

A Comparative Study of Habitat Complexity,
Neuroanatomy, and Cognitive Behavior in *Anolis* Lizards

by

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Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor of Philosophy in the Department of
Biology in the Graduate School
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ABSTRACT

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Abstract

Changing environmental conditions may present substantial challenges to organisms experiencing them. In animals, the fastest way to respond to these changes is often by altering behavior. This ability, called behavioral flexibility, varies among species and can be studied on several levels. First, the extent of behavioral flexibility exhibited by a species can be determined by observation of that species' behavior, either in nature or in experimental settings. Second, because the central nervous system is the substrate determining behavior, neuroanatomy can be studied as the proximate cause of behavioral flexibility. Finally, the ultimate causation can be examined by studying ecological factors that favor the evolution of behavioral flexibility. In this dissertation, I investigate behavioral flexibility across all three levels by examining the relationship between habitat structure, the size of different structures within the brain and total brain size, and behavioral flexibility in six closely-related species of Puerto Rican *Anolis* lizards. *Anolis* lizards provide an excellent taxon for this study as certain species, including those used here, are classified as belonging to different ecomorphs and are morphologically and behaviorally specialized to distinct structural habitat types.

In order to determine the presence of behavioral flexibility in *Anolis*, I first presented *Anolis evermanni* with a series of tasks requiring motor learning and a single

instance of reversal learning. *Anolis evermanni* demonstrated high levels of behavioral flexibility in both tasks.

To address the pattern of brain evolution in the *Anolis* brain, I used a histological approach to measure the volume of the whole brain, telencephalon, dorsal cortex, dorsomedial cortex, medial cortex, dorsal ventricular ridge, cerebellum, and medulla in six closely-related species of Puerto Rican *Anolis* lizards belonging to three ecomorphs. These data were analyzed to determine the relative contribution of concerted and mosaic brain evolution to *Anolis* brain evolution. The cerebellum showed a trend toward mosaic evolution while the remaining brain structures matched the predictions of concerted brain evolution.

I then examined the relationship between the complexity of structural habitat occupied by each species and brain size in order to determine if complex habitats are associated with relatively large brains. I measured brain volume using histological methods and directly measured habitat complexity in all six species. Using Principal Component Analysis, I condensed the measures of habitat structure to a single variable and corrected it for the scale of each lizard species' movement, calling the resulting measurement relevant habitat complexity. I tested the relationship between relative volume of the telencephalon, dorsal cortex, dorsomedial cortex, and whole brain against both relative habitat complexity and ecomorph classification. There was no relationship between the relative volume of any brain structure examined and either relevant habitat

complexity or ecomorph. However, relevant habitat complexities for each species did not completely match their ecomorph classifications.

Finally, I tested the levels of behavioral flexibility of three species of *Anolis*, *A. evermanni*, *A. pulchellus*, and *A. cristatellus*, belonging to three distinct ecomorphs, by presenting them with tasks requiring motor and reversal learning. *Anolis evermanni* performed well in both tasks, while *A. pulchellus* required more trials to learn the motor task. Only a single *Anolis cristatellus* was able to perform either task. *Anolis evermanni* displayed lower levels of neophobia than the other species, which may be related to its superior performance.

In combination, this research suggests that *Anolis* of different ecomorphs display different levels of behavioral flexibility. At the proximate level, this difference in behavioral flexibility cannot be explained by changes in the relative size of the total brain or brain structures associated with cognitive abilities in other taxa. At the ultimate level, the size of the brain and several constituent structures cannot be predicted by habitat complexity. However, behavioral flexibility in certain tasks may be favored by utilization of complex habitats. Flexibility in different tasks is not correlated, rendering broad comparisons to a habitat complexity problematic.

Dedication

This dissertation, and all the work involved in producing it, is dedicated to my mother, Mari Powell, who always supported my love of reptiles, no matter how little she liked them.

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1. Introduction

Behavioral flexibility, defined as the ability to alter individual behavior by developing new responses to novel stimuli or to alter existing responses to familiar stimuli, provides organisms with the ability to behaviorally adapt to changing environments and is commonly associated with cognitive processes such as problem solving, spatial learning, and reversal learning (Shettleworth 1998, Reader and Laland 2002, Delius and Delius 2006). At the proximate level, behavioral flexibility can be explained by neuroanatomical specializations, including large brain size relative to body size, and exaggerated structures within the brain (Clayton 1995, Salas et al. 2003, Sol et al. 2005a, Sol et al. 2005b, Ratcliffe et al. 2006, Healy and Rowe 2007, Chittka and Niven 2009). On the ultimate level, the evolution of behavioral flexibility may be favored by ecological factors, such as complex habitat or unpredictable food sources (Lefebvre and Bolhuis 2003, Reader and Macdonald 2003, Lefebvre and Sol 2008).

The brain provides the substrate that controls behavior, meaning that changes in neuroanatomy can alter its ability to process information and/or respond to the environment. Specifically, increases in the size of the brain and its constituent structures could provide additional cognitive processing ability through the addition of more neurons (Healy and Rowe 2007, Chittka and Niven 2009). For example, increased volume of the brain or structures within the brain are related to differences in spatial learning (Jacobs et al. 1990, Clayton 1995), flexible foraging behavior (Sol et al. 2005a,

Ratcliffe et al. 2006), ability to invade new habitats (Sol et al. 2005b, Amiel et al. 2011), survival (Sol et al. 2007, Gonzalez-Lagos et al. 2010), and ability to occupy complex habitats (Budeau and Verts 1986, Abbott et al. 1999, Safi and Dechmann 2005, Ratcliffe et al. 2006). These differences in the size of structures within the brain can evolve via a combination of two patterns of brain evolution. Changes to the size of any brain structure may occur independently of the rest of the brain through mosaic brain evolution (Barton and Harvey 2000, de Winter and Oxnard, 2001) or they may occur in a coordinated fashion throughout the brain as the result of changes in total brain size through concerted brain evolution (Finlay and Darlington 1995, Clancy et al. 2001, Finlay et al. 2001). In both cases, neuroanatomical changes and associated changes in cognitive abilities are often related to environmental factors.

Several environmental characteristics are associated with behavioral flexibility or increases in brain size. Species occupying harsh environments generally exhibit high levels of behavioral flexibility (Dukas 1998, Shettleworth 1998, Sol et al. 2005a, Roth et al. 2010). In a similar vein, complex structural habitats, particularly those incorporating three-dimensional structures, also seem to favor increases in overall brain size (Budeau and Verts 1986, Abbott et al. 1999, Safi and Dechmann 2005, Ratcliffe et al. 2006). Finally, the sizes of specific structures within the brain, including but not limited to the telencephalon and cerebellum, are related to the type of habitat, such as terrestrial, fossorial, aquatic, or arboreal, occupied by a species (Taylor et al. 1995, de Winter and

Oxnard 2001). Taken together, these studies suggest a complex interplay between selective pressures exerted by habitat complexity and the neuroanatomical and behavioral responses.

In the work described here, I used six species of Puerto Rican *Anolis* to test the predicted relationships between behavioral flexibility, neuroanatomy, and habitat complexity. Like all Greater Antillean anoles, Puerto Rican anoles are categorized by ecomorphs classes, which correspond to the structural habitat in which they are most commonly found (Williams 1972). Species within an ecomorph class exhibit similar morphology, ecology, and behavior (reviewed in Losos 2010). There are dramatic differences in the habitat structure (i.e. vegetation profile including connectivity of vegetation and amount of cluttering) between ecomorph classes (Losos 2010). This repeated pattern of morphological and behavioral specialization to structural habitat preference makes anoles an ideal clade to study the relationship between habitat complexity, brain size, and behavioral flexibility.

In chapter 2, I present the results of an experiment demonstrating high levels of behavioral flexibility in *Anolis evermanni*. Chapter 3 presents an evaluation of the relative contributions of mosaic and concerted brain evolution to *Anolis* neuroanatomy across six species of anoles occupying three distinct structural habitat niches, while chapter 4 examines the relationship between habitat complexity and the size of the brain and several structures within the brain in the same six species. Chapter 5 analyses the

level of behavioral flexibility shown by three species of *Anolis*, each belonging to a different ecomorph class, using two behavioral tasks.

2. Behavioral flexibility and problem solving in a tropical lizard¹

2.1 Introduction

Behavioral flexibility — i.e., the ability of an individual to change its behavior by developing new responses to novel stimuli or altering existing responses to familiar stimuli—has become a central focus in cognitive ecology, and it is commonly associated with advanced cognition (Shettleworth 1998, Reader and Laland 2002), including problem solving. In vertebrates, studies of behavioral flexibility have mostly focused on mammals and birds (e.g., Weir et al. 2002, Lefebvre 2010, Sol et al. 2002, Dunbar and Shultz 2007), because theory predicts that behavioral flexibility is favored in species or clades that exploit diverse food sources, have complex social structure, or inhabit environments with highly unpredictable resources (Reader 2003), attributes not associated with ectothermic vertebrates.

Two types of evidence have been used to document behavioral flexibility. First, studies have examined anecdotal evidence of behavioral plasticity (i.e., behavioral innovations, *sensu* Lefebvre et al. 1997), particularly in feeding behavior within a given clade (Lefebvre 2010). Second, experimental data, usually under laboratory conditions, have been collected to evaluate behavioral flexibility (Shettleworth 1998). In this case,

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studies have addressed a variety of cognitive modules, such as flexibility of motor skills, spatial learning, associative learning, and reversal learning (see Shettleworth 1998, Reader 2003, and references therein). In studies in which multiple cognitive tasks have been used, results indicate that the ability of an individual to solve one cognitive task is not a predictor of success across multiple cognitive tasks (Boogert et al. 2010, Boogert et al. 2011). Thus, a robust demonstration of behavioral flexibility should show that individuals are capable of solving multiple cognitive tasks, an approach that is not commonly used, especially in ectothermic vertebrates (see Day et al. 2002, Manrod et al. 2008, and references therein).

Here, we present the results of a series of experiments designed to test behavioral flexibility by evaluating cognitive abilities on multiple cognitive tasks in the arboreal lizard *Anolis evermanni*. The results not only demonstrate problem solving and behavioral flexibility across multiple tasks, but also indicate that the cognitive abilities of this lizard are comparable to those of some endothermic species, including species recognized as having highly flexible behaviors.

2.2 Materials and Methods

Anolis evermanni, 3 males and 3 females, were collected in Puerto Rico and transported to our laboratory. Lizards were housed independently in cages (29L, 21W and 21H cm), and kept under 12h:12h photoperiod cycle, at 28°C and 60% relative

humidity, watered daily, and fed crickets on a regular schedule throughout the experimental period.

Behavioral experiments were conducted in the cage where the lizards were housed. The behavioral testing apparatus was placed inside the cage, and lizards had 15 minutes to perform the task. The apparatus was an opaque grey platform (12L, 5.5W cm) containing 2 wells, each 1.6 cm in diameter and 1 cm deep. The wells were covered by a tight-fitting 2.5 cm diameter opaque colored circular disc, concealing their contents (fig. 1). A reward (freshly killed, intact larva of *Hermetia illucens*) was placed inside a well, and the lizard had to remove the disc to reach the larva.

Individuals were habituated to the testing apparatus before conducting the experiments. Habituation was performed in 3 sequential steps: (1) the apparatus was placed inside the cage with a reward inside one well; (2) the disc was positioned next to the well containing the reward; (3) the disc was positioned covering half of the well containing the reward. During the habituation period, individuals did not need to manipulate the disc to access the worm. Individuals advanced stages or completed an experiment when they correctly performed the task six consecutive times. Trials were video-recorded.

Once the habituation period was completed, a uniform colored blue disc was tightly-fitted to one of the wells and the lizards were presented with the novel problem

of dislodging the target to access the larva. Only one disc was used and its position was randomly determined before each trial. Individuals conducted one trial per day.

Lizards that learned to dislodge the disc performed two discrimination experiments in which a target (i.e., stimulus used for the motor task) and a distracter (i.e., a solid colored yellow disc for experiment 1 and a disc formed by yellow and blue concentric rings for experiment 2) were presented simultaneously. Position of the stimuli was randomly determined before each trial. The reward was placed under the target, and larva odor was placed inside both wells. Choice was scored as the first stimulus dislodged by the lizard. To further control for smell, in 25 of the trials a larva was placed under both stimuli; each lizard performed a minimum of 5 trials under this condition. In 23 out of the 25 trials ($P < 0.001$, binary choice test), the lizards chose the target.

The same paradigm was used to test reversal learning. However, in these experiments the reward was placed under the disc formed by yellow and blue concentric rings rather than the uniform colored blue disc, reversing the conditions previously presented to the lizards. A lizard was removed from the experiment when it did not attempt to perform the task in six consecutive presentations.

2.3 Results

Four lizards were able to solve the novel problem of removing the disc to access the reward (Table 1). Two distinct strategies were used to dislodge the disc; individuals learned to bite the edge of the disc to dislodge it, or to use their snout as a lever. The two

lizards that could not solve the motor task failed to do so because they continually struck at the target from above, a behavior that was ineffective at dislodging the disc.

All lizards that completed the motor task were able to discriminate between the target and a distracter (Figure 1). Three of four individuals were able to do so without making any mistakes, and the other individual only made one mistake (Table 1, first experiment). Discrimination was further evaluated by testing a second distracter: a disc formed by two concentric rings. The center of the new distracter was of the same color and shape as the target, increasing the similarity between the two stimuli. Again, lizards were able to correctly choose the target without making any mistakes (Table 1, second experiment).

Table 1: Number of trials performed by individuals of *Anolis evermanni* to complete each experiment. (NA= individual stopped performing the experiment; parentheses indicate the number of trials performed).

Lizard	Motor Task	Discrimination		Choice Reversal
		First Experiment	Second Experiment	
Male 3	19	6	6	64
Male 5	36	7	6	65
Female 8	39	6	6	NA(38)
Female 10	19	6	6	NA(60)

Two of four lizards exhibited reversal learning (Table 1). Lizards that did not reverse their association never tried to dislodge the concentric rings; instead, they

consistently removed the uniform disk (i.e., previous target) until they stopped responding due to lack of reinforcement.



Figure 1: *Anolis evermanni* performing a discrimination trial.

2.4 Discussion

Our results show that *A. evermanni* exhibits behavioral flexibility across multiple cognitive tasks, including solving a novel motor task using multiple strategies and reversal learning, plus rapid associative learning. This degree of flexibility is commonly associated with bird or mammal species considered highly flexible (Shettleworth 1998, Reader 2003, Lefebvre et al. 1997), but is not predicted to be exhibited by a species that lacks complex social structure and has a relatively simple foraging strategy (i.e., sit-and-wait), suggesting that behavioral flexibility might be favored by a wider set of life history traits than previously proposed.

The ability of *A. evermanni* to solve the novel motor problem presented here was completely unexpected. The correct response required major changes to what has previously been considered highly stereotyped foraging behavior (Losos 2010), which consists of scanning the environment for moving prey items and striking them from above. In our experiment, motion cues were absent and striking from above was ineffective at dislodging the disc. Lizards used multiple strategies to remove the disc. The first was a modified strike, laterally biting the disc and lifting it away from the reward. The second strategy required the lizard to advance on the disc with its head held against the substrate, using its snout as a lever to push the disc out of the way. This strategy is not a natural foraging behavior that has at least been witnessed, and may demonstrate an entirely novel solution, which is one of the main criteria used to recognize behavioral flexibility (Lefebvre 2010).

The four lizards that successfully completed the motor task were all immediately able to discriminate the rewarded disc from the novel, unrewarded disc (Table 1). The fact that individuals of *A. evermanni* rarely made mistakes during the discrimination tasks shows that individuals were able to quickly form a strong association between a non-ecologically relevant stimulus (i.e., a non-moving colored circle) and a food reward and demonstrates rapid acquisition of non-spatial memory. The behavioral paradigm used in our experiment is similar to that used in studies evaluating cognitive abilities of birds (e.g., Bond et al. 2007, Boogert et al. 2010), which provides an opportunity to

evaluate similarities and/or differences in cognitive performance across the two groups (Lefebvre 2010). However, because distinct taxa might perceive the same cognitive task differently, we only explore the most basic aspect of the task, number of presentations. Although individuals of *A. evermanni* only performed one task per day (i.e., a single presentation), they needed at least three times fewer presentations to successfully complete the associative learning tasks than what is commonly used in studies testing similar abilities in bird species (e.g., Bond et al. 2007, Boogert et al 2010, Roth et al. 2010, Boogert et al. 2011, and references therein). This result was unexpected and suggests a possible difference in the tempo at which associations are formed between these groups; further studies are needed to explore the generality of these results.

The final experiment demonstrated that individuals of *A. evermanni* were capable of reversing their previously learned color associations. Two individuals were able to reverse their association, while two individuals continued to remove the previously rewarded stimulus and never manipulated the new target (Table 1). Studies of reptilian cognition are extremely limited; however, evidence suggests that spatial reversal is an easier cognitive task than reversal based on visual feature cues (reviewed in Day et al. 2002). In our reversal task, the degree of similarity between the cues was higher than that previously used in reptiles (reviewed in Day et al. 2002), suggesting that reptiles might be more efficient than previously reported at discriminating the features of visual stimuli (i.e., color and pattern). Also, our results demonstrate that performance

of a given cognitive task (i.e., motor task and associative learning) was not a predictor of the ability to solve the reversal learning experiment, illustrating the need to measure individuals' cognitive abilities across multiple tasks.

In summary, our results provide evidence that cognitive abilities of *A. evermanni* are comparable to those of some endothermic species known for their behavioral flexibility. As a group, *Anolis* is known for exploiting a diversity of ecological niches and exhibiting complex behavioral repertoires (Losos 2010), attributes that can favor the evolution of behavioral flexibility (Klopfer and MacArthur 1960, Reader 2003).

Alternatively, behavioral flexibility might have contributed to the radiation of this clade by facilitating the exploration of novel environments and promoting diversification of habitat use (Sol and Price 2008). Finally, our findings and those of Wilkinson et al. (2010) force a re-thinking of our understanding of the cognitive abilities of ectothermic tetrapods.

3. Brain evolution across the Puerto Rican anole radiation¹

3.1 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 aspects 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 functional specializations of the brain (Striedter 2005, Sherry 2006). 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

¹ This chapter is currently under review at *Brain, Behavior, and Ecology*.

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). 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).

Two principle 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). It is important to note that there is not a strict dichotomy between these two hypotheses and the two processes can occur simultaneously depending on the strength of the constraints and selective pressures involved (Striedter 2005, Yopak et al. 2010). For example, though both cartilaginous fishes and mammals show primarily concerted brain evolution, both taxa have olfactory bulbs which are allometrically independent of the rest of the brain (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 (Barton and Harvey 2000, de Winter and Oxnard 2001) evolution 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. However, the general question of why concerted evolution appears dominant in some taxa while mosaic evolution occurs in others is an open question.

A recurrent finding across studies describing mosaic 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 or the entire telencephalon. This pattern has been demonstrated in birds (Abbott et al. 1999) and mammals (Safi and Dechmann 2005), and appears to also 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 possibility that the reptile brain may provide valuable insights into the evolution of the heavily studied avian and mammalian brains (Maclean 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 likely 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 correlate with differences in foraging strategies (Day et al. 1999). In the other case, the volume of the dorsal cortex varies between different morphs of side-blotched lizards, *Uta stansburiana*, and the variation is consistent with inter-morph difference in territoriality strategies (LaDage et al. 2009). Because the species used in these studies belong to two distant clades within squamates, the evidence suggests that mosaic brain evolution might be widespread in lizards. 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 high number of species and diversity of habitat use, West Indian *Anolis* lizards have become a model system for studies of evolutionary and behavioral ecology (Losos 2010). 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, 1990b, 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 morphology and are very similar in ecology and behavior (reviewed in Losos 2010). 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 ecomorphs classes (reviewed in Losos 2010). Behaviorally, members of the same ecomorph class have similar foraging strategies, movement patterns, and territorial behavior (Moermond 1979a, Moermond 1981, Johnson et al. 2008, Johnson et al. 2010). In anoles, the repeated pattern of morphological evolution, correlated with a diversity of structural habitat preferences, makes 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 habitats (i.e., forest canopy, tree trunks, and grass). Furthermore, although social system is very

similar across all the species, the species exhibit morphological and behavioral adaptations to effectively exploit the structural habitat in which they are most commonly found (Losos 2010). This pattern of adaptation of multiple 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 2010). 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 of *Anolis* from the island of Puerto Rico (see Figures 2 and 3 for phylogeny), representing a majority of the species forming the Puerto Rican anole radiation (Mahler et al. 2010). The species used 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). For all the species, we measured the volumes of the telencephalon, dorsal cortex, dorsomedial cortex, medial cortex, dorsal ventricular ridge, habenula, medulla, and cerebellum, as well as total brain volume (see Materials

and Methods for a detailed description of the structures measured). These structures were chosen due to their possible role in spatial learning, general cognitive ability, or their position in the brain. If mosaic evolution has played a dominant role in shaping anole brain region size, we predicted that the size of these brain structures relative to overall brain size will differ between species belonging to different ecomorphs.

Alternatively, if the effects of concerted evolution determine brain region size in anoles, we predicted that the size of each brain structure will be highly correlated with overall brain size, regardless of species or ecomorph.

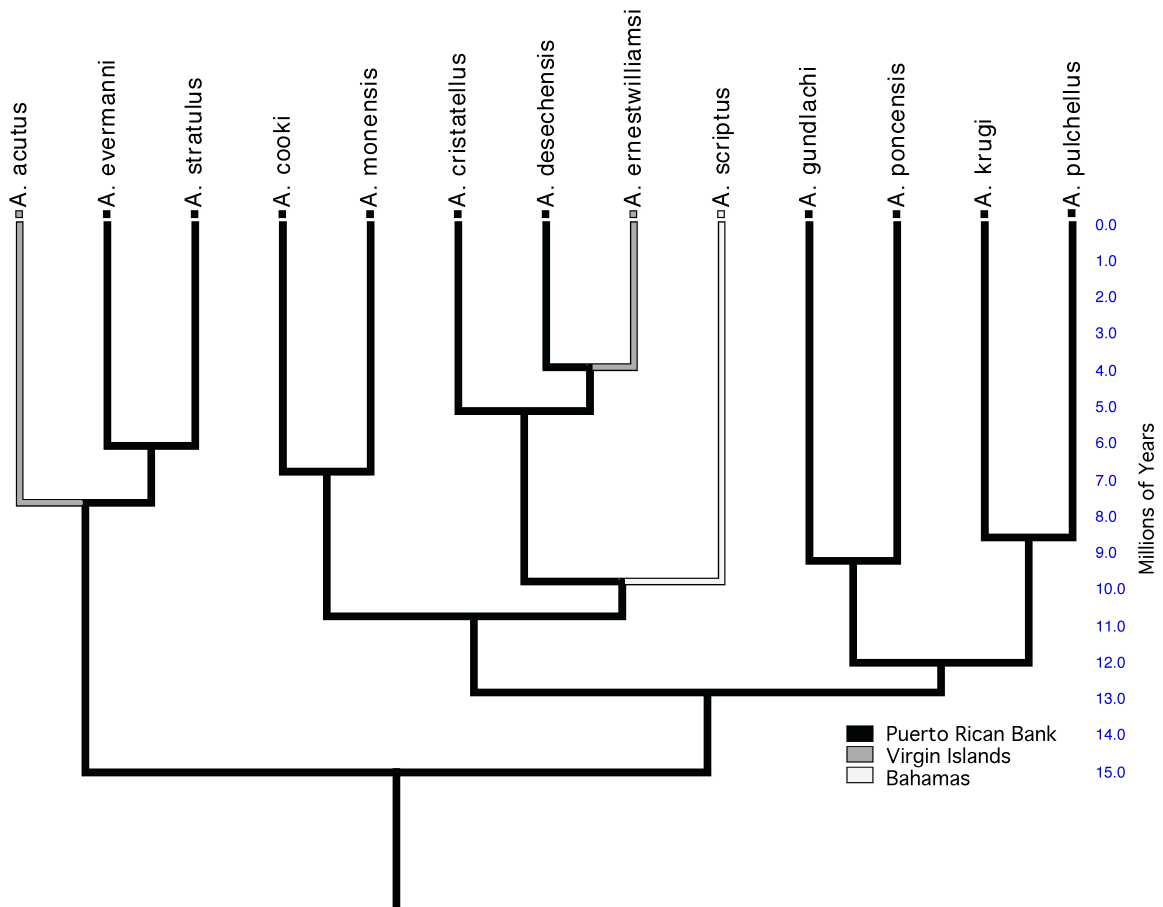


Figure 2: Phylogeny of the *cristatellus* series of *Anolis*, labeled by island. Note that *A. monensis* and *A. desenchensis* are found on small islands off the coast of Puerto Rico, while the other species of the Puerto Rican Bank are found in mainland Puerto Rico. Phylogeny constructed in Mesquite (Maddison and Maddison 2011).

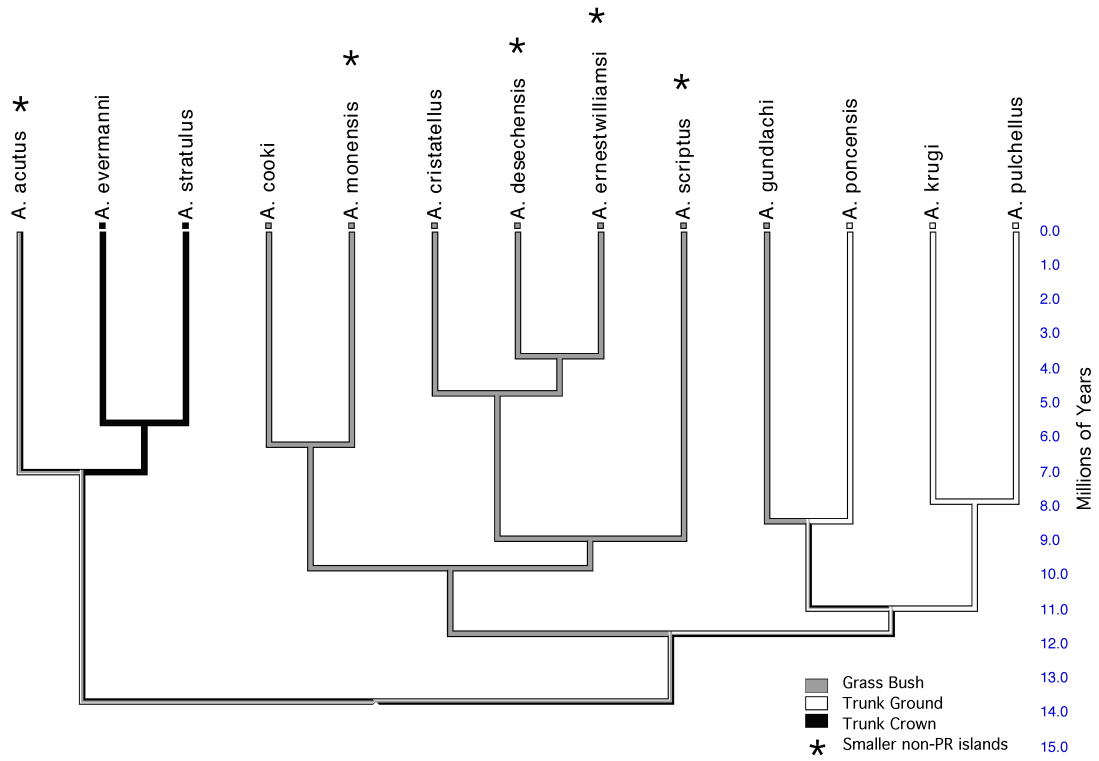


Figure 3: Phylogeny of the *cristatellus* series of *Anolis*, labeled by ecomorph. Species marked with an * are not found on mainland Puerto Rico. *A. acutus* does not fit into any ecomorph class. Phylogeny constructed in Mesquite (Maddison and Maddison 2011).

3.2 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. For each species, we sampled lizards in a habitat in which that species is commonly found. Individuals of *A. cristatellus* ($n=10$) and *A. krugi* ($n=10$) were collected at the forest edge along small roads. *Anolis gundlachi* ($n=10$) was collected in the

understory of the closed-canopy, mature forest. *Anolis pulchellus* ($n=8$) was collected in open grass fields near the station. *Anolis 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 during July and August 2006, July and August 2007, and August 2008. Only adult males were collected for this study. Upon capture, we measured the mass and the snout-vent length (SVL) of all individuals.

Within 24 hours 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 to 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 the brains were postfixed for 4 weeks to 8 months in 4% paraformaldehyde solution. All brains were weighed 4 weeks after dissection from the skull. 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 7 μm thickness. After scoring the first batch, it became evident that thicker sections resulted in less fragile sections 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 cover slipped (Lowe, 1996; modified to replace xylene with Fisherbrand Citrisolv). The resulting sections were photographed at 40X magnification using a Zeiss Axiophot microscope with attached Zeiss Axiocam digital camera. The areas of the dorsal ventricular ridge (DVR), dorsal cortex (DC), dorsomedial cortex (DMC), medial cortex (MC), telencephalon (TEL), cerebellum (CER), medulla (MED), and the whole brain were measured every 70 μm using ImageJ [Rasband 1997-2009] (See Figure 4 and Figure 5). We followed the description of Greenberg (1982) to delimit each area of the telencephalon. To delimit the cerebellum and medulla, we followed the description of the *Tupinambis teguixin* brain in ten Donkelaar (1998); in the case of the medulla, 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.

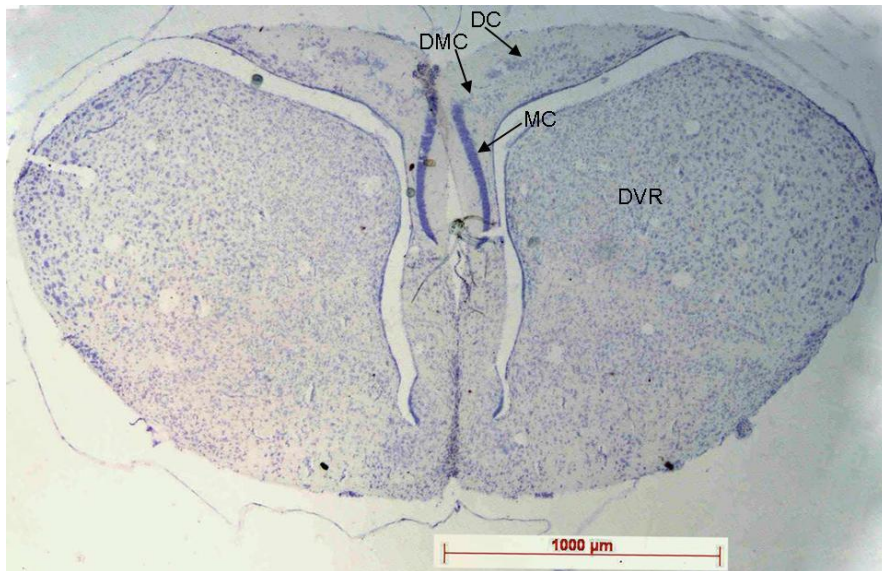


Figure 4: Section through the forebrain of *Anolis cristatellus* showing location of the Dorsal Cortex (DC), Dorsomedial Cortex (DMC), Medial Cortex (MC), and Dorsal Ventricular Ridge (DVR).

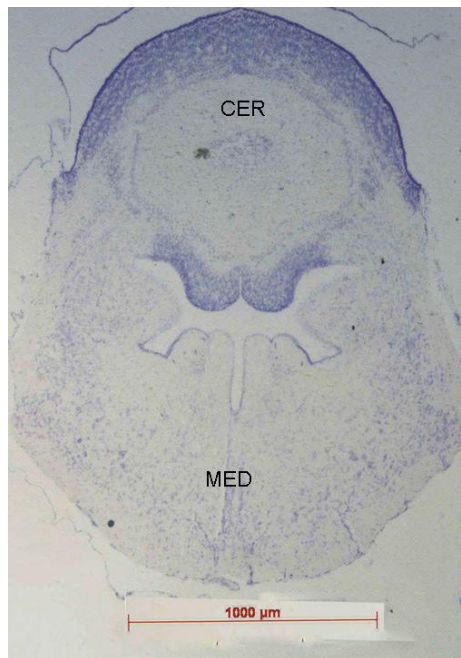


Figure 5: Section through the hindbrain of *Anolis cristatellus* showing location of the Cerebellum (CER) and Medulla (MED).

Structures were chosen based on their putative functions. The dorsal ventricular ridge (DVR) is the main sensory integration structure in the reptilian brain and performs similar functions to the mammalian isocortex (Striedter 2005, Butler and Hodos 2005). Thus, differences in the volume of the DVR may be indicative of differences in general cognitive ability between species. The dorsal cortex (DC) is another potential homolog to the mammalian isocortex, and changes in DC volume may correlate with changes in general cognitive ability (Striedter 1997, Striedter 2005, Butler and Hodos 2005). The dorsomedial cortex (DMC) and medial cortex (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, Striedter 2005, Butler and Hodos 2005, Raby and Clayton 2010). The telencephalon (TEL) is the major brain division that houses all of the previous structures, as well as the lateral cortex and subpallial structures (Striedter 2005, Butler and Hodos 2005). The cerebellum (CER), among many other functions, 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 medulla (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. Under concerted evolution, all structures of the brain are expected to change in proportion with brain size, though more anterior regions of the brain may increase with positive allometry while more posterior regions increase with negative allometry (Finlay et al. 2001).

3.2.1 Statistical Analysis

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 collection and sectioning, we conducted Analysis of Covariance (ANCOVA) with brain volume as a covariate and snout-vent length (SVL) and order of sectioning as independent variables. The ANCOVA 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 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 immeasurable variation exists in both variables (McArdle 1988). In order to test the predictions of the

mosaic evolution hypothesis, these residuals were then compared using ANOVA to determine if each structure's volume differed between species. If the ANOVA reported significant differences, the means were compared using Tukey's range test. Because we are comparing species with no additional grouping variable, we cannot 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 mean volume of each of 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 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 demonstrate 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 DVR, DC, DMC, MC, TEL, CER, MED, and mean total brain volume for each species. We performed a phylogenetically corrected reduced major axis regression using 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). This analysis was conducted in R (R Development Core Team, 2009) using the phytools package (Revell 2011). 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 (see 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, NC, 1989-2011).

3.3 Results

Order 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 collection and sectioning. If undetected shrinkage did occur uniformly across the brain, it should

not affect the results of this study because all measurements are relative to total brain size.

The absolute sizes of the brain and all measured brain structures are shown in Table 2. All species exhibited the same general pattern of brain allometry. No brain structure differed significantly among species in volume relative to the overall volume of the brain, though the cerebellum approaches significance (Table 3). 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 4). In addition, the volume of each brain structure was strongly predicted by brain size (Table 5). Brain size predicted 92.5% of variation in cerebellum volume in the lowest case, ranging up to 99.7% of variance in telencephalon volume.

Table 2: Mean absolute measurements for each lizard species. The measurements for the total brain and each brain structure are volumes given in μm^3 . TEL: Telecephalon, DC: Dorsal Cortex, DMC: Dorsomedial Cortex, MC: Medial Cortex. DVR: Dorsal Ventricular Ridge, CER: Cerebellum, MED: Medulla.

Species	SVL (mm)	Body Mass (g)	TEL	DC	DMC	MC	DVR	CER	MED	Total Brain
<i>A. cristatellus</i>	66.23	9.56	3423.84	131.28	27.44	67.66	1444.44	332.14	1527.36	9467.91
<i>A. evermanni</i>	58.86	4.95	2431.46	77.05	18.26	45.27	1065.94	281.30	1196.93	7004.74
<i>A. gundlachi</i>	62.22	6.29	3128.41	102.29	24.60	53.35	1471.12	357.40	1531.93	8767.14
<i>A. krugi</i>	46.10	2.43	1652.04	46.20	11.53	24.95	722.35	196.35	833.75	4836.76
<i>A. pulchellus</i>	43.62	1.86	1369.81	40.81	10.07	23.06	568.13	158.24	539.63	3958.21
<i>A. stratulus</i>	45.36	2.19	2442.22	80.85	16.24	46.51	1064.23	247.80	1109.51	6855.20

Overall, positive significant correlations were present between the volume of the total brain and each brain structures (TEL, DC, DMC, MC, DVR, CER, and MED). This pattern is present even after correcting for phylogenetic relationship, and is illustrated as a regression against total brain volume (Figure 6). All correlations were positive; thus increases in overall brain size were correlated with an increase in size of all structures. However, the rates of increase varied among structures (Table 5). 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 TEL and DMC approached significance.

Table 3: Results of an analysis of variance (ANOVA) on the relative size of each brain structure across all the species. TEL: Telecephalon, DC: Dorsal Cortex, DMC: Dorsomedial Cortex, MC: Medial Cortex. DVR: Dorsal Ventricular Ridge, CER: Cerebellum, MED: Medulla.

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

Table 4: Results of a phylogenetic analysis of variance (ANOVA) on the relative size of each brain structure across all ecomorphs. TEL: Telecephalon, DC: Dorsal Cortex, DMC: Dorsomedial Cortex, MC: Medial Cortex. DVR: Dorsal Ventricular Ridge, CER: Cerebellum, MED: Medulla.

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

Table 5: Values of a phylogenetically corrected Reduced Major Axis regression on each brain structure across all species. p-values denote confidence that the slope differs from 1, demonstrating either positive or negative allometry.

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

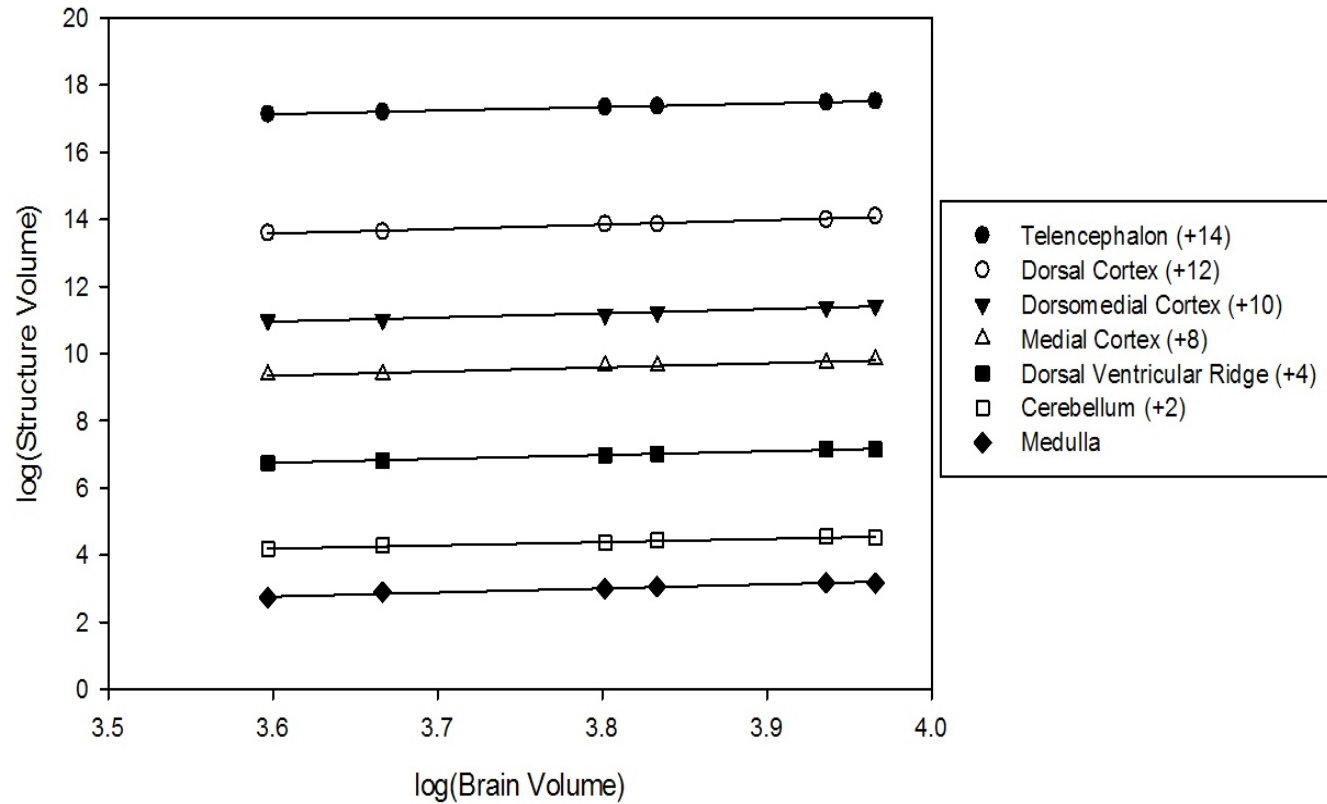


Figure 6: Scaling of the volumes (in μm^3) of seven brain structures plotted as a function of total brain volume. Lines indicated phylogenetically corrected Reduced Major Axis regressions for each brain structure. For purposes of visualization, an arbitrary constant was added to each brain structure, shown in parentheses in the legend.

3.4 Discussion

We found support for predominantly concerted brain evolution across the Puerto Rican anole radiation, though our results are suggestive of mosaic evolution in the cerebellum. The sizes of all other brain structures are consistent with concerted brain evolution. This pattern, in which concerted brain evolution determines the size of the majority of brain structures while a few 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 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 anole ecology, including general aspects of their behavior (Williams 1983, Losos 2009, Johnson et al. 2010). Interspecific differences in habitat complexity in a variety of taxa, including birds (Abbott et al. 1999) and bats (Safi and Dechmann 2005), have been suggested to lead to mosaic brain evolution.

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

telencephalon 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 cerebellum.

Concerted brain evolution has been reported to be dominant in two other clades: mammals, where overall brain volume explains $\approx 96\%$ of the total variance in volumes of constituent brain structures (Finlay et al. 2001); and cartilaginous fishes, where overall brain volume explains $\approx 93\%$ of the total variance in volumes of constituent brain structures (Yopak et al. 2010). The previous 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 telencephalon and cerebellum 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 this pattern, with the telencephalon and its constituent structures scaling with significantly or near significantly positive allometry. The remaining brain structures could not be determined to scale with either positive or negative allometry, but the estimates of their slopes are roughly consistent with previous studies. The largest difference is the medulla, which is estimated to scale with positive allometry in the current study but has been shown to scale with negative allometry in mammals and cartilaginous fishes (Finlay et al. 2001,

Yopak et al. 2010). However, the medulla in anoles does not scale with significantly positive allometry. Adding additional species of Iguanid lizards to the analysis would likely provide improved resolution to the scaling pattern of all areas of the brain, especially the medulla.

We measured four structures in the telencephalon that are putatively related to general cognitive abilities, spatial memory, and spatial problem solving. These included the dorsal cortex and dorsoventricular ridge, which are both potential homologs of the mammalian isocortex, and the dorsomedial cortex and medial cortex, which are both homologs of the mammalian hippocampus (Striedter 1997, Butler and Hodos 2005). All of these structures scale with positive allometry with respect to overall brain size, as does the telencephalon as a whole. However, we estimate that all of these telencephalic structures increase in size at a higher rate than the telencephalon itself. These relationships suggest that some constituents of the telencephalon 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 telencephalon exhibit negative allometric change. The remaining possibilities are the lateral pallium or subpallial structures, including 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 1979a, 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, sensory areas across a wide variety of taxa show strong mosaic evolution related to 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 differences in these sensory areas as they predominantly rely heavily on vision and weakly on chemoreception (Pianka and Vitt 2003, Losos 2010). This area of research would be most productive when adding more strongly chemoreceptive species to those sampled here.

When the effect of overall brain size is removed, none of the structures measured significantly differ in size between species occupying different types of structural habitat (i.e. ecomorphs). However, all of the structures potentially related to learning and navigating habitats of varying complexity scale with positive allometry with respect to total brain size (Figure 6). In our study, we included representatives of the most common ecomorphs found across the Greater Antilles. 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 very distinct structural niches from 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 2010). 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 2010).

While anoles demonstrate considerable diversity in structural habitat used, their foraging behavior (i.e., sit-and-wait) and social systems (i.e., territoriality) are relatively conserved (Losos 2010). 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 semi-aquatic 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, this study is the first to evaluate possible patterns of brain evolution across multiple species of lizards, and reptiles in general. The results are consistent with concerted evolution as the primary mechanism responsible for the evolution of the brain region size in anoles, while the cerebellum 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.

4. Habitat complexity and brain size in Puerto Rican *Anolis*

4.1 Introduction

There is a rich literature in the fields of animal behavior, ecology, and evolution evaluating the role of structural habitat complexity as a selective force on species' ecology. A classical example is the well documented impact of habitat complexity on species diversity, where more structurally complex habitats can result in increased species diversity by providing more niches and/or alternative ways to exploit environmental resources (MacArthur and MacArthur 1964, MacArthur 1965, Tews et al. 2004). However, habitat structure can also impact other aspects of the species' ecology, including survivorship (Stoner 2009), home range area (Longpierre et al. 2001), and species interactions (Jenssen 1973, Moermond 1986, Godbold et al. 2011). Structural habitat complexity can also impact many aspects of behavior, such as locomotion (Moermond 1979a, Mattingly and Jayne 2004), foraging and search behavior (Moermond 1979b, Bezemer et al. 2010), territoriality (Stamps 1977, Jensen et al. 2005), and aggression (Danley 2011, Kobler et al. 2011). Perhaps because of the diverse effects of habitat structure on behavior, much attention has also been given to its effects on various aspects of neuroanatomy, which provides both the physical basis of behavior and a substrate for evolution to directly act upon.

A dominant theme in comparative neuroanatomy centers on the possible advantages resulting from an increase in brain size. It is predicted that larger brains and

brain structures can provide additional cognitive processing ability through the addition of more neurons (Healy and Rowe 2007, Chittka and Niven 2009). The relationship between the size of the brain, including its constituent regions, and habitat complexity has been studied in a variety of species, across a wide range of taxonomic groups. In the seabird Leach's storm-petrel, individuals nesting in forests have larger hippocampal volumes than those nesting in meadows (Abbott et al. 1999). 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, habitat complexity is correlated with the size of the hippocampus (Safi and Dechmann 2005). In addition, bats foraging in more cluttered/complex habitats have relatively larger brains than bats foraging in the open (Ratcliffe et al. 2006). A recurring finding is that interspecific differences in the relative volume of a given brain structure or overall brain volume correlate with interspecific differences in habitat complexity (though see Roth and Thorington 1982 for a case where this did not apply). However, making direct comparison between studies is extremely difficult because methods used to classify habitat complexity vary widely. This hinders identification of potential variables favoring increased brain size as well as the comparison of habitat complexity across studies.

In comparative studies of brain size, several methods have been used to classify habitat complexity. The most common strategy is to divide habitat into discrete

categories, often based on the level of clutter (Budeau and Verts 1986, Abbott et al. 1999, Ratcliffe et al. 2006, Shultz and Dunbar 2006). This method provides no quantitative measure of habitat complexity, but relies on an intuitive estimate of complexity between the categories. However, it allows categorization of a large number of species with relatively little effort. Second, degree of habitat complexity can be inferred from other characteristics of a species, such as wing area in bats (Safi and Dechmann 2005) or number of sympatric species in cichlids (Pollen et al. 2007). This has the advantage of providing a continuous scale of complexity that is relatively easy to measure. However, not all species may have easily measured traits correlated with habitat complexity. Finally, the complexity of the habitat can be directly measured (Pollen et al. 2007, Shumway et al. 2007). This approach provides the most precise measure of habitat complexity, but requires a clear definition of complexity and the scale at which it is relevant (Huston 1994, Tews et al. 2004). Despite these difficulties, this is the only method that allows direct comparison across a range of taxa occupying similar habitats.

West Indian anoles have become a model system for studies of evolutionary and behavioral ecology (Losos 2010). A prominent theme across studies of West Indian anoles has been how selection to effectively exploit distinct habitat types has driven many aspect of anole anatomy and ecology, including morphology, communication, and territoriality (*e.g.*, Williams 1972, 1983; Losos 1990a, 1990b; Losos *et al.* 1994; Leal and Fleishman 2002, 2004; Johnson and Wade 2010). 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 class have the same morphology and are very similar in ecology and behavior (reviewed in Losos 2010). 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 types (reviewed in Losos 2010). Behaviorally, members of the same ecomorph have similar foraging strategies, movement patterns, and territorial behavior (Moermond 1979a, 1979b, Moermond 1981, Johnson et al. 2008, Johnson et al. 2009). This repeated pattern of morphological and behavioral evolution correlated with structural habitat preference makes anoles an ideal clade to study the relationship between brain size and habitat complexity. Furthermore, it provides an opportunity to evaluate possible agreements or disconnects between different methods of characterizing habitat complexity.

The habitat types occupied by each ecomorph 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). The differences in the complexity of navigation between habitats types might favor changes in the size and structure of the brains of species belonging to each ecomorph (Budeau and Verts 1986,

Safi and Dechmann 2005). Here, we examine the relationship between brain size and measures of habitat complexity in six closely related species of *Anolis* lizards from the island of Puerto Rico. In addition, we evaluate if different approaches to characterizing habitat complexity produce concordant results. These species belong to three distinct ecomorphs, with two each belonging to the trunk-crown, trunk-ground, and grass-bush ecomorphs (Williams 1972). While these species all demonstrate similar social systems, they exhibit morphological and behavioral adaptations to effectively exploit their preferred structural habitat; this has been used as evidence to support the role of selection in shaping multiple traits of each ecomorph according to habitat type (reviewed in Losos 2010). In fact, the presence of multiple behavioral and/or morphological adaptations to habitat structure has become the trademark of the *Anolis* radiation and strongly suggests that structural habitat exerts strong selection on this group (reviewed in Losos 2010).

To determine if habitat complexity correlates with the volume of the brain and several constituent structures, we measured the volume of the entire brain, telencephalon, dorsal cortex, and dorsomedial cortex of six species of *Anolis*, comprising a majority of the species forming the Puerto Rican anole radiation (Mahler et al. 2010). The telencephalon, dorsal cortex, and dorsomedial cortex may all exhibit hyperallometry within the anole brain (see Chapter 3). We also directly measured structural habitat complexity for each species utilizing a novel method designed for arboreal organisms.

The species used 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). We predicted that species of different ecomorphs will differ in the relative size of the brain, telencephalon, dorsal cortex, and dorsomedial cortex. In addition, we predicted that species occupying more complex structural habitats will possess relatively larger brains, telencephalons, dorsal cortices, and dorsomedial cortices.

4.2 Materials and Methods

4.2.1 Brain Volumes

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. For each species, we sampled lizards in a habitat in which that species is commonly found. Individuals of *A. cristatellus* ($n=10$) and *A. krugi* ($n=10$) were collected at the forest edge along small roads. *Anolis gundlachi* ($n=10$) was collected in the understory of the closed-canopy, mature forest. *Anolis pulchellus* ($n=8$) was collected in open grass fields near the station. *Anolis 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 during July and August 2006, July and August 2007, and August 2008. Only adult males were

collected for this study. Upon capture, we measured the mass and the snout-vent length (SVL) of all specimens.

Within 24 hours of capture, specimens were anesthetized and intracardially perfused with phosphate buffered saline followed by 4% paraformaldehyde solution (methodology follows Day et al. 2005). Following perfusion, lizards were decapitated and the heads were postfixed in 4% paraformaldehyde solution for 1 to 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 the brains were postfixed for 4 weeks to 8 months in 4% paraformaldehyde solution. All brains were weighed 4 weeks after dissection from the skull. 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.

Whole brains were dehydrated in a graded ethanol series, embedded in paraffin, sectioned in the transverse plane using a microtome. The first 40 brains were sectioned at 7 μm thickness. After scoring the first batch, it became evident that thicker sections resulted in less fragile sections 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 cover slipped (Lowe 1996; modified to replace xylene with Fisherbrand Citrisolv). The resulting sections were photographed at 40X magnification using a Zeiss

Axiophot microscope with attached Zeiss Axiocam digital camera. The areas of the whole brain, telencephalon, dorsal cortex, dorsomedial cortex, and medulla were measured every 70 μm using ImageJ (Rasband 1997-2009). We followed the description of Greenberg (1982) to delimit each area of the telencephalon. The area of the entire brain, including but not limited to the other structures measured and excluding any ventricles, was measured in each section. Brain 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 brain. All volumes were log transformed prior to analysis.

4.2.2 Structural Habitat Complexity

In order to characterize structural habitat complexity, we combined several measurements, which we propose are critical to determine habitat complexity in a biologically relevant context. A major obstacle to measuring habitat complexity is that the measurements should be relevant to the biology of the species, which means that the behavior, locomotion, and general movement patterns of the species must be considered when describing complexity (Huston 1994, Tews et al. 2004). First, we observed individuals of each species in their respective habitats in order to determine the distances moved by each species ($n_{\text{A. cristatellus}} = 20$, $n_{\text{A. evermanni}} = 22$, $n_{\text{A. gundlachi}} = 21$, $n_{\text{A. krugi}} = 20$, $n_{\text{A. pulchellus}} = 20$, $n_{\text{A. stratulus}} = 22$). Only adult males were observed. Each lizard was

observed until it was lost from sight or was observed for 20 minutes. Lizards that were observed for less than 5 minutes 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 10 continuous seconds. The distance of each movement bout was estimated to the nearest cm. We used these data to calculate the 75th percentile value of 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.

In order to measure the vegetation profiles, we walked slowly through the habitats for each species and identified perching adult males (*nA. cristatellus* = 60, *nA. evermanni* = 60, *nA. gundlachi* = 59, *nA. krugi* = 60, *nA. pulchellus* = 60, *nA. stratulus* = 60). Once we found an appropriate lizard, 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 (see Figure 7). Diagrams included the distance and diameter of every branch, as well as the number of branches extending from each choice point. 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 points. A choice point was defined as any point where a lizard was presented with multiple options in order to proceed further away from the perch. 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 varies 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 habitat structure were a simple pole). This value was defined as the corrected distance for each species.

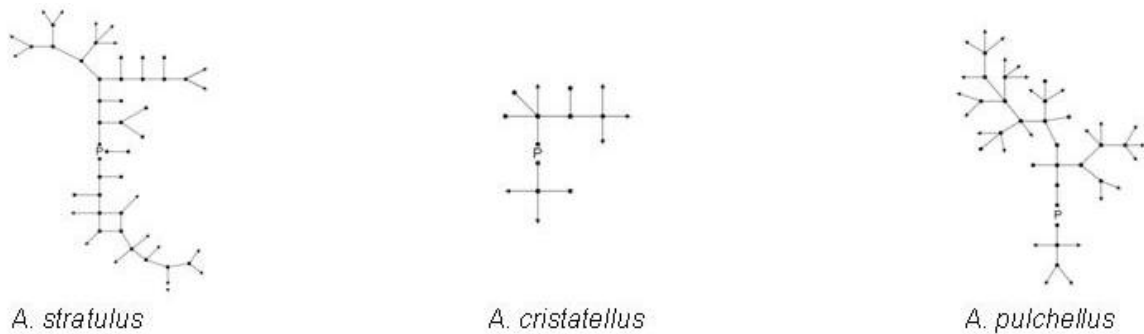


Figure 7: Examples of habitat diagrams for *Anolis stratulus*, *A. cristatellus*, and *A. pulchellus*. P designates the lizard's initial perch. Closed circles designate the ends of branches. Branches ending with arrows indicate that the branch extended beyond the full diagrammed distance.

In order for a measurement of habitat complexity to be relevant to a given organism, it must be measured at a scale appropriate to that organism's movement. To incorporate the scale of each species' movement into our measurements, we observed individuals of each species in their habitat in order to determine the number of branches crossed per meter moved by each species ($n_{A. cristatellus} = 16$, $n_{A. evermanni} = 15$, $n_{A. gundlachi} = 11$,

nA. krugi = 10, nA. pulchellus = 10, nA. stratulus = 19). Only adult males were observed. Each lizard was observed until it was lost from sight or was observed for 20 minutes. Lizards that were observed for less than 10 minutes or continuously moved away from the observer were not included in the analysis. During this observation, we estimated the distance moved by each lizard to the nearest cm 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 habitat structure, while high numbers indicate that the habitat structure occurs at too small a scale to be meaningful to the lizard, as it can step from one branch to another without following each branch to where it joins another.

4.2.3 Statistical Analysis

Differential tissue shrinkage due to variable fixation time is a potentially confounding factor in histological studies of the brain (Healy and Rowe 2007). To test for the possibility of brain volume shrinkage due to variable time between collection and sectioning, we conducted Analysis of Covariance (ANCOVA) with brain volume as a covariate and snout-vent length (SVL) and order of sectioning as independent variables. The ANCOVA was conducted on data from the specimens collected in 2006, as this provided the largest sample size within a single field season.

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 telencephalon, dorsal cortex, and dorsomedial cortex volume for

brain size by conducting a phylogenetically corrected RMA regression of brain volume against each area's volume. 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). Because species are phylogenetically related to varying degrees, they cannot be considered statistically independent of each other (Felsenstein 1985); the aforementioned phylogenetically corrected RMA regressions take this into account. A reduced major axis regression was used because it provides more reliable results than least squares regression in cases such as ours, where unmeasurable variation exists in both variables (McArdle 1988). This analysis was conducted in R (R Development Core Team, 2009) using the phytools package (Revell 2011). Reduced major axis regressions were performed using RMA v. 1.17 (Bohonok 2002). Residuals for each species were used as measures of brain volume corrected for body size.

One potential problem with the different measurements of habitat complexity (i.e., number of choice points, end points, branches, bridges and corrected distances) is lack of independence. To account for this problem and to condense the number of variables we used Principal Component Analysis (PCA), which reduces the numbers of variables to a small number of orthogonal variables (for a detailed discussion of PCA of ecological data, see Losos 1990a, Irschick et al. 1997). We applied PCA to the number of choice points, end points, branches, and bridges as well as the corrected distances as variables. 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 components by the mean number of perches crossed per meter moved.

We determined the relationship between relevant habitat complexity 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 telencephalon, dorsal cortex, and dorsomedial cortex relative to brain size against mean relevant habitat complexity.

We used a phylogenetic analysis of variance (ANOVA) to compare brain size between the different ecomorph types. We first conducted a phylogenetically corrected RMA regression of mean snout vent length (SVL) against 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).

4.3 Results

4.3.1 Behavioral Observations

Movement observations always began when the lizard was perching on vegetation. However, each species exhibited distinctive movement behavior throughout the observation period. For example, during a typical observation of *A. stratulus* (average SVL 43.0 mm), individuals were moving in the forest canopy, where they

perched and moved primarily along larger branches. They would move onto twigs or large leaves in order to cross between larger branches, which they did fairly often (average number of perches moved across per observation = 6.5). 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 observation = 8.5, average distance per movement bout = 59.6 cm). In contrast, individuals of the similarly sized *A. pulchellus* (average SVL = 42.2 mm) were primarily observed in open, grassy fields. Individuals of *A. pulchellus* generally perched on high, solitary blades of grass with 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. *A. pulchellus* moved less often than *A. stratulus* (average number of movements per observation = 4.1) and their movements were generally short (average distance per movement bout = 25.4 cm). In cases where *A. pulchellus* were moving along high, isolated blades of grass, they remained on a single perch for a considerable distance; 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 perches than any other species observed (average number of perches moved across per observation = 50.3). The 75th percentile of movement distance for each species is reported in Table 6, while the mean number of perches crossed per meter movement for each species is reported in Table 8.

4.3.2 Habitat Complexity

The descriptive variables of habitat complexity for each of the species are summarized in Table 6. Species vary widely in all of the measurements except number of bridges, with bridges being rare in all species' habitat. However, the species can broadly be separated into two groups. *A. krugi*, *A. cristatellus*, and *A. gundlachi* all have relatively few choice points, end points, and number of branches within their habitat, while *A. pulchellus*, *A. evermanni*, and *A. stratulus* have roughly twice as many or more choice points, end points, and branches. The PCA yielded a first component explaining 97% of the variance, with large loadings from number of choice points, end points, and branches (Table 7). We used this PC as an index of habitat complexity, in which all the relevant variables are changing in the same direction. High degrees of structural habitat complexity are indicated by strongly negative scores. This component was then divided by the average number of branches crossed per meter moved to yield Relevant Habitat Complexity [RHC] (Table 8).

Table 6: Direct measurements of habitat complexity.

Species	75th Percentile Movement Distance	Mean Number of Choice Points	Mean Number of End Points	Mean Number of Branches	Mean Number of Bridges	Mean Total Distance	Corrected Mean Total Distance
A. cristatellus	620	4.216667	8.683333	13.31667	0.216667	2399.817	1.935336
A. evermanni	577.5	8.04918	14.86885	23.65574	0.344262	3426.508	2.961952
A. gundlachi	650	2.716667	5.566667	8.5	0.3	2026.7	1.560179
A. krugi	550	4.2	8.016667	12.65	0.466667	2057.867	1.873818
A. pulchellus	350	10.21667	16.48333	28	1.266667	2373.8	3.372095
A. stratulus	647.5	12.91667	16.9	30.65	1.05	3596.067	2.777186

Table 7: Principle Component Analysis of habitat complexity.

Variable	Comp.1	Comp.2	Comp.3	Comp.4	Comp.5
Choice Points	-0.33253	0.685002	0.080324	0.098289	0.635676
End Points	-0.46256	-0.71692	0.064922	0.042541	0.515798
# of Branches	-0.82135	0.126277	-0.03365	-0.06463	-0.55149
# of Bridges	-0.01593	0.026465	-0.5505	-0.8189	0.159332
Corrected Total Distance	-0.02457	-0.01249	-0.82774	0.560133	0.018594
Proportion of Variance	0.973931	0.017808	0.005056	0.002295	0.00091

Table 8: Relevant habitat complexity and its constituent variables.

Species	PCA Comp. 1	Mean Branches/m	Relevant Habitat Complexity
A. cristatellus	7.6265854	2.9114	2.619559
A. evermanni	-6.53228073	1.266	-5.15978
A. gundlachi	13.3487398	1.6418	8.130552
A. krugi	8.30381179	2.5601	3.24355
A. pulchellus	-10.5474063	29.2917	-0.36008
A. stratulus	-12.4247678	1.4796	-8.39738

4.3.3 Brain Volume Measurements

Sectioning order 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 collection and sectioning.

The mean SVL, total brain, telencephalon, dorsal cortex, and dorsomedial cortex volume for each species is reported in Table 9. To remove the effect of body size on brain volume, we conducted a phylogenetic RMA regression of SVL against brain volume for all species (y-intercept = 1.0848, slope = 1.5773, $r^2 = 0.6858$, $p_{\text{slope} \neq 0} < 0.05$). We extracted residuals from this regression as measures of brain volume corrected for SVL. In order to remove the effect of total brain volume on the volume of each brain structure, we performed a phylogenetic RMA regression of total brain volume against the volume of the telencephalon (y-intercept = -0.6608, slope = 1.0538, $r^2 = 0.9977$, $p_{\text{slope} \neq 0} < 0.05$), dorsal

cortex (y-intercept = -3.2127, slope = 1.3302, $r^2 = 0.9543$, $p_{\text{slope} \neq 0} < 0.05$), and dorsomedial cortex (y-intercept = -3.5064, slope = 1.2389, $r^2 = 0.9622$, $p_{\text{slope} \neq 0} < 0.05$). We extracted residuals from these regressions as measures of the volume of each area independent of total brain volume.

Table 9: Mean snout-vent length and volumes of all brain structures measured. This table presents a subset of the data found in Table 2.

Species	SVL(mm)	Total Brain (μm^3)	Telencephalon (μm^3)	Dorsal	
				Cortex (μm^3)	Dorsomedial Cortex (μm^3)
A. cristatellus	66.233	9467.913	3423.845	131.2806	27.43814
A. evermanni	58.857	7004.736	2431.458	77.05041	18.25612
A. gundlachi	62.22222	8767.143	3128.413	102.2888	24.59807
A. krugi	46.095	4836.759	1652.042	46.2023	11.52554
A. pulchellus	43.61714	3958.215	1369.807	40.81282	10.06501
A. stratulus	45.36167	6855.204	2442.217	80.85383	16.24106

Table 10: Results of GLM regressions of brain structure volumes against Relevant Habitat Complexity. p-values denote confidence that the slope differs from 0.

Brain Structure	Y-intercept	Slope	R ²	p-value
Total Brain	-0.001516	-0.002992	0.08287	0.5801
Telencephalon	-0.0001562	-0.0003912	0.1806	0.401
Dorsal Cortex	-0.0001851	-0.00008403	0.0002458	0.9765
Dorsomedial Cortex	-0.0006569	0.0003716	0.00537	0.8903

Table 11: Results of phylogenetic ANOVA of brain structure volume, grouped by ecomorph. This table includes some information from Table 4.

Brain Structure	DF	F	P-value
Total Brain	2	1.145572	0.382
Telencephalon	2	0.4421562	0.641
Dorsal Cortex	2	0.03259228	0.962
Dorsomedial Cortex	2	1.524982	0.298

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 10). None of the regression slopes were significantly different from 0, demonstrating no discernable relation between the volume of any brain structure measured and RHC.

There were no significant differences between ecomorphs in brain size relative to body size or in brain structure size relative to brain size (Table 11). Therefore, the species studied exhibit no differences based on ecomorphs in the volumes of the brain structures studied.

4.4 Discussion

Our results support two main conclusions. First, the habitat types occupied by each ecomorph vary in structural complexity. The habitats can be divided into three types, roughly paralleling ecomorph categories. *Anolis evermanni* and *A. stratulus* occupy very complex habitats that constrain their movements to a relatively low number of

perches, while *A. gundlachi*, *A. cristatellus*, and *A. krugi* occupy relatively simpler habitats, although their movement between perches seems to be similarly constrained by the habitat. *Anolis pulchellus* is found in an extremely complex habitat that does not constrain its movement because the complexity exists at a smaller scale than the lizard's movement. In addition, species classified as members of the same ecomorph occupied habitats of different structural complexity. This points to the fact that Relevant Habitat Complexity can be highly variable even within the same general habitat type. Second, our data do not support the prediction that differences in habitat complexity are correlated with differences in overall brain or brain region size. This result was not affected by the method used to measure to measure habitat complexity; that is, direct measurements of complexity or categorization based on ecomorph.

The difference in Relevant Habitat Complexity between the habitats occupied by each ecomorph generally matches an intuitive understanding of the habitat types (Budeau and Verts 1986, Bernard and Nurton 1993). The denser forest canopy is more complex than the more open understory and the bushes along the edge of the forest. While the grass is complex, most of its complexity is at a smaller scale than even the movements of a four centimeter lizard, making it relatively simple to navigate. However, the RHC scores are not entirely in agreement with the habitat-based ecomorph classification of these anoles.

In general, species belonging to a specific ecomorph occupy habitats of similar structural complexity. The two species belonging to the trunk-crown ecomorph occupy habitats with similar RHC scores, as do the two species belonging to the trunk-ground ecomorph. However, this generalization does not hold in all cases. The two species of grass-bush anoles occupy habitats of differing structural complexity. *A. krugi* normally occupies bushes and shrubs (personal observation). This means that it has a RHC score similar to trunk-ground anoles, while its perch height and diameter, the measurements by which ecomorph is assigned, are similar to *A. pulchellus*. *A. pulchellus* is routinely found in open, grassy areas, and has an RHC score reflecting its structurally complex, extremely cluttered habitat. The variability in structural habitat occupied by the grass-bush species has the potential to result in a disconnect between analyses based on ecomorph and analyses based on directly measured RHC. Furthermore, the case of *A. pulchellus* illustrates the fact that the degree of habitat complexity can be greatly influenced by the scale at which the measurements are taken. This demonstrates the importance of verifying that the scale used is relevant to the species in question.

The lack of correlation between Relevant Habitat Complexity and the volume of the brain and any of its constituent structures measured here is surprising because brain size or brain region size is widely associated with habitat complexity (Harvey et al. 1980, Budeau and Verts 1986, Abbott et al. 1999, Safi and Dechmann 2005). However, these studies generally compare species across different habitat categories at a broader scale.

For example, arboreal species tend to have larger brains than terrestrial species (Harvey et al. 1980, Eisenberg and Wilson 1981, Bernard and Nurton 1993), and species living in forests tend to have larger brains than species living in grasslands or deserts (Harvey et al. 1980, Budeau and Verts 1986, Abbott et al. 1999, Safi and Dechmann 2005). While the species addressed in this study vary in structural habitat complexity, they are all arboreal species. This suggests that more substantial differences in habitat complexity, such as those between broad habitat categories, may affect brain size but differences within a habitat type are not sufficient for selection to favor divergence in brain size. For example, living within any arboreal habitat is complicated enough to exert equal influence on brain size regardless of minor differences in structural habitat complexity between different types of arboreality. Alternatively, there may be a continuous gradient of habitat complexity exerting selective pressure on the brain across all habitat types; our study may have lacked the resolution to detect this gradient. The first hypothesis could be addressed by widening the sample to include terrestrial and aquatic iguanid lizards and would be supported if all species within a habitat type have similar relative brain size while species occupying different habitat types have different relative brain sizes. The second hypothesis could be addressed by sampling more species within each habitat type and would be supported if species within a habitat type demonstrate a correlation between habitat complexity and relative brain size. These hypotheses are not mutually exclusive and may both be operating within a given group.

In summary, this study emphasizes the importance of directly evaluating habitat complexity in a consistent manner across all species of interest, rather than assigning them to broader categories. In addition, it demonstrates that brain size may not be related to habitat complexity among species occupying a given habitat type. Further research is necessary to determine how extensive differences in habitat must be in order to result in changes in brain size.

5. Interspecific differences in behavioral flexibility across three ecomorphs

5.1 Introduction

Interspecific differences in cognitive abilities are central to studies in cognitive ecology. Of particular interest is behavioral flexibility, an aspect of cognition that has been widely studied as a measure of general cognitive abilities (Bitterman 1965a, Bitterman 1965b, Shettleworth 1998, Reader and Laland 2002, Delius and Delius 2006), and has been linked to ecological traits such as increased invasion success (Sol et al. 2005a), migratory behavior (Sol et al. 2005b), and evolutionary diversification (Sol et al. 2005c). Behavioral flexibility, defined as the ability to alter individual behavior by developing new responses to novel stimuli or alter existing responses to familiar stimuli, provides organisms with the ability to behaviorally adapt to changing environments and is commonly associated with cognitive processes such as problem solving, spatial learning, and reversal learning (Shettleworth 1998, Reader and Laland 2002, Delius and Delius 2006). A comparative approach to studying behavioral flexibility, analyzing the behavioral flexibility of species with different ecological characteristics across the same task, is particularly useful for identifying ecological correlates of behavioral flexibility (Bitterman 1965a, Wasserman and Zentall 2006).

Behavioral flexibility has been examined in a large variety of taxa, with some studies concentrating on a single species while others compare the abilities of several species. In the case of interspecific comparisons, some studies have focused on a single

cognitive task (Gossette and Hombach 1969, MacLean et al. 2011). However, differences in the performance of a single task between several species can be difficult to interpret, as the differences may result from interspecific variation in non-cognitive traits, such as biomechanics, perception, motivation, or levels of neophobia (Auersberg et al. 2011). Two methodological approaches are commonly used to reduce the possibility that non-cognitive factors can bias interpretation of tasks requiring behavioral flexibility. The first approach is to present multiple species with a variety of tasks, each requiring flexibility in different types of behavior (Bitterman 1965a, Auersberg et al. 2011). The advantage of this approach is that it is unlikely that non-cognitive characteristics of the species being studied will equally affect performance across all tasks and thus bias interpretation of behavioral flexibility, as well as increasing the likelihood of finding species differences (Burghardt 1977). The second approach is to compare performance between closely related species, which are less likely to possess non-cognitive differences (e.g., morphological, sensory, physiological) which may alter interpretations of behavioral flexibility (Rumbaugh et al. 1996, Shettleworth 1998). In order to apply this second approach, it is necessary to identify a group of anatomically and perceptually similar, closely related species that still vary substantially in ecology.

West Indian *Anolis* lizards provide an ideal taxon for studies of comparative behavioral flexibility. Within each island of the West Indies, *Anolis* species have adapted into several ecomorph types, each specialized for a certain type of structural habitat

(Williams 1972). Selection to effectively exploit distinct habitat types has driven many aspects of anole anatomy and ecology, including morphology, communication, and territoriality (e.g., Williams 1972, 1983, Losos 1990a, 1990b, Losos *et al.* 1994, Leal and Fleishman 2002, 2004, Johnson and Wade 2010), and resulted in West Indian *Anolis* becoming a model system for studies of evolutionary and behavioral ecology (Losos 2010). While species of *Anolis* primarily occupy different structural habitat types (Chapter 4), they all exhibit the same general body form, foraging behavior, and sensory abilities (reviewed in Losos 2010). Therefore, interspecific differences in the performance of cognitive tasks most likely reflect differences in behavioral flexibility independent of differences in morphology, physiology, or perception.

Reptiles have a long, if irregular, history of use in studies of cognition (reviewed in Burghardt 1977). In the case of lizards, the ability to modify innate foraging behavior is variable, with some species failing to alter the motor pattern of their strike despite a large number of presentations (Cookson 1962), while others learn the motor pattern required to open a novel problem apparatus quickly (Manrod *et al.* 2008, Leal and Powell 2011). In addition, several species have been demonstrated to be capable of associative and reversal learning based on visual features (Day *et al.* 1999, Gaalema 2011, Leal and Powell 2011). However, only a single study has compared multiple, closely-related species of lizards across the same set of tasks, finding that an active foraging

species was more behaviorally flexible than a sit-and-wait foraging species (Day et al. 1999).

To test the prediction that increased habitat complexity favors the evolution of behavioral flexibility, we presented three species of Puerto Rican *Anolis* with two cognitive tasks. The species chosen belong to three ecomorph categories, and occupy habitats differing in structural complexity (see Chapter 4). These differences in structural complexity may alter the difficulty of navigating through each habitat (Moermond 1986, Johnson et al. 2006), which in turn could favor differences in behavioral flexibility. *Anolis evermanni* is a trunk-crown anole, having a relatively slender body and snout and fore and hind limbs of similar size. *Anolis cristatellus* is a trunk-ground anole, and as such is heavy bodied with robust hind limbs and a blunt snout. *Anolis pulchellus* is a grass-bush anole, possessing an extremely slender body and snout, small limbs, and a long tail. Despite their habitat specializations, the brains, as well as the telencephalon, its constituent structures, the medulla, and the cerebellum, all scale similarly in all three species. All species were presented with a motor task and serial reversal task to test behavioral flexibility. We hypothesize that, due to the differing cognitive demands of navigating their respective habitats, species belonging to different ecomorphs will differ in their performance across tasks.

5.2 Materials and Methods

5.2.1 Subjects and Housing

For these experiments, only adult male lizards were used. 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. *Anolis evermanni* (n = 6), *A. cristatellus* (n = 7), and *A. pulchellus* (n = 6) were collected and transported to our laboratory at Duke University, where they were housed individually in cages (29L, 21W and 21H cm), and kept under 12h:12h photoperiod cycle, at 28°C and 60% relative humidity, watered daily, and fed crickets on a regular schedule throughout the experimental period. All lizards were experimentally naïve. Though the experimental paradigm of this study is similar to that used in Chapter 2, different individuals of *A. evermanni* were used in this set of experiments.

5.2.2 Behavioral Tasks

Behavioral experiments were conducted in the cage where the lizards were housed. The behavioral testing apparatus was placed inside the cage, and lizards had 15 minutes to perform the task. The apparatus was an opaque grey platform (12L, 5.5W cm) containing 2 wells, each 1.6 cm in diameter and 1 cm deep. A reward (intact larva of *Hermetia illucens*; live in the first habituation step, freshly killed in every trial thereafter) was placed inside a well, and the lizard had to remove the disc to reach the larva.

Individuals were habituated to the testing apparatus before conducting the experiments. Habituation was performed in 4 sequential steps: (1) the apparatus was placed inside the cage with a live larva of *Hermetia illucens* inside one well as a reward (2) the apparatus was placed inside the cage with a freshly killed larva of *Hermetia illucens* inside one well as a reward; (3) individuals were randomly assigned a uniform colored blue or yellow disc, which was positioned next to the well containing the reward; (4) the disc was positioned covering half of the well containing the reward. During the habituation period, individuals did not need to manipulate the disc to access the worm. Individuals advanced stages or completed an experiment when they correctly performed the task six consecutive times.

Once the habituation period was completed, the disc was placed over one of the wells and the lizards were presented with the novel problem of dislodging the target to access the larva. The disc was placed over the well at an angle, loosely covering it but completely blocking line of sight into the well. This was necessary to reduce the amount of force required to open the well, as *A. pulchellus* displayed difficulty lifting the full weight of disc. The disc was placed in the same orientation for all species. Only one disc was used and its position was randomly determined before each trial. Individuals conducted one trial per day.

Lizards that learned to dislodge the disc performed a discrimination task in which a target (i.e., stimulus used for the motor task) and a distracter (i.e., a disc of the

alternative color) were presented simultaneously. Positions of the stimuli were randomly determined before each trial. The reward was placed under the target. Anoles show no response to larva odor (Leal and Powell 2011). Choice was scored as the first stimulus dislodged by the lizard.

The same paradigm was used to test reversal learning. However, in these experiments the reward was placed under the alternatively colored disc from that used in the discrimination task, reversing the conditions previously presented to the lizards. Up to five reversals were presented to each lizard, as they completed each task.

Lizards were removed from the experiment if their body condition began deteriorating. The experiment ended after 235 trials, regardless of the task reached by each lizard.

5.2.3 Statistical Analysis

All measurements except for the number of trials to complete the first habituation step and the second reversal task for *A. evermanni* were normally distributed. Because these two measures were not normally distributed, we conducted non-parametric tests on all data.

We performed unequal variance *t*-tests, which takes both unequal sample size and unequal variance into account and is appropriate for non-normally distributed data (this test is discussed in detail in Ruxton 2006), to compare the number of trials necessary to complete the motor task and the first reversal task between species,

including only those individuals that successfully completed each task. Finally, we conducted paired Wilcoxon Signed Rank tests for each species in which at least 3 individuals completed serial reversals, comparing the number of trials necessary for each successive reversal (e.g., 1st reversal to 2nd reversal, 2nd reversal to 3rd reversal).

To test for the possibility of varying neophobia between species, we performed a Kruskal-Wallis test comparing the number of trials to complete the first and third habituation steps (introduction of the apparatus and introduction of the colored disc).

Unequal variance *t*-test were calculated using Microsoft Excel (2003), while all other statistical analyses were performed using JMP (JMP, Version 7. SAS Institute Inc., Cary, NC, 1989-2007).

5.3 Results

A summary of the number of trials required for each individual to complete each task is presented in Table 12. Two individuals of *A. pulchellus*, one individual of *A. evermanni*, and six individuals of *A. cristatellus* were unable to complete the habituation steps. Of those lizards that progressed to the motor task, it was completed by 100% (4 of 4) of *A. pulchellus*, 100% (5 of 5) of *A. evermanni*, and 33.33% (1 of 3) of *A. cristatellus*. There were significant differences between *A. pulchellus* and *A. evermanni* in the number of trials needed to complete the motor task (Figure 8) [Unequal variance *t*-test, degrees of freedom = 6, $t = 10.9058$, $p\text{-value} < 0.005$]. The single *A. cristatellus* completed the motor task in 7 trials, performing similarly to *A. evermanni*.

Table 12: Number of trials performed by individuals of three species of *Anolis* to complete each task. (Brackets indicate the number of trials performed without completing the task).

Lizard	Species	Habituation 1	Habituation 2	Habituation 3	Habituation 4	Motor Task	Disc. Task	1st Reversal	2nd Reversal	3rd Reversal	4th Reversal	5th Reversal
P05	<i>A. pulchellus</i>	13	20	6	6	49	12	24	[101]			
P07	<i>A. pulchellus</i>	36	67	18	[53]							
P03	<i>A. pulchellus</i>	29	40	[113]								
P08	<i>A. pulchellus</i>	13	6	10	16	57	19	[103]				
P04	<i>A. pulchellus</i>	6	6	6	6	40	25	19	41	32	[69]	
P11	<i>A. pulchellus</i>	28	14	6	6	24	33	[121]				
E04	<i>A. evermanni</i>	6	6	6	6	7	6	16	32	25	22	29
E03	<i>A. evermanni</i>	6	6	36	6	19	12	[149]				
E10	<i>A. evermanni</i>	6	18	6	[118]							
E06	<i>A. evermanni</i>	6	6	6	6	6	6	11	18	22	51	18
E08	<i>A. evermanni</i>	6	6	6	6	33	7	38	32	16	29	19
E09	<i>A. evermanni</i>	6	7	11	10	17	19	30	[133]			
C07	<i>A. cristatellus</i>	[235]										
C03	<i>A. cristatellus</i>	24	6	[64]								
C01	<i>A. cristatellus</i>	7	15	6	6	7	8	87	53	[42]		
C11	<i>A. cristatellus</i>	30	13	[192]								
C02	<i>A. cristatellus</i>	22	17	28	73	[70]						
C09	<i>A. cristatellus</i>	[227]										
C10	<i>A. cristatellus</i>	6	6	6	6	[175]						

The first reversal task was completed by 50% (2 of 4) of *A. pulchellus*, 80% (4 of 5) of *A. evermanni*, and 100% (1 of 1) of *A. cristatellus* presented with a reversal task.

Including only those lizards completing the first reversal task, there was no significant difference between *A. pulchellus* and *A. evermanni* in the number of trials necessary to complete the reversal (Figure 9) [Unequal variance *t*-test, degrees of freedom = 6, $t = -1.22507$, $p\text{-value} > 0.10$]. However, the single *A. cristatellus* completed the first reversal task in 87 trials, taking much longer than either *A. pulchellus* or *A. evermanni*.

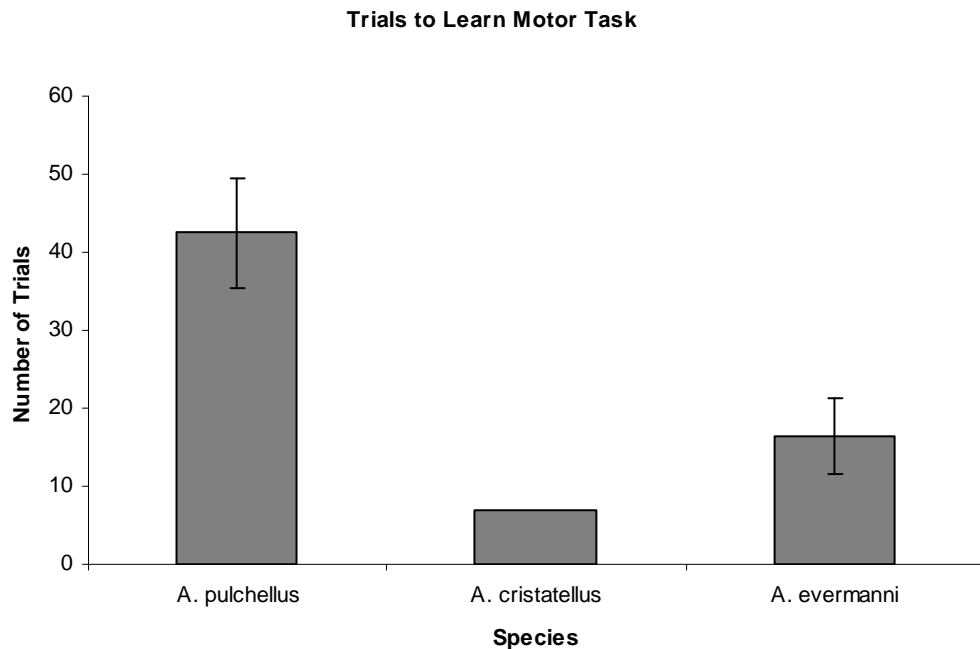


Figure 8: Motor task performance for three *Anolis* species ($n_{\text{pulchellus}} = 4$, $n_{\text{cristatellus}} = 1$, $n_{\text{evermanni}} = 5$).

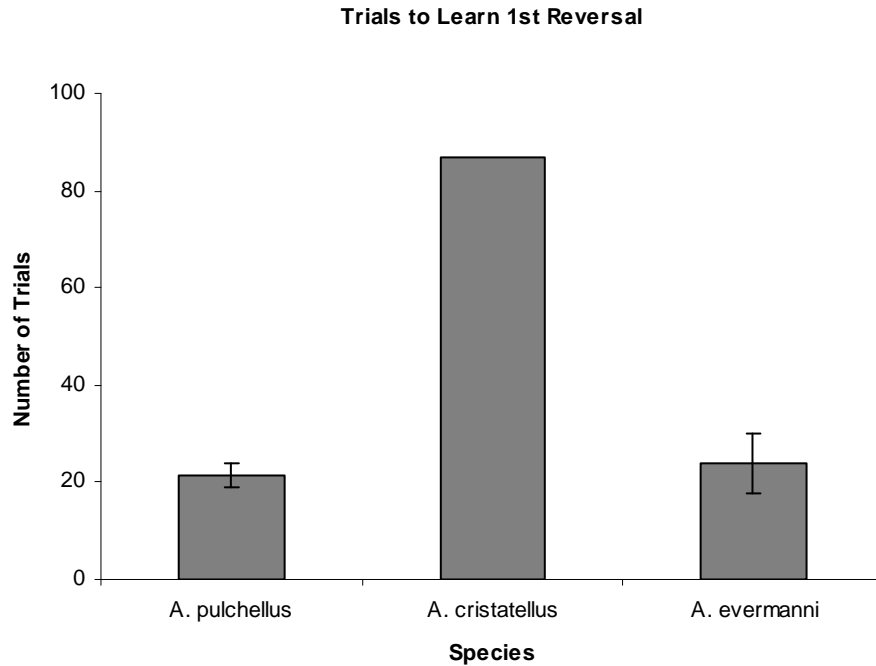


Figure 9: Performance in first reversal task of three *Anolis* species ($n_{\text{pulchellus}} = 2$, $n_{\text{cristatellus}} = 1$, $n_{\text{evermanni}} = 4$).

Few lizards were able to complete multiple reversal tasks. One (of two) *A. pulchellus* presented with serial reversal was able to perform three reversals, while the only *A. cristatellus* presented with serial reversal was able to perform a second reversal. *Anolis pulchellus* did not show a trend toward decreasing numbers of trials to complete successive reversals (Figure 11), while *A. cristatellus* required fewer trials in its second reversal than its first reversal (Figure 10). Three *A. evermanni* completed all five reversal tasks, but also showed no decrease in number of trials required for successive reversals (Figure 12).

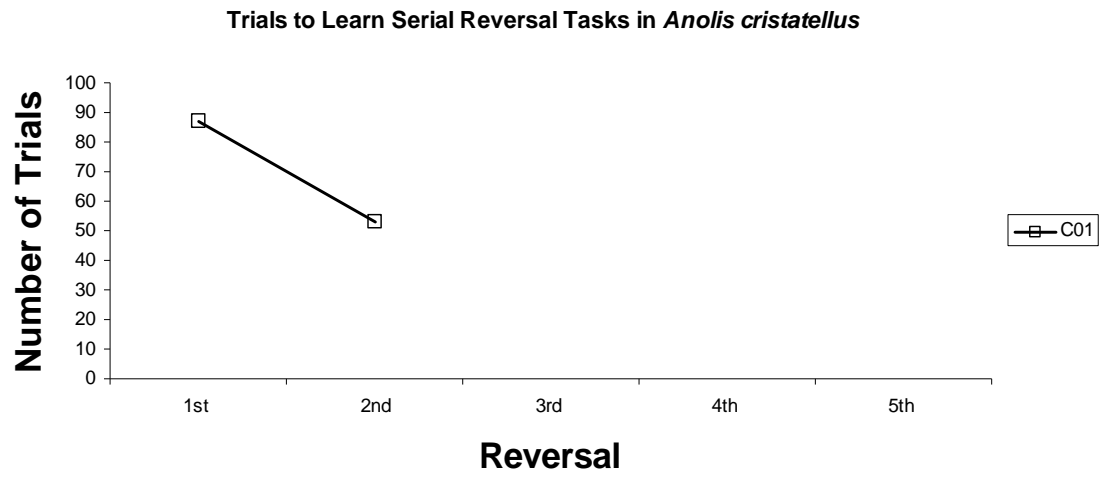


Figure 10: Performance through two serial reversal tasks in *A. cristatellus*.

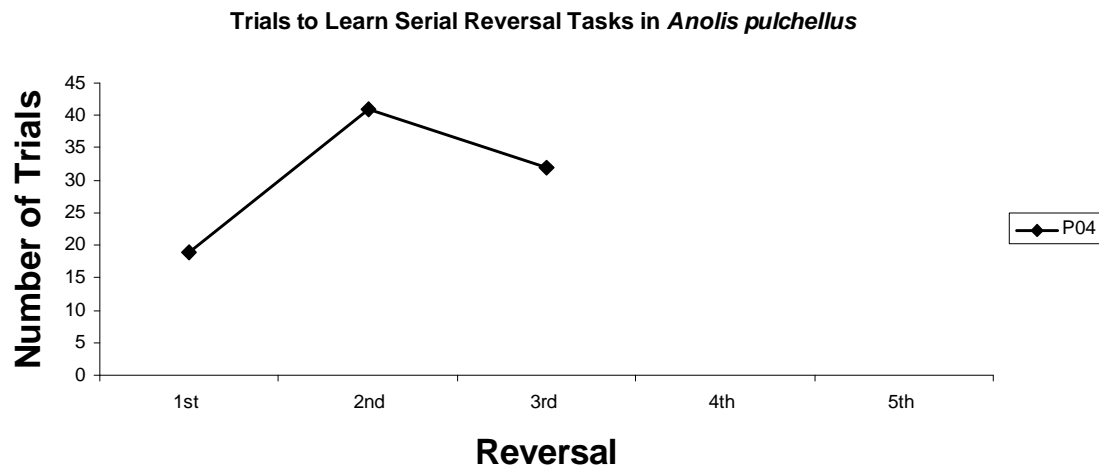


Figure 11: Performance through three serial reversal tasks in *A. pulchellus*.

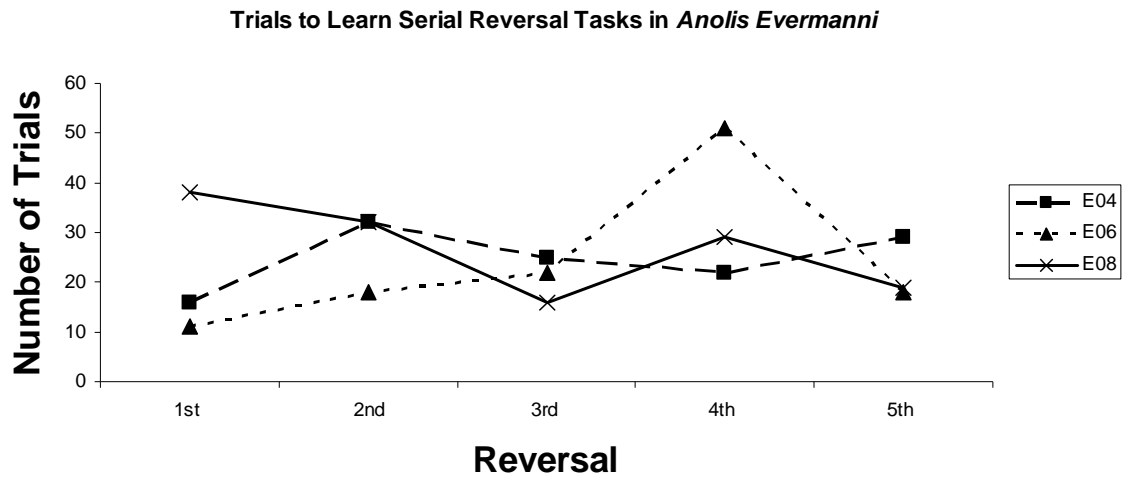


Figure 12: Performance through five serial reversal tasks in *A. evermanni*.

The level of neophobia varied between the species studied. In the first habituation step, in which the apparatus was introduced with a live larva as a reward, there were significant species differences in the number of trials required to finish the task (Figure 13) [Kruskal-Wallis Test, degrees of freedom = 2, $\chi^2 = 8.3433$, p-value = 0.0154], with *A. evermanni* requiring less trials to complete the task than *A. pulchellus* (Wilcoxon Test, $Z = 2.5948$, p-value = 0.0095) or *A. cristatellus* (Wilcoxon Test, $Z = -2.4318$, p-value = 0.0150). However, the introduction of the colored disc in the third habituation step demonstrated no species differences in neophobia (Kruskal-Wallis Test, degrees of freedom = 2, $\chi^2 = 0.0103$, p-value = 0.9948).

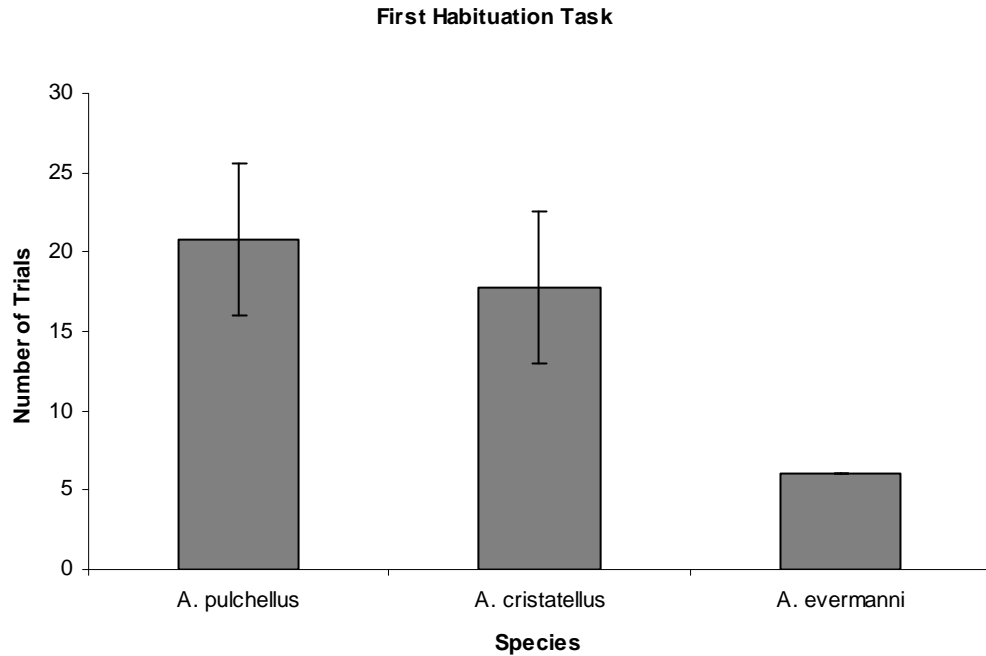


Figure 13: Performance in first habituation step by three *Anolis* species as a measure of neophobia ($n_{\text{pulchellus}} = 6$, $n_{\text{cristatellus}} = 5$, $n_{\text{evermanni}} = 6$).

5.4 Discussion

All the species exhibited associative learning and the ability to learn a motor task. However, the number of individuals that were able to do so differed dramatically between the species. Furthermore, the three species studied demonstrated pronounced differences in their ability to learn both motor and reversal tasks. We also found evidence that performance in one cognitive task is not predictive of performance in the other. Finally, we found interspecific differences in neophobia, a non-cognitive behavioral trait, in the first habituation step, though not in the third habituation step, which may contribute to some of the observed differences in cognitive abilities (Daly et

al. 1982, Seferta et al. 2001 Webster and Lefebvre 2001, Day et al. 2003, Boogert et al. 2006).

The motor task most profoundly demonstrated the differences in learning between these species. The foraging strike of *Anolis* is generally considered to be a highly stereotyped behavior (Losos 2010), and the interspecific differences in learning the motor task may indicate that it is very difficult for anoles to modify their striking behavior. *Anolis evermanni* learned the task quickly in all individuals presented with it. *Anolis pulchellus* also learned the motor task in all individuals presented with it, but they required more trials than *A. evermanni* to learn the task. Finally, only a single *A. cristatellus* was able to learn the motor task; somewhat paradoxically, the single individual that learned the motor task required even less trials than the average for *A. evermanni*. *Anolis evermanni* performed distinctly better at this task than *A. pulchellus*, but the performance of *A. cristatellus* is more difficult to interpret. At least one individual performed just as well as *A. evermanni*, but this individual may be exceptional for *A. cristatellus*.

The first reversal task demonstrates more straight-forward differences in the learning abilities of these species. Once again, *A. evermanni* performed the best at this task, learning it relatively quickly (mean number of trials \pm standard deviation = 23.75 ± 12.45) in the highest proportion (80%) of individuals. A lower proportion of the *A. pulchellus* individuals presented with the reversal task learned to complete it (50%), and

they did so just as quickly (mean number of trials \pm standard deviation = 21.5 ± 3.54) as the *A. evermanni*. The single *A. cristatellus* presented with the reversal task took more trials to learn it than the other species.

Taken together, the performance of these species indicates that ability to learn the motor task was not predictive of ability to solve the reversal task. While *A. evermanni* performed well in both tasks, *A. pulchellus* learned the motor task slowly but learned the reversal task as quickly as *A. evermanni*. Conversely, the solitary *A. cristatellus* that performed both tasks learned the motor task quickly but the reversal task more slowly. This lack of correlation in performance across different cognitive tasks within a single species has been shown across other tasks in several taxa (Boogert et al. 2010, Boogert et al. 2011), and suggests that distinct cognitive processes underlie each type of learning.

Anolis evermanni stands out from the other species as performing well in both the motor and reversal tasks. This may be related to its very low level of neophobia compared to *A. pulchellus* and *A. cristatellus*. All six *A. evermanni* completed the first habituation step in the minimum possible number of trials (six). Given *A. evermanni*'s preference for structurally complex habitats (see chapter 4), this is congruent with findings that species occupying complex habitats display high levels of exploratory behavior (Mettke-Hofmann et al. 2002). Studies have demonstrated that, both within and between species, individuals with lower levels of neophobia tend to perform better at problem solving tasks (Webster and Lefebvre 2001, Day et al. 2003, Boogert et al. 2006)

and learn more quickly (Daly et al. 1982, Seferta et al. 2001). In this case, *A. evermanni*'s higher level of performance across both tasks may be a result of its lower level of neophobia and increased exploratory behavior. However, their performance is unlikely to be totally explained by their lower neophobia, as *A. pulchellus* and *A. cristatellus* displayed equal levels of neophobia but differing ability to perform each task. In addition, all three species showed low levels of neophobia in the third habituation step, which further suggests that interspecific differences in neophobia are insufficient to explain species differences in behavioral flexibility.

The performance of *A. cristatellus* as a species is somewhat puzzling for various reasons. While a single *A. cristatellus* performed well in the motor task and performed two reversals, albeit relatively slowly, the remaining six were unable to perform either task. In fact, four *A. cristatellus* were never able to progress through the habituation period and two of those never completed the first habituation step, requiring only that they approach the apparatus and consume a live larva. The most straightforward explanation for this is that *A. cristatellus* exhibits a high level of neophobia. The large proportion (57%) of individuals unable to complete the habituation steps suggests that *A. cristatellus* is the most neophobic species in the present study. Another possibility is that *A. cristatellus* is the least behaviorally flexible of the species tested in this study, with the single lizard able to perform the motor and reversal tasks representing an exceptional individual. Both of these explanations are interesting, as *A. cristatellus* is the

only invasive species included in this study (Eales et al. 2010). Widely dispersed species as well as successfully invasive species generally demonstrate low levels of neophobia (Greenberg 1989, 1992) and high levels of behavioral flexibility (Sol et al. 2005a, Amiel et al. 2011). Finally, the tasks presented here may not have suitably motivated *A. cristatellus*, resulting in its poor performance even in the undemanding habituation steps. This last explanation seems unlikely given *A. cristatellus*' similar prey preferences and foraging behavior to the other species.

Regardless of the underlying reason for the performance of *A. cristatellus*, the large variability in performance across individuals of *A. cristatellus* highlights the potential pitfalls of small sample size in cognitive studies. Due to the time-consuming nature of many experiments evaluating cognitive abilities, the number of individuals examined is often quite small (e.g. Pepperberg 1991, Wilkinson et al. 2010, Gaalema 2011 and references therein). In many cases, it is typical to have only one or two individuals per species. In the case of species like *A. evermanni* where the majority of individuals seem relatively flexible, limited sample size may not be a major concern. However, the interpretation of behavioral flexibility in a species like *A. cristatellus*, with individuals varying greatly in their ability to perform a single task, is heavily dependent on the individuals sampled. Smaller sample sizes could result in *A. cristatellus* appearing to be totally incapable of learning any of the tasks presented here or appearing to learn the motor task in the fewest trials of any species studied here. Behavioral traits, including

cognitive ones, have the capacity to vary just as much as any other trait; therefore, studies should evaluate multiple individuals from each species in order to gain some measure of the variance within the species (Brodin and Bolhuis 2008).

Serial reversals are widely considered to be a difficult cognitive task, with the most intelligent species across a wide variety of taxa requiring decreasing numbers of trials to complete successive reversals (Bitterman 1965a, Bitterman 1965b, Burghardt 1977, Day et al. 1999). Several species of lizards have demonstrated the ability to improve over the course of serial reversal tasks, including both spatial and feature reversals (Burghardt 1977, Day et al 1999, Gaalema 2011). However, none of the individuals in this study capable of performing multiple reversals exhibited progressive improvement. Even *A. evermanni*, the only species in the present study able to perform five successive reversals, required a relatively stable number of trials to complete all serial reversals. The only exception is *A. cristatellus*, where a single individual required fewer trials in the second reversal than the first. However, this may be an artifact of the relatively large number of trials (the most trials of any lizard on any reversal task) required for this individual to solve the first reversal task. As this lizard performed only two reversals, it is impossible to verify that it shows consistent improvement in serial reversal tasks. The failure of these three species to improve across multiple serial reversals is in contrast to *A. carolinensis*, the only other anole reported to have completed serial reversals, which demonstrated improvement in a spatial reversal task across 5

serial reversals (Norton et al. 1976, cited in Burghardt 1977). This difference may be explained by the fact that spatial reversal is an easier cognitive task than feature-based reversal (Bitterman 1965a, Bitterman 1965b, Day et al. 2002). Therefore, *A. evermanni* may exhibit improvement across successive spatial reversal tasks. It is also possible that five serial reversals were insufficient for *A. evermanni* to learn the task and demonstrate improvement, but that additional reversals may show progressive improvement.

In summary, species of *Anolis* occupying different habitat types exhibit differences in their ability to solve motor and reversal tasks. Although all species were able to learn both the motor and reversal tasks, the grass-bush species required more trials to solve the motor task and the trunk-ground species required the highest number of trials to learn the reversal task. Contrary to expectations, none of the species demonstrated consistent progressive improvement across serial reversal tasks. Future research should present comparable tasks to additional species of each ecomorph in order to determine if species of a given ecomorph show convergent levels of behavioral flexibility. Further, *A. evermanni* should be presented with a spatial reversal task in order to determine if the difficulty of reversal learning in anoles differs between spatial and feature-based tasks.

5.5 Overall Conclusions and Future Directions

Due to the close, mechanistic connection between neuroanatomy and behavior, the relationship between the two has been widely studied. The three species examined

here differed in their ability to solve cognitive tasks, suggesting differences in their levels of behavioral flexibility. However, the relative sizes of structures within their brains did not differ (Chapter 3). Brain structure volume is the coarsest measure of neuroanatomy. Smaller scale neuroanatomical measurements, such as neuron number or neural connectivity, would likely discover differences among the species related to behavioral flexibility. Areas exhibiting positive allometry, such as the dorsal and dorsomedial cortices, as well as the entire telencephalon, provide promising subjects for future studies of *Anolis* neuroanatomy.

Contrary to the relative sizes of brain structures, interspecific differences in habitat complexity are potentially linked to interspecific differences in behavioral flexibility. *Anolis evermanni* performed as well as or better than the other species on every task and also was found in the most structurally complex habitat (Chapter 4). In addition, all but one *A. evermanni* were able to perform all tasks up to and including the first reversal. The majority of *A. cristatellus*, which occupy the least structurally complex habitat, were unable to complete the habituation trials and the motor task. In *A. pulchellus*, which prefer habitat of intermediate complexity, an intermediate proportion of individuals were able to perform all tasks up to and including the first reversal. This suggests that habitat complexity is related not only to individual performance but also to the proportion of individuals of each species able to complete the task.

Behavioral flexibility may be linked to a variety of non-cognitive behavioral traits. In the species examined here, it does not appear to be moderated by neophobia, as *A. cristatellus* and *A. pulchellus* demonstrated similar levels of neophobia. Habitat complexity may be related to level of exploratory behavior, another behavioral trait which is related to high degrees of behavioral flexibility (Mettke-Hofmann et al. 2002, Auersberg et al. 2011). Future studies should analyze the relationship between exploratory behavior and behavioral flexibility. The generality of this relationship between habitat complexity and behavioral flexibility should also be determined by analyzing both characteristics in other species of *Anolis* belonging to the same ecomorphs and in terrestrial Iguanid lizards.

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Biography

Brian James Powell was born in Bel Air, MD, USA, on September 20, 1983. He graduated with a Bachelor of Science degree in Animal Sciences from Cornell University, Magna Cum Laude with distinction in research, in 2005. Brian then joined Manuel Leal's laboratory at Vanderbilt University in 2005. Along with the rest of the laboratory, Brian moved to Duke University, where he received the degree of Doctor of Philosophy in Behavioral Ecology in 2012. During that period, he published "Parental behavior in anguid lizards" in *The Brazilian Journal of Herpetology*, "Behavioral flexibility and problem-solving in a tropical lizard" in *Biology Letters*, and "On the flexibility of lizards' cognition: a response to Vasconcelos et al.," also in *Biology Letters*. During his doctoral research, Brian received the Animal Behavior Society Student Research Grant, the Sigma Xi Sally Hughes Schrader Travel Grant, the Duke University Center for Caribbean and Latin American Studies Travel Grant, the Sigma Xi Grant-in-Aid, and the Duke University Department of Biology Grant-in-Aid. Brian is a member of Sigma Xi, the J.B. Johnston Club, the Animal Behavior Society, and the North Carolina Herpetology Society.