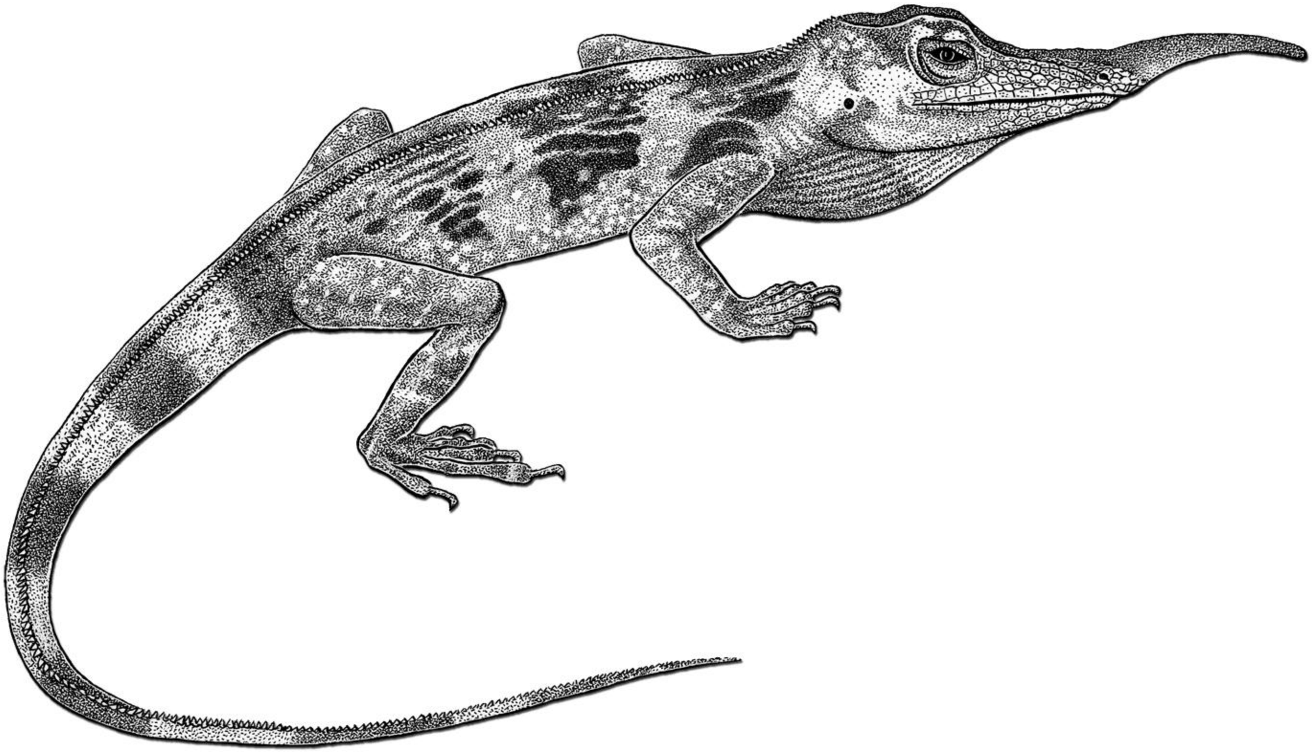
Anolis



Newsletter VI

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June 2, 2010

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Front cover: The enigmatic, rostrally-endowed *Anolis proboscis*, from Ecuador. Reprinted with permission from Williams (1979; Breviora 449:1-19). Illustration by Laszlo Meszoly.

In Memory of A. Stanley Rand (1932-2005)

Stan Rand (left) with his former graduate advisor, Ernest Williams (right) at Soroa, Cuba in 1983.

Preface

On the first weekend of October in 2009, 125 anole biologists traveled from eight countries to Harvard University's Museum of Comparative Zoology to attend the 6th *Anolis* Symposium. It had been 10 years since the previous symposium, and a reunion was long past due. In 2008, as we began to consider how to proceed with such an endeavor, a fortunate thing happened: the Herpetology Department at the MCZ renovated its library and teaching space – the famous lair of the late *pater anolis*, Ernest Williams. The library needed a namesake, and Ernest was under strong consideration (after all, he had been instrumental in filling its shelves!). After a brief period of friendly deliberation, it was decided that the library would be dedicated to Williams, and that the occasion would be the commencement of the 6th *Anolis* Symposium, held at the Museum of Comparative Zoology.

Anole biology has changed considerably in the last decade, and it's been for the better! First and foremost, the field has grown explosively. The number of researchers, the variety of research disciplines, and the number of countries in which anole research is being conducted have all grown considerably in recent years. With nearly 400 named species, an extensive neotropical distribution, and an unmatched diversity of ecologies and morphologies, *Anolis* is well equipped for the increased attention.

Also though, *Anolis* research has blossomed in exciting new directions. Perhaps of greatest note, the genome of *Anolis carolinensis* was recently sequenced, making the Green Anole a genetic model organism and opening the doors for an astounding variety of genetic, developmental, morphological, and evolutionary studies. Perhaps less conspicuously, advances in anole husbandry have enabled a wave of experimental and developmental studies. New tools and techniques have also taken studies of anole functional morphology to new levels of rigor.

While such advances are pushing anole research in new directions, natural history remains at the heart of anole biology. From the surprising observation that anoles can navigate back to their territories from great distances (see contribution by Leal) to the discovery that mainland anole toe pads are less effective for clinging than toe pads in island anoles (see contribution by Crandell et al.), careful study of the basic ecology and biology of anoles continues to form the foundation for top notch science among anole biologists.

In the midst of a decade of progress, we nonetheless bid farewell to one of our most loved colleagues since the last newsletter – Stan Rand. One of Ernest Williams' first students, Rand made a lasting mark with his studies of anoles before blazing trails in numerous other areas of tropical biology and animal communication. Among other things, Stan helped pioneer the ecomorph concept, was among the first to quantify habitat partitioning in anole communities (e.g., the "Rand census"), and carried out early studies of the role of the dewlap in communication. Stan shaped the careers of countless scientists and will be missed tremendously – we dedicate this newsletter to his memory.[1]

As with previous editions, this newsletter is an informal forum for the presentation of data and discussion of theory relevant to anoles. It serves three functions: to allow investigators to inform others of their current and future research; to provide an outlet for speculation and theoretical musings perhaps inappropriate for publication in more formal venues; and to give an opportunity to present data and ideas that otherwise might never be distributed. As with previous newsletters, there is a general request that nothing said herein be quoted without the authors' express permission.

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Cambridge, MA
June 2, 2010

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[1] The previous newsletter (# V) was dedicated to the memory of Ernest Williams.

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Geographic distribution, habitat use, and diet of *Anolis imias* on the Southern Coast of Guantanamo, Cuba

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Abstract.

Anolis imias is a local endemic species, considered Vulnerable.The species is reported from the semiarid south coastline of Guantanamo Province, up to the terraces at Punta de Maisi. There have been few studies of the ecology of this species, with the exception of previous reports of saurophagy. For the first time, we here provide information on the geographic distribution, microhabitat preferences, and feeding ecology of *A. imias*. The species geographical distribution was expanded to nine locations within the semiarid Guantanamo region, and to elevations up to 500m asl. Both sexes preferred rocky substrates, but also utilized tree trunks. The perch height and perch diameter means are 0.94m and 0.36m, respectively. The species is primarily insectivorous, with a diet is composed largely of Hymenoptera (~45%) and Coleoptera (~20%).

Key words: *Anolis imias*, geographic distribution, microhabitat, diet, southern coast, Cuba.

Introduction

The genus *Anolis* Daudin, 1802, has approximately 350 species (Nicholson *et al*. 2005), of which 63 species are reported in Cuba. This represents the largest number of species for a single country (Powell *et al*., 1996; Rodríguez Schettino and Rivalta González, 2003; Navarro and Garrido, 2004). Similar environmental conditions in the macro and microhabitat, climate, and altitude determine the ecogeographic distribution of lizards in Cuba. Most species are endemic (95.2%) and philopatric, and therefore may be considered reliable zoogeographic indicators (Rodriguez-Schettino, 1993).

Habitat selection plays an important role in the evolution, ecology, and behavior of *Anolis* lizards. Differences in microhabitat preference may directly influence the evolutionary dynamics of populations (Morris, 2003). Habitat choice may also have significant effects on foraging behavior and reproduction (Steele, 1993; Doligez *et al*., 2002). Lizards of the Caribbean are an example of adaptive radiation (Schluter, 2000). On the different islands of the Greater Antilles, morphologically similar species (ecomorphs) have originated independently under similar ecological conditions (Losos 1992, 1994). *Anolis* lizards are a model for studies of habitat choice and resource partitioning (Johnson *et al*., 2006). Regardless of the geographical and altitudinal distribution, not all species use the same type of substrate in each community. On the other hand, species are separated vertically on the type of substrate on which they are located (Rodríguez Schettino, 1999a).

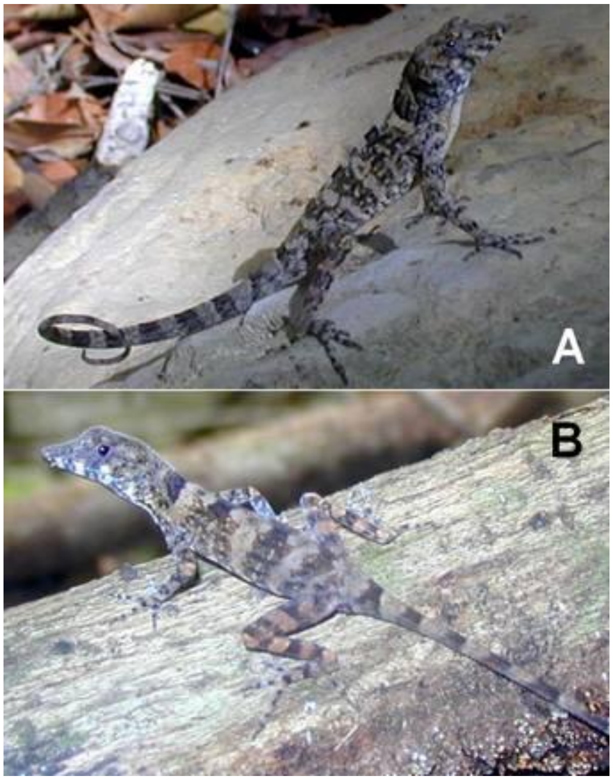

Fig. 1 (A). Adult male *Anolis imias* on a rock. (B). Adult female A. imias on a trunk. Both photographed on the semiarid coast of Guantanamo Province, Cuba.

Ecologists and evolutionary biologists have recently focused on studies of how organisms use their resources and how they have adapted to their environments (Arnold 1983; Huey and Bennett 1986; Garland and Losos, 1994). *Anolis imias* (Fig. 1) is a locally endemic species, considered Vulnerable (Rodríguez Schettino, 1999d), and its ecological habits remain little known. This study extends the geographic distribution of this vulnerable species, provides data on microhabitat preferences and reports new data on diet.

Materials and Methods

Field Study Area

The south coastline of Guantanamo (semiarid region) is located in eastern Cuba, in the Sagua-Baracoa subregion (Núñez *et al.*, 1989) and spans 67999 ha. The vegetation is xeromorphic, with microphylous, thorny elements, and an abundant of succulents - typical given the prevailing climatic and soil conditions. The shadow effect produced by Sierra de Imías causes a semi-arid local climate with the lowest precipitation levels in Cuba, ranging between 600 and 800 mm annually. The vegetation is characterized by a mosaic of forest and shrubs that vary according to soil composition, exposure and altitude. Coastal xeromorphic scrub and dry microphyllous forests are dominant (Capote and Berazaín, 1984; Reyes, 2006).

The coastal hyperxeromorphic semidesert scrub is considered an exclusive feature of this region (Borhidi, 1991; Reyes, 2006). For our study, we divided the macrohabitat into: 1- marine coastal terraces (2, 3 and 4ta); 2- premountains; 3- low mountains; and 4, small flat mountains, following Portela *et al*. (1989).

We accepted the geomorphological classification of Portela *et al*. (1989) in which the study area has structural tectonic rocks (in terraces), abrasive flatlands and hills, and karstic block mountains (h = 200-300 m). To the North, there are flat mountains (~ h = 400-500 m) and block mountains (~ h = 500-750 m).

Geographical distribution

The distribution of the species was previously revised in the literature (Ruibal and Williams, 1961; Schwartz, 1968; Garrido and Jaume, 1984; Schwartz and Henderson, 1991; Rodríguez Schettino, 1999b; Fong, 2000). The main Cuban collections were reviewed: Instituto de Ecología y Sistemática (CZACC), Museo Nacional de Historia Natural de Cuba (MNHNCu), Centro Oriental de Biodiversidad y Ecosistemas (BSC.H) to generate a matrix of the distribution of the species. Nine locations visited by Centro de Aplicaciones Tecnológicas para el Desarrollo Sostenible (CATEDES) researchers were included in the survey. We generated a distribution map using Mapinfo Professional v 8.5.

Habitat

In each location, microhabitat structure was analyzed according to the substrate, perch height and perch diameter where individual specimens were first spotted. All measurements were taken with a diametric tape with precision of 0.05 mm. The observations were conducted between April-May 2008 and February 2009, between 0700-1400h. All days were sunny with temperatures ranging between 30-32 °C.

Diet

We collected eight adult *Anolis imias* (five males and three females), dissected their stomachs, and preserved them through immersion in 70% alcohol for 15 days. The components of the diet were identified in the laboratory under a stereoscopic microscope (Novel XSZ-N207) to the taxonomic level of Order because of partial digestion.

Results

Geographic Distribution

The distribution of the species corresponds to the coastal line from Tortuguilla at the West, to Sierra del Purial in the North, and as far as La Asunción to the East (Fig. 2), and is restricted to rocky areas and xerophytic vegetation. In total, 19 localities were recognized for the species, which includes the reports for the southern coast. The species was restricted in different microhabitats along the entire southern coast (Fig. 3). Based on the localities visited, one can observe *Anolis imias* from sea level to elevations of 500m asl.

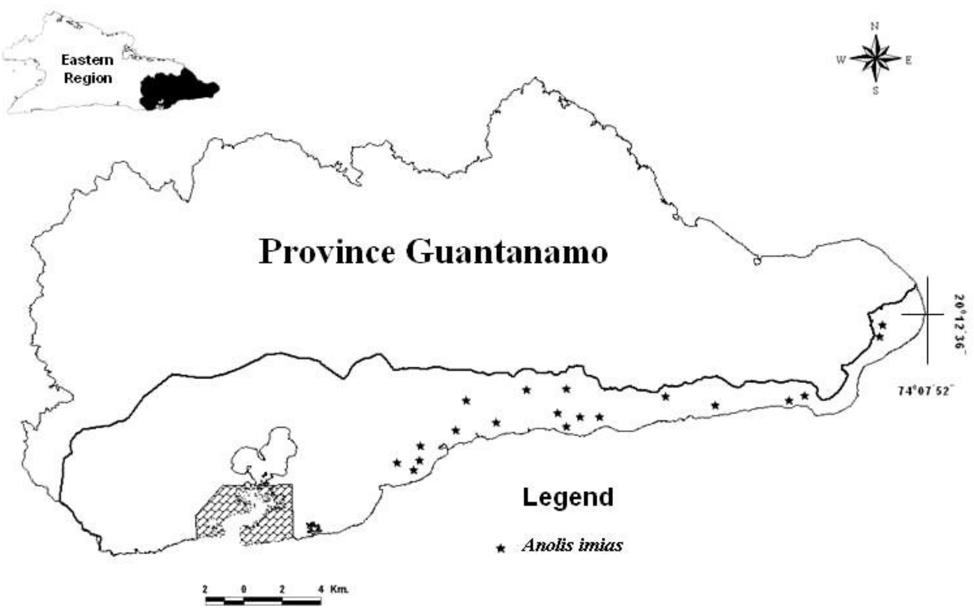

Fig. 2. Geographic distribution of *Anolis imias* on the southern coast of Guantanamo province, Cuba. A bold line demarks the upper boundary of the semiarid region of Guantanamo.

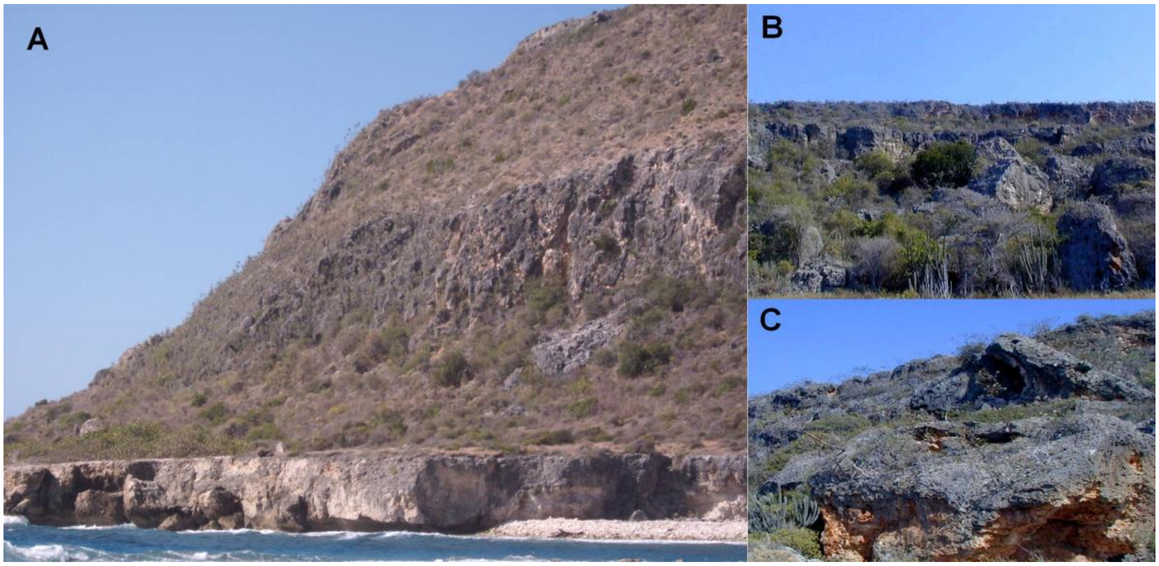

Fig. 3 Macrohabitat in which Anolis imias was found in the semiarid coast of Guantánamo province, Cuba. A: coastal terraces of Bahía de Baitiquírí; B and C: coastal terraces from Tortuguilla to Yacabo Abajo.

Ruibal and Williams (1961) identified Imías, Oriente as the type locality of this species. Schwartz (1968) reports it from the northern mountains of Imías (Sierra del Purial). However, Garrido and Jaume (1984) collected specimens 4.5km west Baitiquirí and east Imías, and suggested that it may also be found in the coastal terraces of Maisí. Rodriguez-Schettino (1999b) states that the species is restricted to the southern coast of Guantanamo (3.5km east of

Tortuguilla). Fong (2000) collected juveniles in dead agave plants around Punta Negra, 22km east of Jauco, Maisi. Other specimens collected in the cliffs of Tacre river and Yacabo Abajo are deposited in the Collection of the National Museum of Natural History of Cuba; others from Baitiquirí, and between Asuncion and Cantillo, Maisi, are deposited in the zoological collection of the Academy of Sciences of Cuba (CZACC).

Habitat

During the survey, 22 individuals of *Anolis imias* (16 males and 6 females) were observed. For both sexes, rocks were preferentially used as substrate (Fig. 4) and dead agave (*Agave albescens* and *A. underwoodii*) as refuge. The use of trunks was similar between sexes. The mean perch height and perch diameter in which the individuals were observed were 0.94 m and 0.36 m, respectively (Table 1).

Fig. 4. Substrate used by *Anolis imias* on southern coast of Guatanamo. T: trunk, R: rock.

Table 1. Descriptive statistics of perch height and perch diameter for males and females of *Anolis imias* in the southeastern coast of Guantanamo Province, Cuba. N = number of individuals; X = mean; SD = standard deviation. All measurments are in mm.

Perch Height	
Males (N = 16)	**Females** (N = 6)
X ± SD (Min.-Max.)	X ± SD (Min-Max.)
92,1± 41,71 (45-214)	95,95 ± 44,02 (53-79)
Perch Diameter	
36,54 ± 39,38 (3,1-149)	36,76± 40,02 (2,5-70)

Diet

In the eight stomachs analyzed, 208 items were found, of which 10 different kinds of food were identified (Table 2). Diets consisted primarily of insects (six orders), with high consumption of hymenopterans in both sexes. The second most common prey was beetles, followed by insect larvae. To a lesser extent, insect larvae of various orders and spider articulations were found. In the stomachs of three males, the remains of lizard skin and a tail of *Anolis imias* were identified.

Table 2. Diet composition of males and females of *Anolis imías* in the southeastern coast of Guantánamo Province, Cuba. N = number of prey ítems; % = percentage of every item in relation to the total number of ítems; in brackets n = number of stomachs analyzed.

Items	Males (n = 5)		Females (n = 3)		Total (n = 8)	
INSECTA	N	%	N	%	N	%
Hymenoptera	57.0	42.9	36.0	48.0	93.0	44.8
Coleoptera	26.0	19.5	15.0	20.0	41.0	19.8
Lepidoptera	8.00	6.01	2.00	2.70	10.0	4.82
Isoptera	1.00	0.75	0.00	0.00	1.00	0.48
Hemiptera	2.00	1.50	1.00	1.33	3.00	1.44
Orthoptera	1.00	0.75	0.00	0.00	1.00	0.48
Insect larvae	19.0	14.2	8.00	10.7	27.0	13.0
Non identified material	14.0	10.5	12.0	16.0	26.0	12.1
ARACHNIDA						
Araneae	2.00	1.50	1.00	1.33	3.0	1.44
SAURIA (Cannibalism)						
Anolis (Remains)	3.00	2.26	0.00	0.00	3.00	1.44
TOTAL	**133**	**100**	**75.0**	**100**	**208**	**100**

Discussion

Geographical Distribution

It was possible to improve the knowledge on the distribution of the species, adding to the work of Ruibal and Williams (1961), Schwartz (1968), Garrido and Jaume (1984), Rodríguez Schettino (1999b) and Fong (2000). Other reports were from specimens in the zoological collections of the country. With our work, the altitudinal range of the species was modified after Rodríguez Schettino (1999a) who included the species in group I (species that only live in flatlands). For that reason, we consider that the species can be included in group II (species that live in flatlands and mountains), the same as stated by Rodriguez Schettino *et al.* (in press).

Habitat

The ecology of *Anolis* lizards in the Greater Antilles (Cuba, Jamaica, Puerto Rico and Hispaniola) are based on how these lizards have specialized in different habitats. Many widespread species occur in numerous environments with variation in vegetation structure. However, previous work has shown these lizards to have evolved adaptations to their environment, and in particular, adaptations for more efficient use of microhabitats (Williams 1983; Losos, 1990). For example, species which typically perch low on tree trunks have long limbs and use the foraging strategy of sit-and-wait, whereas species that perch on twigs or in small shrubs have shorter limbs, and frequently change perches to forage. The preference of rocks for substrate by *Anolis imias* may be an adaptation to the high temperature of its habitat; the lizards also use the rock cavities as protection while the sun is too hot.

Habitat selection patterns are best determined at small spatial scales, such as individual choices in microhabitat conditions – e.g., humidity, temperature, light - (Rodriguez-Robles *et al.*, 2005). *Anolis imias* is a species that has adapted to severe climatic conditions (drought and aridity). Theoretical models predict that individuals choose microhabitats in order to increase and maximize their likelihood for survival and reproduction (Cody 1985, Orians and Wittenberger, 1991). This type of distribution in which both sexes were found allows us to explain a possible structural segregation, although because of the low number of individuals, we could not reach a conclusion on the altitudinal preference that the species might have.

Diet

The diet of this species agree with studies done by other authors (Sampedro *et al.*, 1979, Schoener *et al.*, 1982, Floyd and Jenssen, 1983; Armas, 1987; Martinez Reyes and Rodriguez Schettino, 1994; Fong and Garcés, 2002; Muñoz *et al.*, 2008) who encountered that the preferred food for other species of lizards were ants. Armas (1987), Martinez and Fernandez (1994) reported other similar eating behaviours of terrestrial species of lizards.

Saurophagy and cannibalism are rare events in Cuban reptile diets, but in general, occur in species of snout-vent length greater than 60 mm (Rodriguez Schettino, 1999a). The first has been found in species such as *Anolis vermiculatus, A. porcatus*, *A. allogus*, *A. imias*, *A. equestris,* and *A. luteogularis;* on the other hand, for *A. porcatus*, *A. lucius*, *A. quadriocellifer,* and *A. imias* there is evidence of cannibalism (Socarrás *et al.* 1988; Rodríguez Schettino *et al.*, 1999). Apparently, in the wild, *Anolis imias* commonly consumes lizards.

Acknowledgments

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Phylogenetic relationships of the *Dactyloa* clade, based on molecular and morphological data

The understanding of phylogenetic relationships within *Anolis* has seen great advances in the past ten years due to a significant number of phylogenetic studies based primarily on molecular data. Despite this large effort, there is still a large void of information regarding mainland species. According to recent phylogenies (Poe, 2004; Nicholson et al., 2005) mainland species belong to two non-nested clades: an unnamed clade (designated the M2 clade by Pinto et al. [2008]), which includes the deeply nested Central and South American species derived from Greater Antillean ancestors and *Dactyloa* (same as the *latifrons* series of Etheridge [1959] and the M1 clade of Pinto et al. [2008]), which represents one of the early branches in *Anolis* and includes species from Central and South America and the Lesser Antilles.

The *Dactyloa* clade is composed of 76 currently recognized species distributed from Costa Rica to Peru, including the Amazon region and the southern Lesser Antilles. Species are distributed from sea level to 3000 m of elevation, with most of them found in well-preserved forested habitats. Within *Dactyloa*, six different subgroups (ranked as species groups by Williams [1976*a*] and series by Savage and Guyer [1989]) have been described based on morphological characters (Williams, 1976*b*): *aequatorialis, laevis, latifrons, punctatus, roquet*, and *tigrinus*. More recently, based on phylogenetic studies (e.g., Jackman et al. 1999; Poe 1998, 2004), the species previously placed in the genus *Phenacosaurus* are considered part of *Dactyloa* as well.

To reconstruct the phylogeny of *Dactyloa,* we analyzed new nucleotide sequence data from one nuclear (RAG-1, ~2900b) and two mitochondrial (ND2, ~1500b; COI, ~700b) gene regions. We included molecular data from 42 *Dactyloa* species from Panama, Colombia, Ecuador, Venezuela and the southern Lesser Antilles, as well as 10 outgroup species (3 non-*Anolis* Polychrotinae and 7 non-*Dactyloa Anolis*).

Likelihood and Bayesian (under different data partitions) analyses of each gene region separately, both mitochondrial regions combined, and all three regions combined strongly support *Dactyloa* as a monophyletic group; topology tests (Approximately Unbiased Test and

Bayesian hypothesis tests) further support its monophyly. In addition, data provide strong support–with further support from topology tests–for the monophyly of the *roquet* series of the southern Lesser Antilles. In contrast, the *punctatus, latifrons* and *aequatorialis* series, previously circumscribed based on morphological characters, were not inferred in the optimal trees and their monophyly was rejected by topology tests as well. *Phenacosaurus* species were not inferred as a monophyletic group, though a group composed of all *Phenacosaurus* species except *A. neblininus* was inferred as a clade; topology tests failed to reject the hypothesis of monophyly of this group.

Molecular data were combined with morphological data from 60 species of *Dactyloa* and 6 outgroups species (including non-*Anolis* Polychrotinae and non-*Dactyloa Anolis*). Sixty-six characters: 33 of external morphology and 33 of osteology were analyzed using parsimony and Bayesian methods. For the parsimony analysis, continuous characters were coded using Torres-Carvajal's (2007) modified step-matrix gap weighting method and polymorphic characters were coded using the frequency parsimony method of Berlocher and Swofford (1997). For the Bayesian analysis, continuous characters were coded using Thiele's (1993) gap weighting method and polymorphic characters were coded using the modal condition. Parsimony and Bayesian analyses of the combined dataset inferred *Dactyloa* to be monophyletic, though nodal support was low (bootstrap = 46%, Bayesian posterior probability = 0.80). In agreement with the analysis of the molecular data alone, the *roquet* series was the only subgroup (from those previously described based on morphological characters) inferred with the combined dataset; topology tests further support its monophyly. In addition, topology tests rejected the monophyly of *aequatorialis*, *latifrons* and *punctatus* series, though it failed to reject the monophyly of the *tigrinus* series and the *Phenacosaurus* subgroup.

In addition to the *roquet* series, four other major clades with distinct and coherent geographic ranges were consistently inferred across analyses of the molecular data alone and in combination with morphology: first, a western clade that includes 10 species distributed in the western and central cordilleras of Colombia, the western slope of the Ecuadorian Andes and the Pacific lowlands of Colombia and Ecuador; second, a clade of 13 species for which all males reach a snout to vent length larger than 100 mm, mostly distributed below 1000m of elevation in the pacific lowlands from Costa Rica to Ecuador, Malpelo island and the inter-Andean valleys of Colombia; third, an eastern clade including 12 species distributed in the Amazon region, the northern portion of the eastern cordillera of Colombia, the Sierra Nevada de Santa Marta (Colombia) and the Venezuelan Andes; finally, a clade containing 6 species previously placed in the genus *Phenacosaurus* distributed in high elevations (between 1300-3000m) of the Andes of Colombia, Venezuela and Ecuador.

These five major clades were inferred with strong support with the molecular data alone, and with strong to weak support with the combined dataset. However, the relationships among them remain uncertain as different analyses and/or gene regions inferred different patterns with a wide range of nodal support values.

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Miscellaneous notes on some rare and unusual anoles

In the fall of 2004, as a doctoral student at the University of California, Los Angeles, I began a study of the *trans*-Andean α-anoles (*Dactyloa* spp.) of Central and South America. During the course of my research, I made a few miscellaneous notes on some rare and unusual mainland dactyloids and β-anoles (*Norops* spp.) of Panamá and Ecuador.

Dactyloa purpurescens (Cope) 1899.—**Panamá.** This species is presently known only from the holotype, USNM 4321 (Fig. 1). The specimen was collected during a survey expedition for a proposed ship canal connecting the Atlantic and Pacific Oceans via the valley of the Atrato, in Colombia (Michler, 1861).

Fig. 1. *Dactyloa purpurescens* holotype, ♂, SVL 77 mm (USNM 4321). Río Truando, Departamento de Chocó, Colombia.

USNM 4321 was previously listed as "*Anolis ??reticulatus*" by Cope (1862) and identified as a "♂ jr.," by which Cope presumably meant a juvenile male. Williams (1988) concurred with this, although there is nothing in the proportions of the specimen to suggest it is a subadult. Cope lists a second specimen, USNM 4313, an adult male from Turbo on the coast of the Golfo de Uraba, Colombia. This specimen could not be found by Smithsonian personnel and may be lost (T. Hartsell, pers. comm.). A third specimen, USNM 4318, also from Turbo, was later identified as *Dactyloa latifrons*.

As I was preparing for a trip to the Darién in January of 2006, the staff of Ancon Expeditions in Panama City sent me a photograph of an animal that had been found near Cana (Fig. 2). I believe this individual is either close to or may actually represent *D. purpurescens*. The pattern of spots and vermiculations, as well as the light eyelids, is simlar to the holotype.

One night of searching at Cerro Pirre (1200 m) did not yield any specimens of this species.

Fig. 2. *Dactyloa cf. purpurescens*, sex unknown (uncollected). Cerro Pirre, Parque Nacional Darién, Provincia de Darién. Photo courtesy Ancon Expeditions, Panamá.

According to Williams (1988), the status of *D. purpurescens* remains unconfirmed and the name may be a synonym for *D. frenata* (Williams, 1992). This is unlikely, as specimens of the latter usually have a dark spot in front of the forelimb insertion and more prominent spots on the flanks. Also, there are dark bars radiating from the center of the eye in *D. frenata*, and the light eyelids seen in *D. purpurescens* are absent.

Dactyloa purpurescens is morphologically close to *D. chocorum*, which may itself be composed of more than one species (Williams, 1992). A comparison of the holotype of *D. purpurescens* with 13 specimens of *D. chocorum* (including four of the KU paratypes) reveals close similarity in a number of scale characters.

Anolis casildae (Arosemena, Ibañez D., and de Sousa) 1991. **Panamá.**— The original description of *Dactyloa casildae* states that this species may be recognized by its "brilliant green dorsum with solid brown bands slanted antero-posteriorly on each flank", and that this color pattern is "characteristic and unique for the species" (Arosemena et al., 1991). A subadult male exhibiting this pattern was collected in the Reserva Forestal Fortuna (Fig. 3) and has been deposited in the collections of the Museo de Zoología, Universidad de Panamá.

Fig. 3. *Dactyloa casildae*, ♂, SVL unknown (LACM FS 1087). Quebrada Frank, Reserva Forestal Fortuna, Provincia de Chiriquí, Panamá.

In March of 2007, on the loop trail behind the Smithsonian Tropical Research Institute's field station at Fortuna, an adult male *D. casildae* was collected that represents a unique pattern variant for this species (Fig. 4). Rather than the typical dark bands on a light green background, this specimen exhibited a sprinkling of dark brown spots on a lighter brown background. A series of thin, vertical lines appears at regular intervals along the flanks. Details of squamation in this specimen are in general agreement with *D. casildae* (Arosemena et al., 1991), although a closer comparison should be made.

The specimen has been deposited in the Natural History Museum of Los Angeles County, Section of Herpetology.

Fig. 4. *Dactyloa casildae*, ♂, SVL 101 mm (LACM FS 1084). Reserva Forestal Fortuna, Provincia de Chiriquí, Panamá.

Dactyloa fraseri Günther 1859. **Ecuador.**—This species was encountered at Mindo and at the Reserva Ecológica Bilsa in Provincia Esmeraldas. It appears to be strongly arboreal; in both instances the animals were perched from 3-4 meters above the ground. An adult male was collected during the day in the canopy of a small tree, and an adult female was captured at night while sleeping on a leaf.

Miyata (unpubl.) mentions the reddish head typical of adult males of this species (Fig. 7). This is most striking in living specimens and was not included in the redescription by Williams (1966), although it is mentioned in the *Anolis* Handlist (Williams, 1992).

Dactyloa parilis (Williams) 1975. **Ecuador.**— Much time was spent trying to find the type locality of this species, reported as "Rio Baba, 2.4 km S Sto Domingo de los Colorados, Pichincha, Ecuador" (Williams, 1975). As my field companions and I were to discover, however, 2.4 km south of Santo Domingo the Río Baba is nowhere to be seen. It was not until the holotype was in hand that close inspection of the tag revealed the locality as 24, not 2.4, km south of Santo Domingo de los Colorados (Fig 8). It is easy to see how the dot over the second 'i' in the word 'Pichincha' could have been mistaken for a decimal point.

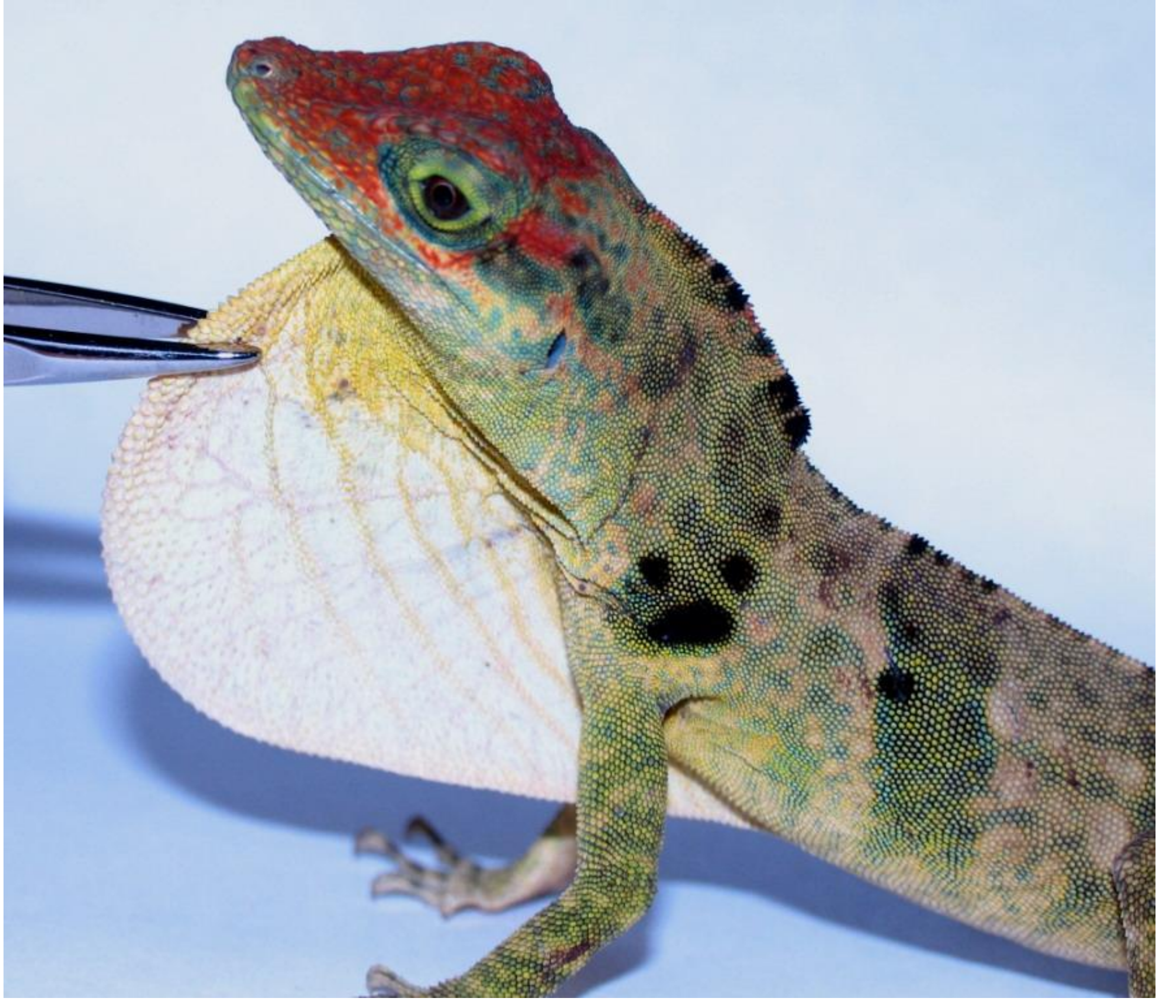

Fig. 7. *Dactyloa fraseri*, ♂, SVL 101 mm (QCAZ 6862). Mindo, Provincia de Pichincha, Ecuador. Photo courtesy of F. Ayala.

Looking at a map, if you take route 25 south out of Santo Domingo for 24 km, you should be somewhere near the settlement of El Esfuerzo, on the opposite bank of the Río Baba. This might be a good place to begin searching for *Dactyloa parilis*.

Norops biporcatus parvauritus (Williams) 1966. **Ecuador.**—This taxon was relatively common at the Tundaloma Hostería, Provincia de Esmeraldas, about 15 min outside the town of San Lorenzo. Here it was sympatric with five other species of anoles: *N. lyra*; *D. chloris*; *D. princeps*; *N. maculiventris*; and *D. festae*. The grounds of this hostería are potentially an ideal study site for investigating the ecology of mainland anoles, as there are a number of readily observed species.

Three adult specimens of *N. b. parvauritus* (QCAZ 6925, 6927-28) were easily collected in mid-afternoon, between 1400 and 1600 hrs. They were perched just 1-2 m above the ground in landscaped areas of low-growing vegetation. Thus, it appears that *N. b. parvauritus* may not always be as strongly arboreal as previously reported (e.g. Miyata, 1977; Williams, 1966).

Fig. 8. *Dactyloa parilis* holotype, ♂, SVL 81 mm (UIMNH 82901). Locality data is clearly seen in this photo.

Color in life was only briefly mentioned by Williams (1966). I have the following notes on QCAZ 6928: "Head bluish. Dewlap small, yellowish orange proximally and reddish distally, with black scales. No blue, but a line of light gray separating dewlap from throat." In contrast, the dewlap of males from more northern parts of the range (e.g. Costa Rica) is mostly powder blue, with white proximally and red-orange distally (Savage, 2002).

The skin of *N. b. parvauritus* is rather fragile and tears easily when the animal is captured, despite careful handling.

Norops lyra (Poe, Velasco, Miyata, and Williams) 2009. **Panamá.**—This species was formally described by Poe et al. (2009) after more than 20 years in obscurity. Its range is given as extending from northwestern Ecuador into the Pacific lowlands of central Colombia.

In February of 2007, an adult male (LACM FS 1083) was collected in the Parque Nacionál Darién, Panamá. This specimen (Fig. 9) has been deposited in the Natural History Museum of Los Angeles County, Section of Herpetology. A second specimen (LACM FS 1082), an adult female from the same area, was deposited in the Museo de Vertebrados at the Universidad de Panamá (Fig. 10). The identification of both specimens should be verified to determine whether or not a range extension is necessary.

Fig. 9. *Norops lyra*, ♂, SVL unknown (LACM FS 1083). Cana Field Station, Provincia de Darién, Panamá.

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Fig. 10. ***Norops lyra*****, ♀, SVL unknown (LACM FS 1082). Cana Field Station, Provincia de Darién, Panamá.**

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Frictional adhesion and toe pad micro-morphology of mainland *Anolis*

Adhesive toe pads are a defining characteristic of the *Anolis* clade, found on all but one described species (Peterson and Williams 1981). Adhesive toe pads may be a key innovation that facilitated occupation of niches unavailable to non-clinging vertebrates (Losos, 2009). The macrostructure of toe pads have been extensively studied, yet little is known about how the pad microstructure, which enables adhesion, contribute to adhesive performance at the organismal level. However, the micromechanics and adhesive performance of the complex, branched adhesive setae of geckos have received considerable attention recently (Autumn 2006).

Anolis toe pads (Fig. 1b) consist of modified subdigital scales termed lamellae (Fig. 1c) that contain setae (Fig. 1d) – microscopic (avg. 20 µm tall) hair-like structures made of beta-keratin (Ruibal and Ernst 1965) that facilitate adhesion. Microscopic spatulae (Fig. 1e) at the tips of the setae interact with the surface. In geckos, adhesion is largely due to intermolecular van der Waals forces (Autumn et al. 2002). Initiation of attachment is reliant on mechanical application to the surface. Setae, lamellae, and whole toes are load dependent and directional; only with correct orientation, preload force, and drag can attachment occur (Autumn 2000). While preliminary research suggests a similar mechanism in *Anolis* (unpublished), the adhesion micromechanics remain untested until now.

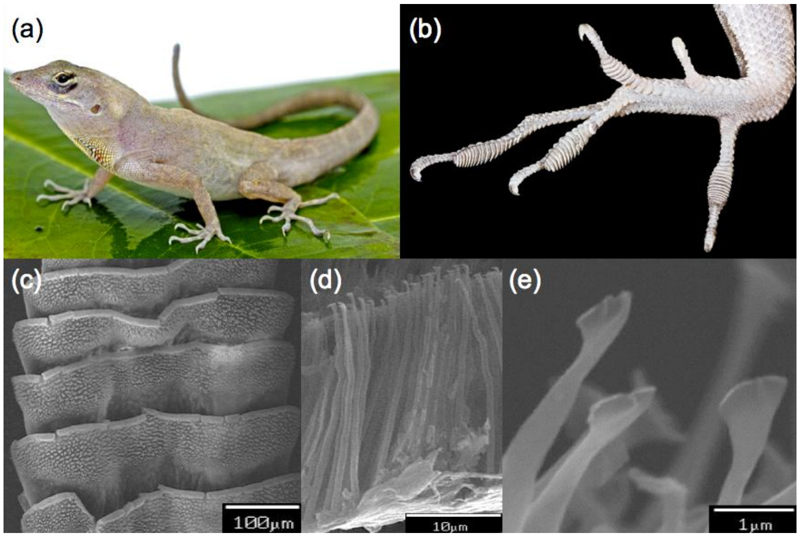

Fig. 1. Anatomy of the *Anolis* toe pad, depicted at increasing levels of detail (see text for explanation).

The specific toe pad microstructure is likely the result of the material requirements of the adhesive, phylogenetic constraint, and adaptation (for review see Autumn 2006; Irschick et al. 2006). Despite considerable variation in morphology of adhesive microstructures (Ruibal and Ernst 1965; Peterson and Williams 1981), a comparison of adhesive structures between *Anolis* species has not been conducted. Models of van der Waals interaction suggest that geometry, including spatula shape, may determine the limits of seta function (Autumn 2006). Spatula shape, seta density and length are important in production of frictional adhesion in geckos (Peattie 2007). Models presented by Campolo et al. (2003) and Sitti and Fearing (2003) suggest that longer setae will be able to conform to rougher surfaces. If we model a single seta as a cantilever beam (as described in Autumn, 2006), the effective stiffness will be inversely proportional to the length squared: longer setae will have lower stiffness. The cantilever model can further be used to examine the angle of the seta to the substrate (Sitti and Fearing, 2003), and ultimately suggests that longer setae, with lower stiffness, may be able to adhere at a lower angle. If this is true, then seta shear force could be greater due to the lower contact angle, and shown by Tian et al. (2006).

In this paper we present preliminary comparative data to examine the consequences of toe pad microstructure on adhesive capabilities in mainland *Anolis* species. To do so, we measured microstructure morphology and organismal-level performance. Specifically, we address the hypothesis that longer setae will allow a higher shear stress (force/area) production.

Methods

In this study we explored adhesive capabilities of 5 *Anolis* species from Costa Rica. The species measured were: *A. capito, A. humilis, A. lemurinus, A. limifrons, A. polylepis,* and *A. cupreus*. On the day of capture in the field, we took shear force measurements using an acetate covered glass microscope slide mounted to a duel-range force sensor (40 Hz; Vernier). I applied the longest hind toe to the acetate sheet, with slight manual preload if necessary. Shear force was recorded as the subject slid at a constant rate until detaching from slide. Each subject underwent three repeated trials for the left and right fourth toes. When necessary, individuals of rare species participated in up to 5 trails. Shear force data was standardized per toe pad area to determine stress (force/area).

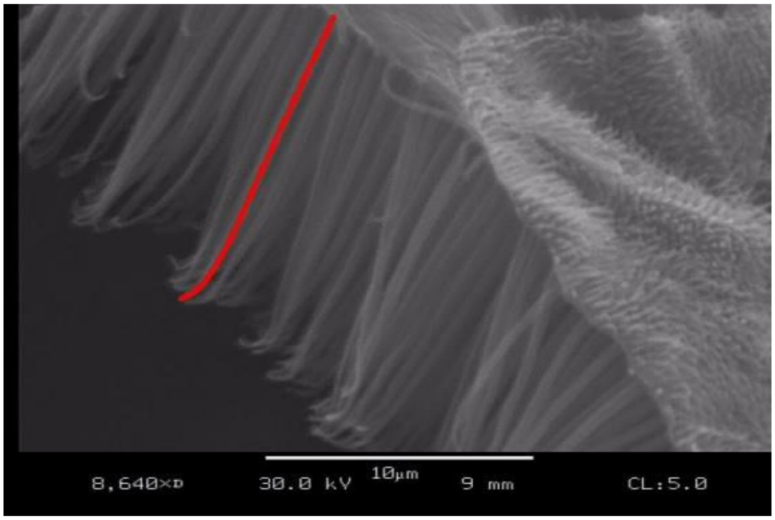

Fig. 2. I measured seta length from images of the proximal tip of the lamella. Setae were traced from the tip of the spatula to the base of the array. The maximum observed value was used in subsequent analysis.

I took a whole toe from individuals in the field post-performance trials. Prior to analyses, toes were stored in 70% ethanol. Using forceps, I isolated and air-dried single lamellae from the center of the preserved pad. I mounted the lamellae on an aluminum SEM stub with LocTite 410 instant adhesive. A Hummer VI sputtering system (Technics) coated all specimens with a thin layer of platinum. I took images of the toe pad microstructure with an Amray 1810 Scanning Electron Microscope (SEM) the day of sputter-coating. Images were digitally recorded with ImageDV (v1.3, Evological), and analyzed using ImageJ (1.40g, Rasband).

I measured seta length from images of the proximal tip of the lamella (see figure). Setae were traced from the tip of the spatula to the base of the array. The maximum observed value was used in subsequent analysis.

We also performed our analysis on independent contrasts (Felsenstien 1985) using PDAP 6.0 (Garland 1999) based on the most complete phylogenetic tree of *Anolis* (Nicholson et al. 2005) to account for potential similarities due to shared ancestry.

Results and Discussion

Microstructure analysis included six mainland anole species from Costa Rica. A strong correlation between body mass and seta length exists (Pearson Correlation = 0.86, P=0.03 [*]). For all subsequent analysis, seta length was log transformed to account for potential non-linear changes of seta length with body size.

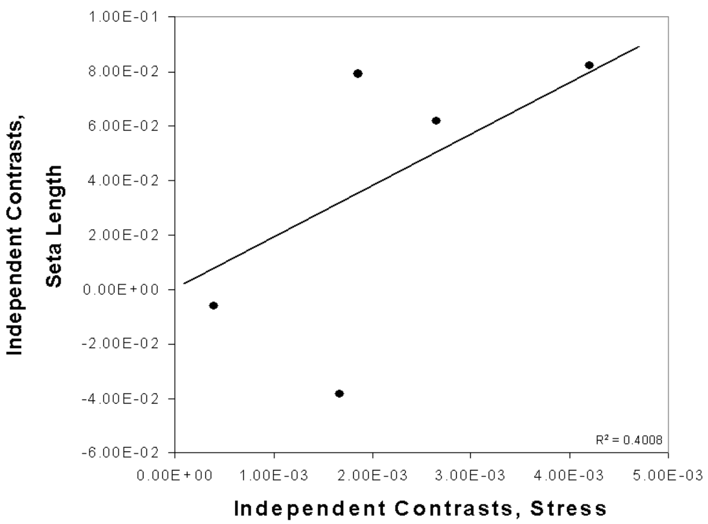

Fig. 3: Relationship between toe pad performance and seta length.

A positive correlation exists between phylogenetically corrected maximum seta length and stress (Fig) (r^2=0.40). However, this relationship is not statistically significant (ANOVA f=1.94, df=1,3, P=0.23 [NS]). Although this correlation supports our hypothesis that microstructure morphology is broadly correlated with performance, further data are needed. In a comparative analysis of single setae of eight gecko species, Peattie (2007) did not find a significant correlation between seta length and shear force in gecko species, but did find a significant correlation between length and width, and width and shear force, likely due to an increase in spatula tips per seta. The microstructure of *Anolis* toe pads, however, allows an analysis of length independent of spatula tip density, because unlike in geckos, each seta has a single spatula. Setae ranged in length from 16 to 30 μm, inconsistent with the hypothesis (Peattie 2007) that *Anolis* are historically constrained from developing longer setae. While the data show a positive correlation between stress and seta length, further analysis of isolated setae and an increase in number of sampled species will be necessary to tease apart the relevance of setal length to attachment force.

Further Research

Data presented here covers one of the relationships between microstructure morphology and adhesive capacity of mainland anoles that we have explored. The varying toe pad microstructure within the *Anolis* clade offers a unique opportunity to test a variety of mechanical models pertaining to fibrillar adhesives, and additional data will allow us to test these models more thoroughly. Further understanding of the *Anolis* adhesive will also allow us to test hypotheses concerning habitat use and mechanical abilities, and will ultimately allow us to test why mainland and Caribbean radiations differ in toe pad structure.

Although the data we report in this paper did not yield a statistically significant result, this is likely due to the small number of species sampled. Over the next year, we hope to increase our sample size by collecting data from additional mainland anole species in Panama.

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Forty hard keratin-associated beta-proteins (beta-keratins) allow the formation of all types of scales, adhesive pads and claws in *Anolis carolinensis*

Introduction

Scales in *Anolis carolinensis* have different consistencies, shapes, pigmentation and dimensions in the various areas of the body (Fig. 1). Furthermore, claws and adhesive pads are present as modifications of scales. The main structural proteins composing the corneous layers of scales, claws and adhersive pads are alpha- and beta-keratins (Maderson et al., 1998). Alpha-keratins of 40-68 kDa differ from beta-keratins in composition, gene structure and other chemical-physical properties. The latter proteins of 12-25 kDa have been recently recognized as equivalent to the keratin-associated proteins of mammalian skin, and are now termed keratin-associated beta-proteins (KAbetaPs) (Alibardi et al., 2007; Toni et al., 2008).

KAbetaPs act like monomers that polymerize into long filaments localized among those of alpha-keratins, and they rapidly coat the latter, forming a hard and mechanically resistant corneous layer in scales (Fig. 2). Also KAbetaPs make up the primary component of avian claws, beaks, and feathers, and the long setae forming the adhesive pads of geckos and *Anolis* lizards.

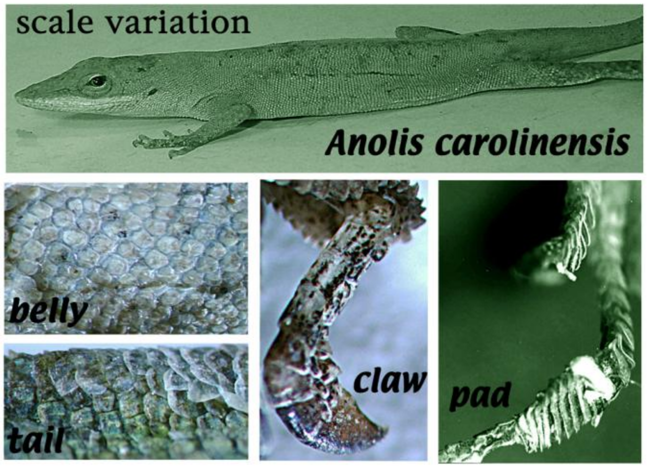

Fig. 1. Examples of scale and other skin appendage variation in *A. carolinensis*.

Recent proteome and genome studies in different reptiles have identified a number of these small proteins, which are responsible for the formation of most of the hard corneous material of reptilian scales (Hallahan et al., 2009; Alibardi et al., 2009). However, we did not previously have any information about the number of genes coding for KAbetaPs (BetaKs) in any reptilian species. After the publication of the first draft genome from a reptile, the

American green anole, *Anolis carolinensis*, by the Broad Institute at MIT and Harvard University in Cambridge, MA, we have initiated this type of analysis. The purpose of the study is the identification of all proteins of the beta-K family that are involved in the formation of scales in this species as a representative of reptiles in general.

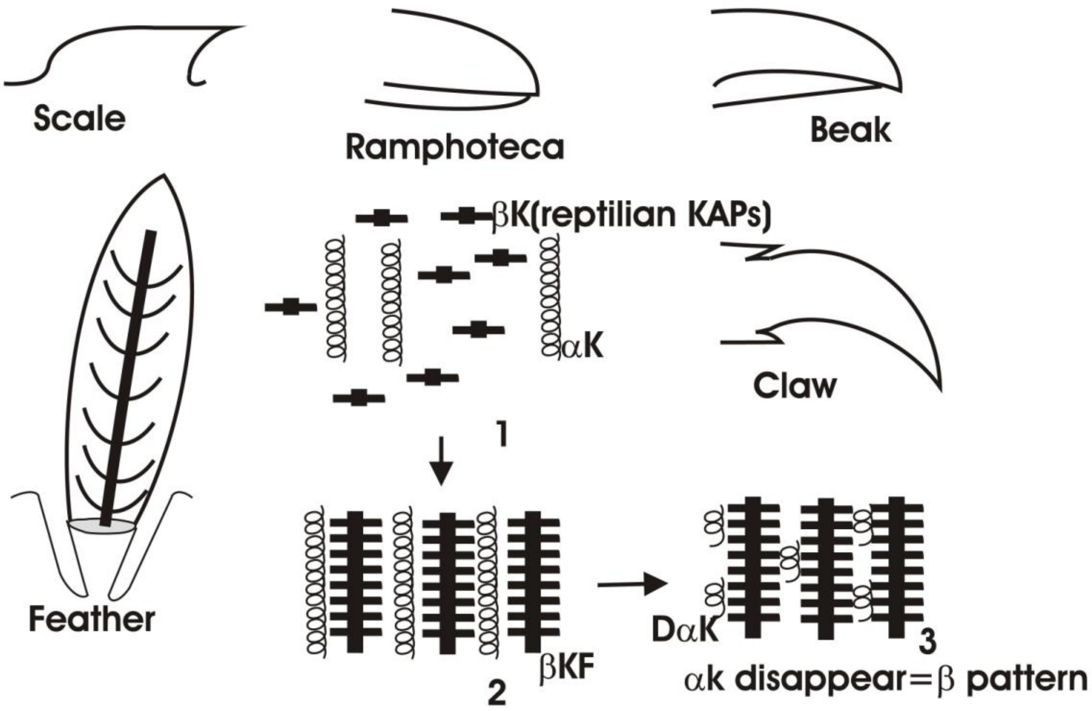

Fig. 2. Schematic drawing showing the process of polymerization of BetaK monomeres (βK) into beta-filaments among alpha-keratins to make the hard corneous material of scales, ramphoteca (beaks), claws, and feathers in birds. In 1, the single monomers aggregate around alpha-keratin filaments (αK, coils). In 2, a beta-keratin polymer (filament) of beta-keratins is formed among alpha-filaments. In 3, alpha-keratin is degraded (DαK) or masked by the prevalent beta-keratins that constitute the hard corneous material.

Materials and Methods

Information from the gene sequences of some lizards has been utilized to probe the recently available genome database for the lizard *Anolis carolinensis* (Broad Institute, Cambridge, MA, USA at UCSC genome browser at www.broad.mit.edu, name G1144).

The study was conducted using bioinformatics methods on the genome of *A. carolinensis* (BLAT program, EST-library analysis), and by RACE-analysis methods on samples derived from organs taken from this species after RNA extraction (see details in Dalla Valle et al., 2010). Using protein prediction programs (Translate tool) we have translated the nucleotide sequences of the coding regions of the different genes into amino acid sequences and then, using the PSIPRED program, we have analyzed the predictions of the secondary structures for these proteins.

Results and Discussion

This analysis has allowed the identification of all of the genes present in this species that code for KAbetaPs. In total, we have identified 40 genes clustered together in scaffold n. 455, which contains 1 065 307 nucleotides) (Fig. 3). The latter figures show that some of these genes are transcribed in opposite directions along the scaffold (see arrow directions in Fig. 3).

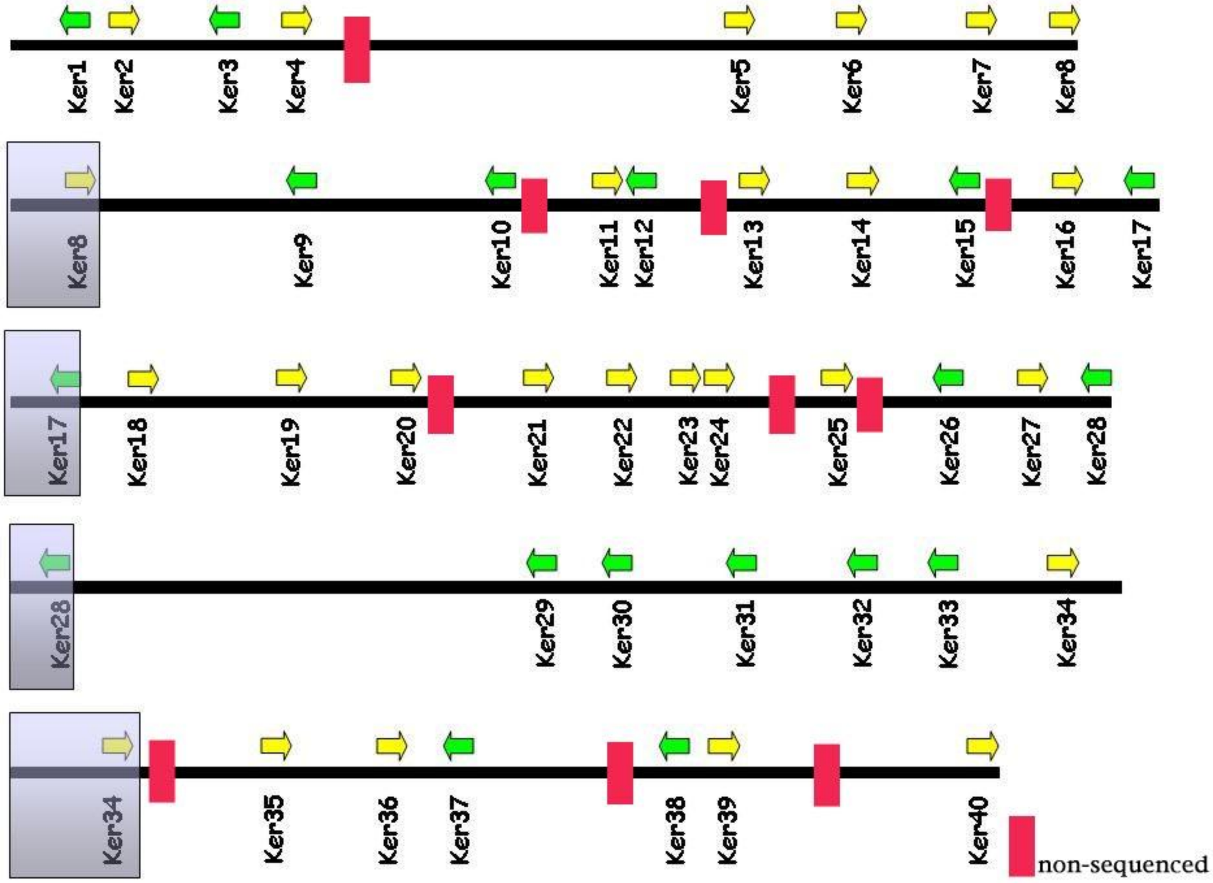

Fig. 3. Schematic representation of all forty genes linearly arranged in scaffold 455 of the draft genome of *A. carolinensis*. Black lines represent known inter-gene sequences.

The gene structure present in each of the 40 genes consists of a 5′-untranslated region with high base conservation among the different genes, and that contains a variably long intron. A short un-translated region precedes the variably long coding region, and is followed by a variably long and diverse 3′-region (Dalla Valle et al., 2010). In general terms, the gene organization of *A. carolinensis* genes reflects that of most reptilian and avian beta-K genes (Gregg and Rogers, 1986).

The deduced sequence for the proteins coded by the 40 genes corresponds to small proteins containing 87–225 amino acids with a Mw ranging from 9 to 23 kDa (Dalla Valle et al., 2010). Based on the percentages of 4 main amino acids (glycine, cysteine, proline and serine) the 40 proteins have been divided into 4 subfamilies, and most proteins are basic (Fig. 4).

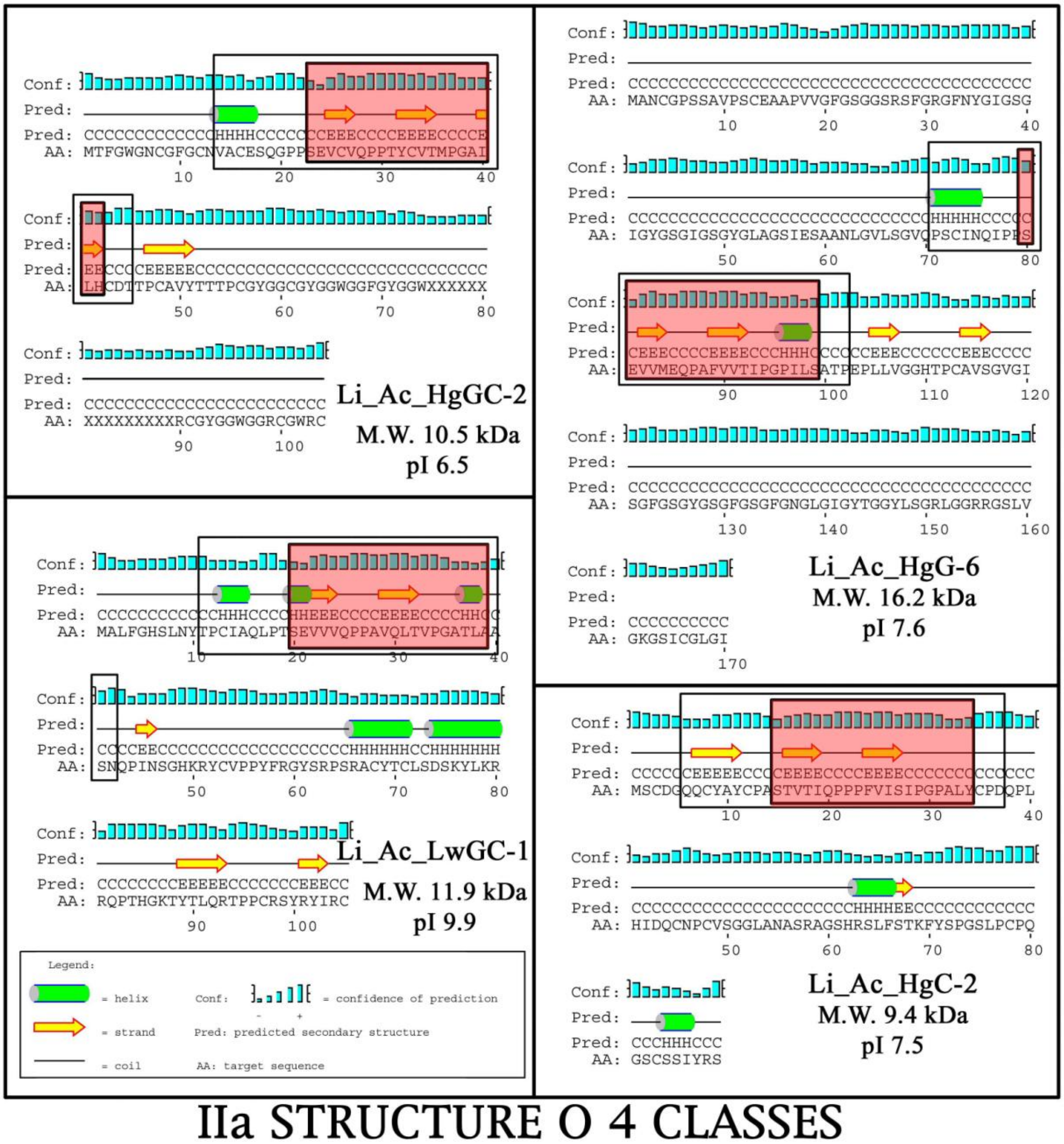

Fig. 4. Schematic representation of the secondary structure of examples of each protein subfamily (Lizard-*Anolis carolinensis* High Glycine Cysteine Protein n. 2= Li-Ac-HgGC-2; Li-Ac-HgGC-1 etc.; see text for explanation). M.W. is molecular weight in kilodaltons; pI is the isoelectric point.

The first family, indicated as High Glycine (HgHG), contains 18 members, the second family, indicated as High Cysteine Glycine (HgHCG), and contains 16 members, the third family contains only two members and is indicated as High Cysteine (HgHC), and the fourth family of two proteins contains low amounts of both glycine and cysteine (LwCG). The computer-predicted secondary structures of the different proteins has shown that most amino acids form a random coiled region (dark lines in Fig. 4), and short alpha-helixes (green cylinders in Fig. 4). All these proteins contain a highly conserved central region indicated as the "core-box" (pink boxed containing yellow arrows in Fig. 4). The core-box and the other, surrounding beta-folded region (boxed in Fig. 4), are responsible for the formation of the filaments of KAbetaPs (Beta-K). These filaments rapidly mask those of alpha-keratin to make most the corneous material for the different scales, adhesive pads, and claws. In general KAbetaPs in *A. carolinensis* share various characteristics with those of similar proteins isolated from other reptiles (Alibardi et al., 2009), and indicate that a limited number of these proteins is necessary to build most scales and claws in reptiles.

The next step of our research will focus on the specific sites of expression for these proteins in order to understand which proteins make softer versus harder parts of scales, adhesive pads, and claws.

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Parasite diversification in Caribbean anoles

Anoles have provided a wealth of biological information, facilitating insight into a broad range of disciplines. The kinds of data extracted from *Anolis* are remarkable, but these lizards harbor a certain kind of data often overlooked by anole biologists: parasites. Indeed, parasitism is an exceptionally common way of life, so much that parasites are estimated to comprise 30-50% of total species diversity. Despite this, the mechanisms underlying parasite diversification remain unclear. Caribbean *Anolis* offer an outstanding study system to examine these mechanisms, and by comparing patterns across multiple parasite groups, we aim to better understand how parasites diversify.

Anoles are hosts to a variety of parasite taxa, including coccidia, pinworm, mite, hemagregarine, and malaria parasites. These groups vary in mode of transmission, prevalence, ploidy, and host specificity. Given these differences, contrasting patterns of diversification may provide insight into how these traits affect parasite diversification, while congruence between groups will reveal potentially common mechanisms of parasite diversification. We will also assess the relative importance of host phylogeny, host ecology, host sympatry, and geography in parasite diversification. Did *Anolis* parasites radiate along with their hosts? Is host ecology more important than host phylogeny in determining parasite infection? What is the effect of host specificity on parasite genetic diversity? Can we use parasites to infer host dispersal (and failed colonization) events between islands?

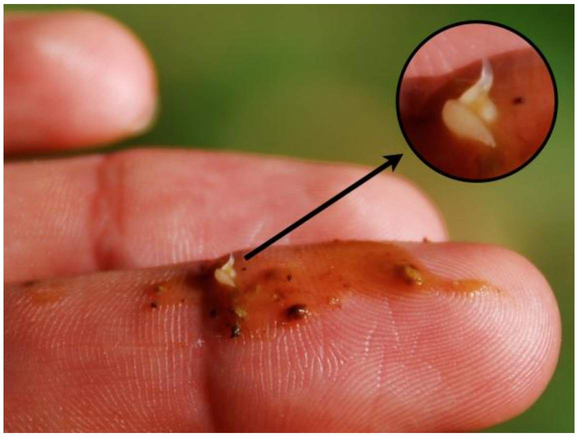

Fig. 1. The pinworm *Parapharongodon cubensis* expelled with *Anolis* excrement.

This research has just begun, but already we have observed cryptic parasite diversity and novel host associations. We have sampled four malaria parasites species (*Plasmodium*) on

Hispaniola; one is a new species, and two of these have relatively high specificity for *Anolis* ecomorphs. We also observed the first *Plasmodium* infection for any member of the “Twig” ecomorph (in *Anolis insolitus*), along with several other new host records. On the Lesser Antillean island of Saba, we sequenced several distinct mitochondrial haplotypes in the pinworm *Parapharongodon cubensis* (Fig. 1), suggesting that this widely distributed Caribbean endemic may be a complex of several species. As we gather data from more islands and more parasites, we expect to continue to uncover cryptic parasite diversity and new host associations. More importantly, these data will be used to address the kinds of questions outlined above, helping us to better understand how parasites diversify.

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Visual motion detection by *Anolis* lizards and its influence on the evolution of visual display motion patterns

Abstract

Anolis lizards respond to motion in the visual periphery by shifting direct gaze and central attention towards the moving object. In the 1980s I tested the relative effectiveness of different motion patterns in eliciting visual attention in *Anolis auratus*. The most effective motion patterns were abrupt, low frequency movements, of roughly 0.3 degrees visual angle. Higher amplitude motion, high frequency motion, motion that included only high acceleration, or motion that contained only high velocity was not particularly effective. These results seemed paradoxical. In my current research I am revisiting this question using a neural- circuit model of peripheral visual motion perception. Behavioral tests show that this model accurately mimics many aspects of *Anolis* visual motion detection. The model responds optimally to a motion pattern that starts from a stationary position, abruptly shifts to a new position a short distance away, and stops. The critical features are the time it takes to shift positions and the distance between the positions. An abrupt movement of 0.3-0.4 degrees visual angle is the most effective pattern. This explains the puzzling results of the earlier studies on *Anolis auratus*. Many anoline species initiate long distance displays with abrupt, moderate amplitude (approximately 10 mm), shifts of head or body position. This pattern appears optimal for stimulating motion vision and drawing the attention of viewers 1.5 to 3 m away.

Introduction

I have a longstanding interest in understanding how sensory processing influences the evolution of communication signals. In this paper I present results from my studies of visual motion perception and how this process appears to have driven the evolution of motion patterns used in anoline displays. I start by reviewing results of some older studies of visual motion detection in *Anolis auratus*, and point out some rather puzzling aspects of these results. I then present results from my current research, which is based on modeling motion detection with simple neural circuits. I show how results from this new approach explain some of the most puzzling results of the earlier research.

In 1979 while working as a field assistant on Barro Colorado Island in Panama I met Stan Rand. Stan convinced me that *Anolis* would be an excellent system for studying the kinds of problems I was interested in, and lured me back to Panama for my Ph.D work. In the 1980's, as a Cornell graduate student, working with Stan as my advisor, I studied visual communication in the Panamanian grass anole *Anolis auratus*. My general interest, then as now, was in the role that sensory response patterns play in the evolution of communication signals.

Fig. 1. A male *Anolis auratus*

An important aspect of *Anolis* behavior is the use of complex up-and-down motion patterns in their displays. From 1980-86, I examined the question of whether the visual system responds more strongly to some patterns of motion than others, and whether this acts as a selective force that shapes the design of visual-display motion patterns. Carpenter (1967) identified the fact that lizard displays can be usefully divided into two distinct contexts. Lizards give "challenge" displays at close range in agonistic and/or courtship situations. Territorial males frequently give "assertion" displays from conspicuous locations when no other individual is close at hand. A key difference is that challenge displays are given to a nearby, attentive receiver whereas the intended receiver of the assertion display is often far away and inattentive (Fleishman 1992). I am particularly interested in the physical design of the assertion display since its effectiveness is likely to be constrained by the receiver's visual perception.

The set-up for the experiments I carried out on *A. auratus* is illustrated in Fig. 2. In each trial a lizard was placed in a small cage and its attention was diverted to a point perpendicular to the long axis of the body (arrow 1 in Fig. 2). A small black bead hanging from a clear thread was set in motion – directly up and down in a periodic fashion. The stimulus motion amplitude was gradually increased until the lizard abruptly shifted its gaze and fixated on the moving object (arrow 2 in Fig. 2). If no gaze shift occurred within a few seconds a negative response was recorded.

The first surprising result from this set of studies was that regardless of the type of stimulus motion used, the amplitude at which response occurred was nearly the same. Lizards often did not respond at all, but when they did, it was nearly always at a peak-peak motion amplitude between 0.2-0.4 degrees visual angle (an average of approximately 0.3 degrees), and there were no significant differences in this amplitude across stimulus motion types (Fleishman 1986).

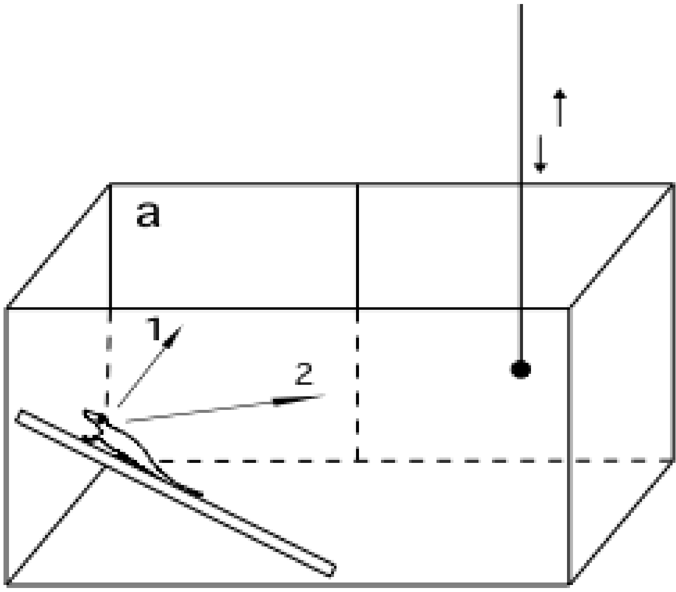

Fig. 2. The basic set-up for behavioral tests of motion detection

There were, however, large differences in the probability of a positive response for different types of motion. I started by testing response to smooth, sinusoidal, up-and-down motion of different frequencies. The probability of response was equal for frequencies from 0.5 to 5 Hz. At higher frequencies of motion response probability declined rapidly, even though these higher frequency movements were well below the flicker fusion frequency. I also compared 1.5 Hz square wave motion (abrupt up and down shifts in position) to 1.5 Hz sine wave motion and found that square wave motion always elicited a much higher response probability.

In order to gain some understanding of why response to a square wave was so strong I created the series of stimuli shown in Fig. 3A. In these experiments amplitude was kept constant, while acceleration and maximum velocity of the up-and-down movement of the stimulus bead were varied. As shown in Fig. 3B, only the combination of high acceleration plus high peak velocity created a significantly greater response. High acceleration alone, or high velocity, alone, was not sufficient to elevate response probability.

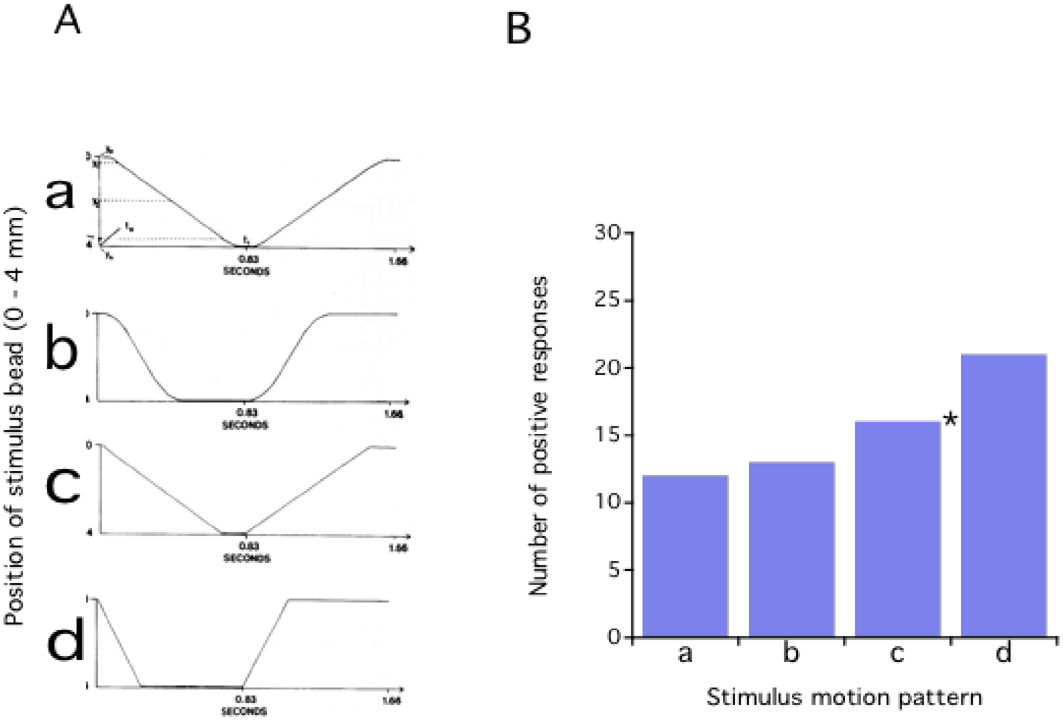

Fig. 3. A. In order to test the importance of acceleration and velocity in making a moving stimulus more detectable four different stimulus motion patterns were created. In each case the stimulus bead (from Fig. 2) was moved up and down periodically. The four different stimuli were (a) low acceleration and low peak velocity, (b) low acceleration and high peak velocity, (c) high acceleration and low peak velocity, and (d) high acceleration and high peak velocity. All stimuli were moved periodically at an amplitude of 4 mm (approximately 0.3 degrees visual angle from the lizard's eye position) for a few seconds. B. Results from the experiment using the stimuli shown in 4A. Only the combination of high acceleration and high velocity significantly increased response probability (P<0.05, Cochran's Q –test). From Fleishman(1986).

Taken together the results of these experiments were difficult to understand. A square-wave movement, which combines very high acceleration and velocity, was a very strong stimulus. However a high frequency sine wave, which also combines high acceleration and velocity was a very poor stimulus. A periodic stimulus that had either high acceleration or high velocity was not particularly effective, while one that combined these was. Finally the amplitude of motion at which response was most likely to occur (if it occurred at all) did not vary across stimulus types. Even though high acceleration and high velocity resulted in an elevated response probability, increasing amplitude, which increases acceleration and velocity, did *not* increase response above an amplitude of 0.3 degrees visual angle.

While I did not fully understand the results at the time, they nevertheless were useful in understanding the evolution of display design. When I compared close range (challenge) displays to long range (assertion) displays, I found that the assertion displays began with a series of abrupt square-wave like movements that, I hypothesized, were designed to elicit the attention of inattentive viewers (Fleishman 1988,1992), while the challenge displays did not. Other anoline species have also been shown to add square-wave-like movements to the beginning of displays intended for distant viewers (Stamps and Barlow 1973; Ord and Stamps 2008).

Modeling the Neurobiology of Motion Detection: the 2DMD Model.

Two decades later, I have again begun to examine the role of sensory response in the evolution of motion patterns. Since the 1980s there has been a great deal of progress by neuroscientists in understanding how visual systems process motion (e.g. see Borst and Egelhaaf 1989). Motion is generally not analyzed at higher brain levels. Rather, it is extracted very early in the process of visual perception. The detection and early analysis of motion is carried out by simple local neural circuits found either in the retina or very early in the brain's visual processing pathway. While this process has been modeled in different ways, there is a growing consensus that for most animals it can be modeled effectively with a simple neural circuit called a "correlation detector" (Reichardt 1961; Borst and Egelhaaf 1989). In collaboration with Adam Pallus, I have developed a computer model of motion detection that uses a grid of interconnected correlation detector circuits to analyze natural scenes captured on video. Our model is based on that described in detail in Zanker and Zeil (2005).

The basic features of our model are illustrated in Fig. 4. The basic unit of our model is a simple correlation-type motion detector like that illustrated in Fig. 4A. When an image moves from right to left across the retina, if the time difference between its arrival at detector 1 and 2 roughly coincides with the delay of the signal from detector 1, a strong output will be experienced from the multiplier. If the image moves in the other direction, or its timing is wrong, it will produce little or no output. The motion detection circuit is characterized by two parameters. The constant τ is the time constant of the low pass filter and determines the delay in output from the first detector. The spacing constant, $\Delta\phi$ is the distance between the centers of the receptive fields of the two interacting detector. An elementary motion detector (EMD) circuit is created by combining two correlation detectors and subtracting their outputs as illustrated in Fig. 4B. To complete our model we created a two dimensional grid of EMDs as illustrated in Fig. 4C. This is referred to as a two-dimensional motion detector (2DMD).

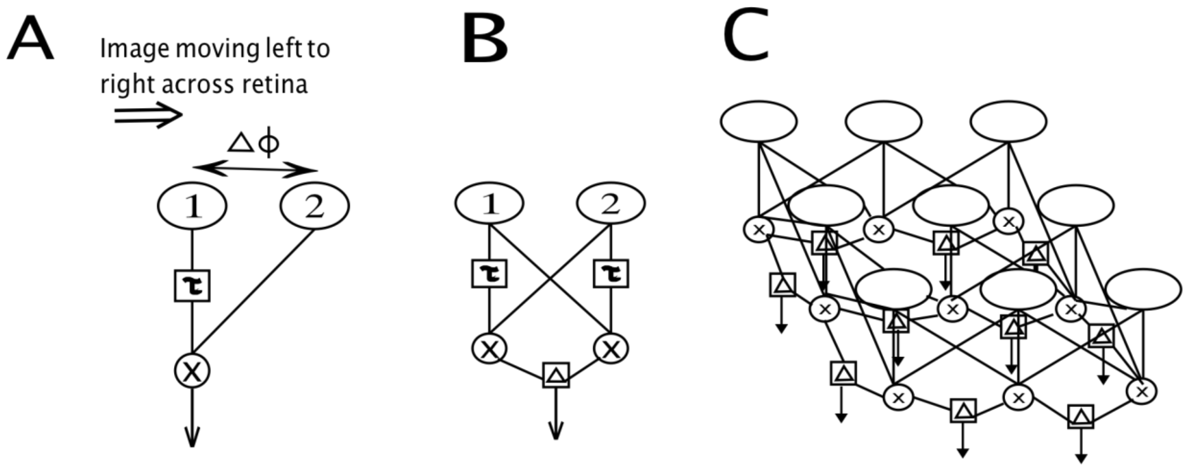

Fig. 4. A. A correlation detector circuit consists of two receptive fields on the retina (1 and 2) a distance Δϕ apart, each of which produces an output proportional to the intensity of light in the image falling on it. The outputs from the two detectors are multiplied together. Output from detector 1 is delayed in time by passage through a low pass filter with a time constant = τ. B. Two correlation detectors are wired together reciprocally, and their outputs are subtracted to create an elementary motion detector (EMD) circuit. C. Our computer model consisted of a simulation of a two dimensional grid of interconnected EMDs referred to as a 2-dimensional motion detector (2DMD).

Our computer program carries out the operations illustrated in Fig. 4 on sequences of grayscale video. The video sequences are input into the program frame-by-frame and, for each successive frame, at each x-y coordinate of the video, motion output is determined. An example of typical input and output to this motion model is shown in Fig. 5. In this case x-y position of the input video (a running cricket) is plotted on the x-y axes of the output, and strength of motion detector model output is shown on the z-axis. A detailed explanation of how this model works along with examples can be found at http://www.union.edu/academic_depts/bioengineering/visual_motion/index.php

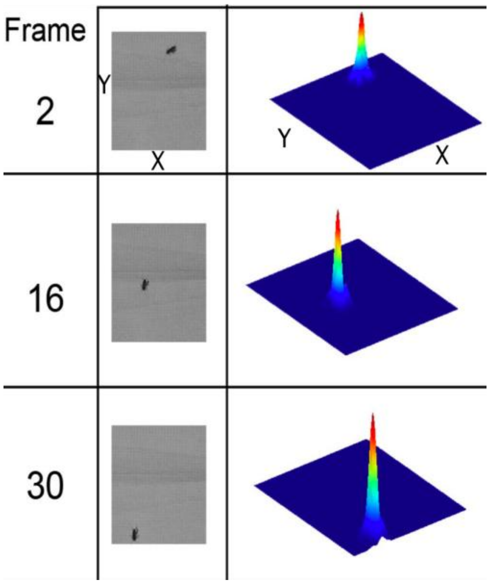

Fig. 5. An illustration of the motion detection model. The input is a video sequence of a running cricket. Three sample frames are shown. The model output from each frame is shown on the right. The x- and y-axes represent x and y positions in the original video. The magnitude of the model output at each frame and each location is plotted in the z-axis. From Pallus et al. (2010).

Behavioral Experiments with Lizards

The next step in our study was to carry out a set of behavioral experiments to (1) test the applicability of the motion detection model to *Anolis* and (2) determine model parameters (Δϕ and τ) that make its performance match that of a living lizard. The behavioral experiments were similar in design to those described above for *A. auratus,* with two important differences: we used *Anolis sagrei* and we created our moving stimuli on a computer screen. Fig. 6 shows the results of an experiment in which we presented lizards with small stimulus squares moving across a computer screen at different velocities. The response of the computer program to the same stimulus pattern is also shown.

Response of lizards closely matched response of the model with τ = 0.1 s. We also tested response probability to small dots that moved abruptly, a short distance, in a single jump. Such stimuli presented to our model produced maximum response when the distance of the jump was equal to Δϕ. Lizard behavioral response was greatest when the jump amplitude was equal to 0.4 degrees visual angle (very close to the amplitude of maximum response we earlier observed in *A. auratus*). From this we were able to conclude that a space constant equivalent to 0.4 degrees visual angle was appropriate to use in our model (Pallus et al. 2010). Thus, behavioral response of lizards closely matched predictions of the model and allowed us to "tune" the model to approximate lizard responses.

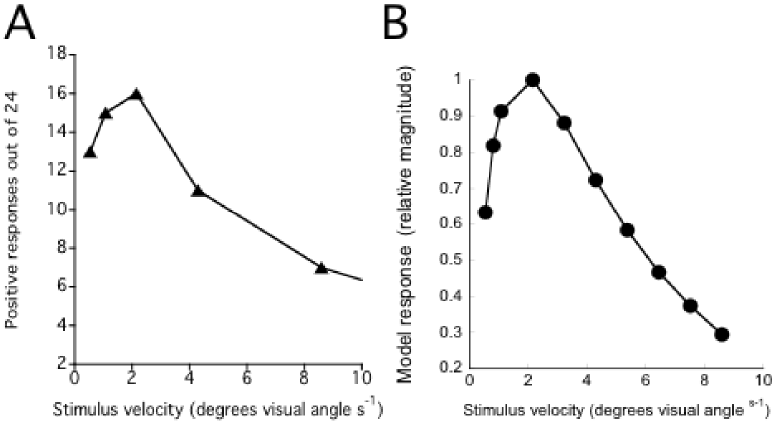

Fig. 6. A. Response of *A. sagrei* to stimulus squares moving across a computer screen at different velocities. The number of lizards (out of 24) that shifted gaze toward the moving stimulus is plotted against the velocity of motion. B. Relative magnitude of maximum output from the motion detector computer model with τ = 0.1 s for stimuli moving at different velocities. From Pallus et al. (2010).

Differential Response of the Model to Different Stimuli

Having established a model of motion response that was consistent with the behavior of live anoles, we were able to test it with different stimuli. We started by testing the model with a variety of simple periodic motion patterns. We used an oscillograph pen motor to create sine wave and square wave motion at a range of frequencies, made videos of these patterns, and ran them through our model. Results are summarized in Fig. 7. The model responds most strongly to low frequency square waves, or, similarly, to ramp stimuli with fairly short rise times, as long as the motion amplitude is approximately equal to the spacing between receptors. The model is not extremely sensitive to motion amplitude, however, and motion ranging from roughly 0.5 to 1.5 times the space constant (ϕ) worked well. Motion patterns with amplitudes of 2 x ϕ or greater were much less effective. Thus the strongest stimulus to the model is low frequency square wave motion of an amplitude approximately equal to the spacing of the receptors in the EMD that serve as model inputs.

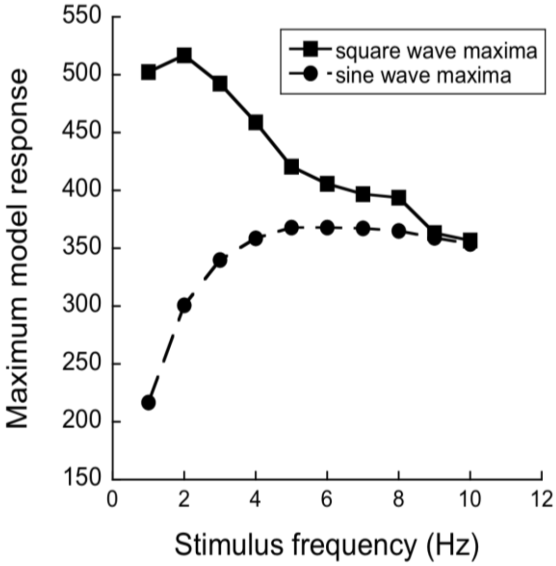

Fig. 7. Response of the computer model to videos of artificially created moving stimuli. Squares show response to square wave motion, and circles show response to sinusoidal motion. Motion amplitude was equal to the spacing constant Δϕ.

Why do square waves work so well with this model? In brief, it can be thought of this way. Consider Fig. 4A. Suppose a bright moving image arrives at detector 1 and stops. Because of the delayed output from detector 1 it takes about 0.1 s for the detector to send out a full signal of the brightness of the image to the multiplier stage. Now suppose the image starts moving. If the image moves very quickly and sits over detector 2, for a brief time detector 1 is still sending out its full output. However, detector 2 has no delay built in to its output, so it instantly begins sending out a strong output. Thus, for a brief moment both detectors are sending maximum output to the multiplier. The response from 1 quickly drops and the high multiplier output stops. Thus it is the near-instantaneous position shift from detector 1 to 2 that produces a maximal output. Slower movement from position 1 to 2 causes a smaller output because the output from detector 1 begins to drop as soon as the image moves away. In summary the most effective way to stimulate a correlation detector is with an image that sits over one detector until the highest output is reached, then moves abruptly to a position one space constant away (the image stimulates the second detector), and stops.

We can now understand quite well the results described at the beginning of this paper. Stimulus velocity and acceleration per se are not important. An effective stimulus is one in which movement starts from a non-moving position, moves a short distance very rapidly, and then stops. We can also understand why maximum response occurs to a small range of motion amplitudes.

Lizard Displays

In Fig. 8, I plot examples of the first 8-10 s of head movements from typical assertion displays by three different species of *Anolis* from Puerto Rico. In all three cases the display starts with abrupt movements of the head followed by a stop. I also show the output from our motion detection model (maximum output per frame). It is very interesting to note that the peak-peak amplitude of the head motion in each case is approximately 10 mm. The distance that an image of an object moves across the retina depends on the actual amplitude of motion and the distance from viewer to object. In Fig. 9, I have plotted perceived amplitude of motion (in degrees visual angle) of a 10 mm movement versus distance. These displays seem well-suited to produce motion of 0.2-0.4 degrees visual angle over the range 1-2.5 m. At closer range the display movements are too large to be maximally effective. This leads to the prediction that display amplitude should be decreased when lizards display at close range. My colleague Manuel Leal is currently carrying out experiments to see if, in fact, anoles do reduce display amplitude as predicted in close range encounters.   We already know that *Anolis auratus* uses lower amplitude movements in challenge displays than in assertion displays.

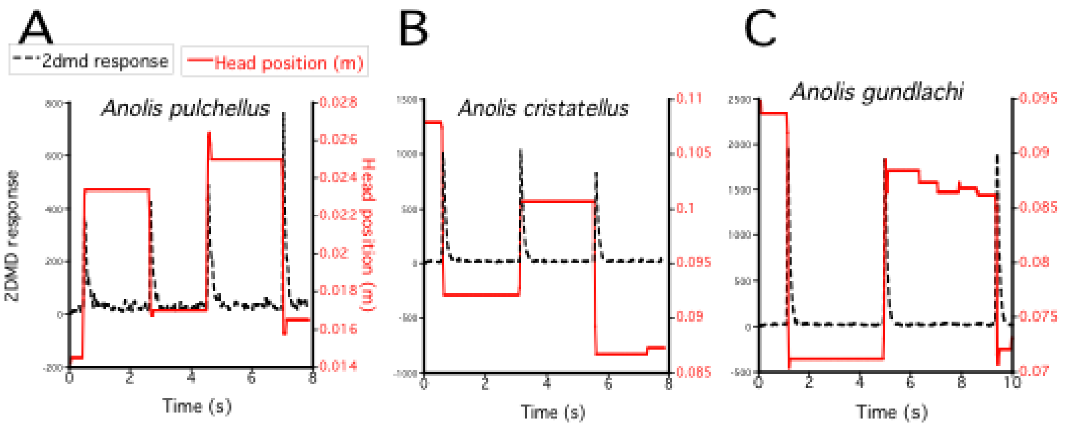

Fig. 8. Plots of head position versus time for the initial 8-10 s of assertion displays by three *Anolis* species from Puerto Rico are shown in red. The responses of the motion detection program to these video sequences are plotted in black.

Display Motion and Visual Noise

A final question of interest is how well motion can be detected in the presence of visual noise. *Anolis* lizards live in complex habitats and the motion of their displays will be more difficult to detect in the presence of windblown vegetation.

We wanted to see how well our *Anolis*-based motion detection model could detect display movements in the presence of windblown vegetation. We did this by recording examples of natural assertion displays of five Puerto Rican *Anolis* species in moderate wind conditions.   In nearly every case the initial movements of the display caused much greater response from the motion detector than did the movement of windblown vegetation. An example of this is illustrated in Fig. 10. It is apparent that the output from the motion detector is much stronger to the display than to the background vegetation movement.

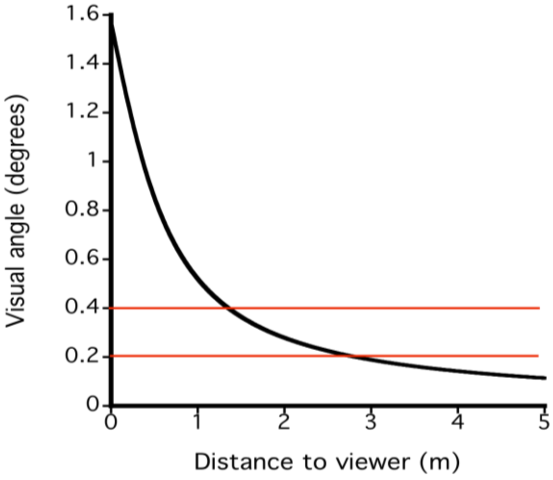

Fig. 9. The apparent size, in degrees of visual angle, of a movement of 10 mm peak-to-peak as a function of the distance between object and viewer. The red lines indicate the range of visual angles that tend to elicit the strongest detection response from anoles.

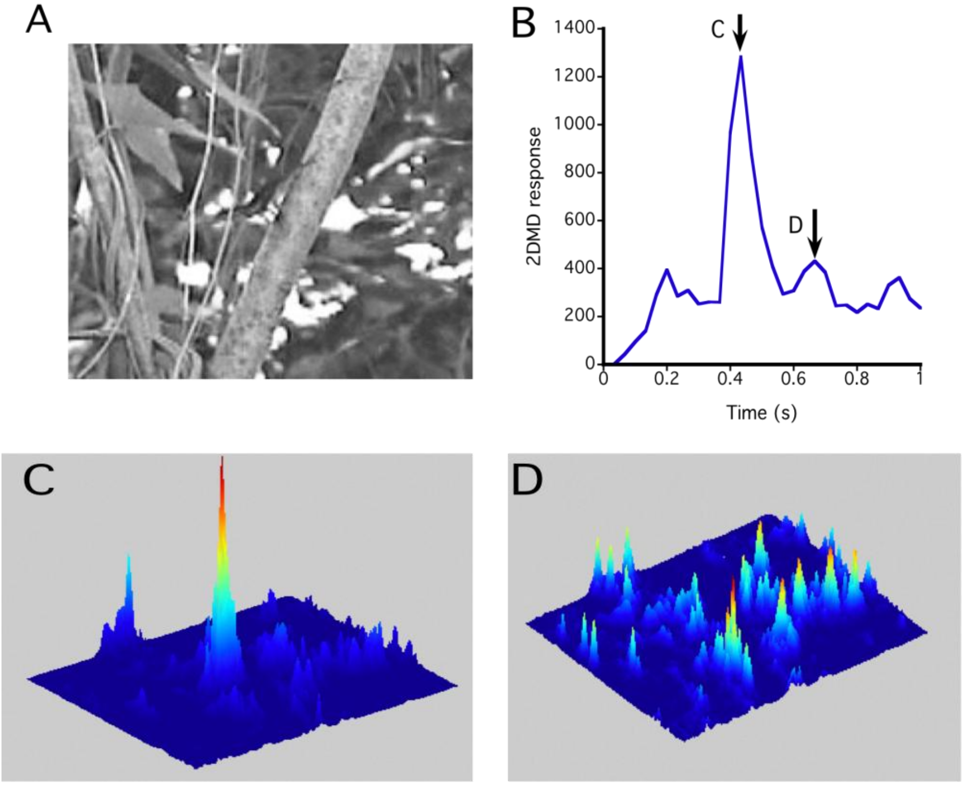

Fig. 10. A. A single frame from a short video sequence at the beginning of an assertion display carried out by *Anolis krugi* in moderate wind. B. A graph of motion detector output (maximum value per frame) versus time from this sequence. The display occurs at point C on the graph. The highest output from the windblown vegetation with no display motion is indicated by point D on the graph. C. A representation of the output from the motion detector model for the frame at which the display motion occurs (C in Fig. 10B). D. Motion detector model output for the frame indicated by D in the Fig. 10B.

Summary

Studies during the 1980s led to the conclusion that abrupt movements of approximately 0.2-0.4 degrees visual angle are the most effective at eliciting the visual attention of anoline lizards. Modeling motion detection using a simple neural network model led to the conclusion that this pattern is due to the nature of the way motion is analyzed by visual systems. In particular, movements that jump abruptly from one detector to its paired detector give a very strong output. The basic design of the lizard motion detection system is similar to that of many other animals, including insects and humans. It appears that motion-detector design is evolutionarily quite conservative. This property of visual motion detectors therefore appears to have been an important selective force in the design of lizard visual display motion. Many species of lizards use abrupt, square-wave-like movements at the beginning of their displays, presumably in order to elicit the attention of potential viewers. These studies show that sensory processing mechanisms tend to favor particular features in communication signals. Thus understanding the neural basis of perception can provide useful insight into the design of communication signals.

Acknowledgments

I am indebted to Stan Rand for introducing me to anoline biology and providing a great deal of mentorship, support and beer during my years in Panama. Adam Pallus and Michael Rudko worked with me to develop the 2DMD motion detector model. Jochen Zeil and Johannes Zanker provided valuable insights into the function of motion detection models.

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Testing for convergence in toepad morphology in mainland and Caribbean *Anolis*

Introduction

Observations of adaptive convergence, in which phylogenetically distant taxa resemble one another more than their ancestors resembled one another, are among the most compelling arguments for evolution by natural selection (Blondel 1991). In the case of Greater Antillean *Anolis*, adaptive radiation has produced similar results on four different islands such that lizards specializing on similar microhabitats on the different islands have converged morphologically (each of these varieties of habitat specialists is called an "ecomorph"; Williams 1983; Losos et al. 1998). This makes *Anolis* lizards an ideal system in which to test hypotheses about convergence.

However, ecomorphs appear to be unique to the Caribbean (Irschick et al. 1997; Velasco and Herrel 2007; Losos 2009). Mainland anoles, despite comprising over half of anole species diversity, are not as well studied and it is unclear whether convergence is as prevalent in the mainland as it is in the Caribbean. Mainland anoles demonstrate as much morphological diversity as Caribbean anoles (Irschick et al. 1997; Macrini et al. 2003; Velasco and Herrel 2007; Pinto et al. 2008). Additionally, in both the Caribbean and mainland radiations, morphological variation is correlated with habitat use, although these relationships differ between the mainland and the Caribbean (Irschick et al. 1997; Macrini et al. 2003).

Given this information, we sought to answer the question: Is morphological convergence, which is a dominant pattern in the Caribbean anole radiation, also an important feature of mainland Central and South American anole radiations?

We chose to examine adaptive convergence in an ecologially important and phenotypically variable anole trait – the adhesive toepad. Toepad morphology is correlated with clinging performance, which is very important in anoles, the majority of which use arboreal substrates (Irschick et al. 1996; Losos 2009). Toepad morphology is also correlated with ecology: variation in toepad lamella number and width has been shown to be correlated with both the height and diameter of the perches typically used (Glossip and Losos 1997; Macrini et al. 2003; Elstrott and Irschick 2004). As a result, we expected toepads to converge with repeated habitat specialization.

In this study we measured toepad characteristics for almost every species included in the most comprehensive molecular phylogeny of anoles to date (Nicholson et al. 2005) in order to examine patterns of convergence among mainland and Caribbean radiations.

We used two different methods to examine convergence. First, we measured phylogenetic signal to assess the extent to which closely related species are morphologically similar. We measured phylogenetic signal separately for mainland and Caribbean anole clades. Evolutionary convergence is expected to depress phylogenetic signal, although convergence is not the only evolutionary scenario which may do so (Losos 2008; Revell et al. 2008).

Second, we conducted a phylogenetic analysis of variance to test whether anoles with similar ecologies were also phenotypically similar (and thus convergent) both in the Caribbean and on the mainland. Besides adaptation, a number of scenarios may result in convergence, including design limitation or random chance (Stayton 2008). This analysis tests whether convergence is associated with adaptation to specific ecological niches. We hypothesized that convergence is more prevalent in the Caribbean because of the early presence of similar, open niches on many different islands, and because the radiations on the different islands occurred largely in isolation and in parallel. In contrast, the greater diversity of competitors on the mainland (and thus lower ecological opportunity) may preclude ecological convergence and produce a pattern of phylogenetic niche conservatism. We also used a large dataset to examine whether there are gross differences in toepad characteristics between island and mainland radiations.

Methods

We examined the toepads of ethanol-preserved anole specimens from several natural history museums. Of the 187 species included in the phylogeny of Nicholson et al. (2005), we were able to analyze 871 individuals representing 175 species. Of these, 37 species were from the M2 clade, 130 were from the Caribbean and eight were from the basal M1 clade (*sensu* Pinto et al. 2008). Because the M2 radiation of mainland anoles is monophyletic and nested within the Caribbean radiation, we pruned the M2 clade and retained it as our phylogeny of mainland anoles. We then used the paraphyletic remainder as our Caribbean tree. We excluded the eight M1 taxa from the phylogenetic convergence analyses because this clade was too poorly sampled to conduct a meaningful analysis (these species were retained in our gross comparison of mainland and island toepad morphology).

To collect data on lizard lamellae, we scanned the forefeet and hindfeet of preserved specimens on a flatbed scanner at high resolution and collected data from the resulting images. We counted the number of lamellae on the fourth toe of the forefoot and hindfoot using the software tpsDIG2 (Rohlf 2006; Fig. 1). We counted all enlarged subdigital scales on the entire toe for this measurement, from the distal end of the pad (before the claw) to the last scale greater than sixty percent the width of the scale distal to it. We measured toepad width at the widest point of the pad (perpendicular to the main axis) of the fourth toe using the software ImageJ (Rasband 1997; Fig. 1). Although toepad area is the measurement that in theory should best predict clinging performance, we measured width because it is tightly correlated with area in anoles (Macrini et al. 2003) and is more reliably obtained from partially damaged specimens.

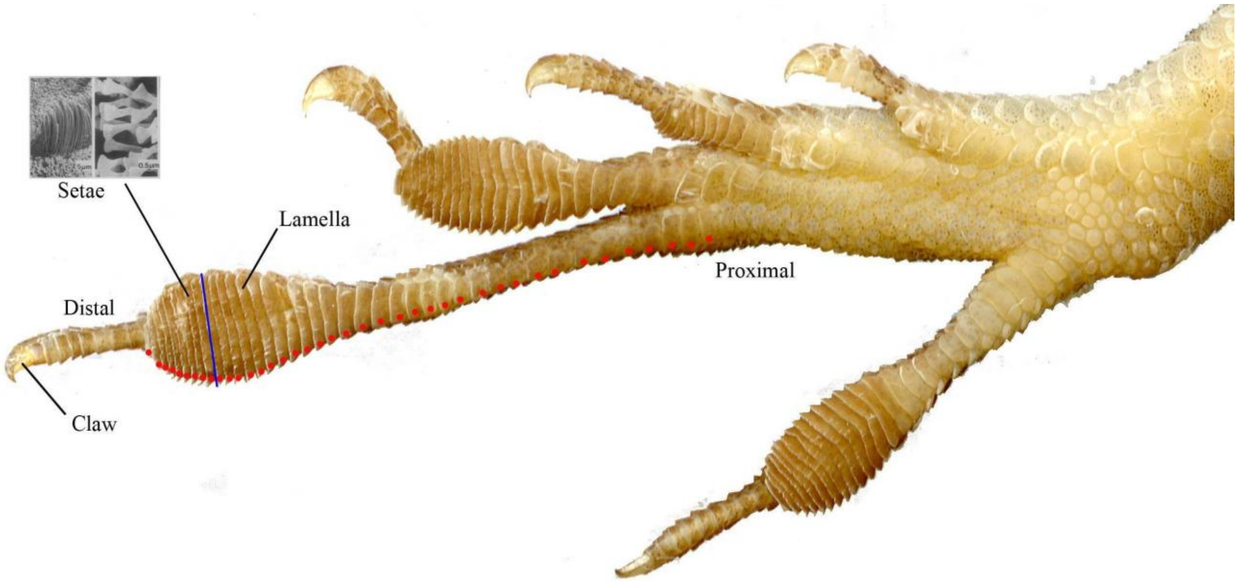

Fig. 1: Image of the ventral surface of the right hindfoot of *Anolis marmoratus*, taken using a flatbed scanner. Each red dot denotes a subdigital scale counted for our study. The blue line denotes the width measurement. Image of setae from Williams and Peterson (1982; figure 1).

For each species, we counted lamellae and measured pad width on the right forefoot and hindfoot of five individuals (when possible) and we then averaged these values. We natural log transformed and size corrected (using lnSVL as body size) the species averages using phylogenetic regression, following the methods of Revell (2009). We then used the residuals of these corrections in a phylogenetic principal component analysis (also following Revell [2009]) and in the calculation of phylogenetic signal. We retained principal components with eigenvalues were greater than one (PC I and PC II) for further analyses (see Results).

We measured phylogenetic signal within the M2 clade and within the Caribbean grade using the K statistic (Blomberg et al. 2003). We generated a null distribution of K values for both mainland and Caribbean radiations by simulating Brownian motion evolution 1000 times on each subtree (Caribbean and M2) using the program multibrownian.c (Revell unpublished). We compared the observed K values for each radiation to the null distributions for K using a two tailed test.

We tested for adaptive convergence in arboreal anoles versus non-arboreal anoles in both the mainland and Caribbean using a phylogenetic ANOVA (Garland et al. 1993). For Caribbean anoles, we considered trunk, trunk-crown, crown-giant and twig ecomorphs as well as other species that perched and foraged at the same heights as these ecomorphs (76 species total) to be arboreal. We classified grass-bush and trunk-ground ecomorphs, as well as the non-ecomorph anoles that perched or foraged at the same heights as these ecomorphs (54) as non-arboreal. Although fewer habitat data were generally available, we were also able to classify almost all the M2 anoles as arboreal or terrestrial, and we were careful to match the Caribbean habitat assignments as closely as possible (we focused primarily on perch height and foraging location). Of the 37 M2 taxa, we were able to assign 34 species to habitat categories: 29 as non-arboreal and five as arboreal.

Given the previous finding that Caribbean anoles have wider toepads with more lamellae than mainland anoles (Macrini et al. 2003; Velasco and Herrel 2007; Pinto et al. 2008), we also tested for this difference using our large sample, which differs in sample, scope, and phylogenetic coverage from previous data sets. Additionally, to test whether these toepad characteristics result from inherited constraint in the M2 lineage, we compared M2 toepad morphology to species in the *sagrei* group (Cuba) and the Jamaican anole radiation, which represent the closest relatives of the M2 clade (Nicholson et al. 2005).

Results

In the principal component analysis (Table 1), PC I and PC II explained 65.8% and 25.8% of the variance respectively. PC I loaded positively and strongly on all four traits. PC II loaded positively on toepad width for both forefeet and hindfeet, and negatively on lamella number for both forefeet and hindfeet.

Table 1: Principal component loadings. Only PC I and PC II had eigenvalues greater than one.

Character	PC I	PC II
Hindfoot Pad Width	0.8297	0.4947
Forefoot Pad Width	0.8353	0.4898
Hindfoot Lamella Number	0.7612	-0.5697
Forefoot Lamella Number	0.8174	-0.4722
Eigenvalue	**2.634**	**1.032**
Percent explained	**65.8**	**25.8**

We calculated phylogenetic signal (Blomberg's K) on the phylogenetically size corrected trait residuals as well as scores on PC I and PC II (Table 2). For both PC I and PC II, phylogenetic signal was higher in the mainland; however, in no cases did signal differ significantly from the expectation under Brownian motion evolution (K = 1).

Table 2: Phylogenetic signal principal components and in phylogenetically size corrected residuals. P-values refer to a simulation test for difference from K = 1.

Trait	Caribbean K	Caribbean P-value	M2 K	M2 P-value
PC I	0.978	0.938 (NS)	1.260	0.292 (NS)
PC II	0.795	0.326 (NS)	1.286	0.272 (NS)
hindfoot pad width	0.721	0.128 (NS)	1.702	0.056 (NS)
forefoot pad width	0.746	0.192 (NS)	1.326	0.234 (NS)
hindfoot lamella number	1.050	0.690 (NS)	0.768	0.384 (NS)
forefoot lamella number	0.986	0.908(NS)	0.815	0.768 (NS)

The four unrotated toepad characteristics also did not show significant differences from the Brownian motion expectation of K = 1 (Table 2). The signal in mainland hindfoot pad width was the highest for any measured trait (K = 1.702) but was not significantly different from K = 1 (p = 0.056). The Caribbean hindfoot pad width had the lowest phylogenetic signal of any trait (K = 0.721) and this was also not significantly different from K = 1 (p = 0.128). Forefoot pad width values were similar in trend although slightly less pronounced, and neither was significantly different from K = 1. Although none of these results were significant, they did reveal interesting differences in toepad phylogenetic signal between mainland and Caribbean anoles. Lamella number exhibited the reverse pattern (although less pronounced), with the Caribbean anoles exhibiting higher levels of phylogenetic signal, although neither radiation had signal that was statistically distinguishable from the null model (Table 2). Unfortunately, as of yet, no one has developed a method to statistically compare K values for different data sets, so we were unable to compare mainland and Caribbean K values directly.

For Caribbean anoles, a phylogenetic ANOVA revealed significant differences between arboreal and non-arboreal specialists in all but one trait (Table 3). Data from three of the four toepad characters and PC I (largely a measure of toepad size and lamella number) indicate that arboreal anoles have significantly wider toepads with more lamellae than non-arboreal anoles. For PC II, the ANOVA indicates that non-arboreal lizards have significantly wider toepads given their number of lamellae than do arboreal lizards (or put differently, arboreal lizards have dense pads relative to their toepad width). The phylogenetic ANOVA on M2 species revealed no significant differences between arboreal and non-arboreal specialists in any toepad traits (either unrotated or rotated; results not shown but available upon request).

Table 3: Phylogenetic ANOVA results for Caribbean species. P-values for traits in which arboreal and non-arboreal lizards were significantly different ($P < 0.05$) are in bold.

Trait	Non-arboreal Mean	Arboreal Mean	F ratio	P-value
PC I	-6.079	9.698	63.709	**<0.001**
PC II	5.559	-0.318	21.615	**0.029**
Hindfoot Pad Width	-0.006	0.097	16.494	0.053
Forefoot Pad Width	-0.010	0.103	24.147	**0.016**
Hindfoot Lamella #	-0.082	0.064	40.916	**0.005**
Forefoot Lamella #	-0.118	0.105	118.780	**<0.001**

Comparison of mainland and island species along PC I showed that in general, Caribbean anoles have wider toepads with more lamellae than mainland anoles (*Chamaelinorops barbouri*, a Caribbean anole with a very low PC I score, is an outlier to this pattern;[2] Fig. 2). Comparisons on PC II revealed a large amount of overlap in the values of Caribbean and

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[2] *Chamaelinorops barbouri* differs from most Caribbean anoles ecologically, in that it is a leaf litter specialist. It is completely terrestrial, which may account for its low PCI score (Flores *et al.* 1994).

mainland taxa. However, there are a few mainland anoles that had higher PC II scores than any Caribbean taxon, indicating these species have wide toepads relative to their lamella number. In contrast, with the exception of a few mainland species (discussed below), most of the species that exhibited highly negative PC II scores are Caribbean. These are taxa that have many lamellae relative to pad width. So while the overall range of values did not differ greatly between the two radiations for PC II, the distribution of species within this range differs. Scatterplots of PC I and PC II in which the M2 clade is compared to its Caribbean sister clades revealed that on one hand, the Cuban *sagrei* radiation is almost completely overlapped by M2 species in toepad morphospace. On the other hand, however, the Jamaican radiation is fairly distinct from both the M2 and *sagrei* radiations along these axes (results not shown but available upon request).

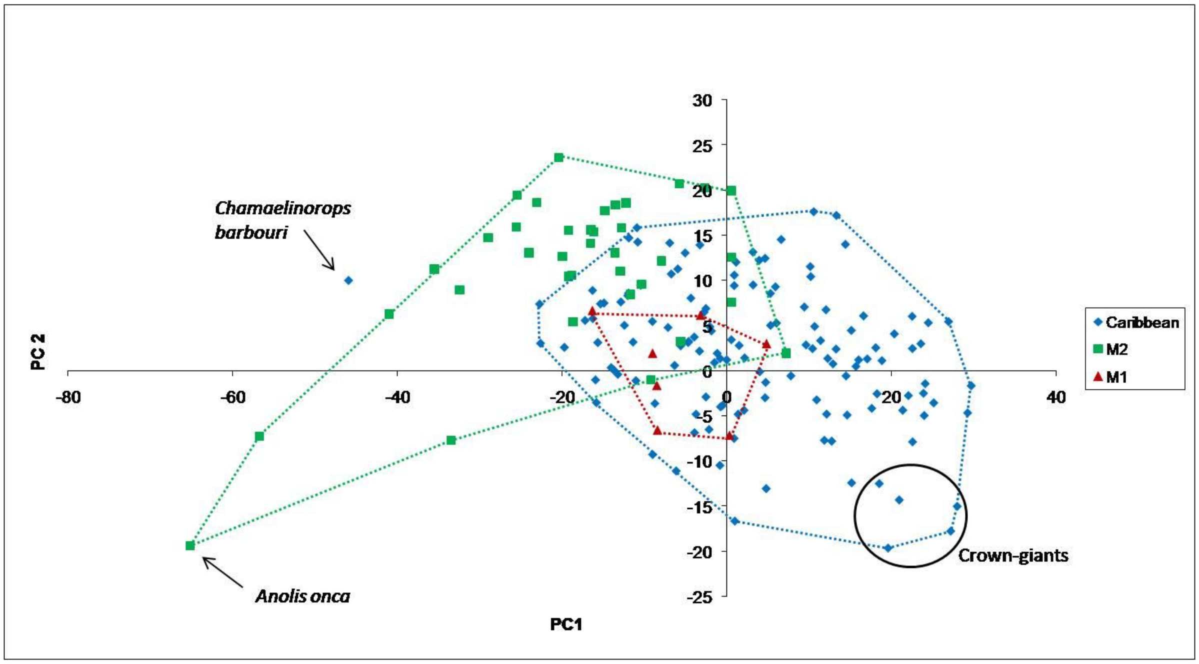

Fig. 2: Scatterplot of PC II vs. PC I in the mainland and Caribbean clades. All four measured toepad traits were positively correlated with PC I. PC II is positively correlated with toepad width and negatively correlated with lamella number.

Discussion

Comparison of phylogenetic signal for toepad traits in mainland versus Caribbean anole radiations supported our hypothesis that convergence is more prevalent in the Caribbean: in general, Caribbean anoles exhibited lower phylogenetic signal than anoles in the M2 clade. Although we did not directly compare the Caribbean and mainland phylogenetic signal values statistically, we observed higher signal in the mainland clade than in the Caribbean clade for most of the traits investigated (Table 2).

However, we did test whether empirical K values were significantly different from Brownian motion evolution (K = 1). Although none of the phylogenetic signal measurements were significantly different from 1, for some traits, the mainland and Caribbean values themselves were very different. This is true of PC I, PC II, and especially hindfoot pad width. For hindfoot pad width, the mainland phylogenetic signal was high ($K = 1.702$) and nearly significantly different from 1 ($p = 0.056$). In contrast, the Caribbean signal was low ($K = 0.721$). By comparison, M2 and Caribbean anoles exhibited more similar levels of phylogenetic signal for lamella number. Although many factors may influence phylogenetic signal, one possibility is that convergence in toepad characteristics among Caribbean species may have depressed phylogenetic signal or at least prevented it from becoming elevated, whereas toepad conservatism may have caused it to be elevated it in the mainland radiation. This is not indicated for lamella number, in which mainland and Caribbean anoles do not differ as strongly.

We note that our investigation of phylogenetic signal likely suffered from low power, as the M2 clade was represented by only 37 species due to phylogenetic sampling limitations. It will be interesting to see if increased future sampling clarifies the pattern we have observed here.

To investigate whether convergence in mainland and island anoles was adaptive, we used a phylogenetic ANOVA to test whether groups of ecological specialists had converged in toepad morphology. The results of the phylogenetic ANOVA confirmed previous findings of significant adaptive convergence in the Caribbean (e.g. Losos 1992; Losos et al. 1998): arboreal lizards (which perch higher) tend to have wider toepads with more lamellae than non-arboreal lizards (see PC I and all toepad traits in Table 3). This result is concordant with previous findings that larger toepads confer increased clinging ability, which is functionally important in arboreal lizards (Elstrott and Irschick 2004). In contrast, the phylogenetic ANOVA of mainland ecological specialists revealed no significant differences between arboreal and non-arboreal M2 anoles in toepad characteristics. Unlike Caribbean anoles, mainland arboreal and terrestrial anoles have not repeatedly converged onto similar, specialist toepad morphologies. As mentioned previously, there are very few habitat data available for many mainland species, and this may have confounded our efforts to classify these species as ecological specialists. However, the lack of convergent arboreal and terrestrial specialists in the mainland is consistent with the results of our analysis of phylogenetic signal.

As with previous studies (Macrini et al. 2003; Velasco and Herrel 2007; Pinto et al. 2008), we found that Caribbean and mainland anoles overlapped somewhat in toepad morphospace, but also exhibited systematic differences, primarily along PC I (lamella number and toepad width), but also to a lesser extent along PC II (lamella density). In general, Caribbean anoles had wider toepads with greater numbers of lamellae, and had denser lamellae too. A few M2 taxa (mostly ground dwelling species) exhibited very high pad width / lamella number ratios that were higher than those found in the Caribbean, probably the result of extreme reduction of lamella number and an absolute constraint on reduction of pad width. Also, some of the Caribbean species showed extremely low values for PC II indicating that their toepads are particularly dense (lots of lamellae) given their width (e.g., the highly arboreal, "sticky fingered" crown giant anoles, circled in Fig. 2). Few mainland taxa have very low values on PC II, although *Anolis onca* and its sister species *A. annectans* are exceptions. The beach-dwelling *Anolis onca* is the only anole without setae or expanded toepads, and *A. annectans* is somewhat intermediate between *A. onca* and other anoles in this regard (Williams 1974; Peterson and Williams 1981; Nicholson et al. 2005). In this case, these anoles also have fairly few lamellae and the low PC II values illustrate just how narrow their toes are.

Taken together, the data suggest that there are fundamental differences in both the prevalence of convergence and in gross toepad morphology between mainland and Caribbean anoles. Compared to the Caribbean, mainland toepads are less specialized for arboreality and apparently exhibit less convergence.

One potential explanation for these patterns is that mainland anoles have radiated with an inherited constraint in toepad morphology. Perhaps the colonizing M2 lineage arose from a Caribbean lineage with poorly developed toepads and reduced evolutionary potential. If this were the case, we would expect the Caribbean relatives of the M2 clade to exhibit “mainland-like” toes. We tested this by investigating the Jamaican anoles, which are likely sister to M2, as well as the Cuban *sagrei*-group anoles, which are the next closest relatives to both. We found little support for the constraint hypothesis. Although the *sagrei*-group anoles largely overlapped with M2, the Jamaican clade exhibited patently “Caribbean” arboreal toepad morphologies. This implicates an ecological cause instead of a phylogenetic constraint.

The question remains then: why are Caribbean anoles more convergent, and apparently better specialized for aboreality?

One factor that may affect the toepad characteristics themselves is the difference in behavior between the mainland and Caribbean lizards. Mainland anoles do not move as much as Caribbean anoles (A. Herrel 2009; personal observation). This could be due to differences in population densities and predator evasion tactics between the mainland and the Caribbean, which Macrini et al. (2003) suggested could select for different toepad morphologies in each radiation. In fact, Losos et al. (2006b) demonstrated that changes in predation regime may trigger behavioral changes in habitat use, which can dramatically alter selection on morphology. Therefore, it is possible that a different predation regime in the mainland is causing mainland anoles to use their habitat differently, which may select for different toepad morphologies.

Another possible explanation for both the observed differences in the toepad characteristics and the prevalence of convergence is that the two radiations are using different morphological strategies to tackle the same ecological problems. If one only examines toepads, it appears that mainland anoles, with their smaller, less dense toepads, do not cling as well as Caribbean anoles. However, mainland anoles may have diversified into arboreal niches by evolving sharper claws (e.g., Crandell 2009) rather than stickier toepads. Perhaps the “missing” convergence in toepad characteristics in mainland anoles is evident in claw diversity, since functional convergence is not always achieved by the evolution of convergent morphologies (Wainwright et al. 2005).

Variance in habitat use might also account for the differences in levels of convergence. Greater Antillean ecomorphs are incredibly specialized to the habitats in which they live. They have evolved to occupy perches of specific heights, diameters, and substrate types, and the most remarkable element of their convergence is the repeated evolution of very specific specialist morphologies. Mainland anoles may be comparatively generalized in their habitat preferences (A. Herrel, 2009; personal observation). It therefore may not benefit mainland anoles to specialize on microhabitats, and this may stymie convergence.

At present, more ecological and behavioral data on mainland anoles are needed before it will be possible to distinguish among these hypotheses. In addition, there is also a tremendous need for additional molecular phylogenetic hypotheses that include a broader diversity of mainland anole species. This will allow better and more accurate tests of phylogenetic signal and will facilitate more sophisticated investigation of convergence hypotheses. Having established that mainland and Caribbean anoles differ in convergence and toepad morphology, the next step will be to determine whether more extensive sampling can help us understand these differences, and to explore whether differences in ecology and function are ultimately responsible.

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George C. Gorman

Rip Van Winkle attends the *Anolis* Symposium, wakes up, and shares some thoughts

Rip Van Winkle went to sleep for twenty years, woke up, and found himself in an unfamiliar world.

I last engaged in primary *Anolis* (or any herpetological) research thirty years ago, suddenly saw the light, woke up, drove to Cambridge, and listened to *Anolis* papers for two days. In many ways it was surprisingly familiar. But DNA hadn't been invented when I left the laboratory, so I have to take it on faith that boots are strapped on correctly; and it never occurred to me that there might be a gizmo to determine how hard an *Anolis* bites. I simply knew the little guys couldn't hurt me, and the big boys could. What surprised me the most were (1) Manuel Leal's story on homing in *A. gundlachi* – I just didn't think that's what anoles would do; (2) the amazing advances in herpetoculture such that anoles can be bred en masse and utilized experimentally for understanding genetics and development; and (3) the sheer number of people engaged in the study of the *Anolis* clade (dare I say "genus"?).

But it was an old piece of unpublished research that got my juices running. Specifically, Robert Holt, a distinguished theoretical ecologist from the University of Florida inadvertently gave me the kick in the rear to re-tell this story. As Bob related to us, he got a great job offer while in graduate school and therefore truncated his thesis, never presenting his provocative results on West Indian anoles.

Much of that work was directly stimulated by the tale that will unfold in this note.

My purpose in this letter is to review the body of my work on the "Mule Lizards of Trinidad" … the story of interspecific competition and hybridization between *Anolis trinitatis* and *Anolis aeneus*. This exercise is not so much to promote my old obscure papers, but to stimulate possible further research. I believe there were interesting, open questions raised by that work that could have been, but have not been followed-up. If one were to ask me about my most important contributions to anolology (and nobody has!), it would have been working out the details of this interspecific interaction and all that it implied.

A more detailed semi-autobiographical version of "The mule lizards of Trinidad" was presented in *Islands and the Sea: Essays in Herpetological Exploration in the West Indies.* Henderson and Powell, Eds. (Gorman 2003). At the risk of redundancy (this version being free and on-line) here's the story.

Anolis trinitatis and *Anolis aeneus* co-occur around homes and gardens in Trinidad. They are reasonably closely related to one another and are part of the southern Lesser Antillean *roquet* clade of *latifrons* series alpha *Anolis* (or *Dactyloa,* take your pick).

A 1959 Bulletin of the MCZ sorted out the fact that there were indeed two common "garden" anoles on Trinidad, a green one (*trinitatis*) and a grey mottled one *(aeneus*) (*A. trinitatis* had been described in 1862 by Reinhardt and Lutken but it was more or less forgotten; most Trinidad species lists included only *aeneus).* In that 1959 bulletin, Underwood (1959) synonomized *A. trinitatis* with *A. vincenti,* of St. Vincent. Quesnel and Kenny (1959) mapped the distribution of these two anoles in Trinidad's urban and garden areas, and found, curiously, that they seemed to exist in pure enclaves of one species or the other. There was no obvious ecological separation between the two. These Trinidad-based scientists also pointed out that there were occasional areas of contact, and even hinted that there might be hybrids.

In October 1963, I went to Trinidad specifically to look for behavioral and ecological isolating mechanisms between these two species (very fashionable at the time). I probed them with the then-popular Schultheiss thermometers; recorded perch heights and perch diameters, and could find nothing that obviously separated the species by thermal or structural niche. I could not suggest why different patches of trees or gardens would house one species and not the other. I did find that the stereotyped territorial displays to intruding males were different between the species. But that fact meant little in terms of interactions. In staged encounters, it seemed to make no differenced whether the "intruder" was a conspecific or a heterospecific in eliciting a display from the resident.

In December, I decided to look at these lizards on the mainland. I flew to nearby eastern Venezuela. It did not bother me that neither species had been recorded from Venezuela. Trinidad is a classic continental island whose northwestern arm is only 20 km or so from the nearest part of mainland South America. Almost every bird, lizard, frog, snake, and mammal species known from Trinidad can be found in Venezuela. On that trip, I saw many of the familiar lizards of Trinidad, the two common *Gonatodes, Cnemidophorus, Ameiva, Thecadactylus* etc. [note: to protect my memory functions, the preceding generic names have not been modernized. I have no idea what they are called these days. Once *Bufo* stopped being *Bufo*, I gave up.] But, I failed to find any *roquet*-group *Anolis* in Venezuela.

Then early in 1964 I began exploring the Lesser Antilles, paying particular attention to St. Vincent (*trinitatis*) and the Grenada bank (*aeneus*).

On those islands, *A. trinitatis* and *A. aeneus* are found around homes and gardens (just as on Trinidad) but they were also found in just about every habitat, natural and disturbed. They also are each sympatric and syntopic with a much larger island endemic - *A. griseus* on St. Vincent and *A. richardi* on the Grenada bank. (Parenthetically, on Grenada the two sympatric species differ in structural niche and thermal niche, per Schoener and Gorman, 1968; the same is almost certainly true on St. Vincent but was not formally documented by us). This means that both Trinidad species can coexist with sympatric anoles at some level.

In the spring of 1964 I had my first "Aha!" moment. I had been attracted to working in Trinidad because of the diversity of charismatic tropical fauna, and hoped to spend lots of time in the montane forests observing anoles while fighting off ocelots and bushmasters. But I could never find "my" anoles in anything approaching a natural situation. (There is a small, somewhat uncommon, "trunk-ground" anole in the Trinidad forests, *Anolis chrysolepis* [a.k.a. *Norops*] but it is not part of our story).

It dawned on me that these were Lesser Antillean endemics that were probably introduced to Trinidad by man, relatively recently. (Later I collected in British Guiana, now Guyana, where Beebe (1944) had correctly recorded *A. aeneus*. They were still present, but again, only in gardens, around homes, and in disturbed sites, not surrounding forest. Since there was substantial inter-island and island-to-Guyana trade, including many flower pots containing ornamental plants (and, presumably lizards or their eggs) moving between territories of the British Caribbean, it is not at all surprising that *A. aeneus* was transported to Trinidad and Guyana).

Now, let's integrate the unpublished study of Robert Holt into this picture. We'll come back to the two-species/hybridization issues soon. What I'm about to explore would be just as valid if there were one garden anole on Trinidad and Guyana.

Digression I: Implications for anoline "communities" on the mainland?

Holt's data on Trinidad showed that even in non-pristine lowland areas, once a certain minimum patch size of forest was reached, the backyard "garden anoles" seemed to be absent. We know it is difficult to prove a negative, but my abundant, if unsystematic, observations on Trinidad also indicated that these lizards had not penetrated forest. This pattern was repeated in Guyana. In any sizeable patch of forested habitat these anoles were excluded.

The important point to reflect upon is that the "garden anoles" are absent from perfectly suitable structural-climatic Anolis *habitat.* On an oceanic West Indian island of comparable-appearing patches, there would be dense populations of anoles. The Trinidad and Guyana forests, of course, would have a much greater number of species of virtually all other taxa from trees to birds to snakes to insects. Yet on the continental island of Trinidad, two oceanic island species that are broadly distributed on their home islands, and can thrive sympatrically with a closely related congener, both fail to find a forest home.

This is a sample size of one (or two) failed forest invasions. But the outcome suggests that the ability of *Anolis* to establish itself on a particular mainland site may not necessarily be driven by the presence or absence of other *Anolis*. The failure to establish populations in "natural" forest conditions is probably explained by some combination of competition from members of unrelated taxa and a host of possible predators that are absent from oceanic islands.

Potential replicate ecological studies:

A quick Google search indicated that *Anolis marmoratus* from the French island of Guadeloupe has been introduced to Cayenne (now French Guiana); and *A. lineatus* of Curacao and Aruba, formerly Dutch colonies, has been introduced

> to (the formerly Dutch) Suriname. Colonizations follow colonial history. It would be interesting to look at the ecology and distribution of these Antillean island anoles to see whether the Trinidad pattern is repeated….inability to penetrate natural habitats.
>
> The underlying explanation of repeated patterns of sympatric ecomorphs on Greater Antillean islands is one of character displacement driven by competition from congeners. We should be open to the possibility that anole "communities" in various mainland sites will have had their independent and complex histories shaped not by congeners but by non-anoline predators and competitors. Thus we might expect much lower similarity of ecomorph communities on the mainland, because of dissimilar histories.

Returning to the narrative:

We confirmed that the two Trinidad garden anoles *tended* to live in pure single-species enclaves, but that indeed there were contact zones, several of which we then studied extensively.

Close observation of overlap areas between *A. aeneus* and *A. trinitatis* revealed "funny looking" individuals that appeared intermediate in color and pattern. In fact, these funny looking specimens tended to be reasonably frequent if both species were abundant. Using gel electrophoresis of proteins in the laboratory of Herbert Dessauer of L.S.U. Medical School, we were able to demonstrate species-specific allelic differences between *trinitatis* and *aeneus*. Our visual identification of funny looking individuals was accurate. They looked odd because they were hybrids, heterozygous for alleles that were species-specific markers (Gorman and Dessauer, 1965)

In the autumn of 1964, I went to the MCZ, presumably to spend one year, and absorb what I could from Ernest Williams. (I ended up spending three years there and finishing my Ph.D under EEW, but that's another story). I thought it might be useful to look at the chromosomes (karyotypes) of *A. trinitatis* and *A. aeneus*, with the thought that I might find another marker to distinguish the species.

There was no reason for optimism. Most of the published literature up until that time implied that karyotypes were conservative within genera and even families of lizards (a notable exception being the true chameleons; Matthey, 1949).

As a naïve beginner I made a truly lucky choice of species to examine. I had a fairly extensive collection of living anoles in the MCZ (those were the days….no permits required to collect or transport lizards in bags on airplanes; no animal welfare nutritionists telling me how many crickets to feed to each juvenile). I decided NOT to choose *trinitatis* or *aeneus* as my first guinea pigs, because they were valuable to me, and I had no idea what I was doing. So I chose unrelated species from outside the *roquet* clade.

Had I chosen within *roquet,* and had I been successful in obtaining karyotypes, I almost certainly would have ended the investigation right there. I would have concluded that the karyotypes were the same as *A. carolinensis* (the only anole that had a published karyotype up to that time). But in starting with members of the *bimaculatus* group of the northern Lesser

Antilles, and "Beta" anoles (now assigned to *Norops),* I found huge differences in karyotype among species (Gorman 1965). This led to a productive line of research that now seems dead...but whose resurrection I will encourage, a few paragraphs from now.

After I became reasonably proficient at extracting karyotype information from lizards, I examined *trinitatis, aeneus* and the hybrids. The parental species differed in diploid number (*aeneus* had one fewer pair of small chromosomes, referred to in the literature as microchromosomes). Much more significant was an examination of meiosis in the hybrid males. It was a mess. The chromosomes could not pair up and seemed, literally, to get "stuck" at meiotic metaphase.

It thus looked like we had a case of two similar sympatric species (both introduced) that differed in body and dewlap color, pattern, and display action patterns…yet none of those traits worked as prezygotic isolating mechanisms. Where they came together they interbred. But, we now had a clue that there were strong post-zygotic isolating mechanisms (Gorman and Atkins, 1968). Note: In the absence of prezygotic isolating mechanisms, and with a high level of sterility in the hybrids, in any given population, the more abundant species would be predicted to drive the less abundant to extinction. This is a pattern that we confirmed qualitatively (Gorman and Boos, 1972), and that Holt demonstrated more formally in his talk at the symposium. This appears to explain the pattern of single species enclaves around the homes and gardens of lowland Trinidad.

Digression II: Why study lizard karyotypes today when we can drown in sequence data?

The amount of "genetic distance" no matter how measured, does not tell us what would happen if two populations that evolved in allopatry come into sympatry. If, under "natural" conditions, they could and would exchange genes, they would belong to the same species (per the late Ernst Mayr). Study of the karyotype and meiosis in the Trinidad garden anoles, and not a measure of the sequences of C's, A's, T's and G's is what told us that these were valid species; and why they were species (strong post-zygotic isolating mechanisms). Chromosome data *may* be able to resolve some species questions.

And it *may* be helpful at higher levels.

In our surveys, we found karyotype very useful within certain species groups. For example, within the *cristatellus* clade, we were able to support the close relationship between Puerto Rico bank *cristatellus* and *scriptus* of the southernmost Bahamas (and Turks and Caicos), the distinctiveness of *cooki* from *cristatellus*, and the absolute separation between the *cristatellus* clade of Puerto Rico and the cybotoids of Hispaniola.

I make no claim that karyotypes will be universally helpful, but their study might provide insights into natural groupings of mainland anoles of the *latifrons* series (*Dactyloa).*

Moreover, it is at least a possibility that the lability of the anoline karyotype - that is...its very ability to evolve - may *enable* speciation. I suggest that there might be some interesting information available to an investigator who employed modern techniques to examine anoline chromosomes.

Back to Trinidad: Reproductive biology

Once we firmly established that hybridization occurred wherever the parental populations overlapped, we followed up with several studies of reproductive biology of the parental species and the hybrids (Gorman et al. 1971; Gorman and Boos. 1972; Gorman and Licht 1973; Gorman and Licht, 1975). It turns out that hybrid males appear perfectly normal in body size and proportions. They also behave as typical males and defend territories. But they have very reduced testis size, and very low sperm production. The female hybrids are most curious. They are much larger than the females of either parental species, although not so large as males, and without well-developed dewlaps. We found a small percentage with oviductal eggs (viability was not assessed), but in general in the height of the wet season when the parental species females are pumping out eggs such that in any collection a large percentage would have at least one oviductal egg, the typical "giant" female hybrids would have enormous abdominal fat pads and no developing egg.

We were not able to solve why the female hybrids were disproportionately large. Now that *Anolis* husbandry and embryology have progressed to degrees unimagined in the 1970s, it might be worth creating *aeneus-trinitatis* hybrids in the lab, to examine development. (Unfortunately, the *roquet* clade did not have readily identifiable sex chromosome heteromorphism, but perhaps advances in karyology since Rip van Winkle plied his trade would allow an investigator to determine whether he/she was looking at a male or female embryo).

Finally, study of reproductive cycles of *A. trinitatis* and *A aeneus* provided the first clue of a eco-physiological difference between the populations that suggests the possibility that *aeneus* may have a slight competitive advantage over *trinitatis*, and may ultimately replace *trinitatis* (Gorman and Licht, 1975).

In summary, the time might be ripe to revisit the interactions of *A. aeneus* and *A. trinitatis* on Trinidad to determine whether there have been changes in geographic distribution and patterns of overlap. Creation of hybrids in captivity could help us better understand the basis of size sexual dimorphism and cytological aspects of reproductive failure. Behavioral studies on territoriality and mate choice could clarify whether dewlap color differences and display differences are "noise" or potentially might serve as isolating mechanisms. Finally, a renewed systematic examination of karyotypes, especially of mainland species, might provide valuable cladistic insights.

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*Addendum

After submitting a draft to the editors of the *Anolis* Newsletter, Rip van Winkle received, from those editors, a PDF of a recent paper (Hailey et al. 2009) that specifically addressed the issues of the micro-distribution and persistence of *Anolis trinitatis* and *A. aeneus* on Trinidad. They specifically re-censused localities sampled about 40 years ago.

Most heartwarming was that the *et als* were, (1) Victor Quesnel, whose published paper 50 years earlier got me started on this problem; and Hans Boos, who along with his brother Julius Boos, were active participants in my field work in the 1960's. This is a remarkable persistence of interest by these naturalists.

What was somewhat distressing to Rip, in terms of his own aging process, was that he had provided information to the authors via personal communication in 2006, and even after the memory jog of seeing their paper in print, he had only the vaguest recall that this research had been in progress.

In Hailey et al., the authors quantified niche overlap, and confirmed almost no difference in structural niche. But the evidence suggests that the population of *A. trinitatis* is declining.

"Th(e) pattern suggests that the decline of *A. trinitatis* is not related to hybridization with or competition from *A. aeneus*. An alternative hypothesis is presented, that *A. trinitatis* requires more vegetated habitats, which have been increasingly lost during urban development especially in northern Trinidad."

Without getting too defensive, I would like to make three comments.

(1) If there are no interspecific prezygotic isolating mechanisms, as appeared to be the case in our early studies, and if the f1 is of greatly reduced, nearly zero, fitness, then the commoner species will always displace the rarer species in overlap areas. Thus hybridization would *contribute* to the decline of the rarer species in overlap areas. The less favorable the habitat for *A. trinitatis,* the greater its decline, the rarer it becomes, and the faster that hybridization would push it to extinction.

(2) Our study of reproductive cycles of the two species on Trinidad (Gorman and Licht, 1975) was referred to but not discussed in the body of the text of this newsletter contribution. Our findings were consistent with the hypothesis of Hailey et al. Specifically, we noted that *Anolis trinitatis* did not seem adapted to the seasonality of Trinidad, and that it wasted reproductive effort by being reproductively active in the dry season, when *A. aeneus* shut down. Thus *A. trinitatis* would be expected to decrease with the decline of shady, moist, vegetated habitats.

(3) Hailey et al. found hybrids but failed to find evidence that female hybrids are significantly larger than females of the parental species. This was very striking in our studies (see Fig. 6, p.13 of Gorman et al. 1971). Creation and captive rearing of hybrids might shed some light on this.

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Hailey, A., Quesnel, V. C., and H. E. A. Boos. 2009. The persistence of *Anolis trinitatis* as a naturalized lizard in Trinidad against hybridization pressure with *Anolis aeneus. Applied Herpetology 6 (2009) 275–294* www.brill.nl/ah

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lizardbase: A new collaborative resource for lizard biology
URL: http://www.lizardbase.org/

Since the publication of the previous *Anolis Newsletter* (V) over a decade ago, much in biology has changed. Novel tools and resources enable a deeper and more sophisticated level of biological exploration than was previously imagined. The recent assembly of the *Anolis carolinensis* genome, developments in molecular approaches, advances in geospatial information systems (GIS), and innovations in communication technologies have provided biologists with an expansive toolbox of resources to approach science in a multi-faceted, multi-disciplinary manner.

While this is an exciting time, we are now facing new challenges of managing and integrating vast quantities of data that continue to be generated at an exponential rate. Lizards are emerging as important biological models: with the availability of new data and data types from an ever-expanding community, a need is quickly becoming apparent to develop new community-driven database resources. The benefits of centralized public resources have been demonstrated by large genomic databanks such as NCBI and Ensembl, as well as the UCSC Genome Browser, and these resources have been instrumental in efficiently providing high-throughput data. Yet for most model taxa, additional genetic resources (e.g., FlyBase, WormBase, TAIR, SGD, MGI), were specially designed to cater to the particular, nuanced needs of a specific community.

With the mission to provide a centralized depository for lizard-related genomic and organismal data that will ultimately facilitate scientific creativity and increased collaboration, we are developing an online resource called *lizardbase*. This database will increase the ease of data access and scientific engagement by a growing community of scientists as well as provide a more comprehensive overview of the diversity of biological questions that are currently being asked by active researchers in the lizard community.

While the diversity of lizard-based research is one of the community's strengths, the previous lack of a relative frame of reference made it difficult to integrate diverse data types. The recent availability of an *Anolis* genome assembly and recent advances in geographic mapping technology enable us to use two common references on to which to map a variety of biological data. On an assembled genome, nearly all genetic features such as gene annotations, genomic resources (ESTs, microarray probes, etc.), miRNAs, transposable elements, SNPs, and orthologs from other species can be readily mapped. On geographic maps, we can geospatially relate an array of organismal and ecological data onto precise geographical coordinates (i.e., longitude and latitude). It is *via* these two gateways or portals that *lizardbase* users can gain access to an assortment of biological data types.

Our "Genomics Portal" offers an entryway to view functional annotations and features as well as comparative and, eventually, population genomics data. New lightweight bioinformatics tools including a genome browser (JBrowse) and a Blast portal (NCBI's Blast+), in addition to an FTP site to download annotated datasets, will be available for users. While there are certainly several other online databases and websites available for geneticists and genomicists (Table 1), *lizardbase*'s Genomics Portal will provide genomic data catered to the broad lizard community, as well as provide a curated platform for the influx of new data directly from the community. Our primary focus is to provide new mapped data that is not available in the other large databanks. We are also working closely with the new lizard gene expression database, *Anolisgenome.org* (see accompanying article by Kusumi and Wilson-Rawls in this *Newsletter*) in a joint effort to create complementary resources for the anole community. Our "Data Mapping Portal" employs an open-source software server (GeoServer, http://geoserver.org/) that provides a GIS interface for rapid selection and visualization of large volumes of data. Using this architecture, data can be easily shared, visualized, and edited using a geospatial platform. Data will include organismal (e.g., physiology, morphology, biomechanics), population-level (e.g., behavioral assays, allele frequencies, environmental), and species-level (e.g., species densities, distributions) information, contributed by individual researchers. These data will be curated and collated in an online database that will be freely accessible to the entire community. We expect to implement database query engines sometime this year (2010) to help facilitate advanced searches. Our goal is to make this portal easily accessible to all levels, so that we can develop outreach curricula and recruit K-12 students to assist in broad-scale data collection. These students can help advance science through "citizen science" while learning about the scientific process as part of their basic education.

With these two portals, *lizardbase* aims to provide a community-driven platform for both collaboration and hypothesis building. Yet *lizardbase*'s most promising strength is that it not only serves as an entryway into various aspects of lizard biology, but it can also *integrate* data of various types in novel ways. For example, behavioral data can readily be correlated to population genetic data, and examined for geographical patterns. Species-level distribution data can be readily compared to urbanization trends using existing layers of GIS data. The potential for these data is enormous and we hope that *lizardbase* will help encourage data exploration and the generation of new hypotheses to test.

lizardbase is presently in its initial development phase. Our first public release is slated for January 2010. We hope that this database will become an integrated repository for novel and unique data types in all squamates, as well as encourage collaborations between different disciplines. Other features in *lizardbase*, including a curated Forum, Meetings directory, and Linkouts will serve to help the community to navigate through an always expanding list of discussion points and resources. We plan on maintaining *lizardbase* as user-friendly and extensible as possible. As high throughput biology takes hold, we hope that *lizardbase* will become a useful domain for the entire lizard community.

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We would like to thank Ed Fuquen for initially setting up the website as well as Kenro Kusumi and Thom Sanger for suggestions in early stages of *lizardbase* development.

Table 1. Current database resources for the lizard community.

Database	*A. carolinensis* Genomic Data	Other squamates?	Community-driven?*	Gene Models	Ecological/ Organismal
NCBI	yes	yes	no	yes	no
Ensembl	yes	no	no	yes	no
Broad	yes	no	no	no	no
UCSC	yes	no	no	yes	no
Anolisgenome	yes	no	yes	yes	no
lizardbase	**yes**	**yes**	**yes**	**yes**	**yes**

* Does the data structure reflect needs of the lizard community?

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Proposed study of potential impacts of climate warming on Puerto Rican *Anolis*

Landmark studies have documented that climate warming is having pronounced effects on the ranges, phenologies, interactions, and survival of organisms (Pounds et al. 1999, Parmesan and Yohe 2003, Root et al. 2003). Effects of warming on terrestrial species have been predicted to increase with latitude (Root et al. 2003, Parmesan 2007, Lee and Jetz 2008) because warming is faster at higher latitudes (IPCC 2007). Nevertheless, several empirical (Pounds et al. 1999, Clark et al. 2003, Burrowes et al. 2004, Whitfield et al. 2007, Raxworthy et al. 2008) and theoretical (Deutsch et al. 2008, Tewksbury et al. 2008, Huey et al. 2009, Kearney et al. 2009) studies have made a surprising prediction: *Although absolute rates of tropical warming may be relatively slow, many tropical organisms will be strongly and negatively affected by warming*.

What explains this apparent paradox? The biological relevance of any rate of climate change depends on the local contemporary variability; and climate variability is of course relatively low in the tropics (Williams et al. 2007). The impact of climate change also depends on the behavior, physiology, ecology, and genetics of organisms. Our recent studies (Deutsch et al. 2008, Tewksbury et al. 2008, Huey et al. 2009) propose that tropical forest ectotherms (those residing beneath the canopy, such as many *Anolis*) are especially vulnerable because they ***(a)*** are thermal specialists and thus relatively sensitive to temperature change, ***(b)*** have surprisingly *low* heat tolerance, ***(c)*** live in 'operative' environments that are already warm relative to their low thermal optima, ***(d)*** have few opportunities for behavioral evasion, **(e)** may have limited acclimation capacities, and ***(f)*** may face increased competition and predation from open-habitat (warm-adapted) ectotherms that can now invade warmed forests.

To test the prediction that warming has already had an impact on tropical ectotherms, we have assembled a team starting with five biologists (Álvarez, Gorman, Hertz, Huey, Lister) who intensively studied the thermal and reproductive ecology of Puerto Rican lizards in the '70s, '80s, and early '90s (e.g., Gorman and Licht 1974, Huey 1974, Lister 1981, Álvarez 1992, Hertz 1992). We add a climatologist (Deutsch), who will elucidate patterns of recent Caribbean warming, as well as three biologists (Pringle, Tewksbury, Wingfield) who bring diverse ecological and physiological expertise.

We are proposing to exploit detailed, baseline eco-physiological data (above) that we collected decades ago on multiple populations of two *Anolis* species in Puerto Rico. We hope to replicate our prior studies, compare old vs. new patterns, and then address the following questions:

(1) *Has female reproduction been reduced by warming summers but increased by warming winters?*
(2) *Are body temperatures (T_b) higher in both summer and winter now, as expected from weather records?*
(3) *Are 'operative' (steady-state) temperatures (T_e) also warmer?*
(4) *Are open-habitat lizards, which require warmer T_b and T_e than forest species, beginning to invade warming forests and therefore increasing competition on forest species?*

We focus on *Anolis* lizards in part because their ecology, behavior, and physiology are exceptionally well known (Roughgarden 1995, Losos 2009) and in part because they play important roles in Caribbean ecosystems (Dial and Roughgarden 1995, Reagan 1996). Importantly, *Anolis* also serve as useful models for other Neotropical forest lizards, with which they share behavioral and thermal traits (Rand and Humphrey 1968, Álvarez 1992, Huey et al. 2009). Because we will replicate our own studies, any changes we detect will not be an artifact of between-worker differences, a potential conundrum for longitudinal studies in conservation (Willis et al. 2007).

We concentrate on *A. gundlachi* and *A. cristatellus*, sit-and-wait predators that are similar in morphology and in perch associations ("trunk-ground ecomorph," Williams 1972, Losos 2009) but are not sister taxa (Poe 2004). They differ in thermal biology (Rand 1964, Heatwole et al. 1969, Huey and Webster 1976, Hertz 1992). They are abundant, easy to observe, and are among the **best-studied lizards in the world** in terms of behavior, ecology, and physiology (reviewed in Losos 2009).

We (Huey et al. 2009) recently used data from the 1970s and 1980s to predict the consequences of warming on the locomotor performance of *A. cristallelus*. Our analyses suggest that a 3°C increase in air temperature will be deleterious to lowland populations, at least in summer. Moreover, observed warming at El Verde may already have been sufficient to cause heat stress to *A. gundlachi* (a forest, low temperature species) and to enable *A. cristatellus* (which requires higher temperatures) to invade the forest.

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The evolution of anole behavior: ecological and physiological approaches

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I am primarily interested in understanding the evolution of behavioral variation in anoles from two perspectives – how behaviors are associated with the ecological contexts in which they are performed, and how variation in the neural, endocrine, and muscular mechanisms that underlie behavior are associated with its evolution. My work integrates these two approaches to address the following questions:

What is the role of habitat use in the evolution of behavior?

Species that occur in similar environments often evolve similar morphological, physiological, and ecological traits; however, whether behaviors evolve as a function of a species' physical environment is not yet clear. In my dissertation work in the lab of Jonathan Losos, I examined whether *Anolis* lizards that use similar habitats evolve similar behavioral strategies using three classes of behavior: territoriality, foraging, and sleep. In our study of territorial behavior, we examined 13 species on four islands and found that structural habitat drives the evolution of territoriality along two axes: species in higher visibility habitats perform more frequent territorial display behaviors, and a species' perch height and diameter (the habitat measures that determine ecomorph categories in this group; Williams 1983) are significant factors in male-male territory overlap (Johnson, Revell, and Losos *in press*). In our study of foraging behavior, we found that ecomorph category (and thus, microhabitat) is strongly associated with rates of movement and prey capture in 31 species (Johnson et al. 2008). In both of these studies, we used phylogenetically-controlled analyses to demonstrat that species utilizing the same microhabitat on different islands have convergently evolved the same behavioral strategies for defending territories and procuring food. Our study of sleep examined sleeping sites of three species of Jamaican anoles. We showed that lizard species that occur in the same locality but are specialized for different microhabitats while active during the day also differ in their sleeping perches and in the dispersal of their sleeping sites; these differences parallel their diurnal habitat use (Singhal et al. 2007).

What neuromuscular and endocrine traits are associated with behavioral evolution?

In collaboration with Juli Wade, I am examining the relationship between sexual dimorphisms in morphology and behavior with dimorphisms in the neural and endocrine traits underlying these traits. In *Anolis* lizards, the dewlap is an important structure used in courtship and territorial behavior. In most species of anoles, including *A. carolinensis*, males have large dewlaps, while females have very small dewlaps. However, species in which males and females have similarly-sized dewlaps also exist, and we have compared dewlap-associated behavior and morphology of these relatively monomorphic species with closely-related species in which the dimorphism is large. Our study included a group of 8 species that included *A. valencienni*, a Jamaican species in which both sexes have large dewlaps, and *A. bahorucoensis*, a species from the Dominican Republic in which both sexes have diminutive dewlaps. We found that within species, males displayed the dewlap more than females, and males generally have larger muscle fibers in the muscles that control dewlap extension and a larger hyoid cartilage supporting the dewlap. However, across species dewlap morphology did not evolve in association with dewlap extension rates (Johnson and Wade *in review*). We are now conducting a parallel study of copulatory structures and behavior in the same group of species.

What behavioral and physiological traits determine reproductive success?

One component of my graduate work was an examination of paternity in the highly territorial Puerto Rican crested anole, *A. cristatellus*. In this study, we tested the hypothesis that males that perform more frequent courtship and territorial displays sire more offspring within their territories than males who display less frequently. In combination with field observational data on display behavior, we used microsatellite markers (Glor et al. 2007) to determine patterns of paternity. We found that in our study population, more than half of females produce offspring with multiple males and “extra-territorial” males account for approximately one-fourth of the paternity. In addition, we found that females appear to preferentially produce offspring with males who are larger and who perform aggressive displays more frequently (Johnson, Fresquez, Kirby, and Wang, *in preparation*).

In addition to determining the behavioral traits that influence reproductive success, I am also working to determine the physiological traits associated with reproductive behavior. In a natural population of *A. carolinensis* from southern Louisiana, during spring 2008 we observed the courtship and copulatory behaviors of adult males, young (but reproductively mature) males, and adult females. After completing the observations, we collected tissue samples from the observed animals to test the hypothesis that variation in neuromuscular and hormonal traits is associated with behavioral differences among individuals. Analyses of these data are underway (Johnson, Vandecar, Cohen, and Wade *in preparation*). Combined with research on multiple species, we hope this work will resolve whether inter- and intraspecific patterns of variation in these traits are consistent, an important step toward understanding how such variation evolves.

Future research: Integrating the study of behavior across multiple levels

In my lab at Trinity University, the main goal of my current and future work is to integrate studies of behavioral mechanisms in both ecological and evolutionary contexts. In particular, I am beginning to examine the interrelationships among neuromuscular morphology and behavior in *A. carolinensis* in differing physical and social environments. We are currently working to determine the extent of natural variation in these traits, and we are planning a series of laboratory and field experiments to determine the role of phenotypic and developmental plasticity in the mechanisms that influence social behaviors.

Also, I am interested in behavioral syndromes in anoles. Behavioral syndromes are correlated suites of behaviors that cause individuals, populations, or species to exhibit consistent differences across ecological situations. For example, one of the most commonly reported such syndromes is an aggression syndrome, in which some animals are consistently more aggressive than others across a range of situations (e.g., feeding, mating, parental care). Given the extensive research performed on some species of anoles, these lizards provide an ideal opportunity to investigate the existence of interspecific syndromes. However, before analyses that determine causal relationships among habitat use and behavioral, performance, and morphological traits can be conducted, we need detailed field behavioral data for many more species. Collecting such data to determine the generality of behavioral relationships is a long-term goal of my research program. Further, by examining the neural, muscular, and endocrine traits that underlie behavioral variation, I hope to determine the extent to which mechanism contributes to behavioral syndromes.

Acknowledgements

I am grateful to Jonathan Losos, my graduate advisor, for sharing with me his enthusiasm for anoles and the exciting evolutionary questions we can address by studying them, and to my postdoctoral advisor, Juli Wade, for introducing me to the wonderful world of behavioral mechanisms in anoles. Also, the students who have worked with me on these projects have taught me a great deal, and much of this work would not have been possible without their dedicated assistance: Jessica Caton, Annie Chen, Carla Fresquez, Becky Kirby, Jason Ladner, Veronica Lopez, Taylor Ramsey, James Shaw, Sonal Singhal, Joe Vandecar, and Su Wang.

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The Culebra Island Giant Anole (*Anolis roosevelti*)

Abstract

The Culebra Island Giant Anole (*Anolis roosevelti*) is known from only two specimens collected for Chapman in the early 1930s, and a handful of additional specimens that had been collected in the 19th century but not correctly identified. In 1977, the federal government listed it as an endangered species and identified its critical habitat. This article reviews what is known about this lizard and the results of a year of field work conducted on Culebra and adjacent islands in 1986. As no specimens were sighted during this study, despite almost daily searches, and none have been reported since, *A. roosevelti* is likely extinct on mainland Culebra and nearby islands.

Introduction

The Culebra Island Giant Anole (*Anolis roosevelti*) is a lizard species of the Puerto Rico bank whose exact native range is unknown. It was named in honor of Theodore Roosevelt, Jr., Governor of Puerto Rico, at the time of its discovery. Only two specimens were originally known to have been collected, both from the main island of Culebra (Grant 1931a, 1931b; Grant 1932). The specimens were collected for Major Chapman Grant in 1931 (1931a, 1931b) and 1932 (Grant 1932) by residents of Culebra. The former specimen (MCZ 36136) is deposited at the Museum of Comparative Zoology, Harvard University, and the latter (UMMZ 73644) in the University of Michigan, Museum of Zoology. *A. roosevelti* was described as brownish-grey in color, with a faint spot on its temple, and two light lines on each side. One line runs from the ear to the groin, and the other from the shoulder to the groin. The eyelids and the posterior quarter of its dewlap are yellow, and the tail is yellowish-brown, with the edge of the tail fin deeply scalloped. Its snout-vent length is approximately 160 mm and it has a bulky head [Grant 1931b; Williams 1962; Rivero 1978; Dodd and Campbell 1982; Dodd 2001), see Figs. 1 & 2].

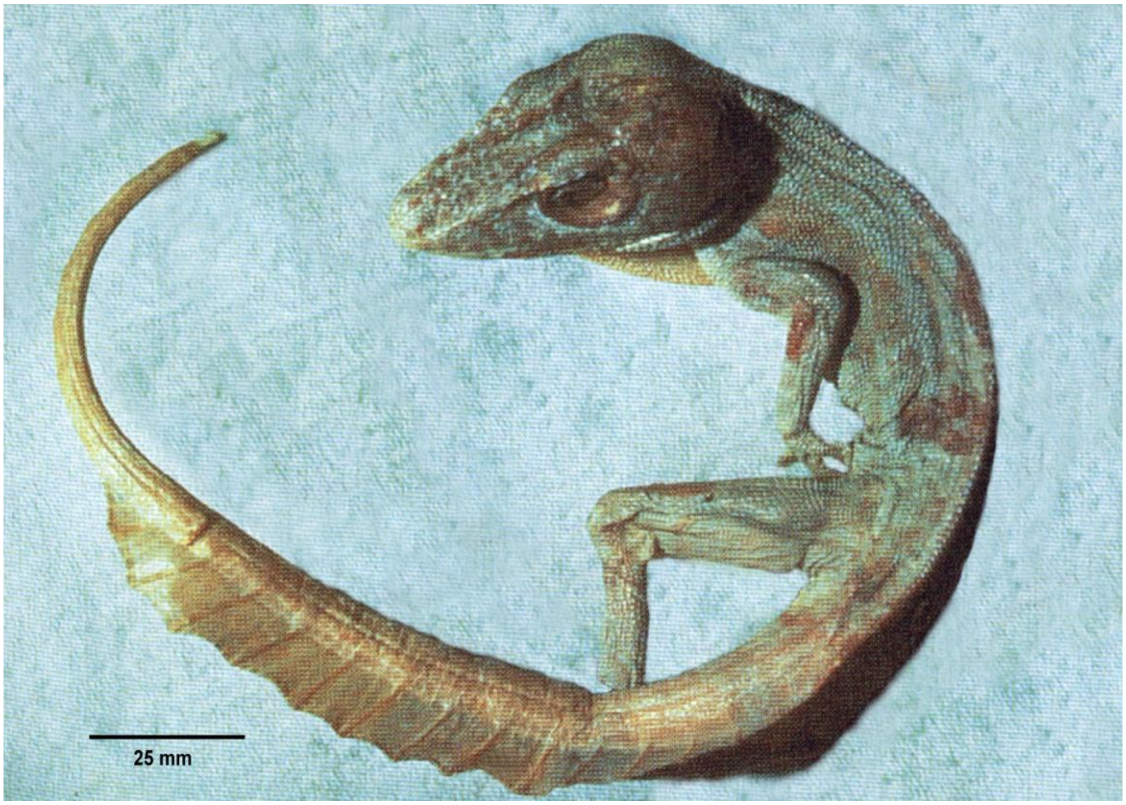

Fig. 1. Culebra Island Giant Anole (*Anolis roosevelti*). Grant's type specimen MCZ 36136 (Grant 1931b) from mainland Culebra. Photograph courtesy of Dr. C. Kenneth Dodd, Jr. and the Museum of Comparative Zoology, Harvard University. Original photograph taken by Dodd appeared in Dodd and Campbell (1982) and Dodd (2001). Photograph was digitally edited by the author to remove flash shadows surrounding the body and tail of this rare specimen.

Data on the life history and behavior of *A. roosevelti* are lacking. Therefore, generalizations based on inferences from evolutionary radiation, ecomorph concepts and anoline phylogeny were used for guidance in the field and for this report (Williams 1962, 1965, 1972, 1978; Losos 2009). Giant anoles are generally canopy inhabitants, although they also forage on the ground and sometimes sleep low on a tree trunk or on low vine tangles at night (Perez-Rivera 1985; Williams 1962, 1965, 1972, 1983). Although the exact niche of this lizard was unknown, it was believed to possibly exist in Culebra's remaining forest in Mount Resaca, its critical habitat. Mount Resaca is part of the Culebra National Wildlife Refuge (CNWR), established in 1909 by President Theodore Roosevelt (see Fig. 3).

In 1977 the U.S. Fish and Wildlife Service (USFWS) declared it endangered under provisions of the Endangered Species Act and identified its critical habitat on the main island of Culebra (USFWS 1977). In 1982, the USFWS developed a recovery plan for this giant anole

(Campbell and Dodd 1982) that was approved in early 1983. However, this plan was never funded (Kenneth Dodd, pers. comm.). In the early- to mid-1980s, there were numerous reports of sightings of this lizard by Culebra residents and visitors from mainland Puerto Rico. In March 1984, a team of biologists from the USFWS and Puerto Rico Department of Natural Resources (PR DNR) spent two days in the presumed critical habitat around Mount Resaca attempting to confirm the giant anole's existence (USFWS memo 12 March 1984). However, no sightings were verified or specimens found. During their time on the island, the team interviewed Mr. Dimas Villanueva who caught the original specimens for Grant (see Results and Discussion sections for details.) In 1985, Dr. Richard Thomas of the University of Puerto Rico and I conducted a field survey for amphibians and reptiles on Cayo Luis Peña with a focus on endangered species, including *A. roosevelti,* but no specimens of this species were found here either. As of 1986, there had not been a long-term systematic search for *A. roosevelti* on the

Fig. 2. Culebra Island Giant Anole (*Anolis roosevelti*). Grant's specimen UMMZ 73644 (Grant 1932) from mainland Culebra. Photograph courtesy of Gregory Schneider and the Museum of Zoology, University of Michigan. (Photographed by Gregory Schneider, September 2009) Photograph was digitally edited by the author to remove flash shadows surrounding the body and tail of this rare specimen.

Culebra Archipelago. The purpose of this article is to review what is known about the original collection and biology of *A. roosevelti*, to summarize the results of my year-long field survey of 1986 (Gaa 1987), and to determine if the Culebra Island Giant Anole still inhabits the main island of Culebra or any of the adjacent keys and islands of the Puerto Rico Bank, including Vieques and St. John (U.S. Virgin Islands).

Materials and Methods

Culebra is the largest island of an archipelago within the larger island chain of the Puerto Rico Bank that extends some 300 km from Puerto Rico in the northwest to Anegada in the southeast. Culebra is located at 18.33°N, 65.33°W or about 27 km east of the big island of Puerto Rico mid-way between it and St. Thomas, U.S. Virgin Islands. Culebra is approximately 11 km long by 5.5 km wide and consists of a main island and 23 smaller islands that lie off its coast. The Culebra National Wildlife Refuge (CNWR) consists of approximately 610 ha including all of the 22 smaller public islands and cays (~ 280 ha) of this 2800 ha archipelago. The largest of these are Culebrita to the east, and Cayo Luis Peña and Cayo Lobo to the west. (Cayo Norte, to the north, is privately-owned and not part of the CNWR.) This archipelago, like other small islands in the area, is arid (rainfall 63-76 cm annually) and contains no rivers or streams. Culebra has an irregular topography and most of the main island is deforested, the main exception being some areas protected by the CNWR. The coast is marked by rocky cliffs, sandy coral beaches, and mangrove (*Rhizophora* spp.) and sea grape (*Coccoloba uvifera)* forests. The interior of the main island consists primarily of grasses, gumbo-limbo (*Bursera simaruba*), Cupey (*Clusia rosea*), fig (*Ficus laevigata*) and thorn brush (*Acacia*) trees, and some areas of semi-xeric woods. The highest point on the island (200 m) is Mount Resaca, part of the lizard's critical habitat (Fig. 3).

I conducted extensive field searches on Culebra and adjacent islands from 1 January through 31 December 1986. The search areas on mainland Culebra and two of its major outlying islands, Cayo Luis Peña and Culebrita are indicated in Fig. 3 and described below, while search areas on Vieques and St. John are only described.

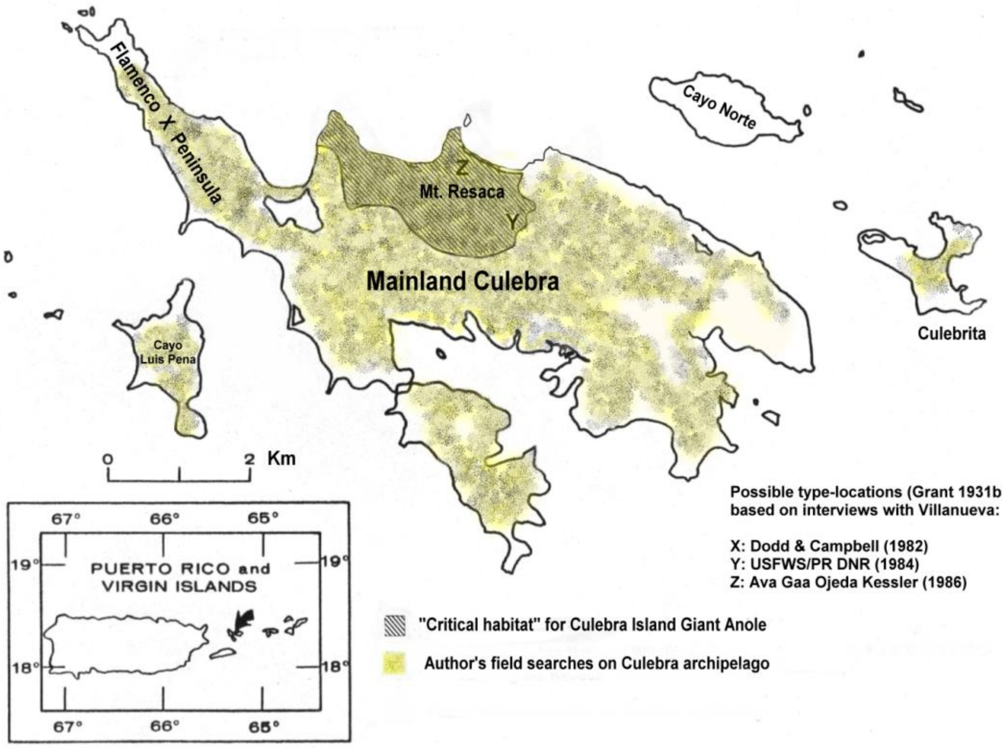

Fig. 3. Map of mainland Culebra showing three of its major outlying islands [Cayo Luis Peña, Cayo Norte (privately-owned) and Culebrita]. Shaded areas indicate section of the Culebra National Wildlife Refuge, including the critical habitat for *Anolis roosevelti*, and yellow/mottled areas searched by the author. Three possible type-locations for Grant's specimen (1931b) are indicated by X, Y and Z (see Discussion section). Original map courtesy of USFWS from Campbell and Dodd (1982) was modified by the author to indicate the above.

The various aspects of the study included the following:

Review of the Literature

Prior to entering the field, I reviewed all available pertinent scientific literature on *A. roosevelti,* convergent evolution, ecomorphs, and the Culebra archipelago.

Interviews

I conducted brief interviews in Spanish and/or English with local residents of Culebra using pictures from Rivero's book (1978), as well as by using live *Anolis* from the island (*A. cristatellus, A. stratulus*, and *A. puichellus*). An immature *Iguana iguana* was shown to interviewees to distinguish between potential sightings of *A. roosevelti* and young *I. iguana.*

Interviews focused on citizens of Culebra who were older than 60 years of age because they would have been youngsters or older during the early 1930s when *A. roosevelti* was discovered and reported by Grant.

Seminar

At the outset of this study, I gave a Spanish-language seminar and slide show on *Anolis* lizards of Puerto Rico at the Culebra public high school to educate students and to point out the differences between anoline lizards and *Iguana iguana* which has been introduced onto Culebra from South America.

Field Searches

Searches for *A. roosevelti* were conducted on mainland Culebra, its adjacent islands of Culebrita and Cayo Luis Peña, Vieques and St. John. Aerial photographs were used to locate potential patches of forested habitats such as mangroves, coastal forest and other subtropical dry forest formations that might support this anoline lizard. A systematic boustrophedon (serpentine) search pattern was used, as well as by selecting random parallel transects oriented from the top of a mountain to sea level and back to the top. This pattern was selected to survey and cover as much area as possible. Diurnal searches were carried out with the aid of binoculars. For safety and efficiency during the nocturnal searches in the "boulder forest" of Mount Resaca, trees were flagged and rocks painted with fluorescent paint during the day to mark the trails. I sometimes returned to the boulder forest at sunset and waited for night fall to start a particular search. For safety and security reasons, night searches in the Mount Resaca area were limited to May, July and August when a field assistant was available from another project.

Field study sites on mainland Culebra:

Mount Resaca: This area was the most extensively searched, for it contains the semi-moist boulder and dry forest believed to be *A. roosevelti*'s critical habitat. The mountains on the north side of the island from Punta Flamenco to Punta Pavimento contain the largest part of what remains of the semi-moist boulder and dry forest habitat on Culebra (Fig. 4). The forest type from Flamenco to that of Mount Resaca facing Playa Resaca is lusher than the side of Mount Resaca facing Playa Brava. This is primarily due to the larger patches of boulders in the large deep ravines, greater rainfall and prevailing easterly winds. The boulder formations also hold moisture and rain water deep in the ravines. During the rainy season, cascading water runs below the boulders behind the Laguna Resaca area facing Bahia del Marejada. Boulder formations on the island are responsible for the semi-moist forest. The dominant trees in this area are gumbo-limbo, Cupey, fig and a thin palm (*Coccothrinax alta*). It also contains various orchids, bromeliads and Anthurium (*Anthurium acaule*). The boulder forest in front of Playa Brava is made up of smaller patches, few rocks, and shallow ravines.

Fig. 4A (left) and B (right). Typical critical habitat of *A. roosevelti* on Mount Resaca, Culebra, Puerto Rico, USA. (Photographed by Matthew J. Kessler, February 1986)

At sea level the dry forest in front of Playa Resaca gives way to a littoral forest, mostly Emajaguilla (*Thespesia populnea*) and vine tangles, before opening to the coast where coconut palms (*Cocos nucifera*), sea grape trees and milkweed (*Calotropis procera*) are abundant. The Laguna Resaca is surrounded by a small interior mangrove swamp except on its seaward side. In back of this lagoon lies the largest patch of semi-moist boulder forest on the island. The other lagoon in front of Playa Brava is surrounded by a smaller mangrove stand fringed by manchineel and sea grape trees. The dry forest that lies behind the mangroves has an area where the trees are covered by a thick canopy of vine tangles extending down to the ground.

<u>*San Isidro and Cerro Balon:*</u> The area from Punta Pavimento to Punta Manchita is very xerophytic. Much of this area along with Cerro Balon was almost entirely deforested to provide grasslands for cattle grazing. This area is also the driest side of the island. Thorn-brush dominates this area and forms a very even canopy of secondary succession forest. Towards the coast on the cliffs, clumps of gumbo-limbo trees still crowd the mountain tops. Various types of cactus (including *Cephalocereus*) are also common in this area. The Laguna Zoni on the northeastern coast is surrounded on its interior side by a mangrove swamp, while its seaward side has a

mixture of an old coconut grove and secondary forest with a dense mass of vine tangles hanging on the canopy. The area in front of the beach is covered with sea grape trees.

Punta Muleros, Punta Almodovar, Punta del Viento Punta Vaca: All of these sea-bound points are fringed with mangroves, except Bahia Mosquito. In the area behind Bahia Mosquito, there is a large watering hole on the lowland. There is a shallow ravine and around this area is a small patch of guayacan (*Guaiacum sanctum*) forest. At the top of the mountains there are patches of manchineel (*Hippomane mancinella*), gumbo-limbo trees, thorn brush and cactus scattered about coastal shrubs. Most of the high and lowlands here were also cleared for cattle grazing, but these areas now contain a solid canopy of *Acacia*.

Punta Carenero and Punta Padilla: This area consists of patches of dry coastal forest, mostly gumbo-limbo trees on the mountaintops and manchineel on the seaward slopes. From Punta Cabras there is a large and extended mangrove system complete with channels. The southeastern peninsula (Playa Sardinas II) of Culebra is the most heavily populated by man and thus the most deforested. There is a very small patch of semi-moist forest opposite the old ferry dock at Punta Aloe. There are extensive mangroves bordering Ensenada Dakity and Ensenada Malena, and on the southwestern side of Laguna Cornelio. Punta Soldado on the southwest coast contains dry coastal forest, mostly composed of manchineel.

Field study sites on Cayo Luis Peña:

Day searches were conducted on this large island off the southeast coast of mainland Culebra in March and April. This Cay contained a moist-type forest on its highest point on the northeastern coast. Most of the remaining Cay consisted of a dry coastal forest, with a small lagoon and mangrove stand on its small Cay.

Field study sites on Culebrita:

The vegetation on this island is generally very shrubby, with cactus and sea grapes. Because Culebrita does not contain the forest type that would sustain a giant anole, it was only searched for one day in June.

Field study sites on St. John, U.S. Virgin Islands:

In August, a few days were spent in St. John performing daytime searches. During this tropical storm season of the year, St. John contained much more lush and forested areas than did Culebra.

Field study sites on Vieques:

A small herpetological expedition team was formed to conduct a week-long search for *A. roosevelti* on Vieques. The team consisted of Dr. Richard Thomas, two of his graduate students, and the author. The survey focused on Mount Pirata on the southwestern end of the island as this area contains the forest types that would most likely harbor giant anoles. The Mount Pirata area held a large moist forest, much more lush, and well-preserved than any on Culebra. The lowlands and coastal zones contained many areas covered with mats of vine tangles hanging from the trees to the ground and over a stream. These areas were considered ideal for nocturnal sighting and collecting any giant anoles that might inhabit the area.

Results

Interviews

The interviews provided no scientific data or evidence of sightings of *A. roosevelti* within the last 50 years. However, they did indicate without any doubt that all those claiming to have seen *A. roosevelti* in recent years had been confusing it with *I. iguana*. Of special interest and historical importance was the author's interview with Mr. Dimas Villanueva (DV), the "Mr. Dumas" (sic) of Dodd and Campbell (1982). At the time, he was in his 70s. DV collected the original specimen of *A. roosevelti* for Grant in the early 1930's. By this time in his life, DV had become bothered by all of the biologists who had come to talk with him over the years about the Culebra Island Giant Anole he collected for Grant. In order to interview him, the author first had to become friends with his family and best friend. When the interview finally occurred, DV said that back in the 1930s Grant paid fifty cents, a significant amount then, for each lizard or snake delivered to him. He and his cousin explored several places on the island together collecting lizards to take advantage of Grant's lucrative offer. DV vividly recalled having caught the "strange" large brownish-gray lizard with a big bulky head. He said that was the first and last time he ever saw this type of lizard or any other one similar to it. Even though Grant paid double for this lizard and offered to buy more, he never saw or was able to capture another specimen despite many searches of arboreal habitats. Contrary to what was reported by Dodd and Campbell (1982), DV said that he did not remember where on mainland Culebra he caught this lizard, as he had collected lizards from several different places that day. He told this author that he believed this *A. roosevelti* was caught around El Vijeya (a local alias for the Mount Resaca area). This is shown as possible type-location Z in Fig. 3. Two other old timers, who also collected lizards and snakes for Grant, also were interviewed. They both talked about the strange lizard DV had collected when they were boys. They also said that they never saw another one like it again. A few other old folks also remembered seeing DV's strange lizard in the early 1930s, and, likewise, they had never seen another since.

Seminar

The seminar and slide show on *Anolis* lizards of Puerto Rico offered at the Culebra public high school was well received and stimulated much interest in the search by the students. It was given in Spanish, the primary language in Puerto Rico, to avoid any misunderstandings. The students and teachers who thought that they had seen the giant anole admitted to having confused it with *I. iguana*.

Field Searches

At the time this investigation was begun, the only known specimens of *A. roosevelti* came from Culebra and the intent was to search its critical habitat for other specimens. Therefore, this study focused on finding *A. roosevelti* in the semi-moist boulder, dry, and littoral forests and mangrove areas on mainland Culebra believed to have the best potential and/or critical habitats. Special, meticulous and repeated searches were made of the Mount Resaca area. During the day and night searches on Culebra and Vieques, the *Anolis* lizards *A. cristatellus*, *A. stratulus*, and *A. puichellus* were the most common and abundant lizards found. They were seen in practically all locations. *Mabuya* and *Ameiva* were less common and were often restricted to certain areas. *Hemidactylus* were seen only at night when they came out to forage. *Anolis* lizards spent the night entwined around the thorns of thorn-brush trees in dry forest areas, while in the semi-moist boulder forest Cupey, Cork (*Pisonia subcordata*), and *Anthurium* leaves or hanging vines were preferred. Very few lizards were found sleeping at night in the vine tangles in the littoral areas on Culebra, perhaps because at night these places were teeming with rats (*Rattus rattus*). Only *A. cristatellus* and *Ameiva* were observed during the day spent searching on Culebrita. On Vieques the rats were less abundant and many *Anolis* lizards were found sleeping on vegetation in these habitats at night. In Hispanola, vine tangles hanging from trees are ideal locations to collect giant anoles at night (Williams, 1962). During the day searches on Cayo Luis Peña and St. John, only the common *Anolis* lizards were seen as well as *Ameiva* and *Hemidactylus.* At no time during this extensive year-long field study was *A. roosevelti* found at any of the study sites, nor was any evidence uncovered that this lizard still exists on mainland Culebra or the other islands searched.

Discussion

Nothing is directly known about the life history or distribution of *A. roosevelti*. However, published literature relating to the biogeography, evolution, and behavior of other giant anoles can be used to make inferences about the Culebra Island Giant Anole. The zoogeographical distribution of *A. roosevelti* would be subject to the changing environment through natural occurrences or by human interference. Geological events, such as the fragmentation of the Puerto Rico Bank by rising sea levels during the Pleistocene accompanied by changing climates would considerably affect the distribution. *A. roosevelti*'s historical distribution could have once included all the islands of the Puerto Rico Bank Complex (except St. Croix which is not part of this bank), as it was a single land mass. The fragmentation of this island bank by rising sea levels would explain how *A. roosevelti* became distributed on all of these islands. The *A. roosevelti* on Culebra were probably a relict of a once widely-distributed giant anole population in xerophytic areas (Mac Arthur and Wilson 1967; Pregill 1981a, 1981b; Williams 1962, 1972, 1983).

On the main island of Puerto Rico, the giant anole, *A. cuvieri,* occurs in mesic environments in widely scattered localities from the mountains to sea level, while its apparent ecomorphic vicar, *A. roosevelti,* seems to have preferred the more xerophytic eastern islands of the Puerto Rico Bank. *A. cuvieri*'s preference for mesic areas is also supported by the fossil record of the late Pleistocene. This fossil record is characterized by its numerous extinctions of species from arid habitats in Puerto Rico and is believed to result from climate changes brought on by rising sea levels (Pregill 1981). Most fossils come from areas now too mesic to have supported these xerophilic species. Thus, those species that are presently restricted to these xeric habitats are thought of as relicts of an arid period as well as being ancestral (Pregill 1981; Pregill and Olson 1981). This hypothesis is supported by Gregory Mayer's discovery of several museum specimens of *A. roosevelti* from the other xeric islands of the Puerto Rico Bank. In 1986, Mayer, who at the time was a doctoral candidate of Dr. Ernest E. Williams at Harvard University, was exploring the British Virgin Islands for giant anoles and other reptiles and amphibians. As part of his dissertation, he compiled records of reptiles from the Puerto Rico Bank and purportedly discovered six additional museum specimens of *A. roosevelti* (originally misidentified as *A. cuvieri*) in two Scandinavian museums that were collected between 1861 and 1863 from Vieques, Tortola (British Virgin Islands) and St. John [(Cope, 1861; Reinhart and Lutken 1863), Gregory Mayer, unpubl. data (Mayer letter to USFWS, 8 July 1986 received by the author at the Puerto Rico Department of Natural Resources on 30 July 1986)]. Thus, there are possibly as many as eight *A. roosevelti* specimens: six in museums originating from Culebra (n=2), Vieques (n=3) and Tortota or St. John (n=1); plus two additional specimens, one from Vieques and one from an unknown island (Gregory Mayer, unpubl. data, *op. cit).*

Human interference and environmental destruction, such as massive deforestation to clear land for cattle and construction, military activities, and major hurricanes have occurred around the islands that compose the Puerto Rico Bank and on mainland Culebra. An increasing human population, increased tourism and housing scattered throughout most of the island, except for the CNWR zones, have significantly reduced the potential habitat of *A. roosevelti* over the years. Culebra, which was the last reported habitat of *A. roosevelti*, now has no virgin forest remaining (not even in the critical habitat of the Mount Resaca reserve), and cattle, goats and chickens roam freely throughout the forests as do a small number of white-tailed deer introduced in 1966. Feral and/or escaped domestic cats are abundant on Culebra; cats are known to prey on lizards as do rats that are also common on the island. As for military activities, in 1903 President Theodore Roosevelt established the Culebra Naval Reservation. With the outbreak of WWII in 1939, the Culebra Archipelago became the primary gunnery, firing range and bombing practice site for the U.S. Navy. These activities lasted until 1976 when the Navy moved some of these operations to Vieques. The U.S. Marines also used Culebra as an amphibious landing training area from 1903 until 1941. The effects, if any, of these military activities in and around Culebra on this and other species is unknown. Finally, Culebra has been hit by hurricanes over the years, the most notable one recently was Hurricane Hugo of September 1989 which caused massive destruction on, and defoliation of, the island.

A. roosevelti appears to have been restricted to the more xerophytic areas on the Puerto Rico Bank Complex and is believed to have been a primitive form of recent giant *Anolis* lizards. To date, as few as two and as many as eight specimens of *A. roosevelti* are known to have been collected. All were collected in the 1860s or early 1930's from Culebra, Vieques, St. John and Tortola. There is no documentation as to exactly where on these islands any of the specimens were collected, but we do know that giant anoles are forest dwellers. Massive deforestation of these islands could have made them or their eggs more susceptible to predation by domestic animals, such as cats and goats, and wild animals such as birds of prey, snakes and rats, as well as to concussions from naval bombardments at sea and on land.

The interview with Dimas Villanueva (DV) and his contemporaries seems to support the belief that *A. roosevelti* was extremely rare on Culebra even in the early 1930s and that the probable type-locality might actually be on Mount Resaca, its designated critical habitat. It is interesting that documents indicate at least three different field survey teams interviewed DV and obtained three different stories about where he discovered the specimen. Information provided to Dodd and Campbell by PR DNR sources indicated that DV had found the specimen on the Flamenco Peninsula, which is reported as the "probable type-locality" in Campbell and Dodd (1982) and Dodd and Campbell (1983). However, in 1984, DV told the USFWS/PR DNR team, including Drs. Geoffrey Hammerson and José Vivaldi, that it came from a hilltop north of San Ildefonso in a cork forest. Finally, in 1986, he told this author that it probably came from Mount Resaca. These three sites are identified as "possible type-locations" X, Y and Z, respectively, in Fig. 3.

Interviews with younger townspeople and with the teenagers at the public high school clearly indicated that all those claiming to have seen the giant anole were confusing it with *Iguana iguana*, a relatively common introduced exotic lizard species on Culebra. In part this confusion may have been due to the fact that iguanas are polymorphic. Young iguanas appear very different from adults and are considered two different types of lizards by untrained observers; they could easily be confused with the giant anole by these individuals as universally seems to have been the case.

During this year-long study an experienced observer would be expected have sighted at least one *A. roosevelti* on Culebra assuming that a reproducing population existed. Like other giant anoles, *A. roosevelti* would be expected to forage for food and carry out other activities in the forest during the daylight hours. All potential forested habitats on Culebra were repeatedly searched during the day and at night. No specimens were spotted. Other islands and Cays surrounding the main island of Culebra that contained forested areas were also searched, albeit briefly, with no success. In view of the intensive field work done on Culebra to find *A. roosevelti* during the year of this study and the lack of any sightings or collections during the intervening almost quarter century (see Mayer 1989; Thomas and Joglar 2006; Losos 2009), *A. roosevelti* should be considered extinct on Culebra. Further investigation seems warranted on Vieques, St. John and Tortola before consideration is given to declaring the Culebra Island Giant Anole, *A. roosevelti*, totally extinct in the Caribbean.

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Anolis conservation: Formation of an IUCN taxon specialist group for anoline lizards

Informal discussions among several people who attended the *Anolis* Symposium raised the issue of *Anolis* conservation. Although many species of *Anolis* are common and abundant, many others have extremely restricted ranges that are subject to threat. The question arose: Is this an appropriate time to plan for the creation of an organization devoted to anoline lizard conservation? If so, what form should the organization take?

The scientists listed below believed that the answer to the first question is yes. Kiester proposed the idea that we should create an IUCN Taxon Specialist Group to promote and focus *Anolis* conservation. Anders Rhodin who is the Chair of the Chairs of the Taxon Specialist Groups has encouraged us to explore this idea further.

Taxon Specialist Groups are the mechanism by which the International Union for the Conservation of Nature systematically obtains advice from the scientific community about the status and conservation of taxa. These groups provide the evaluations that are used in the IUCN Red Listing process and in formulating CITES designations that regulate trade in endangered plants and animals. The Groups also function as international professional societies devoted to science and conservation. They hold annual and other meetings and they frequently publish in the IUCN journal *Species* or their own journals and books.

There are currently 5 Specialist Groups for reptiles. The Iguana Specialist Group is currently the only one devoted to lizards and is very active. Our idea for an Anoline Lizards Specialist Group is for the Group to encompass the entire lineage and not just *Anolis* (however construed).

We would like to assess the need for and the interest in establishing this Group. Two things are especially important in the formation of the Group. The first is that it must self-assemble and reflect the broad *Anolis* community. The second is that it must be genuinely international. Our first task is to create a list of scientists interested in the formation of this Group. We have created the website anolisconservation.org and ask that anyone interested in helping go there to email one of us. If interest warrants, the website will evolve to a general information exchange.

The initial group of interested scientists is: Rich Glor, George Gorman, Blair Hedges, Ross Kiester, Jonathan Losos, Luke Mahler, Greg Mayer and Mason Ryan.

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Development of functional genomic resources, a gene expression database, and cell culture reagents for molecular analysis of *Anolis carolinensis*

http://www.anolisgenome.org

I. Development of functional genomic resources for analysis of musculoskeletal and neuroependymal regeneration in *Anolis carolinensis*

The sequencing of the *Anolis carolinensis* genome gives us an opportunity to identify lizard homologues of mouse genes regulating axial musculoskeletal regeneration. Based on these genomic sequences, we can generate quantitative RT-PCR assays and RNA probes for section-based *in situ* expression analysis. We are focusing our efforts on identifying larger exons (>500 bp) suitable for making RNA probes, and exons separated by short intronic sequences for selecting primers for genomic, RT-PCR, and quantitative RT-PCR, as described previously (William et al., 2007; Sewell et al., 2009). We have focused on assays for gene markers of myogenesis, chondrogenesis, bone and tendon formation, and other key developmental and regenerative genes in *Xenopus*, axolotl, other reptiles, and mammals. Gene assay information is available at the community resources web site http://www.anolisgenome.org/ (further description below). The focus of our efforts is the remarkable ability of lizards to regenerate the skeletal muscle, hyaline cartilage, and spinal cord neuroependyma of the tail (Alibardi, 1995a, 1995b; Chlebowski et al., 1973; Cox, 1968).

II. Development of ANOLISGENOME.ORG as a community resource for *Anolis* gene expression data

We have established a web site (http://www.anolisgenome.org) and associated initial version of a gene expression database, and we have been able to post some of the lab resource genome information to this site. We plan to generate and validate primer sequences for genomic, reverse transcriptase (RT), or quantitative RT-PCR and make this information available on this site before publication. Currently, we are collaborating with Sudhir Kumar (ASU) who has developed the FlyExpress 3.0 (http://flyexpress.net) component of FlyBase to develop the next iteration of the ANOLIGENOME.ORG gene expression database. In addition, we are also closely coordinating with Rob Kulathinal and Tonia Hsieh (Temple University) to develop complementary resources and links with their development of the LIZARDBASE.ORG database.

III. Development of cell culture in *Anolis*: Isolation of satellite stem cell populations from skeletal muscle

Currently, there are no cell lines available for the *Anolis carolinensis* model system. Simpson and colleagues previously isolated myoblast cells (Bayne & Simpson, 1975, 1977, 1980), but these lines do not appear to have been banked anywhere, and apparently are not being used by any current researchers. Availability of *Anolis* cell culture would permit functional studies that could be investigated at the cellular level, such as musculoskeletal and neural regeneration, neurophysiology, and development.

As part of our efforts at ASU to develop primary cell cultures from *Anolis*, we have isolated muscle satellite cells from adult female *Anolis carolinensis*. We have adapted protocols for isolation of mammalian satellite cells with information available for other lizard cell cultures (Allen et al., 1997; Tatsumi et al., 2006). Briefly, large *Anolis* limb muscle groups were dissected in PBS, minced, and cells separated by protease treatment. Cell suspensions were preplated to remove fibroblasts and other debris. Remaining satellite cells in suspension were collected and plated onto Matrigel coated tissue culture plates in growth medium in a 5% humidified chamber at 30°C. We observed that cells with satellite cell morphological characteristics could be isolated and maintained in culture. RNA extracted from these cells is being analyzed for expression of myogenic factors, and differentiation experiments are planned. This demonstrates the feasibility of isolating primary cell cultures from *Anolis*.

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The evolution of testes size in Caribbean *Anolis* lizards

In animal species where females mate with several different males over the course of a breeding season, males are often forced to compete with each other over reproductive access to females (Andersson 1994). This competition may take the form not only of direct male combat, but also of indirect competition between the ejaculates of two or males which may be simultaneously present within a female's reproductive tract. Originally suggested by Parker (1970), a growing number of comparative studies since then have shown sperm competition to be an important and near ubiquitous selection pressure that may affect the size and morphology of male gonadal tissue and of sperm itself (Simmons 2001). Indeed, because of the link between testes size and sperm production, an increased investment in male testes size relative to somatic tissue is a typical response to increased sperm competition in a wide variety of taxa (Møller 1989; Harcourt et al. 1995; Simmons 2001). Despite some notable exceptions (e.g. Emerson 1997), this relationship has shown to be moderately robust, and sperm morphology and relative testes size have been demonstrated to be important correlates of mating system in several taxa, with larger relative testes being characteristic of a polygynous mating system (e.g. Heske and Ostfeld 1990). Species possessing relatively smaller testes are often less polygynous. Consequently, relative testes size has been used as a predictor of mating system (Harcourt et al. 1995).

Although sperm competition and its correlates have been studied in a range of animal taxa from fish to birds, mammals and, especially, insects (reviewed in Simmons, 2001), the evolution and ecology of sperm competition has received far less attention in reptiles in general, and in lizards in particular. Indeed, despite the demonstrated existence of sperm competition and of morphological structures implicated in sperm competition in a range of lizard species (Sever and Hamlett 2002), we currently lack a clear understanding of the ecological factors affecting both sperm competition risk and mating system in lizards as a group. Comprehending these factors is especially important given the growing number of studies reporting incidences of multiple paternity within single clutches in a range of lizard species (e.g. Gulberg et al. 1997; LeBas 2001; Laloi et al. 2004; Calsbeek et al. 2007).

We conducted a comparative study examining testes size in 23species of Caribbean *Anolis* lizards that vary in overall sexual size dimorphism (SSD). Anole species are ideal subjects for a comparative study investigating the evolution of testes size, as they exhibit significant diversity not only in SSD, but also in key characteristics that are likely related to mating system type, such as degree of territoriality and male-male competition. Despite this documented variation, empirical data on the mating systems of most *Anolis* species are lacking (Losos 2009). However, previous researchers have posited, with some support, a general relationship between SSD and intensity of male-male combat (and, consequently, likely degree of polygyny) across various animal taxa, including anoles (Andersson 1994; Blanckenhorn 2005; see also Stamps et al. 1997, who showed a significant relationship between SSD and population density across anole species).

We tested the prediction that relative testes size will be positively related to SSD across a range of *Anolis* species and ecomorphs. We used SSD as a proxy for male combat intensity given the dearth of published data addressing this issue in anoles (see also Lailvaux and Irschick 2007). In addition, we also tested a secondary prediction that testes size will be related to observed copulation rates in several anole species, given that this relationship has been observed in several other taxa. By examining correlations among testes size and these putative explanatory variables while controlling for phylogeny, we hope to gain insight into both the factors driving the intensity of sperm competition in *Anolis*, as well as into the evolution of anole mating systems.

Methods

Testes Size

We dissected and weighed testes from preserved lizards housed in the collection of the Museum of Comparative Zoology at Harvard University, and from A. Herrel's personal collection. We selected species based on the availability of both sufficient intact specimens for examination, and of previously published estimates of SSD (Stamps et al. 1997; Butler et al. 2000). In total, we measured testes from 23 Caribbean Anolis species, comprising all 6 ecomorph classes (3 crown giants, 4 grass-bush, 3 trunk, 6 trunk-crown, 5 trunk-ground and 2 twig anoles) (Fig. 1). We removed both the left and right testes from each individual and weighed them using a Mettler-Toledo electronic balance. We used the average weight of both testes for

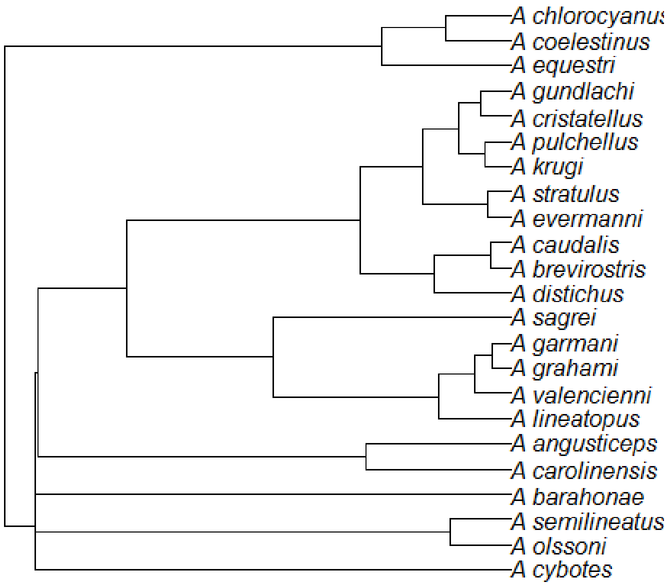

Fig. 1. Phylogenetic relationships of species used in this study (based on Nicholson et al. 2005)

analyses. Because testes have been shown to exhibit recrudescence during periods of non-breeding activity in lizards, including anoles (e.g. Licht 1967; Licht and Gorman 1975; Lee al. 1989), we only measured specimens that were recorded as being captured during the summer breeding season (approximately April-July). For a given species, we also only measured individuals captured within a single locality, given the noted geographic and populational variation in testes size in other animal taxa (see Calhim and Birkhead 2007). We measured at least 5 specimens from each species (Harmon and Losos 2005), except for *Anolis valencienni* and *Anolis carolinensis*, of which we obtained and measured only 4 specimens. For *Anolis sheplani*, we collected usable testes data from only 1 individual, and we consequently excluded this species from the phylogenetic analysis. To control for body size and obtain a measure of relative testes weight, we divided the average testes weight (left and right) for each individual by the snout-vent length. However, note that our results are robust to the type of size correction used.

Copulation rate

Copulation rate data were only available for 12 of the 23 species considered here, and represent the number of copulations observed per hour for each species. Length of observations ranged from 6 hours (for *A. brevirostris*) to 52 hours (for *A. carolinensis*).

Analysis

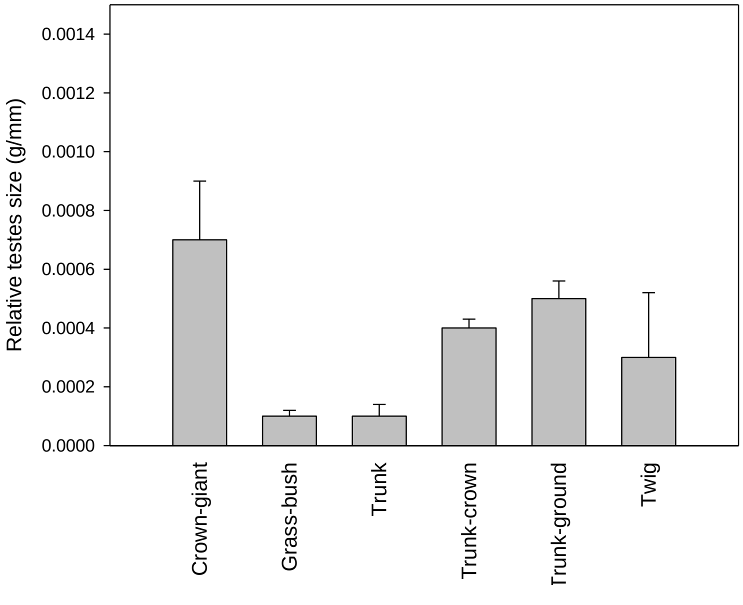

Fig 2. Relative testes weight for each ecomorph class.

All data were $\log_{10}$ transformed as required to meet assumptions of normality. The phylogeny and branch lengths used were drawn from a consensus tree of *Anolis* genomic and mitochondrial DNA. The phylogeny was made ultrametric using nonparametric rate-smoothing (Sanderson 1997) implemented in the **ape** package for R v 2.9.2 (Paradis 2006). We also used **ape** to calculate independent contrasts for SSD, relative testes weight and copulation rates (Garland et al. 1999; Garland and Ives 2000; Paradis 2006). We tested for phylogenetic signal in the tree using Blomberg et al.'s (2003) *K* statistic, implemented in the **picante** R package (Kembel et al. 2009). We used the **geiger** package (Harmon et al. 2008) to compare relative testes weights across all 6 measured ecomorph classes using phylogenetically-controlled ANOVA. Finally, we tested for relationships between contrasts for relative testes weight and contrasts for SSD and copulation rate respectively using OLS regressions forced through the origin (Harvey and Pagel 2001).

Results

Relative testes weight exhibited significant phylogenetic signal (Blomberg et al.'s K = 0.514, P = 0.003), suggesting that convergence in testes size has not occurred across the sampled phylogeny. Nonetheless, the 6 ecomorph classes differed significantly in relative testes size (phylogenetic ANOVA, $F_{5,17}$=7.46, P = 0.008). The relationship between contrasts for relative testes weight and contrasts for SSD was not significant (r^2=0.02048, $F_{1,21}$=0.439 P < 0.5148). Relationships between contrasts for copulation rate and contrasts for SSD (r^2=0.03192, $F_{1,10}$=0.1412, P < 0.715) and contrasts for testes size (r^2=0.1791, $F_{1,10}$=2.181, P < 0.171) respectively were also not significant.

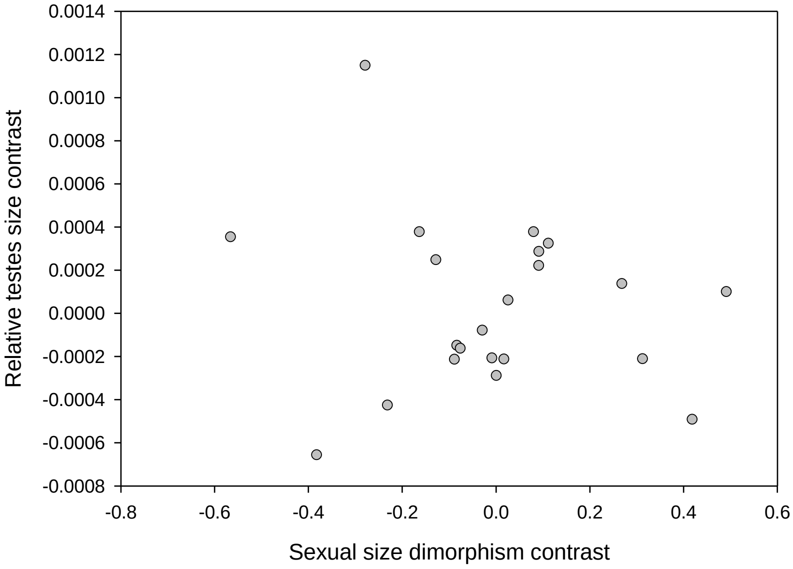

Fig 3. Relative testes weight vs sexual size dimorphism.

Discussion

Relative testes size has been shown to covary with the risk of sperm competition in a variety of animal taxa. Similarly, sexual size dimorphism (SSD) in lizards is thought to be driven in part by the degree of sexual selection and type of mating system (although other factors such as niche differentiation and reproductive separation are likely important as well [Losos 2009]). Indeed, a previous comparative study examining male combat in *Anolis* showed that more dimorphic anole species escalated more readily to physical combat than less dimorphic species (Lailvaux and Irschick 2007). Moreover, territorial species also relied more on physical performance capacities as opposed to displays for settling male-male aggressive interactions suggesting that intrasexual selection is likely more intense in dimorphic species. We consequently tested the prediction that a significant relationship exists between relative testes size and SSD in *Anolis* lizards, such that more dimorphic anole species possess heavier testes for their body size than do species where males and females are more similar.

The 23 *Anolis* species examined exhibited significant phylogenetic signal for relative testes weight, suggesting that closely related species tend to have more similar relative testes weights compared to more distantly related species (Blomberg et al. 2003). Nonetheless, a phylogenetic ANOVA showed significant differences among the 6 ecomorph classes in the relative testes weight, with this difference likely being largely driven by the relatively large testes of the crown giants and the relatively small testes of grass-bush and trunk anoles (Fig 2). The regression between contrasts for relative testes size and those for sexual size dimorphism was not significant for the current sample, and hence our major prediction of a positive relationship between testes size and SSD was falsified. A recent study seeking correlations between testes volume and mating system type in several snake and lizard taxa proved similarly inconclusive (Todd 2008), although sexual size dimorphism was not considered. One possible reason for this null result is that SSD is simply not a good estimator of male competition intensity in *Anolis*; however, this explanation seems unlikely given the previously documented relationships between SSD and both population density (Stamps et al. 1997) and male combat intensity (Lailvaux and Irschick 2007) in several anole species, both of which are considered hallmarks of territorial mating systems. Nonetheless, a recent study showed that habitat use might be among the most important factors affecting territorial behavior in 13 species of anoles (Johnson et al. in press), and our own analyses show no relationship between copulation rate and SSD after controlling for phylogeny. Hence our use of SSD as a proxy for territoriality/mating system type in this study may be questioned. One future goal is therefore to examine relationships between testes size and aspects of anole ecology that might be more indicative of mating system.

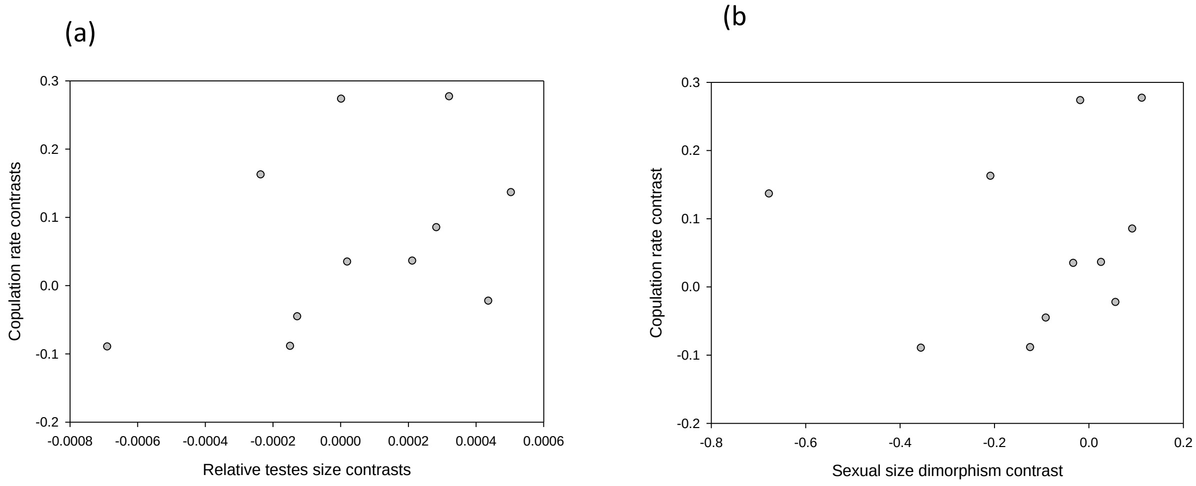

Fig 4. Relationships between contrasts for copulation rate and contrasts for (a) relative testes weight, and (b) sexual size dimorphism.

In addition to our null results when considering SSD, we are also unable to show support for a significant relationship between relative testes size and copulation rate. These findings are in stark contrast to those for other taxa such as birds (e.g. Møller 1991) which typically show a positive relationship between testes size and copulation rate independent of body size. Because our species sample for copulation rate (12 species) was much lower than that for testis size (23 species), one possibility is that our current analyses lack sufficient power to show such a relationship, should one exist. Our aim is to expand on this dataset in the future and to collect copulation rate data on a number of additional species to improve the statistical power of the analyses.

In conclusion, SSD does not explain a significant amount of interspecific variation in testes size in the 23 *Anolis* species studied here. Furthermore, we were unable to show any evidence for relationships between copulation rate and either SSD or testes size. Although our findings for copulation rate are likely influenced by the relatively low statistical power of these analyses, our findings for SSD and testes size are likely robust. Our aim in the future is to consider aspects of ecology other than SSD that might explain testes size variation in anoles.

Acknowledgements

Thanks to Jonathan Losos and José Rosado for granting access to the *Anolis* collection at the MCZ, and the Australian Research Council and the University of New Orleans for funding.

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Homing behavior and the Principle of Unsympathetic Magic

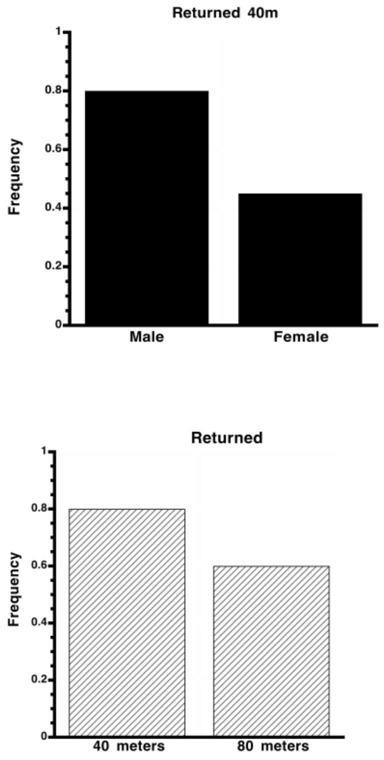
First of all, I want to take this opportunity to THANK Luke Mahler and Anthony Herrel for organizing the VI *Anolis* Symposium and the VI *Anolis* Newsletter.

My research continues to focus on general questions about behavior and evolution in anoles. My work on possible mechanisms shaping the evolution of dewlap diversity and function(s); which is mostly done in collaboration with Leo Fleishman ("El Jefesisimo"), continues to progress at a snail's pace. We recently published a paper (Fleishman et al. 2009; J. Comp. Phys. A), which demonstrates why the Sensory Drive Hypothesis is not sufficient to account for the diversity of dewlaps found in Puerto Rican anoles. Another project, also moving at snail's pace or not moving at all, in collaboration with Rich Glor, evaluates whether divergence in the coloration of the dewlap between populations of *A. cristatellus* can impact gene flow. mtDNA analysis supports the hypothesis that populations are genetically distinct by recovering deeply divergent haplotype clades that are almost perfectly concordant with the observed pattern of dewlap variation. A further evaluation of the predictions of the Sensory Drive using communities on multiple islands should be completed "pronto" and may shed some light onto the multiple mechanisms contributing to the evolution of the dewlap. A related project evaluating geographic variation in the pattern of UV reflection by the dewlaps of *A. krugi* and *A. pulchellus* and its possible implications for signal redundancy was recently finished and a manuscript reporting the results will be submitted shortly. Finally, an experimental paradigm has been developed to address questions about color and pattern discrimination in anoles. The effectiveness of the methodology debunks the traditional assumption that lizards are poor learners. Preliminary results with *A. evermanni* are extremely promising. As expected, based on our understanding of the visual physiology of *Anolis*, individuals are capable of discriminating between distinct colors consistently. Furthermore, individuals can reliably discriminate between patterns that exhibit relatively small differences. Taken together these observations suggest that anoles are able to process both color and pattern information, and to use this information to guide their behavior. My ultimate goal is to derive a model that will predict the degree of divergence in coloration and pattern needed by sympatric dewlaps to effectively convey species identity.

At the symposium, I presented the results from ongoing research addressing homing behavior in anoles. In collaboration with Brian J. Powell, a graduate student in my laboratory, we have carried out a series of field experiments to evaluate possible cues mediating homing behavior in *A. gundlachi* and *A. krugi*. Below I summarize the findings for *A. gundlachi*. A manuscript entitled "Homing Behavior and *The Principle of Unsympathetic Magic*" is currently in the works and should be submitted shortly.

In a series of field experiments, males and females of *A. gundlachi* were displaced without access to familiar landmarks or goal-emanating cues to a distance of 40m or 80m from their "original" perches. Displacement was done in a random compass bearing and following a circuitous route. By displacing the individuals in a random compass bearing, we effectively created a circle with a radius of either 40m or 80m around the original "home" position of the individuals. An individual was scored to successfully return home, if it was found within 2m of its original perch, within a period of nine days.

Fig. 1. Return frequency of *A. gundlachi* from 40m (top panel) and from 80m (bottom panel).



Our findings demonstrate that individuals of *A. gundlachi* can successfully return to their territories when displaced 40m, and that males can successfully return when displaced up to 80m (fig 1).

In a second series of experiments, we evaluated the possibility that homing behavior was partially mediated by the use of polarized light or magnetic field as compasses. To achieve this goal, we manipulated the magnetic field and polarized light perceived by the individuals and displaced them a distance of 40m, using the same methodology described above. Only males were used in this set of studies. Manipulations of the magnetic field and polarized light doesn't diminish the ability of *A. gundlachi* to successfully return "home", suggesting that these cues, which have been commonly proposed to serve as compasses for navigation in diversity of taxa, including lizards, are not necessary at this distance. Studies are currently under way to corroborate these findings at a distance of 80m.

Because displacement was conducted such that lizards had no access to familiar or goal-emanating cues, homing success cannot be explained by the ability of the individuals to integrate information gathered on the outward journey (i.e., path integration). Instead, our findings suggest that *A. gundlachi* may be using a "map-like sense" at the release site to determine its location relative to home, and in combination with a "compass sense" cue orients homewards. To put our finding in context of the natural history of *A. gundlachi*, the mean territory size for male *A. gundlachi* is approximately 3.77m diameter (M.A. Johnson, personal communication). A displacement in a radius of 40m produces a circle with a diameter of 80m, which covers an area of 5,026.5m^2, an area comparable to the area of a football field (5,353.4 m^2);

whereas displacement in a radius of 80m, resulted in a circle with a diameter of 160m, which covers an area of 20,106.2m^2. It seems highly unlikely that an individual of *A. gundlachi* have a cognitive map of such dimensions. However, I have learned to expect the unexpected with regards to the cognitive abilities of anoles.

Prior to conducting these experiments our prediction was that homing success of *A. gundlachi* would be highly reduced when individuals were displaced to areas outside of their immediate territory (i.e., distances of at least 5 times territory size), which should not be visible from the territories boundaries. However, our observations clearly demonstrate that *A. gundlachi* is capable of navigating through a relatively complex habitat, from distances in which familiarity with the habitat as the mechanisms accounting for their behavior seems highly unlikely. Furthermore, every other hypothesis we have derived thus far, based on homing theory, has also been rejected. This is in accordance with what Ernest Williams eloquently described as the Principle of Unsympathetic Magic, (please see *Anolis* Newsletter III, page iv).

> *"It was while walking along a hedge row in the Dominican Republic, listening to a complaint that I and some of my co-workers did not frame hypotheses every day while in the field, that I invented (or recognized) the PRINCIPLE OF UNSYMPATHETIC MAGIC. This states that, if one arrives at any firm and vivid conviction about matters of fact or theory in the field, the NEXT observation will provide a contradiction.........*
>
> *Note, however, that NATURE IS NOT DECEIVED. No opinion merely pretended to, i.e. not held with fierce conviction, will be responded to by a conclusive observation. The MALICE OF NATURE prohibits the PRNCIPLE OF UNSYMPATHETIC MAGIC from being a source of satisfaction to the field worker."*

Finally, our findings also have implications for territoriality - particularly, the possible costs associated with acquiring a territory and the benefits associated with knowing that territory well. As pointed by George Gorman, a male that is displaced 40m or 80m from its territory must cross the territories of numerous males in order to return to his original perch, and doing so most likely results in potentially costly agonistic interactions with territory holders. In fact, several of the males showed signs or recent fights after returning to their territories. Yet individuals are willing to incur such a cost in order to return to their territories, a scenario that suggests the benefits of returning to the territory are equal or greater than the costs associated with traveling. Please stay tuned; we are currently working on a possible experimental design to address this question.

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Hanging on for dear life: A study of *Anolis* claws in arboreal and aquatic species

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Introduction

Aquatic anoles are species of *Anolis* that are found near water and readily enter streams (Leal et al., 2002). These anoles hang onto wet rocks at water's edge to pick up floating food items and to feed on aquatic insects or other small invertebrates. Lizards dive into the water to feed or to escape predators, and may swim to the bottom and hang onto rocks for extended periods. Seven species of aquatic anoles occur in Central and South America and the West Indies.

Leal et al. (2002) examined aquatic anoles and found that they lacked shared morphological traits, concluding that there was little evidence for morphological convergence due to an aquatic lifestyle. The authors suggested that aquatic anoles might exploit different niches in the proximity of water or that anoles have adapted in different ways to a single aquatic habitat type. On a trip to southern Mexico, observations of aquatic anoles (*Anolis barkeri*) hanging onto wet rocks above and in rapidly running water and that individuals in this species had particularly robust claws led us to ask whether aquatic anoles have claws that allow them to grip slick rocks better than arboreal anoles would. Although Elstrott and Irschick (2004) examined clinging performance by anoles, they did not include any aquatic anoles in their study. Consequently, we compared claw size and curvature in aquatic and arboreal anoles to test the following hypotheses:

H_1: Claws of aquatic anoles are larger or differ in shape from those of arboreal anoles.
H_{null}: Claws of aquatic and arboreal anoles do not differ in size or shape.

Materials and Methods

Using digital images of lateral views of the claw on the fourth (longest) toe of the left hind leg (fig. 2), we compared claw size and curvature of aquatic anoles with a variety of anoline ecomorphs (e.g., Rand and Williams, 1969).

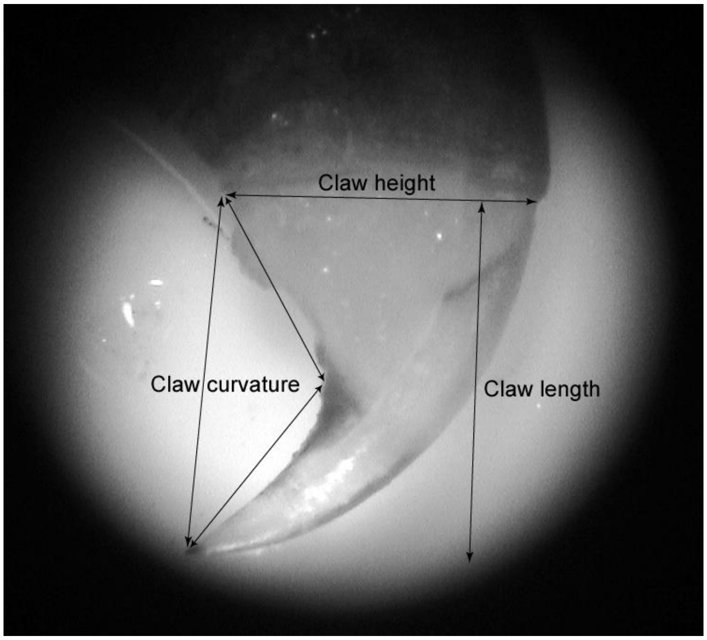

Fig. 1. Claw of *Anolis barahonae*, a crown-giant ecomorph, showing measurements used to evaluate claw size and shape.

Arboreal species studied were: *Anolis barahonae* (crown-giant ecomorph), *A. bimaculatus* (a Lesser Antillean anole of indeterminate ecomorphology), *A. carolinensis* (trunk-crown ecomorph), and *A. cristatellus* (trunk-ground eco-morph). Aquatic species studied were: *Anolis aquaticus*, *A. barkeri*, *A. eugengrahami*, *A. lionotus*, *A. oxylophus*, *A. poecilopus*, and *A. vermiculatus*.

Using the NIH image program (National Institutes of Health, Bethesda, Maryland), we measured claw height, claw length, and claw curvature (fig. 1) following Zani (2000) and Carvalho Ribas et al. (2004). Claw height and length were adjusted for individual body size (i.e., snout-vent length [SVL]). Curvature was calculated using the formula: Claw curvature = $57.296 * (2 * \text{arcsine}(((2 * A^2 * B^2) + (2 * A^2 * C^2) + (2 * B^2 * C^2) – A^4 – B^4 – C^4)^{0.5}/(2 * A * B)))$, in which A and B are the two short measurements and C is the long measurement indicated in Fig. 1. For statistical analyses, we used Statview 5.0 (SAS Inc., Cary, North Carolina). For all statistical tests, $\alpha = 0.05$.

Results and Discussion

Arboreal and aquatic anoles differed significantly on PC1 (increasing length and decreasing curvature; ANOVA, $F_{1,11} = 17.81$, $P = 0.002$), but not along PC2 (increasing height and curvature; $F_{1,11} = 0.66$, $P = 0.44$). Discriminant Function Analysis (DFA) correctly classified 100% of both aquatic and arboreal species.

Aquatic anoles had significantly higher and longer claws with significantly less curvature than arboreal anoles (Figs. 2 & 3), indicative of convergence for this trait in aquatic habitats.

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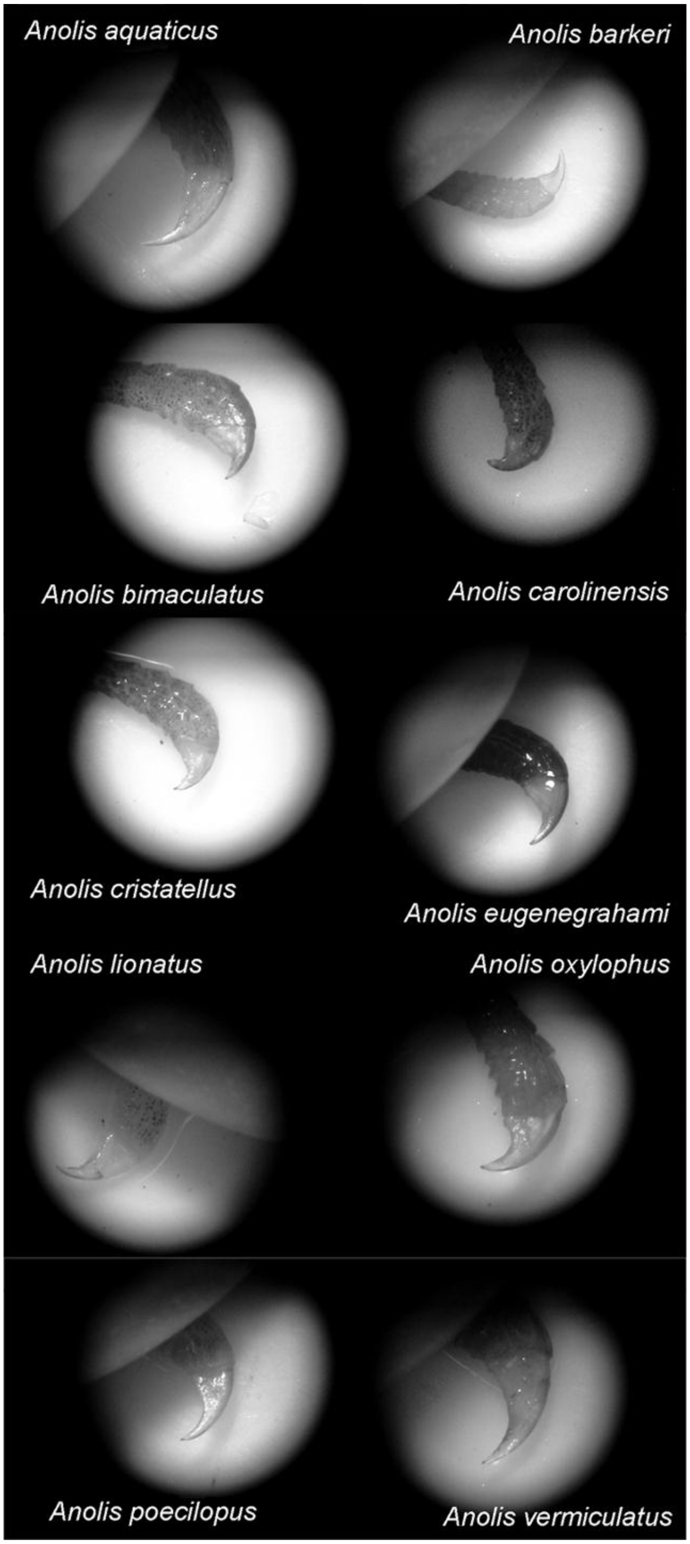

Fig. 2. Representative images of claws of *Anolis* lizards. All are illustrated at the same scale and all, except *A. barkeri*, are large adults.

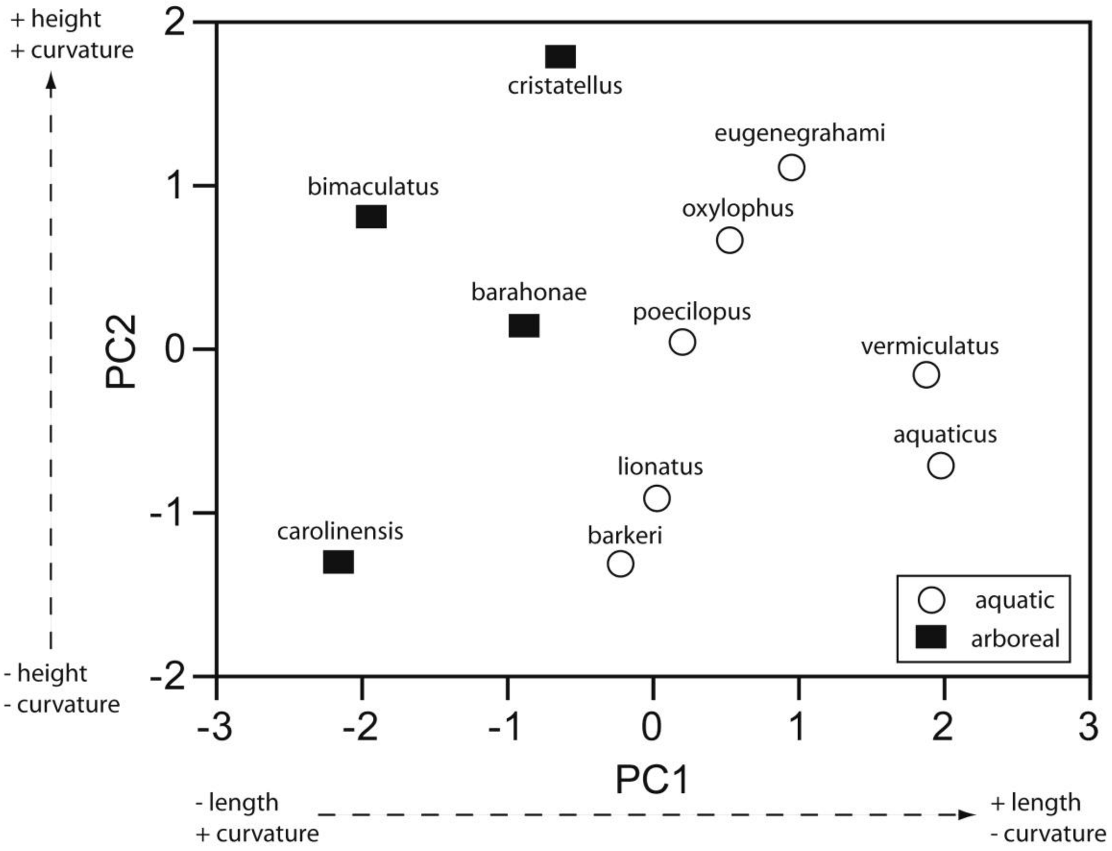

Fig. 3. Plot of PC scores for the first two principal component axes of a principal components analysis of mean claw measurements for aquatic and arboreal anoles.

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Future research on differences in patterns of evolutionary diversification in island and mainland anoles

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Introduction

Caribbean lizards in the genus *Anolis* have become a model system for the study of adaptive radiation and were among the first radiations to be studied in the phenotype ~ performance ~ ecology/behavior framework. On each island in the Greater Antilles (Cuba, Hispaniola, Jamaica, and Puerto Rico), anoles have diversified essentially independently. Despite their evolutionary independence, the end-result of these radiations has been remarkably similar, with more-or-less the same set of habitat specialists, termed ecomorphs and named for the part of the environment they usually occupy (e.g., "trunk-ground," "twig"), evolving on each island. The ecomorph classes differ in morphology (e.g., limb, toepad and head dimensions, tail length), habitat use, and behavior (Moermond, 1979; Williams, 1983; Losos, 1990; reviewed in Losos, 2009). Functional studies have suggested the adaptive basis for this within-island divergence and between-island convergence. For example, the short legs of twig anoles allow them to move with great agility on the narrow and irregular surfaces they utilize, whereas the long legs of trunk-ground anoles provide great sprinting capability on broad or flat surfaces (tree trunks and the ground), which they use to capture prey and confront conspecifics (e.g. Losos and Sinervo, 1989; Losos, 1990b; Irschick and Losos, 1999). Similarly, the large toepads of the more arboreal ecomorphs (trunk-crown and crown-giants) provide greater adhesive ability (Elstrott and Irschick, 2004), and the taller and wider heads of crown-giant and trunk-ground anoles allow them to eat larger prey including fruits and other vertebrates (Bowersox et al., 1994; Herrel et al., 2004).

The synthesis of phylogenetic, functional, and behavioral ecological studies, as well as their unusual four-fold replicated radiation, has made Greater Antillean anoles a textbook case of adaptive radiation. Nonetheless, a little known aspect of anole natural history is that not only do more anole species occur on mainland Central and South America than in the Caribbean (ca.

250 vs. 150), but the ecological and morphological variety of mainland anoles is just as great as that seen in the islands. Despite their evolutionary exuberance, mainland anoles have radiated in fundamentally different ways from island anoles (Irschick et al., 1997; Velasco and Herrel, 2007; Pinto et al., 2008). In particular, very few mainland anoles fit into any of the Greater Antillean ecomorph classes (Irschick et al., 1997; Velasco and Herrel, 2007) and mainland anoles seem to have diversified in different ways, producing morphologies unlike any seen in the Caribbean; mainland and Caribbean anoles occupy partially non-overlapping portions of morphological space (Velasco and Herrel, 2007; Pinto et al., 2008). In addition, preliminary data from relatively few species suggests that the relationship between morphology and habitat use differs fundamentally between mainland and Greater Antillean anoles (Table 1).

Table 1. Differences in ecomorphological correlations between Greater Antillean and mainland anoles (data from Irschick et al., 1997).

	Greater Antilles	Central America
Lamella number *vs.* perch height	0.24	0.65
Forelimb length *vs.* perch height	0.04	-0.59
Forelimb length *vs.* perch diameter	0.79	0.22
Tail length *vs.* perch height	-0.64	0.07

Previously published data suggest that different selective agents may shape evolution in the two areas. In particular, mainland areas host a great number and variety of predatory species, including a wide range of birds, snakes, and mammals. By contrast, the predator faunas of Greater Antillean islands are substantially smaller and less varied (Wright et al., 1984). The result is that predation may have a much greater impact on mainland anoles. By contrast, intraspecific competition for food is likely much more important in island species and a wide variety of ecological, behavioral, and demographic data support this distinction (Andrews, 1979; reviewed in Losos, 2009).

Table 2. Population biology of mainland anoles relative to Caribbean species.

	Caribbean	Mainland
Density	high	low
Food intake	low	high
Prey size	small	large
Growth rate	low	high
Survivorship	high	low

The observations listed above in Table 2 are consistent with predation being a more important factor in regulating anole populations on the mainland, whereas resource competition may be of paramount importance on the islands. As a result, the selective factors affecting mainland and island anoles may be very different and consequently two species - one from the mainland, the other an insular one - might occupy the same habitat, but might have to evolve very different morphological features to adapt to the different circumstances they face there. An alternative possibility is that competition is important in both areas, but on the mainland, it is competition with other lizard genera and small insectivorous birds, whereas on the islands it is competition among anoles that drives diversification. Such differences also plausibly could have driven anoles on the mainland to utilize available resources in different ways (Wright, 1979; Vitt et al., 1999, 2003). Such a scenario, however, would be less consistent with the differences listed in Table 2.

The goals of our future work are four-fold:

1) We will collect data on morphology, habitat use and behavior to test the hypothesis that mainland and Caribbean anoles do indeed differ as suggested by the data available in the literature; we will test whether the relationship between morphology and ecology/behavior differs between species in the two areas.

2) We will test the hypothesis that predation levels are higher on the mainland, that mainland and island anoles differ in response to simulated predation events, and that habitat use differences are consistent with differences in predation level.

3) Assuming that we confirm preliminary findings that the relationship between morphology and ecology/behavior differs between island and mainland anoles, we will investigate whether this results because the relationship between morphology and functional capabilities differs or because the relationship between functional capabilities and ecology/behavior differs (or both).

4) We will test the hypothesis that differences in the functional components of the feeding and locomotor systems drive the observed differences in performance between both radiations. To do so, we will quantify the functional basis underlying observed differences in locomotion and feeding performance between Caribbean and mainland radiations using data on the feeding and locomotor muscles and biomechanical models.

Preliminary results

Goal 1: Are morphology~ecology relationships different?

Our preliminary data indicate significant differences in ecomorphological relationships when comparing Caribbean with mainland anoles. For example, the relationship between hindlimb length and perch diameter differs between Caribbean and mainland anoles (slopes: $F = 6.33$; $P = 0.02$; intercepts: $F = 6.35$; $P = 0.002$; Fig. 3). More dramatically, other relationships are entirely different in the two radiations; the distance to the nearest perch, for example, shows a tendency to be negatively related to residual mass on the mainland ($r = -0.47$; $P = 0.089$) but this relationship is highly significant and positive in the Caribbean ($r = 0.67$; $P = 0.003$). In conjunction with previous studies (Table 1) these data suggest that anoles in the Caribbean and on the mainland have indeed diversified in different ways.

Goal 2: Is predation pressure higher on the mainland?

Our preliminary data on predation pressure suggest that predation pressure may indeed be higher on the mainland with 10% of our models being attacked compared to 5% attacks on models placed in similar habitats in one study conducted in the Caribbean (Gifford et al., 2008). Moreover, our behavioral data suggest that mainland anoles do not move much (time budget data show that mainland anoles sit still during 92% of the time) ; in contrast, Caribbean anoles appear to be more active (e.g., Losos, 1990), although, surprisingly, no time budget data are available for any Caribbean species (but videotapes from which such quantification can be obtained are available; see above) Finally, our data show that mainland anoles wait longer before initiating an escape from a potential predator ($F = 16.22$; $P = 0.001$), even for a given performance level (i.e. given the same sprint speed; ANCOVA, $F = 8.45$; $P < 0.05$) which suggests a difference in escape strategy in which mainland anoles may rely more on acceleration capacity than on sprint speed *per se*.

Goal 3: Morphology ~ performance and/or performance/ecology relationships differ

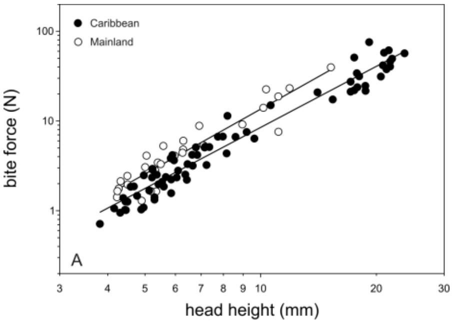

Fig. 2: The relationship between head height and bite force in Caribbean (black circles) and mainland (open circles) anoles. Note how mainland anoles bite harder for a given head dimension compared to Caribbean anoles.

A summary of our preliminary data indicates that the morphology ~ performance relationships are indeed different in the two radiations.

For example, mainland anoles have a higher bite force for a given head size compared to Caribbean anoles (Fig.2). The fact that mainland anoles tend to eat bigger prey (Andrews 1976), and the fact that bite force is tightly related to prey size in anoles (Herrel et al., 2004, 2006; Herrel & O'Reilly, 2006), suggests increased selection on bite force capacity in mainland anoles and may be the result of selection on jaw closing velocity in the Caribbean. Data on muscle structure and function combined into biomechanical models are needed to understand the proximate causes for the observed relationship and to test whether the observed difference is indeed the result of a trade-off between force and velocity at the muscular level. Data on muscle fiber lengths, cross sectional areas and moment arms will be especially insightful in helping to explain the observed differences in the morphology ~ performance relationship. Moreover, bite models (e.g. Herrel et al., 1998; Van Wassenbergh et al., 2005) will allow us to test how observed differences in cranial structure and jaw lever arms (as deduced from CT imaging) may affect the ability to generate bite force or jaw closing velocity in Caribbean and mainland anoles.

Preliminary data suggest that Caribbean anoles have a greater sprint speed for a given hind limb length (Fig. 3b; $F = 4.79$; $P = 0.036$; results using residuals qualitatively unchanged). However, our data also suggest that the relationship between sprint speed and habitat use does not differ between Caribbean and mainland anoles (Fig. 3c; $F = 0.05$, $P = 0.83$) suggesting that it is mainly the different morphology ~ performance relationships that are driving the observed morphology ~ habitat differences (Fig. 3a) between the two radiations. Data on hind limb muscle structure and function are needed to understand the proximate causes for the observed difference in the morphology ~ performance relationship. Moreover, data on acceleration capacity of mainland anoles will allow us to test whether acceleration capacity is higher in mainland anoles (as would be predicted given the shorter approach distances observed in mainland anoles).

Our data on clinging capacity in mainland anoles compared to Caribbean anoles show that mainland anoles are able to generate less shear force per unit toepad area. Interestingly, our data on toepad microstructure based on six mainland species indicate that setal length has an important effect on the amount of stress (i.e., clinging ability) that can be generated by the toepads with longer setae resulting in a greater stress, thus suggesting that quantifying toepad microstructure may be crucial in understanding differences in clinging performance between Caribbean and mainland *Anolis* lizards. Because no data on toepad microstructure are available for Caribbean anoles, we cannot test the hypothesis that Caribbean anoles have longer setae which might explain the higher stresses they can generate.

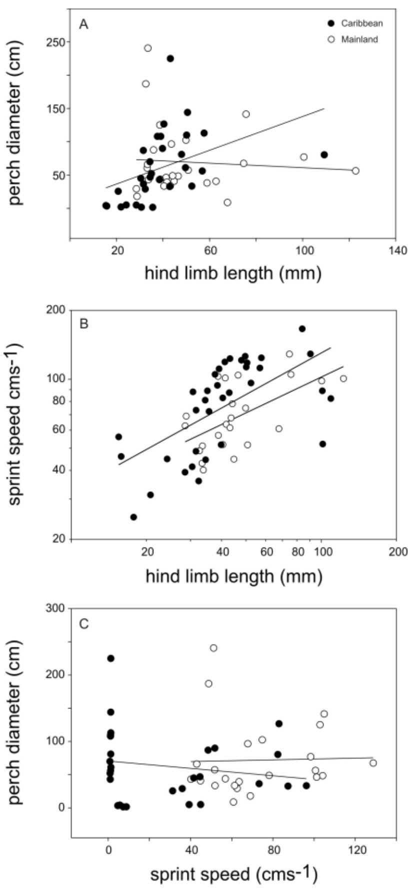

Fig. 3: Figure illustrating the relationships between morphology ~ ecology (A), morphology ~ performance (B) and performance ~ ecology. Whereas relationships are different between Caribbean and mainland anoles in A & B, they are indistinguishable in C, which suggests that differences in the relationship between morphology and performance are responsible for the different morphology ~ habitat relationships. Note that results are qualitatively similar when using residual data.

<u>Goal 4: Functional differences in the locomotor and feeding system drive the observed differences in the morphology ~ performance relationships</u>

These preliminary results clearly show that whereas previously established models on how morphology should be related to performance worked well in Caribbean *Anolis* communities (as discussed in the Introduction), they often may not hold on the mainland. Consequently, a refinement of our understanding of the relationships between morphology and performance will be crucial in explaining the observed differences between mainland and island species. As yet, we have no data of this sort, but we have suggested above several examples of the approach we will take when we identify discordant morphology ~ performance relationships. Moreover, we have taken this approach in understanding biomechanical function with regard to ecologically important interspecific differences in performance in other taxa (Herrel et al., 1998; Aerts et al., 2003; Herrel et al., 2005; Van Wassenbergh et al., 2005).

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Natural history observations for two montane anole species from the Dominican Republic

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Over the last few years, I have visited the Dominican Republic several times to conduct fieldwork on anoles. While the goals of these trips have varied, much work has centered around collection of habitat use and behavioral data for lesser-known anoles that cannot easily be classified within Williams' classic ecomorph paradigm (i.e., the "unique anoles" of Williams [1983] and Losos [2009]). During these trips, my colleagues and I have made several natural history observations that might be of interest to others working on anoles. In this contribution, I'll tell two somewhat different anole stories. The data comprising these observations are a bit scant, but might merit follow-up.

1. Pre-dawn shift in perch location in the montane anole, *Anolis etheridgei*.

In August 2008, I worked for several weeks in the forests of the Cordillera Central in the Dominican Republic, gathering habitat-use data on unique anoles found in these mountains. For help, I brought along my labmate Yoel Stuart as well as Travis Hagey from Luke Harmon's lab at the University of Idaho, and Dominican über-naturalist Miguel Landestoy also joined our team when he could. This particular season, I was after three anoles: *Anolis christophei*, a spindly but feisty little anole that is often found in great densities on the trunks of large shady trees, *Anolis etheridgei*, a small, long-legged denizen of the bushes in well-shaded montane forests, and the ultra-rare *Anolis fowleri* – arguably the crown jewel of Albert Schwartz's numerous Hispaniolan anole discoveries (Glor 2003; see also the next vignette). *Anolis christophei* was a cinch if you knew the right spot, and *fowleri* was pretty much impossible no matter how you cut the deck, but *etheridgei* was something of an enigma. A walk through the forests of the Cordillera Central during the day might lead you to think *etheridgei* was rare. You would stumble across one here or there, but they could be awfully hard to produce on command. Night time, on the other hand, was a completely different story. At night, sleeping *etheridgei* can be as dense as a typical grass anole – in some places, green, dew-covered *etheridgei* sparkle like emeralds on every frond of bracken in sight, and it would be fairly simple to collect well over a hundred on short order. One of my goals in 2008 was to find out where all the *etheridgei* were going during the daytime. These are diurnal animals after all, so where were they?

To figure this out, I took a somewhat 'unherpetological' approach to studying lizards that led to a frustrating, but neat observation. The strategy? I and my assistants woke up hours before dawn, drove up the mountain to my site, and surveyed far and wide for sleeping *etheridgei*, marking the locations of each anole with flagging tape. Then we each set up a video camera on a tripod, aimed it at a focal lizard, and waited. We were going to figure out how these animals were using their habitat by brute force – watching them from the second they woke up, and following their every movement[3].

Or that was the plan anyway. We started taping somewhere in the neighborhood of 30 minutes before dawn – just when it got light enough to use the 'night vision' recording settings on the camcorders. We did this mainly as a precaution – thanks to digital cameras, we were limited by neither battery life nor recording time. However, only about 10 minutes after we started recording, I lost my anole! Without warning, it cracked its eyes open a tad and then hopped from its leaf (about 1m high) and dove underneath the thick understory foliage that carpeted the forest floor by the trail where I had stationed myself. Figuring I had startled the animal, or perhaps was too close, I quickly moved to find one of my other flagged lizards. Then Yoel yelled that he had lost his animal and was also going to move on to another one. I started off towards Yoel, and on the way, was intercepted by Travis, who had *also* lost his lizard. For the next several minutes, all three of us scrambled from flag to flag either to find the lizards gone, or else to start recording for a minute or two only to have the lizard stealthily 'abort mission' (Fig. 1). As best we could tell, they always went deep into the underbrush and what puzzled us was that it was still very dark out. Certainly these lizards weren't starting their days yet!

At dawn, a visitor to this site would have observed three tired, cranky biologists and zero *Anolis etheridgei* (Fig. 1b). They weren't on the stems, the leaves, the branches…. certainly not anywhere visible! (We were never successful in following them when they dove into the thick underbrush.) As we sat around watching the sunrise, pondering how to salvage our morning, I noticed that the forest was bursting with

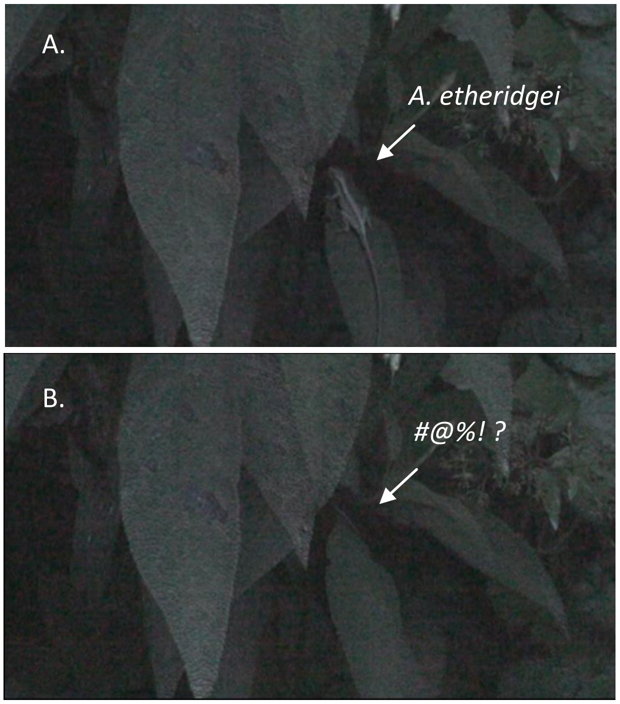

Fig. 1 - *Anolis etheridgei* right before dawn on Aug. 17, 2008. (A) Suddenly awake on leaf at 6:16AM. (B) Nowhere to be found at 6:17AM.

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[3] Credit for this God-forsaken technique is due to my former labmate Michele Johnson, who had great success finding and observing the tiny twig anole *Anolis sheplani* by these means. Michele had me up well before dawn looking for these little buggers on my first Caribbean field trip, and I have paid it forward to my own hapless assistants many times since.

birdsong. Particularly abundant were the foliage-gleaning birds, flitting from bush to bush, carefully inspecting the leaves and branches. Is this why our anoles got out of Dodge before dawn? It certainly seemed possible…

The next day, lacking better ideas and considering the possibility that bad luck was the culprit, we repeated our fruitless survey. Again we awoke at an ungodly hour, trekked up the mountain, and set up our cameras in the cold, damp darkness. Again, lizard after lizard dropped out of sight roughly half an hour before dawn until not a single one remained for observation. This time though, we were paying attention – within ten minutes after the lizards vanished, we observed the earliest of the early birds scanning the foliage, practically in darkness. At dawn, of course, the forest was alive with avian activity.

I suggest that *Anolis etheridgei* living in the central mountains of the Dominican Republic might be wise to the dangers of early-morning foraging birds, and that they might mitigate this danger by trading ideal sleeping perches, such as a leaves, for more hidden refuges several hours before beginning their diurnal activities. In the end, we eventually did have success finding and observing *etheridgei* during the daytime – they're just remarkably cryptic and move very little. Often the best technique is to sit at a good site and scan the bushes leaf-by-leaf and branch-by-branch with binoculars until one is found (they're definitely there!!). If you find a male, he'll probably direct you to his female in due time (and vice versa).

Interestingly, we never observed much *etheridgei* activity before 9:00 or 10:00AM, and we saw the most activity in the middle of the day. Although we were unsuccessful in following lizards when they escaped before dawn, we suspect they were laying low for quite some time since we didn't observe lizard activity until many hours later. A quick, targeted field experiment (e.g., paint sleeping lizards hot pink and THEN follow them) would doubtless solve the matter, but we had bigger fish to fry on that trip. Besides, it was becoming increasingly difficult to rouse my intrepid assistants with each additional pre-dawn failure.

2. Range extensions and first behavioral observations for *Anolis fowleri*, a rare montane Dominican endemic.

In 1971, Danny Fowler, a field collector for Albert Schwartz, discovered a truly remarkable anole while collecting at night along ravines high in the Cordillera Central of the Dominican Republic. Males were patterned like lichen or bracken, with splotches of reddish brown, ashy grey, and black; females were a stunning light green with a giraffe-like network of bold white reticulations along the flanks. The species sported an impossibly long tail and fairly long limbs, and was moderately large in size (smaller than a crown-giant, but a little bigger than most trunk-ground or trunk-crown anoles). Because its morphology provides so few clues, and because Schwartz and his men only collected specimens at night, the ecology of this species has remained completely unknown (Schwartz speculated that it might be a canopy anole, but it has never been observed above 3.1 meters; Williams [1983] considered it a twig anole, citing its morphology, but the basis for this assignment is unclear – it is not similar in shape to other twig anoles). Schwartz (1973) named the beast *Anolis fowleri*, in honor of its discoverer, and of all the species he described, it was reputedly one of his favorites (Henderson 1996; Henderson and Powell 2003). The natural history of *Anolis fowleri* has remained virtually unknown well into the

21st century – the species has only been collected on a handful of occasions (always near Schwartz's localities), and always at night (see Glor 2003 for a particularly entertaining tale of one of these quests). No one had ever directly observed natural behavior or daytime habitat use in this species.

One of the primary goals of my 2008 field season to the DR was to do just that – find *fowleri* and figure out what it was doing! Predictably, this turned out to be an ambitious and naïve endeavor, but by the end of a grueling field season, we managed to throw at least a little bit of light onto the natural history of this mysterious anole.

The last team to find *Anolis fowleri* consisted of Rich Glor, Steve Poe, and Paul Hime – in 2001, those three endured a punishing multi-day quest in the vicinity of the type locality (about an hour up the mountain from the town of Constanza) before stumbling across several sleeping anoles just as they were preparing to give up. This was an epic find, confirming the persistence of the species in the mountains of the DR, but it didn't yield any new information on ecology.

In 2008, *fowleri* lived up to its reputation – it was extremely difficult to find. Things looked good at first, though. While my team (at this point, Travis Hagey, Miguel Landestoy, and AMNH grad student Bryan Falk) searched for several days without turning up a specimen, reinforcements arrived shortly (Yoel Stuart and Susan Perkins [AMNH parisitologist], plus Rich Glor, and his grad students Julienne Ng and Dan Scantlebury) and we found one surprisingly quickly (Fig. 2). During our first night of combined searching, I spotted a gorgeous female perched high in a tangle of vines in a nasty, awful, spiny streamside thicket in the vicinity of the type locality (it took Rich many minutes to traverse the vines to reach my location)! Better still, it had an intact tail – rare for such a long-tailed anole, and a critical feature for my morphometric studies (I am often cruelly ridiculed by field companions due to my noble quest for lizards with a "perfect tail"). We caught this anole at night (it was in a location that would have been prohibitively difficult and risky to observe in the morning) and took it back to our hotel "lab" where we could observe and study it in captivity.

Fig. 2 - The full 2008 *Anolis fowleri* team (with *fowleri*!!) - from back left in 3 rows: Dan Scantlebury, Rich Glor, Bryan Falk, Travis Hagey, Yoel Stuart, Julienne Ng, Susan Perkins, Luke Mahler, Miguel Landestoy.

This was a bittersweet find though. Schwartz had found all of his specimens in the vicinity of two localities on opposite sides of the Valle Nuevo-Alto Bandera-La Nevera ridge. The habitat at the higher elevations of this ridge is actually some of the better protected habitat in the Dominican Republic - the Reserva Cientifica Valle Nuevo protects (at least on paper) the pine forest habitat that is dominant at elevations above 2200m, as well as the coniferous cloud forest that forms a belt right underneath it. Unfortunately, *Anolis fowleri* is only found in patches of montane deciduous hardwood forest that sit just underneath the cloud forest.

Amazingly, on both sides of the mountain, the park's boundary excludes this critical and increasingly scarce hardwood forest by a matter of meters. And sure enough, our *fowleri* female was found on unprotected land near a farm - it was within sight of the park in a small patch of streamside forest that will probably be cleared within a decade. So while were very excited to find this animal, we were discouraged that it turned up on the same threatened land where it had previously been collected.

Nonetheless, this find was encouraging, and we were anxious to observe individuals in the wild, so we redoubled our efforts over the next week. We criss-crossed the entire range encompassing Schwartz's localities, exploring every road, trail, and patch of montane deciduous forest we could find. We stopped by numerous fincas with forest nearby and asked workers if they recognized images of *Anolis fowleri*. To control for the possibility of getting bogus answers, I had printed sheets featuring a diversity of anoles, including species from that area as well as anoles from other parts of the DR, and even one from Jamaica. Responses varied, but more than one field laborer said they occasionally (but very rarely) saw *fowleri*, often saw *etheridgei*, *christophei*, and *cybotes*, but never saw the 'foreign' anoles in these mountains (i.e., their stories checked out). Although we did not conduct many of these interviews, I think *fowleri* probably exists at low densities at more sites than had previously been recognized (although to be fair, Schwartz [1973] also suspected as much).

But we were unable to verify this by finding another animal near Valle Nuevo. After many nights of cold fruitless searches, I terminated the effort in order to move on with the other goals of the trip. This was bitter medicine with just one animal and no observations in the wild, but we had lots more to accomplish and time was getting short.

We didn't leave the mountains of the Cordillera Central altogether though, but moved about an hour's drive northeast to the Ebano Verde Field Station to study *Anolis christophei*. This station sits on the border of another reserve (Reserva Cientifica Ebano Verde), and is actually less than a kilometer from "La Palma," the lower montane streamside site made famous by Stan Rand and Ernest Williams. It was in a seminal paper titled "The anoles of La Palma" that Rand and Williams first presented the underpinnings of their famous ecomorph concept (1969). Naturally, we were excited to be working at this site, and even happier that we could stay at a bona fide field station right down the road.

La Palma and the Ebano Verde Field Station sit at about 1080m - a moderate elevation for anoles - but as you travel east into the Ebano Verde Reserve, you quickly ascend Casabito Mountain, which peaks in cloud forest at just under 1500m. We were quite busy working with the anoles near the field station, but it was hard not to look up the nearby mountains and wonder if there might be *fowleri* in the shrouded hills. This was all a pipe dream of course, but our friend Miguel Landestoy did his best to remind us of the possibility whenever he could ("it's GOTTA be there!"), and we soon learned that a long, steep trail connected the station to the Casabito peak, which featured an observation tower and was famous to birdwatchers. Better *still*, we learned there were cell phone towers on the peak, complete with a private access road leading right to the top (we're herpetologists damnit, not some trail-happy yuppies…)!

We were able to score a key to the gate and late the next night (Aug. 24/25), we figured what the heck - let's see what's up there! It was an easy enough drive along the access road to the peak, and we parked our truck by the satellite antennae at about 12:40AM. After deftly outwitting a very diligent guard dog suffering a bad case of bronchitis, Yoel, Travis, and I located the Casabito Trail and began to descend from the peak into the cloud forest. We made it less than 100 meters.

After just a couple minutes of walking, Travis jumped back, started babbling incoherently (not in his usual way), and waved his finger towards a clustered mass of dead bracken hanging from a tree fern right over the trail… trying without success to blurt complete expletives. Sure enough, it was a sleeping female *Anolis fowleri*! We were so elated we could barely contain ourselves, and we did our best to trade high-fives without making any noise. We couldn't help but wonder how Ernest Williams and Stan Rand might have reacted upon learning that *Anolis fowleri* could be found just up the hill from their La Palma site!

This time the circumstances were a bit different. The animal was in a great position for behavioral observation, it was hanging above a reasonably wide trail, and above all, we were high on a mountain within the secluded confines of a protected reserve. This was an anole we could watch in the morning!

So that's what we did. Since we hadn't actually anticipated finding anything, we had packed light and needed to return to the station to retrieve our video cameras and other gear. We knew that leaving such a special anole unattended would guarantee its escape, so Yoel stayed behind while Travis and I made the run. We sped to the station, threw together the gear, quickly boiled some eggs and cocoa-saturated coffee, and drove back up the mountain. The three of us spent the rest of the night exploring the area for more anoles, but always with one person watching the sleeping *fowleri*. About half an hour before dawn we began videotaping.

Fig. 3 - Female *Anolis fowleri* from Ebano Verde perched in dense arboreal habitat (A), and displaying with dewlap at full extension (B). Note that the dewlap is backlit.

As the night blended into an overcast morning, we recorded the first diurnal observations of *Anolis fowleri*. Over a period of nearly six hours, we ran the tape on one of the slowest, most deliberate, and least active anoles we had ever encountered. Although it's possible that it was simply wary of us (we were careful not to be intrusive), the anole took over an hour to creep just one meter through the tangled matrix of fern leaves and vines in which it was perched, and several times it sat nearly motionless on a single perch for over half an hour. Despite the fact that female *fowleri* are usually a beautiful light green, this individual remained brown for nearly the entire morning, possibly to blend in with the hanging dead leaves that comprised most of its perches. Over the hours, we watched the anole explore a roughly two meter diameter of tangled habitat, feed at least once (we later flushing its stomach and obtained tiny snails and a great variety of arthropods), and slowly display using its dewlap on two occasions (Fig. 3). The full display consisted of a smooth, 5-6 second extension of the dewlap, followed by a short (1 second), sharp headbob once the dewlap was at full extension, and then a slow retraction of the dewlap. In the first instance, it dewlapped three times in slow succession, and in the second instance, it flashed the dewlap briefly, gave a full display pattern, and then extended the dewlap for another 15 seconds without bobbing the head. It's unclear whether these were broadcast displays, or whether it was displaying antagonistically at us, since we were filming it from several meters away. In any case, it's notable in that this was a female animal, and it was deliberately using its large, colorful dewlap for communication. Eventually, at around noon, we were running out of video space and camera batteries, so we noosed the anole and brought it back to the station, where we set it up in a large butterfly cage. This ended up being the last *fowleri* we found that season (and I have not looked for it since). Although we only found two individuals, as we prepared to leave the DR a couple of weeks later, we were thrilled to have found one in a protected cloud forest and to have seen it in the wild during the daytime, even if just for one overcast morning.

One day before I flew home, I spent a few hours checking out the newly renovated herpetological collections at the Museo Nacional de Historia Natural, where ornithologist Robert Ortiz, ichthyologist Peter Sánchez, and herpetologist Christian Marte gave me the grand tour. The anoles were neatly organized by species on the shelves, but I noticed that at the very end, a small jar with a tiny lizard was labeled "*Anolis sp.*" Figuring it was an unidentified hatchling of something common, I picked it up and, lo and behold, it was a tiny female *Anolis fowleri.* The little lizard had been collected by Marcelino Hernández in 1999 in the Jánico municipality (Paraje Cerro Prieto, seccion Franco Bidó), which is roughly 50km NE of Constanza, but still in the Cordillera Central (this specimen was from Santiago province, which is a new province record; all previous localities are within La Vega province). Another additional locality, right before the end of the trip!

In the end, *fowleri* proved as challenging as ever, but we were able to throw a few rays of light onto its biology and natural history. We can now document its occurrence not just at Schwartz's original localities around Valle Nuevo, but also in the deciduous cloud forest habitat within the Ebano Verde reserve (a protected locality!), and somewhere along the Cerro Prieto trail in Santiago province. Furthermore, field laborers at other sites near Valle Nuevo gave realistic accounts of seeing it, although apparently not very often. *Anolis fowleri* is likely a truly rare anole, occurring at low densities across a sizeable portion of the Cordillera Central (an alternative possibility is that it lives high in the trees and is only seen when it is low, but I doubt this given that many specimens have been collected sleeping near each other on fairly low perches in the past). At 1474m, the Ebano Verde specimen was found at a lower elevation than had previously been recorded (1647m, although Schwartz [1973] reported his team seeing but not capturing one at 1586m), so we now know that the species may occur from 1450m to 1769m. Like several previously collected *fowleri*, the Ebano Verde specimen was found in a tree fern in montane deciduous hardwood forest. Tree ferns were abundant at this site, and nearly all of the vegetation was richly covered in hanging moss and epiphytes, as is typical of Hispaniolan cloud forest. Unlike previously collected specimens, the Ebano Verde *fowleri* was not found in association with a stream or ravine – all those described by Schwartz had been found in forest flanking mountain streams, although this may simply be due to fact that ravines are often the last habitats remaining when an area is deforested. During the day, a single female *Anolis fowleri* was incredibly inactive – only when attacking prey did it move with anything resembling urgency. It is likely a thermal conformer – when we noosed it at 11:30AM, both its cloacal temperature and the air temperature read 19.0°C. This female dewlapped in our general direction at two different times, exhibiting a slow, smooth display action pattern with a protracted extension followed by a short, sharp head bob. We could not tell if this display was antagonistic, and directed at us, or whether it was intended as a more general broadcast display. For what it's worth, when we returned to our lab in the United States, both females would promptly dewlap if you approached too closely, so I suspect the display we observed in nature may have been directed at us. Both the Valle Nuevo and the Ebano Verde animals had recently consumed a very diverse variety of minute invertebrates. From investigation of flushed stomach contents and feces, we determined that the former lizard had recently dined on snails (4), spiders, beetles, ants, dipterans, and a caterpillar, while the latter had previously enjoyed snails (3), beetles, dipterans, ants, a wasp, a spider, a tiny caterpillar, and possibly an orthopteran. Nearly all of these prey items were remarkably small, and the largest snail was a tiny cone snail 4.6mm long. All of the snails had been swallowed completely intact, and the shell of one appeared to have already been partially digested. In captivity, these animals have readily eaten larger items such as moths and crickets, lunging at their prey rather clumsily with jaws open and tongue extended. Also in the lab, we measured bite force and conducted sprinting trials, chasing the animals up a 3cm diameter dowel mounted at a 45-degree angle. The maximum recorded bite force measurements were 11.56N and 11.69N for the Valle Nuevo and Ebano Verde females respectively (n = 7 and 4 trials). In three trials, these animals sprinted at maximum speeds of 27.47cm/s and 20.83cm/s respectively at 22°C. This seemed fast given the activity we observed in nature, but it's not particularly speedy for an anole of this size (at a slightly higher temperature of 25°C, they achieved maximum speeds of 24.27cm/s and 32.05cm/s, respectively). The animals were surprisingly clumsy in ascending the dowel. Finally, in early September 2008 (within a week after our return to the United States), the Ebano Verde female laid an egg on top of the dirt substrate of its cage. The egg, which may have been fertile, was elongate and white with a flexible shell, measured 16.2mm by 7.3mm, and was neither buried in the soil nor cemented to a branch or other available substrate. Sadly, these factors contributed to the demise of that egg, as a feeder cricket discovered it before we did and chewed a hole into the shell. Neither female laid another egg in 2008, but in early October of 2009, both began laying eggs fairly frequently (perhaps one every 2-3 weeks) for about two months (eggs appeared very sporadically in later months as well). Although they were nearly identical to the first egg in dimensions, these eggs were probably infertile. We incubated several of them, but they all grew moldy within a week or two. Data are too few, and laboratory conditions differ from conditions in the wild, but it seems possible that *Anolis fowleri* have a late summer or early fall reproductive season. Based on our observations, it's likely that reproduction is seasonal in any case.

This is what we have learned so far in our work on *Anolis fowleri*. Obviously, much more remains to be discovered! High on our list will be to acquire additional wild observation data, as well as to obtain a live male so that we can learn more about mating and reproduction, and perhaps further down the line, ontogeny. Of course if anyone knows anything else about *Anolis fowleri*, and especially if they have encountered one in the wild, please let me know!

Acknowledgements

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Anolis fowleri **female posed in a bromeliad at the Ebano Verde field station.**

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Ecological opportunity and the rate of morphological evolution in the diversification of Greater Antillean anoles

According to the theory of adaptive radiation, ecological opportunity is a primary factor regulating the tempo of diversification (Schluter 2000; Gavrilets and Losos 2009). As lineages proliferate and the diversity of competing species increases, ecological opportunity is expected to decline and the pace of diversification is expected to slow (Sepkoski 1978; Walker and Valentine 1984). Most often, empirical studies of the tempo of evolution during adaptive radiation have focused on patterns of lineage diversification – measuring rates of speciation and extinction over time in a diversifying lineage. These studies test a specific prediction of the ecological theory of adaptive radiation: that greater ecological opportunity will increase the likelihood of lineage divergence. Although such investigations initially fell within the realm of paleontology (e.g., Simpson 1953; Stanley 1979; Raup 1985), more recently it has become possible to test for diversity-dependent patterns of diversification using time-calibrated phylogenies of extant taxa (Nee et al. 1992; Harvey et al. 1994; Mooers and Heard 1997; Phillimore and Price 2009). Several studies have provided support for the ecological opportunity hypothesis by documenting a temporal slowdown in species diversification rate (e.g., Lovette and Bermingham 1999; Weir 2006; Phillimore and Price 2008; McPeek 2008; Rabosky and Lovette 2008). All such studies of lineage diversification, both paleontological and molecular phylogenetic, assume that observed decreases in the rate of species diversification are primarily due to increasing saturation of niche space and a decline of ecological opportunity as a clade acquires more species during the course of its radiation.

However, rates of species diversification may vary for reasons unrelated to ecological opportunity, such as in largely allopatric "non-adaptive radiations," in which species richness does not strongly correlate with ecological diversity (Kozak et al. 2006; Rundell and Price 2009). One potential solution to this problem is to test for the signature of ecological opportunity in patterns of morphological evolution, which may be more directly affected by niche-filling and the erosion of ecological opportunity than species diversification rate (Ricklefs and Travis 1980; Roy and Foote 1997). Indeed, studies of paleontological data have found evidence for a relationship between ecological opportunity and the rate of morphological evolution (e.g., Saunders and Swan 1984; Gould 1989; Foote 1992; Friedman 2010; reviewed in Erwin 2007). Such paleontological investigations, however, have been necessarily restricted to organisms with exceptional fossil records, and rely on methods that are inapplicable to a wealth of recent radiations – including many extant groups for which we have a rich understanding of ecology and microevolution (like anoles!).

In this study, using new statistical methods, we investigate whether the rate of ecological diversification slows during the course of adaptive radiation by estimating rates of morphological evolution in five ecological trait axes in the well-studied adaptive radiation of Greater Antillean anoles. First, we examine whether the rate of phenotypic evolution slows with time (we refer to this as the time model). However, because time may not always correspond closely with ecological opportunity in a geographically complex radiation, we introduce a second method to test for the role of ecological opportunity, which we call the lineage diversity model. This model more explicitly measures ecological opportunity by estimating the number of competing lineages from within a radiation that are present in the same geographic area (see Nee et al. 1992 and Rabosky and Lovette 2008 for application of similar models to patterns of lineage diversification rather than phenotypic evolution). In the radiation of Greater Antillean *Anolis*, the lineage diversity and time models predict different patterns of evolutionary diversification because anoles have colonized and radiated on four large islands for the most part independently, with in situ diversification occurring at different times on different islands (Losos et al. 2006; Losos 2009). For example, when the first lineage to reach Jamaica arrived and began to radiate, diversification was already well underway in Cuba and Hispaniola. Therefore, time since the beginning of the radiation does not capture the difference in the timing of decline of ecological opportunity among the islands of the Greater Antilles.

In the present study, we use new quantitative, likelihood-based phylogenetic methods to test the ecological opportunity hypothesis for the pace of evolutionary diversification in Greater Antillean anoles. We ask if the rate of phenotypic diversification for ecologically relevant traits was constant over time, or whether it varied as a function of the diversity of competing lineages or time since the origin of the radiation.

Greater Antillean Anolis lizards are ideal for a study of ecological opportunity because they are very ecologically diverse, and they have radiated under both high opportunity and low opportunity conditions. Greater Antillean anoles are the result of a single overwater colonization event from a mainland source population (Nicholson et al. 2005). In general terms, diversification began first on the islands of Hispaniola and Cuba, followed fairly rapidly by the occupation of Puerto Rico by two lineages which did not diversify (Fig. 1). Later, diversification proceeded greatly on Puerto Rico after the arrival of a third lineage and on Jamaica, which probably was underwater until some point during the Oligocene (Buskirk 1985). Thus, the pattern of diversification was heterogeneous; as lineage diversity was low at different times on different islands.

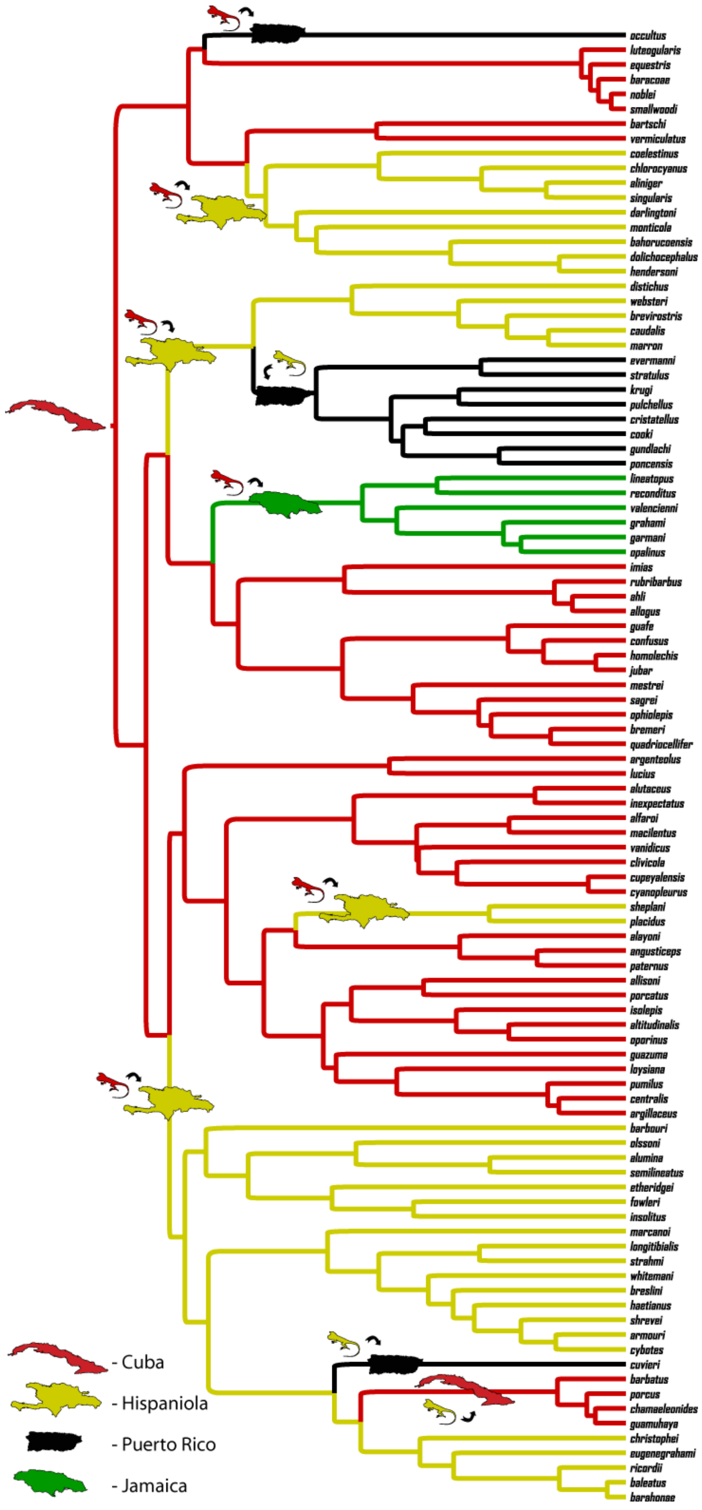


Fig. 4. Phylogeny for Greater Antillean *Anolis* with branch lengths proportional to time. The ML estimate of geographic history is mapped onto the tree, based on current distributions. Branches, lizards, and islands are colored by geography. Arrows and lizards indicate inferred colonization events, in which the colonist is colored to match its source.

Methods

Sampling

We conducted near-comprehensive sampling of Greater Antillean anole species to create phylogenetic and morphometric data sets. We first sampled mtDNA data from 187 anole species occurring throughout the New World tropics to generate a time-calibrated Bayesian phylogenetic estimate for *Anolis*. For the comparative analyses, we pruned the 87 (primarily non- Greater Antillean) species not included in our morphological data set to obtain a tree with 100 species. To ensure that our results were robust to phylogenetic uncertainty, we conducted comparative analyses on a set of 898 trees from the stationary distribution of the Bayesian phylogenetic analysis.

A single investigator (DLM) measured 22 morphological traits from preserved specimens (specimens per species: mean = 7.9; range = 1 – 19), focusing on traits the ecological significance of which is well established (Losos 2009). We log transformed measurements and obtained species mean values for each trait. To control for size and simplify our data, we then performed a phylogenetic linear regression of each trait on ln(SVL), and performed a phylogenetic principal components analysis (PCA) on the residuals (Revell 2009). We used body size and PC scores for the first four PC axes (eigenvalues greater than one) for our analyses of evolutionary rate.

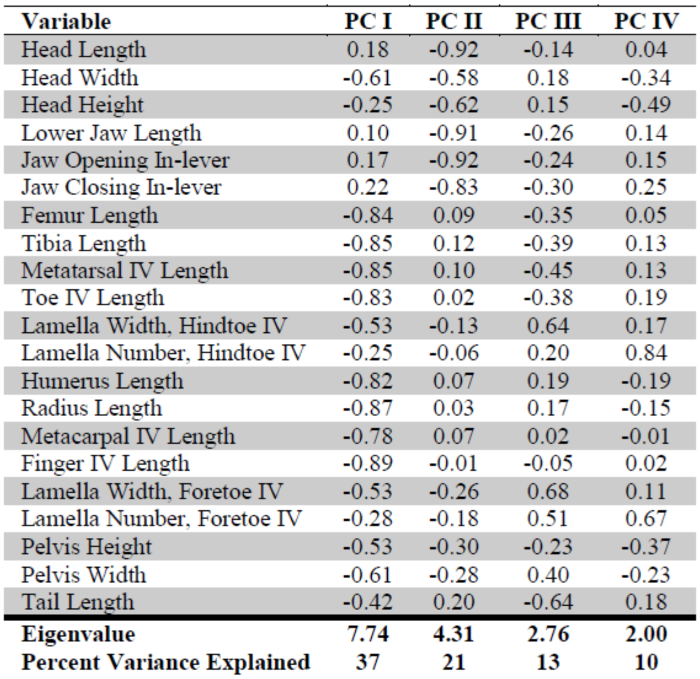
Estimating Ecological Opportunity and Rate

To test the hypothesis that the rate of phenotypic evolution in Caribbean *Anolis* has varied as a function of ecological opportunity, we (1) estimated opportunity as the richness of sympatric lineages at each node in the tree (for the lineage diversity model) or as the relative age of each node (for the time model), (2) calculated independent contrasts for body size and scores on the four PC axes obtained from our morphometric data set, and (3) used a model-fitting analysis to ask if the rate of morphological evolution is best predicted by estimates of lineage diversity, time, or a null model in which rate of evolution are constant throughout the Greater Antillean anole radiation.

To measure lineage diversity at internal nodes, we estimated the number of anole lineages present on an island at the time that a focal lineage on that island was undergoing speciation. Uncertainty exists in the reconstruction of both the geography of the node at which lineage divergence is occurring, as well as the geographic location of all other lineages present at that time, many of which might occur on the same island as the diverging lineage. To incorporate uncertainty in ancestral reconstruction of island occupancy, we developed a new three-step method to estimate island lineage diversity at each internal node in the tree while accounting for the maximum likelihood probabilities that each node occurred at a variety of possible geographic locations. This procedure is described in detail in a manuscript currently in press at Evolution (as are our methods for estimating the parameters and the maximum likelihood score of each model).

At the end of this procedure, for each of our five trait axes (SVL plus shape PCs I-IV), we compared the likelihoods of all three models for the rate of evolution (the lineage diversity model, the time model, and the single-rate model) using AIC$_c$.

We also conducted a simulation analysis, primarily to test the sensitivity of these methods to historical extinction. In brief, we found our methods to be highly effective at identifying the correct model of evolution, even when data were evolved on phylogenies that underwent considerable extinction (see Evolution manuscript for more details). We performed all size correction, PCA, and estimation of opportunity and rate using scripts written in R, whereas we maximized likelihood equations for our models using MATLAB scripts.



Variable	PC I	PC II	PC III	PC IV
Head Length	0.18	-0.92	-0.14	0.04
Head Width	-0.61	-0.58	0.18	-0.34
Head Height	-0.25	-0.62	0.15	-0.49
Lower Jaw Length	0.10	-0.91	-0.26	0.14
Jaw Opening In-lever	0.17	-0.92	-0.24	0.15
Jaw Closing In-lever	0.22	-0.83	-0.30	0.25
Femur Length	-0.84	0.09	-0.35	0.05
Tibia Length	-0.85	0.12	-0.39	0.13
Metatarsal IV Length	-0.85	0.10	-0.45	0.13
Toe IV Length	-0.83	0.02	-0.38	0.19
Lamella Width, Hindtoe IV	-0.53	-0.13	0.64	0.17
Lamella Number, Hindtoe IV	-0.25	-0.06	0.20	0.84
Humerus Length	-0.82	0.07	0.19	-0.19
Radius Length	-0.87	0.03	0.17	-0.15
Metacarpal IV Length	-0.78	0.07	0.02	-0.01
Finger IV Length	-0.89	-0.01	-0.05	0.02
Lamella Width, Foretoe IV	-0.53	-0.26	0.68	0.11
Lamella Number, Foretoe IV	-0.28	-0.18	0.51	0.67
Pelvis Height	-0.53	-0.30	-0.23	-0.37
Pelvis Width	-0.61	-0.28	0.40	-0.23
Tail Length	-0.42	0.20	-0.64	0.18
Eigenvalue	**7.74**	**4.31**	**2.76**	**2.00**
Percent Variance Explained	**37**	**21**	**13**	**10**

Table 1 - Loadings, eigenvalues, and variance explained for a phylogenetic principal component analysis on phylogenetically size-corrected shape variables for Greater Antillean *Anolis*.

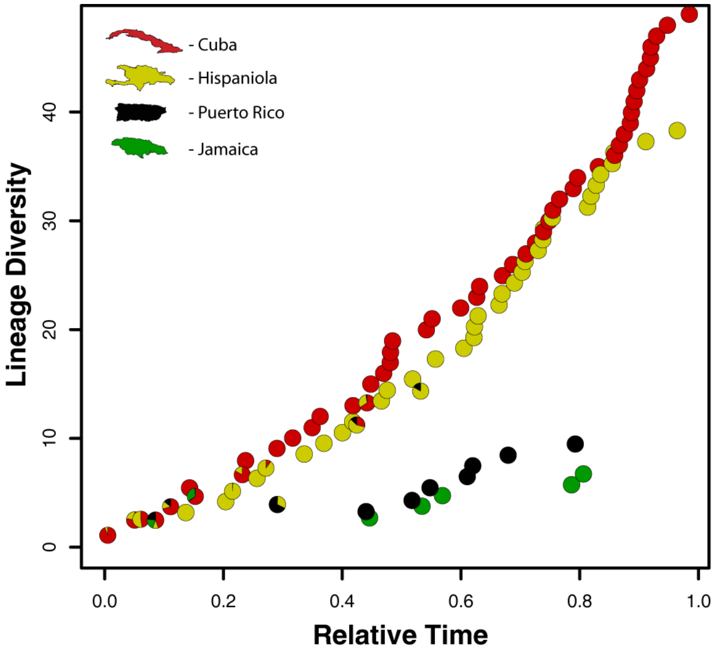


Fig. 2 - Comparison of historical lineage diversity estimates and relative branching time estimates for each node in the phylogenetic topology, colored by island likelihood. Lineage diversity generally increases with increasing time from the root, but the specific pattern of increase depends on geography.

Results

The first four axes produced by PCA of shape residuals represent 80.1% of the total standardized shape variation among species (Table 1). The first PC axis correlates strongly with the lengths of the limb bones; the second correlates primarily with head size and shape; the third axis reveals a contrast between lamella width and tail length; and the fourth axis correlates most strongly with lamella number.

Anolis lineage diversity generally increases with time on each island in the Greater Antilles (Fig. 2). However, species diversification on Puerto Rico and Jamaica begins later than on the other islands such that lineage diversity estimates for the early nodes in these radiations are near-zero even as values are much higher on Cuba and Hispaniola (Fig. 2).

Standardized independent contrasts of body size and PC I are plotted against estimated lineage diversity and time in Fig. 3, and exhibit decreasing rates of phenotypic diversification with increasing values on these axes. For body size, the lineage diversity model fits the data better than either the time model or the single rate model (Table 2). For PC I, both the time and lineage diversity models are favored over the single rate model (Table 2). For the remaining PC axes, the rate of evolution is relatively uniform with increasing lineage diversity and time, and all three models of rate variation exhibit similar levels of support (Table 2).

Table 5 - Comparison of models of rate variation. All values represent averages from 898 topologies sampled randomly from a Bayesian posterior distribution. σ_0^2= starting rate; ψ = rate change.

Trait	Model	σ_0^2	ψ	log(*L*)	ΔAIC_c	AIC_c weight
SVL	single rate	0.14	-	-42.7	7.21	0.04
	time	0.25	-0.18	-40.7	5.34	0.09
	lineage diversity	0.20	-3.5E-3	-38.0	0.00	0.87
PC I	single rate	7.75	-	-241.9	4.04	0.12
	time	13.68	-10.04	-238.8	0.00	0.71
	lineage diversity	10.06	-0.12	-240.2	2.80	0.18
PC II	single rate	4.25	-	-212.1	0.00	0.57
	time	4.09	0.27	-212.1	2.00	0.21
	lineage diversity	4.11	0.01	-212.1	1.97	0.21
PC III	single rate	2.67	-	-189.1	1.08	0.32
	time	3.86	-2.04	-187.6	0.31	0.44
	lineage diversity	3.11	-0.02	-188.2	1.47	0.24
PC IV	single rate	2.02	-	-175.2	0.59	0.40
	time	2.75	-1.26	-174.2	0.72	0.37
	lineage diversity	2.29	-0.01	-174.2	1.32	0.23

Discussion

The ecological theory of adaptive radiation predicts that the tempo of diversification should decline as ecological opportunity diminishes during a radiation. Studies of diversification patterns in extant taxa have primarily investigated the rate of lineage diversification, and many have reported decreasing diversification rates over time (e.g., Lovette and

Bermingham 1999; Phillimore and Price 2008; Rabosky and Lovette 2008), which implies that the process of speciation is influenced by ecological opportunity. This approach, however, offers little insight on how opportunity influences the evolution of the ecological features of these species.

Our study provides a new perspective by (1) estimating rates of evolution in ecomorphological traits rather than the number of lineages, and (2) testing whether such rates are related to ecological opportunity measured using a new metric, the diversity of existing lineages, in addition to the more conventional metric of time since the evolutionary origin of a clade. We find that phenotypic diversification on two important adaptive axes – body size and relative limb length – decreased with time and increasing lineage diversity in the adaptive diversification of Caribbean anoles, which suggests that opportunity affects rates of ecological

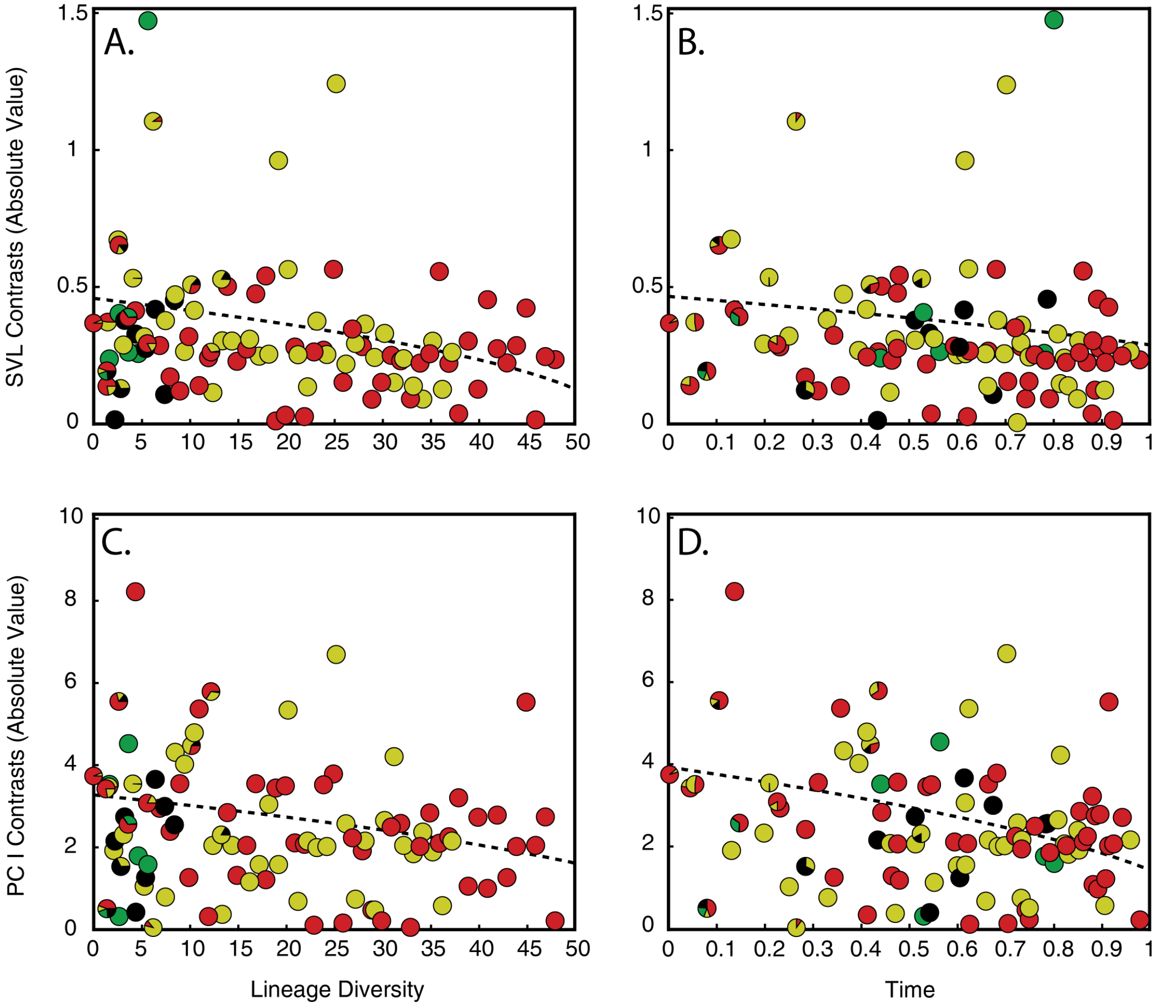


Fig. 3 - Absolute values of standardized phylogenetically independent contrasts of morphological traits for the Bayesian phylogeny of Greater Antillean *Anolis*. (A) and (B) show body size contrasts with increasing lineage diversity and relative time, respectively. (C) and (D) show PC I contrasts with increasing lineage diversity and relative time, respectively. Dashed lines show the expected standard deviation of the contrasts under the best-fit model in which the evolutionary rate varies as a function of the number of lineages (left panels) or time (right panels). Colors represent the islands on which nodes occurred, estimated using maximum likelihood.

diversification, and not just rates of lineage diversification.

Our results corroborate paleontological investigations of morphological disparity over time in fossil lineages radiating under conditions of both high and low ecological opportunity (reviewed in Valentine 2004; Erwin 2007). In general, these studies show initial rapid increases in disparity in lineages presented with new ecological opportunities, followed by a slowdown in morphospace occupation (e.g., Foote 1992). Such patterns match those observed in *Anolis,* in which rates of morphological evolution are high early in the radiation and when the diversity of competing lineages is low. Unlike most of these paleontological studies, however, previous work on the ecology and behavior of anoles allowed us to investigate traits that have been demonstrated to be adaptively important. Consequently, our observation of decreasing rates of evolution in these traits with declining ecological opportunity can be directly interpreted as a decrease in the rate of adaptive differentiation.

Opportunity, rate, and ecomorphology in *Anolis*

Greater Antillean anoles are well known as an example of replicated adaptive radiation, in which diversification on different islands has produced similar outcomes despite largely independent histories. We estimated rates of evolution for a set of ecomorphologically significant traits that vary substantially among species specialized to use different parts of the environment (Williams 1983; Losos 2009). We found support for the ecological opportunity hypothesis in both body size and relative limb length. For body size, we found that the lineage diversity model was most favored, which suggests that increased numbers of competing anole lineages led to a decrease in rates of body size evolution. The ecological opportunity hypothesis was also supported for relative limb lengths (PC I), but in this case, the ecological models of time and lineage diversity both exhibited statistically comparable levels of support (Burnham and Anderson 2002).

The ecological significance of body size and relative limb lengths is well established in anoles. In anole communities, interspecific body size differences are associated with differences in both diet and habitat use (Schoener 1968, 1969, 1970, 1975; Lister 1976; Pacala and Roughgarden 1982; reviewed in Losos 2009), and likely evolved in response to interspecific competition among anole species (Schoener 1970; Roughgarden and Pacala 1989; Losos 1992, 2009; Roughgarden 1995). Similarly, the functional and behavioral significance of variation in limb length is well-established (e.g., Losos 1990; Irschick and Losos 1998, 1999; reviewed in Losos 2009) and sympatric species that occupy dissimilar microhabitats differ in relative limb length (Williams 1983; Losos 2009).

We did not find substantial support for the role of ecological opportunity in the evolution of the remaining three trait axes, primarily representing head and toepad dimensions and tail length. Although variation in these traits is also related to ecological variation (Losos 2009), the lack of a relationship with ecological opportunity may stem from several causes. First, some of these traits, such as head dimensions, are also the target of sexual selection (Lailvaux et al. 2004; Herrel et al. 2007); because the intensity of sexual selection need not be related to ecological opportunity, rates of evolution of such traits might not be expected to be correlated with opportunity. Also, Greater Antillean anoles exhibit a pattern of evolution in which adaptation to different structural habitats (e.g., twigs, canopy, bushes) evolved early in the radiation, but subsequent diversification along other resource axes (e.g., thermal microhabitats) continued throughout the radiation (e.g., Glor et al. 2003; reviewed in Losos 2009). Consequently, some of the traits on these three axes may be involved in this secondary aspect of anole differentiation, and therefore exhibit comparatively constant rates of evolution throughout the radiation. An additional possibility is that we may not have detected a signature of ecological opportunity due to sampling error, which is expected to be more problematic for more minor PC axes.

The mark of ecology on diversification

The result that rates of morphological evolution in anoles are correlated with historical estimates of ecological opportunity suggests that ecological processes may influence patterns of lineage diversification. However, this relationship may be generally difficult to detect, particularly because diversification often occurs over large geographical scales. Under scenarios in which lineage diversity is primarily distributed allopatrically, members of a diversifying lineage will interact little with each other. In such cases, ecological opportunity will usually be determined by a diverse suite of interacting species that are not closely related, and it may therefore be much more difficult to estimate opportunity using phylogenetic methods.

We suggest that the present methods for estimating past ecological opportunity and testing its relationship to patterns of phenotypic evolution will be most powerful when ecological interactions are primarily among members of a single clade, as is often the case in adaptive radiations on islands and in lakes (e.g., Fryer 1996; Grant 1998; Chiba 2004, reviewed in Losos and Ricklefs 2009). On continents, it may be much harder to estimate ecological opportunity. Of course, following mass extinctions, evolutionary recovery is often dominated by a small number of clades that radiate widely and rapidly – even in biogeographically large regions, such as continents (Erwin 2001). In such scenarios, the methods presented here may also detect the signature of declining opportunity in the phenotypic pattern of diversification.

Anoles provide an ideal system to test whether rates of phenotypic evolution exhibit a relationship with historical estimates of ecological opportunity in continental as well as in island radiations. In addition to radiating in the Caribbean, two anole clades have diversified in neotropical mainland habitats (Nicholson et al. 2005; Pinto et al. 2008). Although we presently lack the phylogenetic data necessary to adequately estimate ecological opportunity and rates of evolution in these clades, examination of these taxa is an exciting avenue for future investigation.

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Earliest records of *Anolis bimaculatus* (sensu lato) from the Virgin Islands

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The first specimen of *Anolis bimaculatus* from the Virgin Islands I knew of was the gravid adult female *A.b. leachi* that my wife, Nanette, and I collected on 22 June 1985 in Riise Alley in downtown Charlotte Amalie, St. Thomas. The female and her egg are now MCZ 178132. She was one of several individuals seen in Riise Alley and the adjacent alley to its west. My recollection is that W.P. 'Bill' MacLean of the College of the Virgin Islands had received reports of this species from people who worked in the area, and our search of the downtown area was undertaken at Bill's suggestion.

During St. Thomas's history as an important trading port in the age of sail and steam, commercial enterprises established their warehouses and factories in long low buildings running perpendicular to the waterfront (F.C. Gjessing and W.P. MacLean. 1987. *Historic Buildings of St. Thomas and St. John*. Macmillan Caribbean). In modern times these warehouses have been converted into shops and restaurants serving the tourist trade, with pedestrian walkways or alleys running between the buildings, from the waterfront to Main Street. Riise Alley is named for A.H. Riise, a 19th century Danish apothecary who collected amphibians and reptiles for European museums and E.D. Cope, and who was also the founder of the prominent St. Thomas trading and retail company that still bears his name. The alleys are more or less watered and planted with ornamentals depending upon the businesses along the alley. Riise Alley, at the time of our visit, was well-watered, with numerous plants, leaking hoses, and spigots, and seemed decidedly wetter than other parts of downtown. Other parts of downtown were inhabited by the native *Anolis cristatellus* and *A. stratulus*.

Unknown to us at the time, four months earlier, on 8 February 1985, Alejandro Sanchez, an associate of Juan Rivero of the Universidad de Puerto Rico, Mayaguez, had collected an *A. b. leachi* in St. Thomas "on a tree along street". There is no further locality, but Riise Alley is not excluded by the description. Rivero sent the specimen to the MCZ, and it is now MCZ 173031.

I have recently examined both specimens, and they are *leachi* from the Antigua bank. The population was presumably established by individuals carried in commerce from down island (perhaps even deliberately by tourists - ships visit both places). I am unaware of the current status of the population.

Interestingly, there may have been a much earlier introduction of *Anolis bimaculatus* to the Virgin Islands, although the possibility of locality mislabeling cannot be excluded. In 1918, Henry W. Fowler (Some amphibians and reptiles of Porto Rico and the Virgin Islands. *Papers from the Department of Marine Biology, Carnegie Institution of Washington* 12:1-15) reported on two collections, one from Puerto Rico, the other a smaller and older collection from the Virgin Islands made by A.D. Brown in 1876. Included in the Virgin Island collection were eight anoles that Fowler described as new, naming them *Anolis mayeri*, in honor of Alfred G. Mayer of the Carnegie Institution (no relation to the present writer!).

Anolis mayeri is a synonym of *A. b. bimaculatus* of the St. Kitts bank (J.D. Lazell. 1972. The anoles (Sauria, Iguanidae) of the Lesser Antilles. *Bulletin of the Museum of Comparative Zoology* 143:1-114), and the locality has been assumed to be in error. But might this represent an earlier introduction of a *bimaculatus* group anole to the Virgin Islands?

None of Brown's collection had any published locality data beyond "Virgin Islands". Excluding *A. mayeri*, the species recorded were all unobjectionably potentially Virgin Island in provenance: *Leptodactylus albilabris, Eleutherodactylus lentus, Hemidactylus mabouia, Mabuya sloanii, Anolis cristatellus, A. stratulus, A. pulchellus, Ameiva exsul*, and *Alsophis antillensis* (= *portoricensis*), with the exception of a single snake identified as "*Alsophis rufiventris*".

I attempted to investigate further by examining the Brown collection, deposited at Princeton University. Inquiries made on my behalf by my colleague Marty Kreitman (then at Princeton), especially amongst his more senior departmental colleagues, revealed that, unfortunately, Princeton had retained none of its collections, and no possible recipient of the collection was known to the current faculty.

Fortunately, Fowler kept some specimens for the Academy of Natural Sciences, and others made their way to the MCZ, so at least some paratypes of *A. mayeri* survive, which I have been able to examine, although not recently. Without the context provided by more detailed notes or labels that might have accompanied the original, now apparently lost, collection, it is impossible to support with any conviction the hypothesis of an early unsuccessful introduction, and a labeling error would seem the most likely hypothesis.

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Ecological distribution of the Puerto Rico Crested Anole, *Anolis cristatellus cristatellus*, in Cahuita, Costa Rica

Introduction

Anolis cristatellus, the crested anole of Puerto Rico and the Virgin Islands, was designated a "minor colonizer" by E. E. Williams in his classic paper on the ecology of colonization (1969. The ecology of colonization as seen in the zoogeography of anoline lizards on small islands. *Quarterly Review of Biology* 44:345-389). From its Puerto Rican Bank home, it has colonized the Mona and Monito Banks in the Mona Passage and, considerably farther away, several banks in the Turks and Caicos and southern Bahamas. It has also been successfully introduced into Costa Rica, the Dominican Republic, and twice into south Florida (A. Schwartz and R. Thomas. 1975. A check-list of West Indian amphibians and reptiles. *Carnegie Museum of Natural History Special Publication* 1; Jason J. Kolbe, Richard E. Glor, Lourdes Rodriguez Schettino, Ada Chamizo Lara, Allan Larson, and Jonathan B. Losos. 2007. Multiple sources, admixture, and genetic variation in introduced *Anolis* lizard populations. *Conservation Biology* 21:1612-1625).

In Costa Rica, the initial establishment was at Limon, with the earliest record being in 1970 (J.M. Savage, pers. comm. 2009). Crested anoles have since spread to several other localities (Fig. 1; J.M. Savage. 2002. *The Amphibians and Reptiles of Costa Rica*. University of Chicago Press; pers. comm. 2009). The furthest they have reached is Turrialba in the Central Valley, about 90km by road from Limon. Here I report on observations made on the population in Cahuita.

Fig. 1. Distribution of *Anolis cristatellus* in Costa Rica (pins) after Savage (2002). Map from Google Earth.

Study Site and Methods

Cahuita is a small town on the Caribbean coast of Costa Rica, separated from Cahuita National Park by Kelly's Creek, which is crossed by a foot bridge (Fig. 2). The seaward edge of the town is a littoral forest dominated by coconut palm, seagrape, and almond (all common West Indian species; Fig. 3). The national park is much more floristically diverse (Fig. 4). The trees which dominate the littoral woodland in town are present but much less common, and no species dominates the flora.

Anoles were captured and examined in order to identify them to subspecies. Local residents were queried about their knowledge of the crested anoles. Rand censuses were conducted July 12-13, 2006 in the littoral woodland, and on July 13, a brief survey of the adjacent part of the National Park was made with the assistance of Park personnel.

Fig. 2. Aerial view of Cahuita and vicinity (Google Earth).

Fig. 3. Littoral woodland with coconut palms and almond; a small seagrape is at the base of the most seaward palm. Photo by Sean Murphy.

Fig. 4. Diverse forest along trail just landward of the beach in Cahuita National Park. Photo by Sean Murphy.

Results

Identity

The anoles present were *Anolis cristatellus cristatellus*, the Puerto Rican subspecies, with pale orangish dewlaps with pale green centers. The Virgin Island subspecies, *A. c. wileyae*, is easily distinguished by its deeply colored red dewlap with distinctly green center (Fig. 5; H. Heatwole. 1976. Herpetogeography of Puerto Rico. VII. Geographic variation in the Anolis cristatellus complex in Puerto Rico and the Virgin Islands. *Occasional Papers of the Museum of Natural History, University of Kansas* 46:1-18).

Fig. 5. *Anolis cristatellus cristatellus* from Cahuita, Costa Rica. Photo by Sean Murphy.

Chronology

Cahuita is about 35km from Limon. Sr. Guillermo Mora, owner of the hotel at which we stayed, recalled that the crested anoles had been present for about 20 years (i.e. arriving ca. 1986), and he observed that the tail crest, not found in any local anoles, made them distinctive and noteworthy.

Macrohabitat Distribution

Crested anoles were abundant north of Kelly's Creek (the town side). Of 119 lizards encountered during Rand censuses, 113 were crested anoles; the remainder were *Ameiva quadrilineata* (5) and *Gonatodes albogularis* (1). In addition, two species of introduced geckos (*Hemidactylus frenatus* and *Lepidodactylus lugubris*) were observed north of Kelly's Creek, but not seen during the censuses. East of Kelly's Creek, in the National Park, no crested anoles were found. Although it was a brief visit, 16 lizards of seven species were observed: *Hemidactylus* sp. (4), *Gonatodes albogularis* (2), *Anolis limifrons* (2), *Iguana iguana* (1), *Basiliscus plumifrons* (1), *B. vittatus* (1), and *Ameiva quadrilineata* (5).

Mesohabitat Distribution

Within Cahuita town, anole perch sites were classified as littoral woodland, yard, or hotel. Anoles were much more abundant in the littoral woodland than in the other, highly human-modified areas (Fig. 6). This is not due to sampling bias; considerable time was spent in the town proper looking for anoles.

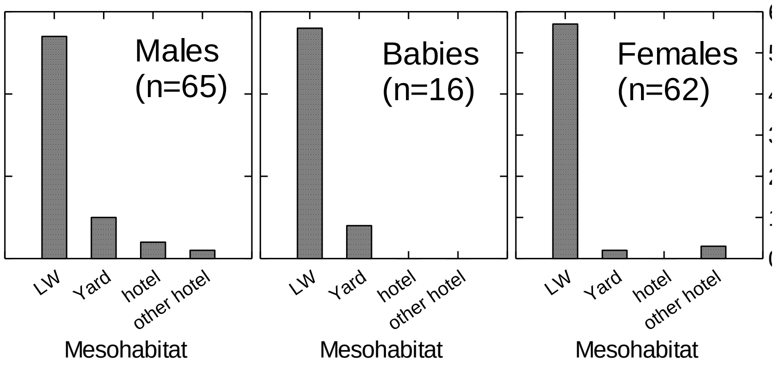

Fig. 6. Mesohabitat distributions of *Anolis cristatellus* at Cahuita, Costa Rica. LW=littoral woodland.

Microhabitat distribution

Perch height distributions were broadly overlapping for males and females, but male perches were on average about twice as high as female perches (Fig. 7). For both sexes the modal and median class for perch diameter was 1-10 cm (Fig. 8). Baby anoles perched lower than females, and had a distinctly bimodal perch diameter distribution, occurring either on very thin perches, or flat surfaces (which have 'infinite' diameter; Fig. 9).

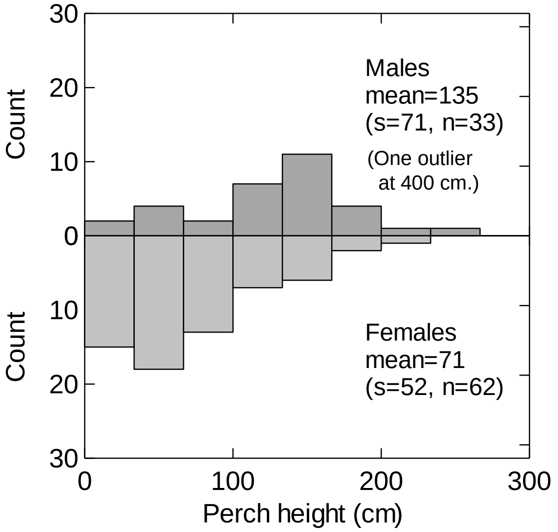

Fig. 7. Perch height distributions of *Anolis cristatellus* at Cahuita, Costa Rica.

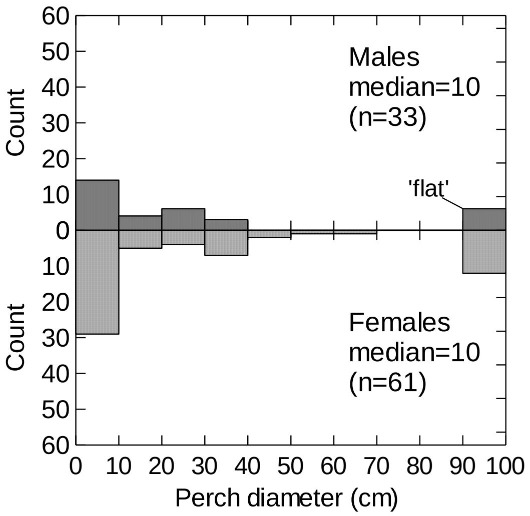

Fig. 8. Perch diameter distributions of *Anolis cristatellus* at Cahuita, Costa Rica. Flat = infinite diameter, arbitrarily plotted as 90-100cm.

Perch type distributions were similar for males and females, the three most frequent types being the same for both sexes; babies were more frequently found on the ground than adults (Fig. 10). Within the arboreal perch types, tree species differed little between the sexes (coconut and almond being most frequent), while babies were again distinctive (Fig. 11).

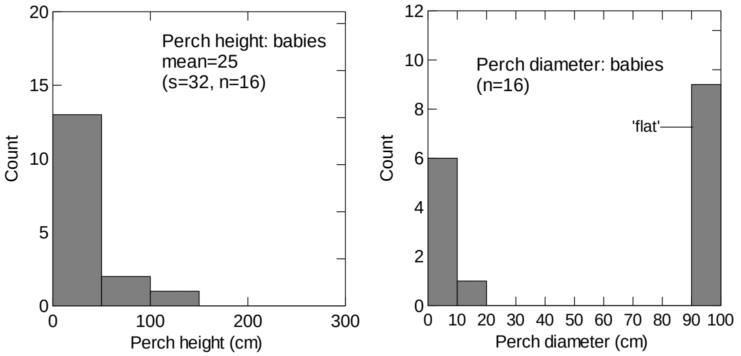

Fig. 9. Perch height and diameter distributions of baby *Anolis cristatellus* at Cahuita, Costa Rica.

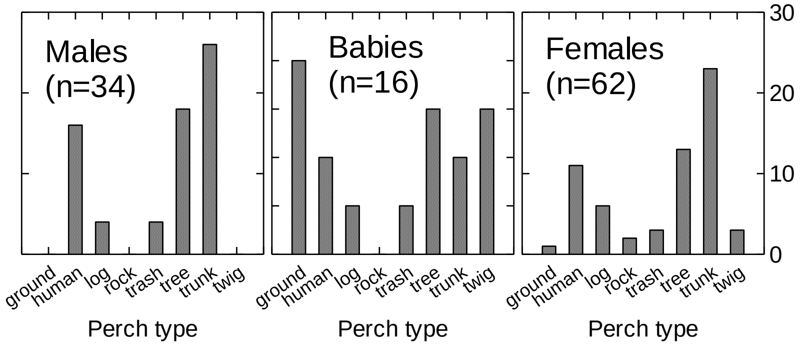

Fig. 10. Perch type distributions of *Anolis cristatellus* at Cahuita, Costa Rica.

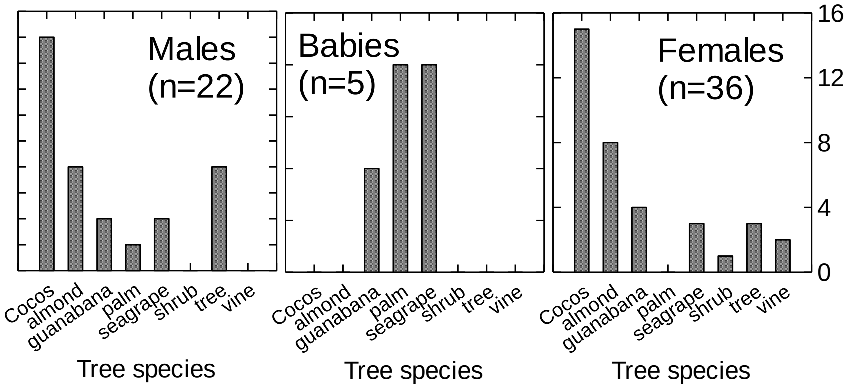

Fig. 11. Tree species distributions of *Anolis cristatellus* at Cahuita, Costa Rica.

Discussion

In Cahuita town, *Anolis cristatellus* is well established and abundant. Its microhabitat distribution is exactly what would be expected in its native range in Puerto Rico or the Virgin Islands (Rand, A.S. 1964. Ecological distribution in anoline lizards of Puerto Rico. *Ecology* 45:745-752; Schoener, T. and A. Schoener. 1971. Structural habitats of West Indian *Anolis* lizards II. Puerto Rican uplands. *Breviora* 375:1-39; Mayer, G. 1989. *Deterministic Aspects of Community Structure in West Indian Amphibians and Reptiles*. Ph.D. thesis, Harvard University). The impression of being in the West Indies is strengthened by the flora, and by the fact that many of the residents of Cahuita are of English-speaking West Indian ancestry.

Upon crossing Kelly's Creek from Cahuita town into the National Park, I felt much as I think Wallace must have felt when crossing between Bali and Lombok. It was like entering another biogeographic region. And, indeed, in many ways it was. In Cahuita town there is a low diversity, high abundance lizard fauna, three of whose five members are introduced. The woodland flora also shows low diversity with dominance of a few common species. It seems just like the West Indies. In the national park, there is a more diverse, but less abundant, lizard fauna: seven species were observed during a minimal survey, only one of which is introduced (and that one, *Hemidactylus*, was found only in the park buildings). That just a small creek separates town and national park is notable, and suggests that the natural forest is difficult for the crested anole to invade. The success of this introduced anole in Cahuita is confined to the anthropogenic environment in the immediate vicinity of the town itself. The close proximity of the town and the national park in Cahuita provides a particularly good test case of whether introduced anoles thrive only in anthropogenic environments, or whether they can insert themselves into natural communities. In this case, it is clearly the former, and not the latter.

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Ernst Mayr, Ernest E. Williams, and the Nondimensional Species Concept

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Ernst Mayr, architect of the Modern Synthesis, director of the Museum of Comparative Zoology, and "the Darwin of the 20th century" (J. Coyne. 2005. Ernst Mayr 1904-2005. *Science* 307:1212-1213), was also a great naturalist. One of his great contributions was the biological species concept: that species are groups of actually or potentially interbreeding populations in nature reproductively isolated from other such groups. Mayr explicated the concept by beginning with the simplest situation in nature: a species inhabiting a single place at a single time. He called the species of such situations, the easiest in which to determine species boundaries, nondimensional species.

Ernest E. Williams, long time dean of *Anolis* studies, Curator of Herpetology and Alexander Agassiz Professor in the Museum of Comparative Zoology, and mentor to innumerable students, was also an adamant advocate of the importance of knowing the animals under study in nature in order to understand evolutionary and ecological phenomena.

Mayr and Williams were for many years colleagues at the MCZ. Mayr once visited St. Croix, where, ever the naturalist, he took note of the diversity in color, pattern, and size of the island's anoles. Upon his return, Mayr, the 20th century's foremost student of species, asked Williams what the three species of anoles were on St. Croix. Williams replied, "The males, the females, and the juveniles."

[Note: I was told this story first by Mayr, and later, I believe, by Williams. For those unfamiliar with St. Croix's anoles, there is one species, *Anolis acutus*.]

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Summer anoles, some are not: differences in the activity patterns of *Anolis equestris* and *A. sagrei* in South Florida

Florida, and especially southern Florida, has become home not only to a diverse exotic herpetofauna but one in which segments of herpetofaunal communities have expanded their geographic ranges as colonizers of Florida. Cuba provides a good example of this phenomenon with two anurans, the Greenhouse Frog (*Eleutherodactylus planirostris*) and Cuban Treefrog (*Osteopilus septentrionalis*) and three lizards, the Knight Anole (*Anolis equestris*), Cuban Green Anole (*A. porcatus*) and Brown Anole (*A. sagrei*) (Meshaka et al., 2004). Often syntopic in southern Florida, these species are not strangers to one another and arrive adapted in some fashion to the competitive or predatory relationships they share with one another.

Among the anoles of this group, perch heights in Florida (Meshaka et al., 2004; Meshaka and Rice, 2005; Meshaka et al., 2008) are similar to those occupied in Cuba (Barbour and Ramsden, 1919; Collette, 1961). All three species can be found close to the ground but, on average, the Brown Anole keeps closest to the ground, especially juveniles and females. The Cuban Green Anole will venture farther up the tree, and the Knight Anole higher still. The latter two species eat other anoles, and the Knight Anole will both root out food and make a fast dash to catch prey such as the Brown Anole (Meshaka et al., 2004; Meshaka and Rice 2005). Analogously considered a "*T. rex* of the trees" (Meshaka, 1999), the ubiquitous and predatory Knight Anole could certainly enforce the perch height separation one sees in this group. However, what about thermal and diel differences? Do they select for similar or different temperatures, times of day, and subsequently seasons for activity?

I wanted to know the answers to these questions, so along with counts and perch height data from my study site in Miramar, Florida (see Meshaka et al., 2008 for site description and methods), I also recorded the air temperature in the shade at the beginning of each of my surveys and extended time and temperature data collection from 2006 to 2009. With 120 observations of the Knight Anole and 2,865 observations of the Brown Anole, I compiled the data on numbers of lizards, months, time of day and temperature and discerned between the cooler months of November-March and the warmer months of April-October. Although some months are missing, testable patterns exist in the data even if the data are preliminary.

First and foremost, these data corroborate earlier findings by Meshaka and Rice (2005) that the Knight Anole is more active in the hotter months (Fig. 1). However active the Brown Anole may be during April-October, numbers of individuals active in the open were consistently highest during the cooler months (Fig. 2). In turn, both the minimum (74 F) and modal (87 F) air temperature at which the Knight Anole was out (Fig. 3) exceeded those of the Brown Anole (57 and 75 F, respectively)(Fig. 4). In selecting for higher air temperatures, the Knight Anole was far more effective at reaching those temperatures during the warmer months (Fig. 5) between approximately noon and 1600 hrs (Fig. 6). Perhaps the optimal ambient temperature range is too limiting for this species for this species to more than occasionally move about in the cooler months. The Brown Anole, on the other hand, was out and about in much higher numbers at its optimal ambient temperatures in the cooler months (Fig. 7) and most especially between approximately 0900 and 1600 hrs (Fig. 8). The greater activity in cooler months by the Brown Anole is contrary to what was found with the Knight Anole, and two testable explanations come to mind that could answer why the Brown Anole was observed in so much greater numbers in the cooler months. One reason could be that the optimal ambient thermal range is a more limiting resource in the winter than in the summer, such that lizards must crowd, as it were, around this winter-limited temperature. If this is the case, then the scarcity of Brown Anoles in the open during the warmer months is a function of animals that need only bask for a shorter time and can be active in shady, less observable areas as well. In this case, seasonal differences in counts are a matter of conspicuousness not abundance. A second explanation is a matter of abundance, whereby more Brown Anoles actually exist during that period of the year from fall recruitment and loss of excess by the following spring-summer to that end, November counts were replete with very small young of the year. Either or both of these explanations could be at play and either or both phenomena could provide a seasonal respite from greater predation pressure from such species as the Knight Anole, which is more active during the warmer months with individuals that are hunting all over the trees. Likewise, during part of the warmer months predatory birds, such as the Northern Mockingbird and Loggerhead Shrike, also intensify predator pressure by having young to feed.

The relevance of these findings is two-fold. First, the broader thermal range and lower optimal thermal range found in the Brown Anole provide this species with a distinct colonizing advantage over its ancient predator, the Knight Anole, as it disperses northward in Florida. Notwithstanding extreme urban heat island effects and climatic changes, the Brown Anole can geographically stay ahead of the Knight Anole where its structural niche constraints can relax. Second, the diel and thermal patterns presented here and the structural patterns known for these two species, provide the sorts of data necessary to quantify changes in niche dimensions by geographically expanding anoles as well as to more effectively measure relative abundances of both species for the purposes of identifying geographic range expansion and assessing status of populations in sensitive areas.

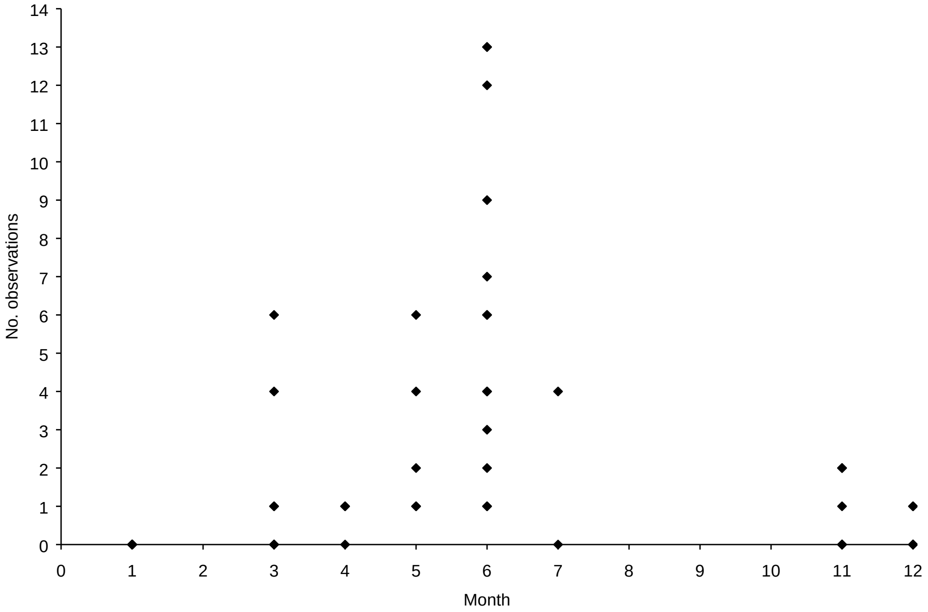

Fig. 1. Seasonal activity of the Knight Anole (*Anolis equestris*) during 2006-2009 in Broward County, Florida.

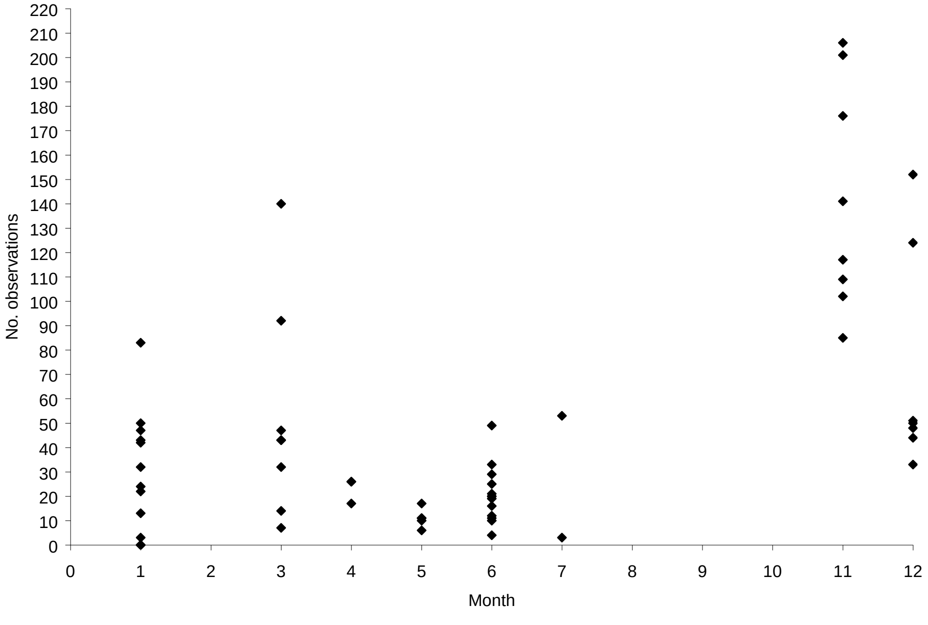

Fig. 2. Seasonal activity of the Brown Anole (*Anolis sagrei*) during 2006-2009 in Broward County, Florida.

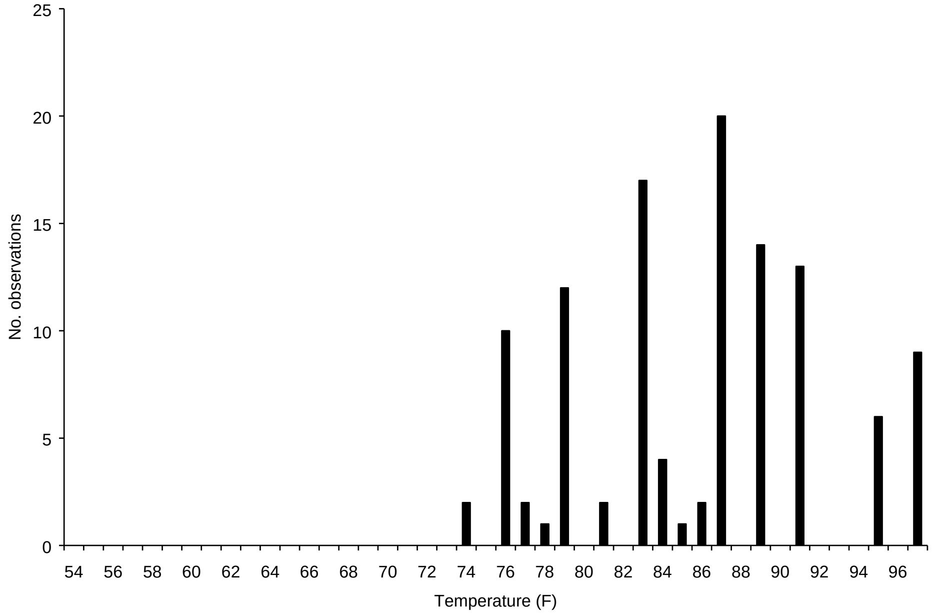

Fig. 3. Distribution of air temperatures associated with observations of the Knight Anole (*Anolis equestris*) during 2006-2009 in Broward County, Florida.

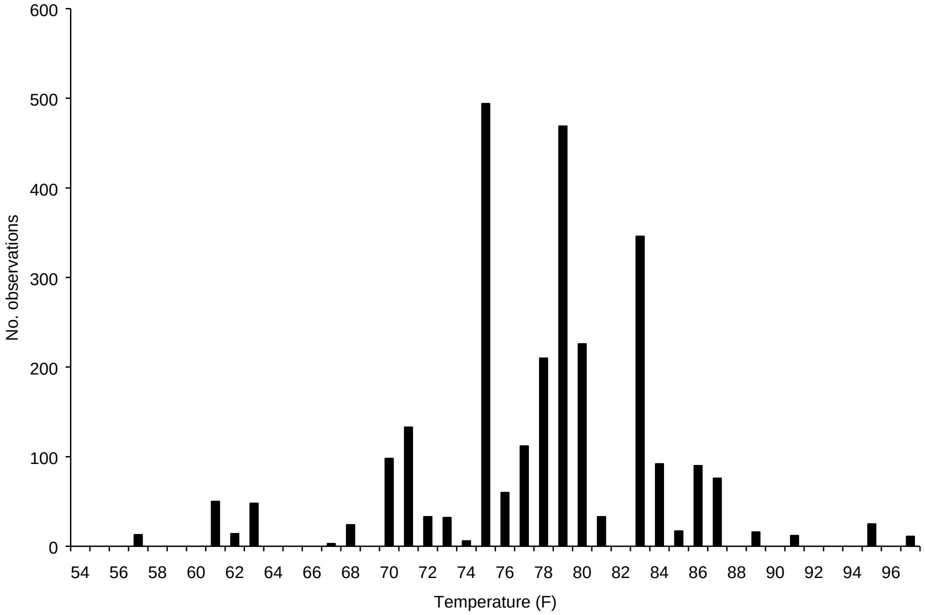

Fig. 4. Distribution of air temperatures associated with observations of the Brown Anole (*Anolis sagrei*) during 2006-2009 in Broward County, Florida.

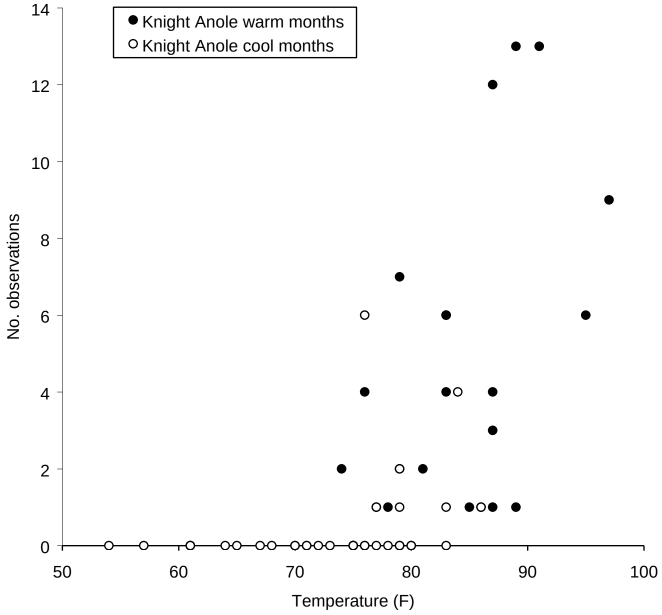

Fig. 5. Numbers of observations of the Knight Anole (*Anolis equestris*) in relation to air temperature during the warm and cool months of 2006-2009 in Broward County, Florida.

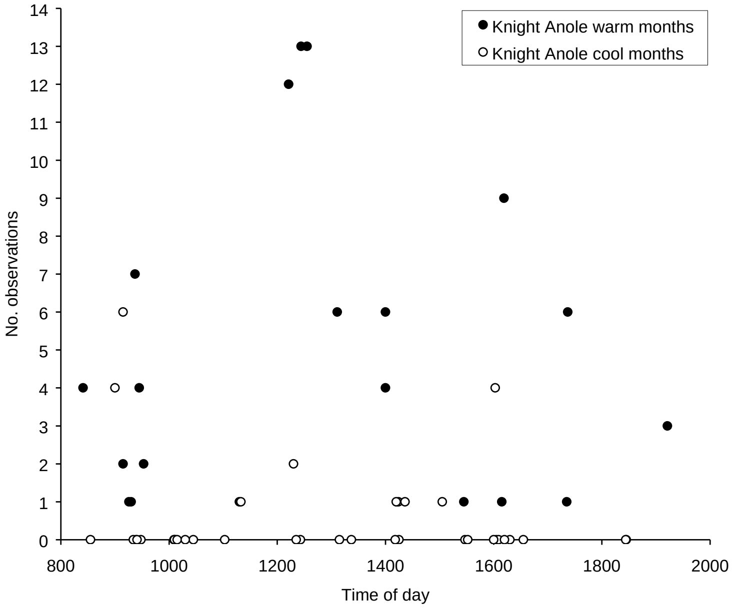

Fig. 6. Numbers of observations of the Knight Anole (*Anolis equestris*) in relation to time of day during the warm and cool months of 2006-2009 in Broward County, Florida.

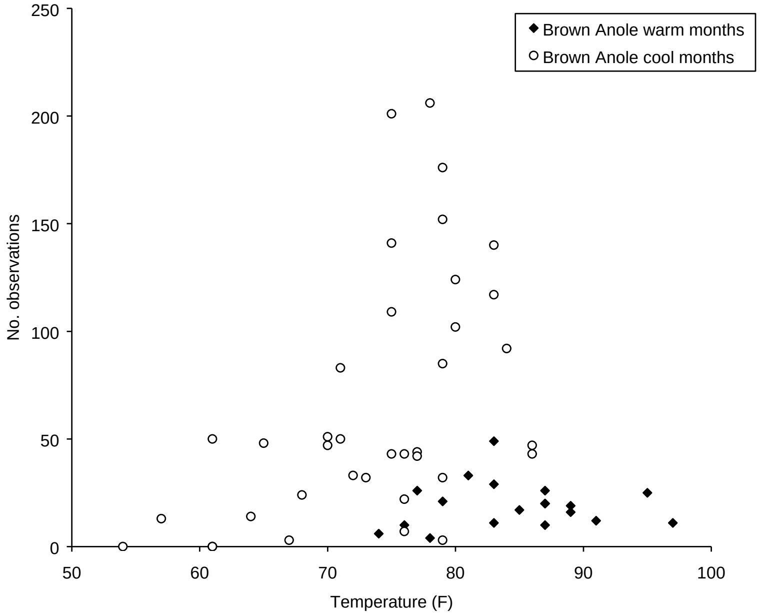

Fig. 7. Numbers of observations of the Brown Anole (*Anolis sagrei*) in relation to air temperature during the warm and cool months of 2006-2009 in Broward County, Florida.

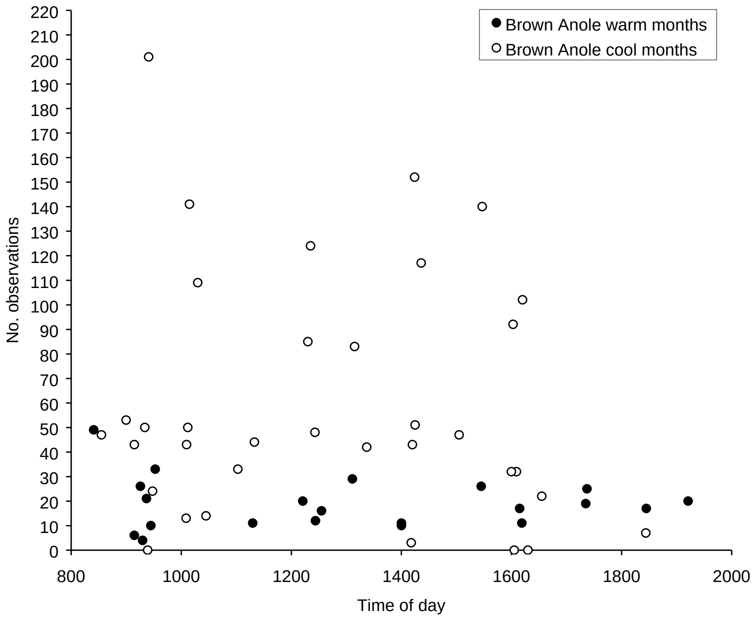

Fig. 8. Numbers of observations of the Brown Anole (*Anolis sagrei*) in relation to time of day during the warm and cool months of 2006-2009 in Broward County, Florida.

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Integration of trophic and locomotor performance during prey-capture behavior in *Anolis carolinensis*: effects of the substrate diameter

Introduction

Feeding is a daily actvity for many living organisms. This activity is directly relevant to individual fitness since it provides the organism with the nutrients and energy needed for correct functioning of the body. Consequently, feeding behavior may be considered key for the survival and the fitness of each individual. Indeed, inefficient feeding activity may result in inadequate energetic intake and may put an animal at a strong disadvantage for survival since a low energy level may affect escape behaviour and performance (Brown and Griffin 2005; Husak et al. 2006; Vanhooydonck et al. 2007). Also, inefficient feeding may ultimately be reflected in external signals of poor health, which are likely to negatively affect mating success (Cellerino and Jannini 2005). Consequently, the study of the performance in activities involved in feeding behavior are relevant to a full understanding of the fitness of living organisms in their ecosystems.

Feeding behavior involves performance of two different anatomical systems: the locomotor system (limbs and vertebral axis) during the approach and the attack of the prey, and the trophic system (jaws, tongue, skull) for prey capture and prey transport (Higham 2007a,b; Montuelle et al. 2008, 2009). *Anolis* lizards provide an interesting model since performance in these systems have been described separately (Moermond 1981; Bels 1990; Bels and Goose 1990; Irschick and Losos 1998). Unfortunately, these performances have been poorly quantified jointly (but see Montuelle et al. 2008), although the functional integration of such complex systems seems to be a relevant parameter to the success of prey-capture behavior in other living organisms (Higham 2007b; Montuelle et al. 2009).

Several locomotor strategies have been observed to be used by *Anolis* lizards in order to successfully attack prey (Moermond 1981). In contrast, tongue prehension is the only mode that is employed by *Anolis* lizards to catch prey (Bels 1990; Bels and Goose 1990). Recently, in *Anolis carolinensis*, the movements of the trophic elements - jaws and tongue - have been demonstrated to be independent from the prey-attack strategy (Montuelle et al. 2008). This study demonstrates that the plasticity of the locomotor performance is associated with the stereotypy of the trophic performance. This study only tests one prey type (crickets; *Acheta domesticus*), for which no size effects were detected, but the activity of the prey is shown to be highly variable and has been suggested to induce the choice of the attack strategy (Montuelle et al. 2008). Consequently, the plasticity of the locomotor performance seems to be a key component of the success of the feeding behavior of *A. carolinensis*.

Within the wide repertoire of prey-attack strategies used by *Anolis carolinensis*, jumping appears to be the most frequently used strategy (Irschick and Losos 1998; Montuelle et al. 2008). Interestingly, jumping performance is known to be affected by the properties of the substrate, especially the perch diameter (Moermond 1979; Losos and Sinervo, 1989; Losos and Irschick 1996; Spezzano and Jayne 2004; Vanhooydonck et al. 2006). However, these data refer to jumping performance in the context of anti-predator response. Indeed, most of the information available on jumping performance in anoles originates from observations recorded while the experimenter induced the lizard to jump by gently tapping on its tail (Bels et al. 1992; Toro et al. 2004; Irschick et al. 2005; Lailvaux and Irschick 2007). Consequently, substrate properties have been demonstrated to have strong effects on the plasticity of the locomotor performance in the context of anti-predator response. In contrast, little is known about the effect of substrate on the locomotor plasticity involved in feeding behavior (but see Irschick and Losos 1998).

This study has two objectives. Our first objective is to test for the effects of the substrate on the elements of physical performance involved in the feeding behavior of *Anolis carolinensis*. Our analysis aims at quantifying the performance of both the trophic (jaw opening-closing cycle and tongue protrusion) and the locomotor systems (jump distance) on two different substrates: a flat and wide substrate versus an 18mm-diameter perch. Our second objective is to examine the functional integration of the trophic and locomotor systems during prey-capture behavior.

Materials and Methods

Six male adult individuals of *Anolis carolinensis* were observed while feeding on live crickets (*Acheta domesticus*) on each substrate. Each feeding event was recorded using a high-speed video camera (250 frames per seconds) set in lateral view. We analyzed the sequences during which the individual performs a jump toward the prey, and successfully catches it. Overall, 55 jump sequences representing the six individuals were observed: 27 on the perch (three, four, five, three, three and nine for each individual respectively; Fig. 1) and 28 on the flat surface (five, three, two, six, six and six for each individual respectively; Fig. 1). For each sequence during which the lizard jumped to capture the prey two jump variables were recorded: the distance of the jump and the jump duration (Table 1). Four points were digitized: the corner of the mouth, the tip of upper jaw, the tip of lower jaw, and the anterior-most point of the tongue (Fig. 2). Based on the coordinates of the digitized points, we generated two kinematic profiles representing the gape angle and the tongue protrusion distance. Gape angle is the angle between upper jaw, the corner of the mouth and lower jaw; tongue protrusion distance is defined as the distance between the tip of the tongue and a line interconnecting the upper and lower jaws (Fig. 3).

From these two kinematic profiles we extracted nine spatio-temporal variables (Fig. 3; Table 1). We analyzed these variables using an ANOVA set to test for the effects of the substrate (fixed factor) and of the individuals (random factor). The functional integration of the trophic and the locomotor systems was analyzed by entering trophic and locomotor variables in a correlation matrix, for each substrate separately.

Results

The effects of the substrate on jumping performance are strong, with jumps being longer on the flat substrate than on the perch (Table 1). In contrast, trophic kinematics are little affected by the substrate. Indeed, only two of the nine variables are significantly different between the perch and theflat substrate. The maximal gape angle occurs later when prey capture is performed on a perch, and the tongue is protruded further on the flat substrate (Table 1).

Interestingly, the coordination of the locomotor and trophic movements appears to be different on perch and on flat substrate (Figs. 4 and 5). On the perch, jump distance is negatively correlated with the time that the tongue is protruded beyond the jaw margin (Fig. 4). This indicates the tongue is protruded sooner when the lizard uses a long-distance jump towards the prey, suggesting the preparation of the tongue occurs earlier when the lizard is about to perform a long jump.

In contrast, on the flat substrate, jump distance and duration are associated with maximal gape and the time to maximal protrusion of the tongue (Fig. 5). In particular, the jaws open wider when the predator performs quick, short-distance approaches. Moreover, the maximal protrusion of the tongue is achieved earlier when associated with slower, long-distance jumps. Consequently, on a flat substrate, the coordination of locomotor and trophic movements is suggested to occur during predator-prey contact.

Discussion

In *Anolis* lizards, tongue prehension is the only capture mode (Bels 1990; Bels and Goose 1990), and the performance of the trophic system is little affected by the substrate (Table 1). In contrast, the performance of the locomotor system during feeding behavior is varies considerably according to substrate (Montuelle et al. 2008). First, several locomotor strategies can be used by each individual to approach and strike the prey (Moermond 1981; Montuelle et al. 2008). Our observations show that locomotor performance involved in prey-capture behavior is modulated according to the substrate diameter (Table 1), a result similar to the locomotor performance involved in anti-predator response and escape behavior (Moermond 1979; Losos and Sinervo, 1989; Losos and Irschick 1996; Spezzano and Jayne 2004; Vanhooydonck et al. 2006). Comparison of our data with those of other studies on jumping in anoles suggests that jump performance is modulated according to the behavioral context, *i.e.* feeding versus escape from predator (in accordance with Irschick and Losos 1998).

The integration of the trophic and the locomotor systems is a key component of feeding success in *Anolis* lizards (Higham 2007ab; Montuelle et al. 2008). In *Anolis carolinensis*, the coordination of trophic and locomotor performance is different on flat substrates versus perches

(Figs. 4 and 5). This coordination occurs primarily during the preparation of tongue protrusion on perches, whereas on flat substrates, it occurs during predator-prey contact. These results demonstrate the relevance of taking into account substrate properties in the study of feeding behavior (Moermond 1979).

Given the importance of habitat effects on each component of prey-capture behavior, *Anolis* lizards are *a priori* a relevant model to investigate functional integration during ecological performance. Our observations indicate that the performance of the locomotor system is a key component of the success of prey-capture behavior in *Anolis carolinensis*, allowing individuals to adapt to varying evasive strategies. In the *Anolis* lineage, morphology and habitat are intimately linked, with body proportions (especially limbs proportions) being similar in different species that live in similar habitats. Consequently, one potential perspective is to investigate the relationships between the morphological convergence of *Anolis* lizards, and their feeding behavior. We propose two future directions: (i) compare species of different ecomorphs to test for the effect of morphological differences, and (ii) compare species within the same ecomorph to test for the effects of the morphological convergence.

Acknowledgements

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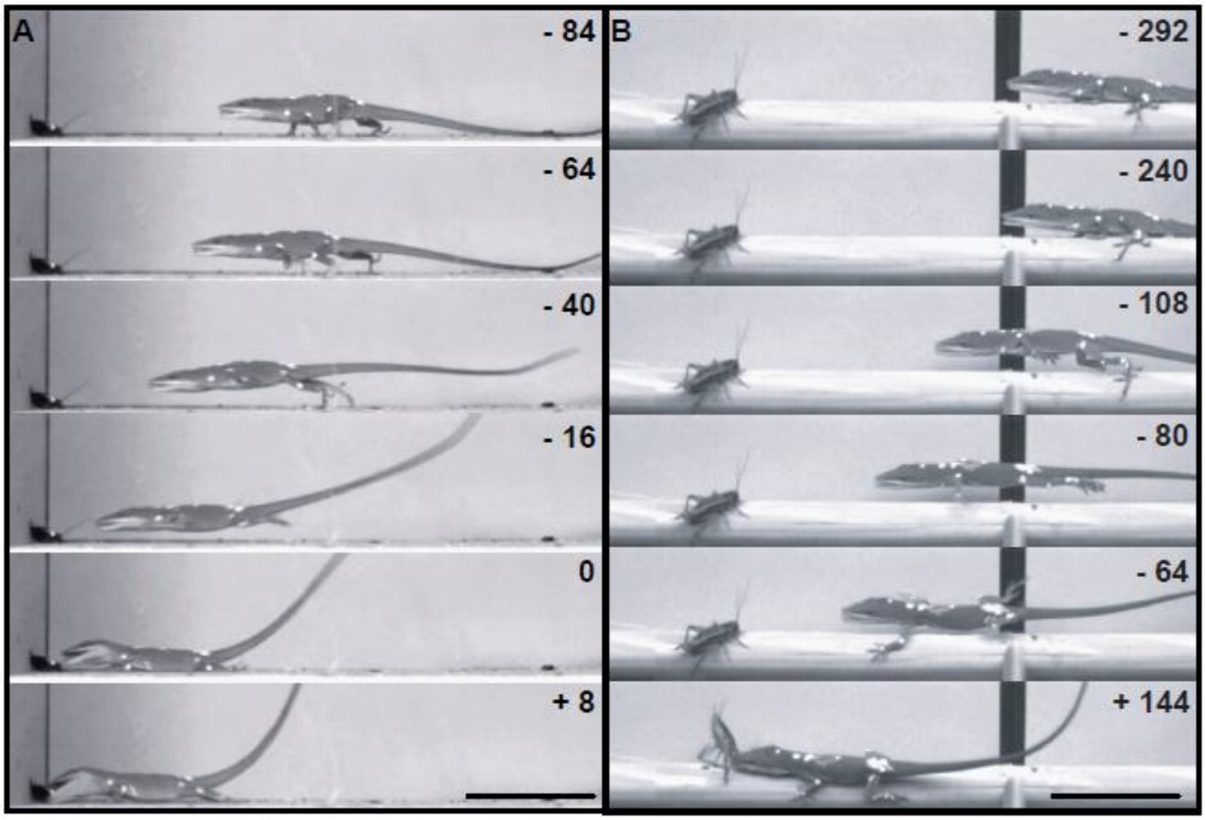

Figure 1. Representative frame sequences of jumping in *Anolis carolinensis* while capturing a cricket on flat substrate (A) and on a perch (B) (recorded at 250 fps). Time is given in ms with t= 0 at tongue-prey contact. The black scaling line represents 50 mm.

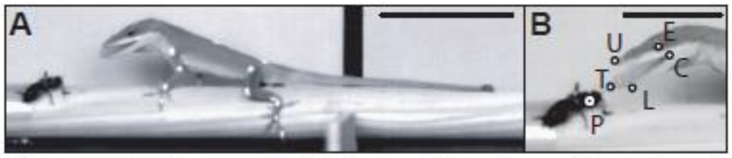

Figure 2. (A) Representative frame of *Anolis carolinensis* capturing a cricket (on a perch). The scaling black line represents 50 mm. (B) Natural spots on the lizard were digitized to quantify prey capture kinematics: eye (E), upper jaw (U), lower jaw (L), corner of the mouth (C), tongue (T) and prey (P). The scaling black line represents 25 mm.

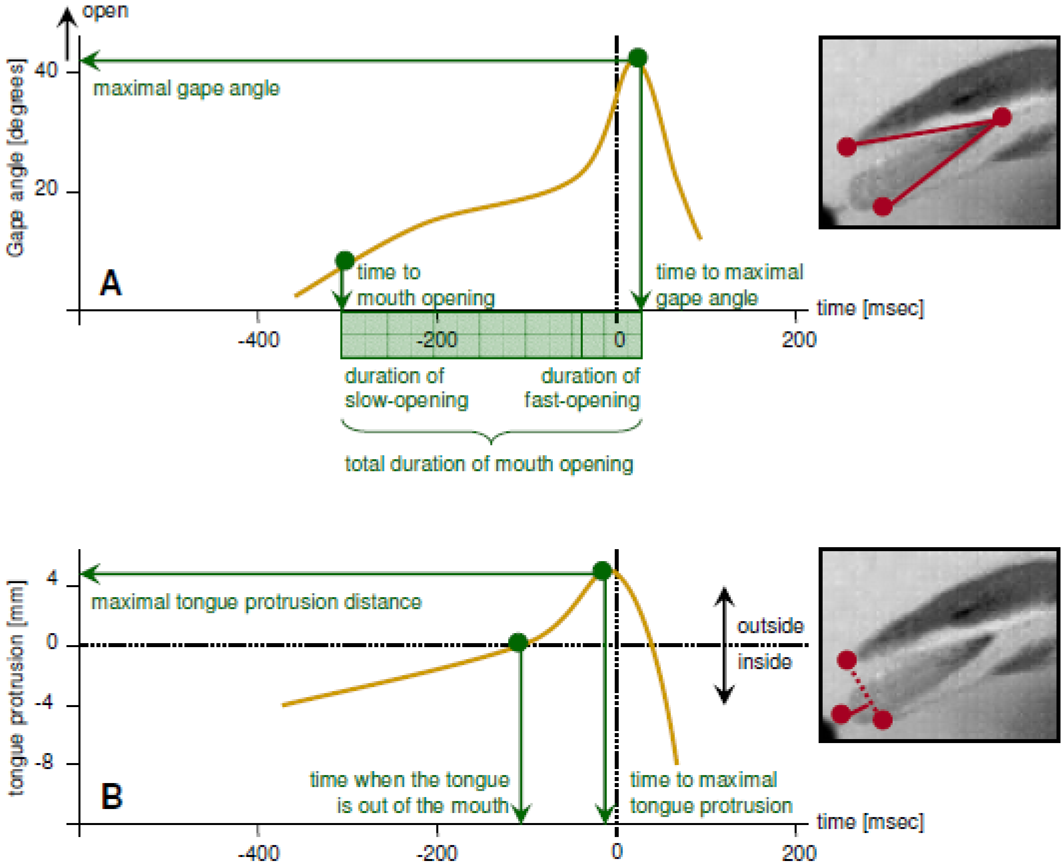

Figure 3. Representative kinematic profiles of the trophic performance during prey-capture behavior in *Anolis carolinensis* (yellow). The kinematic variables used in our analysis are Illustrated (green; see Table 1). (A) Gape angle is the angle between the tip of the upper jaw, The corner of the mouth and the tip of the lower jaw (red). (B) Tongue protrusion distance is the distance between the tip of the tongue and a line interconnecting the upper and lower jaws (red).

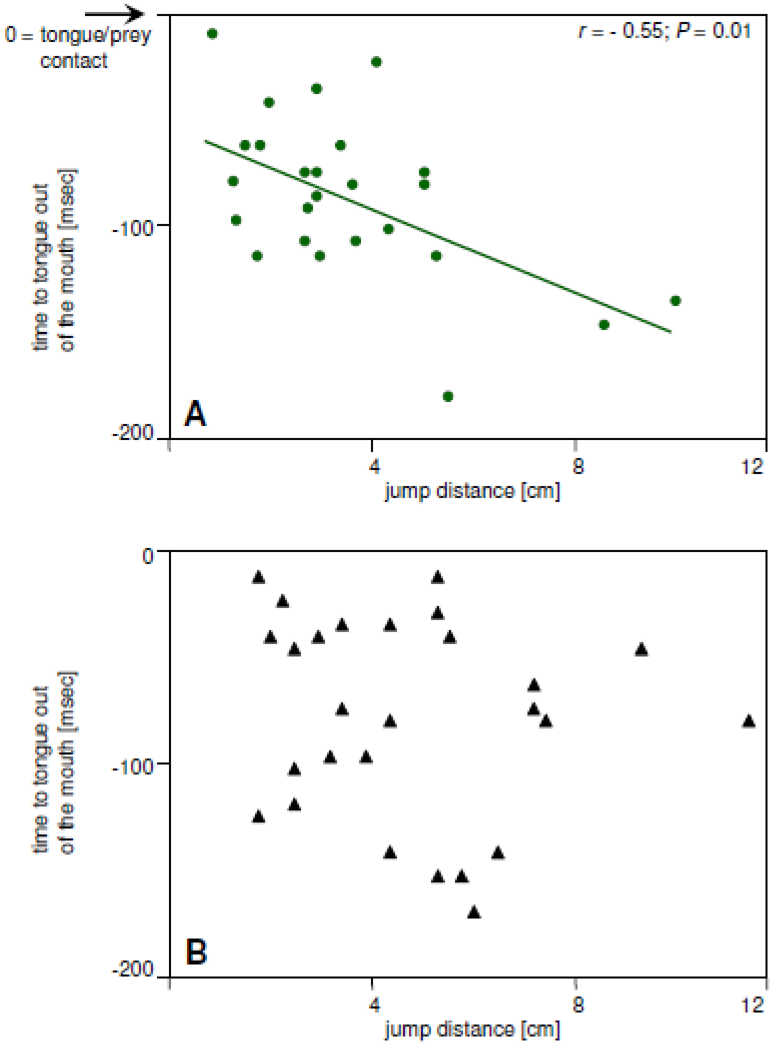

Figure 4. Scatter plots illustrating the correlation of jump performance with the time when the tongue is out of the mouth during prey capture in *Anolis carolinensis* on perch (green dots; A). Jump distance is negatively correlated with time to that the tongue is out of the mouth (*N* = 24; *P* = 0.01), indicating that, on perch, jump performance is associated with the preparation of the tongue. Note such a correlation is not observed on a flat substrate (black triangle; B). Time is given in ms with t= 0 at tongue-prey contact.

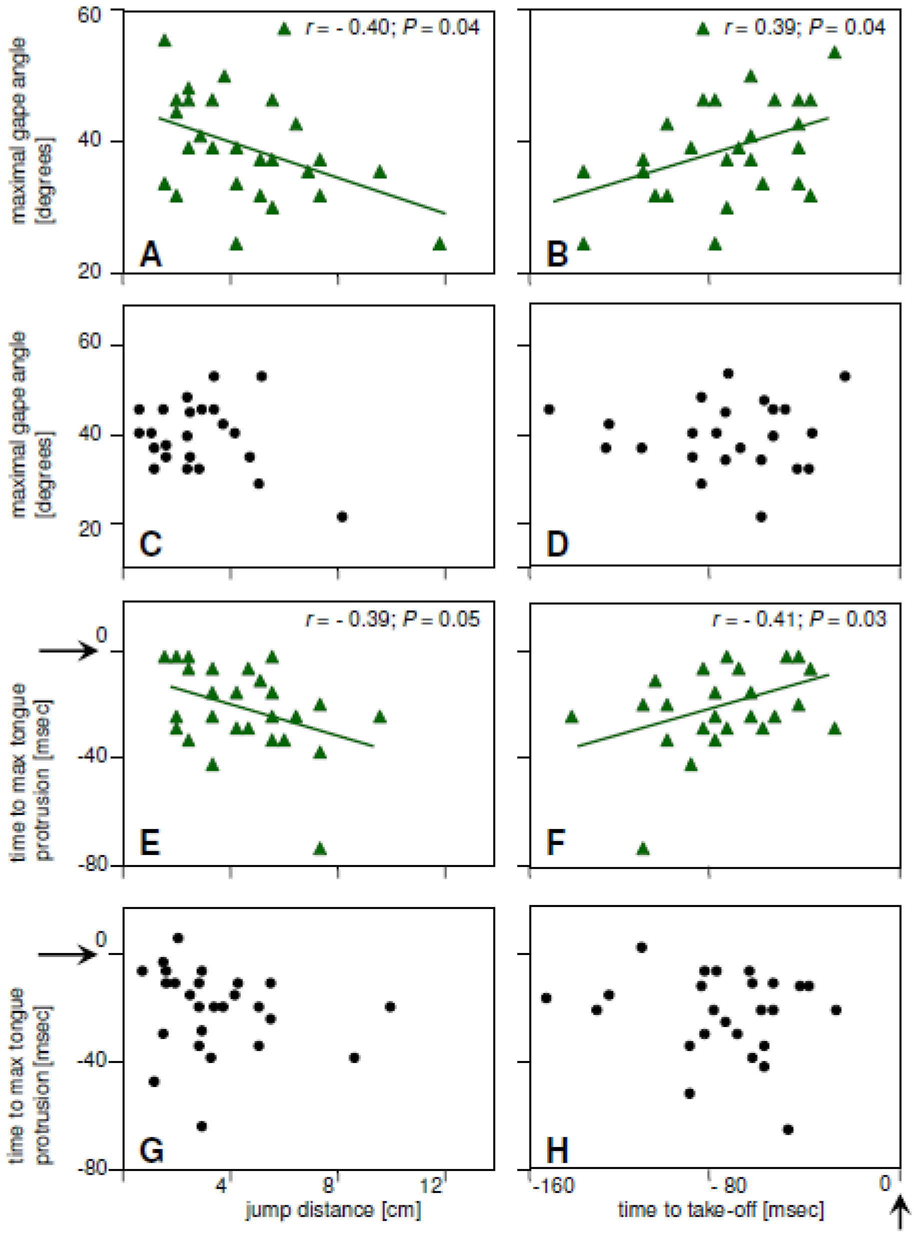

Figure 5. Scatter plots illustrating the correlations of jump performance with trophic kinematics during prey capture in *Anolis carolinensis* on a flat substrate (green triangles; A, B, E and F). Maximal gape is negatively correlated with jump distance (A; $N = 27$; $P = 0.04$) and time to take-off (B; $N = 27$; $P = 0.04$). Note these correlations are not observed on perch (black dots; C and D, respectively). Similarly, time to maximal protrusion of the tongue is negatively correlated with jump distance (E; $N = 27$; $P = 0.05$) and time to take-off (F; $N = 27$; $P = 0.03$). Note these correlations are not observed on perch (black dots; G and H, respectively). This suggests that jump performance on flat substratum is associated with trophic performance relevant to predator-prey contact. Time is given in ms with t= 0 at tongue-prey contact.

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Martha M. Muñoz & James Hewlett

Ecomorphological response of *Anolis lividus* to volcanic activity on Montserrat

Beginning in January 2009 we have been assessing populations of the Montserratian endemic *Anolis lividus*, the only species of anole on this northern Lesser Antillean island. Since 1996, Montserrat has suffered several volcanic eruptions and ash venting episodes that have drastically altered its ecology. We sampled populations of lizards from the relatively undisturbed northern scrubland, where we found them to be quite abundant. However, lizard populations closer to the volcano are patchy in distribution and exhibit generally lower abundances. *Anolis lividus* is not as abundant in Garibaldi Hill and the Woodlands as in the north, southeast, and Centre Hills.

Because travel to southern Montserrat is generally prohibited for safety reasons (more than half of the island is designated as exclusion zone and the boundary of the zone expands and contracts depending on volcanic activity), consistent sampling south of the Woodlands has been difficult. However, we have been able to briefly sample localities in the exclusion zone. In collaboration with the Montserrat Volcano Observatory, we visited six sites in the permanent exclusion zone, including Plymouth, Richmond Hill, St. George's Hill, and sites in between. The habitat at these sites was fragmented and showed signs of acid rain damage. The most suitable habitat at a site a few kilometers north of Plymouth was bereft of lizards. The only two anoles spotted in the exclusion zone were a breeding pair in a tree sporting obvious signs of acid rain damage. This site was, however, the closest of the six visited to the northern boundary of the exclusion zone.

We measured perching preferences and morphological traits for various populations on Montserrat, stretching as far southwest as Garibaldi Hill, Air Studios near Olveston, and the Woodlands. In the southeast of the accessible part of the island, we have sampled at Jack Boy Hill and surrounding environs. In the north we have sampled in the Silver Hills and Carr's Bay. Preliminary analyses show that, while all populations generally perch at 1.5 meters or lower, lizards further north utilize a broader range of perch heights, while populations closer to the volcano (Jack Boy Hill, Air Studios) perch higher up in the trees. Furthermore, populations closer to the volcano possess longer humeri and femora than those in the north, but these data are preliminary. Increased sampling of habitat and morphology in the southern portion of the island is required. Our next trip to Montserrat is planned for January 2010, in which we hope to expand our sampling range and perform habitat transects.

Martha M. Muñoz, Anthony Herrel, Kristen Crandell, & Mahmood Sasa

Natural history of aquatic anoles from Costa Rica and Panamá

There are five known species of mainland aquatic anoles, four of which are located in Costa Rica (*Anolis aquaticus* and *A. oxylophus*) and Panamá (*A. lionotus* and *A. poecilopus*). Relatively little is known about the natural history, morphology, and performance characteristics of these anoles. In the summer of 2008, along with a team of great field assistants, we collected data on habitat preference, behavior, morphology, and jumping and clinging performance for both Costa Rican species.

While perch dimensions did not differ between *A. aquaticus* and *A. oxylophus*, we did find significant differences in substrate preference. Specifically, we found *A. aquaticus* in rockier habitat than that used by *A. oxylophus*, which contained a broad variety of perch types. This species perches on rocks more than any other substrate, even those that are in greater abundance, such as leafy vegetation. In contrast, the habitat for *A. oxylophus* is composed mainly of leafy vegetation, but this species prefers a wide variety of substrates, including those that are less common, such as logs.

Because *A. aquaticus* utilizes rough surfaces like rocks as perches, it cannot rely entirely on its expanded toepads for clinging. However, there are no significant differences in claw morphology, including sharpness, length, or depth, between the two species that would suggest that *A. aquaticus* is more adept at clinging to rough surfaces than is *A. oxylophus*. Furthermore, although *A. aquaticus* possesses longer hindlimbs than does *A. oxylophus*, it is the slower runner of the two species. *A. oxylophus* possesses broader toepads, which may aid in better clinging and, therefore, running on a broad, smooth surface such as the dowels we used in our sprinting trials. In general *A. aquaticus* has relatively longer limbs and a longer jaw than *A. oxylophus*. The longer lower jaw translates into a more powerful bite, and may reflect differences in diet between the two species.

In the summer of 2009, we collected similar data for *A. lionotus* and *A. poecilopus*, the two Panamanian aquatic anoles. We are currently assembling and organizing the complete dataset for all four species in our study.

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Dewlap color variation and reproductive isolation in *Anolis distichus*

Signals important to sexual selection and species recognition play an important role in speciation. Divergence of such signals and associated signal preferences can drive the evolution of prezygotic reproductive isolation, and may underlie the rapid diversification of some species-rich adaptive radiations, such as cichlids (Schluter 2000; Seehausen et al. 2008). With *Anolis* lizards being another classic example of a species-rich adaptive radiation, we are interested in investigating whether the anole dewlap, which is thought to be an important signal for species recognition and sexual selection, plays an important role in speciation and in driving the diversification of anoles. As *Anolis* dewlaps vary remarkably in color and pattern both among and within species (Nicholson et al. 2007), we are particularly interested in testing whether variation in dewlap color is associated with reproductive isolation.

Two species of Hispaniolan trunk anoles – *Anolis distichus* and its putative sister species, *A. brevirostris* – exhibit extensive geographic variation in dewlap color and pattern (Schwartz 1968, Arnold 1980). Webster and Burns' (1972) allozyme-based study of geographic dewlap color variation in Haitian populations of *A. brevirostris* strongly supports the hypothesis that dewlap color is associated with some degree of reproductive isolation. Their results showed that *A. brevirostris* populations differing in dewlap color were genetically differentiated, leading to the division of *A. brevirostris (sensu lato)* into three species (*A. brevirostris*, *A.caudalis* and *A. websteri*) (Webster and Burns 1972; Arnold 1980). We seek to investigate whether dewlap color is associated with reproductive isolation in Dominican populations of *A. distichus* using replicated tests and modern molecular markers. *Anolis distichus* provides us with a unique opportunity as, across its range, multiple instances of dewlap color transitions occur. This intraspecific approach, which will identify patterns at what may be incipient stages of speciation, will be accompanied by comparisons at the interspecific level using *A. brevirostris*, to investigate the pattern seen at the end of the continuum of the speciation process.

Study species

Anolis distichus is a trunk anole found across the Bahamas and Hispaniola that exhibits striking intraspecific dewlap color diversity (Schwartz 1968) (Fig. 1). As most of this variation is distributed allopatrically or parapatrically, the species has been divided into sixteen subspecies that are diagnosed primarily on the basis of dewlap color (Schwartz 1968). This geographic pattern of dewlap color variation has led to speculation that the dewlap serves as a partial prezygotic barrier to mating amongst subspecies (Williams 1977; Williams and Rand 1977; Case 1990). A previous allozyme-based study conducted across a contact zone between two *A. distichus* subspecies shows support for this predicted association between dewlap color and reproductive isolation; restricted gene flow was found between *A. d. dominicensis* (yellow dewlap) and *A. d. ignigularis* (orange dewlap) (Case and Williams 1984).

Studies of sympatric populations of *A. distichus* and *A. brevirostris* also support the hypothesis that the dewlap is a prezygotic isolating mechanism. *Anolis brevirostris* can only be distinguished from *A. distichus* by subtle differences in color and scalation (Arnold 1980). Although the dewlap color of *A. brevirostris* varies across the range in a similar pattern to *A. distichus*, the two species exhibit contrasting dewlap coloration in most areas where they come into contact (Webster and Burns 1972; Webster 1977a). For example, at one sympatric site in Haiti, *A. brevirostris* has an orange dewlap while *A. d. dominicensis* exhibits a pale yellow dewlap (Webster and Burns 1972). At another sympatric site south of this location, the same two species show a reversal in dewlap color (Webster and Burns 1972). Hybridization has been found to be uncommon in areas where the species differ in dewlap color, but in contact zones where both species exhibit similar dewlap coloration, hybridization was frequent (Webster 1977a). In addition, hybrids were found to be sterile due to meiotic abnormalities, and allozyme analyses were unable to recover any evidence for backcrossing (Webster 1977b). These results support an important role for the dewlap in preventing disadvantageous hybridization events. However, a later allozyme-based study of parapatric and sympatric sites (including some of the same localities) showed varying results: at some sites, *A. distichus* and *A. brevirostris* remained distinct despite similar dewlap coloration while at other sites, hybridization was common (Williams and Case 1986). Given these discrepancies, a reassessment is clearly needed.

Field collections

During the summers of 2008 and 2009, we sampled *A. distichus* and *A. brevirostris* from Hispaniola at two spatial scales: (i) broad geographic sampling, which involved a comprehensive sampling of all *A. distichus* subspecies from across its range on Hispaniola, and (ii) detailed sampling at contact zones. Our detailed sampling involved sampling ten transects in the Dominican Republic: five experimental transects across dewlap color transitions (T1-T5, Fig. 1), and five control transects extending across populations exhibiting the same dewlap color (T6-T10, Fig. 1). Each transect was approximately 10km in length, and we sampled five to six populations along each transect, obtaining tissue samples (tail tip or liver) from at least 20 individuals from each population. To test the predicted association of dewlap color and reproductive isolation at the species level, we sampled two sites in the Dominican Republic where *A. distichus* and *A. brevirostris* occur sympatrically: one site where the species exhibit contrasting dewlap colors and another where the two exhibit the same dewlap color. We also re-sampled the Webster and Burns (1972) transect in Haiti, to determine whether the species identified for the first time in that study still remain distinct 40 years later.

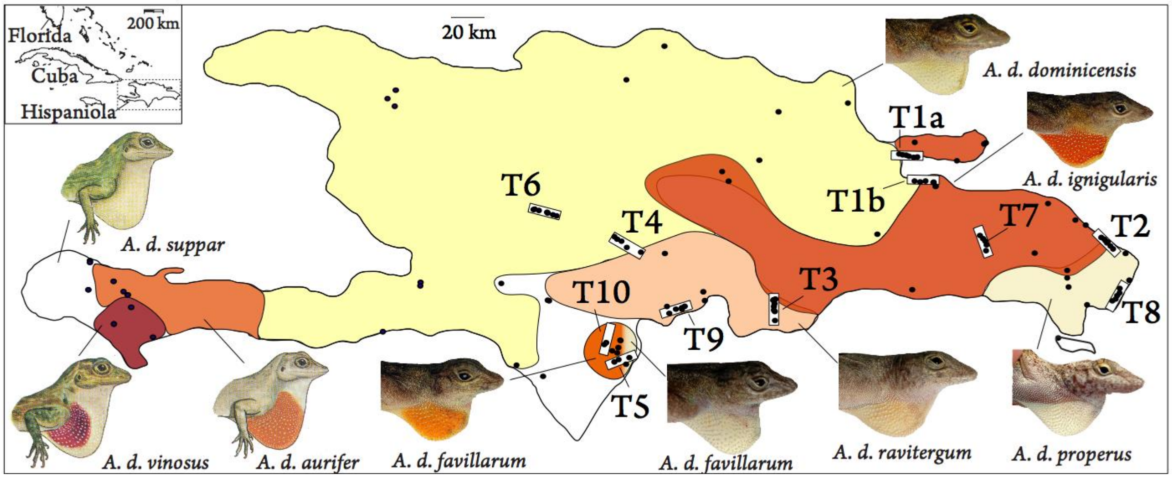

Fig. 6. Range distribution of *Anolis distichus* subspecies on Hispaniola. Colors on map mark the respective ranges of each subspecies (Schwartz 1968) with each color representing the dewlap color of the subspecies. Filled circles represent localities where we have sampled *A. distichus* and *A. brevirostris*. Hollow rectangles represent transects (Experimental: T1-5; control: T6-10). Images of *A. d. suppar*, *A. d. vinosus*, and *A. d. aurifer* are from Schwartz 1968.

In addition to obtaining tissue samples, we also visually quantified the proportion of orange on the dewlap of every male collected using the following categories which allows the diversity of dewlap color and pattern present to be represented: (i) all yellow (<10% orange), (ii) 10-90% orange (in increments of 10%), and (iii) orange with a narrow yellow margin (>90% orange).

Molecular analyses

We expect that if dewlap color is a prezygotic isolating mechanism, populations exhibiting different dewlap colors will be reproductively isolated, and will have diverged through the accumulation of genetic differences. We are testing this hypothesis by employing both phylogenetic and population genetic analyses and are using molecular markers from both nuclear and mitochondrial genomes. For a mitochondrial marker, we sequenced a region that extends from ND2 (subunit two of NADH dehydrogenase) through to tRNA^{Ala} (1147bp). To provide perspective from the nuclear genome, we developed eleven highly polymorphic microsatellite markers for *A. distichus* (Ng et al. 2010) and identified six potential microsatellite markers for *A. brevirostris*. Using these markers, we will investigate areas of contact between *A. distichus* subspecies, and sites in which *A. distichus* is sympatric with *A. brevirostris*. We expect that sympatric and parapatric populations differing in dewlap color will be genetically distinct at both types of markers. We have focused our recent phylogenetic and population genetic analyses on two experimental transects, T1 and T3 (Fig. 1), and here report the results. We also report preliminary phylogenetic results for sympatric *A. distichus* and *A. brevirostris* sites. Our results thus far strongly support our prediction that populations differing in dewlap color remain genetically distinct.

Transect 1

Transect 1 transitions from *A. d. dominicensis* (yellow dewlap) to *A. d. ignigularis* (orange dewlap) populations on the Samana peninsula (T1a) (Fig. 1). Our study of T1 indicates significant genetic differentiation of both the mitochondrial and nuclear genomes between the two subspecies, and the presence of a narrow hybrid zone where the two subspecies come into contact. Our phylogenetic analyses (parsimony and maximum likelihood) show that mtDNA haplotypes from this transect can be assigned to two well-supported (bootstrap=100), deeply divergent (10% average uncorrected sequence divergence) clades that closely correspond with dewlap color. Bayesian clustering analyses using STRUCTURE (Pritchard *et al.* 2000) on microsatellite genotype data further support genetic differentiation between the yellow and orange-dewlapped populations at either end of T1. In addition, pairwise estimates of F_{ST} show significant levels of genetic differentiation where dewlap phenotypes shift from primarily yellow to a greater proportion of orange, suggesting a sharp genetic break. On the other hand, there was no genetic differentiation between populations with similarly colored dewlaps. Both mtDNA and microsatellite markers are also congruent in supporting that a narrow hybrid zone exists: phylogenetic analyses show mixed mtDNA haplotypes and STRUCTURE results indicate a higher proportion of admixed nuclear genomes (Fig. 2c) in the middle populations of the transect.

Transect 3

Transect 3 is along El Recodo Road, where the transition between the same orange-dewlapped subspecies, *A. d. ignigularis*, and another yellow-dewlapped *A. d. ravitergum* was discovered and described in detail by Williams (1977). Our results revealed that *A. d. ignigularis* is also genetically distinct from *A. d. ravitergum*. All analyses on microsatellite data provide evidence that a strong genetic break exists between two middle populations with no evidence of admixture between subspecies. Our mtDNA phylogenetic results support two distinct, well-supported clades, with populations at either end of the transect characterized by divergent mtDNA haplotypes. However, there is evidence of unidirectional introgression of *A. d. ravitergum* mtDNA haplotypes into *A. d. ignigularis*. It is likely that the discrepancy between mtDNA and microsatellite markers is due to mtDNA being more prone to introgression, and hybridization occurring at such a low rate that the signature of hybridization is not seen in nuclear DNA due to backcrossing.

Sympatric sites

A preliminary mtDNA phylogenetic analysis of *A. distichus* and *A. brevirostris* individuals from a sympatric site where the species exhibit divergent dewlap color (5 *A. distichus*, 4 *A. brevirostris*) revealed that *A. brevirostris* haplotypes formed a clade distinct from *A. distichus* haplotypes. However, haplotypes from a site where the species exhibit similarly colored dewlaps (7 *A. brevirostris*, 20 *A. distichus*) showed mtDNA introgression in both directions. While this comparison is based on limited sampling, these results agree with Webster's (1977a) results and provide preliminary support that dewlap color is associated with prezygotic reproductive isolation between *A. distichus* and *A. brevirostris*.

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Steven Poe

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Anolis research in the Poe Lab

We study systematics and evolution of *Anolis*. Our systematic work is concentrated in the phylogenetics of *Anolis* and the discovery and description of species. In recent years we have traveled to Panama, Costa Rica, Colombia, Ecuador, Peru, and Bolivia to collect tissues and search for new and rare species of *Anolis*. Recent highlights of these trips include three new species published from Panama and collection of good series of the rare anoles *A. orcesi* and *A. proboscis* during a trip to Ecuador. All of this work is done in collaboration with Latin American scientists and students.

Our phylogenetic work includes the collection of morphological and molecular data and combined analyses of multiple datasets using Bayesian and other approaches. Our morphological dataset is composed of over 300 species, including 254 that we have coded and are currently incorporating in phylogenetic analyses. We are collecting DNA sequence data with several international and U.S. collaborators thanks to the financial kindness of the National Science Foundation. The long-term goal is a comprehensive phylogeny of *Anolis*. But for now much of our work is concentrated on sequencing the rare species in our freezer for combination with results from the genome project and other groups. We also work on the phylogeny of smaller clades of *Anolis*, with emphasis on mainland groups.

Our evolutionary studies in *Anolis* are concerned with character evolution, adaptation vs. exaptation, and biogeography. We are testing Ernest Williams' idea of a colonizing type of *Anolis* by examining naturalized, solitary, and ancestrally reconstructed *Anolis* lineages. We are working on reconstructing the patterns of dispersal across islands and the mainland, and on examining the traits that favor or inhibit diversification. As with the alpha-taxonomy work, this research is done in collaboration with international and local scientists and students.

Graduate students in the lab are working on various projects in *Anolis*. **Ian Latella** is studying invasive *Anolis*. Recently he helped develop a predictive model to examine which species of *Anolis* are likely to become invaders. **Eric Schaad** studies the comparative evolution of communities of *Anolis*. He is interested in comparing the morphological and phylogenetic diversity of these communities between islands and the mainland. **Mason Ryan** has taken a break from working on nondescript frogs to study the beautiful and interesting solitary anoles—species that are historically endemic to islands lacking congeners. We are finding that solitary species share many unusual characteristics of size, shape, and scalation, and that most of these traits evolved exaptively rather than as adaptive responses to solitary existence. **Levi**

Gray has been working on mainland aquatic anoles and is finding differences in the habitat requirements of these behaviorally similar species. Undergraduate students **Natalie Blea** and **Julian Davis** are currently working on taxonomic issues in mainland *Anolis*. Natalie is elevating *Anolis humilis marsupialis* to species status and Julian is describing a new *fuscoauratus*-like species from Peru and Bolivia.

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Finding *Anolis proboscis*

When we finally reached Mindo it had already been an amazing trip. We had arrived in Quito, Ecuador, two nights earlier at 10:30 PM and immediately drove east down the Amazonian slope of the Andes to the town of Baeza. I had two graduate students with me who I had come to trust to find anoles, Eric Schaad and Ian Latella, plus photographer / birder / weightlifter Dr. Tom Kennedy, neophyte undergrad Natalie Blea, and Ecuadorian student Fernando Ayala. We had driven that slope Sunday night without finding a single herp, in spite of my overexcited encouragement ("this could be the best herping night of your life!"), but had followed that disappointment up with an incredible Monday evening heading north to find innumerable *Anolis fitchi* and eight *A. orcesi*.

Anolis orcesi was described in 1969 by the great field biologist Skip Lazell, and until our evening outside of Baeza, this species was known from just two museum specimens. We had spent barely more than a night herping the eastern Andes but had secured splendid series of *fitchi* and *orcesi*, the first two goals of the trip. Our good fortune with *orcesi* meant we could move on to concentrate on the real prize, the legendary rostrally-endowed species *Anolis proboscis*, last reported from Mindo. This rare form seemed to hold an exalted place among anologists thanks to its ridiculous snout morphology, the tantalizing capture and release of a recent specimen after decades when it was thought possibly to be extinct, and the feature of it by Jonathan Losos in his new anole book. But because a fresh preserved specimen had not been produced, *Anolis proboscis* appeared on the brink of joining *A. roosevelti* in the annals of unobtainable anoles. The recent sighting by an ecotourist birding group notwithstanding, nobody had collected a specimen in over 40 years, and females had never been collected. In spite of my

Fig. 1. Fernando, Steve, and Ian seeking provisions outside of Quito, Ecuador. Photo by Eric Schaad.

admonitions to the newbies along ("we had great luck with *orcesi*, but…it's not always like that"), the *orcesi* success had our group pretty full of itself by the time we arrived in Mindo.

We reached the turnoff down to Mindo at about 1:30 AM after driving up and over the Andes from Baeza and stopping to meet with Fernando's contacts and then get provisions at the 24-hour liquor store just north of Quito (Fig. 1). Mindo sits at about 1400 meters elevation on the edge of cloud forest habitat on the western Andean slope. It is a famous birding site, frequented by gringo ecotourists and Ecuadorians alike, with lovely hotels and restaurants situated around a pleasant town plaza and remnant protected forest just outside of the town limits. We were exhausted during the drive over after herping all night the evening before. Poor Natalie was vomiting out the window from some just-acquired sickness, and everybody else slept intermittently until we reached the turnoff. Once there, I insisted that we drive the 7-kilometer road down to Mindo to check the available roadside forest before starting to herp. I knew we were running out of time (sunup at 6 AM), but thought it most efficient to identify the best possible habitat before proceeding. I had a feeling *proboscis* was a twig anole, like the phenacosaurs (with which it grouped in our recent phylogenetic analyses) and the Caribbean *valencienni*/*insolitus*/*occultus* species. So we searched for twiggy mats and vines below low canopy as we descended the pavement.

At the bottom of the road where the town of Mindo began, we regrouped with boots, snack food, and caffeine in preparation for our quest. Once outfitted, we passed around the rum bottle. We had developed a tradition where at the beginning of a night's searching, each herper takes a pull off the bottle and states which species he/she would like to find that night. There is usually a variety of herpetofaunal desiderata, and each statement of a target species is met with approval or derision from the peanut gallery of other herpers in the group. But on this night everybody said the same thing. "Tonight we herp for one thing and one thing only," went the Blades of Glory reference, "*Anolis proboscis*." We drove back up the road to the good habitat.

I did not think we would find it that night. It was too rare, and things had gone too well, and we were all tired, and it just didn't seem like we had earned it. With nighttime receding, I left the good high parts of the road to the others and moved from twiggy mass to twiggy mass as I worked my way rapidly down towards Mindo. Everybody was energized by the hundreds of *Anolis gemmosus* sleeping splayed on low ferns. But I was getting antsy as 3 AM passed, and then 4 without *proboscis*.

At around 4:30 AM I saw a strange brown anole on a horizontally-oriented twig at about 9 meters up above some trashy understory plants near a big tree. Over time an anole biologist develops a night search image for particular species. *Anolis biporcatus* has a humped back, different from other Central American green species. *Anolis frenatus* sleeps with flexed hindlimbs so you can see its form spreading out laterally from a vine. *Anolis limifrons* has a browner and plumper posterior belly than the undescribed fuscoauratid species from Panama that it resembles. You tune in to these characteristics when you look for anoles at night, and with practice you can identify virtually any sleeping anole definitively before you ever make an attempt to catch it. But I could not place this species. I wondered if it was *proboscis* as I extended the 24-foot telescoping golf ball retriever to its full extent towards the lizard. I tapped it and knocked it down, and caught it out of the air (a textbook solo "shake and bake," in the idiom of our group). It was…something I had never seen before. Drab color…crested body and tail…white dewlap. "I *think* I got it," I yelled, still a bit unsure (nobody knew whether females had the proboscis; my captured specimen lacked one). But everybody was far up the road. Now rejuvenated, I started running back towards the others. Then I slowed and began to look for a male as I worked my way back up the road. I found our first two *Anolis aequatorialis,* a species that later turned out to be common in the area, before meeting Ian and Eric. They were walking way too fast not to have found something cool themselves.

"What's going on?" they yelled.

"*proboscis*," I replied.

"We got a male," they said.

"I've got a female," I shot back.

I had found the first female of the species, but Eric and Ian had collected the trophy of the trip (Fig. 2). They showed me a plastic bag with a 70 mm lizard fumbling about, bending its preposterous snout extension against the transparent walls. It was beautiful, amazing, goofy-looking. I couldn't stop staring at it, marveling at its weirdness. They recounted seeing it on the twig, how it moved its head sideways when disturbed and that was how they knew it was what it was. I could not believe our good fortune.

Fig. 2. *Anolis proboscis*, male. Photo by Tom Kennedy.

We caught three more *Anolis proboscis* over the next two nights, two males and a female, and stayed in Mindo long enough to get good samples of all the anoles known from there (*proboscis, gemmosus, aequatorialis, fraseri*). The remaining ten days of the trip were fantastic (we caught an additional 10 species of *Anolis*), but we never reached the level of excitement of that *proboscis* night. Really, how could we?

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Observations on sleeping behavior of *Anolis occultus* and *A. cuvieri* in Puerto Rico

The intent of this note is to provide some observations on the seasonal behavioral variations in the habits of *Anolis occultus* and *Anolis cuvieri*.

Anolis occultus is a twig anole, and the smallest anole endemic to the Puerto Rican insular bank. In accord with its ecomorph designation, it usually perches on small twigs and branches during the day, although I have observed it on a bromeliad during the day and on a grass blade (about 40 centimeters from the ground) at night.

On regards to spatial arrangement, the species orients itself at night in a rather predictable way, depending on the season.

During fall and winter *A. occultus* almost invariably chooses perches on east-facing sides of shrubs and trees. Such perches are not necessarily exposed, and might indeed be quite hidden in the foliage. However, assuming the lizard's intent is to avail itself of the first available sunlight in the morning in order to warm up through basking, then even if the perch is under cover the animal can move to the closest sunlit spot without having to travel too much and expose itself to predators.

Fig. 1. A male and a female *Anolis occultus* sleeping at night facing the distal ends of their perches. The lengthwise concave rib structure allows them to "hug" their perches, blurring their profiles to a predator's eyes. Photographs taken at the Tayonal National Reserve, and at Cerro Torrecillas, PR.

By contrast, in summer *Anolis occultus* does not follow this pattern as closely, and may be found at night on any thin, brown or gray twig.

A consequence of such seasonally variable behavior, I find it much easier to see them in winter, since I know what side of the vegetation to search.

These observations are mostly from cloud forest habitat. In Puerto Rico this type of floristic community is found almost exclusively at the highest altitudes of the highlands, between about 800 to 1338 meters above sea level, depending on local conditions. Due to the effect of altitude, these forests experience the coldest temperatures in the island, with an average of 64 degrees Fahrenheit during the year, and with rare nocturnal lows in the higher 30's between January and February.

Fig. 2. A male *Anolis gundlachi* sleeps on a perch located about three meters from the ground. It clearly positioned itself towards the base of the perch. El Yunque National Forest, Puerto Rico.

The only three individuals that I've seen at night in lowland karstic forest in northern Puerto Rico (with higher average temperatures) were not resting in any particular position on regards to morning sunlight. One was sleeping on a very exposed twig, while the other two were found on twigs under closed canopy.

Save for only one instance, I have always observed sleeping *Anolis occultus* facing the tip of the twig. (Other species either face the trunk or no particular direction).

Fig. 3. *Anolis krugi* will choose various perches and orientations when sleeping. El Yunque National Forest, Puerto Rico.

Anolis occultus possesses a clearly longitudinally concave belly, which allows it to fuse its silhouette with its thin perch. Presumably this enhances the anole's crypsis, helping it to avoid detection by an approaching predator or human and when it sleeps at night. However, and particularly at night, if it feels further threatened, its typical defensive behavior is simply to let go of its perch and drop to the ground like a dead weight. It does not actively flee by jumping or running away to safety, in the way of many other anoles, but rather allows itself to fall to the ground vertically. This might have the advantage of still keeping it unnoticed by a predator which would otherwise immediately notice an anole running or jumping horizontally or even vertically, but which would not pay attention to what seems to be a falling piece of "debris".

Anolis cuvieri, the Puerto Rican giant anole, belongs to the crown giant ecomorph. It is the second largest Puerto Rican anole, after *A. roosevelti,* found on smaller islands on the eastern end of the insular bank. Save for *A. occultus* itself, it is the least commonly seen anole in mainland Puerto Rico. This is due to its pronounced arboreal habits. It is, however, quite common in appropriate habitat, particularly in humid karstic forests with a closed canopy.

I always find it difficult to see in winter (thus, it shows a seasonal change in behavior similar to that of *A. equestris* in Florida, USA). During the winter, perhaps individuals of *A. cuvieri* stay in the canopy, closer to the warmth-giving sunlight, and are thus able to remain active for longer periods of time due to heightened body temperature. If such is the case, this might allow them to better avoid predators and chase prey. By comparison, in the hot summer, when ambient temperatures are higher and they do not need to spend as much time basking, it is far easier to see them perching low on tree trunks. This is especially true during afternoon hours.

Although during the day *Anolis cuvieri* will most often choose perches of high diameter, including the most massive tree trunks available, it does not do the same at night. When sleeping, it will choose perches of small diameter, including vines and lianas only a few millimeters thick. This might allow it to be alerted in the presence of nocturnal predators, such as the Puerto Rican boa, *Epicrates inornatus*. As such predators approach they will cause the thin perch to vibrate, alerting the lizards to the imminent danger. Unlike *A. occultus*, *A. cuvieri* actively jumps or runs away when threatened at night.

Fig. 4. A female *Anolis cuvieri* found sleeping on the leaves of a thin branch, facing its base. El Tayonal Nature Reserve, Puerto Rico.

Anolis cuvieri seems to be an obligate denizen of closed canopy humid and rain forests. While other Greater Antillean giants like *A. equestris* and *A. garmani* might be easily found on isolated trees in artificial parkland and even urban areas, *A. cuvieri* is seldom, if ever, found far away from dense forest cover. This might explain its apparent rarity compared to other giant species, and may make it more vulnerable to deforestation.

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The *Anolis* Gene Nomenclature Working Group

Anolis lizards are a commonly used model system in studies of ecology, evolutionary biology, behavior, and neuroscience. *Anolis carolinensis* is the first non-avian reptile genome sequenced, and research on *Anolis* is rapidly progressing into novel areas such as genetics, genomics, development, and molecular and cellular biology. The unique phylogenetic position of *A. carolinensis* relative to other species with sequenced genomes means that it will play a critical role in broad-scale comparisons among amniote lineages. Unlike many other model systems, such as the mouse and chicken, additional efforts are being made to develop resources for finer-scale comparisons among different *Anolis* species for studies of evolutionary diversification. Comparative research at any scale, however, will be greatly hampered without guidelines to govern the identification and nomenclature of orthologous loci, homologous regulatory elements, genetic markers, and other functional elements of the genome. We are working towards the development of these guidelines by establishing the *Anolis* Gene Nomenclature Working Group (AGNWG), with members who have broad interests in anole biology. Below we discuss our immediate and future goals with the hope of soliciting further feedback from the *Anolis* community. We view these preliminary guidelines as an evolving document. As a consensus is reached these guidelines will be posted on anolisgenome.org and other community web sites as a reference for the research community.

Overview

Most model organisms, including *Drosophila*, *Xenopus*, chicken, mouse, and human, have established guidelines for gene nomenclature. The AGNWG aims to build the *Anolis* nomenclature system around those that have already been established to avoid the missteps experienced by other groups. Only when necessary will we establish new guidelines that are specific to the anole community – for instance, identifying gene variants associated with a particular species. Due to the primary emphasis on human biology given by the National Institute of Health, special consideration is being given to the well-developed rules and definitions outlined by the Human Gene Nomenclature Committee (http://www.genenames.org/guidelines.html). Therefore, we will not go into extensive detail regarding definitions and terminology here but rather focus on more practical matters that concern the community as a whole. We are regularly conferring with Janet Weber at the NCBI and Martin Grabherr at the Broad Institute. It will be critical to establish these guidelines early in the post-genomic era of anoles to avoid later confusion.

Preliminary objectives of the *Anolis* Gene Nomenclature Working Group

We have identified four issues that have broad applicability to the anole community and have, therefore, taken priority within the working group.

1. Determining orthology and paralogy among anoles and other vertebrates.

Determining gene orthology is not trivial but is critical for comparative analyses at all phylogenetic scales. For many genes, orthologs can be readily identified across multiple species using reciprocal sequence similarity. But, for many others, this is not possible: regions of conservation are limited to narrow functional domains, synteny may or may not be conserved, expression patterns may have evolved, there may be gene loss and gene gain, and some genes may have been co-opted into divergent molecular networks. One of the priorities of the AGNWG is to outline protocols for identifying gene orthologs. Our goal is to design a system where evidence codes can be assigned to a gene indicating how confident one can be in assigned orthology.

2. Nomenclature for novel *Anolis* genes.

Previously published genomes report that 10-15% of functional genes are unique to a particular lineage and cannot be easily assigned to known genes from other species. As novel genes are identified in the *Anolis* genome, how will they be named? To avoid chaos, it is critical that guidelines are established that will govern this process. For example, will they be named based on their function, the phenotype of a mutant, or their location? In addition, how much confidence should be bestowed on a particular gene annotation? Comprehensive lists of previously named genes are available for other taxa and these will need to be referenced to avoid redundancy.

3. Abbreviations for *Anolis* species within studies of functional, population and comparative genomics.

Anolis represent a species-rich genus of lizards with over 370 listed species and many closely related lizard species. It is critical that researchers maintain a common species vocabulary for both clarity and future cross-referencing between data sets. In an effort to curate abbreviated species names, the *Anolis* Species Working Group proposes the following set of rules:

3.1. All genus/species abbreviations for anoles will begin with the capital letter, 'A', followed by three lowercase letters based approximately on the first letters of the species name (see 3.2). Abbreviations will be italicized. Species from other genera will designate another capital letter as their prefix. This system of nomenclature does not address sub-species designations or geographic "races."

Example:
Anolis baracoae = Abar

3.2. The three letter species abbreviation suffix (in lowercase) is generated by the first two letters of the species name and an identifying third letter unique to each species. In cases of redundancy in all of the first three letters of species names, the third letter will be replaced with the subsequent letter of the species name that generates a unique code. If all things are equal, precedence is given to species in alphabetical order.

Examples:
A. baracoae = Abar
A. barahonae = Abaa
A. barbatus = Abab
A. barbouri = Abao

3.3. Abbreviations assigned initially by the working group (~June 2010) shall be maintained for perpetuity.

3.4 Similar to the rules of taxonomy, abbreviations will not change as new species are described or as currently described species are split. Precedent is given to the first abbreviation assigned to a species.

The AGNWG feels that these guidelines objectively generate abbreviations for all 371 *Anolis* species names. However, we also realize that these systematic rules may not reflect the history of a well-established community. In particular, should more commonly known species possess the more obvious genus/species abbreviation? Should precedence be given by number of publications, description date, number of genes described, or some other criteria? We have generated a matrix of data, available on request (please contact RJK), that includes the number of records in each of 30+ NCBI databases (e.g., nucleotides, proteins, SNP, popset, OMIM, etc.) as well as discription dates for each of the 371 listed species. Our final guidelines and list of species abbreviations will be posted anolisgenome.org and distributed by listserve in June 2010 once comments from the community are incorporated.

4. Nomenclature and symbology for *Anolis* genes, proteins, RNA classes, ultra-conserved elements, and functional genomic elements.

How will we refer to genes, their products, and other functional elements in publication? While somewhat subtle and semantic point, consistency within the community is critical if we wish to assure the smooth exchange of information. While we are adapting many of the guidelines outlined by the Human Gene Nomenclature Committee there remain unique aspects of anole genomics that will need to addressed, such as how to identify elements derived from different species.

Future objectives of the *Anolis* Gene Nomenclature Working Group

As work progresses on the *Anolis* genome, new and unforeseen nomenclature issues will certainly arise. The goal of the working group is to foster community-based discussion where these problems can be resolved. We have addressed four short-term objectives for the AGNWG but have several additional objectives that will be addressed subsequently.

1) Nomenclature for populations and treatment of geographic variation. How do we determine the reference or "type" sequence that others are compared to?
2) Creating a common nomenclature for genetic markers such as microsatellites and SNPs
3) Creating a common nomenclature for transposable elements

The AGNWG welcomes feedback from the community. Are there issues that we have overlooked? Have our guidelines inadvertently created conflicts within the naming scheme? We would like to know. Also, would you like to join the AGNWG? Please email one of the AGNWG members to discuss your concerns or to share any comments.

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Presence of lizards of the genus *Anolis* in the protected areas of Cuba

Abstract

Establishment of legally protected areas is one of the most important ways to preserve biodiversity. In Cuba, there is a National System of Protected Areas that includes 253 distinct areas covering 19.93% of the Cuban territory. Six of these are Biosphere Reserves recognized by UNESCO. In general, there are two types of Cuban protected area: National, broad with well-preserved ecosystems, and Local, smaller regions harboring some important floral or faunal resource. In all of the 14 provinces and the Isla de la Juventud there are protected areas of both types. Pinar del Rio, Villa Clara, and Guantanamo have more National areas than the other provinces, whereas Pinar del Rio, Ciudad de La Habana, Matanzas, Camagüey, and Guantánamo have more Local areas than the other provinces. In more than half of the National areas there are species of the genus *Anolis*, but fewer than half of the Local areas contain anoles. Of the 63 species of *Anolis* on Cuba, just four (*Anolis juangundlachi, A. macilentus, A. terueli,* and *A. vescus*) are not protected by the National System. Species vary in the number of protected areas in which they occur: this varies from 1 (14 species) to 65, with *A. porcatus* (57), *A. angusticeps* (58), *A. homolechis* (64), and *A. sagrei* (65) occurring in the highest numbers of protected areas. In general, almost all Cuban anoles occur in at least one protected area, which indicates that the Cuban system of protected areas is useful for the conservation of reptiles.

Introduction

Species of the genus *Anolis* are globally under threat of extinction, like many other species in the world. Fragmentation or destruction of the macro and microhabitat caused by deforestation, the introduction of alien species, and the direct impact of human activities on some species, together with global warming, are the main threats to anole species survivorship (Rodriguez Schettino and Rodriguez Gomez, 2003). That is why it is important to know what species are under threat in order to prevent their extinction. In this sense, scientific literature and herpetological collections are the best ways to obtain such data. Upon this base, there are

some global, regional, or local lists of threatened species. Globally, IUCN (2008) did not include Cuban *Anolis* lizards. Locally, the first attempt to list threatened Cuban species was done by Buide *et al*. (1974) who mentioned eight *Anolis*, and Perera *et al*. (1994) included 26 lizards in a later proposal. Between 1997 and 1999, three Conservation Assessment and Management Plan Workshops for Cuban species (CAMP) were carried out in Cuba, supported by the Conservation Breeding Specialist Group (SSC/IUCN). These workshops produced proposals to list 11 lizards as threatened to different degrees. Rodriguez Schettino and Chamizo Lara (1998) listed 35 threatened *Anolis* according to an evaluation of their endemicity, abundance, and geographic distribution, whereas Vales *et al*. (1998) compiledprevious data and listed 30 species of this genus. Rodriguez Schettino (1999) took into account the several causes threatening Cuban iguanids (*sensu lato*), and concluded that 53 anoline taxa met the criteria of the categories of Critically Endangered, Endangered, Vulnerable, or Near Threatened, according to the IUCN. Fong *et al.* (2005) mentioned seven threatened anolines for the National Park Alejandro de Humboldt. In the web page of the National Center for the Biodiversity (CenBio: http:\\www.ecosis.cu\cenbio\biodiversidadcuba\listarojaiucn_cuba.htm) there are 57 threatened lizards of the genus *Anolis* and *Chamaeleolis* compiled from different sources.
Cuba is trying to achieve nature conservation by means of two main ways: *in situ* and *ex situ* (Rodriguez Schettino and Rodriguez Gomez, 2003). Although zoological parks and other kinds of enclosures for breeding species have an important role for species conservation, the most significant way to preserve biodiversity is the establishment of protected areas that will preserve not only one anole species, but other species as well as their environment (Rodriguez Schettino and Rodriguez Gomez, 2003).

For that reason, we are trying to identify which species are living in the protected areas of Cuba, and are analyzing whether these areas meet the objectives of the preservation of lizards, especially in comparison to lizards occurring outside the system of protected areas.

Materials and Methods

In Cuba, there is a National System of Protected Areas (SNAP) that includes 253 protected areas covering 19.93% of the Cuban territory (CNAP, 2009). There are two types of protected areas: National (91), broad with well preserved ecosystems, and Local (162), narrow places with some important floral or faunal resource. Six of the National protected areas are Biosphere Reserves approved by the UNESCO: Sierra del Rosario, 1985; Guanahacabibes, Cuchilla del Toa and Baconao,1987; Buenavista and Ciénaga de Zapata,1999 (CNAP, 2009).
To date, in Cuba there are 155 described species of reptiles (Rodriguez Schettino *et al*., in press a), of which 63 belong to the clade *Anolis*, and five are included in the subclade *Chamaeleolis* (Hass *et al*., 1993; Jackman *et al*., 1999; Poe, 2004).

We reviewed the literature of all of the Cuban *Anolis,* compiled locality data from several collections of preserved specimens, and conducted several field trips to gather information on geographic distribution. All of this information was incorporated into a database and processed by a GIS (Mapinfo ver. 9) to see which of the localities are contained in any of the protected areas. We considered that a species is covered by the SNAP if at least one of its localities is in one of the protected areas.

Results and Discussion

During a January 2007 workshop for the gap information of the terrestrial vertebrates in the National System of Protected Areas of Cuba, we assessed 12 species of the genus *Anolis* with the result of that all but two have localities in protected areas. However, in a different study using a literature review, Gonzalez *et al.* (2009) pointed out that just two of the 23 analyzed species (*Anolis juangundlachi* and *A. macilentus*) are not covered by the SNAP.

Our present study is more comprehensive than the two previous studies, and we found that in all of the 14 provinces and the Isla de la Juventud there are protected areas of both types (National and Local). Pinar del Rio, Villa Clara, and Guantanamo have more National areas than the other provinces, whereas Pinar del Rio, Ciudad de La Habana, Matanzas, Camagüey, and Guantánamo have more Local areas than the other provinces. In more than half of the National areas (72.5%) there are species of the genus *Anolis*, but in the Local areas the reverse is true (43.2%) (Fig. 1).

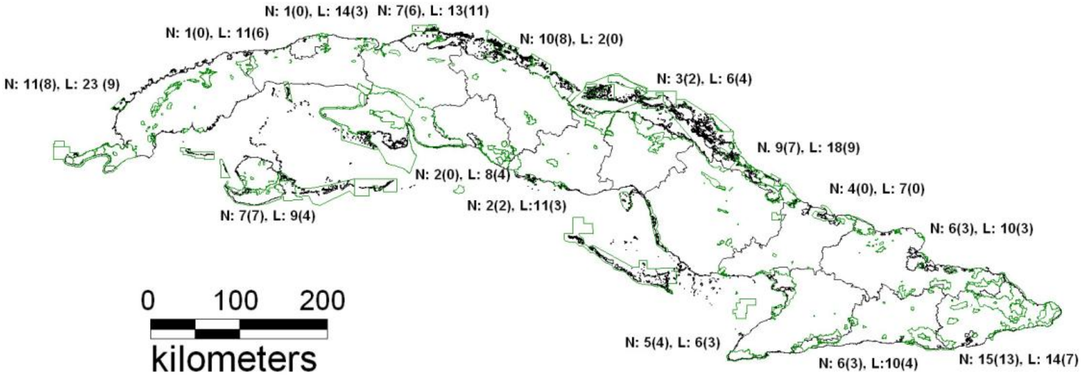

Fig. 1. Number of National (N) and Local (L) protected areas for each province in Cuba.

The amount of protected areas in where species are found ranges from 1 to 65. Of the 63 species of anoles, the four that are found in the greatest number of protected areas are : *A. porcatus* (57), *A. angusticeps* (58), *A. homolechis* (64), and *A. sagrei* (65). On the other hand, four species (*A. juangundlachi, A. terueli, A. macilentus,* and *A. vescus*) have no localities in protected areas. The following 14 species occur in just one protected area each: *A. birama, A. toldo, A. ruibali, A. delafuentei, A. inexpectata, A. alfaroi, A. guafe, A. confusus, A. pigmaequestris, A. spectrum, A. fugitivus, A. incredulus, A. oporinus*, and *A. altitudinalis.* Almost all are local species known from only one or very few localities.

Although, in general, almost all the species of the genus *Anolis* are preserved by at least one protected area, indicating that the Cuban system of protected areas is useful for the conservation of such important reptiles, we think that these 18 species should be the subject of more focused conservation efforts, and that they might serve as flagship species for the preservation of the ecosystems in which they occur.

What will we do next? We will try to locate all the species in every protected area, since we are currently working only with presence and absence data for these areas. More detailed locality information will be very useful for people working in the protected areas. Examples of this work have been presented by Rodriguez Schettino *et al*. (2005), Rodriguez Schettino and Rivalta Gonzalez (2008), and Rodriguez Schettino *et al*. (in press b).

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Ecological character displacement in *Anolis carolinensis*

A long-standing hypothesis in evolutionary ecology is that strong competition between closely related species for similar resources leads to character displacement – correlated ecological and phenotypic divergence between species to reduce competition (Brown & Wilson 1956). Hutchinson (1959) suggested that for traits that govern resource use, there should be a minimum size difference in those traits between two competing species. Subsequently, MacArthur and Levins (1967) proposed the theory of limiting similarity, which stated that competing species must be sufficiently different in resource use or else they will be unable to coexist in sympatry. Hence, natural selection should lead to ecological character displacement by favoring those individuals that reduce competition by exploiting divergent resources using divergent morphologies.

The ecological character displacement hypothesis enjoyed wide popularity following its introduction, and many evolutionary ecologists invoked the evolutionary effects of competition based simply on the co-occurrence of similar closely related species that exhibited some differences in sympatry (Grant 1972). Yet beginning in the late 1970s and early 1980s, community ecologists began to question the accepted dogma of ecological character displacement (reviewed in Dayan & Simberloff 2005). Theoretical models showed that character displacement was only plausible under a restricted set of ecological conditions (Slatkin 1980). Statistical null model tests of empirical data and published results showed that size differences between potential competitors were often no more than expected by chance (Strong et al. 1979; Simberloff & Boecklen 1981). A slew of alternative processes were proposed that could create a pattern like character displacement including interference competition, apparent competition for enemy-free space, and intra-guild predation (reviewed in Schluter 2000).

These critiques crystallized the need for specific criteria that had to be met to conclude that differences between species actually constitute character displacement due to competition. Schluter and McPhail (1992) formalized six tests of a hypothesis of ecological character displacement.

(1) *Chance should not explain the character displacement pattern.* A relationship between phenotypic divergence and competition may occur by chance. Replicate populations exhibiting the character displacement pattern are needed to rule out chance.
(2) *Resource use shifts should match changes in morphology or other phenotypic traits.* If competition selects for divergent resource use, then phenotypic differences should occur in traits important for resource use.
(3) *Character displacement should be the result of an evolutionary shift rather than species sorting*. Species must have diverged *in situ*, rather than allopatrically with subsequent range expansion and coexistence.
(4) *Sites of allopatry and sympatry should not differ in environment.* Environmental differences must be controlled for or removed experimentally to show that interspecific competition, and not other environmental factors, drives phenotypic divergence.
(5) *Independent evidence should show that similar phenotypes compete for resources.* Species must compete for the same resources because the character displacement pattern could be caused by a number of alternative ecological processes (e.g. aggressive interference, apparent competition, and intraguild predation).
(6) *Phenotypic differences between species should be genetically controlled.* Differences between populations or species must be genetic and not the result of phenotypic plasticity.

The debates over ecological character displacement stimulated a wealth of empirical and theoretical studies that combined a wide variety of approaches to test the hypothesis that interspecific resource competition leads to character displacement (Dayan & Simberloff 2005). On the whole, many empirical studies across mammals, reptiles, amphibians, fish, insects, and plants have now provided evidence consistent with ecological character displacement (reviewed in Dayan & Simberloff 2005). In addition, theoretical studies have shown that the conditions required for ecological character displacement are less restrictive than previously thought (Milligan 1985; Taper & Case 1992; Doebeli 1996; Drossel & McKane 1999). As a result, the pendulum has generally swung back to the view that ecological character displacement, driven by interspecific competition, is an important evolutionary force.

Yet despite the research that emerged from the debates of the 1970s and 1980s, the case studies that fulfill the six necessary criteria to show that interspecific resource competition leads to character displacement are surprisingly few (Schluter 2000). In a review of the literature, Schluter (2000) compiled a list of 61 empirical studies meeting at least one of the six criteria. Of these, just five studies met all of the criteria, with most studies failing to address Criterion 5 – experimental evidence for competition (Schluter 2000).

Furthermore, most studies of character displacement are necessarily done in a comparative context, yet a large body of literature is amassing showing that natural selection drives rapid evolution in the wild and that evolution can be studied in an experimental context. Evolutionary experiments specifically designed to test for character displacement are needed to definitively determine whether competition is an important force in adaptive evolution.

Specific research objectives

(1) Test the six criteria for ecological character displacement between two *Anolis* lizard species that have recently come into sympatry, using a natural experiment created by the invasion of a non-native *Anolis* species.
(2) Directly manipulate island populations to experimentally test for adaptive divergence in response to a non-native competitor.

Background

Study System

Anolis: *Anolis* species have similar ecological needs (they are all diurnal insectivores) and have been shown to compete strongly in nature (reviewed in Losos 2009). Evidence suggests that *Anolis* mitigate competition through divergent habitat use, and character displacement may have played a key role in the repeated evolution of similar habitat specialists on the four islands of the Greater Antilles (Rand 1964; Williams 1983; Schoener 1988; Losos 1990a,b; Losos 1992). These "ecomorphs" specialize on subsets of the available vegetation and have distinct limb and toepad morphologies that are adapted to optimize performance in the preferred habitat (Losos 1990b; Losos & Irschick 1996; Irschick & Losos 1998, Glossip & Losos 1997).

Anolis carolinensis* and *A. sagrei: *Anolis carolinensis* arrived in Florida and spread into the southeastern US approximately 3-4 mya (Glor et al. 2005). It is the lone anole native to the US and uses a broader spectrum of the habitat than its two sister species in Cuba, ranging from ground to tree crown (Collette 1961; Campbell 2000). Over the past 60 years, however, *A. sagrei* has invaded the southeastern US from Cuba (Campbell & Echternacht 2003). *Anolis sagrei* primarily inhabits the ground and tree trunks up to 1.5 meters. Wherever *A. sagrei* has come into sympatry with *A. carolinensis*, the latter responds by shifting its habitat use up (Campbell 2000).

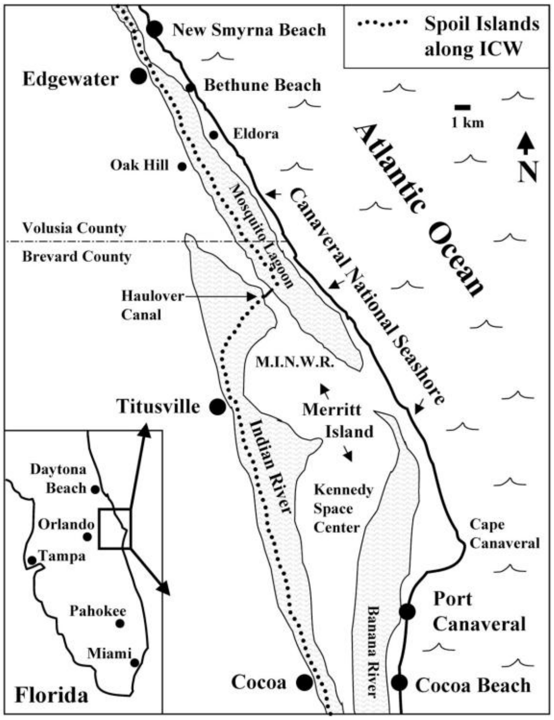

Figure 1: Mosquito Lagoon, Florida. Study islands are located between Oak Hill and Haulover Canal. Modified from Campbell and Echternacht (2003).

Mosquito Lagoon: Approximately 60 years ago, in Mosquito Lagoon, Florida, the Army Corps of Engineers established a series of 80 small islands with sediment generated from the dredging of the Intracoastal Waterway (ICW) (Fig. 1). These islands were colonized quickly by local flora and fauna, including *A. carolinensis*. All the islands are roughly the same in size, shape, floral and faunal species composition, and distance from the mainland (Campbell 2000). In 1995 and 1996 (approximately 20-25 *A. carolinensis* generations ago), Dr. Todd Campbell introduced *A. sagrei* onto several of these islands to study demographic effects of *A. sagrei* on *A. carolinensis*, compared to control islands that had only *A. carolinensis*. Prior to the introductions, he gathered detailed species presence/absence records for many of the islands in the lagoon and has graciously shared this data with us.

A unique opportunity to experimentally study adaptive evolution in nature

Experimental studies of adaptive evolution in nature are rare (but see Endler 1980; Reznick & Bryga 1987). The presence of *A. sagrei* on these islands introduces a putative competitor for *A. carolinensis* that is predicted to lead to character displacement in *A. carolinensis*. Campbell's purposeful introductions constitute a replicated experiment in natural conditions ideal for studying adaptive evolution. Over the course of my dissertation, we will combine study of this unique natural experiment with additional direct experimental manipulations to rigorously test the hypothesis that novel selection from competition for shared resources has led to character displacement and adaptive evolution in *A. carolinensis*.

Preliminary Research

We conducted preliminary research to test the hypothesis that populations of *A. carolinensis* co-occurring with *A. sagrei* will show altered habitat use and morphology compared to populations that do not occur with *A. sagrei.* In 2009, we visited six spoil islands – three with both *A. carolinensis* and *A. sagrei*, and three on which *A. carolinensis* was the only anole. We collected data on two habitat variables and two morphological variables that are functionally related to optimal performance in those habitats: perch height and toe pad area, and perch diameter and femur length (Losos 1990b). We also measured snout-vent length as a proxy for body size. We present preliminary results for male perch height and toe pad size, comparing island means by 'treatment' with ANOVA. Toe pad area data were log-transformed and size-corrected by regressing toe pad area against snout-vent length. After confirming that the populations do not differ in slopes in the relationship between toe pad area and body size, we used the resulting residuals for analysis.

Habitat use: We find significant differences in mean perch height for *A. carolinensis* comparing populations on *A. sagrei*-absent islands (n=3) to populations on *A. sagrei*-present islands (n=3). In the presence of *A. sagrei*, *A. carolinensis* perches nearly a meter higher in the canopy compared to islands where *A. sagrei* is absent (Fig. 2; $F_{1,4} = 13.13$, $P = 0.022$).

Morphology: We find marginally significant differences in *A. carolinensis* populations for toe pad area when comparing *A. sagrei*-absent islands (n=3) to *A. sagrei*-present islands (n=3). *Anolis carolinensis* on *A. sagrei*-present islands have larger toe pad areas relative to body size (Fig. 3; $F_{1,4} = 7.073$, $P = 0.056$), compared to *A. sagrei*-absent islands.

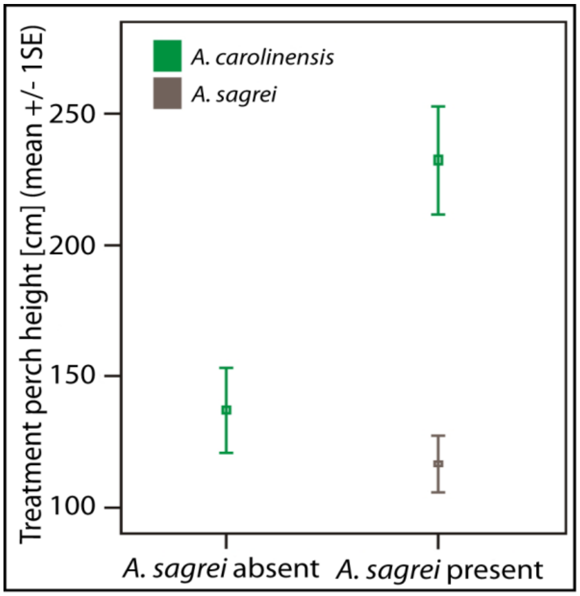

Figure 2: *A. carolinensis* mean male perch height for *A. sagrei*-absent (n=3) and *A. sagrei*-present (n=3) islands. *A. sagrei* mean male perch height shown for comparison.

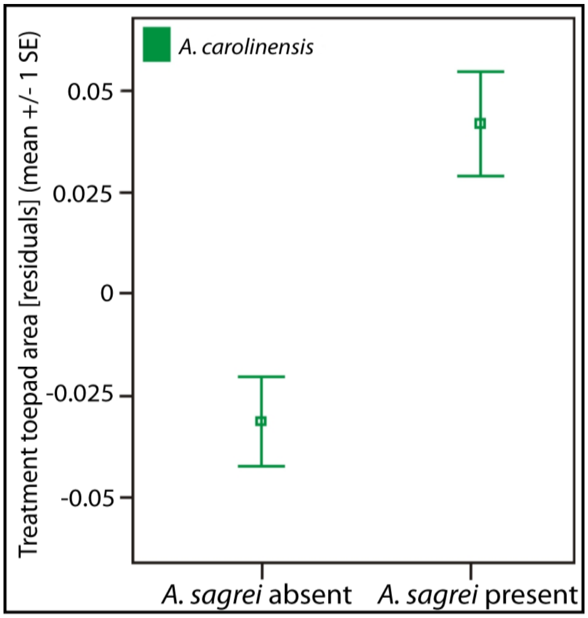

Figure 3: *A. carolinensis* mean male toepad area for *A. sagrei*-absent (n=3) and *A. sagrei*-present islands (n=3).

Future Directions

Future dissertation research will consist of: (1) rigorously addressing the six character displacement criteria in this system to test the hypothesis that *A. carolinensis* populations are undergoing ecological character displacement, (2) directly testing the hypothesis that character displacement in *A. carolinensis* is adaptive with a new introduction experiment, (3) testing for character displacement (using museum specimens) in *A. carolinensis* and its sister species, which have been coevolving with varying numbers of sympatric, congeneric competitors in different *Anolis* communities across Florida, the Bahamas, and Cuba, and (4) using population genomic techniques to isolate regions of the genome that appear to be important in the adaptive response of *A. carolinensis* to *A. sagrei*.

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Higher incidence of the anole predator *Coluber constrictor* following *Anolis sagrei* invasion in Florida

Abstract

In Florida, invading brown anoles (*Anolis sagrei*) often displace populations of the native green anole (*A. carolinensis*) from structurally simple habitats. This effectively changes the identity of the *Anolis* species operating in a food web and may, depending upon the degree to which the two *Anolis* are functionally equivalent in food webs, affect other species in the community. While conducting food web studies on a series of invaded and uninvaded islands, I observed the *Anolis*-eating snake *Coluber constrictor* (black racer) exclusively on invaded islands. I used a contingency table analysis to test the null hypothesis that these racer observations were distributed independently of *A. sagrei* abundance on islands. I found that the incidence of racer observation was not independent of *A. sagrei* abundance, but rather was significantly higher for invaded islands than for uninvaded islands. I suggest that these results provide indirect evidence for a positive racer response to the higher prey densities that result from *A. sagrei* invasion.

Introduction

Food webs in many parts of the world have been altered by the insertion of one or more species of introduced *Anolis* lizards. The effects of these insertions are in most cases unknown, but they may be significant, especially if the introduced anoles attain high densities. My research has focused on the food web effects of the highly invasive and extremely abundant brown anole (*Anolis sagrei*) in Florida, which are coupled to its displacement of a native congener.

In Florida, invading *A. sagrei* displace the native green anole (*A. carolinensis*) from the lower perches of some habitats and the entirety of others, depending upon the degree of habitat openness. Although *A. carolinensis* are in most respects trunk-crown ecomorphs like their Cuban ancestors, *A. porcatus*, they experienced ecological release in North America in the absence of other *Anolis* species and came to occupy nearly all Floridian habitats as well as the spectrum of microhabitats available within them, from crown to ground. In habitats with dense vegetation or a well-developed canopy, invasion of the trunk-ground *A. sagrei* leads to niche partitioning and coexistence of the two species (albeit at reduced *A. carolinensis* densities) as *A. carolinensis* retreat from the lower microhabitats and shift to a higher perch distribution (Campbell 2000, Vincent 2002). In open or structurally simple (often disturbed or edificarian) habitats, however, *A. sagrei* can decimate and effectively replace *A. carolinensis* populations, most likely via intraguild predation of *A. carolinensis* juveniles (Gerber and Echternacht 2000). Such replacements change the identity of the *Anolis* operating in the food web and may, depending upon the extent to which *A. sagrei* and *A. carolinensis* populations are functionally equivalent in food web processes, alter food web dynamics and structure.

Though *A. sagrei* and *A. carolinensis* are similar in both size and diet and likely occupy the same trophic position in food webs, their effects on other food web members may vary due to differences in their abundance or per-capita trophic interaction rates. *Anolis sagrei* attain higher population densities than the *A. carolinensis* they displace (2.1–2.7 times higher after only three years in Campbell's [2000] study), and considering the downstream food web effects of anoles at lesser densities (e.g., Spiller and Schoener 1994), the sheer increase in anole abundance following *A. sagrei* invasion likely impacts (at the least) anole prey taxa. Differences in the rates at which individual *A. sagrei* and *A. carolinensis* interact with predator and prey species could also generate invasion impacts; adult *A. sagrei*, for instance, are more robust than *A. carolinensis* (possessing over 33% more biomass for a given SVL) and may consume more or larger prey (Campbell 2000). Species- or ecomorph-specific differences in behavior, such as perching and foraging behavior, might also cause the two *Anolis* to interact with particular predator or prey species at differing per-capita rates.

I have investigated the relative downstream food web effects of *A. sagrei* and *A. carolinensis* at both the population and individual levels in order to assess, respectively, 1) the impacts of *A. sagrei* invasion on arthropod communities in habitats where *A. carolinensis* are effectively replaced, as well as indirect effects on folivory levels and avian communities that may result, and 2) whether differences in *Anolis* foraging and perching behavior contribute to these impacts. My unpublished work on a series of invaded and uninvaded islands indicates that, as expected, populations of these two *Anolis* species do not have equivalent effects on arthropod communities. Interestingly, in enclosure studies (also unpublished) I found that differences in the perching and foraging behavior of the two *Anolis* did not result in differing per-capita effects on particular arthropod prey taxa, suggesting that these behavioral differences contribute little to the downstream food web effects of *A. sagrei* invasion.

Here I present evidence that *Anolis* predators respond positively to *A. sagrei* displacement of *A. carolinensis*. One of the most common *Anolis* predators in relatively open natural and semi-natural Florida habitats is the diurnal snake *Coluber constrictor* (Black racer). Lizards – including *A. carolinensis* – are one of the largest components of racer diets in the southeastern U.S. (Hamilton and Pollack 1956, Fitch 1963, Halstead et al. 2008), so it seems reasonable to expect that racers would benefit from the displacement of *A. carolinensis* by a more abundant and lower-perching competitor. Racers are known to prey upon *A. sagrei* in Florida (Campbell and Echternacht 2003), but to my knowledge no data are available to assess *C.*

constrictor numerical or behavioral responses to *A. sagrei* invasion. In the course of conducting food web studies on a series of invaded and uninvaded islands in Florida, I observed racers exclusively on invaded islands. I document and analyze this pattern of occurrence as indirect evidence of a positive *C. constrictor* response to *A. sagrei* invasion.

Methods

Study System

Fig. 1. Typical spoils island used in the study (position #12). White bar at left represents 100 m. Digital orthophoto obtained from Florida's Land Boundary Information System.

The data I present come from a set of 34 similar dredge-spoils islands located west of the Intracoastal Waterway (ICW) in Mosquito Lagoon, Volusia and Brevard Counties, Florida. I selected these islands on the basis of shared habitat features from a chain of approximately 60 spoils islands paralleling the ICW through the lagoon; study islands were those possessing an open, xeric interior partially or completely ringed by an inner band of forest habitat and an outer band of seasonally-inundated marsh habitat (Fig. 1; see Campbell 2000 and Turnbough in prep. for more detailed habitat descriptions). For the purposes of my studies, I considered "islands" connected by a narrow strip of marsh habitat to be separate sampling units, equivalent to island pairs with marshes that closely approach but do not physically meet. Islands in the set range in size from 0.6 to 3.6 ha (mean 1.6 ha) and are similar in age (byproducts of ICW dredging in the 1950's), shape, and distance from the mainland.

Anolis sagrei have not yet invaded all of the spoils islands in Mosquito Lagoon, and they ranged from absent to extremely abundant on the study islands. I estimated *A. sagrei* abundance on each island in June 2006 by conducting four 10-minute, methodical visual searches for adult anoles between 0800 and 1025 h along different sections of the interior edge of the forest habitat. Based on my experiences with *A. sagrei* in a variety of mainland and island settings, I qualitatively classified *A. sagrei* abundances on islands as absent (pooled *A. sagrei* count = 0), low (pooled count 1–10), or high (pooled count > 10); for ease of reference, I term islands in these abundance classes "no-*sagrei*", "low-*sagrei*", and "high-*sagrei*" islands. I additionally placed one island (#33) into the low abundance class on the basis of *A. sagrei* individuals observed before and after the visual searches, though none were observed during the searches. *Anolis carolinensis* were observed on all study islands except one (#9), which also lacked *A. sagrei* and was excluded from all analyses. Since *A. sagrei* have been shown to simultaneously reach high densities and displace *A. carolinensis* on these spoils islands (Campbell 2000), I assume that *A. carolinensis* have been effectively replaced on high-*sagrei* islands and that displacement is in-progress on low-*sagrei* islands. I also make the assumption that *A. carolinensis* are and were at typical population densities on spoils islands prior to *A. sagrei* invasion.

Data assembly and analysis

I visited the study islands between May 2004 and August 2008 to collect a variety of data, and in that time span there were seven "trips" in which I both 1) expended equivalent sampling effort among visited islands, and 2) observed at least one *C. constrictor* (Table 1). I did not purposefully search for racers on any occasion, but simply recorded instances where I or my assistants encountered them during routine, study-related activities. Study activities and visit durations differed across trips but were similar within any given trip. I used the environmental conditions present when racers were observed to define a range of visit conditions that were at least minimally suitable for observing racers (temperatures greater than 22.0 C, sunny or partly sunny skies, time of day for summer visits 0800–1800 h), and I excluded from analysis all visits in which one or more conditions were outside the suitable range.

Table 1. Dates, purpose, number of islands visited, and visit statistics for data collection trips to spoils islands. Start time, duration, and mean temperature do not include data from excluded island visits and are reported as overall mean ± standard deviation and range.

Trip	Dates	Purpose	Islands visited[1]	Start time (h)	Duration (min)	Mean temperature (C)
MAY 2004	18/May/04– 08/Jun/04	Preliminary assessment of *A. sagrei* abundances	12 [16]	1211 ± 0229 0905–1735	31 ± 9.9 20–56	___ [2]
JUL 2004	07/Jul/04– 15/Jul/04	Bird point counts and behavioral observations	6 [6]	0701 ± 0004 0656–0708	128 ± 2.8 123–131	29.7 ± 1.0 29.0–31.5
JAN 2006	10/Jan/06– 13/Jan/06	Sweep net sampling of forest edges	11 [34]	1316 ± 0055 1215–1510	16 ± 8.0 5–30	24.2 ± 2.5 22.0–28.3
JUN 2006a	05/Jun/06– 01/Jul/06	Timed searches to estimate *A. sagrei* abundances	33 [34]	0902 ± 0033 0800–0946	37 ± 2.7 33–46	29.2 ± 1.4 25.8–31.9
JUN 2006b	05/Jun/06– 28/Jul/06	Island-wide sampling of arthropod communities	29 [34]	1158 ± 0159 [3] 0945–1730	131 ± 62 [3, 4] 10–230	30.5 ± 2.6 [3] 22.5–33.4
JAN 2007	04/Jan/07– 16/Jan/07	Bird point counts	13 [34]	0909 ± 0023 0843–0955	28 ± 2.9 26–37	23.5 ± 0.7 22.3–24.8
MAR 2007	09/Mar/07– 10/Mar/07	Bird point counts	3 [11]	0914 ± 0024 0847–0934	26 ± 8.4 16–31	23.1 ± 1.1 22.4–24.3

[1] # islands used in data analyses and [total # islands visited]; islands were visited once per trip except for JUN 2006b, in which they were visited 2–3 times

[2] Temperature data not collected on MAY 2004 trip

[3] SD and range are large because islands were visited 2–3 times on different days and at different times in the JUN 2006b trip, and for some islands 1–2 of those visits were excluded from the dataset due to unfavorable environmental conditions. For JUN 2006b, visit start time and mean temperature for each island are the weighted (by visit duration) means of visits remaining in the dataset.

[4] Visit durations for each island in the JUN 2006b trip are the sum of the durations of visits remaining in the dataset

I identified those islands for which racers were detected (observed on at least one trip) and tested the null hypothesis that they were distributed independently of *A. sagrei* abundance class using a Fisher's exact test (3 x 2 contingency table; *A. sagrei* = absent, low, high; racers = detected, not detected). To determine where the observed distribution departed from null expectations, I conducted separate Fisher's exact tests for all pairwise abundance-class combinations (2 x 2 contingency tables). These exact tests implicitly assume equal sampling effort among islands; since the number of trips made to islands varied, I assessed whether the total number of trips to islands differed across *A. sagrei* abundance classes or were greater for racer-detected islands using an ANOVA and a *t*-test, respectively. I also performed a two-way ANOVA with abundance class as a fixed effect and trip as a random effect to test whether the relative number of islands visited in each trip differed across abundance classes (excluded from this test were the two trips containing an unsampled abundance class). Within each trip, I used ANOVAs (or Kruskal-Wallis tests when normality assumptions were not met) to test the assumption that visit duration and conditions (mean time of day, mean insolation, mean temperature) were equivalent across abundance classes (*t*-tests used for JUL 2004 trip, no tests performed for MAR 2007 trip due to low sample sizes). Significant ANOVAs were followed with a Tukey multiple comparison test to determine which *A. sagrei* abundance classes differed. All statistical tests were two-tailed.

Results

I observed a total of twelve racers on nine different islands over the seven data collection trips (Table 2). These nine islands were not distributed independently of *A. sagrei* abundance class (exact test; $P = 0.006$); racers were never observed on no-*sagrei* islands (Fig. 2), significantly less than the null expectation (pairwise exact tests; absent–low $P = 0.014$; absent–high $P = 0.006$). Racer-detected islands were, however, distributed independently of abundance class among invaded islands (pairwise exact test; low–high $P = 1.00$).

With the exception of a single test, I found no significant differences in sampling effort or visit conditions among *A. sagrei* abundance classes. The total number of trips made to islands did not differ among abundance classes ($F_{2,30} = 0.07$, $P = 0.93$) or between racer-detected islands and racer-undetected islands ($t = -0.03$, df = 31, $P = 0.97$). Neither were there any significant differences among abundance classes in the duration, time of day, insolation level, or temperature of island visits within trips (21 independent tests, $P \geq 0.098$ for each test). In contrast, the relative number of islands visited within trips differed significantly among abundance classes ($F_{2,8} = 4.52$, $P = 0.0485$); although the Tukey post-hoc test failed to identify which abundance classes differed, inspection of the means revealed that, on average, more no-*sagrei* islands were visited per trip (mean 8.4) than were low-*sagrei* (mean 5.4) or high-*sagrei* (mean 5.8) islands.

Table 2. Racer observations by island and trip.

			Number of racers observed in trip[2]									
Island position[1]	Latitude, longitude (°)	*A. sagrei* abundance	MAY 2004	JUL 2004	JAN 2006	JUN 2006a	JUN 2006b	JAN 2007	MAR 2007	Racers observed?[3]	Number of trips	Proportion of trips racers observed
3	28.8516 N, 80.8227 W	High	1		–	2	0	0	–	+	4	0.50
4	28.8483 N, 80.8211 W	High	0		–	0	0	–		-	3	0
5	28.8445 N, 80.8184 W	High	–	0	–	0	–	–	–	-	2	0
6	28.8422 N, 80.8169 W	High	0	0	–	0	0	–	–	-	4	0
7	28.8407 N, 80.8160 W	High	1		–	0	0	–		+	3	0.33
13	28.7922 N, 80.7854 W	High	–		–	0	–	0	–	-	2	0
16	28.7828 N, 80.7806 W	High			1	0	0	–	–	+	3	0.33
17	28.7816 N, 80.7793 W	High			0	0	–	0		-	3	0
18	28.7810 N, 80.7785 W	High			–	1	0	–		+	2	0.50
29	28.7563 N, 80.7631 W	High	0	1	0	0	0	0		+	6	0.17
1	28.8571 N, 80.8268 W	Low	0		–	0	0	–	–	-	3	0
2	28.8554 N, 80.8255 W	Low	0		–	0	0	–		-	3	0
11	28.8092 N, 80.7964 W	Low	0		–	0	0	–	1	+	4	0.25
14	28.7884 N, 80.7832 W	Low			–	0	1	–	–	+	2	0.50
15	28.7865 N, 80.7825 W	Low			0	0	0	0		-	4	0
28	28.7589 N, 80.7647 W	Low	0		0	0	0	–		-	4	0
30	28.7535 N, 80.7603 W	Low			0	0	0	–		-	3	0
33	28.7477 N, 80.7543 W	Low			–	1	0	–		+	2	0.50
34	28.7465 N, 80.7526 W	Low			–	0	1	1		+	3	0.67
8	28.8336 N, 80.8120 W	Absent	0	0	–	0	0	0		-	5	0
9[4]	28.8303 N, 80.8103 W	Absent[4]	–		–	–	–	–	–	–[4]	–	–
10	28.8123 N, 80.7981 W	Absent	0		–	0	0	–		-	3	0
12	28.8071 N, 80.7948 W	Absent	0	0	–	0	0	–	0	-	5	0
19	28.7769 N, 80.7765 W	Absent			0	0	0	–		-	3	0
20	28.7752 N, 80.7753 W	Absent			–	0	0	–		-	2	0
21	28.7738 N, 80.7749 W	Absent			0	0	0	0	0	-	5	0
22	28.7724 N, 80.7736 W	Absent			0	0	0	0		-	4	0
23	28.7696 N, 80.7719 W	Absent			–	0	0	0		-	3	0
24	28.7681 N, 80.7712 W	Absent			–	0	0	0		-	3	0
25	28.7655 N, 80.7693 W	Absent			–	0	0	–		-	2	0
26	28.7626 N, 80.7674 W	Absent			–	0	–	–		-	1	0
27	28.7607 N, 80.7662 W	Absent			0	0	0	0		-	3	0
31	28.7523 N, 80.7592 W	Absent			0	0	0	0		-	4	0
32	28.7501 N, 80.7573 W	Absent	–	0	–	0	0	–		-	3	0

Table 2. (continued)

Footnotes:

[1] Islands are ranked by their position in the chain of selected islands, going from north to south

[2] Empty = not visited; dash (–) = racers were not observed and the trip visit was excluded from the dataset due to unfavorable environmental conditions

[3] + = observed on at least one trip; - = never observed

[4] Island #9 lacked both *A. sagrei* and *A. carolinensis* and was excluded from the dataset; racers were never observed on island #9

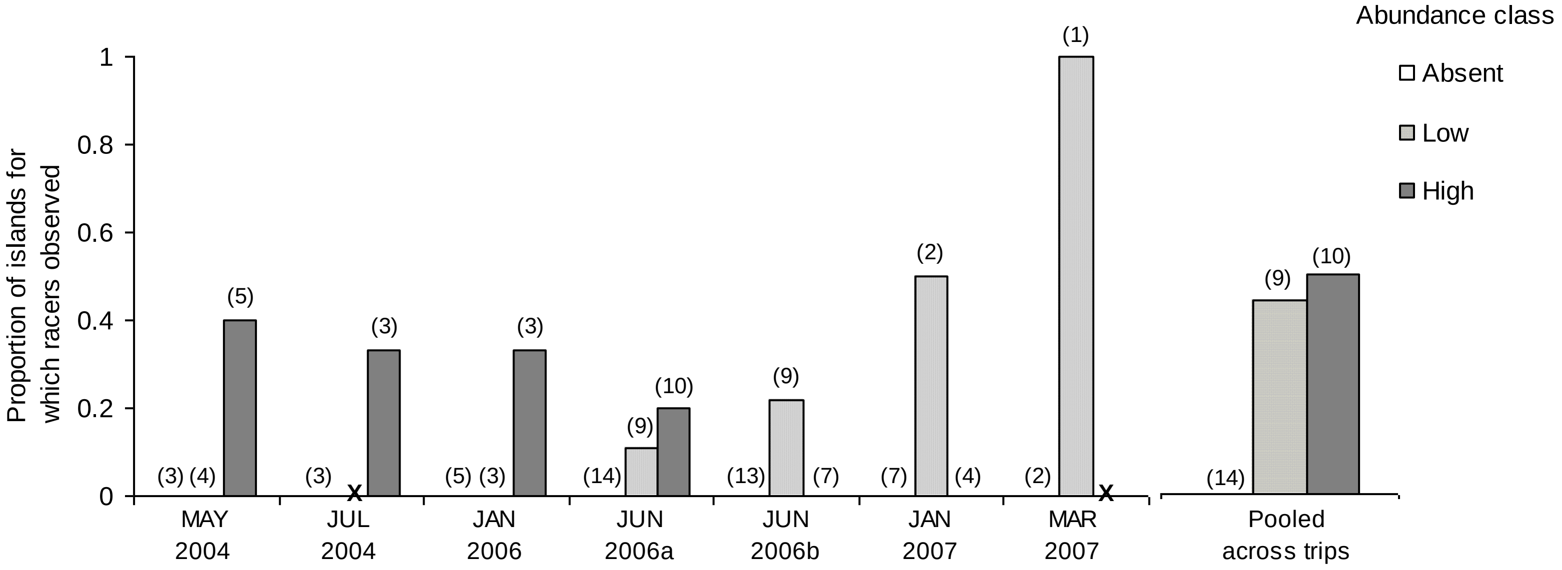

Fig. 2. Proportion of island visits yielding racer observations by trip and *Anolis sagrei* abundance class. Numbers above bars are sample sizes (number of islands visited); unsampled trip-by-abundance class combinations are noted by the symbol "**X**". Pooled-across-trips proportions reflect the number of islands for which racers were observed on at least one trip.

Discussion

I observed racers exclusively on invaded islands during the seven data collection trips, and statistical analysis indicated that this pattern could not be explained by chance. Other than the presence of *A. sagrei*, potential causes for the higher incidence of racer detection among invaded islands include 1) greater sampling effort on invaded islands or more favorable visit conditions, resulting in higher detection probabilities, or 2) a greater tendency for invaded islands to possess certain habitat or locality characteristics that favor higher racer densities, occupancy rates, or detection probabilities. I argue below that these other potential causes are unlikely to have been responsible for the observed distribution of racer detections.

Sampling effort and visit conditions varied among islands but were not biased with respect to *A. sagrei* abundance class or racer detection. Therefore it is highly unlikely that they were causally related to the higher incidence of racer observations on invaded islands. Of the 23 independent tests conducted to assess differences among *A. sagrei* abundance classes in sampling effort (total # of trips, # islands visited per trip, visit duration) or visit conditions (time of day, insolation, temperature), only one indicated a significant difference, the direction of which was conservative with respect to the pattern of racer detections (more no-*sagrei* islands visited per trip). If the low- and high-*sagrei* classes are pooled and the above comparisons are made between invaded and uninvaded islands, all 23 of the tests result in nonsignificant differences (results not shown). Moreover, these factors were similar among racer-detected and racer-undetected islands; no difference existed between the two groups in the number of trips made to islands, and the conditions present when racers were observed served as the basis for admitting all other island visits into the dataset.

Similarly, differences in island habitat characteristics or locality do not appear to have been responsible for the observed pattern of racer observations. I compared island characteristics across *A. sagrei* abundance classes using factor scores from four principal components analyses: one for general island characteristics (e.g., area, isolation, relative habitat composition), and one each for xeric, forest, and marsh habitat characteristics (e.g., vegetation composition and diversity, width of habitat band, total habitat area). I found no differences between the no-*sagrei* and low-*sagrei* classes, but high-*sagrei* islands differed significantly from the other two classes with respect to several factors; together these factors indicated that high-*sagrei* islands tended to be smaller and to have scrubbier, more open habitats (results not shown for the sake of brevity). Although scrubbier and more open habitats may be associated with higher racer densities, occupancy rates, or detection probabilities, the occurrence of these habitat features on high-*sagrei* islands is not sufficient to explain the distribution of racer detections in the dataset – it does not account for racer detections on low-*sagrei* islands. Furthermore, the entire spectrum of island types was represented among racer-detected islands, from relatively small and scrubby to relatively large and heavily forested. Racers were also observed across the range of island positions, from islands at the northern end of the chain to those at the extreme southern end; this fact, along with the lack of differences among abundance classes in distance from the mainland or isolation from neighboring islands, suggests that island locality was unimportant for the distribution of racers detections.

The evidence, though indirect, strongly suggests that the higher incidence of racer detection among invaded islands was caused by a positive racer response to the presence of *A. sagrei* prey. Relative to the mainland, the study islands are depauperate in the small vertebrate prey that typically comprise the bulk of racer diets (Campbell 2000, Turnbough unpubl. obs.); a net increase in prey abundance following *A. sagrei* invasion should, from a racer's perspective, increase the quality of the habitat, especially if the new prey are relatively easy to detect or capture. Racers primarily forage on the ground and in low vegetation (Fitch 1963, Bartlett and Bartlett 2003, Turnbough unpubl. obs.) and thus are probably more likely to encounter *A. sagrei* than *A. carolinensis*, all else being equal, because *A. sagrei* tend to perch lower in the vegetation and more frequently on the ground (Schoener 1968, Campbell 2000). As an anecdotal aside, on the island where I observed the greatest total number of racers (position #3, three racers in this study and one at another point in time), *A. sagrei* seemed to perch ~0.5–1.0 m higher in the vegetation than usual and were noticeably skittish and difficult to find.

Interestingly, the incidence of racer detection did not differ between high-*sagrei* and low-*sagrei* islands. It may be that *A. carolinensis* densities on low-*sagrei* islands were still relatively high, so that densities of the two *Anolis* combined matched the anole densities on high-*sagrei* islands and the two types had similar effects on racers. It is also possible that my *A. sagrei* counts did not accurately reflect densities, or that my classification of low and high abundances did not correspond in a meaningful way to the effects of increasing *A. sagrei* densities on racers. For example, if a racer's home range includes two neighboring, identical islands, then invasion and occupancy of one island by a relatively low-density *A. sagrei* population could cause the racer to forage almost exclusively on the invaded island; subsequent increases in *A. sagrei* density may thus have little impact on the racer's relative occupancy rates among the two islands.

Though my results indicated a positive response of racers to *A. sagrei* invasion, they cannot shed light on whether the nature of that response was behavioral or numerical (or a combination of both). Typical racer home range sizes are larger than the typical areas of these study islands (Fitch 1963), so it is quite possible that racers regularly swim between islands or between the islands and the mainland (on one occasion I observed a racer swimming unhurriedly across the shallow water separating two islands). If racers tend to remain on invaded islands longer than on uninvaded islands because of increased prey availability, then a behavioral response may be responsible for the higher incidence of racers on invaded islands. Alternatively, if greater prey densities on invaded islands support greater racer densities, it may be a numerical response that generates the pattern. Both of types of responses may be operating together, of course, to produce an observed effect.

In summary, the results of this study indicated that *C. constrictor* were more commonly observed on islands invaded by *A. sagrei*, and that this pattern most likely resulted from a positive racer response to higher prey abundances on invaded islands. I hope that these findings will encourage future research into the effects of *A. sagrei* invasion on *C. constrictor* and other *Anolis* predators in Florida.

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Phylogenetic analysis of the *latifrons* series of *Anolis* (Sauria: Iguania) with morphological data

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Anolis has been considered a classic example of adaptive radiation (Schluter 2000, Losos 2007, Losos 2009). The genus is distributed across the islands of the Antilles, as well as in Central America and northern South America. In South America, two major clades occur – the *latifrons* series or Dactyloa clade and the Norops clade– (Losos 2009). The *latifrons* series has been considered as a putative basal clade by several studies (Etheridge 1959, Savage & Guyer 1989, Williams 1989, Poe 2004, Nicholson et al. 2005). This series is composed of ~83 species and belongs to a large clade (the Alpha section) within the genus *Anolis*, and displays great morphological and ecological diversity (Pinto et al. 2008).

Etheridge (1959) diagnosed the *latifrons* series using two osteological characters: four or more parasternal rib chevrons attached to dorsal ribs, and lateral processes of the interclavicle diverging from proximal parts of the clavicle. Additional skeletal characteristics of this series include: large splenial; sculpturing in mandible absent; pineal foramen present at the fronto-parietal suture; parietal roof extending toward the posterior edge of occipital region, forming a trapezoid with the parietal crests and exposing the supraoccipital; free chevron parasternals absent; slivers parasternales present or absent; 23-24 presacral vertebrae (only three species with 23, including *jacare* and *transversalis*); 3-4 lumbar vertebrae; seven to eleven caudal vertebrae without autotomic septa (seven for *bonariensis, chloris, gorgonae, granuliceps* and *peraccae*; eight for *agassizi, fraseri, proboscis, solitarius, richardi, roquet, trinitatus* and *ventrimaculatus*; nine for *boettgeri, boulengeri, buckleyi, fasciatus, latifrons, punctatus* and *transversalis*; ten for *frenatus, insignis* and *microtus*; and eleven for *squamulatus*). Autotomic caudal vertebrae are present, but not fused in *richardi*, *trinitatus, roquet, bonariensis* and *agassizi*; other species lack functional caudal vertebra autotomy due to fusion of septa. Etheridge (1959) noted that the skeletal morphology of this series was highly variable relative to the rest of anoles.

Williams (1976a, b) attempted a comprehensive informal taxonomy of *Anolis* and defined several species groups, complexes, and superspecies within Etheridge's (1959) series.

His *latifrons* series included six species groups: *aequatorialis*, *laevis*, *latifrons*, *punctatus*, *roquet* and *tigrinus*. The genus *Phenacosaurus* was not included as it was assumed to be outside the *Anolis* clade. Although Williams did not list detailed diagnoses for each group, he clearly used external traits and some chromosomal information to delimit groups (Williams, 1976a).

Guyer & Savage (1986) provided the first numerical phylogenetic analysis of *Anolis* and used the resulting estimate to erect a formal taxonomy. Series or groups of series were elevated to genera and Williams' (1976a, b) species groups were recognized as series. Etheridge's (1959) *latifrons* series was called the genus *Dactyloa*. Cannatella & de Queiroz (1992) criticized this paper on analytical grounds, and Williams (1989) found fault with its data. Subsequent authors working on higher level phylogeny of anoles (Poe 1998, 2004; Jackman et al. 1999; Nicholson et al. 2005) did not recognize the Guyer and Savage genera and found additional problems with adopting their taxonomy (e.g., four of their five genera are not monophyletic, and 17 or more additional genera would need to be erected if the single genus that is monophyletic, *Norops*, is recognized).

The history of *Anolis* taxonomy can be confusing, and a paper such as this one must be clear about whether the "*latifrons*" group refers to Etheridge's (=Williams') *latifrons* series, Williams' *latifrons* species group, Guyer and Savage's *Dactyloa*, or one of these groups plus additional taxa or excluding certain taxa. In this paper we use "*latifrons* series" according to Etheridge's (1959) original intention and allowing for recent species descriptions and phylogenetic results. That is, we include all species referred to Etheridge/Williams' *latifrons* series and Guyer and Savage's *Dacytloa*, plus anoles formerly referred to *Phenacosaurus*, plus recently described species that were compared to species in these groups (e.g., *A. umbrivagus* Bernal-Carlo & Roze 2005).

Poe (1998, 2004) used morphological and molecular data to test the monophyly of several species groups within *Anoli*s, including groups within the *latifrons* series. Poe (2004) and Nicholson et al. (2005) provided weak evidence for the monophyly of the *latifrons* series and its sister position to the rest of *Anolis*. However taxonomic sampling in these studies was designed to establish phylogenetic relationships of larger clades of *Anolis*. Thus, the monophyly of species groups within the *latifrons* series was only weakly tested.

The aim of this study was estimate the phylogenetic relationships of the *latifrons* series using meristic, morphometric, coloration and osteological data. We included multiple representatives of all species groups, with a focus on the relatively understudied South American forms. We evaluated phylogenetic information of characters that Williams employed in his taxonomic accounts of *Anolis* species (see Williams et al. 1995).

Materials and Methods

We attempted to include multiple species from each species group of the *latifrons* series. Several species of *latifrons* series anoles are rare, with many known only from type material (e.g., *A. propinquus*), and material for several was thus unobtainable. We scored specimens of the following species groups (Table 1): *aequatorialis* (all species except recently described *A. kunalayae*, plus 3 undescribed species; 12 species total), *latifrons* (6 of 11 species), *punctatus* (12 of 21 species), *tigrinus* (2 of 6 species), *laevis* (1 of 3 species), "*Phenacosaurus*" (5 of 11 species). We chose three species as outgroups according to Frost et al.'s (2001) phylogeny: (*Basiliscus*, (*Polychrus*, *Anolis*)) (Frost et al. 2001). We used two species of *Polychrus* as the first outgroup (*P. gutturosus* and *P. marmoratus*) and one species of *Basiliscus* to root the tree.

We used many characters previously used in species descriptions or phylogenetic studies (e.g., Williams et al. 1995; Etheridge 1959, Etheridge & de Queiroz 1988, Frost & Etheridge 1989, Frost et al. 2001, Brandley & de Queiroz 2004, Poe 1998, 2004) and discovered additional characters during examination of specimens. In total we used 115 characters, 19 continuous and 96 discrete characters. Continuously-varying characters (e.g., loreal counts, ear size, snout length, etc) were analyzed with Thiele's (1993) method of "gap-weighting." This approach assigns ranks to continuous variables and weights the parsimony cost of character changes between states according to the magnitude of the difference between them. Thus, changes between more similar states have lower costs to parsimony. In this study, we used 25 states as the maximum possible (Wiens 1995).

Data were analyzed using parsimony with heuristic search strategies. We performed 100 searches with random taxon addition to obtain starting trees, holding between 30 trees per replicate, with each set of starting trees submitted to branch-swapping using the tree-bisection and reconnection (TBR) algorithm in PAUP (Swofford, 2002). Clade support was evaluated with a decay index using TreeRot (Sorenson 1996).

Results

The analysis with Thiele's method produced four optimal cladograms of 2475 steps (CI= 0.25; RI= 0.51) (Fig. 1). The strict consensus tree exhibits great resolution, but many clades show weak support (Bremer 1988, 1994; Fig. 1). The support values for the recovered clades are relatively lower in Goloboff et al.'s method than Thiele's method (see Fig. 1).

The most parsimonious cladogram recovered *Anolis williamsmittermeierorum*, a phenacosaur anole, as the most basal species of the series (Fig. 1). *Anolis agassizi* is nested in the base of the tree. Three major groupings were recovered in this topology (Fig. 1). The first clade is composed of some species from the *punctatus* group plus the remaining phenacosaur anoles (node 56, Bremer support 4, Fig. 1). Almost all species in the *punctatus* group included in this clade and the phenacosaur anoles have an Andean distribution except *A. transversalis*, which occurs in Amazonian lowlands. A second clade is composed of species from the *latifrons* and *aequatorialis* groups (node 85, Bremer support 4, Fig. 1). These species are distributed in the lowlands of the Chocó region (e.g., *A. mirus*), the Panamanian lowlands (e.g., *A. kunalayae*) and the Andean region (e.g., *A. fraseri*, *A. danieli* "Palmar," and *A. apollinaris*). Finally a third clade is composed of species commonly referred to the *punctatus*, *tigrinus*, *aequatorialis*, and *latifrons* groups (node 81, Bremer support 2, Fig. 1). These species are distributed in the lowlands of the Chocó region (e.g., *A. chocorum*, *A. anchicayae*, *A. peraccae*, *A. gorgonae*, *A. chloris*, *A. latifrons*, *A. frenatus*, and *A. princeps*); the western Andes (e.g., *A. aequatorialis*, *A. gemmosus*, and *A. ventrimaculatus*) and some species occur in the eastern Andes (e.g., *A. ruizi*) and the Sierra Nevada of Santa Marta (e.g., *A. solitarius*).

Table 1. Composition of species groups within the *latifrons* series of *Anolis* (Williams 1976b). Species in bold were used in the phylogenetic analysis of this paper.

Species group	Geographic distribution	Species composition
aequatorialis (9 spp.)	Western Andes of Colombia and Western and Eastern Andes of Ecuador	***aequatorialis, anoriensis***[1], ***antioquiae, eulaemus, fitchi***, ***kunalayae***, ***maculigula***, ***megalopithecus***, ***mirus***, ***otongae***[2], ***parilis***, ***podocarpus***[3], ***ventrimaculatus***
laevis (3)	Western lowlands of Ecuador, eastern of Perú and Amazonia of Brazil	*laevis*, *phyllorhinus*, ***proboscis***
latifrons (12 spp.)	From Costa Rica to Venezuela and western of Ecuador	***apollinaris***, *casildae*, ***danieli***[4], ***fraseri, frenatus***, *insignis*, ***latifrons***, *microtus*, ***princeps***, *pronpiquus*, ***purpurescens***, *squamulatus*
punctatus (23 spp.)	From Costa Rica to western lowlands of Ecuador, northwestern of Venezuela, and northeastern coastal forest of Brazil	***anchicayae***, *boettgeri*, ***calimae***, *caquetae*, ***chloris, chocorum***, *cuscoensis, deltae, dissimilis*, ***fasciatus, festae***, ***gemmosus***, ***huilae***, ***jacare***, *nigrolineatus, nigropunctatus*, ***peraccae***, *philopunctatus*, ***punctatus***, *santamartae, soinii*, ***transversalis***, *vaupesianus*
roquet (9 spp.)	Lesser Antilles, Blanquilla and Bonaire islands	*aeneus, blanquillanus, bonarensis, extrema, griseus, luciae, richardi, roquet, trinitatus.*
tigrinus (9 spp.)	North of South America and southeaster coastal forest of Brazil	*lamari, menta, nasofrontalis, paravertebralis, pseudotigrinus*, ***ruizi***, ***solitarius***, *tigrinus, umbrivagus*
Inserta sedis (1 sp.)	Gorgona Island, Colombia	***gorgonae***
Inserta sedis (1 sp.)	Malpelo Island, Colombia	***agassizi***
Phenacosaur anoles	Andes (Venezuela, Colombia, Ecuador)	*bellipeniculus, carlostoddi, euskalerriari*, ***heterodermus***, ***inderenae***, *neblilinus*, ***nicefori***, ***orcesi***, *tetarii*, ***vanzolini***, ***williamsmittermeierorum***

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[1] A new anole species similar to *A. eulaemus* from Northern Andes of Colombia (Manuscript submitted to Herpetological Journal)

[2] A new anole species similar to *A. gemmosus* from Northern Andes of Ecuador (Manuscript submitted to Zootaxa)

[3] A new anole species similar to *A. fitchi* from Eastern Andes of Ecuador (Fernando Ayala, QCAZ).

[4] We included two putative populations of this species, *A. danieli* Palmar and *A. danieli* Anorí, because of the morphological differences among them and with the type series of *A. danieli*.

Fig. 1. Strict consensus of four most parsimonious cladograms of *Anolis latifrons* series obtained using Thiele's method for coding continuous characters. The numbers below the nodes correspond to Bremer support values (left) and clade numbers (right).

This study is the first to explicitly evaluate phylogenetic relationships within one of the most basal clades of *Anolis* - the *Anolis latifrons* series, or Dactyloa clade (Etheridge 1959, Williams 1976b, Guyer & Savage 1986, Poe 2004, Nicholson et al. 2005). Although this work was done principally using morphological characters (and some coloration characters) it represents the first step towards the combination of this dataset with data for higher level phylogenies of anoles (Poe 2004, Nicholson et al. 2005). In this paper we have demonstrated that all of the phenetic species groups of the *Anolis latifrons* series are non-monophyletic and therefore do not represent historical lineages. Williams (1976) proposed six species groups within the *latifrons* series, plus the phenacosaur clade (Table 1). The *aequatorialis, latifrons, punctatus,* and *tigrinus* groups were remarkably non-monophyletic. The phenacosur anoles were polyphyletic although the position of *Anolis williamsmittermeierorum* was probably due to long branch attraction (see above). Most likely, the inclusion of more taxa for some poorly sampled species groups will improve the relationships within the *Anolis latifrons* series. For instance, for the *tigrinus* group, we only included two representatives (*A. ruizi* and *A. solitarius*). Also, we were unable to test the monophyly of the *laevis* group because we only included one species in our analysis – *A. proboscis*. The *laevis* group was considered monophyletic due to the presence of a nasal appendage projecting forward from the rostrum. However, Williams (1979) considered that the distinction between *A. proboscis* and *A. punctatus* on the basis of this characteristic could be artificial because a slight swelling is present in the rostral scale in males of *A. punctatus*. In this work, we found that *A. proboscis* is nested within a group of phenacosaur anoles (Fig. 1).

Our analysis suggests that phenotypic characters used by Williams (1976) to define species groups were in some cases not phylogenetically informative. In this paper, we have tried to use all diagnostic characters used by Williams (e.g., body size, head size, tail size, scale counts, color pattern, etc.) in a cladistic way to test the monophyly of *Anolis latifrons* species groups. However, individual morphological characters by themselves provide little support for accurate relationships between species in *Anolis latifrons* series. In this way, a combination of these morphological characters with molecular sequence data will provide a phylogenetic tree with higher resolution and greater support.

Although the *latifrons* series was defined by Etheridge (1959) on the basis of two osteological characters (four parasternal chevrons attached to dorsal ribs and lateral process of the interclavicle diverging from proximal parts of the clavicle) these characters are not synapomorphies of the *latifrons* series. The species included in this study showed a great variation in these characters. Some others characters described by Etheridge (1959) exhibited a great variation in the *latifrons* series. For instance, presacral, lumbar and caudal vertebrae exhibit greater variation than reported by Etheridge (1959) and Poe (2004). Guyer & Savage (1986) considered the *latifrons* series as a valid genus (*Dactyloa*) without inclusion of phenacosaur species. However, Canatella & de Queiroz (1989) and Williams (1989) reviewed their data and they did not find support for their conclusions. Poe (1998, 2004) found evidence justifying inclusion of phenacosaur anoles within *Anolis*, and in particular within the *latifrons* series. This paper found that phenacosaur anoles are nested in the *latifrons* series with the exception of *A. williamsmittermeierorum*, which is nested in the base of the tree, probably as a consequence of long-branch attraction (Fig. 1) (see Poe 1998, 2003).

The phenacosaur anole group was divided into three groups by Barros et al. (1996): the *heterodermus* group, the *orcesi* group, and the *neblilinus* group. The *heterodermus* group has two subgroupings: the first one, the *heterodermus* subgroup, includes the large species *A. heterodermus, A. inderenae*, and *A. vanzolini*; the second is called the *nicefori* subgroup, and includes *A. nicefori* and *A. tetarii*. The *orcesi* group includes *A. orcesi*, *A. euskalerriari*, and the recently described *A. williamsmmittermeierorum*. Finally, the *neblilinus* group contains *A. neblilinus*, *A. carlostoddi*, and *A. bellipeniculus*, which was considered to be very close to *A. neblilinus* (Myers & Donnelly 1996). Our results show some consistency with these previous taxonomic arrangements (Fig. 1). *Anolis orcesi* and *A. nicefori* are a sister group of the *heterodermus* clade which is formed by *A. heterodermus*, *A. inderenae* and *A. vanzolini* (Fig. 1) plus *A. proboscis*.

The position of *A. calimae*, a species endemic to the cloud forests of the Western Andes of Colombia, deserves an additional comment here (Figs. 1 and 2). Ayala et al. (1983) described *A. calimae* from six specimens and they assigned it to the *punctatus* group superficially (sensu Williams 1976b). *Anolis calimae* exhibits several characters that those authors considered to be unique to this species, such as a superciliar margin composed of granular scales or a short superciliar scale followed by granular scales. However, some species referred formerly to *Phenacosaurus* exhibit granular superciliar scales (e.g., *A. tetarii*). Presence of the dewlap in both sexes in *A. calimae* was considered to be a primitive condition but not unique to species of the

Fig. 2. *Anolis calimae* Ayala et al. 1983. Collected recently in the Western Andes of Colombia.

punctatus group (e.g., *A. tetarii* exhibits a dewlap in both sexes). Another character that these authors interpreted equivocally to the assignment of this species to the *punctatus* group was the absence of interparietal scale. In all species examined that belong to the *punctatus* group, an interparietal scale is present. In several *Anolis calimae* specimens examined, the interparietal is deductible by size, position and shape, but the pineal eye is absent. Although the pineal eye is absent in other species, the interparietal scale was deductible by size and shape (e.g., *A. vanzolini* see Williams et al. 1996). The re-evaluation of these characters in a cladistic context and inclusion of continuous characters in the analysis placed *A. calimae* as the sister species of all phenacosaur anoles except *A. williamsmittermeierorum* (Fig. 1, node 53).

In our analysis, we considered *Anolis danieli* as a composite species containing at least two species (Fig. 3). We have checked specimens of two localities that correspond closely to *A. danieli* (San Jose del Palmar, Valle del Cauca, Colombia – referred as *A. danieli* Palmar; and Anorí, Antioquia, Colombia –referred as *Anolis danieli* Anorí). We made comparisons between our specimens and type material of *A. danieli* and we were unable to assign any of these populations with absolute confidence to *A. danieli*. The principal differences among our specimens are color and head scales, and for that reason we considered these populations as two putative different species. However, considerate will be necessary to conduct additional analyses to establish the correct taxonomic affinities of the specimens from both localities.

Fig. 3. Specimens tentatively assigned to *Anolis danieli* from Colombia. A) *A. danieli* Palmar (photo Julián Velasco); B) *Anolis danieli* Anorí (photo Rosario Castañeda).

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Inferring factors of lineage differentiation in widespread *Anolis* species

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Recently I joined Jonathan Losos' lab for a three-year postdoc project. This meant a transition in two ways for me: First, having mostly worked with amphibians before, I hopped up a step on the evolutionary scale (as Jonathan pointed out). Second, I changed the level of inference from phylogenetic comparative methods to inferring within-species differentiation. In switching, I in part hoped to make my work less prone to the argument that whatever pattern I find was the outcome of post-speciation processes (although I anticipate that from now on, I will hear that lineage diversification need not end in speciation).

During my project I plan to infer the effects of various abiotic and biotic factors (such as geomorphological structure, or interactions with more or less closely related species) on lineage differentiation. Although this may sound trivial, patterns and processes of lineage diversification are still challenging to study – but who reading the *Anolis* Newsletter would disagree that anoles provide a fabulous system in which to study these processes.

Although I initially planned to sample a widespread anole species in Honduras, the recent political turmoil there has led me to develop a "Plan B." Luckily, I found almost the same situation and setting as I proposed for "Plan A" on Hispaniola with the cybotoid trunk-ground anoles. Pioneering work by Rich Glor et al. (2003) found the widespread species *Anolis cybotes* to exhibit high genetic differentiation among populations; several highly differentiated clades exist which exhibit sharp geographical boundaries. In their phylogeny, clades from the southern paleoisland of Hispaniola seem to be monophyletic with respect to clades from the northern paleoisland.

Thinking about this, I wondered

**Is genetic differentiation associated with morphological, micro or macrohabitat shifts?*

**When anoles occur in sympatry with other species of the same ecomorph, do genetic structure, morphology & ecology (micro & macrohabitat) shift in comparison to allopatric sites? If so, how?*

I spent October 2009 on a reconnaissance trip, travelling the Dominican Republic to sample clade boundaries between northern and southern DNA clades. I sampled about 250 waypoints on transects between known clade boundaries (Glor et al., 2003), roughly following the political borders of the country and cutting through the eastern part of the island, aiming for

5-kilometer intervals between sites. I collected specimens, took tissue samples, recorded dewlap reflectance, and measured ecological parameters of habitat use (if only sporadically due to time constraints). Furthermore I identified sites where *Anolis cybotes* co-occurs syntopically with other cybotoid anole species. A very preliminary screening of the sporadically recorded ecological data from this trip showed that some clades clearly seem to be allopatric, while others seem sympatric (pending genetic identification). Interestingly, habitat use of *Anolis cybotes* differs between clade localities; in total, 66% of lizard observations were made on trunks and poles, 14% on logs, ground and the leaf litter, and 8% of observations were made on rock faces (the latter was a bit unexpected but quite exciting).

Between all transects (each of which contains a clade contact zone), I found perch height (Kruskal-Wallis ANOVA KW-H=17.8; $p = 0.0032$), percent humidity (KW-H = 45.0; $p < 0.0001$), and air temperature (KW-H = 12.2; $p = 0.03$) to vary significantly. This reflects the highly structured environment in the large area that is occupied by *Anolis cybotes* and could influence clade differentiation.

I found lizard SVL (KW-H = 13.0; $p = 0.02$), perch diameter (KW-H = 18.1; $p = 0.003$, and relative perch height (although only a trend, KW-H = 9. 5; $p = 0.09$) to vary among transects which reflects the strong genetic differentiation among *cybotes* clades.

Within one of these transects in the eastern Dominican Republic containing a clade contact zone, a preliminary comparison of sampling localities containing specimens from one clade (clade O of Glor et al., 2003) sampled on the Samana Peninsula and Los Haitises National Park and localities containing both specimens of its sister clade (P, from Glor et al., 2003) and a contact zone between OP, there was a significant difference between these localities in % humidity (Mann Whitney U test $U=1.0$, $Z= 3.9$ $p < 0.0001$). Such differences in microclimate on the Samana Peninsula and Los Haitises National Park could explain the prevalence of a different lineage (O) of *Anolis cybotes* here versus on the surrounding part of the island (P, and the contact zone), although this requires more detailed, long-term study. However, large scale GIS maps do not show any apparent zonation in either altitude (no differences > 200m) or vegetation (all Hispaniolan broadleaf forests) - although in this case, biogeographical history of the region needs to be considered as well.

However, to conduct a more detailed analysis I need to assign specimens conclusively to genetic clades (using mitochondrial and nuclear markers, and microsatellites), and reanalyze the ecological data on this basis. Dewlap size and colour seem to vary a lot among localities, as does cranial morphology, so detailed morphometric analyses may yield interesting results in the future.

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