

Brain Evolution across the Puerto Rican Anole Radiation

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Key Words

Brain evolution · Reptile · Comparative neuroanatomy · Allometry

Abstract

Patterns of brain evolution have been widely studied across vertebrates, with the bulk of studies using mammals and/or birds as model systems. Within these groups, species occupying different habitats have been shown to have divergent neuroanatomy, particularly with regard to differences in the relative size of different brain structures, correlated with differences in habitat complexity. We examined the pattern of allometric scaling across the telencephalon, dorsal cortex, dorsomedial cortex, medial cortex, dorsal ventricular ridge, medulla and cerebellum in six species of Puerto Rican *Anolis* lizards, which are grouped in three distinct ecomorphs (i.e. ecological types) according to interspecific differences in preferred habitat type. The differences in habitat preferences are accompanied by morphological and behavioral adaptations for effective use of each habitat type. Our results challenge this trend and demonstrate a lack of convergence in the relative size of different brain structures between species belonging to the same ecomorph type. Overall brain volume explained between 92.5 and 99.8% of the variance in the volume of each of the brain regions measured and 93.8 and 98.5% of the variance in the volume of each component measured within the telencephalon. This pattern of brain al-

lometry is consistent with concerted brain evolution. However, in the case of the cerebellum, interspecific differences in volume exhibit a trend in accordance with mosaic brain evolution. This suggests that both concerted and mosaic brain evolution have shaped the anole brain, with the former playing a dominant role. Concerted brain evolution is the primary mechanism shaping the brain in mammals and cartilaginous fishes, and its presence in *Anolis* lizards provides additional evidence supporting the hypothesis that concerted brain evolution might result from a conserved pattern of brain development common to all vertebrates. More generally, our findings highlight the necessity of further studies of brain evolution in reptiles as they can provide valuable insights into the mechanisms underlying vertebrate brain evolution.

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Introduction

In the last decade, behavioral and evolutionary biologists have renewed their interest in understanding the patterns and processes underlying brain evolution [Sherry, 2006]. This resurgence has been triggered by a series of studies evaluating possible commonalities of brain evolution across a diverse set of taxa and comparative studies suggesting that interspecific differences in brain region size are associated with differences in many as-

pects of species' ecology [e.g. Abbott et al., 1999; de Winter and Oxnard, 2001; Safi and Dechmann, 2005; Amiel et al., 2011]. Although the vertebrate brain is composed of functionally distinct but interconnected structures [Striedter, 2005], a given cognitive function can be regulated by several structures. The general consensus is that different types of information are processed by specific structures of the brain [Garamszegi and Eens, 2004; Butler and Hodos, 2005], which may allow for functional specializations of the brain [Striedter, 2005; Sherry, 2006]. Furthermore, an increase in volume of a given brain structure may increase its processing power, which may improve the ability of an organism to process ecologically relevant information [Roth et al., 2010]. For example, bird species that cache food and vole species that move between multiple burrows both possess large hippocampal complexes, the brain structure most related to spatial memory, compared to closely related species without spatially demanding behaviors [Jacobs et al., 1990; Raby and Clayton, 2010]. Echolocating bats have a larger inferior colliculus, the primary auditory processing structure in the mammalian brain, than non-echolocating bats [Baron et al., 1996], while weakly electric fish have a tremendously expanded valvula cerebellum, which processes information from electroreceptors [Bell and Szabo, 1986].

Two principal hypotheses have been proposed to explain how selection favoring an increase in processing power can shape brain evolution. The first is the concerted evolution model, which predicts that development constrains the entire brain to change in a coordinated manner [Finlay and Darlington, 1995; Clancy et al., 2001; Finlay et al., 2001]. Under this model, selection favoring the increase in size of a given structure would result in an increase in size of the entire brain, resulting in correlated changes between overall brain size and the size of all constituent brain structures. However, the allometric rates of increase of different brain regions may vary, meaning that some brain structures can become disproportionately large in larger brains [Finlay et al., 2001]. The second model is mosaic evolution, which states that individual brain structures can change in size independently [Barton and Harvey, 2000; de Winter and Oxnard, 2001]. Therefore, selection favoring an increase in size of a given brain structure would not alter the size of other brain structures. Changes in the size of one structure independent of other structures would result in changes in the relative proportions of the brain's constituent structures. Mosaic brain evolution is most commonly found in relatively specialized structures, such as sensory areas and

the hippocampus [Bell and Szabo, 1986; Jacobs et al., 1990; Baron et al., 1996; Striedter, 2005; Raby and Clayton, 2010]. However, it should be noted that concerted and mosaic evolution are two ends of a continuum, and these processes are not mutually exclusive [Striedter, 2005; Yopak et al., 2010]. For example, cartilaginous fishes and mammals show primarily concerted brain evolution. However, both taxa have olfactory bulbs which are allometrically independent of the rest of the brain, displaying mosaic evolution [Finlay and Darlington, 1995; Finlay et al., 2001; Yopak et al., 2010].

The contribution of these processes to the observed pattern of brain evolution has been examined in a relatively small but increasing diversity of taxa. Initial support for both concerted [Finlay and Darlington, 1995; Finlay et al., 2001] and mosaic evolution [Barton and Harvey, 2000; de Winter and Oxnard, 2001] was based on studies of mammals. Neuroanatomical studies in birds [Iwaniuk et al., 2004] and African cichlid fishes [Gonzalez-Voyer et al., 2009] found evidence supporting mosaic evolution. More recently, Yopak et al. [2010] found that the brains of cartilaginous fishes evolved following the predictions of concerted evolution. Taken together, these results suggest that patterns of brain evolution are relatively labile across vertebrates.

A recurrent finding across studies describing brain evolution is that interspecific differences in the relative volume of a given brain structure correlate with interspecific differences in habitat complexity [e.g. Abbott et al., 1999; Safi and Dechmann, 2005]. In general, more complex, three-dimensional habitats require greater spatial cognitive ability and are related to expansion of certain brain structures, such as the dorsomedial cortex (DMC) or the entire telencephalon (TEL). This pattern has been demonstrated in birds [Abbott et al., 1999] and mammals [Safi and Dechmann, 2005] and also appears to be present in lizards [Day et al., 1999; LaDage et al., 2009].

Compared to other clades, the patterns of brain evolution in amphibians and reptiles have largely been overlooked. Studies in amphibians suggest that mosaic evolution contributes to changes in the size of major brain subdivisions and, more noticeably, at the level of specific nuclei [Taylor et al., 1995], while studies on reptiles tend to concentrate on the relationship between brain and body size [Platel, 1975; Timmel and Platel, 1988; Platel, 1989]. The lack of research on these clades is surprising when considering their location within the phylogenetic history of tetrapods and the likelihood that the reptile brain can provide valuable insights into the evolution of the heavily studied avian and mammalian brains [Mac-

lean, 1978; Northcutt, 1978]. Unlike both birds and mammals, reptiles have brains that are considerably smaller than the skull cavity. In addition, the walls of the brain case are frequently not completely ossified [Liem et al., 2001]. These characteristics might remove the brain case as a constraint on brain shape and may favor mosaic brain evolution [Kotrschal et al., 1998; Shumway, 2008]. In the case of lizards, at present there are only two studies addressing brain evolution as it relates to species ecology and/or life history [Day et al., 1999; LaDage et al., 2009]. Both studies have evaluated a possible correlation between brain region size and behavior, and their findings are consistent with the predictions of the mosaic evolution hypothesis. In one case, interspecific differences in the relative sizes of the dorsal and medial cortices between two congeneric species of Lacertidae correlated with differences in foraging strategies [Day et al., 1999]. In the other case, the volume of the dorsal cortex (DC) varied between different morphs of side-blotched lizards, *Uta stansburiana*, and the variation is consistent with intermorph differences in territorial behavior [LaDage et al., 2009]. However, a study explicitly designed to test patterns of brain evolution across multiple brain regions using multiple species is currently lacking in lizards and reptiles in general.

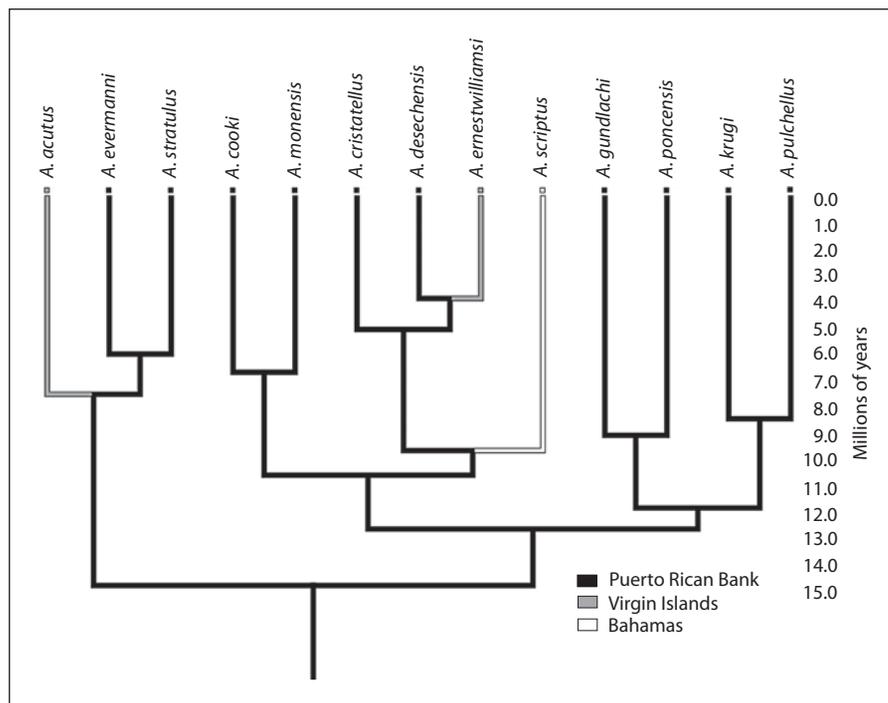
Due to their species richness and diversity of habitat use, West Indian *Anolis* lizards have become a model system for studies of evolutionary and behavioral ecology [Losos, 2009]. Anoles have been used to study a variety of topics, including adaptation, convergence, communication, territoriality and adaptive radiation [e.g. Williams, 1972, 1983; Losos 1990a, b; Losos et al., 1998; Leal and Fleishman, 2002, 2004; Johnson and Wade, 2010]. Although not as extensive, several species of anoles, in particular *Anolis carolinensis*, have been used to study neuroanatomy and neurophysiology [Deckel, 1998; Baxter et al., 2001; O'Bryant and Wade, 2002; Meyer et al., 2004; Neal and Wade, 2007]. Greater Antillean anoles are grouped into ecological types or 'ecomorphs', which correspond to the structural habitat in which they are most commonly found [Williams, 1972]. Members of the same ecomorph have the same body morphology and are very similar in ecology and behavior [reviewed in Losos, 2009]. For example, a trunk-ground anole generally occupies the tree trunk from ground level up to 2 m, while a trunk-crown anole primarily inhabits the canopy and highest sections of the trunk. There are dramatic differences in the habitat structure (i.e. vegetation profile, including connectivity of vegetation and amount of cluttering) between ecomorph classes [reviewed in Losos, 2009].

Behaviorally, members of the same ecomorph class have similar foraging strategies, movement patterns and territorial behavior [Moermond, 1979, 1981; Johnson et al., 2008, 2010]. This pattern of convergent evolution of behavioral and/or morphological traits has become the trademark of the *Anolis* radiation and strongly suggests that habitat preferences exert strong selection in this group [reviewed in Losos, 2009], making them an ideal clade to address possible patterns of brain evolution.

Here, we evaluate the pattern of brain evolution in six closely related species of *Anolis* lizards from the island of Puerto Rico which inhabit three distinct habitat types (i.e. forest canopy, tree trunks and grass). These habitat types can differ in their spatial complexity [Johnson et al., 2006], which might affect the ability of the species to navigate through their respective habitats [Moermond, 1986; Johnson et al., 2006]. Based on the extensive knowledge of the natural history of anoles, we predict that the relative size of different structures of the brain, particularly structures responsible for processing spatial information, will differ between species inhabiting distinct habitat types.

To examine the pattern of brain evolution in anoles, we measured the volume of multiple brain structures of six closely related species which are representative of three ecomorphs: *Anolis evermanni* and *A. stratulus*, trunk-crown ecomorphs; *A. gundlachi* and *A. cristatellus*, trunk-ground ecomorphs, and *A. krugi* and *A. pulchellus*, grass-bush ecomorphs [Williams, 1972]. These species represent the majority of the cristatellus clade and a significant portion of the Puerto Rican anole radiation [Mahler et al., 2010] (fig. 1). For all the species, we measured the volumes of the TEL, DC, DMC, medial cortex (MC), dorsal ventricular ridge (DVR), habenula, medulla (MED) and cerebellum (CER), as well as total brain volume. These structures were chosen due to their possible role in spatial learning and general cognitive ability and/or their position in the brain. If mosaic evolution has played a dominant role in shaping the size of different structures of the anole brain, we predicted that the size of brain structures related to spatial learning relative to overall brain size will differ between species belonging to different ecomorph types. However, if the effects of concerted evolution dominated the evolution of the anole brain, we predicted that all structures of the brain will change in proportion with overall brain size, regardless of species or ecomorph. Furthermore, anterior regions of the brain may increase with positive allometry while more posterior regions increase with negative allometry [Finlay et al., 2001].

Fig. 1. Phylogenetic relationships among the species of the cristatellus series of *Anolis*, which includes the six species used in this study, based on sequences from Nicholson et al. [2005]. The species are labelled by island. In the case of species from the Puerto Rican Bank, *A. monensis* and *A. desenchensis* are found on small islands off the coast of Puerto Rico, while the other species of the Puerto Rican Bank are found in mainland Puerto Rico. The phylogeny was constructed in Mesquite [Maddison and Maddison, 2011].



Materials and Methods

Individuals from all species were collected in the general vicinity of El Verde Biological Station, located in the northeastern mountain range of the island of Puerto Rico. Individuals from each species were collected in their preferred habitat. *Anolis cristatellus* (n = 10) and *A. krugi* (n = 10) were collected at the forest edge along small roads. *A. gundlachi* (n = 10) was collected in the understory of the closed-canopy, mature forest. *A. pulchellus* (n = 8) was collected in open grass fields near the station. *A. evermanni* (n = 10) was collected where the canopy descends along the edges of streams running through the rainforest, whereas *A. stratulus* (n = 10) was collected in the rainforest canopy, from a canopy tower. Lizards were collected by hand or noose between July and August 2006, between July and August 2007, and in August 2008. Only adult males were collected for this study. Upon capture, we measured the mass and the snout-vent length of all individuals. The original research reported herein was performed under guidelines established by the Duke University Institutional Animal Care and Use Committee.

Within 24 h of capture, individuals were anesthetized and intracardially perfused with phosphate-buffered saline followed by 4% paraformaldehyde solution (methodology follows Day et al. [2005]). Lizards were perfused in groups of mixed species. Following perfusion, lizards were decapitated and the heads were postfixed in 4% paraformaldehyde solution for 1–8 weeks, depending on when they were captured during the field season. After returning to the laboratory, we dissected the brains from the skulls and postfixed them in 4% paraformaldehyde solution. All brains were weighed 4 weeks after dissection from the skull. Due to logistical constraints, the time of postfixation prior to embedding ranged

from 4 weeks to 8 months (see below). The olfactory bulbs are extremely fragile in these species and could not be reliably dissected and measured. For this reason, olfactory bulbs were removed from all brains and are not included in measurements of overall brain mass or volume.

Brains were embedded and sectioned in the order in which they were perfused. Whole brains were dehydrated in a graded ethanol series, embedded in paraffin and sectioned in the transverse plane using a microtome. The first 40 brains were sectioned at a thickness of 7 μm . After scoring the first batch, it became evident that thicker sections were less fragile without loss of resolution. Thus, the remaining 18 brains were sectioned at 10 μm . Sections were mounted on albumin-coated slides, stained with cresyl violet and coverslipped [Lowe, 1996; modified to replace xylene with Fisherbrand Citrisolv]. The resulting sections were photographed at $\times 40$ magnification using a Zeiss Axiophot microscope with attached Zeiss Axiocam digital camera. The areas of the DVR, DC, DMC, MC, TEL, CER, MED and the whole brain were measured every 70 μm using ImageJ [Rasband, 1997–2009] (fig. 2). We followed the description of Greenberg [1982] to delimit each area of the TEL. To delimit the CER and MED, we followed the description of the *Tupinambis teguixin* brain in ten Donkelaar [1998]; in the case of the MED, we began measuring its volume in the first section that did not include any areas of the mesencephalon. The area of the entire brain, including but not limited to the other structures measured and excluding any ventricles, was measured in each section. Structure volumes were calculated by averaging subsequent measurements and multiplying that area by the distance between the measurements, following the method used by Kabelik et al. [2006]. These volumes were then summed across the length of the structure. All volumes were log transformed prior to analysis.

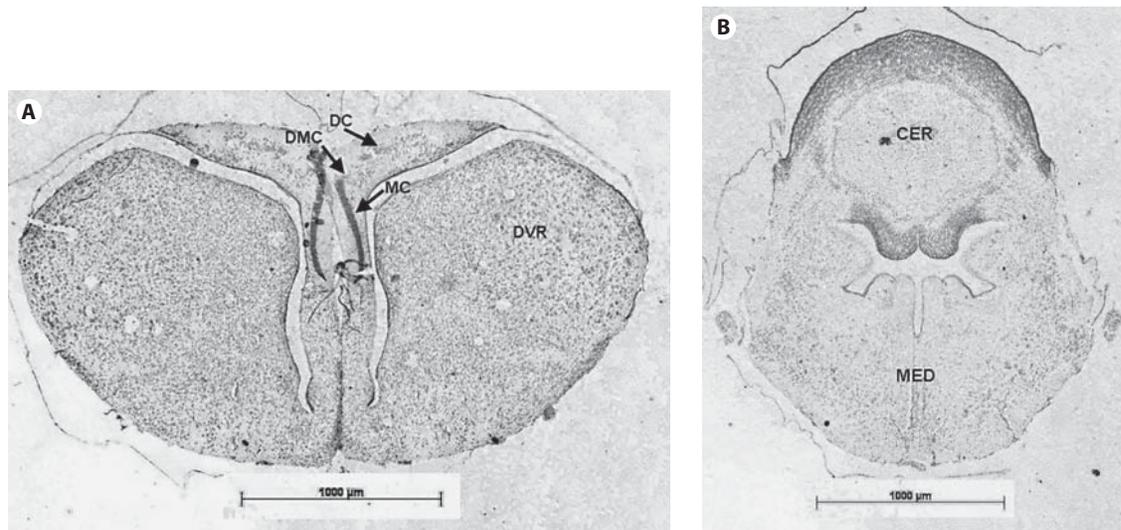


Fig. 2. **A** Transverse section through the forebrain of *A. cristatellus* showing the location of the DC, DMC, MC and DVR. **B** Transverse section through the hindbrain of *A. cristatellus* showing the location of the CER and MED.

Structures were chosen based on their putative functions. The DVR is the main sensory integration structure in the reptilian brain and performs similar functions to the mammalian isocortex [Butler and Hodos, 2005; Striedter, 2005]. Thus, differences in the volume of the DVR may be indicative of differences in general cognitive ability between species. The DC is another potential homolog to the mammalian isocortex, and changes in DC volume may correlate with changes in general cognitive ability [Striedter, 1997, 2005; Butler and Hodos, 2005]. The DMC and MC are homologous to areas of the mammalian and avian hippocampal formations and can play a role in 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 TEL is the major brain division that houses all of the above-mentioned structures, as well as the lateral cortex and subpallial structures [Butler and Hodos, 2005; Striedter, 2005]. Among many other functions, the CER is related to maintenance of the balance of the body, coordination of muscular activity and learning motor strategies [Butler and Hodos, 2005]; movement on different substrates, which vary by habitat, may result in changes in cerebellar volume or structure [Yopak et al., 2010].

We also evaluated the predictions of the concerted evolution hypothesis. To do so, we measured the volume of the MED, which is a highly conserved structure of the vertebrate brain [Butler and Hodos, 2005] and was not expected to experience selection based on ecology. In combination with the other structures measured, this allowed us to examine the relationship between the volume of the whole brain and brain regions across the entire length of the brain.

Differential tissue shrinkage due to variable fixation time is a potentially confounding factor in histological studies of the brain [Healy and Rowe, 2007]. In order to test for the possibility of brain volume shrinkage due to variable time between fixation and embedding, we conducted analysis of covariance (ANCOVA) with

brain volume as a covariate and snout-vent length and order of sectioning as independent variables. The analysis of covariance was conducted on data from the specimens collected in 2006, as this provided the largest sample size within a single field season.

To remove the effect of total brain size on the size of each structure measured, we conducted a reduced major axis regression of total brain volume against the volume of each of the DVR, DC, DMC, MC, TEL, CER and MED for all individuals and computed residuals for every individual. Reduced major axis regression provides more reliable results than least squares regression in cases such as this one where the potential for error exists in the measurements of both variables [McArdle, 1988]. In order to test the predictions of the mosaic evolution hypothesis, these residuals were then compared using analysis of variance (ANOVA) to determine if each structure's volume differed between species. If the ANOVA demonstrated significant differences, the means were compared using Tukey's range test. Because we were comparing species with no additional grouping variable, we could not phylogenetically correct this ANOVA. However, we conducted the following analysis to take into account the effect of phylogeny.

In order to compare brain structures between the different ecomorphs, we conducted a phylogenetic ANOVA. We first conducted a phylogenetically corrected reduced major axis regression of mean total brain volume against the mean volume of each of the DVR, DC, DMC, MC, TEL, CER and MED for each species and computed residuals for every species. Because species are phylogenetically related to varying degrees, they cannot be considered statistically independent of each other [Felsenstein, 1985]. To correct for this, we then conducted a phylogenetic ANOVA on these residuals, grouped by ecomorph. We used the phylogeny from Mahler et al. [2010], pruned to include only the species in the data set and with branch lengths made ultrametric using the program r8s [Sanderson, 2003]. The analysis was conducted using the Geiger package [Harmon et al., 2008] in R [R Development

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

Species	SVL mm	Body mass, g	TEL μm^3	DC μm^3	DMC μm^3	MC μm^3	DVR μm^3	CER μm^3	MED μm^3	Total brain, μm^3
<i>A. cristatellus</i>	66.23	9.56	3,423.84	131.28	27.44	67.66	1,444.44	332.14	1,527.36	9,467.91
<i>A. evermanni</i>	58.86	4.95	2,431.46	77.05	18.26	45.27	1,065.94	281.30	1,196.93	7,004.74
<i>A. gundlachi</i>	62.22	6.29	3,128.41	102.29	24.60	53.35	1,471.12	357.40	1,531.93	8,767.14
<i>A. krugi</i>	46.10	2.43	1,652.04	46.20	11.53	24.95	722.35	196.35	833.75	4,836.76
<i>A. pulchellus</i>	43.62	1.86	1,369.81	40.81	10.07	23.06	568.13	158.24	539.63	3,958.21
<i>A. stratulus</i>	45.36	2.19	2,442.22	80.85	16.24	46.51	1,064.23	247.80	1,109.51	6,855.20

SVL = Snout-vent length.

Core Team, 2009]. In both of the above analyses, significant differences between species in the volume of a brain structure relative to overall brain volume demonstrates the presence of mosaic brain evolution in that structure.

To determine the potential contribution of concerted brain evolution to producing the observed pattern of brain evolution, we calculated the mean volume of the DVR, DC, DMC, MC, TEL, CER and MED and mean total brain volume for each species. We performed a phylogenetically corrected reduced major axis regression using the phytools package [Revell, 2011] in R [R Development Core Team, 2009]. For this analysis, we used the same phylogeny described above. We performed a phylogenetically corrected reduced major axis regression of the mean volumes of all species for each structure against mean total brain volume. The proportion of variance in the volume of a brain structure explained by total brain volume provides a measure of the contribution of concerted evolution to brain region size [Finlay and Darlington, 1995, Finlay et al., 2001; Yopak et al., 2010]. In this analysis, consistent high correlations between brain region volume and overall brain volume across multiple brain structures are indicative of concerted brain evolution. All other statistical tests were two-tailed at $\alpha = 0.05$ and were performed using JMP 8 (JMP, version 8; SAS Institute Inc., Cary, N.C., USA, 1989–2011).

Results

Order of sectioning did not have a significant effect on brain volume (DF = 1, SS = 0.0019, F = 0.1980, $p = 0.6595$), demonstrating no significant effect of variable time between fixation and embedding. Therefore, observed differences in brain volume were not artifacts resulting from variable time between fixation and embedding. Also, if undetected shrinkage did occur uniformly across the brain, it should not affect the findings of this study because all measurements are relative to total brain size.

The absolute sizes of the brains and all measured brain structures are given in table 1. All species exhibited the same general pattern of brain allometry. No brain struc-

Table 2. Results of ANOVA on the relative size of each brain structure across the six species sampled in this study

Brain structure	DF	SS	F	p value
TEL	5	0.00161277	0.3922	0.8519
DC	5	0.05194033	0.9188	0.4764
DMC	5	0.05306348	0.9125	0.4804
MC	5	0.07323759	1.3654	0.2528
DVR	5	0.01614322	0.9430	0.4612
CER	5	0.04610492	2.2414	0.0642
MED	5	0.02351090	0.9700	0.4450

DF = Degrees of freedom; SS = sum of squares.

ture differed significantly among species in terms of volume relative to the overall volume of the brain, though the CER approaches significance (table 2). Areas which do not show significant differences, such as the DVR, occupy a similar proportion of overall brain volume regardless of brain size or species. Ecomorph type also did not have a significant association with differences in any of the brain structures, after correcting for phylogeny and total brain volume (table 3). In addition, the volume of each brain structure was strongly predicted by brain size (table 4). Brain size predicted 92.5% of variation in CER volume in the lowest case, ranging up to 99.7% of variance in TEL volume.

Overall, positive significant correlations were present between the volume of the total brain and each brain structure (TEL, DC, DMC, MC, DVR, CER and MED). This pattern was also present after correcting for phylogenetic relationships and is illustrated as a regression against total brain volume (fig. 3). All correlations were

Table 3. Results of a phylogenetic ANOVA on the relative size of each brain structure across the ecomorph types sampled in this study

Brain structure	DF	F	p value
TEL	2	0.4421562	0.641
DC	2	0.03259228	0.962
DMC	2	1.524982	0.298
MC	2	0.4281584	0.658
DVR	2	0.4006081	0.687
CER	2	0.3592306	0.675
MED	2	3.583708	0.111

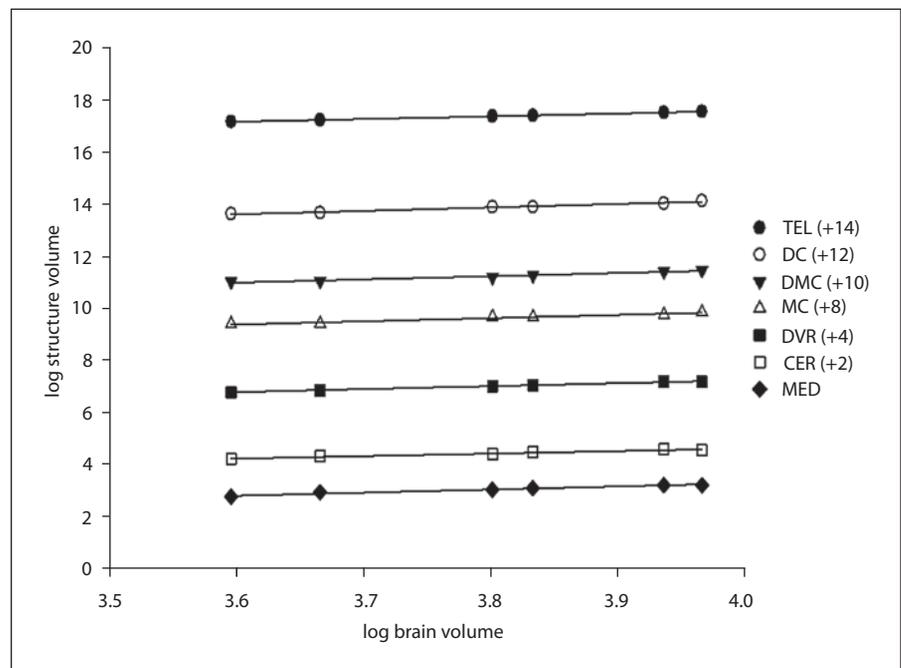
DF = Degrees of freedom.

Table 4. Values of a phylogenetically corrected reduced major axis regression on each brain structure across the six species sampled in this study

Brain structure	R ²	Slope	Intercept	p value
TEL	0.99765940	1.0537795	-0.6607591	0.08649284
DC	0.95431786	1.330191	-3.212678	0.04715161
DMC	0.96217811	1.238872	-3.506419	0.08225825
MC	0.9376744	1.238792	-3.118403	0.1503395
DVR	0.9849842	1.131286	-1.321468	0.1041009
CER	0.9254201	0.968047	-1.293486	0.8219610
MED	0.9480136	1.202486	-1.564671	0.1702390

p values denote confidence that the slope differs from 1, demonstrating either positive or negative allometry.

Fig. 3. Scaling of the volumes (in cubic micrometers) of seven brain structures plotted as a function of total brain volume. Lines indicate phylogenetically corrected reduced major axis regressions for each brain structure. From left to right on each regression, the points represent *A. pulchellus*, *A. krugi*, *A. stratulus*, *A. evermanni*, *A. gundlachi* and *A. cristatellus*. For purposes of visualization, an arbitrary constant was added to each brain structure, shown in parentheses in the legend.



positive; thus, increases in overall brain size were correlated with an increase in the size of all structures. However, the rates of increase varied among structures (table 4). Only the DC exhibited positive allometry relative to the overall brain, with a slope significantly greater than 1 when plotted against overall brain volume, though the rates of increase for both the TEL and DMC approached significance.

Discussion

We found support for concerted brain evolution as the main process shaping the brain areas examined across the Puerto Rican anole radiation (table 4). In addition, our results show near significant interspecific differences in the relative size of the CER, raising the possibility of mosaic evolution in the CER (table 2). This pattern, in which concerted brain evolution determines

the size of the majority of brain structures while a few structures show mosaic evolution, is congruent with that found in cartilaginous fishes and mammals [Finlay and Darlington, 1995; Clancy et al., 2001; Finlay et al., 2001; Yopak et al., 2010]. The potential presence of mosaic evolution in only one of the areas measured is somewhat unexpected in light of the fact that there are considerable differences in the structural habitat most commonly used between the species studied, which have been suggested to select for differences in many aspects of their morphology and behavior [Williams, 1983; Losos, 2009; Johnson et al., 2010]. This raises the possibility that the brain, at least at the relatively broad scale measured in this study, might be less labile than morphology or behavior in anoles.

Consistent with the predictions of concerted evolution, the volume of each brain structure measured was highly correlated with overall brain volume. Overall brain volume explained between 92.5 and 99.8% of the variance in volume of each of the brain regions we measured (TEL, CER and MED). In addition, overall brain volume explained between 93.8 and 98.5% of the variance in volume of each component of the TEL measured (DC, DMC, MC and DVR). Taken together, these results strongly support concerted evolution as the main mechanism shaping the evolution of brain region size in anoles, with mosaic evolution potentially affecting the CER.

Concerted brain evolution has been reported to be dominant in two other clades, namely mammals, where overall brain volume explains approximately 96% of the total variance in volumes of constituent brain structures [Finlay et al., 2001], and cartilaginous fishes, where overall brain volume explains approximately 93% of the total variance in volumes of constituent brain structures [Yopak et al., 2010]. These values, which can be interpreted as the signature of concerted evolution, are very similar to the values reported in this study, suggesting that concerted evolution has played a similar role in shaping the pattern of brain evolution of anoles.

Previous studies have found that both the TEL and CER increase in size more rapidly than other structures in response to increases in total brain size [Finlay et al., 2001; Yopak et al., 2010]. Our results are consistent with these findings, with the TEL and its constituent structures scaling with significant or near significant positive allometry. In our study, the remaining brain structures could not be determined to scale with either positive or negative allometry (table 4), but the estimates of their slopes are roughly consistent with previous studies [Fin-

lay et al., 2001; Yopak et al., 2010]. In particular, these studies have found that the MED scales with negative allometry. Due to the large variance in the estimates of the allometric slopes in our study, we could not assign with confidence either positive or negative allometry to the MED (table 4). Including additional species of iguanid lizards in the analysis would likely provide improved resolution to the scaling pattern of all areas of the brain, especially the MED.

We measured four structures in the TEL that are putatively related to general cognitive abilities, spatial memory and spatial problem solving. These included the DC and DVR, which are both potential homologs of the mammalian isocortex, and the DMC and MC, which are homologs of mammalian and avian hippocampal structures [Striedter, 1997; Butler and Hodos, 2005]. All of these structures scale with positive allometry with respect to overall brain size, as does the TEL as a whole. However, we estimate that all of these telencephalic structures increase in size at a higher rate than the TEL itself. These relationships suggest that some constituents of the TEL must be scaling with negative allometry, resulting in the slower rate of overall change in telencephalic volume. Based on our measurements, we are unable to identify which structures of the TEL exhibit negative allometric change. Possibilities include the lateral pallium and subpallial structures such as the striatum and paleostriatum. The lateral pallium, which analyzes olfactory information, may be a particularly promising structure for further investigation, as *Anolis* are extremely visually oriented lizards and rely very little on olfaction [Moermond, 1979; Leal and Fleishman, 2002].

As we were concerned primarily with brain structures potentially related to navigating habitats of varying complexity, we did not measure any structure primarily involved in sensory processing. However, mosaic evolution has been documented in sensory areas across a wide variety of taxa, driven by sensory specializations [Bell and Szabo, 1986; Baron et al., 1996]. Even in taxa showing predominantly concerted brain evolution, the size of the olfactory bulb is independent from the rest of the brain [Finlay and Darlington, 1995; Finlay et al., 2001; Yopak et al., 2010]. Therefore, further analysis of the lizard brain extending to the olfactory bulb, lateral cortex and optic tectum may reveal the presence of additional mosaic evolution. However, anoles and iguanian lizards in general are unlikely to show interspecific differences in these sensory areas related to vision and chemoreception as they predominantly rely heavily on vision and weakly on chemoreception [Pianka and Vitt, 2003; Losos, 2009]. This

area of research would be most productive if more strongly chemoreceptive species were added to those sampled here.

When the effect of overall brain size is removed, none of the structures measured differ significantly in size between species occupying different types of structural habitat (i.e. ecomorphs). Upon closer examination, all of the structures potentially related to learning and navigating habitats of varying complexity trend toward positive allometry with respect to total brain size (fig. 3). In our study, we included representatives of the most common ecomorphs found across the Greater Antilles. The lack of convergence in the volume of brain structures within an ecomorph suggests that the brain is less evolutionarily labile than morphology or behavior, at least at the level of structure volume. To confirm the generality of our results, future studies should include other less common habitat specialists such as the aquatic and twig ecomorphs, which use structural niches very distinct from those of the species sampled in this study and possibly different foraging strategies. In the case of aquatic anoles, they are only found in close proximity to small rivers, forage on the boulders at the water edge and are the only ecomorph to commonly capture aquatic prey items [Leal et al., 2002; Losos, 2009]. Twig anoles are restricted to relatively cluttered vegetation, consisting of small-diameter perches (i.e. twigs), and, unlike other anoles, might not exhibit territoriality and might actively search for prey [Losos, 2009].

While anoles demonstrate considerable diversity with regard to their structural habitat, their foraging behavior (i.e. sit-and-wait) and social systems (i.e. territoriality) are relatively conserved [Losos, 2009]. Our findings suggest that habitat differences are not sufficient, at least in isolation, to favor mosaic brain evolution as the dominant process shaping the anole brain. It is currently an open question if this pattern is common across lizards and reptiles in general. Therefore, to gain a complete understanding of brain evolution in lizards, it is necessary to examine the neuroanatomy of a wider array of lizard species. Several lizard families may be particularly promising in this regard. Varanids occupy a wide diversity of structural habitats, including terrestrial, arboreal and semiaquatic species, display diverse diets and possess relatively high basal metabolic rates [Losos and Greene, 1988; Clemente et al., 2009]. In addition, several species of varanids have been used in cognitive behavior studies, demonstrating problem solving [Manrod et al., 2008] and reversal learning [Gaalema, 2011]. Anguids demonstrate widespread and extensive parental care compared

to other families of lizards [Greene et al., 2006]. Both families provide excellent opportunities to further examine the effects of behavior, ecology and life history on lizard neuroanatomy and brain evolution. In addition, the frequency of mosaic evolution increases with the taxonomic level analyzed [Striedter, 2005]. The addition of further families to broaden the analysis would likely uncover additional examples of mosaic brain evolution in lizards.

In summary, our results demonstrated that concerted brain evolution is the primary mechanism shaping the areas of the *Anolis* brain analyzed in this study. The results are consistent with concerted evolution as the primary mechanism responsible for the evolution of the brain region size in anoles, while the CER may display evidence of mosaic evolution. This pattern of correlated allometric scaling has also been proposed as the mechanism driving brain evolution in chondrichthyans and mammals [Finlay et al., 2001; Yopak et al., 2010]. Given the phylogenetic position of lizards with regard to chondrichthyans and mammals, the commonality in brain evolution across these three distantly related groups of vertebrates provides further evidence in support of a general pattern of brain evolution across vertebrates. More generally, our findings demonstrate the necessity to further study patterns of brain evolution in reptiles, as they can provide valuable insights into the mechanisms underlying brain evolution.

Acknowledgements

We thank K. Sewall, S. Burmeister, S. Nowicki, C. Cunningham, S. Johnsen, G. Striedter and two anonymous reviewers for providing helpful comments and suggestions on the manuscript. S. Burmeister and G. Striedter also provided extremely helpful advice on displaying brain section images. We are very grateful to K. Smith for providing laboratory space and equipment to conduct the histology work. N. Dautel provided valuable assistance performing histology. We also thank the Departamento de Recursos Naturales y Ambientales of Puerto Rico, which provided all the necessary permits to complete this work. Our work in Puerto Rico would not have been possible without the logistical support provided by El Verde Biological Station (University of Puerto Rico, Río Piedras) as well as field assistance from J.D. Daza, A. Herrera Martinez and D. Steinberg. Funding was provided by grants from the Duke Center for Latin American and Caribbean Studies, Animal Behavior Society and Sigma Xi to B.J.P.

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