Leach's storm petrel, individuals nesting in forests have larger hippocampal volumes than those nesting in meadows [Abbott et al., 1999]. Similarly, chipmunks dwelling in dense forests with extensive understories have larger brains than those living in more open forests, which have larger brains than those living in shrub-steppe habitats [Budeau and Verts, 1986]. In bats, which have been extensively studied due to their specialized sensory systems, habitat complexity is correlated with the size of the hippocampus [Safi and Dechmann, 2005]. In addition, bats foraging in more cluttered habitats have relatively larger brains than those foraging in open habitats [Ratcliffe et al., 2006]. The previous examples illustrate a recurring finding, which is that interspecific differences in the relative volume of a given brain structure or overall brain volume correlate with interspecific differences in habitat complexity. More generally, these findings support a dominant theme in comparative neuroanatomy: relatively larger brains and brain structures can provide the additional cognitive processing power necessary to effectively exploit more complex habitats [Chittka and Niven, 2009].

Although reptiles have been studied less than mammals, birds, or fishes, it has been suggested that they too exhibit an association between species ecology and neuroanatomy [Day et al., 1999; LaDage et al., 2009]. The adaptive radiation of West Indian anoles has become a model system for studies of behavioral and evolutionary ecology. In anoles, a predominant line of inquiry was and continues to be focused on how selection to effectively exploit distinct habitat types has driven many aspects of anole anatomy and ecology, including behavior, morphology, and physiology [e.g. Williams, 1972, 1983; Losos, 1990a, b; Johnson and Wade, 2010; Gunderson and Leal, 2012; Mahler et al., 2013]. Collectively, this research has demonstrated that Greater Antillean anoles can be grouped into ecological types or 'ecomorphs' which broadly describe the structural habitat in which species are most commonly found [Williams, 1972]. Furthermore, the same ecomorph types have evolved independently multiple times and constitute the trademark of the anole adaptive radiation, which is characterized by convergent evolution in behavior, ecology, and morphology [see Losos, 2009; Mahler et al., 2013].

In the case of behavior, it has been documented that species of anoles belonging to the same ecomorph exhibit similar foraging strategies, movement patterns, and territorial behavior [Moermond, 1979a, b, 1981; Johnson et al., 2008, 2009]. However, the structural habitats occupied by different ecomorphs can differ in their respective spatial complexity [Johnson et al., 2006], which may alter the difficulty of navigating through each habitat [Moermond, 1986; Johnson et al., 2006]. This finding makes anoles an ideal group to evaluate the hypothesis that interspecific differences in habitat complexity would favor interspecific differences in overall brain structure [Budeau and Verts, 1986; Safi and Dechmann, 2005].

To test this hypothesis, we collected two types of data for six species of Puerto Rican anoles (*Anolis cristatellus*, *A. evermanni*, *A. gundlachi*, *A. krugi*, *A. pulchellus*, and *A. stratulus*), which are representative of three ecomorph types from the Puerto Rican anole radiation [Mahler et al., 2010]. First, we measured the volumes of six structures within the brain as well as the whole brain. The structures measured are all putatively concerned with learning and navigating complex habitats (see Materials and Methods), and several may exhibit hyperallometry within the anole brain [Powell and Leal, 2012]. Second, we quantified the structural habitat complexity of the habitat utilized by each of these species. If habitat complexity selects for differences in the relative volume of the brain or its constituent structures, we predict a positive correlation of the relative volume of brain structures related to spatial learning and memory with structural habitat complexity.

## Materials and Methods

All species were collected in the general vicinity of El Verde Biological Station, located in the northeast mountain range of the island of Puerto Rico. Data were collected in the habitat most commonly used by each species, and only for males. Individuals of *A. cristatellus* and *A. krugi* were sampled at the forest edge along small roads or in forest gaps. *A. gundlachi* was sampled in the understory of the closed-canopy, mature forest. *A. pulchellus* was sampled in open grass fields near the station. *A. evermanni* was sampled where the canopy descends along the edges of streams running through the rainforest, whereas *A. stratulus* was sampled in the rainforest canopy from a canopy tower.

### Brain Volumes

The specimens were collected in the areas described above. For neuroanatomical measurements, we collected individuals of each of the following species: *A. cristatellus* (n = 10), *A. gundlachi* (n = 10), *A. krugi* (n = 10), *A. pulchellus* (n = 8), *A. evermanni* (n = 10), and *A. stratulus* (n = 10) by hand or noose during July and August

2006, July and August 2007, and August 2008. Upon capture, we measured the mass and the snout-vent length (SVL) of all individuals.

From all specimens, we removed the brains and fixed them in paraformaldehyde. We embedded the brains in paraffin and sectioned them at 7 and 10  $\mu\text{m}$  thickness. The sections were stained in cresyl violet and photographed. For each brain, we used the photographs to measure the volume of six structures within the brain as well as the whole brain volume (see Materials and Methods in Powell and Leal [2012] for a detailed description of brain removal, sectioning, and measurements).

We chose to measure six structures within the brain based on their putative relationship to spatial learning and memory. The telencephalon (TEL) is a major division of the forebrain that has been related to a variety of higher cognitive functions; all other measured structures except for the cerebellum (CER) are part of the TEL [Butler and Hodos, 2005; Striedter, 2005]. The dorsal cortex (DC) is a potential homologue to the mammalian isocortex, and thus may be related to general cognitive ability [Striedter, 1997, 2005; Butler and Hodos, 2005]. The dorsomedial cortex (DMC) and medial cortex (MC) are homologous to areas of the mammalian and avian hippocampal formations, which are integral to spatial memory and learning [Striedter, 1997; Day et al., 2001; Butler and Hodos, 2005; Striedter, 2005; LaDage et al., 2009; Raby and Clayton, 2010]. The dorsal ventricular ridge (DVR) is primarily responsible for sensory integration in the reptilian brain and functions similarly to the mammalian isocortex [Butler and Hodos, 2005; Striedter, 2005]. The CER performs a large number of roles in the brain, but was chosen for this study based on its function in maintaining balance, coordinating muscular activity, and learning motor strategies [Butler and Hodos, 2005]. Moving over different substrates in varying habitats may result in changes in cerebellar volume or structure [Yopak et al., 2010].

### Structural Habitat Complexity

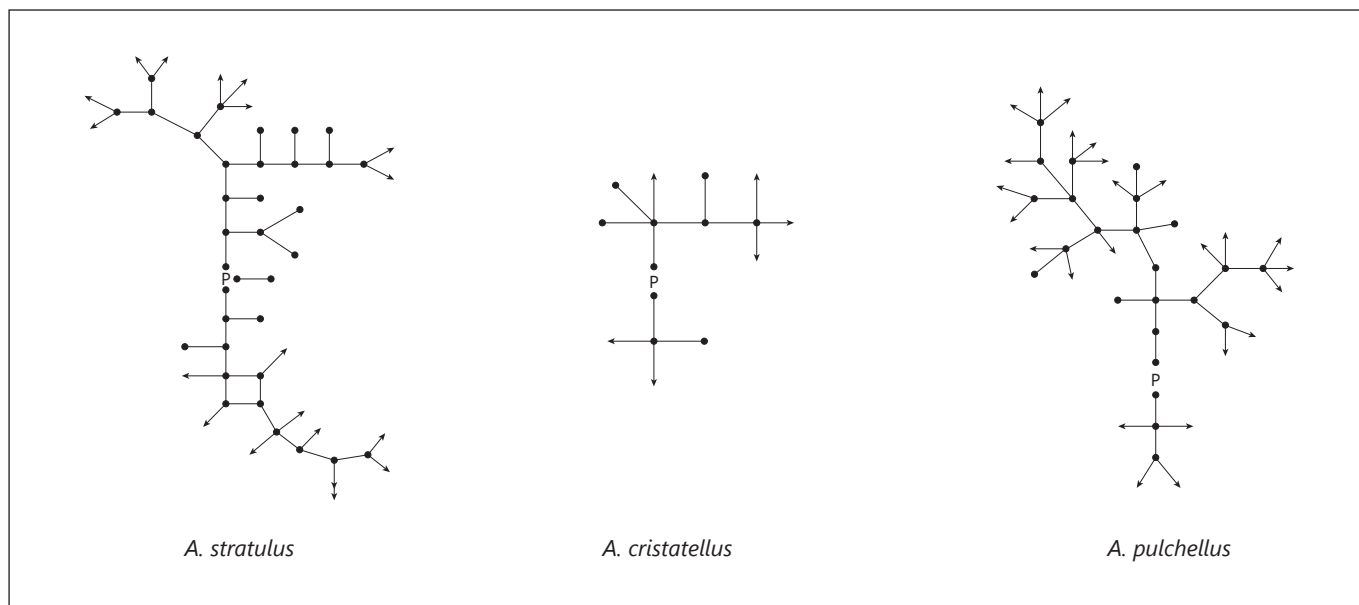
A primary goal in describing habitat complexity is to do so at a scale relevant to the biology of the species [Huston, 1994]. Therefore, an informative description of habitat complexity must take into account the morphology and behavior of the species and how those traits can affect behaviors such as locomotion and general movement patterns [Huston, 1994; Tews et al., 2004]. Failure to do so may result in documenting complexity that, while quantifiable from a human perspective, might not be relevant to the organisms.

To measure habitat complexity, we collected three types of data, which together provide a biologically relevant approximation of the habitat used by each species and the complexity of that habitat at each species' appropriate scale. First, we conducted focal observations of individuals from each of the species to determine the species' relevant movement distances. Second, we diagrammed the habitat structure surrounding perching lizards. Each diagram encompassed 75% of the movement distances for that species in all directions. Finally, we conducted another set of focal observations to evaluate how the habitat structure influences each species' movement patterns. This measurement determined the degree to which the habitat structure constrained a lizard's movement; in other words, it described how many possible paths connect two points along the lizard's immediate movement substrate. This was used to correct the scale of our habitat complexity measurement to a scale that was relevant for each of the species' movement.

To determine movement patterns, we conducted focal observations on adult males of each species (*A. cristatellus*:  $n = 20$ ; *A. evermanni*:  $n = 22$ ; *A. gundlachi*:  $n = 21$ ; *A. krugi*:  $n = 20$ ; *A. pulchellus*:  $n = 20$ ; *A. stratulus*:  $n = 22$ ). We followed each individual for 20 min or until it was lost from sight. Lizards that were observed for less than 5 min or continuously moved away from the observer were not included in the analysis. We defined a movement bout as any series of movements not interrupted by the lizard remaining stationary for at least 10 s. The distance of each movement bout was estimated to the nearest centimeter. We used these data to calculate the 75th percentile value of the movement distance within a single movement bout for each species, henceforth referred to as diagrammed distance. The diagrammed distance was used to inform our second set of measurements, which we called vegetation profiles.

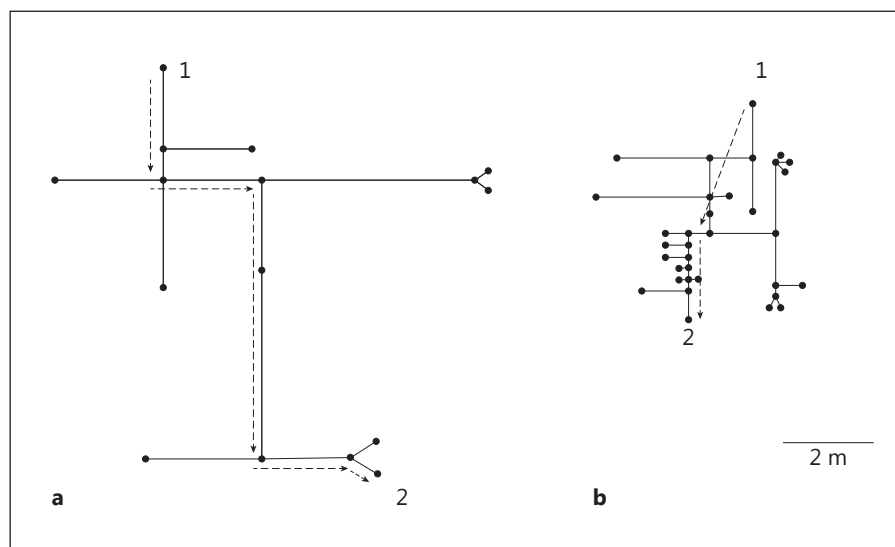
To measure the structure of the vegetation (i.e. vegetation profile) at perch sites selected by lizards, we walked slowly through the habitat searching for perching adult males. Once we found a perching lizard (*A. cristatellus*:  $n = 60$ ; *A. evermanni*:  $n = 60$ ; *A. gundlachi*:  $n = 59$ ; *A. krugi*:  $n = 60$ ; *A. pulchellus*:  $n = 60$ , *A. stratulus*:  $n = 60$ ), we diagrammed the structure of the habitat, beginning from the point where each lizard was perching and expanding in all directions out to the diagrammed distance or the end of the structure. The diagrams included the length and diameter of every branch as well as the number of branches extending from each choice point (fig. 1). A choice point was defined as any point where a lizard was presented with multiple branches proceeding further away from the perch and thus had the option to change the direction of its movement. From each diagram, we counted the number of branches, choice points, end points, and bridges. A branch was defined as any continuous structure between choice and end points. An end point was defined as any point where the structure ends or that is the full diagrammed distance away from the perch. A bridge was defined as any point where paths that have previously diverged come back together. We also determined the total distance of all branches in the diagram. Because the diagrammed distance varied for each species, we divided the total distance by twice the diagrammed distance for that species (i.e. the distance that would be comprised by a diagram if the lizard were perching on a straight pole and could only move directly up or down). This value was defined as the corrected distance for each species.

To evaluate the effect of scale on our measures of habitat complexity, we conducted a second set of focal observations to determine the number of branches crossed per meter moved by each species (*A. cristatellus*:  $n = 16$ ; *A. evermanni*:  $n = 15$ ; *A. gundlachi*:  $n = 11$ ; *A. krugi*:  $n = 10$ ; *A. pulchellus*:  $n = 10$ ; *A. stratulus*:  $n = 19$ ). We followed the same procedure described above for our focal observations. As part of the observations, we estimated the distance moved by each lizard to the nearest centimeter and counted the number of branches over which each lizard moved. Low numbers of branches crossed per meter moved indicate that movement is highly constrained by the habitat structure and a lizard must follow a branch until it reaches a choice point in order to cross to a new branch. High numbers indicate that the habitat structure occurs at too small a scale to be meaningful to the lizard. In this case, the branches are tightly clustered and a lizard can move between them without following the branches to a choice point (see fig. 2 for an example). We used the mean number of branches crossed per meter moved for each species to correct our habitat complexity measurements for the scale of each species' movement.



**Fig. 1.** Representative diagrams of the vegetation profiles for *A. stratulus*, *A. cristatellus*, and *A. pulchellus*. P: lizard's initial perch. Dots: ends of branches. Branches ending in arrows indicate that the branch extended beyond the full diagrammed distance.

**Fig. 2.** Diagrammatic representation of how path choices through the habitat determine the number of branches crossed per meter moved. Paths leading from point 1 to point 2 are drawn across the vegetation profile with a dashed line, based on the movement of each species. Sections of the path that cross over branches without moving along them represent the lizard jumping. Both vegetation profiles are drawn to the same scale. **a** Path through a vegetation profile from *A. gundlachi*, which crosses 7 branches moving over 13 m (0.54 branches/m). **b** Path through a vegetation profile from *A. pulchellus*, which crosses 11 branches over 5 m (2.2 branches/m).



#### Statistical Analysis

To test for the possibility of brain volume shrinkage due to variable time between collection and sectioning, we conducted an ANCOVA with brain volume as a covariate and SVL and order of sectioning as independent variables [see Powell and Leal, 2012, for a detailed discussion of this analysis]. Sectioning order did not have a significant effect on brain volume (d.f. = 1, SS = 0.0019,  $F = 0.1980$ ,  $p = 0.6595$ ).

We used phylogenetic methods to conduct the statistical analysis [Felsenstein, 1985]. The phylogeny from Mahler et al. [2010] was

pruned to include only the species in the data set, and with branch lengths made ultrametric using the program r8s [Sanderson, 2003]. To correct overall brain size relative to body size, we performed a phylogenetically corrected reduced major axis (RMA) regression of SVL against brain volume. We corrected the TEL, DC, DMC, MC, DVR, and CER volume for brain size by conducting a phylogenetically corrected RMA regression of brain volume against each structure's volume. An RMA regression was used because it provides more reliable results than least-squares regression in cases such as ours, where the potential for error exists in the measure-

**Table 1.** Mean values of the absolute measurements of brain structures for each of the anole species

	SVL, mm	Body mass, g	TEL, mm <sup>3</sup>	DC, mm <sup>3</sup>	DMC, mm <sup>3</sup>	MC, mm <sup>3</sup>	DVR, mm <sup>3</sup>	CER, mm <sup>3</sup>	Total brain, mm <sup>3</sup>
<i>A. cristatellus</i>	66.2	9.6	34.2384	1.3128	0.2744	0.6766	14.4444	3.3214	94.6791
<i>A. evermanni</i>	58.9	4.9	24.3146	0.7705	0.1826	0.4527	10.6594	2.8130	70.0474
<i>A. gundlachi</i>	62.2	6.3	31.2841	1.0229	0.2460	0.5335	14.7112	3.5740	87.6714
<i>A. krugi</i>	46.1	2.4	16.5204	0.4620	0.1153	0.2495	7.2235	1.9635	48.3676
<i>A. pulchellus</i>	43.6	1.9	13.6981	0.4081	0.1007	0.2306	5.6813	1.5824	39.5821
<i>A. stratulus</i>	45.4	2.2	24.4222	0.8085	0.1624	0.4651	10.6423	2.4780	68.5520

**Table 2.** Results of general linear model regressions of brain structure volumes against RHC

	Y-intercept	Slope	R <sup>2</sup>	p value
Total brain	0.0001	-0.0111	0.2334	0.3317
TEL	-0.0012	-0.0	0.0019	0.9345
DC	-0.0010	-0.0014	0.0627	0.6322
DMC	0.0067	0.0019	0.1092	0.5223
MC	-0.0007	-0.0041	0.4371	0.1528
DVR	0.0019	0.0008	0.0490	0.6733
CER	0.0010	0.0014	0.0750	0.5994

p values denote confidence that the slope differs from 0.

**Table 3.** Results of phylogenetic ANOVA of brain structure relative volume, grouped by ecomorph

	d.f.	F	p value
Total brain	2	1.4006	0.3087
TEL	2	0.4422	0.6254
DC	2	0.0326	0.9670
DMC	2	1.525	0.2897
MC	2	0.4282	0.6643
DVR	2	0.4006	0.6733
CER	2	0.3592	0.6923

ment of both variables [McArdle, 1988]. This analysis was conducted in R [R Development Core Team, 2009] using the phytools package [Revell, 2011]. The RMA regressions were performed using RMA version 1.17 [Bohonak and van der Linde, 2004].

Residuals for each species were used as measures of brain volume corrected for body size. We used a phylogenetic ANOVA to compare the brain size between the different ecomorph types. We first conducted a phylogenetically corrected RMA regression of the mean SVL against the mean total brain volume for each species and computed the residuals for every species. We then conducted a phylogenetic ANOVA on these residuals, grouped by ecomorphs. The analysis was conducted using the phytools package [Revell, 2011] in R [R Development Core Team, 2009].

We compared the average movement distance per movement bout and the number of movement bouts per minute using ANOVA. Significant results were analyzed using the pairwise Student t test. To account for the possibility that the different indices used to characterize habitat complexity (i.e. number of choice points, end points, branches, bridges, and corrected distances) could be correlated, we used principal component analysis (PCA). The number of branches crossed per meter moved was not included in the PCA. The analysis was conducted in R [R Development Core Team, 2009].

In order to scale our measure of habitat complexity to the relevant space used by each species, we divided the principle component scores by the mean number of branches crossed per meter

moved. This final measurement, corrected for scale, will be referred to as relevant habitat complexity (RHC). We determined the relationship between RHC and relative brain size by performing a general linear model regression of phylogenetically corrected brain size relative to body size as well as the volumes of the TEL, DC, DMC, MC, DVR, and CER relative to brain size against the mean RHC.

In order to compare RHC scores between species, we corrected scores for each individual by dividing the first principal component for that individual by the mean number of branches crossed per meter moved for the relevant species. These scale-corrected scores were then analyzed using ANOVA, followed by a pairwise Student t test, if appropriate.

## Results

### Brain Volumes

The mean values for SVL and the volumes for each brain structure measured for each species are reported in table 1 [as presented in Powell and Leal, 2012]. We performed a general linear model regression of RHC against corrected brain volume as well as the volume of each brain structure corrected for total brain volume (table 2).

**Table 4.** Direct measurements of habitat complexity

	75th percentile of movement distance, cm	Mean number of				Mean total distance, cm	Corrected mean total distance
		Choice points	End points	Branches	Bridges		
<i>A. cristatellus</i>	62	4.2	8.7	13.3	0.2	240.0	1.9
<i>A. evermanni</i>	58	8.0	14.9	23.7	0.3	342.7	3.0
<i>A. gundlachi</i>	65	2.7	5.6	8.5	0.3	202.7	1.6
<i>A. krugi</i>	55	4.2	8.0	12.7	0.5	205.8	1.9
<i>A. pulchellus</i>	35	10.2	16.5	28.0	1.3	237.4	3.4
<i>A. stratulus</i>	65	12.9	16.9	30.7	1.05	359.6	2.8

**Table 5.** PCA of habitat complexity

	Component 1
Choice points	-0.3325
End points	-0.4626
Number of branches	-0.8214
Number of bridges	-0.0159
Corrected total distance	-0.0246
Proportion of variance	0.9739

None of the regression slopes were significantly different from 0, demonstrating no significant relationship between the volume of any brain structure measured in this study and RHC. However, MC volume trended toward a negative correlation with RHC. This suggests that species occupying more complex habitats tend to have larger MCs.

At a broader scale, there were no significant differences between ecomorph categories in brain size relative to body size or in the size of brain structures relative to overall brain size (table 3). Therefore, the species' differences in preferred structural niche and use of the related microhabitats do not favor divergence in the volume of the neuroanatomical structures measured in this study.

#### Behavioral Observations

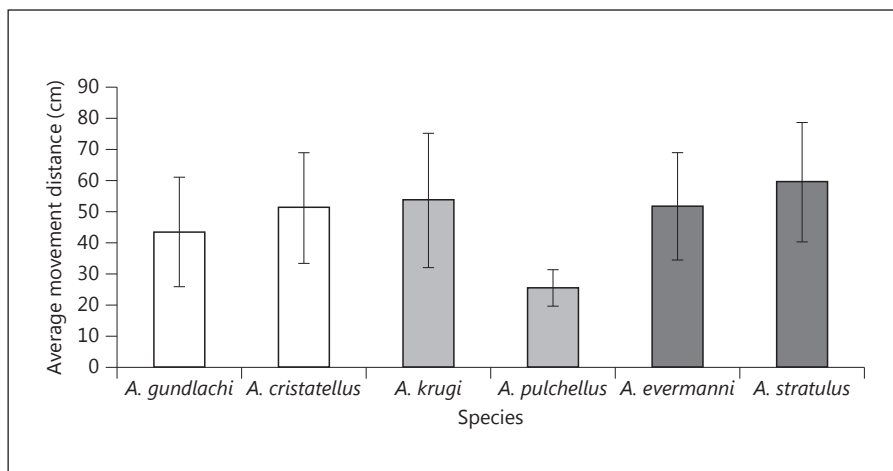
Focal observations always began when the lizard was perching on vegetation. However, each species exhibited a distinctive movement behavior throughout the observation period. The 75th percentile of the movement distance for each species is reported in table 4, while the mean number of branches crossed per meter moved for each species is reported in table 5. Figure 3 shows that the

species did not differ in their average movement distance (d.f. = 5, SS = 15,494.82, F = 1.8618, p = 0.1059). However, as reported in figure 4, the species did differ in movement bouts per minute (d.f. = 5, SS = 1.4278755, F = 11.7002, p < 0.0001).

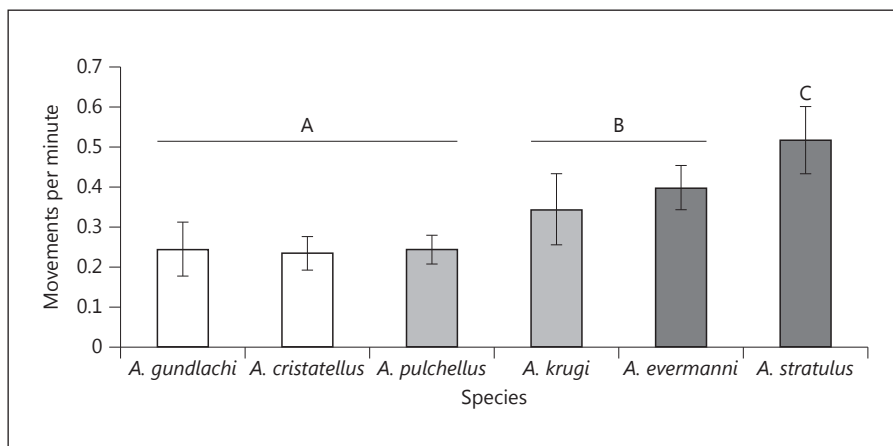
For example, during a typical observation of *A. stratulus*, individuals were moving in the forest canopy, where they perched and moved primarily along larger-diameter branches. They would move onto twigs or large leaves in order to cross between larger branches, which they did fairly often (average number of branches moved across per meter moved = 1.48). Also, individuals of *A. stratulus* moved almost continuously throughout each observation, making a relatively large number of movements, generally of a long distance (average number of movements per minute = 0.52, average distance per movement bout = 60 cm).

In contrast, individuals of the similarly sized *A. pulchellus* were primarily observed in open, grassy fields. They generally perched on high, solitary blades of grass, which can provide good visibility. They would descend such stalks until they reached the tangle of shorter grass stalks near the ground, at which point they would walk across the surface of the grass. Individuals of *A. pulchellus* moved less often than those of *A. stratulus* (average number of movements per minute = 0.24), and their movements were generally short (average distance per movement bout = 25 cm). In cases where individuals of *A. pulchellus* were moving along high, isolated blades of grass, they commonly remained on a single perch until they reached the ground; once they descended into the grasses near the ground, they moved readily across the many tightly clustered blades of grass. This resulted in them crossing a much higher number of branches than any other species observed (average number of branches moved across per meter moved = 29.29).

**Fig. 3.** Average movement distance per movement bout for each of the species. There were no interspecific differences in movement distance per movement bout (d.f. = 5, SS = 15,494.82, F = 1.8618, p = 0.1059). Open bars: trunk-ground species. Light grey bars: grass-bush species. Dark grey bars: trunk-crown species. Error bars: 95% CI.



**Fig. 4.** Number of movement bouts per minute for the six species of anoles sampled in this study. There were interspecific differences in movement pattern (d.f. = 5, SS = 1.4279, F = 11.7002, p < 0.0001). Species labeled with different letters (A–C) are significantly different at p < 0.05, based on pairwise t tests. Open bars: trunk-ground species. Light grey bars: grass-bush species. Dark grey bars: trunk-crown species. Error bars: 95% CI.



### Habitat Complexity

The descriptive variables of habitat complexity for each of the species are summarized in table 4. The PCA yielded a first component explaining 97% of the variance, with large loadings for number of choice points, end points, and branches (table 5). We used this principle component as an index of habitat complexity, in which high levels of structural habitat complexity are indicated by strongly negative scores. The principle component 1 was then divided by the average number of branches crossed per meter moved to yield an RHC (table 6; fig. 5).

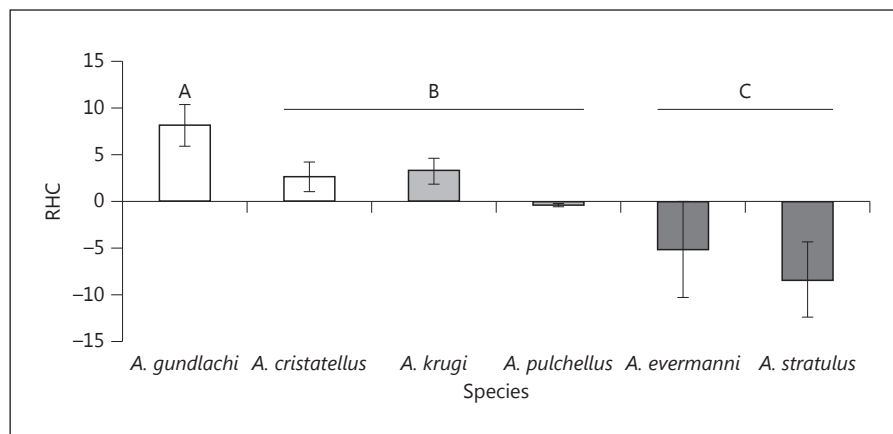
The habitats varied widely in all variables, except the number of bridges, with bridges being rare in all species' habitats. There were significant interspecific differences in RHC (d.f. = 5, SS = 10,808.123, F = 15.8911, p < 0.0001), suggesting that the species prefer habitats of different levels of complexity (fig. 5). The species can be broadly divided into three groups. *A. gundlachi* occupies relatively

**Table 6.** RHC and its constituent variables

	PCA Component 1	Mean number of branches/m	RHC
<i>A. cristatellus</i>	7.6266	2.9	2.6196
<i>A. evermanni</i>	-6.5323	1.3	-5.1598
<i>A. gundlachi</i>	13.3487	1.6	8.1306
<i>A. krugi</i>	8.3038	2.6	3.2436
<i>A. pulchellus</i>	-10.5474	29.3	-0.3601
<i>A. stratulus</i>	-12.4248	1.5	-8.3974

simple habitats, while *A. cristatellus*, *A. pulchellus*, and *A. krugi* all have habitats of varying complexity, but at a small enough scale that the lizards' movements tend to be relatively unhindered. The extreme case of this is *A. pulchellus*, which lives in an extremely complex habitat at a small enough scale that the lizards are able to ignore it

**Fig. 5.** RHC for the six species of anoles sampled in this study, which differed significantly between species (d.f. = 5, SS = 10,808.123,  $F = 15.8911$ ,  $p < 0.0001$ ). Negative values signify a higher-complexity habitat. Species labeled with different letters (A–C) are significantly different at  $p < 0.05$ , based on pairwise  $t$  tests. Open bars: trunk-ground species. Light grey bars: grass-bush species. Dark grey bars: trunk-crown species. Error bars: 95% CI.



during most movements. Finally, *A. evermanni* and *A. stratulus* occupy habitats roughly twice as complex as that of any other species.

## Discussion

A common finding in studies evaluating the possible effect of habitat complexity on neuroanatomy is that species occupying structurally complex habitats have larger brains. This pattern is proposed to result from selection favoring an increase in cognitive power to deal with the higher cognitive demands of a complex habitat and has been shown both for whole brains [Budeau and Verts, 1986; Ratcliffe et al., 2006] as well as for structures of the brain related to spatial memory and learning [Abbott et al., 1999; Safi and Dechmann, 2005]. The results of our study did not support this trend. Instead, we found a lack of correlation between the species' neuroanatomy and RHC for overall brain size as well as all measured brain structures.

The lack of correlation between neuroanatomy and RHC across the six species of anoles sampled in this study was unexpected, particularly in light of the rich literature showing that habitat preference has a major effect on many aspects of anole biology [review in Losos, 2009; but see Leal et al., 2002]. Why neuroanatomy does not follow the same trend as other morphological and behavioral traits in anoles is an open question. However, based on what is known with respect to differences in neuroanatomy between species or populations inhabiting different habitat types, we propose two nonmutually exclusive hypotheses.

First, previous studies have compared species across different habitat categories at a broader scale. For example, arboreal species usually have larger brains than ter-

restrial species [Harvey et al., 1980; Eisenberg and Wilson, 1981; Bernard and Nurton, 1993], and forest species commonly have larger brains than grassland or desert species [Harvey et al., 1980; Budeau and Verts, 1986; Abbott et al., 1999; Safi and Dechmann, 2005]. Although there are interspecific differences in structural habitat complexity between the anoles sampled in this study, all the species can be categorized as arboreal. This suggests that differences in RHC within a given habitat type might not be sufficient to favor divergence in the volume of the overall brain or its constituent structures. In other words, being an arboreal species may be sufficiently cognitively demanding to exert an equal influence on brain size regardless of differences in structural habitat complexity between different types of arboreal habitats.

Second, it is not uncommon for interspecific differences in neuroanatomy and habitat complexity to be accompanied by a divergence in other aspects of the species' biology [e.g. Roth and Thorington, 1982; Shultz and Dunbar, 2006; LaDage et al., 2009; Gonzalez-Voyer and Kolm, 2010]. Such differences include differences in social structure, foraging behavior, and sensory systems. However, all anoles that were used in this study exhibit consistent but only relatively subtle differences in behavior. They are all territorial, exhibit sit-and-wait foraging behavior, are insectivorous, and possess similar sensory systems. The lack of neuroanatomical differences in these anoles raises the possibility that differences beyond habitat complexity are necessary to favor changes in brain organization. For example, recent work with Tanganyikan cichlid species, which demonstrate species diversity and diverse habitat preferences similar to *Anolis*, may shed some light on our findings [Gonzalez-Voyer and Kolm, 2010, and references therein]. In the case of Tanganyikan cichlids, species



divergence in neuroanatomy tends to be correlated with species differences in social structure, parental care, and foraging behavior [reviewed in Gonzalez-Voyer and Kolm, 2010]. This suggests that in the case of closely related species, which are relatively young and can be found syntopically, differences in relative habitat complexity might not be sufficient for selecting differences in neuroanatomy. Alternatively, differences might be present at a finer neuroanatomical scale than we used in this study. Further studies evaluating the possible contributions of habitat type and species ecology and the outcome of their interactions in selecting for differences in neuroanatomy in reptiles, and more generally across taxa, are needed [Healy and Rowe, 2007].

The observed differences in our measure of RHC between the structural habitats occupied by the different ecomorph types generally match an intuitive understanding of expected differences at a relatively coarse level [Budeau and Verts, 1986; Bernard and Nurton, 1993]. For example, the denser forest canopy, i.e. the preferred habitat for *A. evermanni* and *A. stratulus*, is more complex than the more open understory, i.e. the preferred habitat of *A. cristatellus* and *A. gundlachi*. The canopy is also more complex than the bushes along the edge of the forest, where *A. krugi* is commonly found. While grass, the preferred habitat for *A. pulchellus*, is complex, most of its complexity is at a smaller scale than even the movements of a 4-cm lizard, making it relatively simple to navigate. However, the RHC and ecomorph methods of habitat description are not interchangeable.

With the exception of *A. gundlachi*, at a coarse level, species belonging to a given ecomorph occupy habitats that are more similar in RHC than species belonging to different ecomorphs. Nevertheless, even within the same ecomorph category, there is still substantial variation in RHC (fig. 5). Such variability might further contribute to community structures in anoles, by providing a finer scale of habitat partitioning by which sympatric species with similar behavior and morphology can decrease their amount of resource overlap. This seems to be the case for *A. krugi* and *A. pulchellus*, the two species of grass-bush anoles which occupy habitats of differing structural complexity. Although *A. krugi* and *A. pulchellus* are commonly found sympatrically, *A. krugi* seems to prefer bushes and shrubs, although it is occasionally found perching on grass blades (pers. observation). *A. pulchellus* is routinely found in open, grassy areas and has an RHC score reflecting its structurally complex, extremely cluttered habitat. Both species have RHC scores similar to that of the trunk-ground species *A. cristatellus*, which also occupies the

edge of forests, where there are often large amounts of undergrowth. The other trunk-ground species, *A. gundlachi*, has a significantly different RHC score that expresses its relatively simple but very open habitat under the forest canopy. The possibility that anole species prefer habitats with different levels of complexity has been suggested for *A. evermanni* and *A. stratulus*; in this case, the species occupy trees, particularly the canopies, which may differ in their general structure [Dial and Roughgarden, 1994]. Structural habitat partitioning at a finer scale has also been suggested as a mechanism to avoid intersexual competition within anole species [Butler et al., 2007]. Overall, the variation in RHC within ecomorphs opens the possibility for sympatric species to share resources by occupying what has been previously recognized as the same structural niche, and might contribute to the observation that many communities contain multiple sympatric species of the same ecomorph [Losos, 2009].

The impetus of this study was partially based on the general trend that species occupying different levels of habitat complexity also exhibit a divergent neuroanatomy (see references above). Our findings do not support this trend at the level of the whole brain or its constituent structures. Nevertheless, this study illustrates the value of combining a detailed understanding of the natural history of species with a theoretical framework in which to evaluate those observations. Currently, there are no other studies in reptiles with a similar number of species and detailed data of their habitat characteristics to evaluate if our results are limited to anoles or are a shared feature of reptile brain evolution. Further studies on reptiles are needed to evaluate the generality of our findings. More broadly, regardless of the taxonomic group, studies are needed in which both the ecological and neuroanatomical variables are collected at a relatively fine scale using consistent techniques [Healy and Rowe, 2007]. This type of approach should provide a clearer picture of the possible factors contributing to brain evolution.

Finally, much has been written about the fact that, until this study, convergence due to habitat preference apparently permeated every aspect of the biology of West Indian anoles [see Losos, 2009]. Neuroanatomy, the physical substrate through which selection can shape behavioral differences, seemingly does not follow this trend. This conspicuous exception is ripe for further investigation. For example, is the brain less labile than morphological and physiological traits in anoles? If so, does this account for the observation that foraging and movement patterns are relatively similar across a radiation that has over 300 species?

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