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Anolis Newsletter VII

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Anolis Newsletter VII

Edited by
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Front cover: The established *Anolis* community of Miami, FL. From top; Jamaican giant anole (*Anolis garmani*), Cuban knight anole (*A. equestris*), [right] Hispaniolan bark anole (*A. distichus*), [left] American green anoles (*A. carolinensis*), Puerto Rican crested anole (*A. cristatellus*), Cuban brown anole (*A. sagrei*). Illustration generously provided by Gabriel Ugueto, Gabriel is a scientific illustrator and paleoartist whose work is second to none; contact him for commissions! www.GabrielUgueto.com

The attendees of the 7th *Anolis* Symposium.
Fairchild Tropical Botanic Gardens, Miami FL.
17-18th March 2018.

Preface

It had been nearly a decade since the previous *Anolis* symposium was held in Cambridge, MA, at the Museum for Comparative Zoology, Harvard. A reunion of anole biologists en masse was long past due and it was decided that this symposium would be slightly different – we were going to hold it somewhere with anoles! And so, on the weekend of 17-18th March, 2018, nearly 70 anole biologists traveled to sunny south Florida to attend the 7th *Anolis* Symposium held at the beautiful Fairchild Tropical Botanic Gardens in Miami. In the grounds of the botanical gardens, attendees were presented with a diverse community of six (!) species of anole, both native and non-native, representing four distinct ecomorphs.
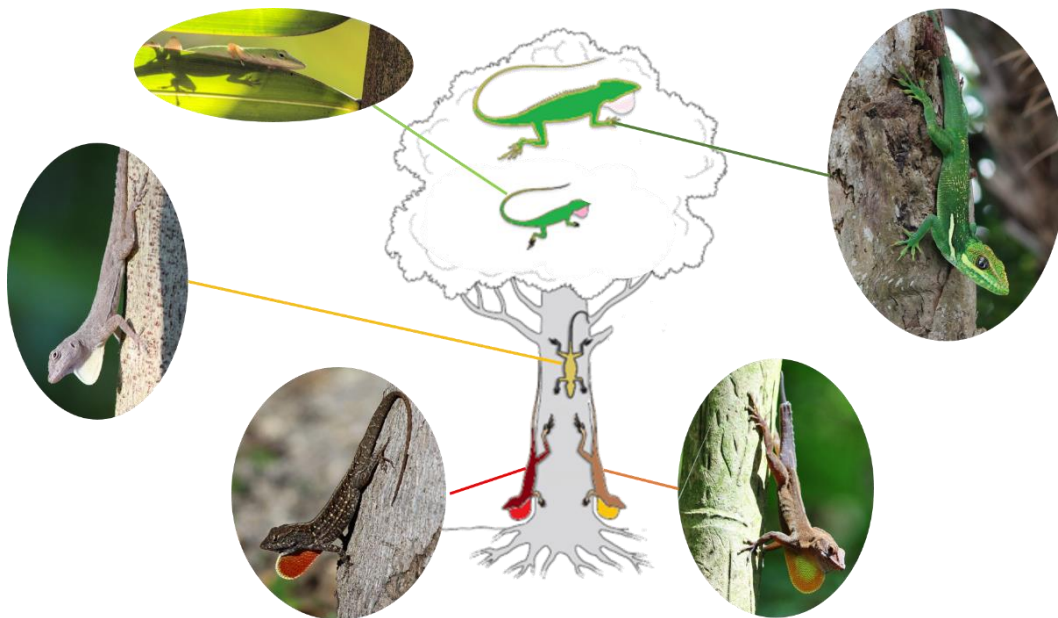
The anole community of Fairchild Tropical Botanic Gardens, clockwise from top right; Cuban knight anoles (*Anolis equestris*; Crown-Giant), Puerto Rican crested anoles (*A. cristatellus*; Trunk-Ground), Cuban brown anoles (*A. sagrei*; Trunk-Ground), Hispaniolan bark anoles (*A. distichus*; Trunk), and – the sole native – American green anole (*A. carolinensis*; Trunk-Crown).
Photos: James Stroud.

The early Spring timing of the symposium was chosen for two reasons, (i) Miami's famous, yet unforgiving, heat is particularly sweltering in the Summer period when conferences and symposia are usually held, but more importantly, (ii) March marks the commencement of the anole reproductive season in Miami! Attendees gathered just as anoles were jostling for societal positions in the early Spring sunshine and were rewarded with a plethora of showy dewlap displays and behavioral interactions. As true royalty passed within earshot[1], attendees were

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[1]At one point on Saturday, all attendees were locked inside the symposium room while personnel from both the US and Japanese Secret Service escorted the Crown Prince of Japan on a private tour of the garden…. seriously!

regaled with 34 research presentations spanning a broad range of topics on anole biology, from insightful natural history to the latest advances in genome editing.

So, what's changed in anole biology since the 6th symposium in 2009? Well, the field continues to grow at an explosive rate! Web of Science returns 1,345 "*Anolis*" articles from 2009-2018. Most notably, a lot of recent research has focused on how human stressors, such as urbanization and contemporary climate change, are affecting the lives of anoles. Similarly, the human-assisted dispersal of anoles to areas outside of their native ranges continues to yield exciting developments in ecology and evolution. Anole invasion biology led the charge in uncovering evidence for rapid evolutionary change driven by species interactions, after the introduction of brown anoles (*A. sagrei*) to spoil islands in central Florida drove native green anoles (*A. carolinensis*) to evolve larger toepads in response to an increased arboreal lifestyle; findings first presented in *AN VI*. Back in 2009 we were also excited to announce that the newest direction of anole biology was upon us; the sequencing of the *Anolis carolinensis* genome. Now, a decade later, three more species have faced the same fate, with many more underway! These accessions will continue to fuel a swathe of studies of anoles and further cement their position as a model system in ecology and evolution.

There are also now many new anoles to enjoy! A recent study, providing the most comprehensive review of *Anolis* systematics to date, put the number of species at 379, a number which might yet change as anole systematics continues to be a hotly debated topic.

While technological advances are expanding the breadth of research on anoles, we continue to echo the previous newsletter in saying that some of the most exciting results still stem from observations of basic natural history. Who knew that natural selection can act on behavior (see contribution from Lapiedra)? Or, that anole eggs hatch early when they're tickled (see contribution from Doody)? Just how important is it to still study anole behavior (see contribution from Johnson)? And what of those remarkable diving anoles, able stay submerged for up to 15 minutes while appearing to re-circulate oxygen from air bubbles attached to their nostrils (see contribution from Swierk)?

As with previous editions, we reiterate the underlying ethos of the *Anolis* Newsletter, "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."

James T. Stroud, Anthony J. Geneva, and Jonathan B. Losos
Washington University
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January 31, 2019

Fairchild's anole (*Anolis fairchildi*)

Hosting the *Anolis* meeting at Fairchild Tropical Botanic Garden's had an added special significance to anole biologists. On a 1930 expedition to the tiny island of Cay Sal, a small remnant from a once-larger Cay Sal Bank that sits in the ocean passage between Cuba and the southern tip of Florida, malacologist Paul Bartsch came across two anoles; the ubiquitous brown anole (*A. sagrei*) and a curiously large green anole, found to sport white speckling along its flanks. Bartsch collected specimens and passed them on to the famous Caribbean herpetologist, Thomas Barbour. Upon investigating the specimens, Barbour noted that differences in dorsal and temporal scales separated this island population from two morphologically similar species; the Cuban green anole (*A. porcatus*) and the Bahamian green anole (*A. smaragdinus*). In 1935, Barbour, along with a fellow herpetologist Benjamin Shreve, published their description of this curious new lizard. The authors chose to patronize the species *Anolis fairchildi*, in honor of their good friend, expedition companion, and occasional financier, David Fairchild. Aboard a legendary research vessel owned by Allison Armour, the 1315-ton steamer *Utowana*, Barbour and Fairchild, himself a prolific botanist, had explored the flora and fauna of the Caribbean throughout much of the early 20th century.

Fairchild's anole (*Anolis fairchildi*). Cay Sal, 30 May 2012. Courtesy of Michael Sorenson.

And so, by hosting the VII *Anolis* Symposium at the Fairchild Tropical Botanical Garden, the former private botanical collection of David Fairchild – including many original collections from his Caribbean expeditions, we provided a 21st century update to the ongoing *Anolis*- Fairchild relationship. We would like to thank the current Director of Fairchild's Gardens, Dr. Carl Lewis, for his enthusiastic support of both *Anolis* research in the gardens themselves, and for providing such an exciting and historic venue for the VII *Anolis* Symposium.

Graham Reynolds provides a charming and more detailed account of the history of Fairchild's anole on *Anole Annals* and in his recent *Breviora* paper, in which he and colleagues document their own exploration of Cay Sal and, for the first time, explore the genetic relationship of *A. fairchildi* to the rest of the *carolinensis* green anole clade.

http://www.anoleannals.org/2018/06/24/anole-outpost-the-cay-sal-bank-part-ii/

Reynolds, R.G., Puente-Rolón, A.R., Castle, A.L., Van De Schoot, M. and Geneva, A.J., 2018. Herpetofauna of Cay Sal Bank, Bahamas and Phylogenetic Relationships of *Anolis fairchildi*, *Anolis sagrei*, and *Tropidophis curtus* from the Region. *Breviora*, *560*(1), pp.1-19.

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Ectoparasite-host relationships: a case study of *Anolis* lizard ecomorphs on southeastern Cuba

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Ectoparasites often utilize different portions of a host's body as potential microhabitats (Carvalho et al., 2006; Rocha et al., 2008; Conover et al., 2015). The ectoparasite fauna of Cuban lizards has been little studied, with the parasite-host relationships of only twelve lizard species having been described (Rodriguez-Schettino, 1999 and literature therein; Daniel and Stekolnikov, 2004). On eastern Cuba, ectoparasite infections have been reported from isolated lizard populations that inhabit coastal areas (Daniel and Stekolnikov, op. cit.). In our study, we investigated three questions regarding parasite-host relationships: 1) Does the corporal infestation pattern on anole lizards differ between different ecomorphs? 2) Does parasite infestation represent opportunism or preference for a particular host? 3) Does ectoparasite infestation induce any immunological or behavioral responses in the host? To address these questions, we collected data on parasite-ecomorph relationships, infestation intensity, and abundance per body region between different anole ecomorphs.

Fieldwork observations started in May 2017 and continued for several years around the southeastern Cuban coast in Marea del Portillo (Granma province), Siboney (Santiago de Cuba province) and Baitiquiri (Guantanamo province). All microhabitats along the coastline were similar in climate conditions (~30ºC, 749 mm rainfall annually) with coastal and pre-coastal xeromorphic scrub, semideciduous microphyll forest, rocky coastal vegetation complex and semidesertic thorny bush. We first included eight anoles species from different ecomorphs CG (1), TC (1), TG (3) and three unique species (no ecomorph assigned) [Fig. 1]. The bodies of lizards were divided into nine morphological regions following previous sites of mite infestation documented on squamate lizards (Carvalho et. al., 2006; Rocha et. al., 2008; Conover et. al., 2015).

Although this study has just begun, we have already quantified three species of trombiculid mites on anoles we captured (*Eutrombicula anguliscuta*, *Eutrombicula leiocephali* and *Eutrombicula* sp.) [Fig. 2A]. After carefully inspection on wild-caught individuals, we never recorded any mites on the ventral or dorsal regions of the body. The most frequent infestation area on the body was the ear opening (E) and dewlap area (DA) [Fig. 2B]. Body infestation did

not shown differences in laterality for almost any variables except DA (Fig. 2C), for which a larger mite infestation was found on the left side of the dewlap relative to the right side.

   As we collect data from additional coastal localities on southeastern Cuba, we expect to continue to reveal more details regarding the relationship between ectoparasites and their hosts. These data will be used to further address the questions outlined above.
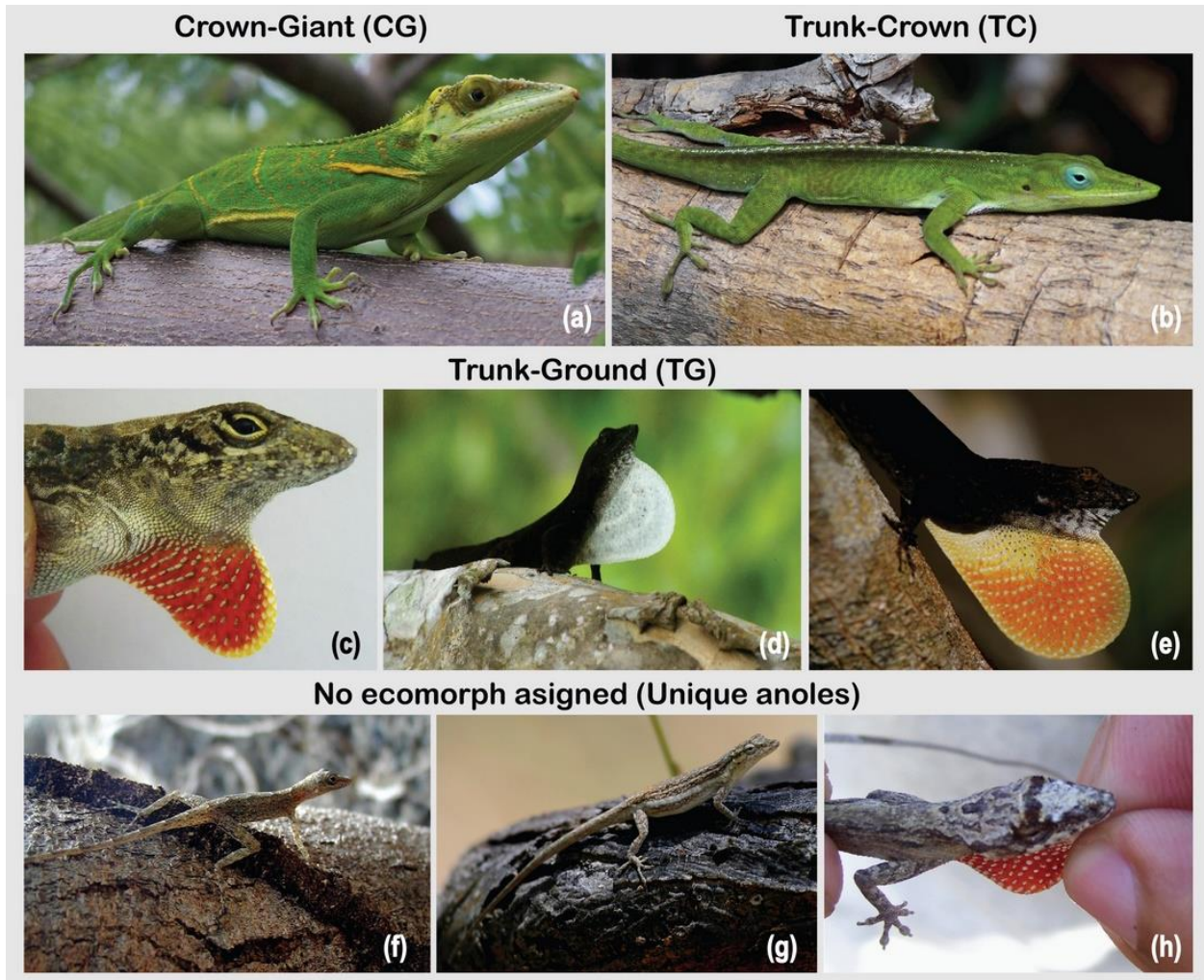
Fig. 1 – Anole species included in this study on southeastern Cuba. *Anolis smallwoodi* (a), *Anolis porcatus* (b), *Anolis sagrei* (c), *Anolis homolechis* (d), *Anolis jubar* (e), *Anolis argenteolus* (f), *Anolis litoralis* (g) and *Anolis ruibali* (h).
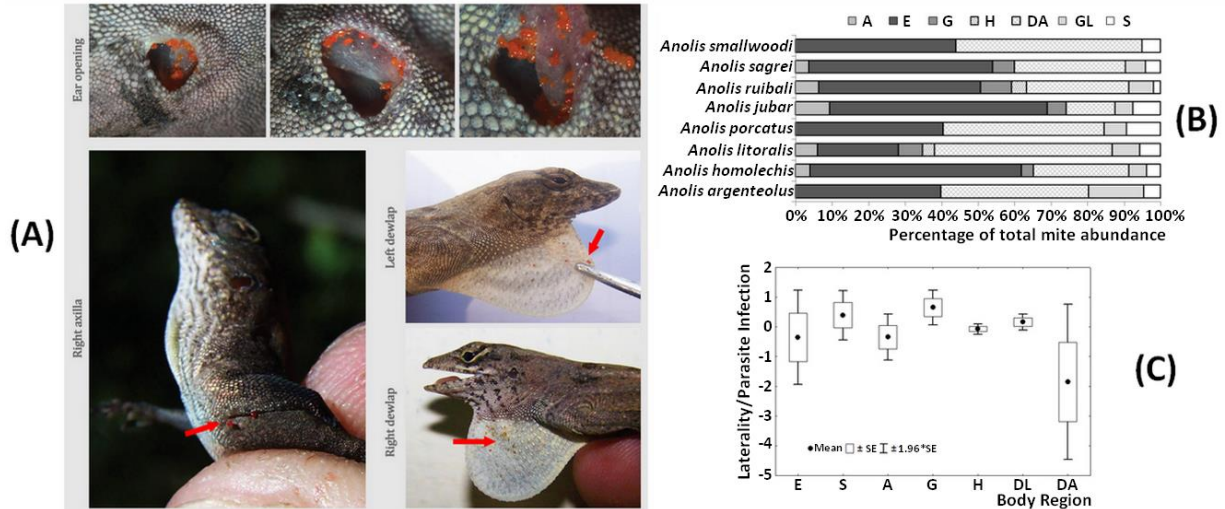
Fig. 2 – (A): Different body regions of anole lizards infected by mites. (B): Percentage of infection per body region and species. (C): Analysis of laterality between right and left side of the body. A (axilla), E (ear opening), G (groin), H (hind-leg), DA (dewlap area), GL (gular region), S (shoulder), DL (dorsolateral area). GL was only considered on females except for *Anolis smallwoodi* where both sexes have a large dewlap area.

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The bizarre false-chameleons (clade Chamaeleolis, *Anolis*) from Cuba

Cuba and Hispaniola are characterized by the presence of "unique anoles" not found on any of the other Antillean islands (Losos 2009, Mahler et. al. 2016). Cuban twig–giant anoles from the "Chamaeleolis" clade (Poe et al. 2017) have been of interest to anole biologists due their highly derived morphology, aberrant way of life, and extreme camouflage (Fig. 1). Moreover, these species are poorly understood; very few papers regarding their taxonomy, evolution, and ecology have been published (Rodríguez-Schettino 1999 and literature therein; Losos 2009; Mahler et. al. 2016; Cádiz et. al. 2018).

However, over the past three decades, multiple researchers have gathered enough specimens and tissue samples to investigate the species delimitation, biogeography, and evolution within this understudied group of anoles. Anoles of the Chamaeleolis clade have been historically diagnosed based on few morphological characters and the most recent description (*A. sierramaestrae*) lacks proper diagnosis.

We are recognizing species based on the evolutionary species concept, utilizing quantitative analyses on large morphological datasets in combination with molecular phylogenetic analysis (mtDNA, nDNA) of several populations for each described taxon along the Cuban archipelago. We are currently testing several hypotheses involving potential adaptive radiation scenarios in the Cuban archipelago by considering the Cuban paleo–island and current terrain accidents. We are also analyzing the dentition morphology between species and clades using high-resolution X-ray micro-computed tomography (Fig. 2) to better understand the evolution of heterodonty within the group.

Recently, Prötzel et al. (2017) reported that true chameleon species (*Calumma* spp.) have bony tubercles on the skull that are visible through their scales and fluoresce under UV light. After examining Cuban false-chameleons under UV light, we have identified similar fluorescent tubercles associated with hyperossified regions of the skull (Fig. 3). We will need to review this finding more thoroughly to determine if there is any correlation between the fluorescent

tubercles, aberrant lifestyle, and extreme camouflage present in these two highly divergent lineages of lizards.

Fig. 1 – (a) *Anolis chamaeleonides* (perching on a branch, note the similar coloration between the anole and bark), (b) *Anolis barbatus*, (c) *Anolis porcus* and (d) Osvaldo López (biologist) holding a female of *Anolis porcus* in eastern Cuba. Photographs by Nils Navarro (a, c), Raimundo López-Silvero (b) and Yasel U. Alfonso (d).

Fig. 2 – Micro-computed tomography scan of *Anolis chamaeleonides* USNM 51891 collected on 1914 from La Mulata, Pinar del Rio, Cuba.
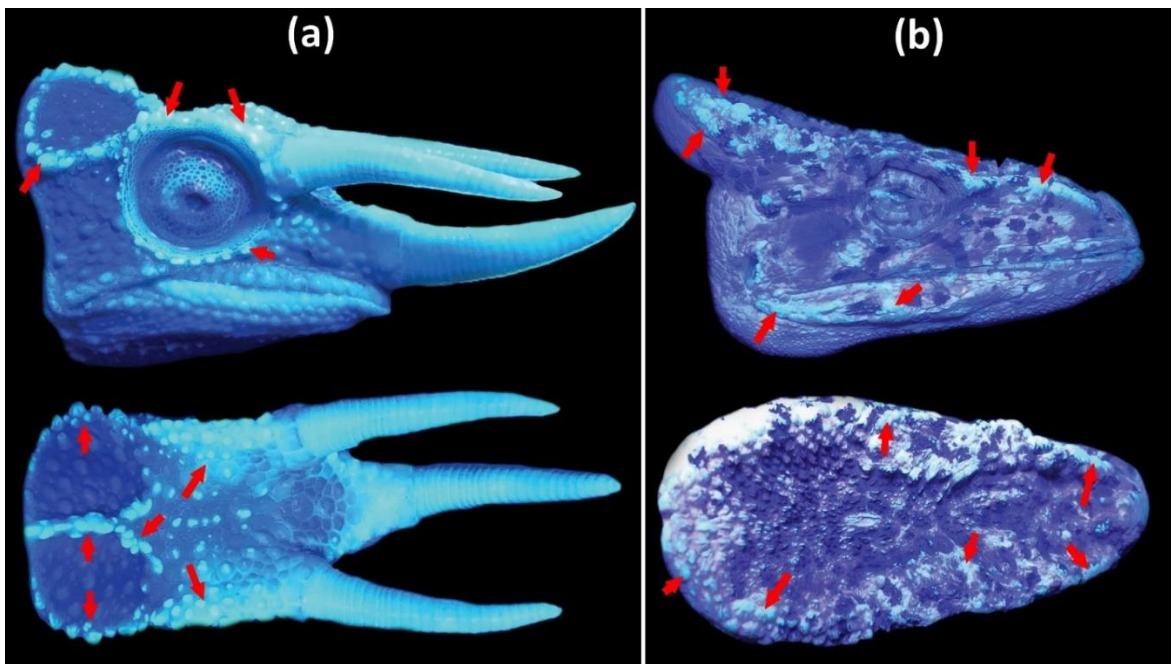
Fig. 3 – Vouchers museum specimens examined under UV light. (a) *Trioceros jacksonii* UF 174349 and (b) *Anolis chamaeleonides* USNM 51891. Fluorescent tubercles are indicated with a red arrow. Photographs by Yasel U. Alfonso.

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How do anoles respond to urbanization?
A summary of Ph.D. research on ecology and thermal biology in *Anolis* in Miami, FL.

My dissertation evaluated several important ways that urbanization alters habitats for arboreal ectotherms. Together, the first two chapters constitute a complete evaluation of one mechanism behind persistence in urban habitats, from how the urban structural habitat differs from that of the ecologically and evolutionary historical natural habitat, to how they express preferences for habitat elements and the resultant performance consequences. The third chapter explores another mechanism influencing persistence that is critically important for ectotherms, environmental temperature.

Urbanization is a global change phenomenon that is increasing in frequency and magnitude worldwide. As a greater proportion of the human population resides in urban areas, cities must grow, therefore developing natural environments and exposing an increasing number of species to human-modified habitat. While some species become extirpated when their habitat is urbanized, others persist and even spread throughout our cities. Furthermore, human activity increases the rates of species invasions around the world, and many of the introductions occur in urban areas. The objective of this dissertation is to evaluate some of the most prominent differences between urban and natural habitat and how they affect non-human urban dwellers. Specifically, I measure the effects of changes in the structural and thermal properties of urban habitats on two species of *Anolis* lizards introduced to Miami, FL: the Cuban brown anole (*Anolis sagrei*) and the Puerto Rican crested anole (*Anolis cristatellus*). These species, and anoles in general, are arboreal and ectothermic (i.e., cold-blooded), and so are an excellent system to study some of the more profound habitat changes caused by urban development.

One of the most apparent effects of urbanization is the change in the structural habitat. In order to develop a landscape for human use, vegetation is removed and modified, while artificial structures are added. I evaluated the qualities and magnitude of differences in the structural habitat between natural and urban habitats, and how lizards expressed their habitat preferences, given the changes in the urban environment. First, in the lab at URI, I assessed lizard preference for perch diameter using individuals from natural populations of both species. I allowed lizards to choose between vertical perches of three different diameters and recorded the proportion of

time spent on each perch. Second, in four urban and four natural sites in the field in Miami, FL, I measured the diameter, height, and type (e.g., "tree trunk", "branch", "metal pole") of available vegetation and artificial structures upon which lizards may perch. I also recorded these values for perches that lizards used. From the preference trials, I discovered that lizards of both species prefer the largest available perches. In the field, I found that both vegetation and artificial structures in urban areas were broader compared to vegetation in natural areas. Lizards expressed their preference for broad diameter by using broader perches than were randomly available in both habitat types, including using artificial structures. *Anolis sagrei* used artificial structures at the rate of their availability, while *A. cristatellus* could sometimes avoid artificial structures. Therefore, in urban habitats, lizards used broader perches than they did in natural habitats, demonstrating an expansion of the structural niche axis in urban areas.

Because use of the broadest perches in urban areas means that lizards often use artificial structures (the broadest available), urban populations are exposed to substrate properties they rarely encounter in natural habitats. Artificial structures, such as metal poles and painted walls, can be extremely smooth, and could prove challenging to species that primarily utilize vegetation (and man-made structures in cities) for daily activity. So, I assessed how lizard sprint performance was impacted by substrate smoothness and whether urban populations, more often exposed to smooth, artificial substrates, performed better. I measured the velocity and two-dimensional hindlimb kinematics of lizards running on three substrates of increasing smoothness (rough bark, concrete, and smooth wood) for two inclinations (inclined: 37°; vertical: 90°). I filmed lizards from urban and natural populations of both species using a high-speed video camera to capture specific points during a sprint. I found that on vertical tracks, lizards ran slower, took shorter strides, moved their bodies shorter distances with a single step, kept the foot in contact with the substrate for longer (duty factor), and exhibited more contracted limb postures upon finishing a stride than when running on the inclined track. I also observed these kinematic effects on the smooth wood substrate compared to the rough bark, though this effect was not as strong as with incline. I did not find an overall effect of habitat type, such that urban lizards did not run faster or use different gait characteristics or hindlimb positions than natural lizards.

Another effect of the structural changes caused by urbanization, along with increased impervious coverage (e.g., rooftops, parking lots, roads), is increased ambient temperatures in cities. Known as the urban heat island effect, warmer urban temperatures could have great impact on ectothermic organisms, such as the anoles studied in my dissertation. To regulate their internal body temperature (T_b), ectotherms depend on not only ambient conditions, but also the presence of warm and cool microhabitat between which they can shuttle to raise or lower their temperature. I assesed how the structural changes of urbanization affect thermal conditions and in turn, the body temperatures of lizards. I first measured operative temperatures (T_e), the body temperatures lizards would have if they did not actively thermoregulate, using copper models

distributed randomly throughout each of four urban and four natural sites. Then, while the operative temperature models were recording data, I captured lizards and took internal body temperature measurements. Next, in the lab, thermal preferences were established for lizards of both species from urban and natural areas. Finally, I evaluated temperature-dependent sprint performance by measuring thermal performance curves for sprint speed. I found that urban areas had more open canopies compared to natural areas, which led to higher T_e in urban sites than in natural habitats. I also found that lizards actively thermoregulated, maintaining T_b higher than T_e in all sites. While neither species differed in thermal preference or performance between urban and natural populations, *A. sagrei* preferred warmer temperatures and sprinted faster at higher temperatures than did *A. cristatellus*. Urban sites may lower thermoregulatory costs for both species, but I found only *A. sagrei* T_b more often within their preferred temperature range in urban compared to natural habitat. Furthermore, based on available T_e within each species' preferred temperature range, urban sites with only *A. sagrei* appear less-suitable to *A. cristatellus*, and vice versa for natural sites with only *A. cristatellus*. While *A. sagrei* may find opportunities for dispersal in many urban locations, *A. cristatellus* is likely constrained to forested locations or those with higher canopy coverage.

As urban areas grow and more species are introduced to areas outside of their native ranges, studies such as these are important to understand and predict persistence and invasion dynamics. Look out for each of these chapters in journals! Right now, the first chapter is available from *Urban Ecosystems*: https://link.springer.com/article/10.1007/s11252-018-0787-1

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Variation in habitat lighting may mediate the persistence of dewlap color polymorphism in South Florida bark anoles

Project description/rationale

While colorful dewlap displays are the hallmark trait of anoles, we still don't know why or how dewlaps have evolved to be quite so diverse. Despite this mystery, selection on dewlap design likely follows the established principles of visual signal evolution in other taxa, favoring signal components that best stimulate the sensory systems of intended receivers, such as potential mates or competitors (Guilford & Dawkins, 1991; Endler, 1992; Endler 1993). As such, it is possible to use information about the visual systems of anoles and the habitats they occupy during displays to test if diversification in signaling traits is the result of selection for improved efficacy across variable visual environments. For my dissertation, I am investigating this question by studying the bark anole *Anolis distichus*, a phenotypically variable anole from Hispaniola and the Bahamas (Fig 1).

Across the Caribbean, bark anoles exhibit a greater variety of dewlap color morphs than most other anole species, with populations of red, orange, pale yellow, or even two-toned dewlaps found across Hispaniola and the Bahamas (Schwartz, 1968). Over the past century, bark anoles from several of these populations have been introduced to Florida (Kolbe et al., 2007), forming a mixed-phenotype population across a

Figure 1: Above, a bark anole displaying its dewlap. Below, images of Caribbean bark anoles exhibiting dewlap variation.
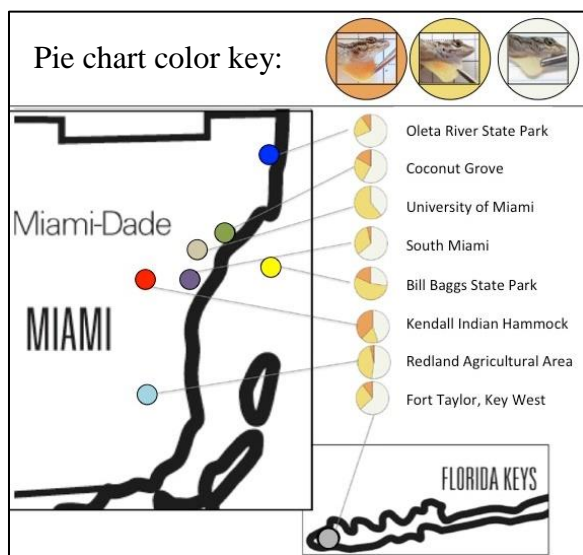
Figure 2: Pie charts representing the relative abundance of orange, two-toned, and yellow dewlaps across eight sites in South Florida.

mosaic of new habitats (Fig 2). These introductions provide us with an evolutionary experiment, in which we can ask if the diversity of dewlap colors exhibited by bark anoles is an adaptation across variable visual environments.

Summary of results

We first determined that variation in dewlap color of South Florida bark anoles is poorly predicted by their putative source populations, as assigned by mitochondrial haplotype comparisons with Caribbean bark anoles. This finding suggests that dewlap variation among South Florida bark anoles is not purely the result of evolutionary history (i.e., multiple source populations). We then analyzed field-collected spectral data from over 200 bark anole home perches across eight sites in South Florida, as well as the color and brightness measured from the dewlaps of each anole. Across the eight sites we sampled in South Florida, we found that the relative abundance of ultraviolet (UV) light at each site is significantly and positively correlated with bark anole dewlap UV reflectance at those same sites. As most visual signals require an external light source for illumination, signal efficacy will be increased by more efficiently reflecting the available ambient light spectra. These data suggest that dewlap variation could be driven by selection for efficacy across variable signaling environments.

A Natural Experiment

In an unexpected turn of events, South Florida bark anole habitats were abruptly transformed in September of 2017 by category-5 Hurricane Irma, a storm that wreaked havoc across the Caribbean and parts of Florida. The resulting habitat changes (Fig 3) immediately altered each habitat's light profile, providing us an opportunity to test our hypothesis on selection for signal efficacy among bark anoles introduced to new signaling environments. We hurried to repeat our data collection at the eight sites in the weeks after the storm to promptly characterize the changes in ambient light profiles, and identified changes in the relative distribution of dewlap colors across sites. Upon analysis of the new spectral data, we found the correlation between dewlap reflectance and ambient

Figure 3: Satellite imagery of the University of Miami Gifford Arboretum (field site 1) before (above) and immediately after (below) Hurricane Irma. Images from Google Maps.

ultraviolet light was no longer present after the storm.

Moving forward

As a full year has now passed since the destruction caused by Hurricane Irma, we are currently collecting a third set of habitat and bark anole data from across the same eight field sites. It will be exciting to see whether light profiles have returned to their pre-hurricane state, and, more importantly, if the relationships between dewlap color and ambient light profiles are recovered over time.

Conclusion

In summary, results from this research suggest that variation in habitat lighting may mediate the persistence of signal polymorphism among anoles. Using Hurricane Irma as a natural experiment, we have a unique opportunity to test how abrupt changes in visual conditions influence signal design among bark anole populations in South Florida. This research can provide insights into the evolutionary processes responsible for the genesis and maintenance of the incredible diversity dewlap colors displayed by male anoles.

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Conservation concerns for loyal lizards: Habitat specificity, site fidelity, a localised territory and in-situ growth rates for *Anolis bicaorum* (Squamata; Dactyloidae), endemic to Isla de Utila, Honduras

Figure 1. An endemic male *Anolis bicaorum* displays his dewlap proudly from his perch in the forest. Unfortunately, forest habitat on Utila is increasingly threatened owing to development and urbanization.

Introduction - This short annotation documents some preliminary results of a capture-mark-resight study in the endemic anole lizard *Anolis* (alternatively *Norops) bicaorum,* found only on Isla de Utila, Honduras (Figure 1.). The species currently remains un-accessed by the IUCN Redlist, though was listed by Johnson et al. (2015) with an Environmental Vulnerability Score (EVS) of 17, placing *A. bicaorum* in the middle portion of the highest vulnerability category. The following focuses on reporting noteworthy recapture data for three individuals (2 female, 1 male) across a 6-10 month timespan. This reencounter data provides information on in-situ growth rates, and evidence that *A. bicaorum* exhibit a high site fidelity and localized individual territory. It is believed that the presented data is crucial in forming an understanding of this species life

history, and indeed informing future conservation management of this anole. Ongoing research at KURCF suggests populations are increasingly threatened and currently the project seeks to inform a valid IUCN Redlist classification.

On the eastern side of Utila, a capture-mark-resight study of *A. bicaorum* (see Brown, 2017) was conducted at a broad-leaf/ palm forest site known as Pumpkin Hill (16.12003°N, -86. 88223°W (WGS84) 74m asl). This locality was previously noted as an important site and 'hotspot' for large populations of this endemic anole (Brown et al, 2017[1]). As reported prior, the habitat consists of Tique Palm (*Acoelorrhaphe wrightii)* dominated broad-leaf forest (Fickert and Gruninger, 2010), presently directly threatened by private sale and deforestation for development (Brown et al, 2017 [1, 2]).

Method - From July - August 2017, we commenced Visual Encounter Surveys (VES) in a ca. 9000m^2 survey plot (150 x 60 m) at the summit of Pumpkin Hill. The site was trisected with three ca. 150 m transects separated by 20 m (10 m perpendicular either side), providing sufficient survey coverage of the area. On 12 occasions during both the day and night, we systematically looked for *A. bicaorum* in all available microhabitats along each transect. When encountering anoles, standard morphological data, e.g. SVL (Snout-Vent Length mm), TL (Tail Length mm), Weight (g), Sex (Male or Female) and ecological data (e.g. perch height, perch diameter, perch substrate) was collected. Individuals captured of a suitable size (>50 mm SVL) were marked semi-permanently by attaching one or two 2mm coloured plastic beads to the base of the tail using 5/0 Nylon Monofilament sutures; a marking method adapted from that which is detailed for lizards by Galdino et al (2014). This method facilitated the identification of individual anoles on subsequent visits to the site, for the most part effective up to 2 months post attachment (Figure 2.). Outstandingly however, the three individuals we precede to detail were encountered with beads still attached after a period of ca. 6, 8 and 12 months; allowing for the collection of valuable in-situ growth and home range data.

Results - While analysis of the extensive dataset and results obtained during this large population study is still pending, I can report that at a single Pumpkin Hill site between July - August 2017, 129 individuals were captured and marked, with an overall total of 283 re-sight observations on 12 survey occasions (T. Brown unpub.data). Throughout this period of intensive research, individuals were found to exhibit high site fidelity and often a preference to certain perches and positioning. Of these marked individuals, revisits to the site in 2018 unexpectedly discovered three anoles with I'D beads still attached. All these individuals were within 5-10 meters (m) of their initial recorded positions 6 - 10 months prior. Upon capturing these anoles, standard measurements were taken, and records of their capture history data was collated to provide information on their growth and movements.

Figure 2. Photographic examples of *Anolis bicaorum* marked using the beading methodology adapted from Galdino et al (2014), considered an effective semi-permanent means of identifying individuals' in-situ.

Observations - The first reencountered adult female (#62) was initially captured on the 12 August 17, and marked with two 2 mm beads (Colours – Light Pink/Purple). Throughout 12 revisits to the site in 2017, this individual was reencountered on one more occasion. This same individual was then encountered 5 m from its original capture point, 0 m from its last capture point, exactly 6 months and 13 days later on the 24 February 18. In this total of 197 days, a positive growth rate of + 7 mm in SVL, +23.5 mm in tail length and +0.6 g in body mass was recorded. The capture history and overview of data collected for this individual is shown in Table 1.

The second adult female (#06) I report, was initially captured on 25 July 17 and marked with a single 2 mm bead (Colour – Light Pink). In 2017, this individual was reencountered on three more occasions. During an opportunistic revisit to the site on 07 April 2018, the final observation was made exactly 8 months and 14 days after the initial capture. During this time span, the individual moved ca. 9 m from its initial and 7 m from last capture point. Additionally, growth rates of +4.2 mm in SVL and +0.6 g in body mass were documented, and a reduction of 30.5 mm in tail length perhaps owing to predator spurred caudal autonomy (such an event may of caused this individual to move greater distances). The capture history and data collected for this individual is shown in Table. 2.

Table 1. A summary of the data collected on a female *A. bicaorum* individual (#62), and calculation of total growth and approximate movement. **Columns and abbreviations**: Time (00:00), Date, Location (Distance/meters along transect), SVL (Snout-Vent length mm), T-L (Tail length mm), WT (Weight g), P-H (Perch Height cm), P-D (Perch Diameter mm – if applicable), Substrate (the perch of choice e.g. Leaf-litter, Volcanic Rock, Buttress root, Dead Stump, Fallen branch, Plant stem, Plant leaves, Palm leaf, Palm stem, Palm trunk, Broad-leaf trunk, Branch, Mature vine).

Time	Date	Location	SVL	T-L	WT	P-H	P-D	Substrate
10:50	12 August 17	35	61	120.5	5.9	161	265	Palm Trunk
22:37	24 August 17	40	N/A	N/A	N/A	173	N/A	Palm Leaf
10:40	24 February 18	40	68	144	6.5	154	240	Palm Trunk
Total	197 days	0-5	+7	+23.5	+0.6			

Table 2. A summary of the data collected on a female *A. bicaorum* individual (#06), and calculation of total growth and approximate movement. **Columns and abbreviations**: Time (00:00), Date, Location (Distance/meters along transect), SVL (Snout-Vent length mm), T-L (Tail length mm), WT (Weight g), P-H (Perch Height cm), P-D (Perch Diameter mm), Substrate (the perch of choice).

Time	Date	Location	SVL	T-L	WT	P-H	P-D	Substrate
11:37	25 July 17	149	62.9	131.8	6.9	230	317	Branch
10:03	26 July 17	149	N/A	N/A	N/A	140	82	Palm Stem
11:56	02 August 17	149	N/A	N/A	N/A	109	94	Palm Stem
11:13	16 August 17	147	N/A	N/A	N/A	90	92	Palm Stem
09:30	07 April 18	140	67.1	101.3	7.5	136	520	Tree Trunk
Total	257 days	0-9	+4.2	-30.5	+0.6			

Lastly, the male individual (#07) was first encountered on the 12 August 17, and marked with two 2 mm beads (Colours –Pink/Pink). Throughout revisits to the site in 2017, this individual was reencountered on three more occasions. In 2018, we again made three more observations, the final encounter location being 0 m from its original capture point (on the same tree), exactly 1 year and 3 days later on the 14 August 18. In this total of 368 days, a positive growth rate of + 4.4 mm in SVL, +8.6 mm in tail length and +0.9 g in body mass was recorded. The capture history and overview of data collected for this individual in shown in Table 3.

Table 3. A summary of the data collected on a male *A. bicaorum* individual (#07), and calculation of total growth and approximate movement. **Columns and abbreviations**: Time (00:00), Date, Location (Distance/meters along transect), SVL (Snout-Vent length mm), T-L (Tail length mm), WT (Weight g), P-H (Perch Height cm), P-D (Perch Diameter mm), Substrate (the perch of choice).

Time	Date	Location	SVL	T-L	WT	P-H	P-D	Substrate
10:53	12 August 17	43	70.6	142.6	8.1	162	127	Palm Trunk
14:17	13 August 17	42	N/A	N/A	N/A	140	132	Palm Trunk
12:51	22 August 17	44	N/A	N/A	N/A	158	84	Palm Stem
11:25	23 August 17	46	N/A	N/A	N/A	115	105	Palm Trunk
15:25	22 June 18	44	N/A	N/A	N/A	240	90	Palm Stem
11:15	4 August 18	40	N/A	N/A	N/A	172	130	Palm Trunk
11:43	14 August 18	43	75	151.2	9	171	132	Palm Trunk
Total	368 days	0-6	+4.4	+8.6	+0.9			

Discussion - While data collected on three individuals is not considered representative of a population, the presented results do strongly suggest females and males exhibit high site fidelity, potentially maintaining territories of less than 5-10 m^2. Though species movements cannot be accounted for across the entire timespan, short term re-encounter data for both male and female territories indicates that movement of adults outside their territories is minimal. While intensively surveying numerous sites, individuals were continually found to exhibit preference to a certain perch or tree in their domain, providing solid evidence for earlier anecdotal observations of their behaviour and ecology (e.g. consecutive use of individual sleeping sites – Brown et al. 2017[1]). These combined observations support the proposition that within the dense populations observed, the territory of males overlap those of one or several females, and competition rates demand that individuals protect personal resources, maintain small territories and their relations with closely neighbouring anoles which they encounter and interact with near daily. This ideology corresponds with Nicholson and Richards (2011) discussion of the current understandings of spatial ecology in polygynous lizards whilst comparing home range data for numerous anole species.

Conservation research in reptiles shows that habitat specificity, a small home range and limited distribution, correlates with species vulnerability to extinction (e.g. Waldron et al. 2006; Johnson et al 2015). Accordingly, a small home range may be a detrimental attribute, as *A. bicaorum* is already imperilled owing to its limited geographic distribution and numerous

additional pressures (for threats to *A. bicaorum* see Brown et al. 2017[1, 2]). Overall, it was found *A. bicaorum* exhibits a high dependency and fidelity to its locally restricted hardwood and palm forest habitats; the sheer abundance of this anole in the forest interior contrasts its utter absence from alternative habitats. Accordingly, it seems probable that drastic local habitat fragmentation and degradation will spell doom for resident anoles.

Concluding remarks - As I write this listening to the sound of chainsaws, construction and tree-fall, I genuinely worry about the large areas of forest habitat at Pumpkin Hill (and indeed island wide) being progressively sold and developed on each day. This unfortunate trend of deforestation is now set to ever-increase following the rapid placement of new roads in July 2018, which now makes Pumpkin Hill (a previously remote area) very accessible to private developers, construction machinery, public and tourist influxes; an accumulative driver for the urbanisation of this region. Our observations of localised abundance and site fidelity in *A. bicaorum*, suggest that displaced anoles may not survive outside their territories, and certainly wouldn't maintain as high abundance in degraded habitats. This documentation echoes the forewarnings of McCranie & Kohler (2015), in that *A. bicaorum* appears to be declining in altered habitat, and that populations are 'seemingly down from their former exceedingly abundant category'. A secondary associated issue is that, with increased urbanisation of previously remote island regions, the spread of the invasive and competitive *Anolis sagrei* (as well as *Anolis allisoni*) from Utila town is assisted; both are now well established occupants of disturbed urban areas. *Anolis sagrei* specifically, is a considerable threat to the endemic anoles, as currently its distribution is expanding in synergism with the pace of island development; now beginning its spread towards remote areas of primary forest habitats. Even if *A. bicaorum* did show tolerance to degraded or alternative habitats, the likelihood of the species successfully competing with *A. sagrei* in such edificarian environments seems very unlikely. Considering the apparent extirpation or simple absence of *A. bicaorum* from Utila Town, it seems the best hope of this endemic anole enduring the invasion is within its stronghold and home turf – the undisturbed hardwood palm forests.

Sadly though, mature, pristine and undisturbed hardwood palm forest habitats are an ever rarer commodity on Utila. Following 3 years of personal observations, it seems evident that to preserve the remaining high abundance of *A. bicaorum*, land purchase and conservation action is required to safeguard *A. bicaorum* populations and its remaining core forest habitats. While unmentioned until now, this same action is also needed to conserve its sympatric endemic 'canopy dwelling' congener *Anolis utilensis,* which relies primarily on the same mature growth hardwood palm forests (Brown et al. 2017[2]). I feel conservation action is of the upmost urgency, as many of the anole survey sites established in 2016 have already been entirely lost or impacted by development activities, and despite intense study, little is known regarding these anoles behaviour and ecology. Alongside active research, it is with great hope and optimism that by increasing local and international awareness through community outreach, media and publication

(e.g. see Brown 2018[1, 2, 3]; MBZ 2018), we can generate the interest and momentum to protect areas of key forest habitat for the active conservation of these species and indeed much of Utila's incredible biodiversity.

All methods and data collection approved and performed under valid biological research and collection permits (Resolución DE-MP-054-2017 – Dictamen técnico ICF-DVS-169-2017; Dictamen técnico DAP-068-2017); for the project "Conservación de los reptiles y anfibios de Utila, Honduras" issued in part to TWB (myself) of Kanahau (KURCF) by General Director Misael León and General Secretary Gudit Mariel Muñoz of Instituto Nacional de Conservación y Desarrollo Forestal, Áreas Protegidas y Vida Silvestre (ICF), Tegucicalpa, Honduras.

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Non-native species dominate herpetofaunal community patterns in both native and non-native habitat patches in urban Miami-Dade County

Introduction

Land use change and invasive species are two of the greatest threats to biodiversity on a global scale (Clavero and García-Berthou 2005; Davies et al. 2006). Not only do these anthropogenic changes individually affect native ecosystems, but the two may also interact to further threaten native biodiversity (Byers 2002; Didham et al. 2007). Ecosystems in Miami-Dade County, South Florida, USA, are threatened by both substantial habitat loss/degradation and the introduction of numerous non-native species (Enge et al. 2004; Smith 2006), providing an opportunity to investigate community composition under these potentially interacting threats.

South Florida is notorious for the presence of non-native species from a broad array of taxa (Gordon 1998; Forys and Allen, 1999; Smith 2006), with the large number of introduced reptiles and amphibians providing a unique opportunity to investigate herpetofaunal community composition in the presence of non-native species. One hundred and thirty-seven non-native reptile and amphibian species have been introduced to Florida, and 56 of these species are now established in the state, making Florida home to more established species of non-native herpetofauna than anywhere else in the world (Krysko et al. 2011). South Florida is susceptible to invasion due to a number of interacting factors. The peninsular nature of Florida makes it similar to an island in terms of being geographically isolated and having a relatively depauperate native fauna (Smith 2006; Kraus 2015), and islands are known to be particularly susceptible to invasions (Gimeno et al. 2006; Okamoto et al. 2013; Kraus 2015). In addition, the tropical climate in South Florida provides an environment to which many of the non-native herpetofauna are already well-adapted (Forys and Allen 1999; Smith 2006). The pet trade is the primary pathway of introductions (~84% of all introductions) as Miami is the center for the exotic reptile and amphibian pet trade (Krysko et al. 2011). Finally, South Florida, especially Miami-Dade County, is heavily populated and has suffered intense anthropogenic pressures over the past 100 years (Diamond and Heinen 2016). The number of non-native species introduced to an area generally increases with human presence, partly due to humans acting as dispersal agents and partly due to the increase in disturbances that frequently accompany human presence (Mack et al. 2000; Smith 2006).

As the human population in South Florida has increased, so has the amount of anthropogenic disturbance, jeopardizing native ecosystems such as the pine rocklands and tropical hardwood hammocks. Because of the pine rocklands' position at higher elevations along the Miami Rock Ridge (Bradley and Martin 2012), they have been targeted for development to avoid more flood-prone regions (Diamond and Heinen 2016). As such, only 2% of the historic extent of pine rocklands remain in Miami-Dade County, primarily in small isolated patches surrounded by an urban matrix (Bradley and Martin 2012).

To protect the native diversity of these fragmented habitats, the Environmentally Endangered Lands (EEL) Program has established a network of preserved native ecosystem patches. Previous studies suggest that native species should persist in native habitats to which they are well-adapted (Byers 2002; Didham et al. 2007), while non-native species tend to be habitat generalists (D'Amore et al. 2010) and may be better able to persist in disturbed landscapes than native species (Byers 2002; Didham et al. 2007; Maskell et al. 2006). Therefore, we sought to investigate how the herpetofauna community differs between preserved native habitat patches and non-native habitat areas.

Our primary question was whether preserved native habitat patches hold a higher relative abundance and richness of native herpetofauna than that found in non-native habitat patches. In addition, we wanted to examine important elements of the native preserve network, such as how the area and isolation of the preserves influence the species richness found within them. Finally, we were interested in overall herpetofauna community structure and composition. As different habitat types (native and non-native) may alter the community within them, we investigated community divergence between these two habitat types and indicator species associated with each.

Methods*
   *Contact slclements@miami.edu for more detailed methods

Site Selection
   We selected 15 native/non-native pairs of parks throughout Miami-Dade County (Figure 1). We classified native parks as those for which the majority of habitat was native vegetation (i.e., pine rockland, tropical hardwood hammock, and mangrove). Non-native parks were county parks, green spaces, and recreation areas that did not have primarily native vegetation. We paired parks based on their location within the county to ensure that both native and non-native parks represented the same geographic areas. Parks varied in size, ranging from 0.6 to 229 hectares, and native and non-native parks spanned similar size ranges. All selected parks were isolated

fragments surrounded by an urban matrix, such that none of the selected parks were contiguous with other parks or preserves or with the more extensive natural habitat surrounding the Miami Metropolitan Area.

Survey Techniques

We conducted diurnal visual encounter surveys (Enge et al. 2004) from March-May of 2017, which corresponds with the season of maximum herpetofaunal activity in this region (Diaz, R. Personal communication). Our surveys used the active search technique (searching through microhabitats), as this was determined to yield the most species per unit time (Enge et al. 2004). Time spent surveying in each park was scaled to the log of the area of the park to ensure that the larger parks, which could not be completely surveyed due to time constraints, were sampled to the same depth on the species-abundance curve as the smaller parks.
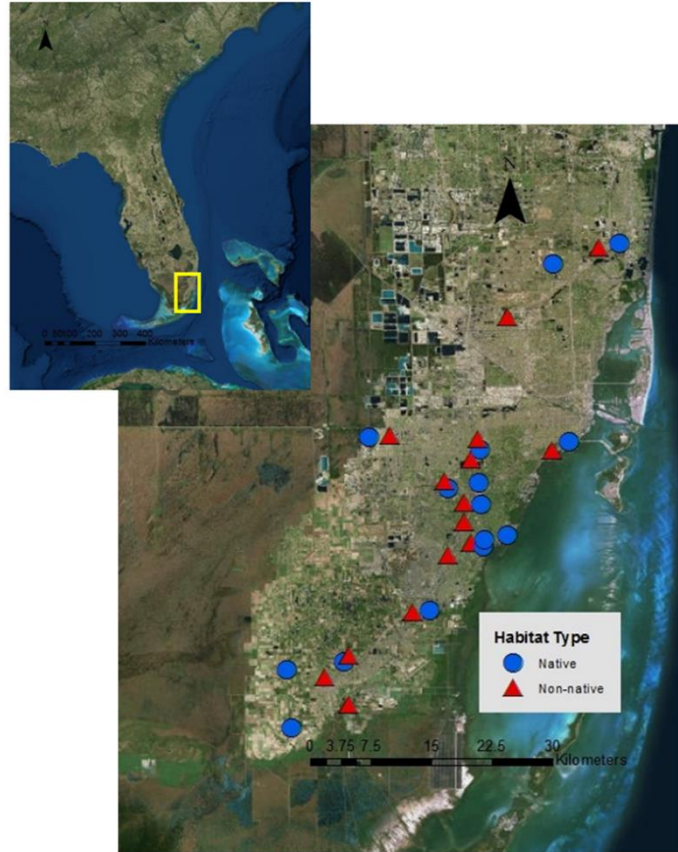
Fig. 1 Map of survey sites. Top left inset shows the location of Miami-Dade County in Florida, USA. Map in bottom right shows survey sites in Miami-Dade County. Blue circles represent native parks and red triangles represent non-native parks.

Statistical Analyses

Native vs non-native patch analyses

We conducted *t*-tests to compare the herpetofauna of native vs. non-native parks for the following responses: 1) total abundance (divided by person-hours), 2) estimated species richness (from the second-order jackknife), 3) proportion of native individuals, 4) proportion of native *Anolis* individuals (this genus included both the most abundant native and non-native species), and 5) proportion of native species.

Area and isolation analyses

We used ANCOVA to determine whether there was a difference between the rate at which native and non-native species richness increased with log(park area). We also used ANCOVA to determine if there was a difference in the rate at which overall species richness increased with log(park area) between native and non-native parks.

We used a patch-based weighted sum (Winfree et al. 2005) to calculate a native habitat connectivity metric for each park. The patch-based weighted sum is calculated as:

$$H_x = \sum_{i \neq x} A_i e^{-\frac{d_i}{D}},$$

where x is the focal patch, H is connectivity, A_i is the area of patch i, d_i is the distance between patch x and patch i, and D is the mobility constant of the organism in question. All native habitat patches owned by EEL or surveyed by the Institute for Regional Conservation were included in the connectivity calculations (M-D Parks 2010; Gann 2017). Since there is no data on the average dispersal of herpetofauna through this habitat matrix, we used a range (0.1, 0.2, 0.5, 1, 2) of mobility constants to determine whether they affected the outcome of these analyses. We used linear regression to determine whether there was a relationship between native habitat connectivity and $\log_{10}$(native species richness). We only considered native species in this analysis because we expect native habitat connectivity to matter for these species, while non-native species are most likely able to persist in the urban matrix as well as the native habitat patches.

Because there may be competition between native and non-native herpetofauna (Losos et al. 1993; Gerber and Echternacht 2000; Maron and Marler 2008), we sought to examine how the abundance of non-natives within a patch impacts native abundance. To investigate the simultaneous effects of multiple environmental factors, we used a model with native species abundance (divided by person hours) as the response and patch area, non-native abundance (divided by person hours), connectivity, and habitat type as predictors. We also ran this same model for our most common herpetofaunal guild, the *Anolis* lizards. In this model, the only native anole, *Anolis carolinensis*, was used as the response, and the abundance of the most common non-native anole, *Anolis sagrei*, was used as the metric of non-native competition. Area, all abundances, and connectivity were log-transformed for normality.

Community structure analyses

We conducted a PERMANOVA using the Bray-Curtis distance metric to determine whether there was a difference in community structure between native and non-native parks. We relativized each row (park) of the community matrix by the number of person-hours spent surveying and relativized each column (species) by its total abundance in order to upweight the contribution of rare species to the analysis. This was deemed necessary because *Anolis* species accounted for 86% of individuals in the dataset and would obscure any changes in the rest of the community without the correction. We followed the PERMANOVA with an indicator species analysis to determine which species were most indicative of native versus non-native parks.

Results

Across the 30 study parks, we recorded 7318 individuals from 33 different species (15 native species and 18 non-native species). Of the 7318 individuals found, only 9.4% were native ($n = 692$). Non-native species were very common across the parks. For example, the non-native *Anolis sagrei* accounted for 57% of all individuals found and was present in every surveyed park. Non-natives *A. distichus* (11%) and *A. cristatellus* (8.4%) were the next most abundant species. Overall, *Anolis* species (one

Fig. 2 Strip plot showing the relative abundance of native individuals in native and non-native parks. There was no significant difference between the two habitat types ($P = 0.28$).

native and four non-native) comprised 86% of the observed individuals. *Anolis carolinensis* was the most abundant native species, accounting for 8.0% of all individuals found, and was present in 87% of the parks that were surveyed. The most widespread species after *A. sagrei* and *A. carolinensis* were *A. distichus* (73% of parks), *Hemidactylus mabouia* (70%), *A. equestris* (57%), *B. vittatus* (50%), *C. constrictor* (43%), *Ramphotyphlops braminus* (43%), *R. marina* (30%), *I. iguana* (27%), *A. cristatellus* (23%), *Pseudemys nelsoni* (23%), and *Trachemys scripta* (20%). Out of these 13 species that were found in five or more parks, only *A. carolinensis*, *C. constrictor*, and *P. nelsoni* are native.

Native vs non-native patch analyses

The relative abundance of native herpetofauna did not differ between native and non-native parks ($P = 0.28$; Figure 2). Nor did the total abundance ($P = 0.09$), estimated total species richness ($P = 0.71$), or proportion of native species ($P = 0.31$). The relative abundance of the most common native, *A. carolinensis*, also did not differ between native and non-native parks ($P = 0.28$).

Area and isolation analyses

Herpetofaunal species richness increased with park area for both native and non-native species ($P = 0.004$), and mean non-native species richness was greater than mean native species richness across all parks ($P = 0.0001$). The slope of the species-area curve was the same for both species types (species type*area interaction: $P = 0.42$), indicating that both native and non-native

species increase at approximately the same rate with area (Figure 3). The slope of the species-area curve was also the same for both native and non-native parks (habitat type*area interaction: $P = 0.64$), indicating that species richness increases at approximately the same rate with area in both native and non-native parks, and overall species richness increased with area ($P = 0.03$) but did not differ between habitat types ($P = 0.53$). There was no relationship between native habitat connectivity and native species richness using any of the dispersal constants (D = 0.1, 0.2, 0.5, 1, 2), indicating that the network of native reserves is not functioning as a metapopulation.
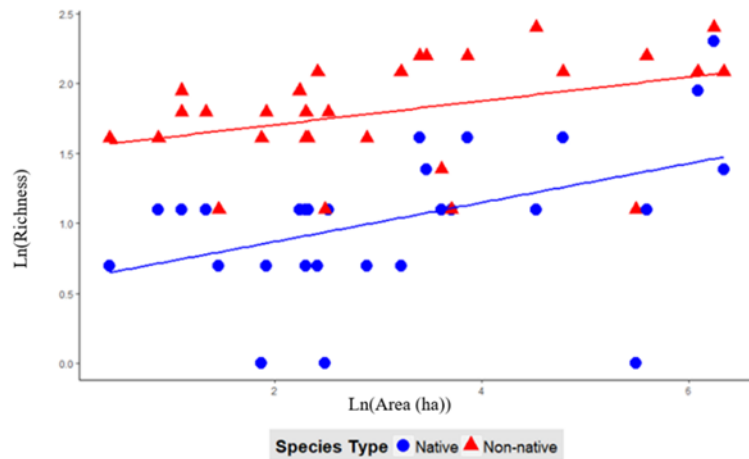
Fig. 3 Native and non-native species richness both increase with park area ($P = 0.002$) and non-native species richness is uniformly greater than native species richness across parks ($P < 0.0001$). The rate of species accumulation does not vary between native and non-native species (species type*area interaction: $P = 0.19$). Each park is represented by one blue dot for its native species richness and one red triangle for its non-native species richness.

In the global model taking area, connectivity, habitat type, and competition into account simultaneously, the only significant term was the one for competition (non-native abundance: $P = 0.0001$). Similarly, the only significant term in the global *Anolis* model was the one for competition (*A. sagrei* abundance: $P= 0.011$). For both of these models, however, abundance of the non-native taxa was positively correlated with native abundance, indicating that competition with non-natives is not driving abundance of native species (Figure 4). There was no evidence of spatial autocorrelation for any of the above models ($P > 0.07$) except for total native abundance in the global model ($P = 0.03$). For this model, the reported P-value includes a term for spatial autoregression error.

Year of introduction was negatively correlated with the number of parks where a species was recorded ($P = 0.04$), meaning that non-native species that have been present in Florida for a longer period are more widespread. For example, *A. sagrei* was introduced in 1887, making it one of the earliest introductions, and was found in every park.

Using a PERMANOVA, we found that there was a significant difference in community composition between native and non-native parks ($P = 0.0002$) with park type accounting for 9.0% of the total variation in community composition (Figure 5). Our indicator species analysis revealed that *Anolis equestris* ($P = 0.0058$) and *A. sagrei* ($P = 0.041$) were indicators of non-native habitat and that *Ramphotyphlops braminus* ($P = 0.0094$) was an indicator of native habitat. *Anolis sagrei* was more abundant in non-native than in native parks ($P = 0.043$). Eighty-eight percent of *A. equestris* individuals were in non-native parks and 77% of *R. braminus* individuals were in native parks.
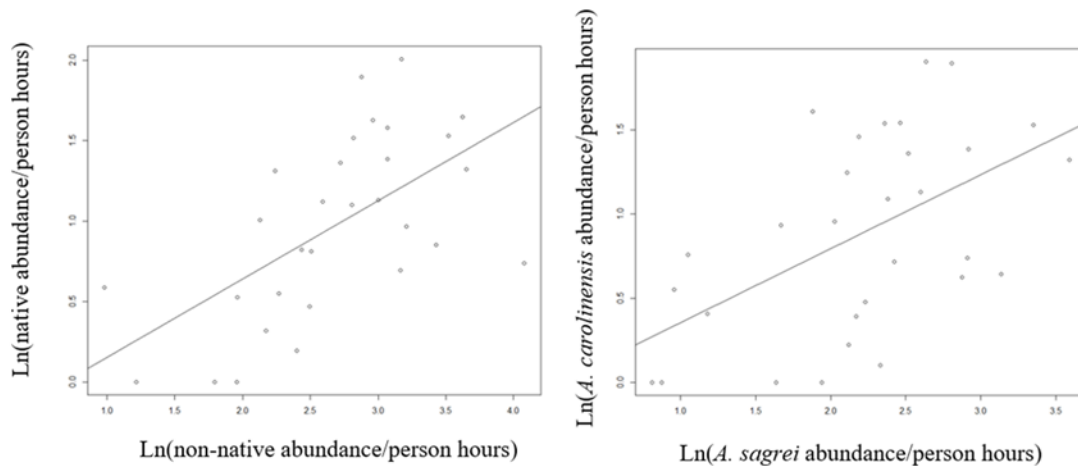
Fig. 4 Significant positive correlation between native and non-native abundance. In a simple linear regression, there was a positive relationship between native and non-native abundance ($P = 0.04$, $R^2 = 0.14$), and between *A. carolinensis* and *A. sagrei* abundance ($P = 0.002$, $R^2 = 0.28$), indicating that both natives and non-natives are most successful in the same parks.

Discussion

The results of all analyses indicate that non-native species dominate herpetofaunal communities in Miami-Dade County, both in abundance and species richness. Overall, less than 10% of the recorded individuals were of native species, and within individual parks non-natives accounted for an average of 74% of species identified, significantly more than natives ($P = 0.0001$). It is also worth mentioning that *A. carolinensis*, the most abundant native species (8% of observed individuals), is suspected to now be hybridized with the non-native Cuban green anole (*A. porcatus*; Wegener 2017). If this is correct and *A. carolinensis* is no longer classified as being native, the overwhelming proportion of non-native individuals is even more apparent, with only 2% of observed individuals being truly native.
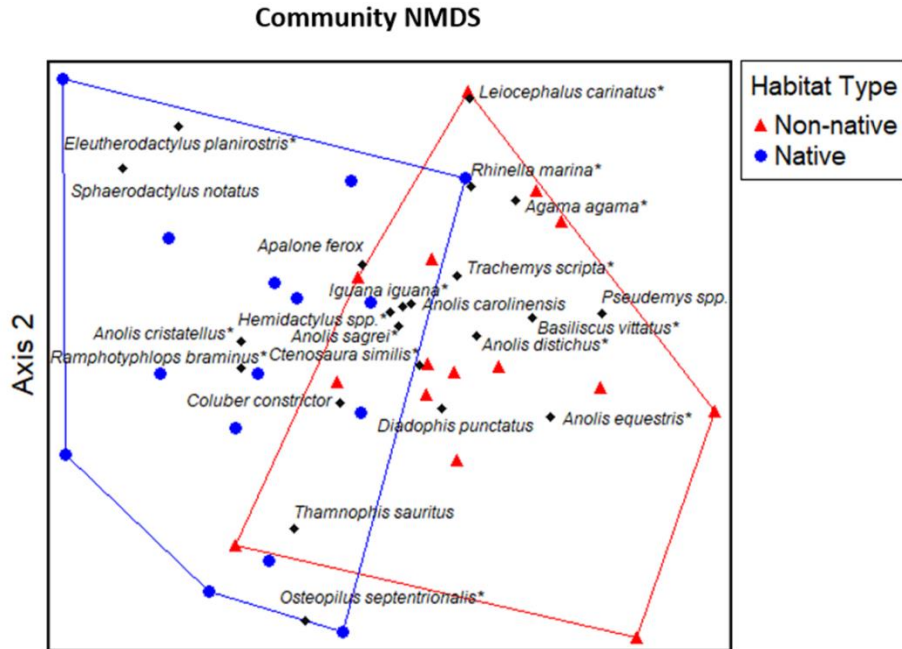
Fig. 5 Non-metric multi-dimensional scaling plot of herpetofaunal community composition in Miami-Dade County. Red triangles represent non-native parks and blue circles represent native parks. Each park type is surrounded by its minimum convex hull. Black diamonds represent species, which are located central to the parks in which they were found. An asterisk at the end of the name indicates a non-native species. *A. equestris* and *A. sagrei* were indicator species of non-native parks ($P = 0.0058$ and $P = 0.041$, respectively), while *Ramphotyphlops braminus* was an indicator species of native parks ($P = 0.0094$).

Many green spaces are and will continue to be within metropolitan areas, so understanding how to conserve species within this altered landscape is critical for conservation efforts. Our study agrees with others that have shown area of habitat to be one of the most important predictors of species richness within urban landscapes (Drinnan 2005; Bickford et al. 2010; Beninde et al. 2015). While patch size does have an influence on the number of species found, it does not seem to matter whether the habitat is comprised of native or non-native vegetation, or whether the taxa being considered are native or non-native herpetofauna. However, it should also be noted that our largest habitat patch was approximately 200 hectares and that native habitats that exceed this size may exhibit different patterns. Area did not influence abundance, however, which is in line with other studies that demonstrated a relationship between richness and area, but no relationship between abundance and area (Bickford et al. 2010).

Invasion ecology suggests that non-native species are more likely to dominate in human-modified areas with altered ecosystems (Colautti et al. 2006; Maskell et al. 2006; Smith 2006; D'Amore et al. 2010). Our findings show that non-native species dominate not only in human-

altered green spaces, but also within the remaining native habitat patches in urban Miami-Dade County. Due to the small size of native habitat fragments, no portion of the native habitat is more than 700 m from the urban matrix, and many sections of native habitat fragments now constitute edge habitat. Thus, the urban matrix may be serving as a source of non-native individuals moving into native habitat patches (With 2002). In contrast, native species may not be able to move through an urban matrix, which then limits their population sizes and increases probabilities of extinction (With 2002). Our isolation analysis supported this hypothesis in that native species richness did not increase with connectivity, indicating that these native habitat patches are not functioning as a metapopulation.

While there was a significant difference in community composition between native and non-native parks, this difference accounted for only 9% of the total variance in community composition. An indicator species analysis revealed that all indicator species are non-natives (*A. sagrei* and *A. equestris* for non-native parks) and that even native parks are indicated by a non-native species (*R. braminus*). *Ramphotyphlops braminus* is the most widely introduced snake in the world (Lever 2003), and the fact that it is now the reptile/amphibian species most representative of native habitat in urban Miami is further evidence that non-native herpetofauna dominate even native habitat patches.

We did not find any evidence of competition driving community assembly in this system. Instead, we observed a positive correlation between native and non-native abundance. This is somewhat surprising due to the vast amount of literature discussing competition between native and non-native species (Losos et al. 1993; Gordon 1998; Simberloff and Von Holle 1999; Gerber and Echternacht 2000; Gibbon et al. 2000; Mack et al. 2000; Cole et al. 2005; MacDougall and Turkington 2005; Maron and Marler 2008; Kraus 2015). However, competition between native and non-native *Anolis* lizards seems to result in differential use of microhabitats rather than competitive exclusion from entire patches (Losos et al. 1993; Gerber and Echternacht 2000). Perhaps this is the main consequence of competition in our patches as well: microhabitat partitioning but patch-level coexistence. At the patch-scale, it seems that native and non-native herpetofauna have similar habitat preferences and therefore have positively correlated abundances. The habitat parameters driving this, however, remain unknown as the locations with the highest and lowest abundances of both natives and non-natives do not have any striking differences that are apparent to the researchers.

From a conservation standpoint, our results are a cause for concern. Non-native species constituted the majority of species and over 90% of individuals that were recorded in Miami-Dade County. It seems probable that non-native species will continue to expand in number and geographic extent, while native species will continue to decrease. We should not conclude, however, that native habitat fragments provide no conservation value to native herpetofauna. As we did not survey within the urban matrix itself, it is possible that both park types (native and

non-native) increase the abundance and richness of native herpetofauna, and that Miami-Dade County parks in general are beneficial for the conservation of herpetofauna in the county. Green spaces within cities can offer a variety of microhabitats and structures that support multiple species (Nielsen et al. 2014). Prior studies indicate that city parks and green spaces can serve as hotspots of biodiversity within urban areas, although this diversity is frequently comprised of both native and non-native species (Nielsen et al. 2014).

Additionally, while the pine rocklands do not appear to host a higher abundance or richness of native herpetofauna, the same pattern may not hold true of other taxonomic groups. The size and distribution of the native habitat fragments may be sufficient to maintain populations of native plants and other taxa that are not facing the additional threat of invasive species to the same extent as the herpetofauna. In South Florida, there are more established non-native herpetofauna than anywhere else in the world (Krysko et al. 2011), and as a result, the herpetofaunal communities are dominated by non-native species in all habitat types. Future research that identifies the mechanisms that lead to non-native dominance in this system may be beneficial. For example, are non-native species competitively dominant over native species within native habitats, or are the dynamics within native habitat patches overwhelmed by a continuous influx from the much larger urban matrix? Hopefully, an increased understanding of the mechanisms behind the increasing abundance and diversity of invasive herpetofauna in both native and non-native habits will help guide strategies for conserving native diversity.

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Anole behavioral neuroendocrinology

My fundamental research interests lie in the interplay between the nervous and endocrine systems. Specifically, we are interested in the role hormones have in regulating changes to neural plasticity and behavior, especially in the context of reproduction. Additionally, we are examining the brain's influence in regulating seasonal reproduction through the hypothalamic-pituitary-gonadal (HPG) axis. Our lab uses the green anole lizard (*Anolis carolinensis*) to address various research questions:

How do seasonal changes in steroid hormones impact brain morphology?

It has been well established that the morphology of certain parts of the brain in seasonally breeding animals are altered between breeding and non-breeding seasons. In anoles, areas of the brain related to reproduction, such as the preoptic area and ventromedial hypothalamus, are larger in breeding compared to non-breeding individuals (Beck et al., 2008). Sex steroid hormones, such as testosterone and estradiol, also fluctuate seasonally and are likely candidates for influencing these morphological changes. Furthermore, adult neurogenesis can alter neuron number in the brain and sex steroid hormones have been shown to play a role in the survival and integration of new adult-born neurons in a variety of animals (e.g., Cohen et al., 2016). In lizards there are recent reports of the addition of adult-born neurons to the brain (LaDage et al., 2017; McDonald and Vickaryous, 2018), but no information is currently available on adult neurogenesis for *Anolis* species. We are examining whether sex steroid hormones impact neurogenesis in brain areas controlling reproduction in steroid hormone-treated *A. carolinensis*.

How does the brain seasonally regulate steroid hormone levels?

Anole lizards breed seasonally, with high levels of circulating sex steroid hormones in the breeding season, and low levels during the non-breeding season. Thus, the regulation of steroid hormone production likely differs across seasons in this lizard, as it does in other animals. Recent work from graduate student Christine Peek has shown that steroidogenic enzyme mRNAs are differentially expressed across seasons in *A. carolinensis* gonads and brain, with steroidogenic acute regulatory protein (considered the rate limiting step in steroidogenesis) highly expressed in breeding gonads and aromatase (estrogen production) highly expressed in

non-breeding brains (Peek and Cohen, 2018). Current work in the lab is examining the gene expression of peptides that control HPG axis function. For example, kisspeptin is a known positive regulator of gonadotropin releasing hormone (GnRH) in mammals, but its expression has only been documented in non-breeding *A. carolinensis* (Dunham et al., 2009). Graduate student Sawan Talwar is characterizing the expression of kisspeptin and gonadotropin inhibitory hormone (an inhibitor of GnRH) in hypothalamic dissections from breeding and non-breeding *A. carolinensis* using quantitative PCR. We are also using *in situ* hybridization to localize the parts of the brain where these peptides are expressed to determine where these peptides are expressed and if expression patterns differ seasonally.

How does the interaction between the HPG axis and thyroid hormone regulate seasonal breeding?

Thyroid hormone is important for gonadal development during puberty, and may also play a role in gonadal growth during the breeding season in seasonally breeding birds (Perez et al., 2018). Similarly, thyroid hormone in the brain may have a role in regulating the seasonal control of the HPG axis in birds (Yoshimura, 2013). In *A. carolinensis*, testicular recrudescence is delayed in hyperthyroid lizards exposed to breeding-like photoperiod and temperature (Turner, 1972), suggesting that appropriate thyroid hormone levels are necessary for normal gonadal growth in this species. Our lab is beginning to examine the interaction between thyroid hormone and the HPG axis by characterizing the seasonal expression patterns of deiodinase 2 (activates thyroid hormone) and deiodinase 3 (inactivates thyroid hormone) mRNA in breeding and non-breeding gonads. Graduate student Hyejoo Kang is also investigating whether the expression patterns of these enzymes are altered in HPG axis-stimulated lizards.

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Protocol for setting up and rearing a successful lizard room

1: Introduction

     Anolis lizards are among the best-known examples of adaptive radiation and convergent evolution. As such, *Anolis* lizards represent one of the prime models for understanding evolutionary biology (Losos, 2011) and behavioral ecology (Huey *et al*., 2004). Housing and breeding experiments with anoles provide an excellent means for estimating heritability and can be a valuable resource for the study of development, adaptation, and speciation. For example, Cox *et al*. (2017) and Ng *et al*. (2013) investigated the effect of environmental and genetic factors on dewlap size and pigmentation with breeding experiments. In addition, by controlling the environment, housing experiments allow scientists to test the effect of specific parameters on a specimen's phenotype or performance. For example, Lailvaux *et al*. (2012) studied the effect of high vs. low food availability on the morphology, dewlap size and bite force of *Anolis carolinensis*. Similarly, Delaney *et al*. (2016) tested whether perch availability affected reproduction in *Anolis sagrei*. Finally, breeding and housing experiments could allow scientists to study phenotypic and developmental plasticity, as the morphology and/or behavior of hatchlings in response to certain conditions can be studied during their ontogeny.

     For my current experiment, we plan to raise *Anolis sagrei* hatchlings on different feeding regimes (hard vs soft diet) and different levels of competition (no contact between males, regular contact, and continuous contact) to see how it affects their head shape and feeding performance by measuring several aspects of head shape (head width, height, etc.) and bite force during the development of the hatchlings to adult. This will allow us to directly evaluate whether differences in diet and/or aggressiveness are influencing the frequently observed sexual dimorphism in head shape and size in *Anolis* lizards. To set up lizard room in Ghent, Belgium, I visited the animal care facility in the Losos lab. There, I was guided and assisted by Anthony Geneva, Colin Donihue, Matthew Gage, Cory Hahn and Jeff Breeze, who shared with me their experience establishing and maintaining an *Anolis* lizard breeding colony.

     This research visit resulted in this document, which details protocols for establishing, maintaining, and conducting research in an Anole breeding colony and can serve as the basis for

creating new facilities. It gives a detailed and comprehensive overview of the specific requirements for setting up a lizard room. In addition, the document provides guidelines and tips on general lizard room maintenance, cage building, hatchling handling, lizard transportation and identification and cricket housing.

The general design of the animal care room was developed for the care of *Anolis distichus* and *A. sagrei* and some portions of this protocol are most useful to these species. The same procedures and facilities have also been used to house *A. brevirostris*, *A. carolinensis*, *A. extremus*, and *A. grahami*. Modification of the cage design described in this document has been used to house larger bodied anole species such as *A. equestris* and *A. leachii*.

2: Creating an animal facility for Anoles

2.1 Room Design

Facilities requirements:
  RO or distilled water
    Reverse osmosis (RO) water is ideal for keeping the lizards healthy. Distilled water is an acceptable alternative. The water is used for general cleaning as well as twice daily misting of lizard cages, which provide drinking water for captive anoles and maintains a humid microenvironment.

  Lighting
    For proper lighting, water-vapor resistant lights (F32T8 fluorescent bulb fixture) should be used. For bulbs, we recommend UV lizard bulbs or, the less expensive, full-spectrum bulbs (32W 6500K). These light bulbs can lose their ability to produce a portion of the UV light spectrum over time and should be replaced annually.
    In general, we employ a 14h daylight/10h darkness scheme during breeding season and 12h light/12h dark for winter cycling. Sanger *et al*. (2008) used a 13h light/11h dark during summer, shifting to 11h light /13h dark during winter months (early October – late March). A short winter period (one-two month) might stimulate reproduction. Some species will eat less during this simulated winter and therefore should be fed less often. To ensure complete darkness, windows must be covered.

  Temperature and Humidity
    We currently maintain room temperature at 84 ± 1° F (28-29° C). For this, a programmable temperature control should be used, ideally with the potential for two settings per day (day and night). Our target for minimum humidity is 40% in the lizard room and minimum of 60% in the cage. A programmable humidity control can be used as well, although high ambient humidity is often difficult to maintain due to air exchange requirements of university vivaria. Our cage design maintains a far more humid microenvironment than ambient room humidity. For most species the higher the

cage humidity, the better (around 80% is optimal). If humidity is too low in the cages, misting more than twice per day may be necessary.

Shelving

The shelves should be made of rust-resistant and UV-resistant material, such as stainless steel with a chrome coating. The space below the bottom shelf is convenient for cricket storage (red arrow in Figure 1), with no light fixture above. The lowest shelf is placed just above the cricket boxes. Each shelf contains several cages next to each other (see picture), with a light installed on the bottom of each. Make sure there is enough space between the lights and the cages themselves, as water and food are provided through the top of each cage (see "Section 2.2 cage building"). Due to this set-up, a light will be above and below the two upper-most shelves (blue arrow), while only one is present above the cages on the lowest shelf (green arrow). Consequently, the cages on the two uppermost cages can be slightly warmer and less humid then the cages on the lower shelves. To prevent a cage effect in experiments, cages could be switched between each shelf on a regular basis. Alternatively, all different treatments can be put on the lower shelf as well, in order to control for any systematic bias.

Figure 1: Lizard facility shelving units. The three uppermost shelves are used for lizard housing (blue and orange arrow); the cages on the ground floor for cricket storage (red arrow).

Figure 2: Building scheme for shelves
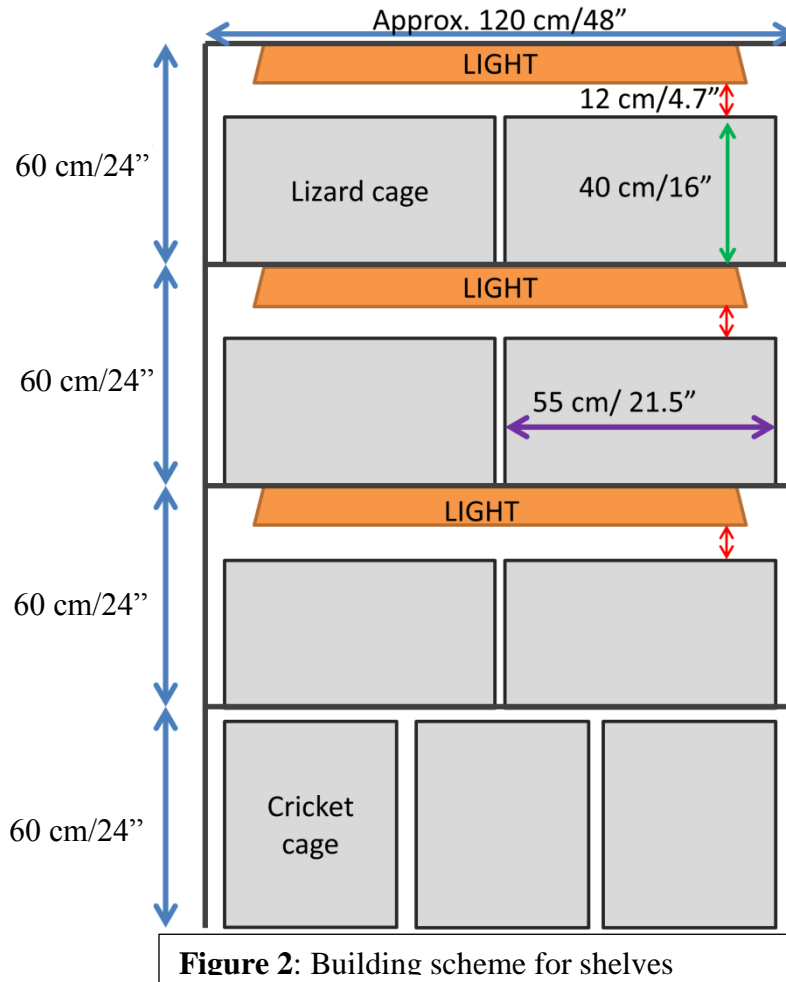
Work areas

Ideally, the room should have floor drains and moisture resistant floor, wall, and ceiling coverings. It's useful to have large standing-height countertop space for work areas and any animal care procedures that may arise.

Procedure area

A separate space for cleaning, specimen preparation, and general storage (consisting of shelving units as well as drawers). This room can be situated within the animal care area or an adjoining room. The temperature in this area does not need to be the same as the animal care area. This area requires approximately 10ft of counter space including a deep basin sink with RO, hot, and cold water taps. This space also contains small 4℃ refrigerator and -20℃ and freezer (can be a single unit as long as -20℃ space in not frost-free).

Autoclave

Cage dressings and soil are autoclaved before use. Autoclave access near to the lizard facility is needed, but a dedicated autoclave is not necessary.

Entry

Ideally at least two sets of doors separated the animal care facility and public space, with room access being controlled via card or key if possible. The intervening space

should have storage cabinets and wall hooks for hanging personal items. Doors should have tight seals on all sides, and a sweep underneath to prevent cricket escapes.

Cricket storage

Crickets are stored in separate containers depending on their size (pinheads, ¼ in, 1/8 in and 3/8 in; or in mm: pinheads, 32mm, 64 mm, 96 mm). Containers should be high enough to prevent them from jumping out. Several large holes should be made in the cover of each container (3"/ 7.5 cm diameter). These holes should be covered with a screen to prevent cricket escape. Each container is labeled with cricket size, date of arrival and, if necessary, a box number. We have found it useful to color-code the labels of cricket tubs by size and lizard cages so it is clear which lizards receive which size crickets. These containers are placed on the ground underneath the lowest shelf (see above). For a lizard room of approximately 1000 lizards: consider 38 ft^2 of storage space (12m²).

Signage

It is recommended to have the most useful files printed out and always visible in the lizard room. These signs include: toe clipping scheme, sexing guide, vermiculite mix, and feeding color labels.

Cage labelling

Cage labels contain the following information: cage number, species, locality, origin (wild-captive), specimen ID, sex, introduction date/date of birth and parental information (if required)

Figure 3: Cricket cage, with holes covered by a screen in order to avoid escape.
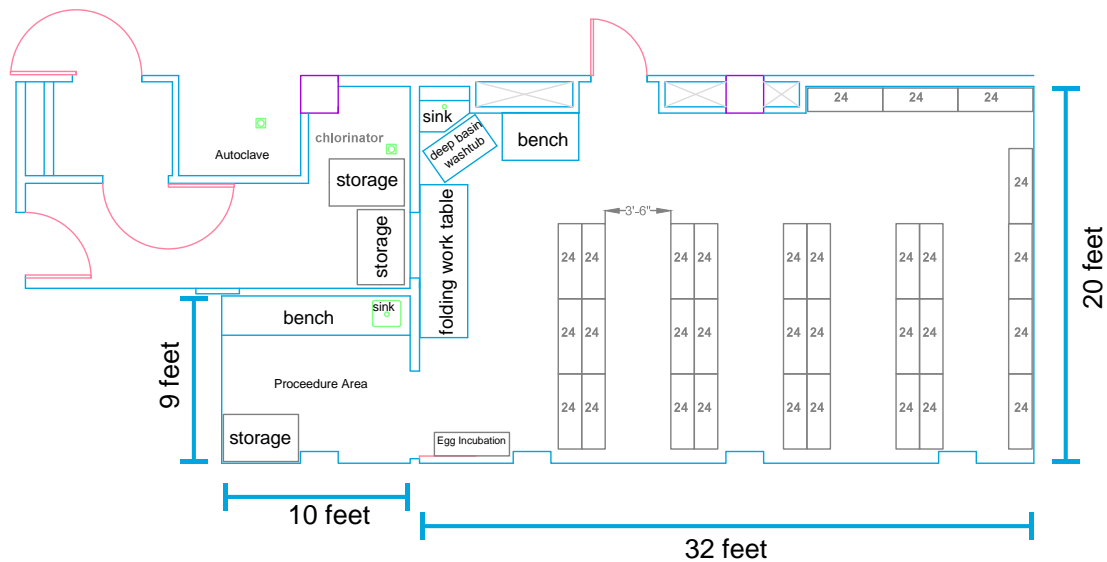
Figure 4: Example of the annotated floor plan of a lizard facility

2.2 Cage building

Materials
  Screen
      Aluminum mesh screen is used to cover the top of the cage. This allows air exchange
      and for misting cages without the need of opening them.
  Acrylic panels
      Cages are constructed from relatively inexpensive, sturdy acrylic panels. Opaque
      acrylic sheets are used for the sides and bottom (to limit interaction between cages).
      Transparent acrylic sheets are used for front and top panels.
  Acrylic Cement
      Sci-Grip thin set acrylic cement (highly volatile, close when not in use).
  Magnets
      Easy, automatic closing system. Should be large enough as larger lizards might be able
      to open the cage when magnets are too small. In case this doesn't suffice, you can use
      two magnets or an additional closing mechanism such as a hook.
  Hinges
      Used to open the door and upper lid. Normally, 1 hinge for the upper lid and 2 for the
      door suffice. As each cage will contain 4 enclosures, 12 hinges suffice for one complete
      cage construction.

Building protocol
1. Connect outer panels with tape (see Figure 5). Make sure they are perfectly perpendicular to each other and edges are lined up!
2. Wear gloves for all gluing steps. Use a syringe and put a small amount of acrylic glue in the corners (Figure 5: blue arrows) and let dry for 1 minute
3. Glue the remaining contact surfaces (Figure 5: Green arrows)

42
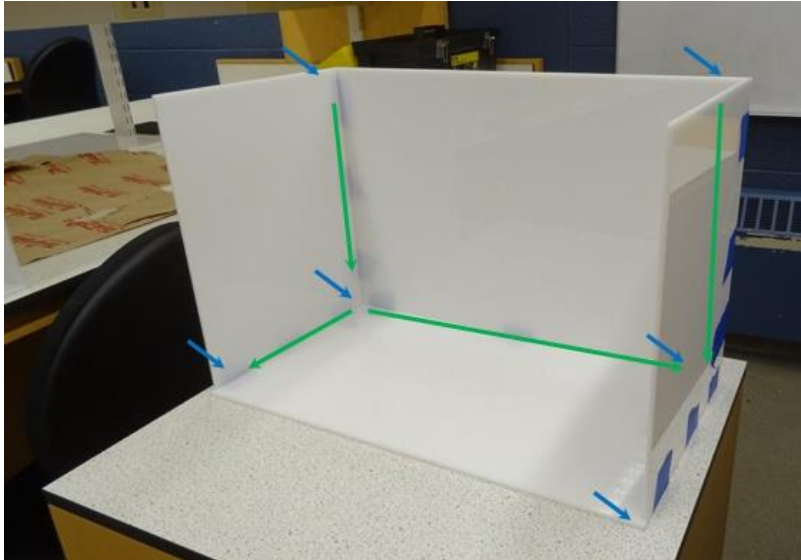
Figure 5

4. Insert cross sections using wooden blocks (use the block on top first, then the one in front). The block on top should be parallel with the acrylic panel at the back.

5. Fix the bottom of each cross section with glue (application to 1 side suffices; Figure 6: blue arrows), subsequently the corners at the upper edges. For gluing these: fist glue the middle cross-section (this makes it easier to press the remaining cross-section panels against the border panel). Add glue through the remaining unglued parts (Figure 6: red arrows). Remove the blocks and glue the parts that were covered by the blocks (Figure 6: orange arrows)
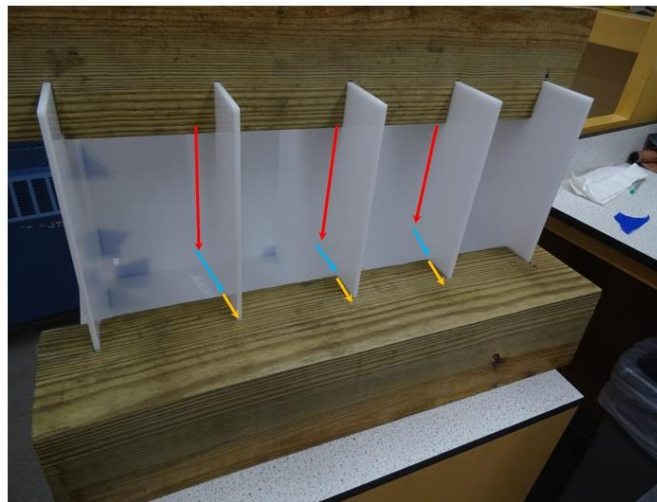
Figure 6

6. Take transparent acrylic parts and sand the sides that will be touched during feeding and cleaning. Wear a mask during this step.

7. Use ethanol to remove dust from the sanded sides.

8. Attach the panels as shown in Figure 7 using tape. Once the panels are stable, glue the lower panels (yellow arrow indicates sanded side) in the following order: First, blue, then

red and finally the panels at the green arrows (Figure 7). Then do the same for the upper panels. Remove tape once the glue is dried.
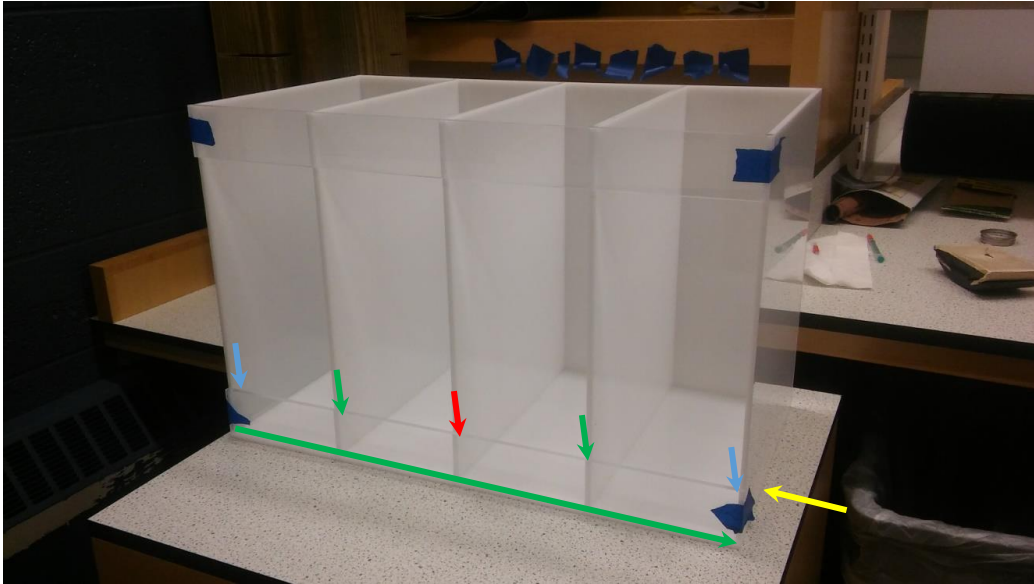
Figure 7

9. Prepare the top of the cage a shown in Figure 8 in the following order: blue, red, green. The panels on the cross-sections (light red) shouldn't be perfectly in the middle, as screen mesh will be covering the openings. Yellow arrow indicates stranded edge. To attach these panels, you will put the syringe upward, so make sure to wear goggles.
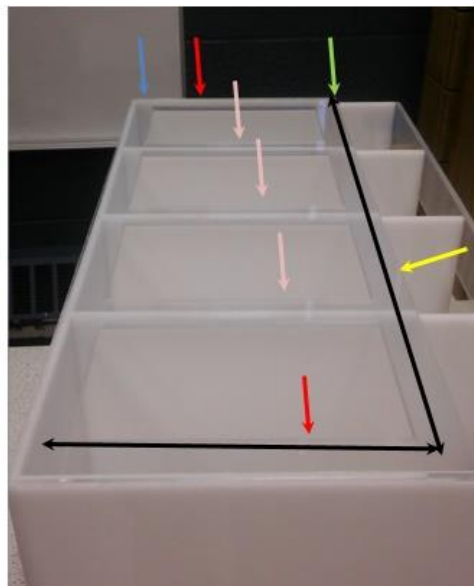
Figure 8

10. Measure the required size of the mesh screen, the screen should normally reach only to half of the transparent upper panels (black arrows in Figure 8). Normally, the size should be 20.5X53.5 cm (8 X 21 in).

11. Cut the screen mesh to the proper size, remove any loose ends.
12. Put screen on upper layer and attach the first transparent panel. Align this panel as best as possible with the one below using tape. Add glue on both sides of the panel (see arrows in Figure 9).
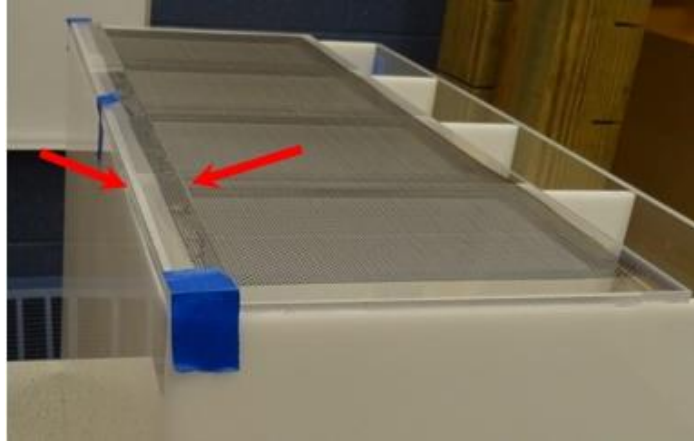
Figure 9

13. Now you can build up the other panels as shown in Figure 8. Make sure all panels are aligned as best as possible and again, make sure the screen mesh has a wet look by using a lot of glue. The screen mesh should only bulge in a little when being touched after gluing. The cage should now look like Figure 10.

Figure 10

14. Put some large, heavy objects on the upper borders to improve glue bonding.
15. While the glue is drying, take the final transparent panels used as "front door" and "upper lid". Sand three sides of these panels (the upper side that won't be touched can be left alone). Don't forget to wear a mask! Clean the panels with ethanol to remove dust.
16. Remove the blocks from the cage and put the upper lids in line with the cross-sections (the sides of the lids should be on the white of the cross-sections). You can also leave a very tiny space between the sides of each of the upper lids (approx. 1 mm), which allows to easily open and close these lids.
17. Put one hinge in the middle of each panel. Now you can glue the hinges to the panels. Only apply glue on the front and the back side of the hinge as shown below (green arrow in Figure 11). If glue would come between the wrong panels (red arrow), immediately open the lid and remove the glue with paper towels. Yellow arrows indicate stranded sides.
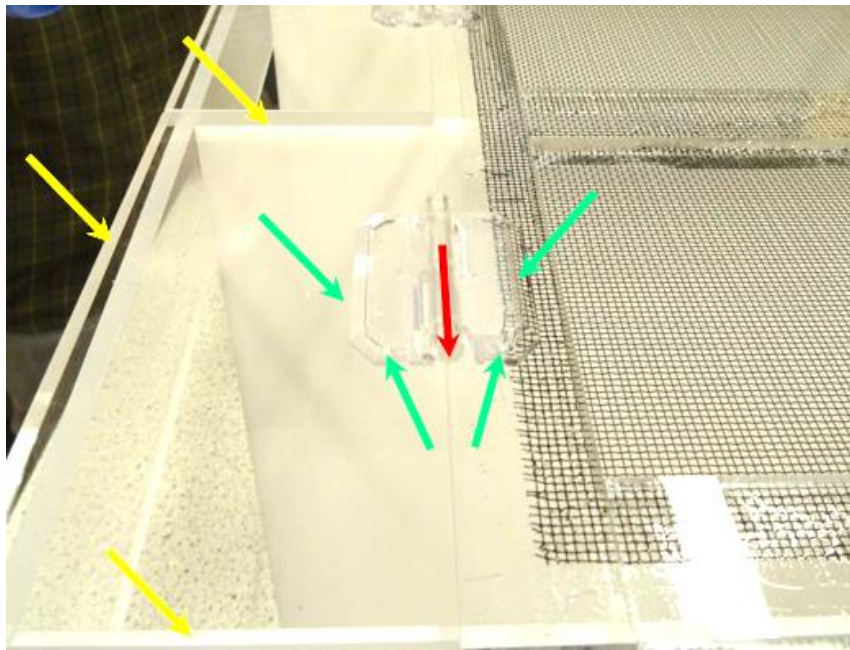
Figure 11

18. Align the four doors and attach them to each other by tape as shown in Figure 12 (makes it easy to move them). You can add more tape if you want. Make sure the sides of each door covers the white edge of a cross-section.
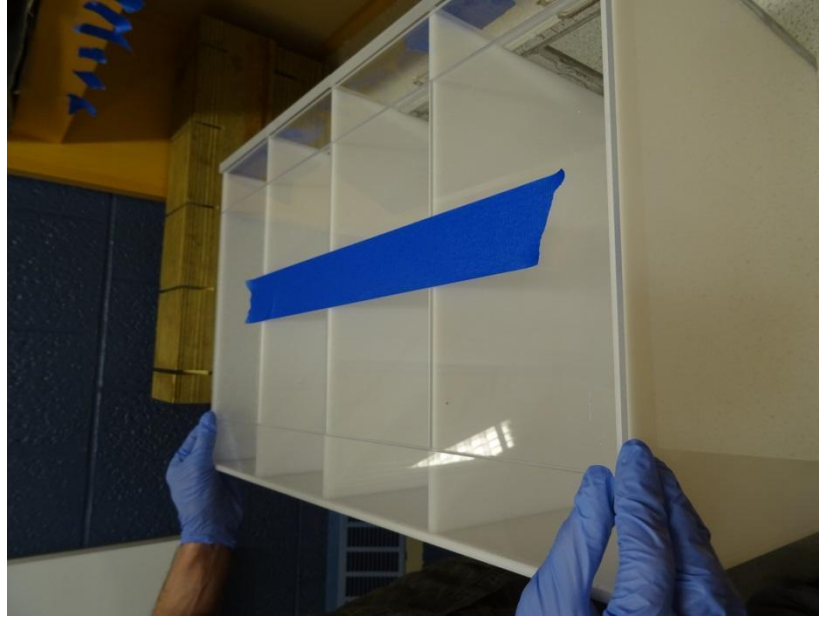
Figure 12

19. Attach two hinges per door as was done in step 17. You can leave a small gap between the door and the lower transparent panel as shown in the figure below (but make sure that a hatchling can't fit through).

20. Make sure your magnets are built as in Figure 13. The rectangular block should make a straight line with the magnet when closed!!

Figure 13

21. Place the upper part of the magnet such that it is in the middle of the door, with the edge with the green arrow above being perfectly in line with the lower edge of the door. Glue the magnet at the position of the green arrows as shown in Figure 14.
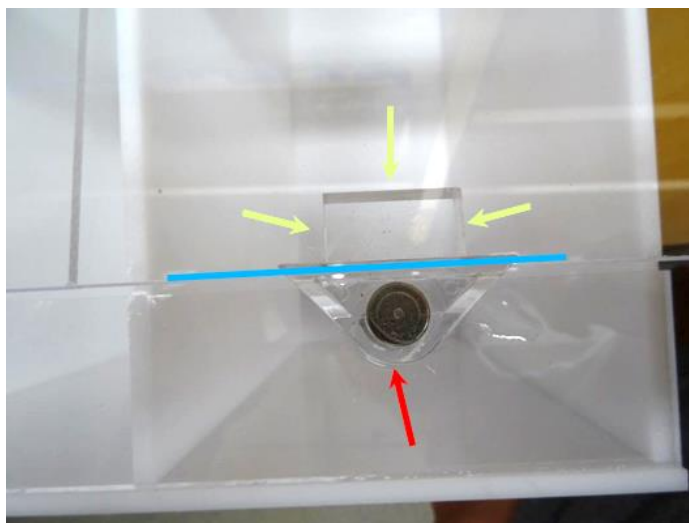
Figure 14

22. Once the glue has dried, prop the doors open to attach the lower part of the magnet.
23. Glue the lower part of the magnet. Make sure the magnet doesn't hang over the edge, so position it as shown below. Put a mark at the edge of the magnet (red arrow in Figure 14 and 15) so you can easily determine its position.
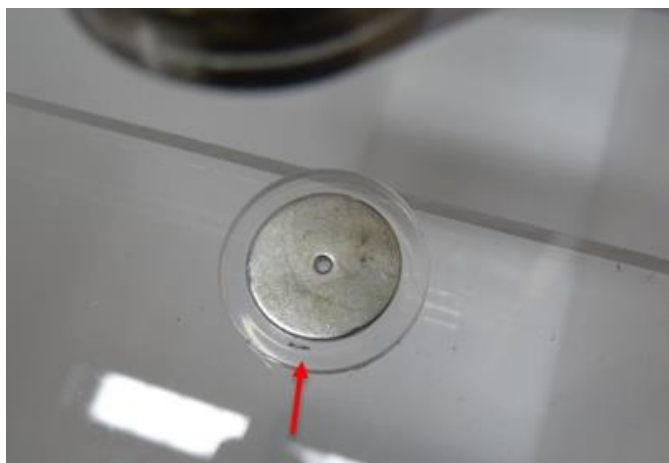
Figure 15

Protocol and hints for building cages (sanding, gluing, etc.)
- Use goggles when applying the acrylic parts on top.
- Make sure all acrylic panels are completely on the shelf (so no parts hanging over edges) as this can warp acrylic panels over the long-term
- Put a piece of cloth or paper underneath the transparent acrylic panels. This prevents them from being scratched when moved, which reduces visibility.
- Use all-plastic syringes. Rubber gaskets are dissolved by acrylic cement.
- If glue accidentally drips on the transparent panels allow it to dry in place. Do NOT try to remove this as it will make a blurry stain on the panels, impairing visibility.

2.3 Cage dressings

Soil

Use "Organic potting mix" as this does not contain any added fertilizer. Autoclave all soil before use. Add 475 cc soil per cage, which provides a thin layer covering the base of the cage which helps maintain humidity but is too shallow for egg laying. Soil should only be changed every three months as this is stressful for the lizards.

Dowels - Preparation (remove adhesive)

Dowels should be similar in size and diameter (0.5 in/ 1 cm) in each cage, as differences in dowel size can cause a plastic response in limb size (Losos et al., 2000). Dowels should be placed across each other to form and "X". Dowels are sanded to remove sharp edges and adhesive labels.

Plants

Plastic foliage can be used for each cage. These can be reused and cleaned by soaking in 10% bleach solution for 30 minutes followed by thorough rinsing with RO water.

Egg cups

Polypropylene yogurt cups (1 liter) are used as egg cups. A small hole (diameter: approx. 1 in or 2-3 cm) is made in the lid of these cups where females can dig in and lay their egg. The cups are completely filled with a vermiculite-water mix (18:11 water to vermiculite by weight). Label each cup with the cage number. If the eggs are being laid in the soil instead of the egg cup, this

Figure 16: Completed cage with cage dressings

indicates that the mixture in the egg cup is either too dry or too moist. Replace or remoisten the vermiculite mix in this case.

3: Establishing an Anole colony

3.1 Collecting animals from the field

In field

Butterfly cage

Females can be put together in a large butterfly cage. This cage can easily be sprayed so that the lizards have water.

Cloth bags

Males should be kept separate. For this, you can use a cloth bag with a string that allows you to close it. You can spray these bags so that the lizards have water.

Ice Chests

Put the butterfly cage/cloth bags in an ice chest to move the lizards from the field to your place of stay. Make sure that the size of the ice chest is suitable for the number of lizards you want to transport. Also check the isolating capability of the chest, the more it holds the temperature the better.

Thermal Blocks

Thermal blocks (phase change material) are used to buffer the temperature in the ice chest from extreme highs and lows. The example shown below holds the temperature at 22°C. You can put the thermal blocks outside of the chest while working in the field.

Padding

Use cloths or other soft material to steady the cages and thermal blocks.

Figure 17: Thermal block used to keep a constant temperature in the ice chest

Moving the lizards

Individual Containers

For moving, the lizards are transferred from the cloth bags/butterfly cage to individual containers. We use egg incubation cups for this (for picture, see "Section 4.4 Egg check"). Place a sheet of soft paper in the cup and lightly spray. Also spray the sides of the cup so that the lizard has enough water. Lizards can live over a week without food, so no food needs to be supplied. Put the cups into the ice chest, together with the thermal blocks and proper padding.

3.2 Anole Biology in Captivity

Sperm storage

Female lizards can store sperm (and thus lay eggs) for >4 months on average after copulation. This has the following consequences:

- If females are collected from the field, sexes should be kept separate initially. If you put males and females together before that, paternity with be uncertain. Provide egg laying cups in female cages and check for eggs from wild-caught females. When the ratio infertile/fertile eggs becomes large, you can introduce the male (for genetic experiments, a maximum 1% of the eggs should be fertile; for a non-genetic experiment, the ratio can be higher, but introduces error).
- To start breeding lizards, males should only be put together for a short period (48h – 1 week), after this, the female can reliably lay eggs for 2-4 months.

Reproductive period

West Indian anoles in nature lay eggs from about March through October. Wild-caught females keep following this rhythm even when brought to the lab. Lab-bred lizards are less prone to this. However, both for wild-caught and lab-bred lizards, it is good to induce a winter period of 6 to 8 weeks. For this, the temperature should be dropped by minimum 2° and the light-dark period should be shifted so it is longer dark than light (see also "Lighting" in Section 2.1). After returning to summer, wait 1-2 weeks before establishing breeding pairs.

Generation time

The generation time differs between males and females and among species. In *A. sagrei* females are usually reproductive 6 months after hatching. For males, this is approximately 7-8 months. Once the male develops secondary sexual characteristics, they can be used for breeding.

Aggression (M-M, M-F, F-F)

- General: Hatchlings are housed together if they are born within the same week.
- Male-male aggression: In our experience, males are normally not aggressive if they grew up together. However, aggression might be observed if A) there is not enough food or/and B) a female is visible (e.g. in the cages on the opposing side). In this case, males should be separated. Also make sure that the males that are put together are similar in size.
- Male-female aggression: Breeding pairs can generally be kept together. However, make sure to check the health of the female regularly. While there might not be bite wounds, she might still be stressed.
- Female-female aggression: We have only occasionally observed female-female aggression. If bite wounds are observed, separate the females.

Pairing animals (considerations and best practices)

- Introduce females first and let them acclimate for a week before introducing males.
- In many cases mating occurs immediately upon introduction.
- While normally 6 adult crickets suffice per lizard, this amount should be ~2.5 times as much when pairing animals as males eat substantially more and no food would remain for the females. When feeding breeding pairs, only a few crickets should remain after 2 days. In case all crickets are gone, supply more crickets.

3.3: Setting up cages

Sex segregation

- Males and females can be readily distinguished about 1 month after hatching (see "Section 4.7 Handling cohorts")
- You can keep up to 3 males together in one cage (regularly check for bite wounds). If males grew up in the same cage, they should not interact with each other. Possible interaction might be caused by the visibility of a female or by food shortage.
- Up to four females can be kept in one cage if they are not laying eggs. If females are egg laying, keep them separate.
- There should only be 1 breeding pair per cage.
- The above amounts are appropriate for smaller lizards (*A. sagrei, distichus*, etc.). Larger lizards (such as crown giants) should be kept individually.

Identification: toe clipping

Toe clipping allows for the unique identification of up to 9999 animals (see Appendix for clipping scheme). This protocol results in no more than two toes to be removed from any one extremity and also ensures that adjacent toes are never removed. Toes should be removed at the base of the knuckle using heat-sterilized micro dissecting spring scissors.

4: Maintaining a breeding colony
4.1 Cricket care and maintenance
Cricket ordering
- Check the current stock of crickets before ordering new crickets in order to determine the required amount of new crickets
- When new boxes of crickets arrive, open them immediately and empty each box of crickets into a new plastic bin. Remove any excess paper or cardboard.

Cricket food & water
- Orange cricket cubes (red arrow in Figure 18): enhance the nutritive value of crickets by supplementing calcium and other vitamins. Administer a few of these cubes to the crickets.
- Chicken feed pellet (blue arrow): Add one dose (covers approx. one corner of the box)
- Sweet potato (completely eaten in picture): cut into small pieces and put a few of those on top of the chicken feed.
- Use water crystals (soil moisture granules; green arrow) to provide water to the crickets. This prevents the crickets from drowning. Cover the bottom of a cup and fill it with water, the crystals will absorb the water in a minute. Subsequently, place a lid with a 1cm layer of moistened water crystals in the box. Make sure the water crystals are not overflowing and coming into contact with the food or egg crates.
- Check cricket food & water regularly (each 2-3 days). Add food if this is finished. You can just put new wet crystals over the older crystals when dried (crystals are dried in Figure 18)

Setting up cricket boxes
- For built-up, see Figure 18. Up to 3 egg crates can be kept in the box on one side of the bin.
- Use as many crickets as possible in each box. Around 6000 crickets can be kept per container (pinheads, 1/8" and 3/8") and around 5000 per cage for the largest crickets (1/4").
- Cricket die offs can occur as a result of excess moisture in the cricket bins.
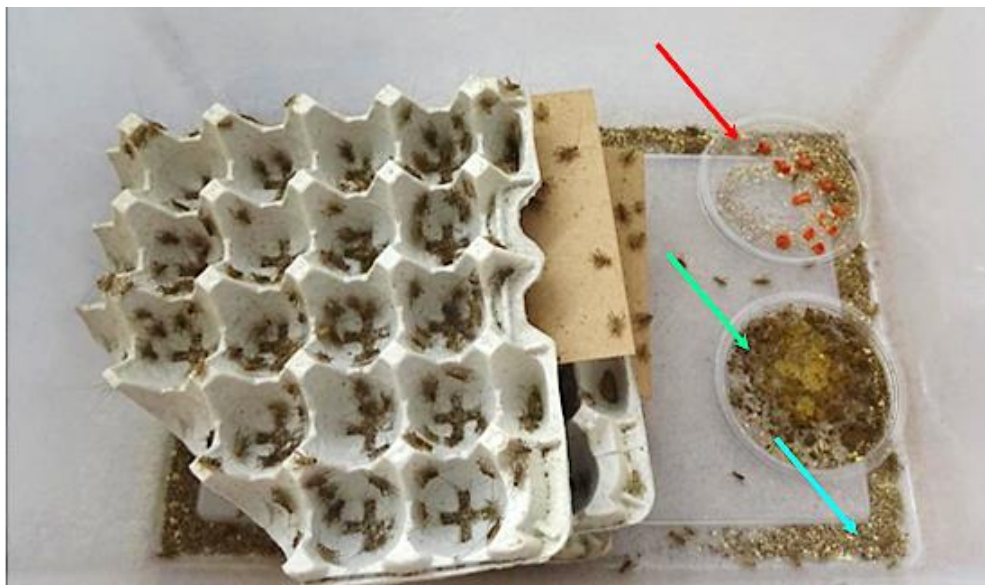
Figure 18: Cage dressing in cricket box

Cleaning cricket boxes
Discard food, egg crates, and water crystals. Rinse the box in the floor sink with hot water to remove as much material as possible, then soak with hot water and dish detergent for at least 10 minutes (and up to overnight). After soaking, wipe down walls with sudsy water then rinse with RO water and dry completely before reusing.

Escaped crickets and other pest arthropods (mites, spiders, etc.)
Crickets may escape while feeding the lizards. In addition, other insects and spiders may be present in the room. Therefore, 8 – 12 cricket traps are placed around the room at least twice a week. The date when the trap is placed is mentioned on each trap. A color code can be used for traps that are placed on the same day. Make sure to also put traps just outside the room for crickets or other insects that might escape. IMPORTANT: Escaped lizards can get stuck in these traps. Therefore, it is important to close of the openings of the traps with some tape as shown in Figure 19 This way, the opening of the trap is still large enough for insects to enter, but too small for lizards.

Figure 19: Entrance to cricket trap is partially blocked in order to avoid lizards getting stuck.

4.2 Feeding Procedures
How to prepare the feed (+ tips & tricks)
- To ensure that the lizards obtain enough vitamins, vitamin D and calcium, these are added to the cricket diet. Fill a separate cup with vitamin mix (50%) and vitaminD+Ca mix (50%). The vitaminD+Ca mix should be phosphorus-free! If excess bone growth is observed in lizards, reduce the frequency of calcium supplementation.
- Crickets can be collected most easily by emptying the egg crates in a separate bucket (for pinheads, this can be immediately in a small deli cup, for larger crickets use a flexible bucket that is high enough so crickets cannot escape. When removing the egg crates, be sure to prevent that the egg crates come into contact with the water crystals.
- Dust the pinheads and crickets with the vitamin-mixture
- Use the flexible bucket as a funnel to put the dusted larger crickets in a smaller deli cup. Fill this cup to around 1/3 its depth (more might cause the crickets to die before being given to the lizards)
- The crickets can now be given to the lizards

How to add lizards in cage
- Before lifting the cage lid, make sure that no lizards are near to prevent escape. In case lizards are close to the lid, tap the cage so they move away.
- In case any dead animal is present in the cage, remove it prior to feeding.
- Use the deli cup to drop the crickets into the cage.
- Before closing the lid, make sure the tail of the lizard will not be crushed! Sometimes, it is better to let the lizard escape than to hurt it by dropping the lid.

How many crickets and how often
- Hatchlings are fed daily. They can get 6-12 pinheads per individual. All (or at least most) pinheads should be eaten the day after. If there are still pinheads present, give a lower dose (pinheads can bite and wound hatchlings)
- For older/larger lizards: give crickets appropriate for their head size. If the cricket fits their mouth, they will eat it. These lizards are fed 6-12 adult crickets per lizard 3 times a week. Make sure to check whether most crickets are gone from previous feeding. While this level of feeding is appropriate for *Anolis sagrei,* differently sized species may need more or fewer crickets.
- If lizards are hungry, they start eating immediately.

Cage labeling
Feeding is simplified if each cage is labeled with a color that corresponds to the cricket size that the lizards should get. The labels on the cricket storage boxes should match this color.

Drosophila for hatchlings:
When a cricket shipment goes wrong, it is especially important that the hatchlings get their food. One can hold a *Drosophila* population as a reserve in case this happens.

4.2 Animal health check
- Feeding time is the perfect opportunity to check on the health of all animals. When providing food, be sure to check that all animals are present and healthy.
- Following signs indicate that the lizard may have health issues:
  1. Lizard is laying on its back
  2. Lizard is sitting on the soil
  3. Lizard has a darker color (stress)
  4. Lizard has been eating soil (will die very soon)
  5. Visible tumor growth
- Check older animals for bite wounds: female bite wounds often indicate a shortage of food; male bite wounds might indicate that there is a shortage of food or that they can observe females
- Sick animals can be placed in separate bins with paper on the bottom. In case necessary, the animals can be fed by hand.

4.3 Misting
Setup and equipment
MANUALLY: hose and a nozzle with multiple settings (should include mist-setting). Taps for daily spraying must be able to remain open without being held.

AUTOMATIC: Large basin from which water can be pumped. Automatic misting system, including timer, pump, hose and a nozzle for each cage. Make sure the nozzle points downward towards each cage. Depending on the number of cages, multiple pumps should be used.

RO water

Use RO or distilled water to spray the cages. Set the nozzle to the "mist"-setting and try to spray on each of the walls of the cage, while avoiding to saturate the soil.

Frequency

Each cage should be sprayed twice a day. Once before 10 AM and once after 4 PM. If the humidity in the cages is too low, spray more frequently.

4.4 Egg checks

Division of labor

Depending on the amount of eggs, the work can be subdivided among different people. First, collect all the egg cups. Subsequently, one or two people empty the egg cups in a separate bin and check the vermiculite for eggs. In case an egg is present, it is moved to an incubation cup which should be labelled immediately. Used vermiculite is thrown away. A third person subsequently cleans the empty yogurt cups. Once cleaned, one or two people refill the egg cups with the vermiculite mix. Finally, one person is responsible for putting the egg cups back in the right cage.

Making egg incubation cups

Incubation cups can be made in transparent, plastic boxes as shown in the picture below. Fill each cup with a 130g vermiculite/water mix (18:11 water to vermiculite by weight). Eggs should be put on top of the vermiculite/water mix. In case not present, make small holes (appr; 2-3 mm) in the cup to provide oxygen. Close the cups once finished.
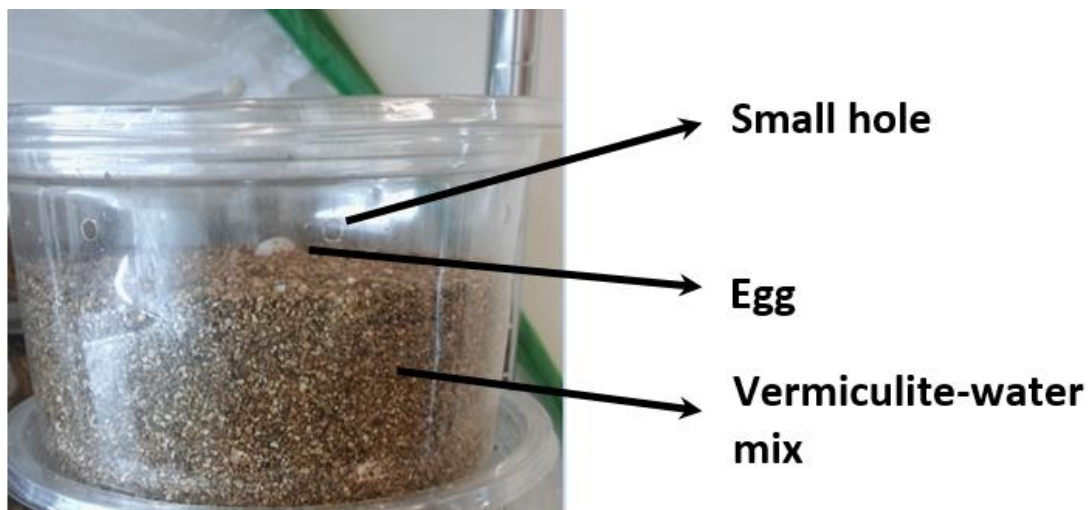
Figure 20: Template for egg incubation cup

Labels

Each egg is labeled with a specific number (which will also be the ID of the hatchling). Put this number both on the lid as the cup itself. This way, eggs can be linked to their origin (see "Record keeping on fertile and infertile eggs").

Distinguishing fertile and infertile eggs

Infertile eggs are typically yellow, small and uncalcified, whereas fertile eggs are white, large and calcified (see Figure 21).
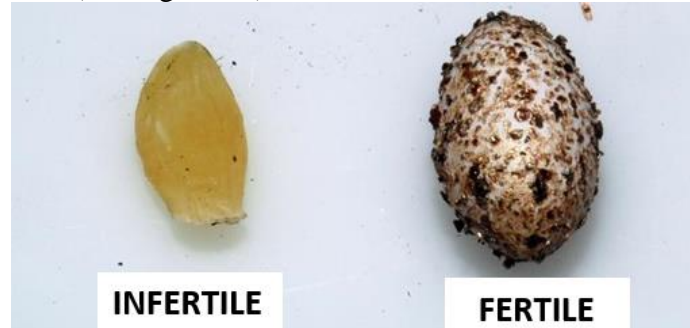
Figure 21: Infertile vs fertile lizard egg

Cleaning, checking, rehydrating egg laying cups (yogurt cups)

Gloves should be worn and washed or changed throughout egg checks to prevent disease spread among cages. The yogurt cups are checked once each week. All vermiculite should be sorted and rehydrated if too dry. Wash egg cup and lid before returning the cup to the cage. If a female is laying her eggs on top of the yogurt cup or in the soil, this usually indicates that there is something wrong with the vermiculite mix (either too dry or too moist). In that case, remove the yogurt cup and replace the vermiculite.

Record keeping on fertile and infertile eggs

The relative production of fertile and infertile eggs is often informative, and critical data for measures of reproductive isolation. Make sure that in your record keeping file contains a column in which the state of the egg can be mentioned: Y (Yellow or Infertile) or W (White or fertile). Keep track of the amount of infertile eggs compared to fertile eggs.

Animal record keeping: Paper and digital

Make sure both a hard copy with all information of the egg/hatchling and a digital version of this info are present

4.5 Managing incubating eggs

Considerations and best practices

- Check incubating eggs daily for hatchlings or failed incubations. It takes approximately 1 month for *Anolis sagrei* eggs to hatch.
- If a dent is present in the egg, this indicates that the vermiculite mix was made incorrectly (too dry). Record the dent and mist or add a drop of water to dented eggs to try to restore it.
- Just before hatching, condensation might be observed on the egg. This is commonly called "sweating".

4.6 Newborn lizards

Introduction of newborns

- Check the egg cup daily for new hatchlings, they will often be running on top of the vermiculite mix.
- Provide an empty incubation cup with folded, moistened paper towel. Transfer the hatchling to the new cup and leave the hatchling there for a few minutes, so it can remove vermiculite that is attached to its body.
- After ~5 minutes, you can grab the hatchling gently (best to hold it at its thigh) and remove remaining vermiculite around the eye or body with a paper towel.
- Weigh the hatchling and report its weight in the data log.
- Then toe clip following the toe clipping scheme posted in the lizard room (see Appendix).

Tips and remarks

- Hatchlings are easily stressed due to excessive handling. If toe clipping takes too long, one might put the hatchling back in the cup for a few minutes so it can relax again.
- Try not to hold the hatchling at its body cavity, they might overheat! Again, holding them around the thigh is the best strategy.
- For toe clipping, either use your bare hands or gloves that are one size too small so they are stretched. This makes it easier to spread the toes for toe clipping. In case using your bare hands, do not forget to wash these first!
- Remember: lizards might play dead. In that case, also put them back in the cup to relax them again.

4.7 Handling cohorts

Sexing juveniles

Male *Anolis sagrei* develop a dewlap around 3 months of age. Determining the sex of lizards can be complicated for young animals. The most reliable method to determine the sex of an animal is the presence or absence of enlarged post-anal scales. Males have two enlarged scales a few rows below their cloaca whereas females will have more or less evenly sized scales in each row. Even hatchlings will show a difference in this trait when observed under a microscope. The scales become more easily observable when the lizards get older. The post-anal scales of males should be easily visible by scope after 1 month.

Grouping of hatchlings, juveniles and adults

- HATCHLINGS: 4-6 hatchlings can be kept together in one cage, as long as they are born in the same week.
- JUVENILES and especially ADULTS should be separated by size and sex. A cage can contain either 3 males, 4 females or 1 breeding pair. Make sure that the lizards in one cage are the same size.

4.8 Cage Cleaning

Cages are generally cleaned as follows:

- Remove enrichment: plastic plants and dowels can be kept in a separate cleaning bin.
- Vacuum the cage to remove soil.
- Rinse the cage with a bleach solution (10% bleach – 90% water).
- Using a paper towels remove any all traces of dirt and feces from cage.

- Rinse cage multiple times with RO water to remove residual bleach and let the cage dry for 24 hours.
- For the plastic plants and dowels: fill a basin with a 10% bleach solution and put the material in the basin. Keep it in there for a couple of hours. Then remove the solution and rinse the material several times with water. This material can be reused once dried.

4.9 Managing Lizard Issues
Escapes
- All walls, ceiling, and spaces under cabinetry must be sealed to prevent lizards or crickets from escaping. All air vents and floor drains should be covered with fine mesh screening. Even small holes should be covered as hatchlings might get in these.
- It might be better to let a lizard escape rather than closing the lid quickly as the lizard might get stuck between the cage and closing lid, causing large wounds.
- In case a lizard escapes, try to capture it immediately!

Disease
- In case a lizard is sick (see also "Section 4.2 Animal health check"), you should separate it from the other lizards.
- As long as a lizard is able to feed, there should be no problem. As the lizard is no longer able to feed, you may try to hand feed using a syringe.
- In case the animal is too sick, one might want to contact the local vet or euthanize the animal (See section "5.4 Euthanasia and preparing museum specimens").

Mortality
- It is critically important that you properly identify and label dead animals. It is very difficult to undo mistakes made at this stage!
- FAILED EGGS: See "Section 4.5 Managing incubating eggs"
- HATCHLING: Remove hatchling from cage. Record death date in data log. Store in 2 mL tube with hatchling number labeled on the outside in sharpie and inside the tube in pencil on a piece of paper.
- DEAD JUVENILES/ADULTS: Carefully identify animals using toe clipping. If there is any ambiguity in the toe clipping due to decay, identify every other animal remaining in the cage and determine the number of the dead animal by elimination. Using a single line to cross off the dead animal from the cage, leaving the information legible. Enter death data for each animal in the binder. Remove ½ of the tail and put it in 2 mL tube filled with 100% Ethanol with the specimen ID on the outside of the tape and inside the tube on a piece of paper, written in pencil. The rest of the dead animal should be placed in a ziplock bag and placed in the refrigerator. Using a sharpie writing the following information on the outside of the bag: Specimen number, Death date e.g. (05 July 2016) and your initials.

5: Protocols for research in a colony

5.1: Establishing an appropriate block design
Different shelves often have different temperature/humidity conditions
  As mentioned in "Section 2.1 Room set-up" under "Shelving", different shelves might have different conditions. The upper shelves will have a light both above and below, whereas cages on the lower shelf only have a light above. Consequently, cages on the lower shelf could have a lower temperature and higher humidity. This could lead to a cage effect. It is

possible to change the cages between the different shelves regularly, but this is unhandy and stressful for the lizards. A proper block design allows us to take into account a potential cage effect. An example of a proper design (3 different conditions) is shown below.

	CAGE 1	CAGE 2	CAGE 3	CAGE 4	CAGE 5	CAGE 6	CAGE 7	CAGE 8
ROW 1	Cond 1	Cond 2	Cond 3	Cond 1	Cond 2	Cond 3	Cond 1	Cond 3
ROW 2	Cond 3	Cond 1	Cond 2	Cond 3	Cond 1	Cond 2	Cond 3	Cond 1
ROW 3	Cond 2	Cond 3	Cond 1	Cond 2	Cond 3	Cond 1	Cond 2	Cond 3

6: Record keeping and common room-wide metrics for calculation

6.1: Updating online records
As mentioned before, each new hatching, egg find or death should be immediately noted on a hard copy file. It is, however, important to update this information in the online-file as well. Do this at least once a week, in order to have a backup when one of both files goes missing.

6.2: Consistent identifiers
To make identification easier, each new egg immediately gets an ID and keeps this ID throughout its whole life. This makes it easy to track all information related to a certain specimen.

6.3: Counts of fertile and infertile eggs
As mentioned previously, it is very useful to determine the amount of yellow eggs vs white eggs. This ratio can be used as a proxy for the fertility of a female. Once the yellow egg/white egg ratio becomes low, a male should be introduced. Furthermore, for genetic experiments, one should wait for the yellow egg/white egg ratio to be very high. When approximately 99% of the eggs are infertile, a male can be introduced with a very high chance that the fertile eggs produced by the female afterwards stemming from the introduced male.

6.4: Survival etc.
Two interesting metrics to determine are "incubation period" and "survival period". The incubation period can generally be calculated by "date of egg find – date of hatching". However, remember that this only an approximation, as egg cups are only checked once a week for new eggs. Survival period, on the other hand, can be determined by "Date of death – Date of hatching".

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Toe clipping scheme (modified from Ferner 2007)
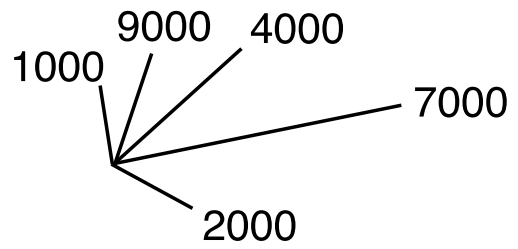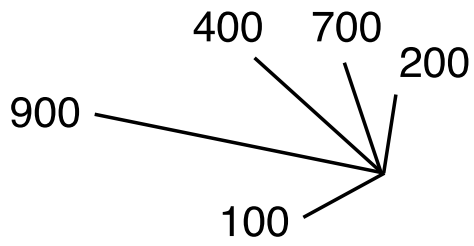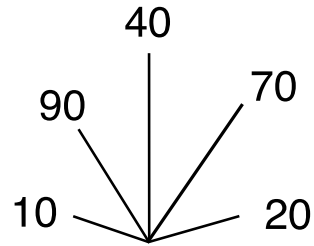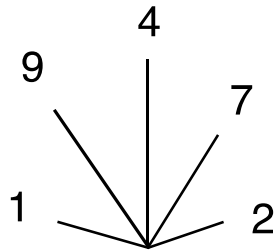
Appendix: Size of cage components (in inches)

Part name	color	thickness	Length	width	quantity per unit	100 units	200 units
Lid long	clear	0.25	22	1	4	400	800
Lid short	clear	0.25	7	1	10	1000	2000
Trap Door	clear	0.5	5.5	3	4	400	800
Bottom	white	0.25	22	12	1	100	200
Back	white	0.25	22	14	1	100	200
Sides	white	0.25	14	11.5	5	500	1000
Anchor/Dam	clear	0.25	22	2	2	200	400
Doors	clear	0.25	9.875	5.5	4	400	800
door knobs	clear	0.5	1	0.5	4	400	800
trap door knobs	clear	0.25	1	0.5	4	400	800

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A report on *Anolis nubilus* from the now rat-free island of Redonda

The island of Redonda is rugged, remote, and unique. It is surrounded on all sides by tall cliffs, some 400 m above sea level, and separated by deep water from the islands of Nevis, Montserrat, and Antigua, in the Lesser Antillean island chain. Redonda is home to large colonies of brown, masked, and red-footed boobies, magnificent frigate birds, and red-billed tropicbirds and its isolation has facilitated the evolution of three endemic lizard species, the Redonda ground lizard (*Pholidoscelis atrata*), an as-yet unnamed dwarf gecko (*Sphaerodactylus sp.*), and the Redonda tree lizard (*Anolis nubilus*). Despite the imposing cliffs, humans inadvertently introduced the Black Rat (*Rattus rattus*) to the island, and, much like elsewhere in the world, the rats have negatively impacted the native flora and fauna, contributing to the extinction of an endemic skink and extirpation of the Antiguan burrowing owl. Goats were intentionally brought to the island around the turn of the 20th century and have had an even more devastating effect on the vegetation, turning the island into a dusty, dry, moonscape.

In 2017, the Government of Antigua and Barbuda decided to eradicate the non-native mammals from the island in hopes of protecting the bird colonies and lizard populations and restoring the vegetation. While these eradication efforts are being carefully monitored to assess whether the ecosystem is restored and the native populations are recovering, such an invasive removal provides a unique opportunity for experimental evolutionary studies investigating how local populations evolve following release from these pests and a return to a more natural food web. We took the opportunity to collect baseline data on the natural history of *P. atrata*, and *A. nubilus*. In 2018, we returned to the island to resurvey the lizard populations.

We intend to publish the results from our studies on changes in the demographics, behavior, morphology, performance, and diet of these species following the eradication in the future. So, here, we will instead update one of the few published accounts of accessing Redonda in search of *A. nubilus,* with our own natural history observations in an attempt to share information on this

Redonda from afar.

magnificent island and completely not-too-shabby lizard.

Accessing Redonda

In the 1972 species description, Skip Lazell colorfully related making the harrowing jump from a bouncing boat to Redonda's rocky shore in 1964. The shore is some 300 m below the spine of Redonda where the lizards are typically found, so he then climbed up a guano-coated sluiceway in order to get to *A. nubilus*. Lazell was on the island for a few hours, noted the distinct lack of trees for the tree lizard, and caught a number of individuals in and around a concrete bunker-like ruin which belonged to the operations manager for the abandoned mining operation that was on the island at the turn of the 20th century. Another researcher who attempted a revisit in the 2000s tried to swim from a boat to the island twice but never managed to climb the steep slopes. The sluiceway from the 60s has now become impassably treacherous due to rock slides and loose soil, and so we opted to get on the island by helicopter. In 2017, we stayed on Redonda for eight days; for the revisit in 2018, we stayed for seven.

Photos from the same position on Redonda showing the difference in vegetation between March 2017 (left) and March 2018 (right) following the eradication of rats and removal of goats from the island.

Notes on *Anolis nubilus* from the field:

Anolis nubilus is at first blush a relatively innocuous member of the genus. They're perfectly camouflaged in the dry, dusty environment of Redonda (which has been present at least for the last century), which is to say they're drab gray and brown. Their dewlaps are cream-colored (which is to say drab gray-yellow), and the most elaborate of the females sport faint dorsal stripes. Males did regularly display impressive crests behind their heads, but nonetheless, the species is considerably less flashy than many of their cousins on nearby islands.

Woody vegetation on Redonda is limited to a non-native *Casuarina* tree planted next to the managers house (which in 2018 was poisoned by the conservation and eradication team to prevent its spread), and a handful of *Ficus* trees that managed to survive the ravenous goats that had the run of the island for decades. While a lucky few anoles have made their homes on these trees, well out of the reach of rats and the predatory ground lizard, most of the anoles on Redonda can be found on boulders.

Average perch height was 150 cm, and the average diameter of the boulders they were perched on was just over a meter (106 cm; n=60). Perch choice did not differ between the sexes. Our perch height data, however, did have a long tail; the maximum perch height we recorded was a death-defying lizard perched on a branch jutting over a cliff's edge, 40,000 cm (we anticipate some margin of error in that approximation) above the sea. AH caught that fellow.

Anolis nubilus *male (left) and female (right) on Redonda.*

Male *A. nubilus* are substantially larger than females; average male SVL was 77 mm while females averaged 54 mm (30 of each sex). Males were thereby also heavier (average of 13.7 g as opposed to 4.2 g), and they had substantially stronger bite forces (male average 51.6 N as compared to female average of 15.3 N). Fourteen of the 30 adult females we captured on March 3rd, 2018, were gravid. The diet of both males and females was largely composed of small insects, particularly ants.

The Redonda tree lizard, while waiting for their trees to regrow now that goats have been extirpated, are at risk from predation by the roving ground lizards. During our week on Redonda in 2018 we observed anole predation by ground lizards on three occasions, including the capture and killing of a large adult male. Now that rats are off the island, the ground lizards pose the greatest threat to anole individuals. That said, despite the predation threat, the populations of both species have increased substantially post-eradication.

Perch height for this male A. nubilus *was 400 m. Photo Credit: Geoffrey Giller*

Future directions:

   Our hope is to continue revisiting Redonda to track the populations of *A. nubilus* through time. We predict that as the vegetation on Redonda recovers from the rat and goat pests, *nubilus* populations will increase in numbers and density. These populations will eventually move from boulders to saplings, and ultimately trees, as woody vegetation becomes established again. Until this happens, earthbound *A. nubilus* will increase their flight initiation distances in response to the increased threat of the ground lizards, whose population should also increase. Furthermore, the eventual shift from rock-hopping to tree-climbing may ultimately be accompanied by shifts in limb morphology.

Additional photos and videos are available at colindonihue.com/rat-eradication-on-redonda/

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Plasticity in hatching of anoles

Remarkably, animal behavior starts in the embryo. Embryos can increase their survival by hatching early, delaying hatching, or hatching synchronously in response to a variety of environmental cues (Warkentin and Caldwell, 2009). One type of environmentally cued hatching involves embryos hatching early in response to an imminent threat. For example, embryos of the frog *Agalychnis callidryas*, which normally hatch spontaneously in about seven days, can hatch up to three days early when threatened by predation from snakes or wasps; embryos can distinguish between vibrations given off by predators and those given off by benign sources such as wind and rain (Warkentin, 2011a).

The taxonomic distribution of early hatching in response to predators is virtually unknown, however, leading to an excess of possibilities for how it might evolve in different organisms. Recently, a review and a spate of published anecdotes have revealed early hatching in lizards, including anoles (reviewed in Doody, 2011; Doody and Paull, 2013; Doody and Schembri, 2014a, b; Doody et al., 2015; Hernandez et al., 2017; Doody et al., 2018), raising the possibility that early hatching is common and widespread in lizards. This has important research implications; early hatching appears to be uncommon in frogs (Warkentin, 2011b; S. Doody, unpubl. data), reducing our ability to track its evolution in that group. In contrast, the presence of early hatching in skinks, geckos, whiptails and anoles increases the likelihood that a diversity of mechanisms and contexts await discovery! Moreover, the lizard life cycle is much different than that of frogs; this fact changes the context within which predator-induced early hatching can evolve. For example, *A. callidryas* embryos hatch and fall from leaves into the pond below where they face very different predators as tadpoles than they did as embryos, while reptile embryos and hatchlings are likely the target of the same predators. A disadvantage of working with early hatching in reptiles is that we

Figure 1. A delicate skink (*Lampropholis delicata*) hatching early in response to its egg being prodded by the back end of a bamboo skewer. Photograph by N. Pezaro.

have not identified which egg predators same predators. A disadvantage of working with early hatching in reptiles is that we have not identified which egg predators are driving the evolution and maintenance of early hatching in lizards. It is likely, however, that lizard eggs are routinely consumed by insects and other invertebrates, mice, rats, snakes and other lizards.

My lab is thus focused on early hatching in response to predation in lizards, including anoles. Our current main thrusts are (1) What is the taxonomic distribution of early hatching in lizards? In anoles? (2) What are the predators of anole eggs, and which predators might have driven the evolution of, or be driving the maintenance of, early hatching in anoles? (3) How early can anoles hatch, in terms of age and developmental stage? (4) What are the costs of early hatching, in terms of body size, performance and survival? (5) What cue(s) do embryos use to perceive predation risk?

We currently have gravid mothers and incubating eggs of 10 species, including five species of anoles, in the lab at University of Florida – St. Petersburg. We are excited to begin answering research questions this Summer and Autumn, and over the next several years. Sean Sullivan's Masters thesis will contain much of the initial research, and we have a swaggle of volunteers getting work experience.

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Claire Dufour

Ongoing and future research on *Anolis*

Invasive species are a global threat to biodiversity, driving species to extinction and imperiling ecosystems. Therefore, understanding how invasive species successfully establish in new environments and their impacts on native species have become some of the main contemporary challenges. Cases where the invasion has been tracked since its beginning are rare, however, such that the first interactions between invasive and native species remain poorly understood. During my postdoctoral research, I study the recent interaction between two closely related species of *Anolis* on the island of Dominica: the sole native species *Anolis oculatus* and the invasive species *A. cristatellus* (native from Puerto Rico). With an empirical approach comparing monospecific populations (allopatry) and co-occurring populations (sympatry), my postdoctoral research revealed the impact of the interspecific competition on the behavioral (Dufour, Herrel & Losos, 2018), ecological (Dufour, Herrel & Losos, 2017), morphological (Dufour, Herrel & Losos, 2017; Dufour, Losos & Herrel, 2018) and physiological traits (Dufour, Losos & Herrel, 2018) and the role of hurricanes in the selection of physiological and morphological phenotypes (Dufour, Donihue, Losos, Herrel, in prep).

My ongoing research asks whether the interspecific competition drives differences on the display behavior towards conspecifics and heterospecifics. To determine the display behavior under natural conditions and towards a standardized signal directly *in natura*, we built lizard robots representing an averaging of *A. oculatus* vs *A. cristatellus* allopatric males in terms of display behavior, morphology and dewlap color (Dufour, Herrel, Clark, Losos, in prep). This experiment reveals the impact of interspecific competition on species recognition and agonistic and social behaviors of species.

A second aspect of my research focuses on the invasion process in the island vs mainland contexts. *Anolis cristatellus* also invaded Costa Rica. To have a complementary view of the role of agonistic behavior in the invasion process under the island versus mainland contexts, we will compare the behavioral display recorded towards the two robots in Dominica with the ones from the exact same experiment performed in 2017 in Costa Rica. Finally, the same behavioral experiment in the native range of *A. cristatellus* (i.e. Puerto Rico), would give us the initial behavioral state.

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Anolis research in the Echternacht Lab

Much of the anole-related research in our lab has focused on a) the ecology of *Anolis carolinensis*, b) the ecological and behavioral interactions between native *A. carolinensis* and introduced *A. sagrei* in the southeastern United States and between *A. sagrei* and *A. conspersus* in the Cayman Islands, and c) habitat use by introduced *A. carolinensis* and native *Lamprolepis smaragdina* in Palau. However, the "we" has become "me" as, in preparation for retirement, I am no longer accepting new graduate students into my group. The last three fledged the nest in May or December of 2016. What I describe below is a brief description of two personal research projects which are ongoing.

The deliberate introduction of *Anolis carolinensis* on Eastern Island, Midway Atoll, Hawaiian Archipelago: the history of a failed invasion.

Figure 1. Midway Atoll, Hawaiian Archipelago. Foreground: Sand Island; Background: Eastern Island. Source: Woody, T. 2013. We may be waving goodbye to Pacific island nations sooner than we thought. Quartz, online, 15 April 2013.

The presence of *Anolis carolinensis*, originally identified as *A. c. porcatus*, in the Hawaiian Islands, on Oahu, was published in 1950 (Shaw and Breese 1950). It was probably introduced in the mid to late 1940's. The source of the introduction was said to have been via the pet trade. In subsequent years, the species spread to all of the major islands in the archipelago (summarized by Kraus, 2009). In 1978, it was deliberately introduced to Sand Island, Midway Atoll by a U.S. Navy Preventative Medicine Specialist who was stationed on Sand Island (L.J. Pinter, pers. com. 8 June 1999). He had purchased the lizards from a pet store in Honolulu. Multiple individuals, both males and females, were introduced around his quarters. No mention of the presence of *A. carolinensis* in Midway Atoll made by Sean McKeown (1978) in his field guide to the Hawaiian reptiles and amphibians but it was noted in a second field guide published 18 years later (McKeown 1996). The species apparently did not disperse far from its original introduction between 1978 and 1980 and there are no reports of its occurrence on the island after the latter date (L.J. Pinter, pers. com. 8 June 1999). However, to my knowledge, no formal recognition of its disappearance has been published by the time in xxxxx that I visited Midway intent on documenting the presence of the species on the atoll and study its habitat use. An extensive search of Sand Island, and a less extensive search of part of Eastern Island, revealed no anoles, and none of the U.S. Fish & Wildlife personnel based at the atoll with whom I spoke reported having seen the anole on either island. Neither had any of the few contract laborers employed on the atoll to whom I showed photographs. All of this shifted my interest from studying the habitat use of the (non-existent) species to gathering more information on the original introduction and on possible reasons for its failure to become established. This led to a second visit to the atoll. That trip further confirmed the absence of the species and led to a search for the individual who had originally introduced the species. This took several years and began with contacting Tom Fritts, a herpetologist employed by the U.S. Geological Survey who had worked in the Hawaiian Islands and an old friend having received his PhD. Degree from the University of Kansas a few years after I did. Tom suggested that I contact Lawrence Pinter and then found him listed in the phone book for Maui. Mr. Pinter had known the individual who introduced *A. carolinensis* to Sand Island when both were stationed on the island and he was there when the introduction occurred. He remembered the man's first name, but not his last. It took a few more years to find that, and then his email address in the U.S. When I contacted him, he agreed to provide the details of the introduction but not in writing. Our communication was by telephone. He was a bit embarrassed about his role in all of this. He had come to know that the deliberate introduction of exotic species was now at least frowned upon if not considered illegal. He has asked that I not publish his name.

There are at least two viable hypotheses explaining the failure of the introduction of *A. carolinensis* to Midway Atoll. Both are indirectly related to human activities and these may have acted in concert. A manuscript is in preparation which discusses the rationale for the lizards being introduced in the first placed and two reasons why I believe that it failed.

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A comparative study of habitat use by native *Lamprolepis smaragdina* (Scincidae) and introduced *Anolis carolinensis* in the Republic of Palau (Belau).

Beginning with their introduction to the Hawaiian Islands in the late 1940's (first record published in 1950; Shaw and Breese 1950), *Anolis carolinensis* has been introduced to many islands in the western Pacific region. Exactly when it was introduced to Palau is not known but Owen (1977) suggested that it had arrived by at least 1947.

Figure 2. Left: Adult male *Anolis carolinensis* (Dactyloidae), Blount Co., Tennessee, USA. ©A.C. Echternacht. Right: *Lamprolepis smaragdina* (Scincidae). This species is widely distributed on islands in the Philippines, New Guinea, and the Indonesian Archipelago. Over its range, it is represented by many different color morphs, some restricted to small geographic areas, such as a single island, or by multiple morphs on a single (larger) island. The photograph is of a specimen from Waigeo (Raja Ampat), Indonesia but its appearance is very close to that of the species in my study area in Palau. Photo courtesy of Amir Hamidy (©Amir Hamidy).

The point of entry was the Port of Palau on Malakal, a small island connected by a causeway to the city of Koror (Oreor) on the larger island of the same name. In term of area and population, the city of Koror is the largest municipality in Palau. In 1980, in a personal communication to Ronald L. Crombie, Owen noted that the local distribution of the species was still limited to the vicinity of the Port and adjacent docks (Crombie and Pregill 1999). Crombie and Pregill (1999, p. 51) observed that the species was, "… 15 years later, now found 50-100

meters east and west of the Port along the road, but it is not abundant." Their observations and mine confirm that there is ample suitable disturbed habitat on Malakal and Koror to support *A. carolinensis* and that there are no obvious barriers to their dispersal. The first indication that the species was expanding it range was with the discovery in 1996 of a single individual at a site in an agricultural setting on the side of a hill some distance from the docks, and a large population at a site at the Malakal end of the causeway leading to Koror. In 1998, the species was, having crossed the causeway, found on the edge of the city itself (Crombie and Pregill 1999). Since then, and except for a few instances of jump dispersal, probably hitch-hiking on vehicles or in their cargo, *A. carolinensis* has dispersed only about 7 km along the main road through Koror. Crombie and Pregill (1999) were probably correct that this slow advance is at least partly because the native and very abundant Emerald Tree Skink (*Lamprolepis smaragdina*) is both a predator on and a competitor of the introduced species. Crombie has observed multiple instances, and I a few, of predation or attempted predation on the anole by the skink. In addition, I have noted, as did Crombie and Pregill (1999), that the anoles are behaviorally different from those in populations we are familiar with elsewhere in the Pacific or the United States. They are much more secretive than I have observed and are rarely seen far from dense vegetation into which they disappear on the approach of a skink or a human.

In six trips to Palau, I have seen an adult male displaying on an open perch only twice. Females can be especially difficult to locate and catch. However, size matters. I have observed adult male anoles and adult skinks perches in close proximity while apparently ignoring one another. Adults skinks can reach snout-vent length slightly greater than that of the anoles, and they are bulkier, but an adult male anole may be difficult for a nearly equal-sized skink to subdue and swallow. As for competition, the two species occupy similar edge habitats but anoles appear to prefer smaller trees (height and trunk diameter breast-high [DBH]) than do the skinks. Although my data have yet to be analyzed statistically, it appears that adult male anoles prefer trees with a trunk diameter of about 7-8 cm whereas the skinks prefer substantially larger trees; DBH of at least 25 cm and often much greater. Also, skinks of all sizes prefer perch heights substantially higher than do the anoles. These figures, however, are based only on data from areas where the two species are syntopic. I have not yet spent much time looking at the data for skinks which occur in areas not occupied by anoles. This calls attention to two negative aspects of my study: 1) the study is asymmetrical. Whereas it is possible to locate sites where skinks occur in the presence of anoles, and where skinks occur in the absence of anoles, there are no sites where anoles occur in the absence of skinks. 2) I can, with a high degree of accuracy, tell the sex of all but hatchling and small young-of-the year anoles but I cannot distinguish male skinks from female skinks of any age. In this species, sexing based on external characteristics is extremely difficult or impossible, and definitely so at a distance. Fortunately, the anoles seem not to have adjusted their reproductive cycle from that pattern I am familiar with in the U.S. By collecting data in March, only large subadults and adults are present. Hatchling-sized skinks are present but in very small numbers. Another problem with the study is incontrollable; the weather. I was able to obtain data during my first four trips to Palau on large numbers of skinks and much smaller but still usable numbers of anoles. Despite the fact that Palau is supposed to be outside of the typhoon belt, one hit my study area in 2013 four months before I arrived and did considerable damage, falling many of the larger trees in my study area. That was followed by a severe drought that persisted through my entire visit. Then, in 2016, drought had set in several months before my arrival and had reached the point by the time I arrived that there was a

serious water crisis. The countries only reservoir of fresh water, which usually maintained a pool depth ~4.5 meters had dropped to only ~1 meter above the conduit that fed water to homes and businesses. Water was being rationed, the supply cut off from 10:00 am until 6:00 pm and from 10:00 pm until 6:00 am. The hours of tap water availability was later further reduced. Taiwan and Japan were shipping in bottled water. The bimodal diurnal activity period normally exhibited by both species had been reduced to a unimodal pattern beginning soon after sunrise and terminating by noon or a little before. My sample sizes for those two trips, especially for the anoles, were abysmal. So … it's back to Palau, hopefully for the last time.

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Geographic variation in trophic ecology of the Brown anole (*Anolis sagrei*): species-rich communities are composed of more diverse populations

ABSTRACT

Here we analyze a large dataset of the diet of the brown anole (*Anolis sagrei*). We asked how the trophic niche varies among populations with the specific goal of testing a long-standing model of adaptive diversification – ecological release. Our results do not support the predicted inverse relationship between community richness and niche breadth. Instead, we find that population niche breadth increases with increasing community richness. Using a subset of data for which we have individual-level data we also find that variation in niche structure along this community richness gradient is driven by increasing variation within and among individuals. Our results show that a widely cited dynamic underpinning ecological models of adaptive diversification – ecological release – does not appear to explain interpopulation niche variation in *A. sagrei*. While we do not have data sufficient to explain this incongruence between theory and observation, we briefly discuss some ideas worth exploring. Ultimately, we hope our findings stimulate new ideas and further evaluation of the relationship between community richness, competition, and the origins of intrapopulation diversity.

INTRODUCTION

Burgeoning interest in intrapopulation diversification (e.g., individual specialization) and ecological speciation has fueled a wave of research into the processes of ecological and phenotypic diversification (Dieckmann and Doebeli 1999, Schluter 2000, Bolnick et al. 2003, Ackermann and Doebeli 2004, Bolnick and Fitzpatrick 2007, Nosil 2012). Indeed, much of this work has focused on understanding the behavioral and ecological mechanisms that reduce geneflow within populations subject to divergent selection – that is, reinforcement. Besides some well investigated model systems (e.g. Galapagos finches: Grant and Grant 1989, three-spined stickleback: Hendry et al. 2009, Timema stick insect: Farkas et al. 2013), advances in divergence with-gene-flow models have tended to overlook earlier stages in the diversification process that generate phenotypically diverse populations. Consequently, a general model describing how

phenotypcially (or ecologically) diversified populations arise is lacking. Instead, a rather limited set of eco-evolutionary predictions seems to dominate the way evolutionary ecologists currently think about the origin of ecological diversity within populations (Yoder et al. 2010, Wellborn and Langerhans 2015).

The prevailing model of adaptive ecological diversification generally includes some version of *ecological release*. While the ideas underlying ecological release existed prior (Mayr 1942, Simpson 1944, 1953, Lack 1947), it seems Wilson was the first to name it (Wilson 1961). In his 1961 paper on taxon cycling in ant communities of southeast Asia, Wilson used the term to describe what happens when species from species-rich habitats (e.g., mainlands) colonize species-poor ones (e.g., oceanic islands). Wilson's simple verbal model articulates a clear prediction, "…*the ecological amplitude of both expanding and endemic species should be negatively correlated ... with the size of the local fauna to which they belong.".* In contemporary language, the immediate, or non-evolutionary, consequences of ecological release consist of increased population size (density compensation) and increased population variance in resource use (expanded population niche width). Essentially, the model describes what happens when a species encounters and exploits what we would now recognize as ecological opportunity arising from altered heterospecific interactions such as competition and predation (Stroud and Losos 2016).

It's important to note that evolution was not integral to Wilson's ecological release model. Nevertheless, the evolutionary implications were clear – filling an important gap in developing theory about the ecological dynamics of adaptive evolutionary diversification (Losos and Queiroz 1997, Schluter 2000). Specifically, ecological release suggested that population niche expansion (increased phenotypic variance) emerged when and where relaxed heterospecific interactions prevailed. But niche expansion alone does not explain how species or populations diversify. Rather, it simply posits that a population's niche would expand, not diversify per se. This limitation, famously outlined in Van Valen (1965) is important because the next stage in the ecological model of adaptive diversification is disruptive selection – a discriminating ecological force hungry for intrapopulation variation (Roughgarden 1972). Without intrapopulation phenotypic variation, any form or strength of selection would simply depress population mean fitness rather than promote adaptive diversification (Ackermann and Doebeli 2004). While not part of Wilson's ecological release hypothesis, the niche variation hypothesis – broader niches are also more diverse – has become a core component of adaptive diversification theory (Roughgarden 1972, Lister 1976a, 1976b, Bolnick et al. 2010, Yoder et al. 2010).

Understanding how ecological release promotes ecological diversification requires an understanding of the ecological mechanisms that generate intrapopulation niche diversification (Bolnick et al. 2003, Rueffler et al. 2006, Araújo et al. 2011). The answer is somewhat counterintuitive; diversification does not arise directly from niche expansion (as might seem an appealing route). Instead, ecological diversification comes from the other symptom of ecological

release - density compensation (Crowell 1962, MacArthur et al. 1972, Case 1975, Wright 1981, Buckley and Roughgarden 2006, Buckley and Jetz 2007). Density compensation, the numerical response to ecological opportunity, is the critical ecological link between heterospecific interactions and evolutionary diversification that has fueled most recent work in this area. The model works as follows: 1) low interspecific competition drives density compensation, 2) increased population density increases intraspecific resource competition, 3) negative frequency dependent selection favors extreme (or specialist) phenotypes resulting in a diversified population (Roughgarden 1972, Bolnick 2001, 2004, Rueffler et al. 2006, Svanback and Bolnick 2007, Nosil 2012, Martin and Wainwright 2013). Reinforcement by phenotype or geographic isolation may subsequently drive the evolutionary side of the process towards reproductive isolation and speciation.

Together, ecological release and negative frequency dependent selection by intraspecific competition form the prevailing hypothesis for ecological diversification. We call this integrative model the ecological release paradigm. In whole or part, this model figures prominently in adaptive diversification theory and is a fixture of speciation with gene-flow dynamics. The crucial role of ecological release derives from a mechanistic ecological linkage between ecological opportunity and a diversified population – interspecific competition has a negative effect on intrapopulation variation. The history of this idea goes back quite far yet a review of the ecological release paradigm does not exist, as far as the authors know. However, in one of a series of papers questioning components of the paradigm, Abrams (2008b) traces its roots back to MacArthur and Levin's analysis of limiting similarity (MacArthur and Levin 1967). In his brief review Abrams (2008b) undermines the assumptions of niche theory propping up the ecological release paradigm. This thorough deconstruction raises new questions about the effects of interspecific competition and the ecological conditions favoring adaptive diversification.

As far as we can tell, few studies assessed the ecological release paradigm in *Anolis* lizards. All are observational, comparing phenotypic variation across a gradient of community richness – a proxy for interspecific competition. The evidence in support of the ecological release paradigm is mixed. Indeed, while quite a few studies clearly show evidence that congeneric competitors can drive niche shifts at macroevolutionary (Losos and Queiroz 1997), microevolutionary (Lister 1976a, 1976b, Losos et al. 1994), and ecological timescales (Jenssen et al. 1984, Stuart et al. 2014), evidence for the predicted effects of ecological release on resource use variation (niche expansion and specialization) is rather thin (Roughgarden 1974, Lister 1976a, 1976b, Mesquita et al. 2007, Costa et al. 2008). At the very least, a role for ecological release in the diversification of *Anolis* remains unclear. And while many important questions

Figure 1. Conceptual diagram outlining how niche structure is described (panel 1) and predicted effects of community richness on niche structure (panel 2). Four hypothetical populations depicting individual resource use distributions (grey dotted lines) and the population niche width (black line) (a). These niches can be described by the contribution of individual niche width (WIC) and between individual niche width (BIC) to the total population niche (TNW) such that WIC + BIC = TNW (b). The two populations depicted in (b) have equivalent BIC values, but differ in TNW because of the larger WIC component (broader mean individual niche width) of the lower population. These metrics can be plotted in niche space to visualize how variation among populations arises – that is, whether populations differ in TNW due to proportional increases in WIC and BIC components or whether one component increases disproportionately than the other (BIC/TNW). Here, the hypothetical populations from (a) are plotted in niche space to illustrate how variation in niche structure can be described in this two-dimensional niche space (note, other depictions of niche space often use WIC on the y-axis). Isoclines represent increasing TNW with constant contributions of BIC (and therefore WIC). In this sense, increasing TNW parallel to an isocline represents a population varying in BIC, but not in the relative contribution of BIC (and WIC) to the total niche width (BIC/TNW). In panel 2, we illustrate some possible effects of ecological release on niche structure using the same niche metrics (d-h). *Individual specialization* (d), the total niche width stays the same, while the BIC and WIC components change (an increase in mean variation among individuals and a corresponding decrease in variation within: BIC/TNW). Note that an increase in BIC/TNW is equivalent to a decreasing WIC/TNW, a commonly used metric of individual specialization. *Population generalization* (e), population niche expands while BIC remains the same (and WIC increases). *Parallel expansion (f)*, population niche expands while the relative contributions of BIC and WIC remain unchanged; they both increase in proportion to each other. *Convergent generalization (g)*, TNW increases but the BIC component decreases. Population diversification (h), the increase in TNW is driven by an absolute increase in BIC as well as a shift towards increasing BIC relative to WIC.

remain regarding this textbook case of adaptive radiation, one that has remained for decades is: does ecological release facilitate the evolution of ecological diversity within *Anolis* populations?

We approach this question by examining intraspecific ecological variation within the brown anole, *Anolis sagrei.* In many ways, this study is an extension of earlier work that yielded mixed results as to the role of ecological release in niche diversification in this system (Lister 1976b, 1976a). However, here we include a more extensive diet dataset to more rigorously evaluate the predictions of ecological release (Table 1, Figure 1). Specifically, we test whether *1) population niche width is inversely correlated with species richness,* and *2) whether intrapopulation niche variation decreases with species richness.* Note that results reported here are part of an ongoing effort to address these questions. Consequently, these unpublished results are provisional in the sense that our inference may change once additional resource axes are added, morphology is included, and reviewer comments are heeded. For now, we report our results for dietary data, confident those encompass the geographic variation in the trophic niche of *A. sagrei* and the potential effect of interspecific competition on adaptive diversification.

METHODS

Focal organism - *Anolis sagrei* is a geographically widespread species native to the West Indies and introduced broadly (Bermuda, Taiwan, Singapore, Ascension Island, Ecuador, Hawaii, Brazil, Costa Rica, California, Texas, the southeastern US, and several countries in Central America) (Kolbe et al. 2007, Huang et al. 2008, Stroud et al. 2017, 2018). Throughout this geographic range *A. sagrei* succeeds in a variety of ecological contexts and coexists with a number of ecologically similar lizard species. A trunk-ground ecomorph, *A. sagrei* is known to use a variety of habitats, from sparsely vegetated rocky coastlines, to cities and dense forest. This breadth of habitat use, coupled with its huge geographic range, means that *A. sagrei* are components of many different communities. In some habitats, *A. sagrei* are the sole lizard species present, and in others, they are syntopic with several species of *Anolis* and a range of other diurnal insectivorous lizards (Table 1 & 2). *Anolis sagrei* has been subjected to several dietary studies. As with other small invertivore lizards, including *Anolis*, the diet generally consists of small arthropods such as ants, cockroaches, caterpillars, and spiders. As a whole, the diets of *A. sagrei* are unremarkable, and at coarse taxonomic levels (e.g., Order), the diets of *A. sagrei* are not much different from other semi- arboreal anoles such as *A. cristatellus* (Stroud 2018).

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Table 1. Study locations and sources of data used in our analysis. 1 – same as diet data, 2 – Powell et al. 2012, 3 – Personal Observation, 4 – iNaturalist.

Region	Site	Latitude	Longitude	Diet	Richness
Bahamas	Abaco	26.403	-77.095	Lister 1976	1
	Abaco – Marsh Harbour	26.532	-77.058	Giery unpub.	3
	Abaco – Pine forest	26.217	-77.212	Giery unpub.	3
	Abaco – Robinson's bight	26.332	-77.027	Giery unpub.	3
	Abaco – Wilson City	26.376	-77.003	Giery unpub.	3
	Exuma – Georgetown	23.503	-75.869	Lister 1976	1, 2
	Exuma – Moss Cay	23.505	-75.759	Wright 2009	1
	Exuma – North Gaulin Cay	24.198	-76.462	Wright 2009	1
	Exuma – Staniel Cay	24.167	-76.442	Wright 2009	1
	South Bimini	25.708	-79.290	Schoener 1968	1, 2
Bermuda	Paget Parish	32.292	-64.772	Stroud et al. 2017	3
	Pembroke Parish	32.300	-64.792	Stroud et al. 2017	3
Cayman Islands	Cayman Brac	19.724	-79.780	Lister 1976	1, 2
	Little Cayman	19.692	-80.035	Lister 1976	2
	Little Cayman – N	19.690	-80.066	Wright 2009	1
	Little Cayman – S	19.677	-80.062	Wright 2009	1
Florida	FL. Keys – Big Pine (hammock)	24.705	-81.391	Giery unpub.	3 4
	FL. Keys – Big Pine (pine)	24.701	-81.376	Giery unpub.	3, 4
	Gainesville – FNHM	29.644	-82.344	Wright 2009	4
	Gainesville – Neighborhood	29.634	-82.426	Wright 2009	4
	Gainesville – University garden	29.645	-82.357	Wright 2009	4
	Gainesville – McCarty Woods	29.646	-82.344	Wright 2009	4
	Miami – Banyan Drive	25.688	-80.284	Stroud 2018	3
	Miami – Doug Barnes Park	25.738	-80.310	Stroud 2018	3
	Miami – Fairchild Garden	25.677	-80.272	Stroud 2018	3
	Miami – Florida International University	25.758	-80.381	Stroud 2018	3
	Miami – Kendallwood Park	25.693	-80.345	Stroud 2018	3
	Miami – Matheson Hammock	25.682	-80.281	Stroud 2018	3, †
	Miami – Red Road Canal	25.682	-80.284	Stroud 2018	3
	FL. Keys – No Name Key	24.695	-81.328	Giery unpub.	3, †
	North Miami – Biscayne Bay	25.906	-80.137	Giery et al. 2013	3, †
	Tampa – Hillsborough Preserve	28.070	-82.391	Wright 2009	†
Jamaica	Savanna – La-Mar	18.221	-78.135	Lister 1976	1
Swan Islands	Great Swan Island	17.411	-83.900	Lister 1976	2

Community composition - To estimate the number of species coexisting and presumably interacting with *A. sagrei* we used a variety of data sources. In many cases the lizard community was described by authors. However, in many cases the community was determined from direct observation in collection localities by the authors, occasionally being supplemented by photographic observation records from iNaturalist (www.inaturalist.org) and museum records in VertNet (vertnet.org). Searches were performed in July 2018. These sources are detailed in Table 2. We considered a species as coexisting with *A. sagrei* if they can be found in the same habitat and potentially competing for space and/or food resources, that is, they are diurnal, invertivore lizards. For example, *Hemidactylus* spp. geckos were excluded from inclusion, as were large, herbivorous species such as *Iguana iguana* and *Cyclura* spp.. Note that we did not consider other species of vertebrates such as birds in our dataset. While birds likely interact with *A. sagrei* as predators and perhaps competitors (Wright 1979, 1981, Buckley and Roughgarden 2006, Buckley and Jetz 2007) we chose to restrict the analysis to the lizard community at this time.

Also note that although intraguild predation can strongly affect how *A. sagrei* use habitats and food resources we did not differentially treat species that might also eat *A. sagrei* (e.g., *Leiocephalus* spp.). Further partitioning of the effect of predation on resource use is an obvious next step.

Figure 2. Map of study sites where *A. sagrei* used in this study were sampled. Each red dot indicates a collection location within the region of study. Names within inset study regions correspond to labels in Figures 2 and 3.
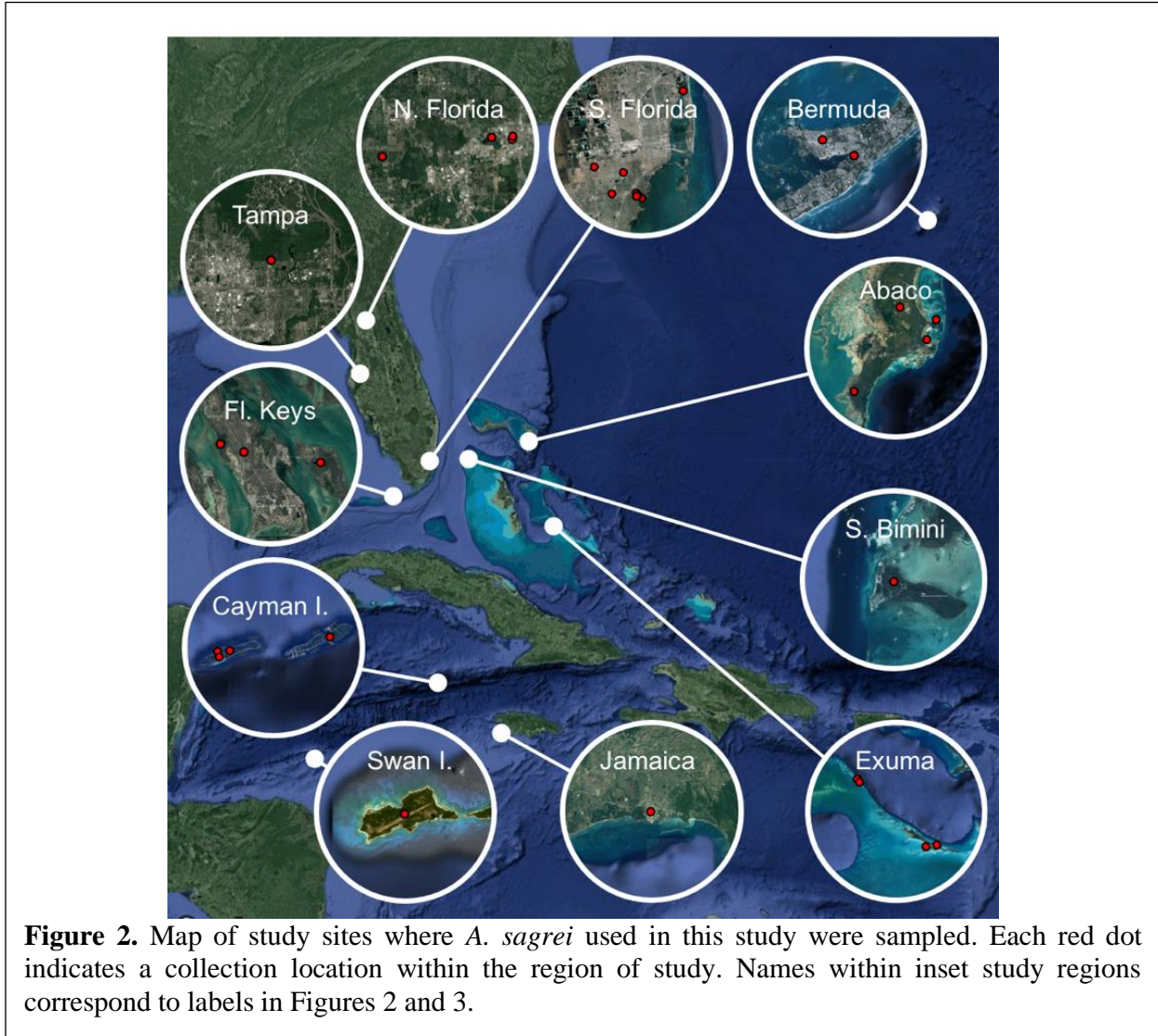
Diet data **-** Our primary dataset consists of the diet of *A. sagrei* as inferred from analysis of their stomach contents. We searched the literature for published data on *A. sagrei* diets – often presented in summary tables. We also included unpublished diet data collected by the authors. Given the diverse origin of data included in this analysis and the various schemes used to report and categorize them, we analyzed these data at a rather course level. While some studies identified diets to a finer taxonomic level, most examined diet at a taxonomic level corresponding with Order and a few more inclusive categories (e.g., miscellaneous arthropods). While it may obscure some detail, we chose to collapse finer resolved data (family, genus or

even species) into the coarsest, that is, lowest resolution categorization to allow comparison across a wider range of population. Because many earlier studies of *A. sagrei* only included adult males, here we restricted our analysis to adult males.

From these data we calculated several metrics summarizing population and individual-level diet variation. Population niche width (Total Niche Width - TNW) was estimated as the inverse Shannon-Weaver index following Bolnick et al. (2002). A subset of our data allowed measurement of within-population components of population niche width, BIC and WIC. BIC is the amount of niche variation explained by among individual variance. WIC is the variance explained by individual niche width (Roughgarden 1972, 1974, Bolnick et al. 2002). We used these intrapopulation metrics to describe variation in niche structure among populations and compare them to possible diversifying responses (Figure 1). Niche structure can diversify in various ways. Individuals may become more dissimilar from each other without an expanded population niche – *individual specialization* (Figure 1d). Population diversification also includes scenarios whereby the population niche expands from a combination of increased individual niche width and / or increased among individual variance: individuals may become more generalized – *population generalization* (Figure 1e), individuals may become more generalized and more dissimilar from one another - *parallel expansion* (Figure 1f), individual niches may expand and become more similar - *convergent generalization* (Figure 1g), and finally, individual niches may become more dissimilar - *population diversification* (Figure 1h). Population diversification is also known as the niche variation hypothesis (Bolnick et al. 2010). After examining how niche structure varies, we tested the effect of community richness on niche structure by examining the relationship between species richness and three measures of niche variation: BIC, WIC, and TNW.

Non-independence of samples **-** We collected diet data for populations spanning the natural and introduced geographic range of *A. sagrei* (Table 1, Figure 2). This dataset consists of fieldwork done by a variety of different authors for a diversity of ecological aims which complicates analysis and inference. First, data are unevenly distributed within the range of *A. sagrei* – meaning that samples are spatially non-independent in some cases (Figure 2). For example, we have several samples geographically clumped in South Florida while we have only one sample from the entire island of Jamaica. Second, we lack detailed quantitative data on ecological conditions for each sampling location – notably lizard community composition, *A. sagrei* population density, and prey community composition – all of which should influence the trophic ecology of *A. sagrei*. Last, the ecological and evolutionary history of each population differs drastically – some populations have long been isolated on small islands such as the Swan Islands, some have recently (decades – century) established on continental mainlands such as Florida (Giery et al. 2013), and yet others have very recently (~ 2014) invaded small islands such as Bermuda (Stroud et al. 2017). Further analysis of these divergent histories might yield interesting caveats to our analysis and interpretation. However, we do not address these aspects

here. Nevertheless, we attempt to account for a few of these issues analytically.

Table 2. Community composition of study sites used in this study. N = Native, I = Introduced.

Species	Abaco	Abaco – Marsh Harbour	Abaco – Pine forest	Abaco – Robinson's bight	Abaco – Wilson City	Exuma – Georgetown	Exuma – Moss Cay	Exuma – North Gaulin Cay	Exuma – Staniel Cay	South Bimini	Paget Parish	Pembroke Parish	Cayman Brac	Little Cayman	Little Cayman – north	Little Cayman – south	Big Pine Key (hammock)	Big Pine Key (pine)	Gainesville – FNHM	Gainesville – Neighborhood	Gainesville – University	Gainesville – McCarty Woods	Miami – Banyan Drive	Miami – Doug Barnes Park	Miami – Fairchild Garden	Miami – Florida International	Miami – Kendallwood Park	Miami – Matheson Hammock	Miami – Red Road Canal	No Name Key	North Miami – BBC	Tampa – Lower Hillsborough	Savanna-la-Mar	Great Swan Island
Anolis sagrei	N	N	N	N	N	N	N	N	N	N	I	I	N	N	N	N	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	N	N
A. carolinensis																	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		
A. distichus		I				N			N	N							I	I					I		I	I	I		I	I	I			
A. equestris*																								I	I			I	I		I			
A. cristatellus																								I	I			I	I					
A. grahami											I																						N	
A. smagdarinus		I				N	N	N	N	N																								
A. angusticeps						N				N																								
A. lineatopus																																	N	
A. valencienni																																	N	
A. opalinus																																	N	
A. leachi											I																							
A. maynardi														N	N	N																		
A. conspersus													N																					
Agama agama																									I									
Celestus maculatus													N	N	N	N																		
C. crusculus																																	N	
Ophiasauris ventralis																			N	N	N	N										N		
Basiliscus vittatus																								I	I	I		I						
Leiocephalus varius																	I	I																I
L. cairinatus	N	N	N	N		N		N		N			N	N	N	N																		
Pleistodon laticeps																			N	N	N	N												
P. inexpectatus																		N	N	N		N			N				N	N	N	N		
P. fasciatus																			N	N	N	N												
Scincella lateralis																			N	N	N	N										N		
Spondylurus fulgidus																																	N	
Aspidoscelis sexlineatus																					N											N		
Pholidoscelis auberi						N		N	N	N																								
P. dorsalis																																	N	
Anolis richness	1	3	1	1	1	4	2	2	3	4	3	1	2	2	2	2	3	3	2	2	2	2	3	4	5	3	3	4	5	3	4	2	5	1
Total Richness	2	4	2	2	1	6	2	4	4	6	3	1	4	4	4	4	4	5	7	7	7	7	3	5	8	4	3	5	6	4	5	6	8	2

Three variables important to our study are likely to vary in a spatially autocorrelated fashion: lizard community richness, the composition of prey communities, and *A. sagrei* genetics. Spatial covariance among these variables could yield causally spurious relationships if, for example, lizard community richness and *A. sagrei* phenotypes respond similarly (or dissimilarly) to an underlying spatial gradient. Spatial autocorrelation between prey community composition (i.e., taxonomic richness of prey) and lizard community richness could also yield a

false impression of causality if, as we predict, lizard community richness is correlated with *A. sagrei* phenotypes.

Our approach to accounting for autocorrelation was to first assess the degree to which geographic distance underlies similarity in our focal variables. We used Mantel tests and Moran's I to check for spatial autocorrelation in our predictor (lizard community richness) and response variables (TNW). Second, we used spatial regression to analyze the relationship between community richness and TNW. Because spatial regression includes the geographic distance between sample points, it accounts for spatial autocorrelation between samples while testing our overall hypothesis.

We first used AIC to choose among several model structures. Our base model was a linear model including TNW as the response variable and community richness as the predictor. We then fit three spatial regression models with different distribution structures: Gaussian, spherical, and ratio. We repeated the model selection procedure with three additional base models including the number of individual lizards in each sample (n lizards) as a covariate. We also included a series of models in which community richness was log-transformed. Not part of our initial prediction, the log-transformation was included after examining the residuals of a linear fit to the data. Best fit models from each base model set were then compared by AIC. Note that because of our small sample of individual-level data used to explore niche variation components, BIC and WIC, we only applied this spatial regression analysis for our analysis of range-wide variation in TNW. Mantel tests, Moran's I, and spatial regression were performed in nlme and vegan packages in R. All geographic distances were calculated using rdist.earth in the Fields package.

RESULTS

Our dataset included dietary data for 875 adult male *A. sagrei* and more than 8200 prey items from 32 populations (Table 1, Figure 2). For 13 populations we had individual-level diet data allowing us to examine intrapopulation niche variation. Among all study sites, *A. sagrei* cooccurred with at least 30 different species of lizard from eight families (Table 2). The number of lizard species in the community varied substantially among sampling locations. Several of the communities consisted of single species (i.e., only *A. sagrei*); the richest communities included up to eight (mean = 3.7, mode = 3).

Prediction 1: Population niche width is inversely correlated with species richness – Our test of this prediction yielded significant, but counterintuitive results. That is, the observed relationship between TNW and community richness was positive – opposite our prediction – even after accounting for spatial autocorrelation.

Indeed, while Mantel tests showed that spatial autocorrelation existed, it did not explain the positive relationship between community richness and TNW. Specifically, samples geographically near each other were more similar in TNW and lizard community richness as indicated by positive and significant spatial autocorrelation for TNW and community richness ($r = 0.25$ and $r = 0.3$, respectively). However, a partial Mantel test showed a positive correlation between TNW and community richness despite spatial autocorrelation, presumably arising from shared ecological and genetic backgrounds among near samples ($r = 0.18$, p. $= 0.06$). Similarly, Moran's I showed spatial autocorrelation for lizard community richness ($p < 0.01$), but no spatial autocorrelation for TNW ($p = 0.56$). Further, analysis of residual TNW derived from a linear model in which community richness was the independent variable also failed to reveal evidence of autocorrelation ($p = 0.63$)
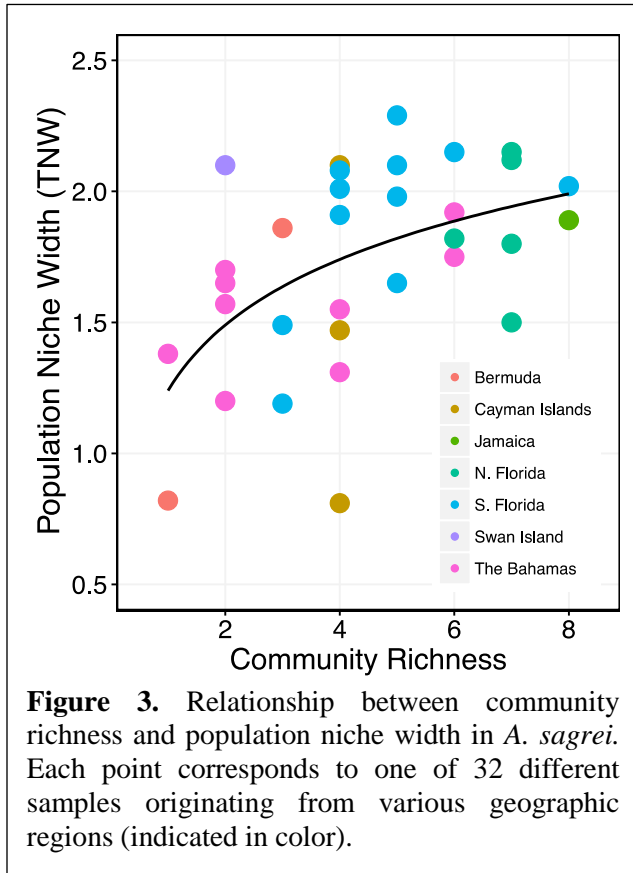
Figure 3. Relationship between community richness and population niche width in *A. sagrei.* Each point corresponds to one of 32 different samples originating from various geographic regions (indicated in color).

Ultimately, *A. sagrei* population niche width (TNW) increased with the natural log of community richness and spatial regression models showed that geographic distance was a poor predictor of TNW. Within each base model structure, spatial models performed worse than base models and tended to perform worse overall as judged by AIC (Table 3). In all models, community richness was a significant predictor of TNW regardless of model structure and whether space was included in the regression. The best overall model included a nonlinear, log(community richness) predictor indicating a strong positive and saturating effect of community richness on *A. sagrei* population niche width whether or not spatial variance – our proxy measure of underlying, and unaccounted for, ecological and genetic autocorrelation – is included in the model or not. (Table 3, Figure 3).

Prediction 2: Intrapopulation niche variation decreases with species richness –
We tested this prediction with several metrics of intrapopulation niche variation, the within individual component. Our analysis of the structure of *A. sagrei* trophic niche showed that WIC and BIC both contributed to TNW expansion. The significant relationship between TNW and WIC (slope = 0.35, p = 0.010) indicates a slight increase in individual niche breadth contributes to population expansion. Similarly, the significant positive relationship between TNW and BIC (slope = 0.63, p < 0.001) indicates a moderate - strong increase in interindividual niche variation (~ low individual overlap) contributes to population expansion. In combination with no significant increase in BIC/TNW across the TNW range, these data clearly suggest that *A. sagrei* niche structure follows a pattern of parallel niche expansion roughly parallel to the BIC/TNW = 0.6 isocline (Figures 1 & 4).

Our analysis of ecological release revealed a similar result; WIC, BIC, and TNW increased along the community richness gradient, although the WIC

Figure 4. Interpopulation variation in *A. sagrei* trophic niche structure. Because WIC + BIC = TNW the upper limit (slope = 1) is where BIC = TNW. Dotted lines represent values of these ratios and are provided as interpretive guides following (Bolnick et al. 2003; 2010). The regression line is included to show how increasing between individual niche (and expanding individual niche variation) components contribute to population niche expansion. The positive slope (0.64) indicates that BIC increases with population niche (TNW) - a relationship indicating interindividual niche variation contributes strongly to the population niche width. Study region indicated by color.

relationship was not significant (Figure 5). BIC/TNW (a measure analogous to individual specialization) was not correlated with community richness. These data show that total niche width expands with increasing species richness, primarily from greater niche difference among individuals (Figure 5). While the within individual component did not show a significant increase along the species richness gradient, a positive correlation between richness and WIC suggests a moderate contribution of individual niche expansion to the total niche width – parallel expansion (Figure 1f)
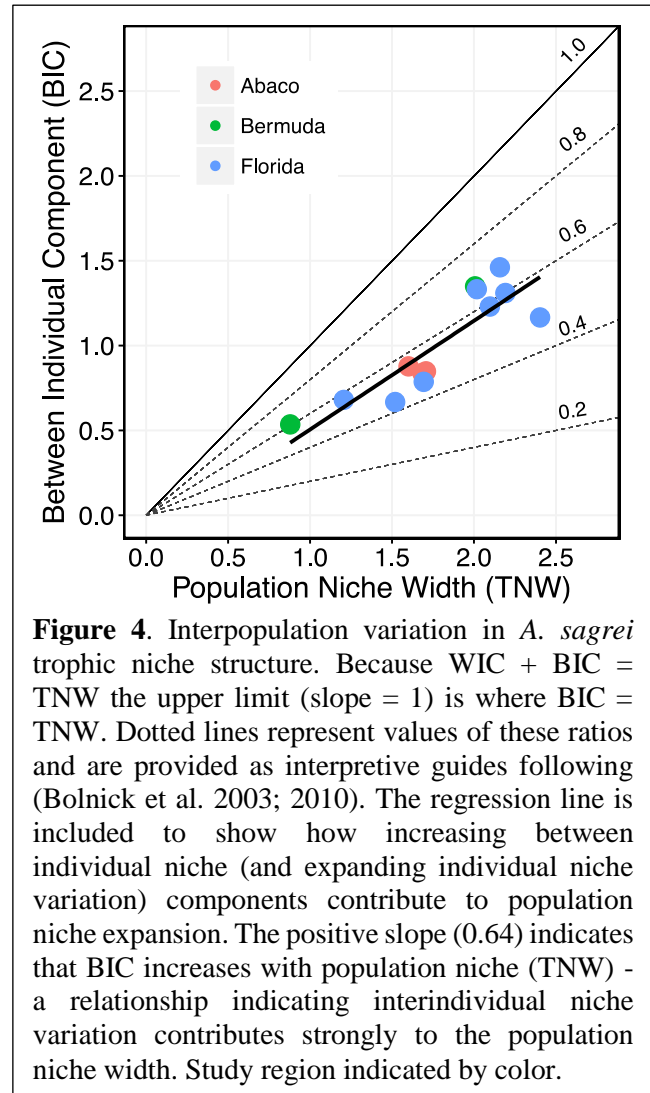
DISCUSSION

Trophic niche variation in *A. sagrei* does not match the predictions of the ecological release model. Our data clearly show that population niche width expands with increasing community richness (Figure 3). In effect, *A. sagrei* populations in species-poor areas (Bermuda, Abaco, Cayman Islands) tend to have narrow population niche widths while those from species-rich sites (Florida, Jamaica, Exuma Islands) have broad ones. Further, our data suggest that intrapopulation niche structure varies predictably with community richness (Figure 5). Specifically, individuals tend to be more generalized (higher WIC) and less similar (higher BIC) in species rich communities. This latter result matches a pattern of parallel expansion of niche components – wider population niches are composed of more dissimilar, and perhaps more generalized individuals – a result qualitatively similar to the niche variation hypothesis, but in the direction opposite that predicted by the ecological release paradigm (Van Valen 1965, Roughgarden 1972, Bolnick et al. 2007, Svanback and Bolnick 2007, Yoder et al. 2010). The pattern of geographic niche variation in *A. sagrei* emerging from our data is clear, the ecology underpinning it is not. Indeed, without additional analyses and experimental tests, we can only speculate as to the eco-evolutionary mechanism(s) underlying the observed pattern. Below, we examine and discuss potential drivers of geographic variation in *A. sagrei* resource use in hopes of stimulating new research directions in the evolutionary ecology of adaptive diversification.

Exploitative Competition

Anolis lizards are often food limited. A series of studies on *A. sagrei* in The Bahamas routinely show that subsidies can boost population size and individual growth rates (Spiller et al. 2010, Wright et al. 2013). They also demonstrate that *A. sagrei* can deplete prey abundances and alter prey community composition in favor of small, low-value prey (Schoener and Toft 1983, Schoener and Spiller 1987, 1999, Spiller et al. 2016). Food limitation and depletion by *Anolis* lizards strongly suggests the potential for exploitative competition to shape resource use. But outside of character displacement, evolutionary theory has little to say about a diversifying role for interspecific competition (for a review of the assumptions underpinning adaptive diversification theory see Abrams et al. 2008b). However, ecological theory does, and meta-analyses show that consumer richness tends to exacerbate resource depletion (Cardinale et al. 2006, Griffin et al. 2013). One might expect that resource partitioning, such as that characterizing *Anolis* ecomorphs, might alleviate some of this interspecific pressure on shared resources (Schoener 1968, Giery et al. 2013). However, empirical studies routinely show that resource depletion is stronger when competing species partition resources (Snyder et al. 2006, Finke and Snyder 2008, Northfield et al. 2010) – a finding in accord with theoretical analysis (Abrams and Rueffler 2009). To the authors' knowledge no study has investigated this in *Anolis* lizards. However, experimental removals of *Anolis* has
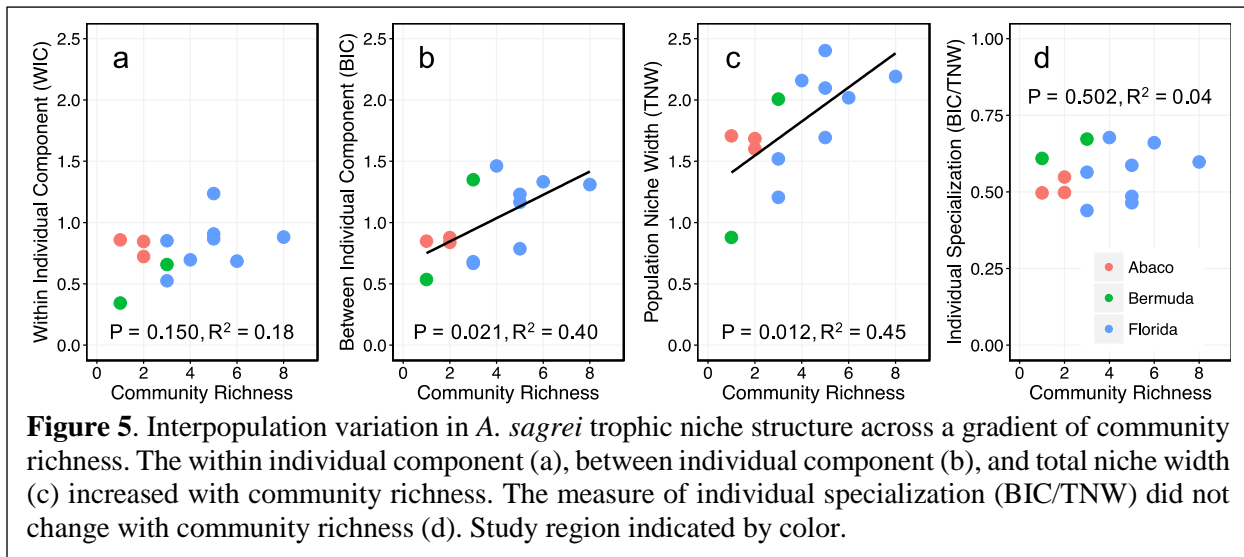
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Table 3. Results of spatial regression including alternative models evaluated with AIC. Among all alternative models, model 3, which included the log of community richness proved the best fit overall. Comparisons among models including spatial information (Gaussian (G), Ratio (R), and Spherical (S) residual structures) and one without (Base (B)) showed that the base model performed best, as judged by AIC.

Model	Base	G	R	S	Beta	St Err	df	F	P	R^2
			AIC							
3 Intercept	**31.1**	36.1	36.1	36.1	1.24	0.16	1,30	882.7	0.000	0.33
log(community richness)					0.36	0.11		11.5	0.002	
1 intercept	**35.1**	39.9	39.9	40	1.32	0.15	1,30	841.6	0.000	0.34
community richness					0.09	0.03		9.5	0.004	
4 Intercept	**43.5**	49.1	49.1	49.1	1.20	0.17	1,29	868.7	0.000	0.33
log(community richness)					0.36	0.11		11.3	0.002	
n individuals					0.00	0.00		0.5	0.475	
2 intercept	**47.4**	52.8	52.8	52.9	1.28	0.16	1,29	828.5	0.000	0.34
community richness					0.09	0.03		9.4	0.005	
n individuals					0.00	0.00		0.5	0.471	

shown that prey depletion is at least as strong in multispecies communities as it is in single species ones (Pacala and Roughgarden 1984, Dial and Roughgarden 1995). In sum, all things equal, empirical data and theoretical analysis suggests resource depletion should be most severe in diverse lizard communities including species that partition resources.

How does resource depletion affect niche width? Efforts to understand the effects of competition on the evolution of population niche breadth have spanned decades, typically geared towards understanding how individuals exploit resources depleted by heterospecific and conspecific consumers (Case 1981, Connell 1983, Taper and Case 1985, Futuyma and Moreno 1988). Two of these models make predictions consistent with our results – niche compression (MacArthur and Wilson 1967), and intermediate competition diversification hypotheses (Jones and Post 2013, 2016). The niche compression hypothesis formulated in (MacArthur and Wilson 1967) extends the basic ecological release scenario by incorporating foraging theory developed in (MacArthur and Pianka 1966) to explore optimal resource use in populations experiencing varying degrees of interspecific competition, among other things. The critical difference between Wilson's earlier ecological release hypothesis and niche compression is that the population-level niche response to competition depends on the attributes of the limiting resource and the behavior of the focal species. As discussed in (MacArthur and Wilson 1967) niche compression makes several predictions about how a generalist should respond to an increase in interspecific

exploitative competition. First, habitat use should narrow. Second, and more relevant to our study, the population trophic niche should expand as resource depletion forces active, generalist foragers to consume a larger fraction of less-preferred taxa and/or forage over a larger area – effects that would increase WIC and BIC, respectively. This is a clear parallel to the ecological model of adaptive diversification in that interspecific competition also drives negative frequency dependent selection on resource use when consumer niches overlap and resources can be depleted. Heretofore, the niche compression hypothesis has only occasionally interested theoreticians (Schoener 1974, Schoener et al. 1979), and has yet to receive more than a modicum of empirical support (Crowell 1962, Huey and Pianka 1977). Nevertheless, population niche expansion via amendment of resource subsets to the population niche in species-rich communities (increased BIC) suggests optimal foraging by generalist consumers for depleted resources might explain the geographic niche diversification in *A. sagrei* we observe here (Figures 3 & 5).

Figure 5. Interpopulation variation in *A. sagrei* trophic niche structure across a gradient of community richness. The within individual component (a), between individual component (b), and total niche width (c) increased with community richness. The measure of individual specialization (BIC/TNW) did not change with community richness (d). Study region indicated by color.

A newer model making similar predictions has been termed the intermediate competitive diversification hypothesis (Jones and Post 2016). In many ways this model echoes several aspects of niche compression. Specifically, population niche width expands as increasing exploitative competition depletes preferred resources subsequently driving consumers towards less-preferred taxa. However, the model differs in that it explores the extreme upper end of the competition gradient at which all preferred prey are depleted, leaving only non-preferred taxa. The result is a non-monotonic function with TNW increasing and then decreasing across the resource depletion gradient (Jones and Post 2013). Interestingly, the hump-shaped pattern described in the verbal model seems to reflect the highly contingent nature of ecological release effects seen in nature. Jones and Post originally developed their verbal model for intraspecific

competition, but the model is adaptable enough to encompass interspecific competition based on the assumption that species richness increases resource depletion and the overall intensity of competition when consumers are general and resources are fine-grained. Further testing of this model is needed, however the incorporation of nonlinearities in niche theory is clearly worth investigating (Abrams et al. 2008b, 2008a).

Behavioral Interference

Another route by which interspecific interactions shapes resource use is interference competition (Peiman and Robinson 2010). To date, ecological release models have focused on exploitative competition as the critical ecological link between competitors. But a surging interest in behavioral, non-consumptive effects of interspecific interactions has begun to quantify the role of interference competition in resource use. As defined in Grether (2017), interference competition is, "*any costly interaction between individuals over access to a resource, aside from resource depletion, regardless of whether the resource is shared or limiting; includes fighting, dominance, territoriality, and allelopathy (chemical inhibition)*". Conceptual models of interspecific competition incorporating behavioral interference are beginning to emerge (Peiman and Robinson 2010, Grether et al. 2013, 2017). Behavioral interference is well known among *Anolis*, however, the consequences of interference for niche variation are still rather vague (Jenssen et al. 1984, Hess and Losos 1991, Edwards and Lailvaux 2013, Kamath and Stuart 2015). Fortunately, a series of detailed studies of *Anolis* lizards in South Florida has generated insights into the direct interactions among and within *Anolis* species and their effect on resource use. Briefly, the system consists of two ecologically and morphologically similar species introduced into South Florida, the brown anole *A. sagrei*, and the Puerto Rican Crested Anole (*Anolis cristatellus*). Short-term density reduction experiments conducted by (Losin 2012) in this system suggests weak exploitative competition between these species (as well as within). However, a recent comparative study by (Stroud 2018) that included detailed behavioral observations and dietary analysis shows that when sympatric, the behaviorally subordinate *A. sagrei* moves more frequently, perches lower, consumes more terrestrial prey, and has a wider population niche width. These data suggest that the community richness effect we observe here could arise, in part, from persistent behavioral interference between *A. sagrei* and other members of the community such as *A. cristatellus* (Stroud 2018). These data clearly show that the nature of interspecific interactions between *A. sagrei* and other community members includes direct, behaviorally mediated interactions that change how *A. sagrei* forage without invoking prey depletion (i.e., exploitative competition). This rare coupling of detailed interference behavior, habitat use, diet, and prey depletion provides good evidence that interspecific behavioral interference – an increasingly recognized interaction in *Anolis* and other animals – is likely to shape how resource use responds to interspecific competition. These data show that interference intensity probably increases with species richness and drives a corresponding expansion of the

population niche.

<center>*Covarying Diversity Gradients*</center>

Because our study is a pattern-based analysis we cannot exclude a host of confounding variables that might explain the observed result. These variables include geographic variation in prey and predators. First, we do not account directly for the composition of prey communities. It is quite possible that geographic variation in trophic niche we observe is ultimately determined by geographic variation in prey communities. If prey diversity is correlated with lizard diversity, then the observed pattern may simply reflect consumption of prey in proportion to their availability. While we cannot exclude this possibility, we find it unlikely that an underlying gradient in prey diversity could explain our results. Perhaps the biggest reason we doubt this effect is that our dietary analysis is done at a course taxonomic level – order. Geographic variation in the richness of higher taxonomic levels such as order should be rather low (Gaston et al. 1995). Second, predation can also affect how consumers interact with prey (Roughgarden and Feldman 1975, Chase et al. 2002). Several of the lizards we include in our community richness gradient are predators of *A. sagrei* as well as competitors. For example, the curly-tailed lizard (*Leiocephalus carinatus*) is a well-known predator of *A. sagrei* where they co-occur (e.g., Bahamas and Florida) (Giery *unpublished data*). In this analysis we did not differentiate between species based on the types of interaction with *A. sagrei*. Given the diverse effects of intraguild predators on prey we could not speculate as to how this gross categorization might affect our observed patterns. However, terrestrial predators such as *L. carinatus* are known to affect *A. sagrei* behavior and population density (Schoener and Spiller 1999, Chejanovski et al. 2017, Lapiedra et al. 2018). Indeed, altered behaviors and density should influence the trophic niche and deserve further consideration. In addition to these two interspecific interactions, climatological and productivity gradients might also influence niche breadth (Roughgarden 1974, Gainsbury and Meiri 2017). We did not assess them here.

<center>*Ecological Release: Shifting Ideas and New Opportunities*</center>

In sum, our results clearly do not fit the ecological model of adaptive diversification putting them at odds with initial data for *Anolis* (Roughgarden 1974, Lister 1976a, 1976b), as well as more recent research on stickleback (Svanback and Bolnick 2007, Bolnick et al. 2010), yellow perch (Svanbäck and Persson 2004), and Bahamian mosquitofish (Araujo et al. 2014). Instead, the data presented here indicate that interspecific interactions may generate patterns of niche variation that differ from the classic ecological release scenarios that dominate adaptive diversification models such as that depicted in Yoder et al. (2010) and Wellborn and Langerhans (2015). Clearly, there is still much to be explored and explained about why population niche breath increases with community richness in *A. sagrei*. What is clear however, is that a foundational assumption of adaptive diversification does not hold for our data on the trophic

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niche of *A. sagrei*. Further, previous studies of the ecological release paradigm in *Anolis* have yielded mixed results (Lister 1976b, 1976a). Clearly, additional work is needed to understand the processes generating the observed pattern. The contrast between theory and our data leads us to suppose that the ecological release paradigm insufficiently explains how populations respond to variation in interspecific interactions. We believe, this clear incongruity demands a closer examination of the mechanistic links between ecological opportunity and diversification.

Along those lines, emerging models, new data, and an increasing appreciation for behavioral interference provide exciting opportunities for understanding consumer competition and community dynamics (Fukami et al. 2007, Grether et al. 2013, Fukami 2015, McPeek 2017). In the case of adaptive diversification, evolutionary models have largely failed to integrate the contingent ecological and evolutionary dynamics that could provide alternatives to the ecological release paradigm (discussed in Abrams et al. 2009). Predicting how populations respond to ecological variation should not overlook these complexities. For example, as judged by our data, models of interpopulation niche variation that consider optimal foraging in heterogeneous environments (e.g., niche compression) and nonlinearities (e.g., intermediate competition diversification) better predict geographic niche variation in *A. sagrei*.

Building evidence for and general appeal of the ecological release paradigm has made it the favored eco-evolutionary bridge between ecological opportunity and adaptive diversification (Schluter 2000, Yoder et al. 2010, Nosil 2012). The crucial ecological mechanism linking ecological release to adaptive diversification is an eco-evolutionary response (negative frequency dependent selection) to intensified intrapopulation resource competition arising from a reduction of interspecific exploitative competition and density compensation (Bolnick 2001, 2004, Bolnick and Lau 2008). However, pattern-based evaluations of ecological release and niche variation hypotheses reveal diverse responses (Crowell 1962, Roughgarden 1974, Lister 1976b, Huey and Pianka 1977, Vassallo and Rice 1981, Losos et al. 1994, Losos and Queiroz 1997, Mesquita et al. 2007, Svanback and Bolnick 2007, Costa et al. 2008, Araújo et al. 2009, Thomas et al. 2009, Nimmo et al. 2011, Jones and Post 2013, 2016, Araujo et al. 2014). This diversity suggests that intensified intraspecific competition attending release from interspecific competition is not the only way to generate phenotypic diversity in populations. As discussed in Abrams et al. (2008b), the conditions underpinning the ecological model of adaptive diversification are rather narrow and unlikely to reflect how generalist consumers interact with resource arrays. Indeed, a wide range of conditions are likely to generate disruptive selection on consumer resource use and a comprehensive survey of existing data is sorely needed to better summarize the evidence. We specifically recommend that behavioral interference, interspecific competition, foraging behavior, and predation need more attention in the ecological release literature. Ultimately, a unified conceptual synthesis is needed for the field to advance.

How could such modifications alter the dynamics of the ecological theory of adaptive radiation? First, it would shift the model away from an ecological release paradigm. What we mean here is that the initial stages of adaptive diversification would not rely on an inverse

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relationship between competitor richness and niche breadth to generate phenotypic diversity. Rather it would broaden the range of ecological components in the direction of species interactions in general – which are obviously much more diverse than the ecological release paradigm suggests. Doing so would uncover important new dynamics. For example, if the effect of community richness on *A. sagrei* diversification we illustrate here is reflective of initial ecological stages of adaptive diversification, it suggests that adaptive diversification processes are subject to a positive feedback (i.e., diversity begets diversity) driven by adaptive responses to increasing competition (i.e., not just intraspecific competition). Ultimately, it seems that niche diversification is not just a phenomenon attending low community richness (the classic model of ecological release), but a more general pattern emerging under a broad range of ecological circumstances. Stated succinctly, the pattern we uncover here suggests that more diverse communities are composed of more diverse populations.

In conclusion, the ecological release paradigm underpinning the ecological theory of adaptive radiation seems incongruent with our findings. Surprised by our results, we find ourselves without a satisfying explanation of their origin. Nevertheless, we find the overall pattern compelling. Our search for an explanation has identified new opportunities for exploration – in particular, the eco-evolutionary dynamics at the root of adaptive diversification. Much remains to be explored in the dataset we've assembled. For example, analysis of variation in prey size, prey habitat, and *A. sagrei* habitat use along the community richness gradient will help identify the mechanisms of niche evolution in *Anolis*. Nevertheless, we hope that our foray into the geographic variation in *A. sagrei* trophic niche stimulates new ideas about the adaptive diversification of *Anolis* lizards and a closer look at the ecological release paradigm.

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The brown anole (*Anolis sagrei*) as a model for studying life-history adaptation to seasonality

Introduction

In seasonal environments, the timing of reproduction can impact offspring fitness. Offspring produced late in the season often experience decreased survival and lower growth rates than earlier-produced individuals. This trend has been studied across a variety of taxa (e.g. Varpe et al. 2007; Warner and Shine 2007; Öberg et al. 2014; Pearson and Warner 2018). The seasonal decline in fitness-relevant phenotypes of offspring may be due to a concomitant decline in the quality of the offspring environment. Late-produced offspring may suffer from increased

competition from earlier-produced conspecifics that are larger and better able to acquire resources. Late-produced offspring may, independent of competition, have access to a poorer pool of resources during a critical early-life stage or simply have less time to grow prior to winter or dry seasons. Moreover, during winter, environmental conditions (e.g. reduced temperature and rainfall) may favor survival of larger individuals. Thus, late-produced offspring may not attain a body size or fat reserves/water supply that would ensure overwinter survival. For this reason, late-produced offspring tend to be of lesser reproductive value to their

Figure 1. A brown anole egg uncovered in the field. Eggs are often laid underneath cover objects (e.g. rocks. leaf litter) and left to develop under prevailing environmental conditions.

parents than earlier-produced offspring (see Varpe 2017 for review of adaptation to seasonality). When the reproductive value of offspring is season-dependent, life-history theory predicts that females will invest differently in early- vs late-produced offspring. This may be accomplished by altering the investment in offspring size vs number as the season progresses (Lack 1947; Smith and Fretwell 1974; Brockelman 1975). Early in the season, females should invest lots of energy

in many, smaller offspring; however, late in the season, they should invest less total energy but divide it among fewer, better provisioned (i.e. larger) offspring (Nussbaum 1981).

Reptiles have played an important role in studying seasonal shifts of parental investment towards offspring size versus number (e.g. Nussbaum 1981; DeMarco 1989; Du et al. 2014).
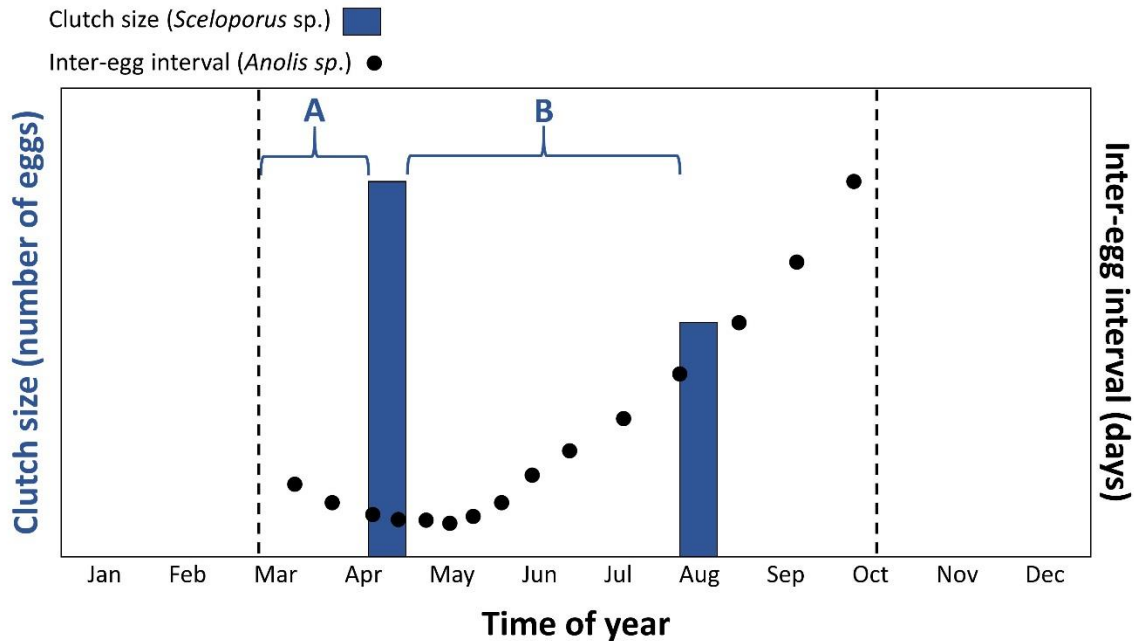

Figure 22. Hypothetical seasonal change in clutch size for an individual Sceloporus lizard (e.g. Sceloporus woodi; Jackson and Telford 1974; blue bars) and hypothetical seasonal change in inter-egg interval for an individual Anolis lizard (black circles). A greater inter-egg interval equates to a slower rate of egg production. Vertical dashed lines represent the hypothetical beginning (March) and end (October) of the breeding season for both species. Both individuals display the same general trend in reproduction: more eggs are produced earlier in the season than later. However, for the Sceloporus female, there are long periods of time during the breeding season (periods A and B) during which there is great uncertainty in how the environment impacts reproduction. The continuous reproduction of anoles, however, allows researchers to monitor changes in reproduction at a finer scale.

Unlike birds and mammals, oviparous reptiles rarely exhibit parental care (Fig 1). For this reason, measuring maternal investment into offspring is as straightforward as quantifying the amount of energy that a female invests into a single egg vs a clutch of eggs. Often, a simple measure of egg mass vs clutch mass will suffice. Although many oviparous reptiles have a prolonged reproductive season, there are often long intervals between reproductive events for a given individual. For example, seasonal shifts in offspring size vs number have been well studied in *Sceloporus* lizards (e.g. DeMarco 1989; Du et al. 2014); however, females may only produce 1 or 2 clutches (rarely 3) per year. Large periods of time pass between each clutch, making it difficult to determine which seasonally-shifting environmental factors (e.g. temperature,

photoperiod, food availability) drive seasonal changes in reproduction and precisely how those factors impact reproductive physiology (Fig 2).

The unique reproductive biology of *Anolis* lizards makes this group an excellent model for studying seasonal shifts in maternal investment. Anoles lay a single-egg clutch once every 4-14 days (depending on the species) across a broad reproductive season. They alternate egg production between ovaries, so each egg is yolked, shelled, and laid separate from every other egg (Crews 1977). The rapid, independent production of eggs allows females to adjust their reproductive effort among offspring as the environment changes. This continuous production of eggs has great potential to demonstrate how changes in maternal investment subtly shift through the season. In contrast, for lizards that produce 2-3 multi-egg clutches per season (e.g. *Sceloporus*), changes in maternal investment can only be measured discretely by observing mean differences between early and late season clutches (Fig 2). Furthermore, species that produce multiple eggs in a clutch are limited in their ability to differentially allocate resources among individual offspring within each clutch.

Figure 3. Differences in key reproductive traits between three seasonal cohorts of captive-bred A. sagrei. Cohorts 1, 2, and 3 were collected early-, mid-, and late-season, respectively. This figure was taken from Mitchell et al. (2018).

Recent published results from the Warner lab

Recently-published work from the Warner Lab strongly suggests that the quality of the offspring environment declines seasonally for brown anoles (*Anolis sagrei*) in Florida: survival is higher for early-produced offspring and lower for late-produced offspring (in the field - Pearson and Warner 2018; Mitchell and Warner unpublished data; and in the lab - Warner and Lovern 2014). Thus, we predicted that reproductive investment should shift seasonally in ways predicted by life-history theory. In a recently published study (Mitchell et al. 2018), we found that females produced more but smaller eggs early in the season and fewer, but better provisioned, higher quality eggs later in the season
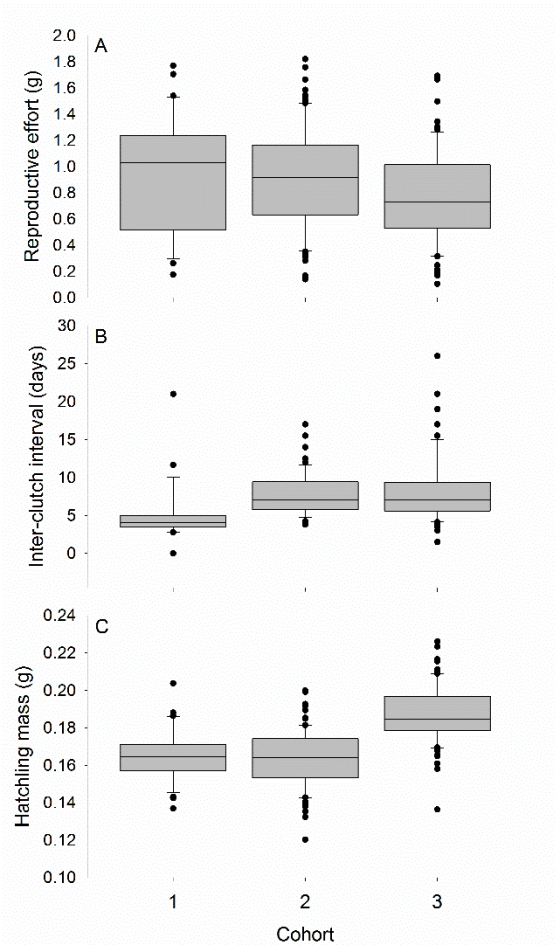
(Fig 3). Despite the larger size of late-produced eggs, reproductive effort was greatest early on. These are the patterns we would expect if selection favors females that shift investment in offspring size vs number throughout the season because the quality of the offspring environment declines. This result was also independently produced by another Warner Lab study (Pearson and Warner 2018).

Although these studies demonstrate that females shift reproductive investment in ways predicted by life-history theory, many important questions remain. For example, we still don't know to what extent these seasonal changes in reproduction are due to intrinsic factors (e.g. genes) and to what extent they are induced by proximate environmental cues. In these studies, we used separate cohorts of females that were each captured at different times during the same reproductive season (early-, mid-, and late-season cohorts in Mitchell et al 2018; early- and late-season cohorts in Pearson and Warner 2018). Although both studies controlled for factors that influence reproduction once the animals arrived in the lab (e.g. food abundance, temperature, humidity), each of these cohorts experienced a different environment in the field prior to capture. Thus, we can't say if these patterns are intrinsic or wholly induced by the environment. Additionally, existing studies have not explored how reproductive shifts differ among individual females. Such inter-individual variation is necessary for phenotypes to evolve via selection.

Current and future studies and preliminary results

To build upon these recent studies, we have another project underway that expands our knowledge of seasonal variation in reproduction of brown anoles. This project will determine if seasonal patterns of reproduction persist when females are housed in the lab for the entire
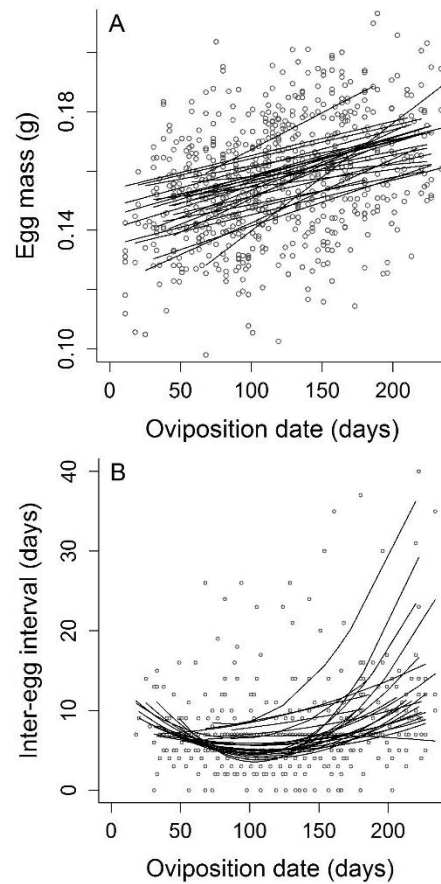
Figure 4. Changes in egg mass (A) and inter-egg interval (B) of female brown anoles across the reproductive season. Open circles show raw data from all females. Lines show fitted values for each female. Day 0 of oviposition date is March 10, 2017. Values of 0 for inter-egg interval indicate that 2 eggs were collected from a nest pot on the same day – thus, one egg was assigned an inter-egg interval of 0 days. In this study, we collected eggs 3 times per week, so we cannot be certain that two eggs were laid on the same day. However, in a current, unrelated study, JMH is collecting eggs from brown anoles daily and on many occasions (n=25) has collected 2 eggs from a nest pot on the same day. This indicates that female brown anoles sometimes oviposit 2 eggs within a 24-hour period.

reproductive season. We collected females at the beginning of the breeding season (early March; Lee et al. 1989) and tracked their reproduction in the lab until the end of October when egg-laying ceases in the field (Mitchell et al. 2018). We carefully monitored reproduction and growth during this time. We anticipated that the patterns observed in previous studies (e.g. a seasonal shift toward fewer, better provisioned offspring) would be observed in the lab if these patterns were somehow intrinsic; however, if the expected patterns were not observed, they may only be induced by conditions in the field. We also considered that these patterns may differ among females. Such individual variation is suggestive of a genetic basis for reproductive traits, which is necessary for phenotypes to evolve in response to selection.

We observed that, even when females are housed in the lab for the entire season, relative egg size (egg mass relative to maternal body mass at oviposition) increases through time independent of snout-vent length (oviposition date: $t_{1,631}=3.49$; p=0.005; SVL: $t_{1,631}=0.36$; p=0.72; Fig 4A). Thus, females are increasing the relative effort per offspring as the reproductive season progresses. At the same time, the rate of egg production is lowest (i.e. highest inter-egg interval) at the end of the season (oviposition date: $t_{1,625}=3.93$; p <0.001; SVL: $t_{1,625}=0.46$; p=0.65; Fig 4B.). These data support our hypothesis; however, the trends observed in this study do not appear as strong as those observed in other studies that leveraged temporally separated cohorts of breeding adults (e.g. Mitchell et al. 2018). Likely, seasonal shifts in reproductive traits are strongest in the field where both extrinsic (e.g. temperature, photoperiod, diet) and intrinsic (e.g. genes) factors may work additively.

We also observed among-individual variation in how reproductive traits shift through time (Fig 4); however, there is remarkable consistency in the patterns. For example, although some females increased egg size more than others through the experiment, all the slopes for this trait are positive. Regardless, seasonal shifts in key reproductive traits seem to persist when females were kept in the lab for the entire season. Thus, we think that brown anoles have great potential to make important contributions to our understanding of life-history adaptations to seasonal environments.

One final (and monumental) challenge remains. We need to assess how seasonal shifts in reproduction occur in the field. Due to the inconspicuous nesting behavior of anoles, it is difficult to locate large numbers of freshly laid eggs in the wild. For perspective, JMH estimates that he has checked over 5980 nest pots for eggs (in the lab) over the last 3 years. He has only once observed a female anole in the process of digging a nest. Currently, Christopher Thawley, James Stroud, and JMH are collecting reproductive data on brown anoles and crested anoles (*Anolis cristatellus*) in Florida (via dissection). This study can potentially determine how egg size shifts seasonally for both species. Two major drawbacks to such a study are that euthanasia precludes the ability to obtain longitudinal reproductive data on individual females and egg size measurements from dissection will not perfectly reflect egg size at oviposition. Thus, to better

assess how reproduction shifts in the field, other experimental designs may need to be employed (e.g. outdoor caging and egg collection; use of an ultrasound to monitor reproduction during a mark-recapture study).

In conclusion, multiple studies from the Warner Lab demonstrate that seasonal shifts in reproduction of brown anoles conform to predictions from life-history theory: when the quality of the offspring environment declines through the year, females shift from producing many, smaller offspring to fewer, larger offspring as the season progresses. The nearly unique reproduction of anoles (compared to other lizards; Fig 2) should allow us to formulate studies that explore how seasonal shifts in reproduction evolve and determine how a changing environment can impact reproduction in ways that might drive or constrain evolution. Some of these studies are already underway in the Warner Lab and, hopefully, we will have many more answers (and questions) to present at the next *Anolis* Symposium.

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Revealing Controls on Abundance and Microhabitat use of Anolis Lizards in a Changing Island Landscape using Airborne Remote Sensing

Project Summary

In these times of rapid environmental change and species extinction, understanding the drivers and mechanisms governing species' abundance is more important than ever. The major goal of this work is to further our understanding of what drives variation in species' abundance and microhabitat use through space, particularly in the context of rapid land cover change and human habitat conversion, using the little explored anole fauna of the Honduran island of Utila as a natural ecological laboratory. By pioneering emerging technologies in unmanned airborne remote sensing for predicting animal abundance, this project is designed to improve our ability to predict species' ecological responses to habitat conversion and identify key ecological interactions between habitat structure, microclimate, prey availability and species' abundance and distribution. The project will allow us to improve our understanding and the public appreciation of Utila's little known Anolis fauna, promote its conservation and demonstrate how emerging technologies can help us understand and preserve the natural world.
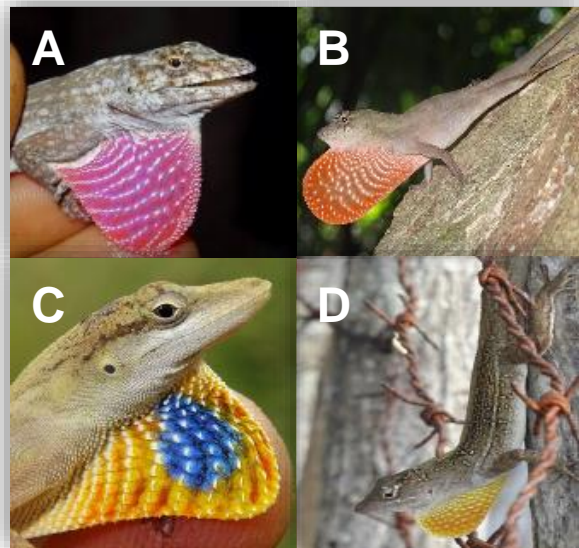
Figure 1: Anoles of Utila A) *Anolis utilensis*, B) *A. bicaorum*, C) *A. sericeus*, D) *A. sagrei*. Images courtesy of Kanahau Research and Conservation Facility. Used with permission.

Research Site

The research will take place on Utila, a small island (40 km^2) off the northern coast of Honduras, which hosts a number of land cover types including natural and degraded habitats and developed areas. The island is one of the protected Bay Islands and features a rich mosaic of habitats, including mangrove, tropical dry forest, neotropical savannah and volcanic rock exposures (Schulte and Köhler, 2010; Fawcett *et al.*, 2016), all of which contribute to the islands high biodiversity. To date a total of 42 amphibian and reptile species have been

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recorded on the island (McCranie and Orellana, 2014), including five species of anole: *A. sericeus, A. utilensis* (endemic; fig1), *A. bicaorum* (endemic; fig1), *A. sagrei*, a recent invader, and *A. allisoni.* An expanding tourism industry along with illegal housing developments has led to ongoing habitat fragmentation and degradation.

Figure 2. Location of Utila, Isla de Bahia, Honduras

Emerging remote sensing technologies have the potential to transform our understanding of the link between species' abundances, habitat use, and environmental change. Unmanned aerial vehicles (UAVs), such as the DJI Phantom 4 Advanced (Figure 3), now make it possible to capture highly detailed information on canopy structure from plot to landscape scales. However, field tests of the capacity of remote sensing to capture habitat characteristics relevant to animals living below the canopy are limited. We still know little about the mechanisms linking remotely-sensed habitat characteristics and animal abundance.

Figure 3. DJI Phantom 4 Advanced with attached RGB and MAPIR Near Infrared Cameras.

We will test the ability of remote sensing to predict variation in anole abundance and habitat use at the landscape scale across different land cover types, providing the first systematic exploration of the abundance and ecology of Utila's anoles. We will test hypotheses for the mechanisms linking canopy structure and species' abundance by integrating UAV-captured canopy data with field data on below-canopy habitat structure, prey availability, thermal environment, anole microhabitat use and abundance. This will not only help reveal the secrets of this little understood fauna but identify general principles underlying limits on animal abundance and factors that inhibit or promote the use of human-modified environments by native species.

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The potential for large-scale behavioral studies: A call to *Anolis* field biologists

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The history of *Anolis* lizard research is firmly rooted in studies of behavior. Indeed, some of the earliest studies of anoles were intensive examinations of behavior, exemplified by the large body of work by Evans in the 1930s (e.g., Evans 1936, 1938) and the classic greenhouse study of *Anolis carolinensis* by Greenberg and Noble (1944). Behavioral studies in the lab and field have continued to be a focus of anole researchers ever since, including the work of scientists such as Robin Andrews, David Crews, Neil Greenberg, Tom Jenssen, Manuel Leal, Jonathan Losos, Stan Rand, Judy Stamps, Robert Tokarz, Robert Trivers, Juli Wade, and their students and collaborators. With such a large body of work on anole behavior, you might think that we now know everything there is to know about how these lizards behave. What is the value, then, of continued studies of anole behavior?

When Charles Snowdon was president of the Animal Behavior Society in 1990, he wrote that "Behavior is the link between organisms and environment, and between the nervous system and the ecosystem." In other words, studies of animal behavior provide the context for all of the other traits we anole biologists study. If we're studying the development of a morphological structure, an understanding of how animals use that structure is critical. If we're studying population structure, an understanding of how animals move through time and space is informative. If we're studying adaptation to climate change, an understanding of how animals behaviorally thermoregulate is important. And so on. Without an understanding of the natural history of our study organisms (and their behavior, in particular), our findings will be limited. Further, species in our changing world are increasingly affronted with novel environmental conditions, both abiotic and biotic, and behavior is the first way that *all* species respond to new challenges. If we hope to be able to understand the ecological and evolutionary impacts of human-induced global change, then we need to understand the proximate changes in behavior that may reveal the new selection regimes that each population or species is encountering.

Yet, despite the rich history of behavioral work in anoles, there are many basic questions about anole behavior that remain unanswered. Further, most behavioral studies of anoles focus on a single population, such that we know very little about the intraspecific variation in behavior across habitats. Likewise, we know little about behavioral consistency over time; the same

behavioral measures are rarely collected in a previously-studied population, and the development of behaviors across the anole lifespan is rarely considered. We also have almost no data on juvenile behavior in most anole species, and studies on female behavior remain rare (although female anoles are receiving more attention in recent and ongoing work). Further, even in many well-studied species, we know little about behaviors that are relatively rarely observed, such as copulation, oviposition, escape from predation, or dispersal. For example, I recently wanted to determine the average copulation duration for the 30 anole species that I have studied, for a total of approximately 1600 hours of focal observations during the summer breeding season. In compiling data from my field notes, I found that I (or my students) have observed a total of 64 complete copulation events in 17 of those species (with 24 of those copulations occurring in the two species I have most commonly observed). Despite hundreds of hours of observation, I do not have any copulation data for 13 species (and I have not found this information in the primary literature). Thus, there remains a clear need for further behavioral studies in anoles, and there is great potential for combining quantified behavioral data from multiple research teams to conduct larger-scale analyses.

For behavioral data to be combined from multiple studies, a standardized methodology to quantify behavioral events is needed. The two most commonly measured behavioral traits are the frequency at which a behavior occurs, and the duration of that behavior. For those new to behavioral work, I describe my own methodology below, which is generally consistent with the work of many other anole behavioral biologists.

Figure 1. Field assistants Amy Payne (left, Texas) and Annie Chen (right, Dominican Republic) collect anole behavioral data in the field.

Focal behavioral data (i.e., the record of an observation of a single, focal animal) are relatively straightforward to collect, as you basically watch an animal and record what it does. At

a minimum, recording these data requires only an attentive observer, a wristwatch, a pencil, a field notebook, and perhaps (depending on the wariness or perch height of the species, or the sharp-eyed vision of the observer) a pair of close-focus binoculars (Figure 1). (This is also a particularly attractive toolkit for researchers working with a small budget.) There are many ways to enhance this basic toolkit, of course, and some researchers use video or voice recordings to document observations. Video, in particular, can provide a remarkable wealth of valuable behavioral information, but as much of my work occurs in remote Caribbean locations with unreliable electricity for recharging electronics and uploading large files, I use pencils and Rite-in-the-Rain notebooks in my own research. Another powerful technology that I have not yet explored in my own work, but is becoming far more common (and inexpensive), is the use of smart phone or tablet apps to collect time-stamped data on each behavior recorded, potentially for multiple animals at the same time. These apps make collecting behavior data *even* easier, and while their development has been primarily driven by research on other taxa (e.g., primates, birds, and guppies), they should be readily adaptable to anole behavioral research.

Yet, behavioral observation can take a lot of time, which may be the main deterrent preventing more field biologists from conducting focal behavioral studies. And, as in other fields, some kinds of behavioral data are easier to collect than others. Just as collecting snout-vent length (SVL) is easier that collecting dewlap reflectance data, determining the number of a lizard's locomotor movements per minute is more straightforward than assessing its spatial location over time. On the other hand, large groups of students (or others) can be employed to collect most types of behavioral data with relatively little training. And while some species are so active that it can be difficult for one observer to record all of their movements, others (particularly mainland anoles) appear to do very little, which makes for generally boring observations. However, my own most interesting discoveries have often resulted from these careful observations!

In our fieldbooks (Figure 2), at the beginning of each observation we record the individual's identify (species, sex, and ID number if applicable), the location of the observation, and the date. We then record each behavior by each minute of the observation, with abbreviations for the most commonly observed behaviors. Our work has generally focused on social and locomotor behaviors, and we record those as follows. During displays, we record each time the dewlap is extended (noted as D), and each time the lizard performs a pushup or headbob (P). Early in my behavioral work, we recorded pushups and headbobs separately, but I found that it was difficult for my student assistants to consistently distinguish between the two movements, especially across species with dramatically different display patterns. In the field, we can far more consistently count the total number of up-and-down movements, whether they are headbobs or pushups, and so I now use the combined total of pushups and headbobs in analyses (we call these "pushbobs"). For locomotor movements, we record each movement as a run (R), crawl (C), jump (J), or a change of position but not location, such as when a lizard moves to face

the ground instead of the canopy (we record this movement as @). Other researchers may denote this kind of movement as a crawl. We also describe any other behavioral events in words, such as changes in body color, defecation, raising the nuchal crest, developing an eyespot, consuming prey, copulating, or licking the substrate. Thus, our field notes often look like this:

> 9:32 C   C   C   J
> 9:33 eat   R
> 9:35 D, 4P   D, 4P   C

This notation would indicate that the lizard crawled three times and jumped at 9:32, ate and ran at 9:33, did not perform any (recorded) behaviors at 9:34, and displayed (for a total of 2 dewlap extensions and 8 pushbobs) and crawled at 9:35.
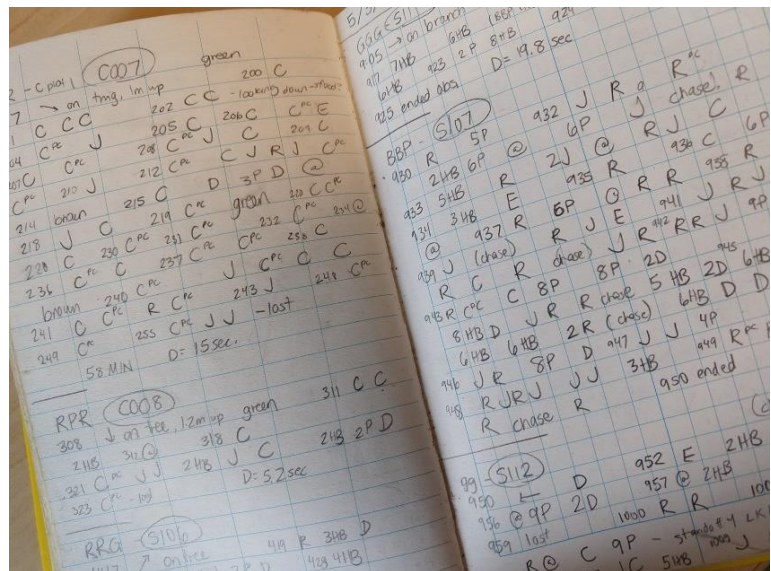

Figure 2. Sample field notes from anole observations. (In these notes, "PC" indicates a perch change, "E" indicates eating prey, and "HB" indicates headbobbing.)

Our general rule is to try to record as much behavioral information as possible during each observation. Depending on the focus of the current project, we may add more information to our notes, such as movements of the head, the orientation of the lizard on its perch, or the duration of time (in seconds) the dewlap is extended. In addition, several highly detailed ethograms of anole behavior are also available in the literature, which greatly extend the simple behavioral repertoire I describe here (e.g., Greenberg 1977; Jenssen et al. 1995). Generally at the end of each field day, and no later than several days after an observation, all observers tally their observational data in a spreadsheet, such that we have both a hard copy and a digital version of each observation. We have used this general approach to observe both marked and unmarked lizards, and for observations of varying lengths of time. Our observations generally last 30 minutes, but for species that occur at low densities or are highly cryptic, we have conducted focal observations for durations of up to 180 minutes.

I have begun each of my behavioral studies with a particular goal in mind, or a specific hypothesis to test. However, throughout my career (from my work as a graduate student with Jonathan Losos, a postdoctoral researcher with Juli Wade, and now in my own lab at Trinity University), because I have collected all of my focal behavioral data in a generally consistent way, I can make comparisons across different species, populations, and years of study. I have also been able to combine my behavioral data with those of others to address larger-scale questions. For example, we combined my field data with field data collected by Jonathan Losos, Manuel Leal, Lourdes Rodríguez Schettino, and Ada Chamizo Lara to examine anole foraging behaviors across 8 field seasons and 5 islands (Johnson et al. 2008). I have also found that my extensive behavioral data have been useful in new and unexpected ways. For example, in my dissertation work, I marked all of the lizards within each of a series of study plots to study territorial defense and territory overlap in 14 species of anoles (Johnson et al. 2010). By marking each lizard in a study plot in order to repeatedly measure its behavior, we had (unintentionally) also effectively censused the population in each plot. Recently, Pavitra Muralidhar and I examined these data to measure variation in population sex ratios across species (Muralidhar and Johnson 2017). I am now working with Ambika Kamath and James Stroud to reexamine data from these same marked lizards to determine if individual anoles exhibit different degrees of specialization to particular microhabitats (Kamath et al. in prep.). Finally, I have also been able to repeatedly mine my own behavioral data to address new questions (such as whether the duration of copulation across species, mentioned above, is associated with the male copulatory morphologies I have measured more recently). Thus, my own experiences have shown me that there can be exciting long-term payoffs for collecting detailed behavioral data.

In conclusion, despite a long history of valuable anole behavioral studies, there remain so many important issues to address. By sharing data with new collaborators to address new areas of study, and with a larger group of researchers collecting focal behavioral data on diverse populations, our community will continue to tackle both classic and innovative questions in anole biology.

Acknowledgements

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Beneath the Spanish moss: Growing up with *Anolis* in Florida
A photographic naturalist's perspective

From March through November of 1774, two years before Thomas Jefferson composed the Declaration of Independence, American naturalist William Bartram sojourned beneath and through the Spanish moss and scattered palms of the Floridian peninsula, recording his encounters of flora, fauna, and people. Nearly twenty years after his journey, on the flip-side of the American Revolution, Bartram published his 1791 naturalist travelogue, *Bartram's Travels*[2].

Bartram's Travels is, of course, rife with dynamic descriptions of Floridian wildlife. While some details are consistent with what we know to be true today, others fall somewhere between fiction and fancy. Historical naturalist writing is prone to dramatism of the observed by the observer. As for *Travels*, Bartram describes a number of the reptiles he encountered during his journeys, no doubt fascinating material to and for his more-northern audience. As one might expect, the American alligator (*Alligator mississippiensis*) looms dramatically large and somewhat menacingly in several passages. The Eastern diamondback (*Crotalus adamanteus*), Timber (*C. horridus*), and Dusky pigmy (*Sistrurus miliarius barbouri*) rattlesnakes also make brief appearances. So too does the Florida cottonmouth, *Agkistrodon (piscivorus) conanti*.

Despite the seductive allure of Florida's larger, more dramatic reptilian biota, Bartram does pause to note a curious little lizard we might recognize. In Chapter 5 of *Travels*, Bartram writes,

> The green lizard or little green chameleon is a pretty innocent creature; the largest I have seen were not more than seven inches in length; they appear commonly of a fine green colour, having a large red gill under their throat; they have the faculty of changing colour, which, notwithstanding the specious reasoning of physiologists, is a very surprising phenomenon. (Bartram, 1791, Ch. 5)

Bartram is clearly describing *Anolis carolinensis*, the Carolina green anole, the sole species of *Anolis* ranging throughout Florida during the 1700s (*so far as we know*). In his writing and in relation to what we know today, Bartram seems to have a better handle on alligators and

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[2] The full title is *Travels through North & South Carolina, East & West Florida, the Cherokee Country, the Extensive Territories of the Muscogulges, or Creek Confederacy, and the Country of the Chactaws, Containing an Account of the Soil and Natural Productions of Those Regions, Together with Observations on the Manners of the Indians*. http://docsouth.unc.edu/nc/bartram/bartram.html

rattlesnakes than he does the reality of the "little green chameleon" with the "red gill." Unfortunately for us, however, that's about it for *Anolis carolinensis* in *Bartram's Travels*. Indeed, for the reading audience of the late 1700s, there were bigger critters to focus on in his narrative travelogue.

One hundred and four years after the publication of *Bartram's Travels*, Bradford Torrey published his own Floridian travelogue, *A Florida Sketchbook*, in 1895. Like his predecessor, Torrey, a New England native, spent much of his time traveling across North America and (*even more dramatically*) recording what he too observed. A writer for *Atlantic Monthly* and *Youth's Companion*, a popular children's magazine, Torrey's representations of Floridian biota are at times rather hyper-realistic, over-saturated, and somewhat exaggerated. His is a text meant more to entertain and enthrall than to inform and analyze — the looser, more imaginative side of the naturalist writing spectrum.

As with Bartram, Torrey also finds himself in the company of Floridian lizards while exploring sugar mill ruins near New Smyrna Beach in what is now Volusia county, Florida:

> The morning is cloudless and warm, till suddenly, as if a door had been opened eastward, the sea breeze strikes me. Henceforth the temperature is perfect as I sit in the shadow. I think neither of heat nor of cold. I catch a glimpse of a beautiful leaf-green lizard on the gray trunk of an orange-tree, but it is gone (I wonder where) almost before I can say I saw it. Presently a brown one, with light-colored stripes and a bluish tail, is seen traveling over the crumbling wall, running into crannies and out again. Now it stops to look at me with its jewel of an eye. And there, on the rustic arbor, is a third one, matching the unpainted wood in hue. Its throat is white, but when it is inflated, as happens every few seconds, it turns to the loveliest rose color. This inflated membrane should be a vocal sac, I think, but I hear no sound. Perhaps the chameleon's voice is too fine for dull human sense. (Torrey, 1895, Ch. 5)

By his description, it seems Torrey may have observed two *Anolis* lizards and perhaps one of Florida's toothy, *Plestiodon sp.* skinks. The first anole, clearly *A. carolinensis*, sports its standard "leaf-green" coat, but the other anole is described as matching "unpainted wood in hue." This second lizard inflates its throat to reveal "the loveliest rose color." Such subjective descriptions of color can be interpreted a number of ways. This third lizard was likely also *A. carolinensis*, but it's worth remembering that *Anolis sagrei* in Florida arguably dates back to the 1880s — a decade prior to Torrey's publication. Regardless of species, the third lizard is clearly

Fig. 1. *Anolis carolinensis*, the Carolina green anole. Top: Broward county, FL, 21 Jan. 2017; Left: Lake county, FL, 03 Mar. 2012; Right: Lowndes county, GA, 31 Aug. 2011.

an anole extending its dewlap. Whereas Bartram described the anole's dewlap as a "gill" of sorts, Torrey describes it as possibly a "vocal sac." Unsurprisingly, Torrey does not actually *hear* the anole make any sounds with its extendable "vocal sac."

I often reflect on Torrey and Bartram's respective travels through Florida and imagine them trying to get a handle on the postmodern ecology of my home state today. Florida is, of course, a peninsula sporting a fluid, turbulent, unpredictable, and *rapidly* changing network of ecological systems. Non-native species come and go, and much of the state's biota is perpetually

Fig. 2. *Anolis sagrei*, the Cuban brown anole. Top: Lake county, FL, 11 Feb. 2016; Left: Broward county, FL, 23 Apr. 2013; Right: Collier county, FL, 25 May 2012.

negotiating new challenges to almost (*but-not-quite*) established patterns. Southeast Florida is particularly dynamic on this front, though the entirety of the peninsula is undergoing rapid-fire, dynamic change in one way or another.

Growing up in Volusia county, Florida, during the 1970s and early 1980s, *A. carolinensis* was a common staple on the exterior of my family's Ormond Beach home (*also in Volusia county*). Our shrubs, windows, screens, and panels seethed with Carolina green anoles posturing, bobbing, and displaying. Every now and then, however, I'd see a *different* kind of anole when

my family went to buy groceries at the Trails Shopping Center. The shopping center was rife with shrubs and elaborate decorations. An artificial system of interconnected, flowing pools of water wove through the outdoor complex of wood-paneled buildings. It was in these shrubs and on the lower reaches of the wood-paneled walls of the Trails Shopping Center that I first saw *Anolis sagrei* as a child. Back then, Cuban brown anoles seemed rare, *precious* even. They were faster and more skittish than the greens that dominated our home a mere mile away. Catching these low-riding brown anoles was far trickier than getting my hands on the Carolina greens. It was also more fun. They made you *work* for it, those Cuban brown anoles.

Nowadays, the spread of *Anolis sagrei* throughout the entire Floridian peninsula (*and beyond*) is well documented. The Carolina greens have moved a bit higher, *back into the trees and higher on the walls*, while the Cuban browns now scratch out a living along the edges of nearly every shrub-lined sidewalk and driveway in the state. It's hard to go *anywhere* without seeing Cuban brown anoles darting about in front of your feet.

Though I've never lived in South Florida, I've made it a habit to try to get down there a few times every year to observe and photograph the ever-shifting maelstrom of biota trying to find its place among the banyans, palms, and Spanish moss. Taking advantage of the miracle of mechanized transportation, I'm able to skip to the south side of the peninsula and continue tracing out my own little sketchbooks and travels, so to speak, in a fashion far more hit-and-run and rapid-fire than what Bartram and Torrey were able to do in their respective times. Whereas Bartram and Torrey had to rely primarily on the written word and the mental image, I, like many others, carry with me my trusty and handy DSLR, eager to catch lizards and conduct macro studies of each lizard's scaling and patterning. It never gets old.

When I head down south, some locales feel different from prior visits. One species will have moved into a new area, while another will have seemingly vanished. Fluidity is the currency of these South Florida ecological battlefields. In other locations, however, relative stability adorns the passage of time — *for now, at least*. While some populations and communities in Florida can change faster than the weather during the late summer months, others somehow stubbornly resist change over time and persist in their micro-habitat domains.

At home in Volusia county, *A. carolinensis* and *A. sagrei* remain our two resident anoles species. Though many locals will claim the Carolina greens are are "gone," I still readily find *A. carolinensis* in my home turf; they've simply moved higher into the trees and, interestingly, lower into the inundated wetlands. Indeed, I now often find Carolina green anoles utilizing lily pads and grassy reeds within local wetland and pond habitats — environments not really utilized by the the Cuban brown anoles. Though they may not be as obvious as they once were, the Carolina greens continue to march forward.

As for *A. sagrei*, as noted prior and well documented elsewhere, they have been extraordinarily successful through the Floridian peninsula. Whether homes, malls, theaters,

stores, restaurants, or you name it, if there's a shrub, there's a few dozen Cuban brown anoles darting about in close proximity, dominating their low-riding kingdoms with vigor and veracity. The battle for the Floridian peninsula has already been won, and Cuban brown anoles have very much earned their place in *La Florida*. They are, in my view, as ubiquitous as great blue herons, sunburns, Disney advertisements, and "Florida Man" news reports.

When I was younger, *still a kid flipping through my Audubon field guides and dreaming of the day I'd be able to drive south to Miami*, the Cuban knight anole, *Anolis equestris*, was my Holy Grail non-native species in Florida — the one species I knew I would one day be lucky enough to observe first hand. Of course, I've now worked with more than a few in south Florida. Though my youthful fantasies of dragons in the trees, as I once imagined them, have been somewhat grounded by reality and experience, I still find the knight anoles to be tremendously fascinating beyond reason. Though still limited to South Florida, they *have* expanded north along the Atlantic coastline over the years — as far north as St. Lucie county, just on the edge of what

Fig. 3. *Anolis equestris*, the Cuban knight anole. Top: Miami-Dade county, FL, 11 June 2016; Left: Miami-Dade county, FL, 18 Mar. 2017; Right: Miami-Dade county, FL, 11 June 2016.

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is considered to be central Florida. I even occasionally receive isolated accounts of *A. equestris* from friends and colleagues here in central Florida, though I haven't found any established populations. I suspect people head to south Florida and bring Knights back, perhaps hoping they'll spring up in their yards. This is Florida, after all, and *anything goes*.

Far more focused in Miami-Dade and Broward county is *Anolis cristatellus*, the Puerto Rican crested anole. In the Coral Gables area, *A. cristatellus* can be ridiculously abundant — darting about the lower trunks of trees and swirling around competing *A. sagrei*. Given the density of *A. cristatellus* in Miami-Dade and Broward counties, as you move north out of their introduced range, their absence can suddenly feel dramatic and jarring; I suspect the more-northern Cuban brown anoles don't mind their absence. To date, I have not seen or heard any

Fig. 4. *Anolis cristatellus*, the Puerto Rican crested anole. Top-Left: Miami-Dade county, FL, 02 Sep. 2011; Bottom-Left: Miami-Dade county, FL, 11 June 2016. *Anolis distichus*, the Bark anole. Top-Right: Monroe county, FL, 10 June 2011; Bottom-Right: Monroe county, 08 July 2011.

personal accounts of *A. cristatellus* in central Florida, though many amateur naturalists confuse cresting *A. sagrei* as Puerto Rican crested anoles.

Also quite common in southeastern Florida is *Anolis distichus*, the Bark anole, an adorable but extremely frustrating little non-native species. Currently ranging from Key West north to Stuart (in St. Lucie county), this small trunk-ecomorph is particularly frisky and reactive. Unlike *A. sagrei* and *A. cristatellus*, Bark anoles don't *cooperate*, so to speak, with lumbering hominids carrying cameras or lizard gigs. Their hyper-defensiveness and ultra-agility are understandable,

Fig. 5. Left column: *Anolis chlorocyanus*, the Hispaniolan green anole, Broward county, FL, 21 Jan. 2017. Right column: *Anolis cybotes*, the Large-head anole, Broward county, FL, 21 Jan. 2017.

however, as this species seems to have brought a knife to the gunfight of competition in south Florida. Flanked by larger species from both below *and* above the ecomorphology scale, Bark anoles spend much of their time wrapping around tree trunks, trying to avoid perpetual drama. I do, however, find them easier to work with in the Florida Keys. In the Florida Keys, almost *everything* is more relaxed. *Almost* everything.

In Broward county, I was able to observe and photograph a fairly active and seemingly dense population of both *Anolis cybotes*, the Large-headed anole, and *Anolis chlorocyanus*, the Hispaniolan green anole. Though each species has been observed elsewhere in the Broward county area, I've only seen these two species in one focused location, an area also rife with *A. sagrei* and *A. carolinensis*. It was most certainly a packed micro-habitat for these lizards, and our little group of lizard-hunters was able to get our hands-on seven *A. cybotes* and five *A. chrorocyanus* in well under two hours (*not to mention those who weren't caught*). For the casual photographic naturalist such as myself, this was a day of ridiculous abundance and overkill, though I'm not complaining.

Fig. 6. *Anolis garmani*, the Jamaican giant anole. Left-column: Miami-Dade county, FL, 11 June 2016; Right: Miami-Dade county, FL, 06 Aug. 2017.

From a Floridian perspective, *Anolis garmani*, the Jamaican giant anole, is the stuff of legend. *A thunderdome of a species*. *The holiest of grails*. Stories and reports of isolated populations in south Florida continue to drift about the layman's internet and unanswered comments are repeatedly posted, "Hey, where'd you see that?" Most aren't willing to say, of course. As for myself, I'd been clued in on a few spots which repeatedly failed to deliver… *until they did deliver*. Truly, *A. garmani* did not disappoint.

A crown-giant ecomorph, *A. garmani* is (*subjectively speaking, of course*) the most beautiful species of wild anole we have in the tangles of south Florida. I was able to photograph two specimens in-hand during my first encounter, both fairly small. I've never seen such dynamic color displays and rapid color changes in an anole before. Both individuals put on quite a show, eye candy to the max.

On a later visit down south, I was able to snag some decent shots of an adult perched fairly low on a tree, but the capture didn't quite go as well as hoped. It was one of those failed catches that stings and burns. As the long strip of emerald green escaped to the foliage above, the deep, sustained feeling of *actually-missing-that-lizard* settled in for the long haul. To this day, missing that lizard still hurts. One day the wrong will be righted, and my camera will find justice on the other side of persistence. Jamaican giant anoles are not easy to find (*or catch*) in south Florida. Know hope.

Of course, there are other species of Anolis known to be in Florida at one time or another.

I've never been able to resolve differentiating between *A. porcatus* from *A. carolinensis* in Miami-Dade county, so the Cuban green anole remains a species somewhat off my radar for the time being. It's possible I've photographed a hundred of them and have no idea. It's also possible *A. porcatus* and *A. carolinensis* are, at this point, one and the same in south Florida. I can't really tell them apart.

Another non-native species, *Anolis allisoni*, has also been recorded in Florida. A few months back, I traveled to Naples, Florida, to look for *A. allisoni* based on some fairly specific accounts. I managed to find the actual reported location *and* an anole I suspected was *A. allisoni*, but I *also* managed to catch the ire of a paranoid police officer who didn't like the looks of a guy creeping about the edge of the shrubs with a fairly large camera. Before I could catch or clearly photograph my suspect, I was interrupted by this Naples officer who (*for reasons I still don't entirely understand*) told me I had to leave the property (*public* property, mind you) because I had a camera with a big lens <u>and</u> because of "all the stuff going on." I'm still not sure what all

that stuff was, but cameras are now apparently a threat on public property (*hide your phones*)! With the situation escalating, I retreated fairly quickly as a second office joined in the fray. I'll return more conservatively at some point and look for those blue-headed wonders once again.

Then there's *A. trinitatis*, St. Vincent's bush anole, another blue-accented anole species recorded in Florida. It looks like this one, however, may have been extirpated from its primary home base near Miami Beach. Maybe, or maybe not. I'm sure they'll show up somewhere else at some point. Non-native species tend to do that in Florida, and every day is a day for something unexpected on the ecological front.

Truly, in Florida you just *never really know*. Things change pretty quickly, and I never know what I'll find on the next trip south. I variously see reports of many other *Anolis* species throughout Florida, *mostly in south Florida*, but many of these are likely escapees from either the domestic or commercial pet trade — not necessarily established colonies or populations. Still, in Florida the golden rule of identification should always be "Never say 'Can't Be'" based on conventional range mapping or species descriptions. The second golden rule should be: "When in doubt, get a sample!"

In July 2018, I jaunted south to the Big Pine Key stretch of the Florida Keys — nearly a year after Hurricane Irma devastated the region. My objectives were to survey the damage dealt to the mangroves in that area and to check out any *Anolis* or *Nerodia* action. The damage to the mangroves was indeed still considerable, unfortunately. At one point, however, I spotted a curious green anole ducking about the foliage fairly high off the ground near a parking lot. I only had my iPhone on me, but I did manage to snag a quick reference shot of its dewlap. At this

Fig. 7. *Anolis equesris*, the Cuban knight anole. Top: Miami-Dade county, FL, 11 June 2016.

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point, I high-tailed it back to my Jeep and snagged my Nikon. This was a curious looking green anole. After a few minutes, I was able to find it again. Though I wasn't able to get my hands on the lizard (*another unfortunate 'miss'*), I did at least get some decent reference shots.

At first, I assumed it was simply *Anolis carolinensis* with a fairly excessive amount of yellow. Only later was the idea brought up that it could be, in fact, *A. smaragdinus*, the Bahamanian green anole. I later solicited identification feedback, and the dominant responses fell on the *Anolis carolinensis* line (based on head shape). Still, nearly all respondents remarked on those bold, strong yellows and that fantastic dewlap. Now, I *really* wish I'd been able to snag that lizard. Sometimes photographs simply aren't enough. Sometimes photographs ask better questions than find answers. Perhaps that's one key value to layman naturalism in general: It inductively finds the questions while science deductively seeks the answers.

For Bartram and Torrey, the idea of hopping back south to double-check something wasn't really an option. For me, on the other hand, *it is*. Being regional and local has its advantages, and it's something I'm often grateful for.

At this point in my life, I've settled into my own unique sojourn, my own little dance beneath the Spanish moss, camera in hand, eyes darting about, questions rattling off every which way but loose. Like so many amateur naturalists and ecology enthusiasts, I'm eager to photograph and record the changing world around me, and in Florida, every day holds the potential of asking some damn fine questions. I find no greater source for inspiration than the tangled biodiversity of *Anolis* in the Sunshine State, *La Florida*.

Fig. 8. The mystery green anole; likely *Anolis carolinensis*, but could it be *A. smaragdinus*? Monroe county, FL, 26 July 2018.

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Collaborative research projects on *Anolis* lizards in Cuba

We have been collaborating with Dr. Antonio Cádiz (Havana University until 2016, and Queens College at the present) and Dr. Luis M. Díaz (the National Museum and Natural History of Cuba) since 2009 to investigate the ecological and evolutionary aspects of *Anolis* lizards in Cuba. Our researches have conducted under the collaboration agreements between Tohoku University and Havana University 2010-2016), and between Tohoku University and the National Museum and Natural History of Cuba (2017-). Our Japanese members have conducted fieldwork throughout Cuba every September since 2010. Herein, we would like to describe the focus of our ongoing research.

Evolution of thermal adaptation in Anolis lizards in Cuba

We hypothesize that ancestral *Anolis* species of Cuba might have inhabited forest interiors, where dense canopy cover limits direct sunlight, allowing ambient air temperatures to remain relatively cool. However, some *Anolis* species, such as *A. sagrei* and *A. porcatus*, inhabit open habitats and human-developed areas where direct sunlight leads to much higher air and substrate temperatures. We estimate that the evolution of *Anolis* from shade-adapted, interior-forest species to open-habitat species has occurred at five independent times in Cuba (Kanamori et al. manuscript under preparation).

We are currently examining the genetic factors responsible for facilitating the evolution to different thermal environments, particularly from cool-shaded habitat to hot-open habitat. In one of our previous studies (Akashi et al. 2016), we detected a deferentially expressed gene associated with circadian regulation, *Nr1d1*, which exhibits opposite expression patterns in the cool- adapted *A. allogus* and the hot- adapted *A. sagrei*. In that study, we also focused on heat avoidance behavior and the sensor genes that might be responsible for that behavior. We showed that temperatures triggering behavioral and TRPA1 responses are significantly lower in the shade- dwelling species *A. allogus* than in the sun- dwelling species *A. homolechi*s and *A. sagrei*. Similarly, the TRPV1 and TRPM3 genes are believed to be involved in acute noxious heat sensing (Vandewauw et al. 2018; Nature, 662-666). Therefore, we are planning to examine the sequence evolution and thermal sensitivity of TRPV1 and TRPM3 as well as TRPA1 genes.

Based on previous results, we are now pursuing the following lines of research:

(1) Using coding sequences determined with RNA-seq, we are attempting to detect positively selected genes in *Anolis* lineages to determine which species have evolved from exploiting cool-shaded habitats to hot-open habitats (Kanamori et al. in prep.).

(2) We are comparing the gene expression and genomic sequences between forest and semi-desert populations of *Anoli*s to detect candidate genes associated with adaptation to hot and dry habitats (Ishii et al. manuscript under preparation). Our focus is on *A. homolechi*s, which usually inhabits forest edges. However, in eastern Cuba, we found a population of *A. homolechis* living in semi-arid areas where the annual average temperature is 5° higher than nearby forest habitats.

(3) Because thermal environments differ among populations even within a species, we plan to compare whole-genome sequences among *Anolis* populations living in different thermal habitats to detect gene sequences related to thermal regulation. At present, whole genome sequences of only a few species (e.g., *A. carolinensis*) have been reported (e.g., Tollis et al. 2018; Genome Biology and Evolution, 10:489-506). We are attempting to determine whole genome sequences for several Cuban species using Chromium systems.

Adaptation to hot-open habitat might be related to invasion ability. *A. carolinensis* and *A. sagrei* (both species native to Cuba) are known to be invasive, and both species have exerted significant negative impacts on habitats in regions where they have been introduced. *A. carolinensis* evolved from within a clade of *A. procatus*, which also inhabits open-hot habitats. However, *A. procatus* and *A. sagrei* might have evolved from ancestral species that inhabited cool-shaded habitats. Thus, we hypothesize that the ability to exploit hot-open environments might be related to the evolution of invasiveness, and therefore, we are searching a genomic basis that could facilitate both adaptation to hot-open habitat and invasiveness.

Phylogeny of Cuban Anolis *lizards*
Cádiz et al. (2013) constructed a phylogeny using 13 trunk-ground species from 34 locations throughout Cuba and analyzed factors affecting species differences in genetic variation within species (Cádiz et al. 2018). We estimated that there are 33 species belonging to more than 219 populations. Our results provide the most comprehensive sampling of Cuban *Anolis* species to date.

Literature published by the project
Thermal adaptation
Akashi, H., S. Saito, A. Cádiz , T. Makino, M .Tominaga, M. Kawata. (2018) Comparisons of behavioral and TRPA1 heat sensitivities in three sympatric Cuban Anolis lizards. ***Molecular Ecology*** 27:2234–2242.

Akashi, H. D., A. Cádiz, S. Shigenobu, T. Makino and M. Kawata (2016) Differentially expressed genes associated with adaptation to different thermal environments in three sympatric Cuban Anolis lizards. *Molecular Ecology* 25:2273-2285

Development of hind limb

Wakasa, H., A. Cadiz, L. M. Echenique-Diaz, W. M. Iwasaki, N. Kamiyama, Y. Nishimura, H. Yokoyama, K. Tamura and M. Kawata (2015) Developmental stages for the divergence of relative limb length between a twig and a trunk-ground Anolis lizard species. *Journal of Experimental Zoology* (Part B: Mol. Dev. Evol.) 324:410–42

Phylogeny and diversity

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Invasion by **Anolis carolinensis**

Tamate, S, W. M. Iwasaki., K. L. Krysko, B. J. Camposano, H. Mori., R. Funayama., K. Nakayama., T. Makino and M. Kawata. (2017) Inferring evolutionary responses of *Anolis carolinensis* introduced into the Ogasawara archipelago using whole genome sequence data. *Scientific Reports*, 7: 18008

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The ecological and evolutionary consequences of behavior in a changing Planet

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My research stands at the interface of behavioral ecology, evolutionary ecology, and global change biology. I am particularly interested in studying the association between behavior, ecology, and evolution to unravel the processes behind the early stages of adaptation to changing environments—a question of major relevance in a changing Planet.

Recent research:

During my time as a postdoctoral researcher, I have used *Anolis* lizards as a model to study the idea that among-individual differences in behavior could influence the chances of animals to persist under new selective pressures. The brown anole *Anolis sagrei* is an ideal species to study this question because a vast knowledge exists on their biology (Losos 2009). In addition, previous studies showed it is possible to conduct manipulative experiments in the wild with this species (e.g. Losos et al. 2004; Kolbe et al. 2012). This has allowed my collaborators and I to behavioral assays to quantify ecologically relevant variation in behavior and to carry out manipulative experiments in which free-ranging animals are subjected to new environmental conditions.

In Jason Kolbe's lab (University of Rhode Island), we investigated how two major components of global change—urbanization and biological invasions—shaped variation in behavior in wild populations of *A. sagrei* lizards in Florida. To this end, we designed novel behavioral experiments that allowed studying consistent among-individual variation in ecologically relevant behavioral traits in *Anolis* lizards for the first time (see Figure 1). We showed that both urbanization and biological invasions are shaping the behavior of *A. sagrei* populations in different directions (Lapiedra et al. 2017). In a recent piece I highlighted that *Anolis* lizards can be a suitable study model to help achieve an integrative perspective on the role of behavior in facilitating the persistence of animal populations in urban areas (Lapiedra 2018).

In recent times, much attention has been paid to the importance of among-individual variation in behavior. However, empirical evidence that natural selection acts on behavior under novel

selective pressures has remained more elusive. The ability to individually quantify behavior in *Anolis* lizards in the previous study paved the way to address this major gap in behavioral and evolutionary ecology. While in the lab of Jonathan Losos at Harvard University, I set up a large-scale field experiment in the Bahamas to explicitly quantify natural selection on among-individual variation in ecologically relevant behavioral traits. In this experiment, conducted in collaboration with Jason Kolbe, Jonathan Losos, Tom Schoener, and Manuel Leal—and the help of many other colleagues and a fantastic team of field assistants—we manipulated predation pressure (presence vs. absence of the ground predator *Leiocephalus carinatus,* the curly tailed lizard; Figure 1) in a set of experimentally established island populations of *A. sagrei*. This study provided evidence that among-individual variation in risk-taking behavior determines differential survival of brown anoles under different ecological conditions. In addition, we found that selection on behavior occurs simultaneously, and independently, to selection in morphological traits (Lapiedra et al. 2018). In the near future, I will extend this experimental set up to a long-term study to address a few additional research questions.

Future research questions:

Biologists have long debated the role of behavior in evolution. My research program will continue to investigate this question by taking advantage of the ongoing large-scale ecological experiment my collaborators and I recently set up in Great Abaco, the Bahamas. Our recent finding that natural selection favors certain behaviors over others under different ecological conditions has paved the way to elucidate two major questions:

-How does natural selection on behavior affect the ecological and evolutionary dynamics of populations of *A. sagrei* under rapid environmental changes?

-Do these changes have cascading effects that reshape biological communities of the ecosystem?

To address these questions, my research plan has three more specific aims:

1. Examine evolutionary change in risk-taking behavior of anoles

Determining whether and how behavior evolves in response of new selective pressures remains an open question (Baldwin 1896). In my lab, we will assess whether previously observed differences in survival between individuals with different risk-taking behaviors under different predation regimes have a genetic basis. This will show if the described natural selection on risk-taking behavior has evolutionary consequences. These data should allow testing in natural conditions if individual variation in behavior evolves in response to rapidly changing ecological conditions.
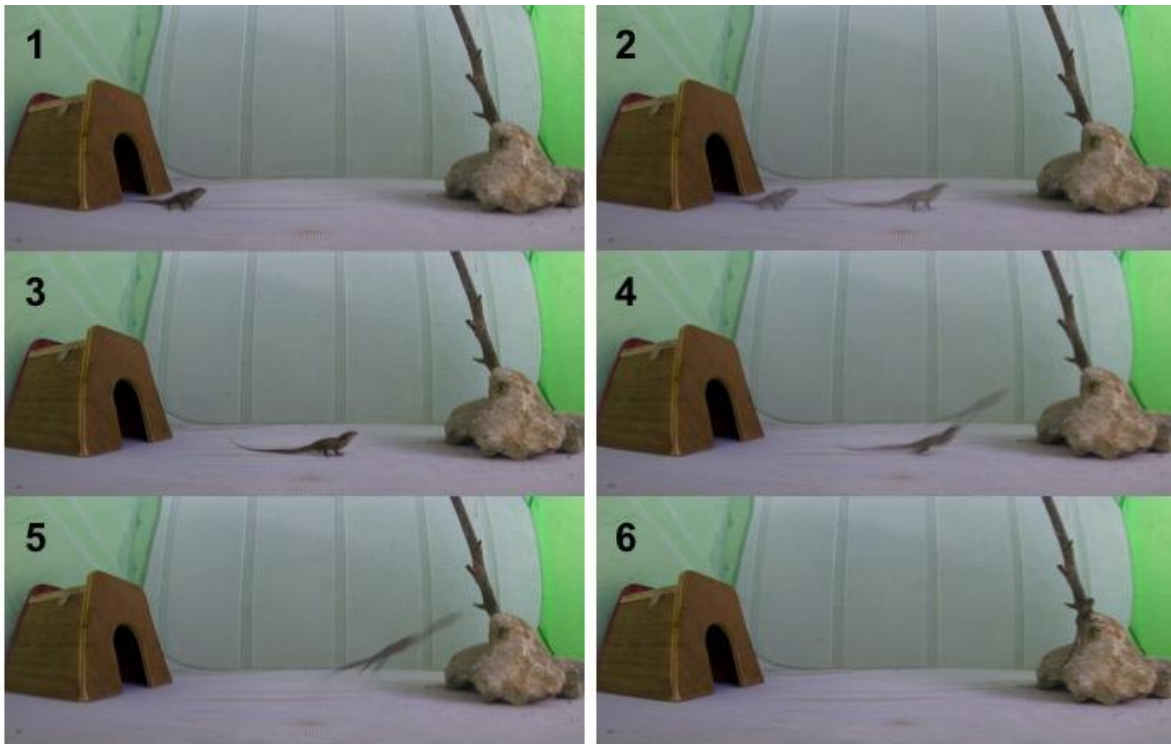
Figure 1: Top: Species of study (*A. sagrei*, left, and *L. carinatus*, right). Bottom: detail of a behavioral assay designed to quantify individual variation in risk-taking behavior. In this particular experiment, risk taking is quantified as the time spent by each individual on the ground before seeking refuge.

2. Unravel the link between ecological variation and among-individual variation in behavior

Assessing the ecological consequences of among-individual variation in risk-taking behavior can help unravel whether and how selection on behavior modifies ecological processes and drives novel evolutionary trajectories (Bolnick et al. 2011; Wolff and Weissing 2012). To investigate the connection between behavioral and ecological variation, we plan to assess if individuals with different risk-taking behaviors play different ecological roles by examining two key components of anoles ecological niche: habitat use and diet. We will use this link between behavior and ecology to study if eco-evolutionary changes in *A. sagrei* populations in natural conditions have cascading effects on trophic dynamics across the biological community.

3. Assess if changes in eco-evolutionary dynamics modify the functioning of biological communities

Finally, does natural selection in among-individual variation in behavior affect population dynamics? And, do these changes spur cascading effects across trophic levels in impoverished biological communities? To this end, my lab will carry out a large-scale characterization of the diet and the trophic relationships among species on experimental islands with different selective regimes. We will study trophic relationships across trophic levels on our small experimental islands. Conducting this sort of study is possible because these experimental islands are impoverished biological communities with low diversity of species. This research could provide relevant empirical information to inform management and decision planning directed to minimize the alteration of ecosystem services in a rapidly changing Planet.

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Little evidence for size-structured habitat use in a diverse *Anolis* community

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Introduction

The partitioning of structural microhabitat among *Anolis* lizards is a well-studied phenomenon, with replicate patterns observable across independent island radiations (Losos 2011). Though a substantial body of literature describes the predictable nature of habitat partitioning *between* species, fewer studies have investigated how partitioning *within* species (and within sexes) may also be consistent among species.

One theory of intraspecific habitat partitioning is that patterns of habitat use may be driven by body size (Werner & Gilliam 1984), as optimal habitat may be preferentially used by larger individuals most capable of winning agonistic interactions. In anoles, complex behavioral intraspecific interactions are characterized by dewlap displays, lateral body presses (head bobs and push ups), which can escalate to aggressive physical confrontations, particularly among males (Johnson et al. 2010, Losos 2011). Body size correlates positively with success in agonistic interactions, in other words social dominance increases with body size (Tokarz 1985), which suggests that larger lizards should use perches which are most preferential. This is known as the size-structured habitat use (SSHU) hypothesis, and evidence for it has been found in some anole studies (Tokarz 1985, Jenssen et al. 1998, Kamath and Losos 2017). It is possible that intraspecific size-structured habitat use may be the underlying mechanism driving interspecific divergence in perch use, if intraspecific relationships exist to different perch optima. Therefore, testing the SSHU hypothesis may be incredibly important in identifying the mechanisms that underlie interspecific habitat partitioning and community structure.

In this study, we test the SSHU hypothesis in anoles by examining perch height and diameter use among four different species of the anole community of Fairchild Tropical Botanic Gardens, Miami FL USA. We tested two specific hypotheses; (i) anoles of different ecomorphs used different portions of the structural habitat, and (ii) that a relationship exists between perch use and body size in each species.

Methods

Fairchild Tropical Botanical Garden (FTBG) is located in Miami FL USA (25.403°N, 80.163°W, WGS 84; < 1 m elev.), and hosts a diverse, lizard assemblage, which includes 5

species of native and non-native anoles: *Anolis carolinensis* (native), *A. cristatellus* (Puerto Rico), *A. distichus* (Hispaniola), *A. equestris* (Cuba), and *A. sagrei* (Cuba and the Bahamas). We examined SSHU for all anole species present in the FTBG assemblage with the exception of *Anolis equestris* because of low efficacy in matching body size to perch use; *A. equestris* are large and highly arboreal, meaning that while perch use data may be empirically collected, it can be difficult to accurately estimate body size from a far distance. *Anolis carolinensis* are trunk-crown ecomorphs, primarily utilizing the upper portions of tree trunks and canopy branches. *Anolis cristatellus* and *A. sagrei* are both trunk-ground ecomorphs, primarily using the lower portions of tree trunks for perching and display, while actively foraging on the ground. *Anolis distichus* are trunk specialists, utilizing the full strata of broad perches – primarily trunks of palm trees (especially palm, such as *Roystonea* sp.).

Data collection was conducted opportunistically from 12/12/14 to 22/10/15, between 0800h – 1600h. Lizards were found by walking paths in FTBG while visually scanning all trees and vegetation from ground level to approximately 6 meters above the ground. On observation, lizards were quickly identified to species-level, and perch data were recorded empirically. Data were only collected for adult male lizards, and only on those individuals whereby perch use could be determined from distance, prior to any effect from the observer. Perch height was determined as the direct vertical distance from the mid-point of the perching lizard to the ground, while perch diameter was the width of the perching substrate. All perch use data were recorded empirically using a metric tape measure, although the diameter of perches of lizards observed at >2.5m in height were estimated.

After recording perch height and width, lizards were captured using a 10ft Cabela's telescopic fishing pole with a dental floss noose at the end. Snout vent length was measured for each individual using 15cm digital calipers accurate to 0.01 mm (Neiko 01407A) by measuring from the anterior tip of the snout to the cloaca. Data were log transformed. Single factor analysis of variance (ANOVA) was used to assess differences in body size (SVL) among the different anole species, and differences in perch use (both perch height and perch diameter). We tested for differences in perch height and perch width independently, significant ANOVAs being followed by Student's t-tests. We performed linear regressions, with perch height and perch diameter being the response variable against snout-vent length to investigate SSHU relationships.

Results

We recorded perch use data of 330 lizards during the course of our sampling. In general, structural habitat use was consistent among species as expected under the ecomorph hypothesis, such that Trunk-Ground species (*A. sagrei* and *A. cristatellus*) perched lowest, Trunk species (*A. distichus*) perched at an intermediate height on very broad perches (e.g. tree trunks), and Trunk-Crown species (*A. carolinensis*) most frequently used high, thin perches (Table 1).

We found significant difference in body size among the four anoles assessed in this community (ANOVA, $p < 0.0001$); *A. cristatellus* were generally the largest followed by *A. carolinensis*, *A. sagrei*, and *A. distichus* (Table 1). We also found significant differences in both perch height (ANOVA, $p < 0.0001$), and perch diameter (ANOVA, $p < 0.0001$) among species. *Anolis cristatellus* and *A. sagrei* did not differ significantly in perch height ($p = 0.341$), nor did *A. carolinensis* and *A. distichus* ($p = 0.729$). *Anolis cristatellus* and *A. sagrei* perched significantly lower than *A. carolinensis* and *A. distichus* ($p < 0.0001$ for all comparisons) (Table 1). Only *A. distichus* differed significantly in perch diameter ($p < 0.0001$ for all comparisons), occupying significantly wider perches than the other three species (Table 1); removal of *A. distichus* showed no significant difference in perch diameter among the three remaining anoles (ANOVA, $p = 0.366$) (Table 1).

Table 1. Size-structured habitat use (SSHU) for four species of anole coexisting in the same community at Fairchild Tropical Botanic Gardens in Miami FL. All quantative data are means ± 1 S.E. Ecomorph categories follow each species name in parentheses as follows: TG, "*trunk-ground*", T, "*trunk*", TC, "*trunk-crown*". Significant relationships at α = 0.05 are presented in bold and at α = 0.1 in italic.

Species	N	SVL (mm)	Perch height (cm)	R^2	P value	Perch diameter (cm)	R^2	P value
A. sagrei (TG)	72	58.4 ± 0.30	107.8 ± 8.30	0.001	0.742	14.4 ± 1.52	0.001	0.850
A. cristatellus (TG)	81	66.7 ± 0.36	110.5 ± 5.26	*0.036*	*0.091*	16.8 ± 1.86	0.004	0.568
A. distichus (T)	89	49.1 ± 0.38	193.0 ± 11.14	0.022	0.183	33.9 ± 3.12	0.001	0.819
A. carolinensis (TC)	88	62.5 ± 0.52	194.1 ± 9.52	0.000	0.885	15.3 ± 2.51	**0.069**	**0.015**

We found no evidence in support of SSHU in any of the species examined in this study in either perch height (Fig. 1) or perch diameter (Fig. 2), with all R^2 values explaining less than 5% of variation (Table 1), with the exception of a significant positive SSHU relationship in *A. carolinensis* for perch diameter ($p = 0.015$). Incidentally, there is also a negative SSHU relationship of *A. cristatellus* in perch height ($p = 0.091$).
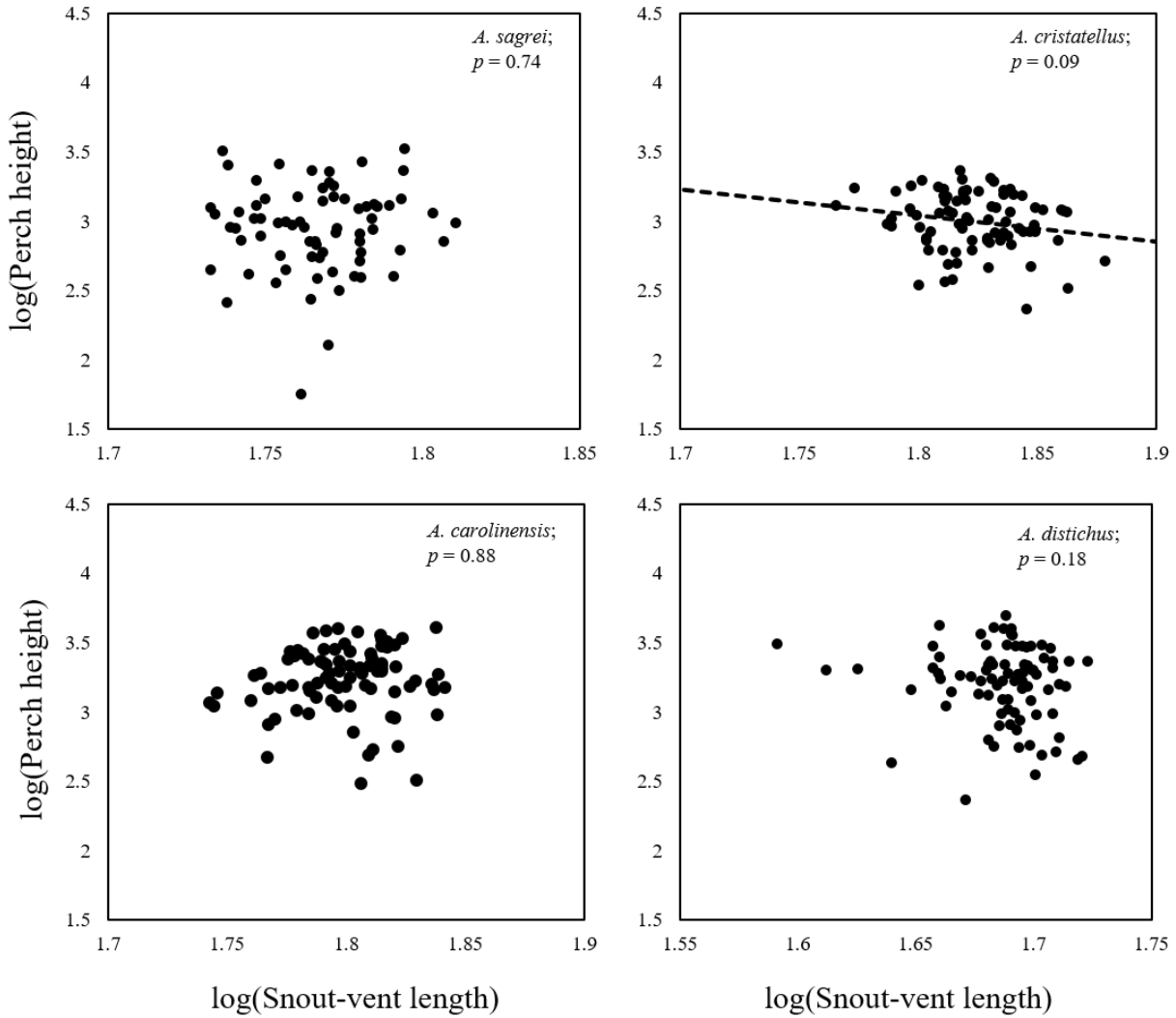
Figure 1. The Size Structured Habitat Use (SSHU) relationship between body size (snout-vent length) and perch height for four species of *Anolis* lizard in Fairchild Tropical Botanic Gardens, Miami FL. Solid lines represents significance at α = 0.05, while dashed lines at α = 0.1. Note that the x-axis scale of *A. distichus* is not the same as the other species due to a relatively smaller body size.
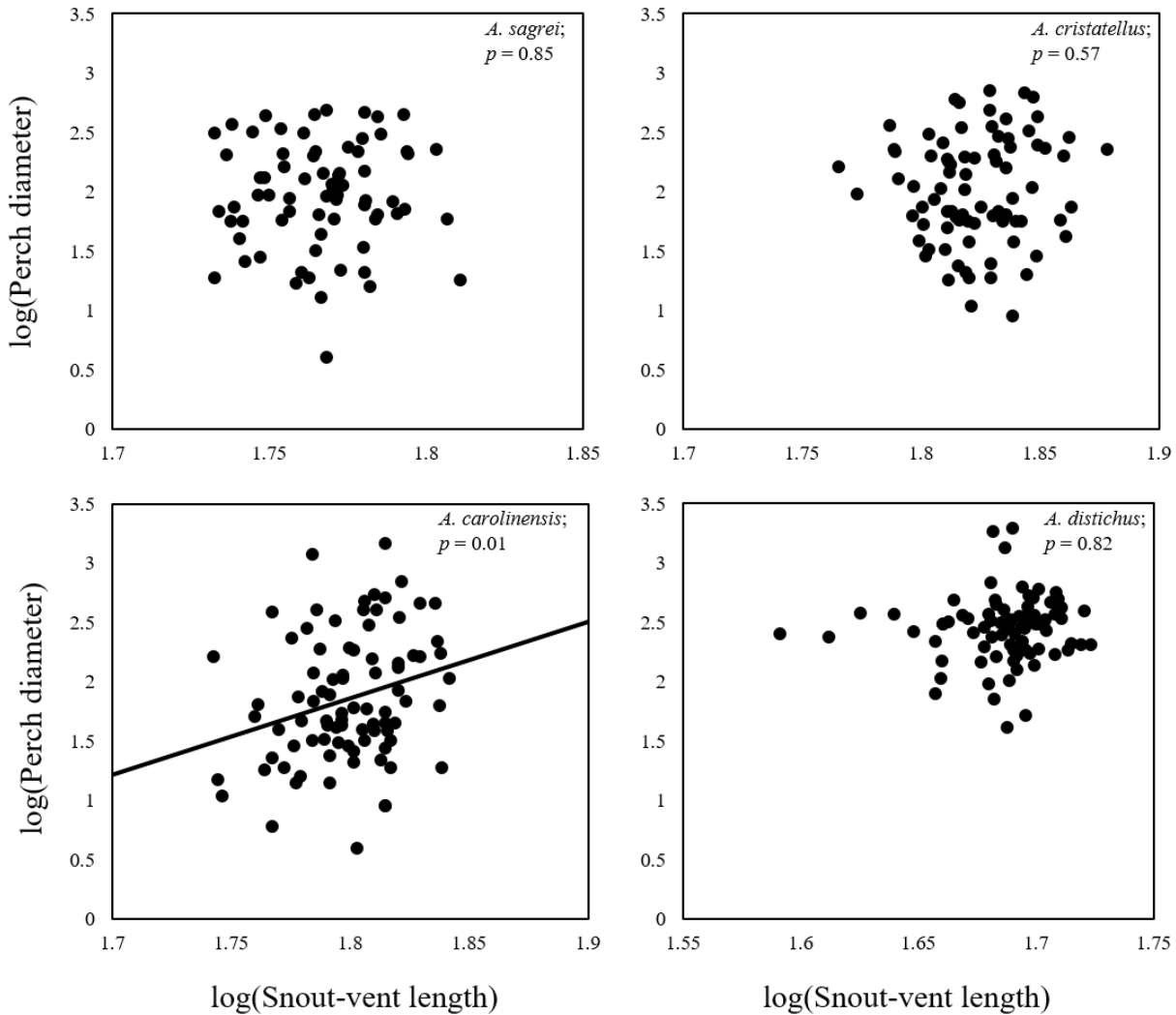
Figure 2. The SSHU relationship between body size (snout-vent length) and perch diameter for four species of *Anolis* lizard in Fairchild Tropical Botanic Gardens, Miami FL. Solid lines represents significance at α = 0.05, while dashed lines at α = 0.1. Note that the x-axis scale of *A. distichus* is not the same as the other species due to a relatively smaller body size.

Discussion

This study provides little support for the hypothesis of size-structure habitat use (SSHU) in an assemblage of anoles in Miami FL. *Anolis carolinensis* was the only species that demonstrated any significant SSHU relationship; a positive correlation between body size and perch diameter. However, the percent of variation explained by this relationship is extremely low ($R^2 = 0.069$; Table 1), and so, despite being statistically significant, may have little bearing in describing any ecologically relevant patterns. Similarly, a negative relationship between perch height and body size exists for *A. cristatellus,* although this also explained a low proportion of the variation ($R^2 = 0.036$; Table 1). These results suggest that either habitat use is not partitioned

within species by the size of individuals in this community, or that perhaps perches are so abundant that intraspecific interactions for perches are not strong enough to drive a pattern of usage. In other words, perches are not so limiting that interactions have driven a size-structured pattern of usage. Alternatively, perch preference may instead be highly idiosyncratic to individuals and not a conserved behavior throughout the population. In this way, a preferential perch for one large lizard may not have the same characteristics as a preferred perch of another. This inter-individual variation, sometimes called individual specialization, has received relatively little attention so far in the anole literature (but see Kamath & Losos 2017). Further studies would benefit from exploring variation in perch use of focal individuals to tease apart this alternative hypothesis.

It is also possible that, in this anole community, perch height and diameter may not be the ecological axes which best reflect the perceived habitat quality which an individual is inhabiting. For example, future studies would benefit from examining whether a relationship exists with body size and other environmental qualities of microhabitats, such as thermal microsite characteristics or prey abundance and diversity. This may be especially important for *A. cristatellus* which generally occupy shaded microhabitats in Miami FL, such that it can be accurately used to predict the species distribution at the landscape scale (Kolbe et al. 2016). In this situation it is possible that more dominant (i.e. larger) males may drive smaller males to occupy habitats with less desirable thermal profiles, providing an alternative axis for which SSHU to operate. Similarly, for trunk-crown species such as *A. carolinensis*, variation in crown structure between different tree species may be a more important predictor of habitat quality than perch height or diameter. Variations in tree canopies may be especially pronounced in botanical gardens, such as in Fairchild Gardens (which has approx. 2,400 species), given the artificially high ecological and taxonomic diversity of tree species in the collection.

Future studies of SSHU may also benefit from examining natural anole assemblages, as it is possible the dynamics involved in this novel assemblage of primarily introduced non-native species may not be reflective of patterns occurring in the natural range of these species. It would also be beneficial to consider the SSHU hypothesis in other non-natural settings. For example, many anoles in urban environments utilize artificial perches (e.g. Kolbe et al. 2016, Winchell et al. 2016 2018, , Battles et al. 2018), which are generally less structurally complex than natural environments.

It is worth noting that these results contradict those of previous studies finding significant relationships between body size and perch height for *A. carolinensis* and *A. sagrei* (e.g. see Tokarz 1985, Jenssen et al. 1998, Kamath and Losos 2017). This may be attributable to the relatively narrow range in body size of individuals utilized in this study. These data were collected only from fairly large adult male lizards as they are easy to identify from afar; in many species, smaller males can look incredibly similar to mature females and so were excluded.

Examining individuals across a wider body size range may demonstrate varying patterns of habitat use in relation to body size more clearly as smaller males may be displaced by larger males (Jenssen et al. 1998, and Tokarz 1985). Similarly, further studies would also benefit from exploring this hypothesis in females, as well as across the entire body size range of all individuals (i.e. including juveniles).

Acknowledgements

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Using transplant experiments to understand adaptation and speciation in anoles

It is no secret that the environments experienced by *Anolis* lizards are changing rapidly. Nearly every location on the planet has been touched by global climate change, of course, but other anthropogenic stressors such as habitat destruction, invasive species, and pollution are also generating novel environments that anoles must deal with. These tiny lizards are not known as particularly good dispersers, so how will they cope with changing environments? Could rapid genetic or plastic change rescue them from extinction? As part of a large collaboration (including Christian Cox, Jonathan Losos, W. Owen McMillan, Daniel Nicholson, Lauren Neel, Albert Chung, Christina Miller, John David Curlis, Timothy Thurmond, Michael Angilletta, and Michael Sears), we have transplanted hundreds of *Anolis sagrei* (brown anoles) and *A. apletophallus* (slender anoles) to small islands in The Bahamas and the Panama Canal, respectively. We are following in the footsteps of previous scientists (namely Jonathan Losos, Tom Schoener, and David Spiller) who first developed the idea of moving anoles to small islands to study adaptation. Unlike many of these previous experiments, however, we are not solely using the islands as physically isolated substrates upon which other environmental variables can be manipulated to study anole evolution. In our case, we specifically chose islands that vary in habitat structure, substrate, and topographic complexity such that the structural and biophysical environments of the islands themselves generate divergent selection (Figure 1).

We have now released 70 slender anoles (equal sex ratios) to each of 10 islands in Lake Gatun, Panama, and 40 brown anoles (equal sex ratios) to each of 17 islands in Exuma, The Bahamas. In The Bahamas, we measured a comprehensive suite of morphological traits, as well as lower and upper thermal tolerances and the thermal sensitivity of metabolic rate (oxygen consumption at different body temperatures) in every lizard we released. In Panama, we measured the same suite of morphological traits and thermal tolerance in every lizard we released, and we have subsequently measured preferred temperatures in a laboratory thermal gradient, as well as the thermal sensitivity of metabolic rate and sprint speed in a subsample of mainland lizards so that we can compare the ancestral population to phenotypic changes that may occur on the islands. Lastly, in Panama, we have conducted gene expression experiments to understand which genes are upregulated during exposure to cold and heat shock.

We predicted that many of these traits will change in response to differing structural and

thermal environments among islands. Interestingly, early results suggest that many of the traits are indeed changing, and fast! For example, relative to mainland slender anoles in Panama, head size has decreased across all islands, and in only a single generation (Figure 2).

We took a tissue sample (tail tip) from every lizard in both experiments. Starting early next year, we will use genome scans to develop pedigrees for our populations in Panama, where we have uniquely marked every single individual and conducted intensive mark-recapture over the past two years (we have tissue samples from nearly every individual in each population over two generations, soon to be three). These pedigrees can be used to estimate selection via variation in lifetime reproductive success in each population. We will also use the SNP data from our genome scans to conduct genome-wide-association studies (GWAS) that can help us identify regions of the genome (may even specific genes) that give rise to variation in our traits of interest, and then track changes in allele frequencies on the islands. With comprehensive data on both genotypes and phenotypes, we can test for convergent evolution at multiple levels of biological complexity and begin to reveal important genes underlying adaptation to rapid environmental change. Additionally, we will combine our field-estimates of natural selection
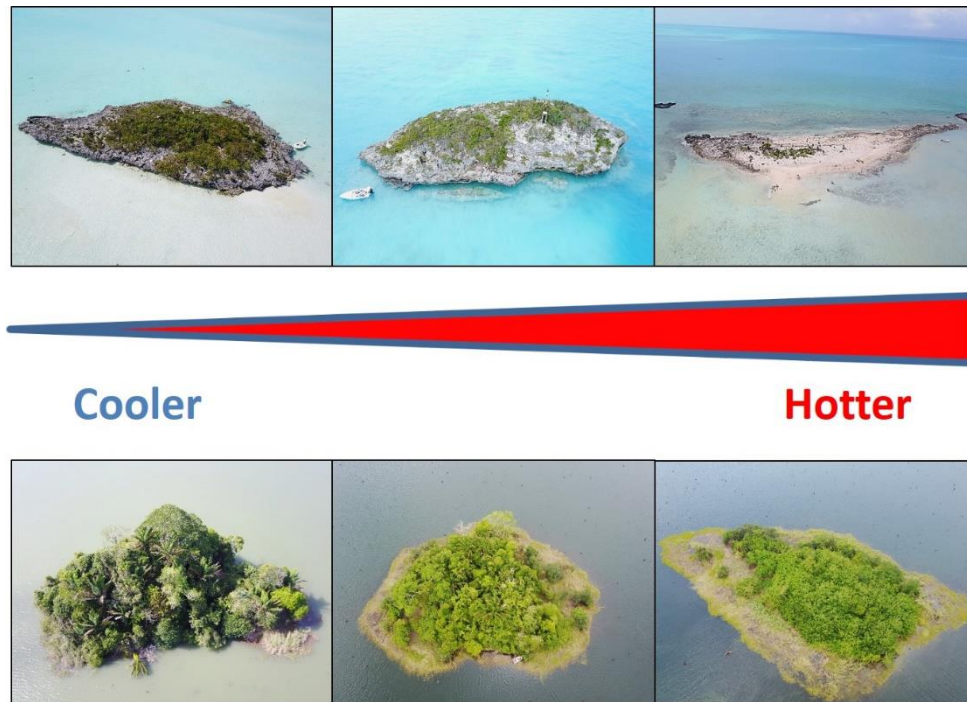

Figure 1. Our experimental islands in The Bahamas (selected examples, top row) and Lake Gatun, Panama (selected examples, bottom row), differ in local environments such that we expect strong and divergent selection on morphological, physiological, and behavioral traits. Differences in canopy cover, substrate type, exposure (e.g. sheltered in a cove or out in the open ocean/lake), and topographic complexity among islands conspire to generate variation in local climate, while differences in plant communities result in variable habitat structure.

with gene expression and laboratory acclimation experiments to explore the ways that phenotypic plasticity and genetic change interact to mediate local adaptation.
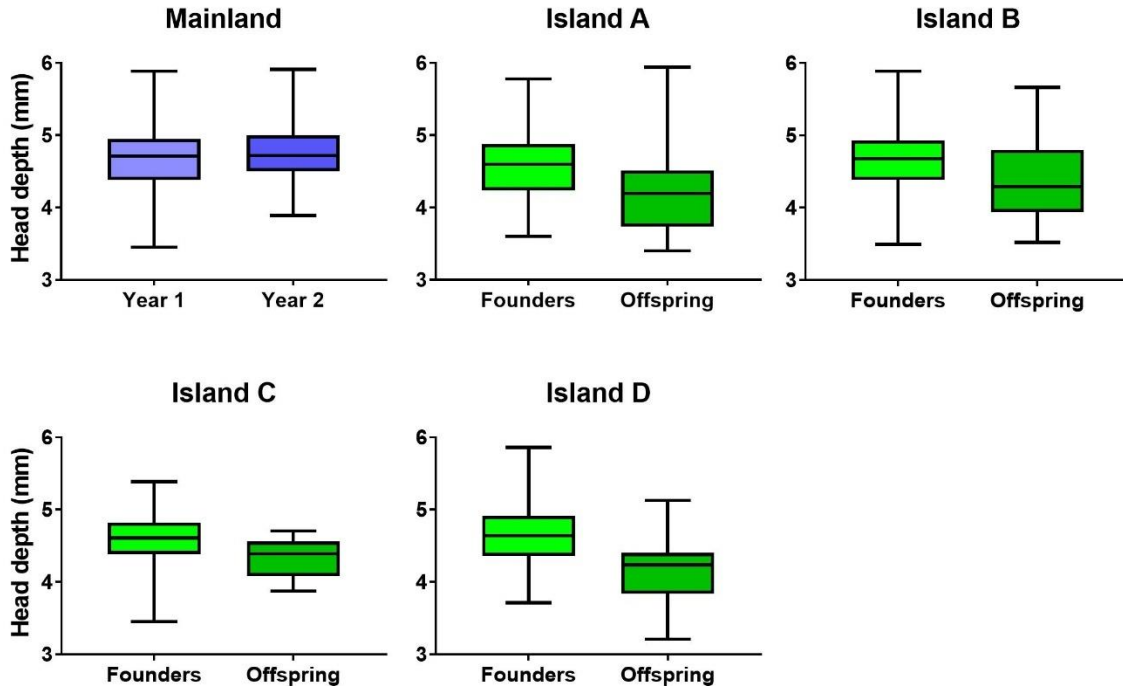

Figure 2. An example of one trait that has changed rapidly on our experimental islands in Panama. Head depth did not change on the mainland (blue boxes) from 2017 to 2018, but it decreased significantly (even after correcting for body size) on each island (green boxes) after only a single generation. It is possible that selection or plasticity has occurred in response to smaller prey or reduced competition (lower densities resulting in fewer of the fights between individuals that require strong jaw muscles) on the islands. The boxes display the mean and interquartile range of the data, whereas the whiskers display the full range of the data. Four islands are not included in this figure because we only transplanted lizards to them this year. Two additional islands were not included because one went under water (the Panama Canal Authority raised lake levels) and the second experienced an unexplained population die-off.

These experiments give us a highly replicated, powerful way to understand how anoles may adapt to environmental change, but they also provide the opportunity to explore the ecological forces that lead to the evolution of pre-zygotic isolation mechanisms and incipient speciation. After our populations have diverged for several generations in response to differing island environments, we will conduct mate choice experiments by exposing males and females from different islands to each other in the lab and testing whether there is mating preference for local individuals. But we can go further, forcing lizards from different islands to mate and then releasing hybrid offspring back into the wild to track their lifetime reproductive success in different environments. Ultimately, we hope to dive deep into the drivers of ecological

speciation, testing for potential mitochondrial-nuclear genome mismatches between populations that arise from rapid adaptation of mitochondria to local thermal environments.

Finally, in addition to studies of speciation, there are a number of experiments we plan doing in the near future. We plan on manipulating competition and predation on some of our islands to examine the ways that biotic and abiotic factors interact to shape fitness surfaces. We plan on manipulating dispersal between pairs of islands to test the role of gene flow in either constraining or facilitating local adaptation, as well as its role in favoring the evolution of phenotypic plasticity. In collaboration with Jordan Kueneman (STRI postdoc), we have already "clean-caught" several dozen slender anoles from mainland Panama in order to sequence their microbiome. Eventually, we hope to document evolutionary changes in the microbiomes of lizards on each island and discover ways in which these changes drive population dynamics and structure fitness landscapes for other traits. We want to collaborate with specialists on other taxonomic groups (e.g. birds, mammals, arthropods, plants) to track how changes in lizard phenotypes and genotypes give rise to eco-evolutionary feedbacks in association with other members of the community.

Lots of interesting things to come. Stay tuned!

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Anolis Research in the Losos Lab

The Losos Laboratory continues to focus its efforts on studying the evolutionary diversification of *Anolis* lizards. Many of these efforts are detailed in other contributions to this volume, and so will only be mentioned briefly here.

In *Anolis Newsletter VI*, Anthony Herrel and I detailed a relatively new project to study the diversification of mainland anoles and compare it to the Caribbean radiations. This decade-long effort has led to field work through the Neotropics (including in Mexico, Honduras, Costa Rica, Panama, Colombia, Ecuador, and Venezuela). Several smaller papers have been published, including our *Breviora* paper on *Anolis proboscis* (right), but now we are working to pull together larger, synthetic papers. Hopefully these will get out in 2019 and 2020.

An adult male *Anolis proboscis*.

Experimental evolution studies also continue in the Bahamas. This work has been repeatedly pummeled by hurricanes in the last decade (e.g., Irene, Sandy). Hopefully, the hurricanes will stay away and allow evolution to take its course. Oriol Lapiedra's contribution gives more details on one aspect of this work.

What Other Lab Members Are up to

Current Grad Students:

Nick Herrmann is a 4th-year Ph.D. student studying the process of niche expansion following a reduction in interspecific competition, commonly referred to as ecological release. He is currently conducting a manipulative experiment on small islands in southern Florida, where invasive *Anolis sagrei* and native *A. carolinensis* have co-occurred for several decades. By removing *A. sagrei* and tracking behavioral responses in uniquely marked *A. carolinensis*, Nick is investigating 1) how changes in habitat use during ecological release vary across individuals within a population, and 2) whether inter-individual variation in habitat shifts correlate with inter-individual differences in morphology.

Inbar Mayaan has been studying *Anolis conspersus* on Grand Cayman and its interactions with the invasive *A. sagrei*. She is now beginning a phylogeographic study of the Jamaican radiation of anoles (for more details, see her contribution).

Pavitra Muralidhar is a 5th-year Ph.D. student interested in the genetic basis of adaptive evolution in *Anolis sagrei*. She is using next-generation sequencing of individuals across multiple generations on small Bahamian islands to identify the genes underlying rapid decreases in hind-limb length.

Sofia Prado-Irwin is a 4th-year Ph.D. student interested in the evolution and ecology of mainland *Anolis* species. In particular, she is focusing on a mainland species complex, *Anolis lemurinus*, and its recently diverged island relatives. Sofia plans to resolve the relationships between *A. lemurinus* group populations throughout Central America, explore population history and demographics in several recently-split island populations, and describe mainland-island divergence and adaptation using a combination of genetic, morphological, and ecological evidence from populations throughout the species' range. This project will provide unique insight into both mainland Central American biogeography and diversification, as well as island biogeography and adaptation.

Current and Recent Post-Doctoral Fellows:

Simon Baeckens (now a postdoc in the Laboratory of Functional Morphology at the University of Antwerp) is investigating the functional and adaptive role of variation in skin scale morphology in anole lizards. Working together with fish scale specialist Dylan Wainwright (Lauder Lab, Harvard University) and functional morphologist Duncan Irschick (University of Massachusetts), Simon is imaging and quantifying the skin surface topography of different anole species using the latest techniques in gel-based profilometry. By doing so, Simon is, for example, able to accurately determine the degree of skin that is covered by scales. Knowing that these keratinized scales protect the underlying skin from water loss, Simon examines whether anole species living in dissimilar thermal and hydric environments evolved disparate skin scale features to adapt to the local conditions. Aside from an among-species comparative approach, Simon also studies the form and function of anole scale morphology from an experimental viewpoint. Working side-by-side with lab-mate Dan G. Bock, Simon is looking at the skin scale morphology and physiology (e.g., evaporative water loss) of *Anolis sagrei* lizards across an environmental gradient, with the aim to unravel the invasive success of *A. sagrei* in the southeast of the US.

For his postdoctoral project with Jonathan Losos and Jason Kolbe, **Dan Bock** is investigating the contribution of admixture to the success of the *Anolis sagrei* invasion in Florida. Using *A. sagrei* males obtained along three latitudinal transects, Dan is attempting to establish whether invasive populations are locally adapted to climate. In collaboration with Simon Baeckens, he is focusing on tolerance to extremes in temperature and water availability. As well, Dan is performing large-scale genotyping of native (Cuban) and invasive populations. He will combine trait data and genomic data to clarify the genetic architecture of climate

adaptation in this system. Ultimately, the project's aim is establish whether native range alleles that were brought together by hybridization allowed this species to adapt to a novel climate during range expansion.

Colin Donihue and Anthony Herrel are studying how the endemic Redonda anole is rebounding after rat eradication (for more details, see their contribution).

Claire Dufour (now at the University of Montpellier) is studying interactions between invasive *Anolis cristatellus* and native species on Dominica and Costa Rica (for more details, see her contribution).

Anthony Geneva is interested in the genomics of anole adaptation and speciation. He is currently leading two large collaborative projects, one on speciation and the other on *Anolis* comparative genomics. The speciation project comprises three separate datasets. First, Anthony and his team established experimental crosses between *Anolis sagrei* populations that vary in their degree of genetic and morphological divergence (see the protocol used for maintaining an *Anolis* breeding colony in this newsletter). Two generations of crosses were then used to estimate reproductive isolation between these populations. Anthony will then compare the measure of reproductive isolation with estimates of whole-genome genetic divergence and ecomorphological divergence using morphometric measurements of traits known to be involved in ecomorph divergence. By comparing these three measures, Anthony and his collaborators will be able to assess the relative importance of genetic divergence and ecological adaption in driving the process of speciation and provide a glimpse into the early stages of anole speciation.

The comparative genomics project involves the generation of nine new *Anolis* reference genomes. When completed, Anthony will use these to investigate a variety of genus-wide questions, including resolving relationships at the root of the *Anolis* phylogeny, testing for convergent molecular evolution, and investigating the evolution of *Anolis* sex chromosomes. The first of these new genomes, for *Anolis sagrei*, is completed and is the most complete reptile genome assembled.

Over the past few years, **Melissa Kemp** has been evaluating the stability of *Anolis* communities through time and asking whether changes in species composition correspond to environmental changes, such as human-mediated habitat modification. A major component of this research has been the identification and excavation of paleontological sites throughout Puerto Rico, as such sites provide a baseline for what *Anolis* communities looked like prior to human colonization. Melissa excavated a cave site in central Puerto Rico, and radiocarbon dates indicate that the oldest material have a corrected age range of 40829 - 42509 years before present. This means that the faunal assemblage provides a glimpse of diversity in central Puerto Rico before and after the Last Glacial Maximum, as well as before and after both Indigenous and European colonization events. Thousands of bones from a variety of taxonomic groups (birds, amphibians, reptiles, and mammals) have been identified. Using osteological characters, Melissa and collaborators have successfully established the prehistoric presence of at least three *Anolis* species: *A. cuvieri, A. cristatellus*, and *A. evermanni*. Species-level identifications are still underway and surveys of the modern-day *Anolis* community at the site are being planned.

James Stroud is continuing his studies of natural selection on anoles in Miami and beginning a new project to extend those studies to anole communities in the Greater Antilles (for more details, see his contribution).

Kristin Winchell is extending her studies of urban evolution of anoles to other islands, after studying the topic in Puerto Rico for PhD thesis (for more details, see her contribution).

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A case study of character displacement and phylogeography of Jamaican anoles

Following my fieldwork this summer to study the effects of introduced *A. sagrei* on the ecology and morphology of the endemic Grand Cayman anole (*A. conspersus*), I have been analyzing the data I collected and working to incorporate them into a historical framework consisting of data from museum specimens. My specific goals for my fieldwork were to assess if an *A. sagrei* has altered the ecology of *A. conspersus*, and to investigate whether this shift in ecology led to adaptive morphological change. I was surprised to find that *A. sagrei* were found at much lower densities across the island than I had expected, but I was still able to collect data at a pair of sites with similar habitat composition but differing concentrations of *A. sagrei*. I found no significant difference between the two sites in how male and female *A. conspersus* use

Fig 1. A male Grand Cayman anole (*A. conspersus*), a member of the Jamaican anole clade. Males exhibit a range of colors and patterning, from deep reddish brown to brilliant emerald, while females tend to be a drab range of browns and tans.

the habitat, and found that *A. sagrei* tend to perch much lower than *A. conspersus*. I also found no morphological differences between *A. conspersus* at the two sites, a predictable outcome given the lack of difference in habitat use.

These findings suggested that interactions with *A. sagrei* have not led to a meaningful change in the ecology or morphology of *A. conspersus*. However, given my small sample size and earlier reports showing a difference in *A. conspersus* habitat use in the presence of *A. sagrei*, it is possible that the morphology and ecology seen in present-day populations is the legacy of this shift. I will use museum specimens collected during the decades before and after the initial introduction to test the hypothesis that *A. conspersus* morphology has changed since *A. sagrei* was first introduced to the island. Now that I have a better idea of where *A. sagrei* are found across the island, and have collection data on presence in the past, I will re-sort through historical

collections to compare the most relevant *A. conspersus* specimens. I also collected data on *A. conspersus* across Grant Cayman, which will be useful for my work on Jamaican anoles.

Second, I have been working on my project on the phylogeography of Jamaican anoles, which will be the focus of my dissertation. In the course of this project, I plan to investigate the interspecific relationships within the relatively young, monophyletic radiation of Jamaican anoles, as well as intraspecific relationships among populations across the island. Previous reconstructions have not conclusively resolved the Jamaican anole tree, and relationships within and between species remain especially unclear (e.g. two of the seven species are rendered paraphyletic by others). Published accounts and anecdotes suggest that there is a fair amount of undescribed morphological and ecological diversity, as well as genetic diversity. Two of the species exhibit substantial variation between populations and have named subspecies; the status of these and variation within the rest of the species will be particularly interesting to study further. Because this is a relatively young radiation and species boundaries appear to span the continuum, this will also be an exciting opportunity to study the process of speciation, and to do so against the backdrop of the mosaic geological history of Jamaica.

Fig 2. Catching Grand Cayman anoles and collecting ecological data at Collier's Reserve in the summer of 2018.

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Histopathology of large epidermal cysts on the invasive Puerto Rican Crested Anole (*Anolis cristatellus*) in Miami, Florida, USA.

Abstract

Large masses were observed on the head and bodies of non-native Puerto Rican crested anoles (*Anolis cristatellus*) in Miami, Florida USA. Following examination, the masses were found to be epidermal inclusion cysts. The cysts did not appear to interfere with body condition or behavior. This is the first record of epidermal inclusion cysts in *A. cristatellus* in either the native (Puerto Rico) or non-native (Florida) range.

Keywords: invasive species, Florida, *Anolis*, cyst.

Introduction

Emerging human activities are having detrimental consequences in wildlife ecosystems, increasing the frequency and effects of biological invasions and introduction of novel diseases. Globalization and increased connectedness are causal factors for the prevalence of invasive species, which represent the second leading cause of extinction in the US (Crowl *et al.*, 2008). Recognizing and recording any alteration in basic behavioral and morphological conditions of any wildlife organism is important if we are to accurately document how novel stressors are influencing biodiversity (McNamara, 2015). To understand dispersion of invasive species and diseases, observational and experimental approaches are required at local, regional, continental and global scales, with which biotic and abiotic effects and impacts can be evaluated (Crowl *et al.*, 2008).

Here, we report the first histopathological examination of large, external cyst-like masses observed on a non-native exotic lizard – Puerto Rican crested anoles (*Anolis cristatellus*) – in Miami, Florida USA. Epidermal inclusion cysts refer to an epidermoid cyst resulting from the implantation of epidermal cells in the dermis. They are benign lesions that can be of sebaceous or follicular origin, commonly reported in mammals, specifically in humans, dogs, sheeps, cats and horses. Lesions can be caused by several mechanisms such as sequestration of epidermal rests in embryonic life, occlusion of the pilosebaceous unit, or trauma/injuries(Parker, 1995; Fomm, 2018). In reptiles, cases of epidermal inclusion cysts have been reported in Painted

Turtles (*Chysemys picta*), which were located around the tympanum and seem to have arisen from epidermal pockets similar to sebaceous cysts reported in mammals (Christiansen *et al.* 2004).

Case Study

Puerto Rican crested anoles (*A. crisatellus*) were originally introduced to two independent locations in Miami FL in the 1970s (Kolbe *et al.* 2016). Genetic analyses confirmed that the two populations – Key Biscayne and South Miami/Pinecrest – were the result of independent introductions (Kolbe *et al.* 2007). The Key Biscayne population was first detected in 1975 and originates from the capital of Puerto Rico, San Juan (Schwartz & Thomas 1975, Bartlett & Bartlett 1999), while the South Miami population was discovered in 1976 on the Red Road canal (Snapper Creek) and originates from northeast Puerto Rico (Wilson & Porras 1983). While the population on Key Biscayne has remained relatively constrained, dispersal of the South Miami population is ongoing and *A. cristatellus* may now be found throughout the South Miami/Pinecrest/Coral Gables/Coconut Grove neighborhoods (Kolbe *et al.* 2016).

Tumor-like external masses were first observed on adult male individuals of *A. cristatellus* in 2013 in the South Miami population. The masses are soft to touch, ovoid, and appear as a swollen protrusion from the skin (see Fig 1A-D). In some populations in the South Miami region, almost all individuals will possess at least one facial mass (and an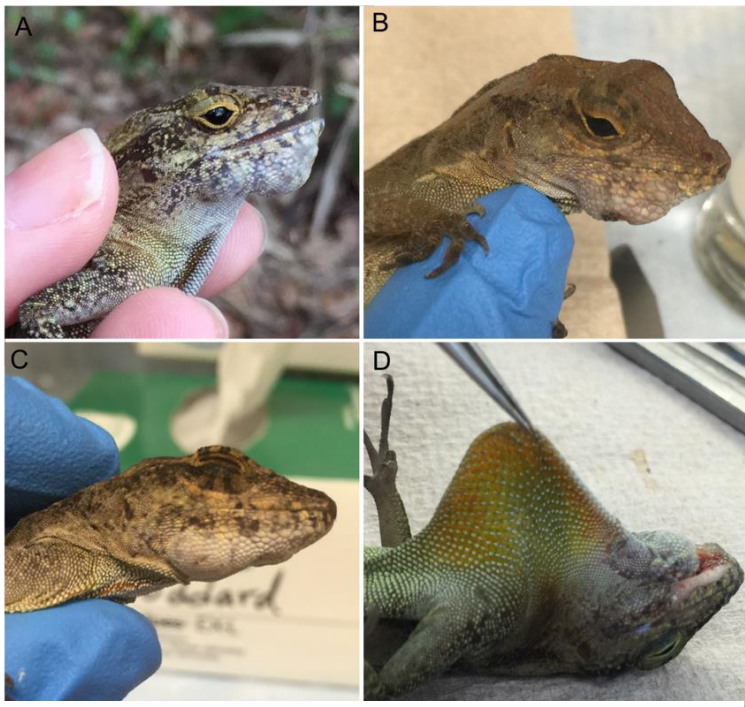ecdotally this is heavily skewed towards males). Individuals appeared healthy, with no discernable effects on body condition or behavior. Some individuals have been observed foraging successfully and in entering agonistic interactions with conspecifics. A review of the literature revealed no similar cases to this in other *Anolis* lizards, although some anecdotal reports exist from other anole biologists[3] and in other anole species (*A. carolinensis*, *A. smaragdinus*) from the herpeto-cultural industry (e.g. *Reptile Boards*, *A Reptile and Amphibian Community* blog).

We collected six male individuals with obvious external masses from Fairchild Tropical Botanic Garden, Coral Gables FL

Figure 1. Epidermal inclusion cysts on male individuals of *A. cristatellus* in Miami, FL**.**

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[3] For example, Brian Magnier writes on *Anole Annals*, "Parasitic Fly Larva in *Anolis cristatellus*?" http://www.anoleannals.org/2016/09/28/parasitic-fly-larva-in-anolis-cristatellus/

(25.677°N, 80.276°W). All individuals were anesthetized via intracoelomic injections of 0.2-0.4ml of liquid lidocaine diluted to 0.5 g in 10ml of distilled water. Once individuals were sedated, an injection of 0.5 ml of lidocaine was administered intracardially to euthanize the lizard. Procedures were performed following the American Veterinary Medical Association (AVMA) guidelines for the euthanasia of animals (2013 edition).

Following euthanasia, all masses on all individuals were dissected and preserved in 10% neutral buffered formalin. Samples were fixed using 10% neutral buffered formalin for 24 hours and preserved in 70% ethanol and then sent for histopathological analyses in Histopat Laboratory (Bogota, Colombia).
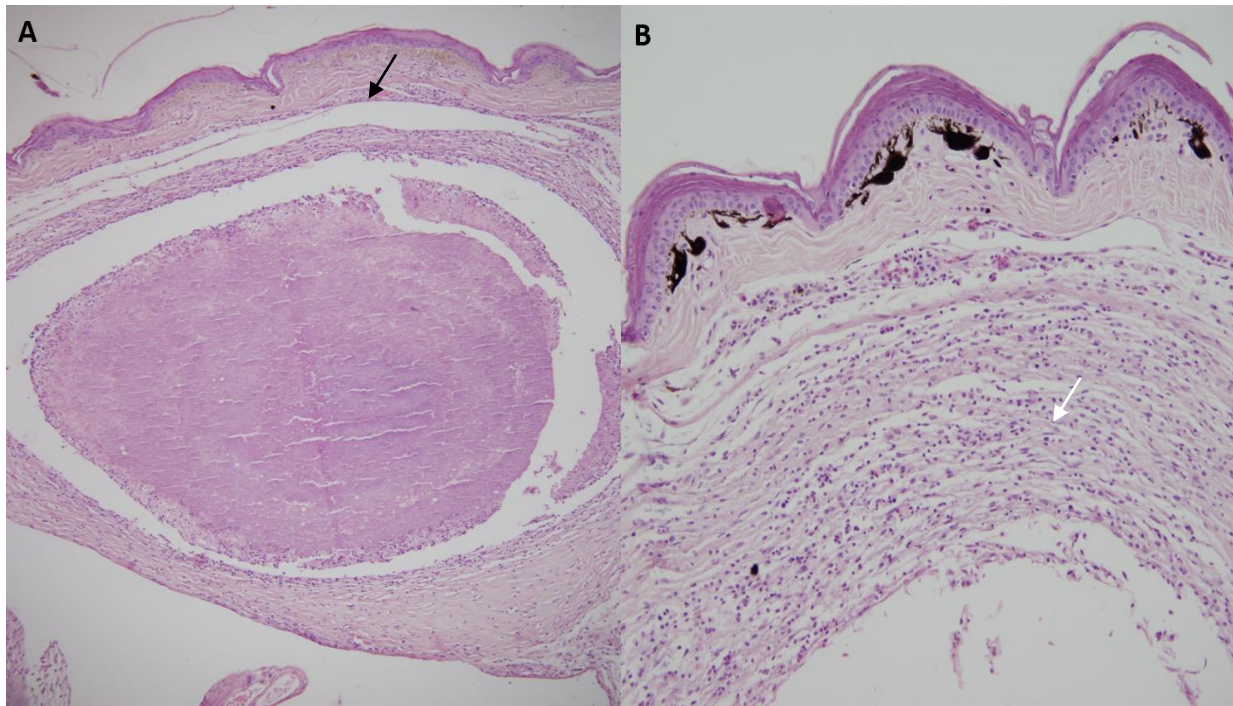

Figure 2. Cyst lined (black arrow) by keratinized epithelium with a distinct granular layer without nuclear atypia. In addition, we observed inflammation due to cyst rupture with presence of histiocytes, lymphocytes and scattered eosinophils (white arrow). **A**. (4x). **B**. (10x). H&E (Hematoxylin and Eosin staining).

Histopathological Description

We processed skin biopsies from six *A. cristatellus* specimens with obvious epiderminal lesions. Laminar and ellipsoid fragments were generally ca. 1-1.5 cm long, ca. 0.8 cm wide, and ca. 0.5-0.7 cm thick. The skin surface was corrugated and tan grey. Following a tangential cut, diameter cystic lesions varying in diameter were found with white and oily content. For the histopathology, formalin-fixed tissues were de-paraffinized in xylene and alcohol, embedded in paraffin, sectioned at 2 μm and stained with hematoxylin and eosin (H&E).

We observed that all cysts were lined by a keratinized epithelium with distinct granular layers without nuclear atypia. In addition, we observed inflammation due to cyst rupture with presence of histiocytes, lymphocytes, and scattered eosinophils (see Fig 2). No presence or remains of microorganisms or parasites associated with the cysts were observed in any of the samples.

Conclusions

Here, following our investigations, we report of epidermal inclusion cysts on populations of Puerto Rican crested anoles (*A. cristatellus*) in Miami FL are not associated to microorganisms. We are still unsure of the causal factors of the masses and that avenue represents ongoing research. Further questions include ascertaining whether masses on the Key Biscayne population are the same as the masses assessed in the South Miami population in this study.

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Using archival DNA to elucidate anole phylogeny

In 1984, Rusell Higuchi and colleagues (Higuchi et al., 1984) published a path-breaking paper on the quagga, a zebra-like equid from southern Africa that had gone extinct about a century earlier. In the paper they reported that they had obtained DNA from a dried museum skin, and had been able to sequence 229 bp of mitochondrial DNA. The ability to obtain DNA from specimens such as the quagga—preserved, if consciously preserved at all, without any intent to preserve the DNA—soon gave rise to a now flourishing field of study: the study of evolutionary history using "ancient" DNA sequences. The development of the field has not been without hiccups. In some of his earliest studies, on Egyptian mummies, Svante Pääbo, now a leader in ancient DNA studies, turned out to have sequenced modern human contaminants (Pääbo, 2014). But the methodology of sequencing DNA in general, and ancient DNA in particular, has advanced greatly, and has now been successfully applied to a great diversity of extinct taxa, from Vegas Valley leopard frogs (Hekkala et al., 2011) and Bahamian tortoises (Kehlmaier et al., 2017), to Mascarene skinks (Austin and Arnold, 2006) and Tasmanian tigers (White et al., 2018). For DNA obtained from museum specimens that are no more than a century or two old, we prefer the term "archival DNA"—defined as DNA extracted from specimens that were not preserved with the intent of preserving the specimens' DNA—leaving the term "ancient DNA" to refer to the sorts of serendipitous and non-scientific preservation found in much older specimens such as mummies, Neanderthals, and wooly mammoths.

Before new experimental methods can be accepted, they must be validated by showing that new results comport with well-confirmed earlier findings. As Sir Arthur Eddington (1935) quipped, no new experimental finding can be accepted until it is confirmed by theory. Equally important in the case of extinct species, it must be shown that the risks of destructive sampling of irreplaceable specimens are outweighed by the rewards of new and otherwise unobtainable data. Thus, the second figure in Higuchi et al. (1984)—the first was the sequence of A's, G's, C's, and T's themselves—is a phylogenetic tree showing that the quagga, just as was already well-confirmed by morphological data, was indeed a member of the horse family, and nearer to a cow than a human. The exact relations of the quagga have been confirmed and further elucidated by later sequencing work by Leonard et al. (2005), which shows that quaggas are most closely related to plains zebras. As this and other examples show, archival DNA has proven to be a

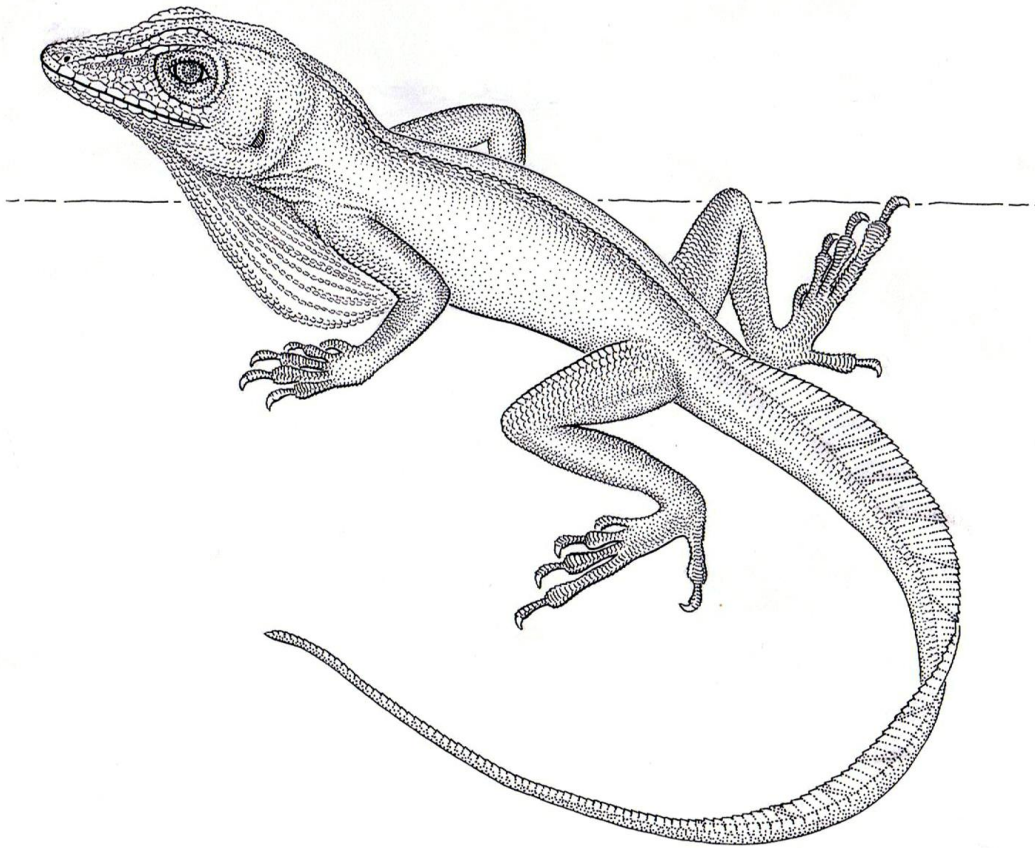valuable source of data for the study of extinct species and populations.

Figure 1. MCZ 36138, the holotype of *Anolis roosevelti*. Laszlo Meszoly, del.

For anoles, we are fortunate in that, of the 400 or so species known from living specimens, there has been little extinction (Böhm et al., 2013). Although some poorly known anole species have not been recently collected, other poorly known species—for example, *Anolis proboscis* (Poe et al. 2012)—have been recently rediscovered and more thoroughly studied. The only species of anole widely acknowledged to be likely to have gone extinct in historical times is *Anolis roosevelti* (Fig. 1), which inhabited the eastern islands of the Puerto Rican Bank, where it is known to have occurred on Vieques, Culebra, St. John, and Tortola (Fig. 2). Based on the reports obtained by Chapman Grant (1931, 1932), the species' describer, and its morphology, *roosevelti* has been interpreted as the crown-giant ecomorph of the eastern Puerto Rican Bank, where it would have been the ecological vicar of *Anolis cuvieri* of the Puerto Rican main (Fig. 3; Mayer, 1989). Last collected on Culebra in 1932, a number of searches in its known range, most notably heroic endeavors by Ava Gaa Ojeda Kessler (2010), in and around its last known haunts on Culebra, have turned up nothing; and though we still hold out some hope for its survival, especially in the still little explored former naval reservation on eastern Vieques, the species is

usually considered extinct.

Figure 2. Puerto Rico and the Virgin Islands, showing the known distribution of *Anolis roosevelti* (stars). From west to east, the islands are Vieques, Culebra, St. John, and Tortola (north at top). Base map: Google Earth.

With no fresh specimens available, phylogenetic studies of *roosevelti* have necessarily been morphology-based. Using the morphological characters then available—primarily Richard Etheridge's (1959) osteological characters—Ernest Williams (1972) attempted to place *roosevelti* in a phylogenetic context amongst the other Puerto Rican anoles. Osteologically, *roosevelti* is an alpha-anole (lacking transverse processes on the caudal vertebrae), with an arrow-shaped interclavicle, and three fixed and two free inscriptional ribs. This places it near the base of the tree constructed by Williams, but the overall evidence is not strong.

Liam Revell, Luke Mahler, Graham Reynolds, and Graham Slater (2015) tried a novel method using metric characters to infer *roosevelti's* relationships. They found it to be near the Cuban crown giants, not the Puerto Rican giant, *cuvieri*. But for some time the standard for phylogenetic estimation in anoles has been DNA sequence data. With no recently collected specimens, the only option for study of *roosevelti* is archival DNA. As noted earlier, in the case of extinct species, it must be shown that the risks of destructive sampling of irreplaceable specimens are outweighed by the rewards of new and otherwise unobtainable data. This consideration is clearly of concern with *roosevelti*, since only six extant specimens are known: four collected by A.H. Riise in the 1860s, and two by Chapman Grant in the 1930s. An additional consideration is that it is very difficult to get DNA from specimens fixed in formalin, and since Stejneger's promotion of formalin as a fixative in 1911, most collectors have used it. Riise's specimens, collected in the 1860s, would be more likely to have been fixed in ethanol. (Not to mention that Riise founded what is now the largest liquor store in the Virgin Islands, and

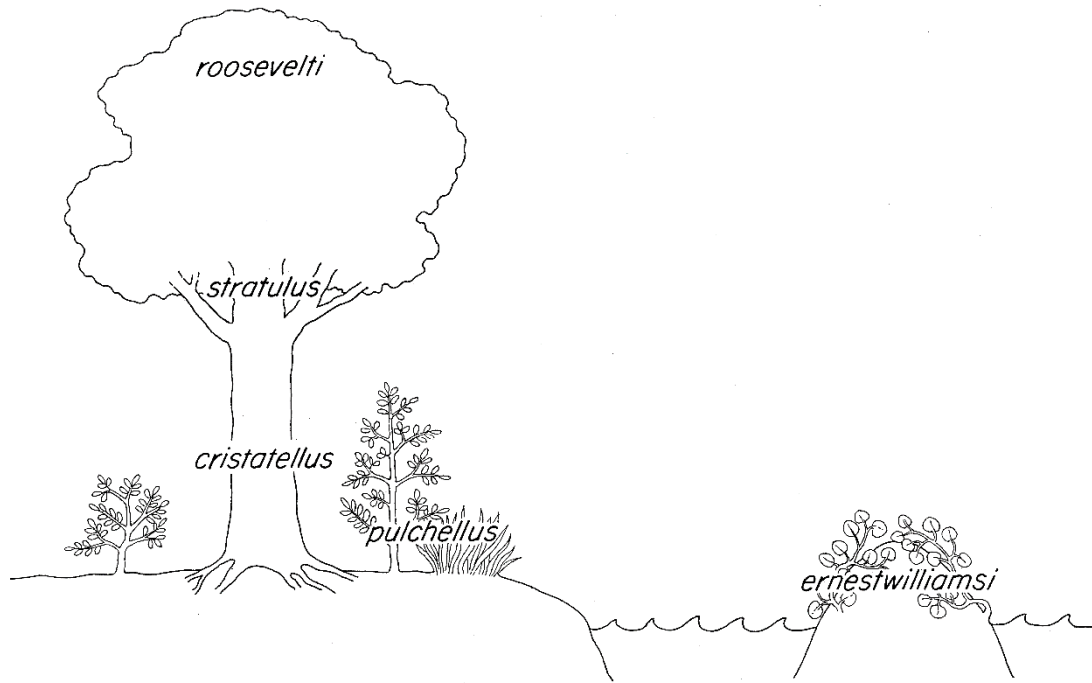so seems to have had an affinity for alcohol!)

Figure 3. Ecological distribution of the anoles of the eastern Puerto Rican Bank (Virgin and Passage Islands). *Anolis roosevelti* is the crown-giant ecomorph, *A. stratulus* is the trunk-crown ecomorph, *A. cristatellus* is the trunk-ground ecomorph, and *A. pulchellus* is the grass-bush ecomorph. *A. ernestwilliamsi* is a *cristatellus* derivative endemic to the largely *Coccoloba*-covered Carrot Rock. Laszlo Meszoly, del.

So, preliminary to study of *roosevelti*, we have attempted the extraction of archival DNA from specimens of *Anolis cristatellus*, which is abundant and distributed throughout the Puerto Rican Bank—literally from one end to the other— and with close relatives on off-lying island banks. By showing that we can extract and sequence archival DNA from this species, and that the results obtained comport with what is known about this well-studies species, we can pass the "Eddington test", and thus have greater justification in consumptively sampling from the irreplaceable *roosevelti* specimens, and greater confidence in the results of that sampling.

Methods

Our goal was to utilize specimens that are as similar as possible in their history to the extant specimens of *roosevelti*. We have studied three specimens of *cristatellus* collected on Vieques by Riise in about 1861, five specimens collected on Vieques and Culebra by Grant in 1931, three more recent specimens collected by Skip Lazell in the Virgins in 2000, and one specimen collected by one of us (TG) in Puerto Rico in 2014. This last one, unlike the others, was fixed in ethanol with the intent to preserve its DNA. For archival DNA we thus have eight

specimens of *cristatellus* collected approximately coincident in time and place with Riise and Grant's specimens of *roosevelti*, plus three more recent ones; as well as a single 'modern' specimen (Table 1).

Table 1. Specimens used and the results of DNA extraction and sequencing.

Specimen	Year	Locality	fixative	DNA (ng/ml)	Library	mtDNA assembly	ND2 phylogeny
ZMUC R37381	1861	Vieques	ethanol	<50	good	fail	no
ZMUC R37383	1861	Vieques	ethanol	<50	good	good	yes
ZMUC R37386	1861	Vieques	ethanol	<50	good	good	yes
MCZ R35732	1931	Vieques	ethanol	68	good	partial	yes
MCZ R35735	1931	Vieques	ethanol	145	good	good	yes
MCZ R35739	1931	Vieques	ethanol	55	good	good	yes
MCZ R35953	1931	Culebra	ethanol	54	poor	fail	no
MCZ R35959	1931	Culebra	ethanol	<50	poor	fail	no
MCZ Z28485	2000	Necker Id.	isopropanol	416	good	partial	yes
MCZ Z28486	2000	Necker Id.	isopropanol	385	good	good	yes
MCZ Z28585	2000	Tortola	isopropanol	267	good	partial	yes
TG 2223	2014	Puerto Rico	ethanol	520	good	good	yes

We expected that Riise's three *cristatellus* from the Zoological Musuem in Copenhagen (ZMUC), collected about 1861, would have been fixed in ethanol, and we can confirm this, as they all had the opaque white pupils characteristic of ethanol fixation (Fig. 4; Simmons, 2014). Unexpectedly, Grant's specimens were also ethanol fixed, as shown by their also having opaque, white pupils, as kindly confirmed for us by Jose Rosado. Skip Lazell's specimens (MCZ Z numbers in Table 1) were fixed primarily in isopropanol (which is available by retail sale throughout the West Indies). For the cataloged Museum of Comparative Zoology (MCZ) specimens (MCZ R numbers in Table 1), Breda Zimkus took thigh muscle and liver tissue for us; for the others, we took liver tissue from a ventral incision, little different from that made in a fresh specimen from which tissue is taken (Fig. 5).

Figure 4. ZMUC R 37381, *Anolis cristatellus*, showing the opaque white pupil indicative of ethanol fixation.

Figure 5. ZMUC R 37383, *Anolis cristatellus*, showing the ventral incision for removal of liver tissue.

DNA was extracted from the tissues following the protocol of Ruane & Austin (2017). llumina libraries were prepared using NEBNext Paired-end library kit, and sequenced with Illumina HiSeq 2500 at the Medical College of Wisconsin. Reads were cleaned and trimmed, and PCR duplicates removed. *De novo* assembly was performed using CLC Genomics Workbench for whole mitogenome assembly. Reads were mapped to assembled *A. cristatellus* mitogenome using Geneious for partial mitogenome assembly. The mitogenome was annotated using mitoAnnotator. We performed two phylogenetic analyses, one using whole mitogenomes with our archival and modern DNA, plus multiple anole taxa, including *cristatellus*, from Gen Bank; and a second phylogenetic analysis using just ND2. Alignment was done with MUSCLE (using data from Reynolds at al., 2017 for ND2), and trees estimated using RaxML.

Results and Discussion

We obtained quantifiable DNA from 8 of 12 samples (detection limit 50 ng/ml), and good Illumina libraries from 10 of 12 samples. *De novo* assembly produced complete or near complete (>80% complete) mitogenomes in 6 of 12 samples, and partial mitogenomes from 3 of the 6 remaining samples (Table 1). Unfortunately, sequences could not be recovered from either of the Culebra samples, both of which were collected by Grant.

The assembled whole mitogenome (Fig. 6) appears as would be expected, with a fairly typical genome size and arrangement of the genes, with the exception of ATPase 8, which is found at about 16.2 kb; normally it's at about 8.5 kb, near ATPase 6.

The mitogenome phylogenetic analysis was designed to demonstrate that the archival sequences were what would be expected of *cristatellus*, and utilized a number of other anole mitogenomes, either from GenBank or generated in TG's lab. The results (Fig. 7) clearly show that the archival *cristatellus* DNA samples—shown boxed in gray—form a clade with the two modern samples of *cristatellus*— ours (TG 2223), and another from GenBank. Note that several of the archival samples form their own subclade, but that another is within the adjacent, otherwise modern, subclade of *cristatellus*. This result confirms that the archival DNA is indeed *Anolis cristatellus* DNA.
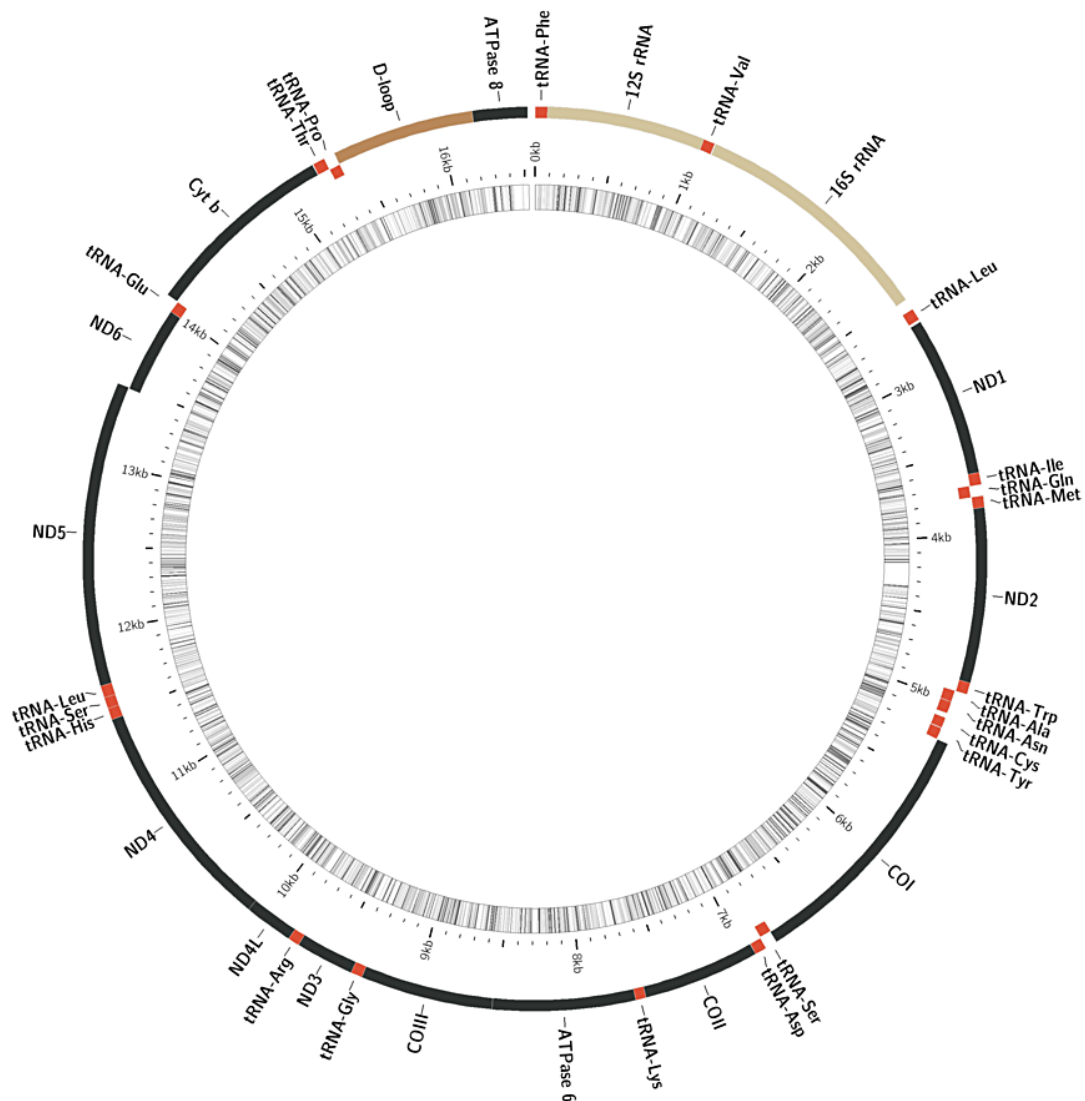
Figure 6. Annotated mitogenome of *Anolis cristatellus*.

The ND2 phylogenetic analysis was designed to look at the placement of the archival samples within *cristatellus*, and utilized a large number of sequences of *cristatellus* and its close relatives, most from Graham Reynolds and colleagues' recent paper (2017). There are three things to note in the estimated phylogeny (Fig. 8). First, Skip Lazell's British Virgin Island specimens (MCZ Z numbers) fall amongst other British Virgin Island samples, as expected. Second, all the archival Vieques samples (MCZ R numbers) are either sister to another Vieques sample, nested within a Vieques clade, or nested within a clade that includes Vieques specimens.
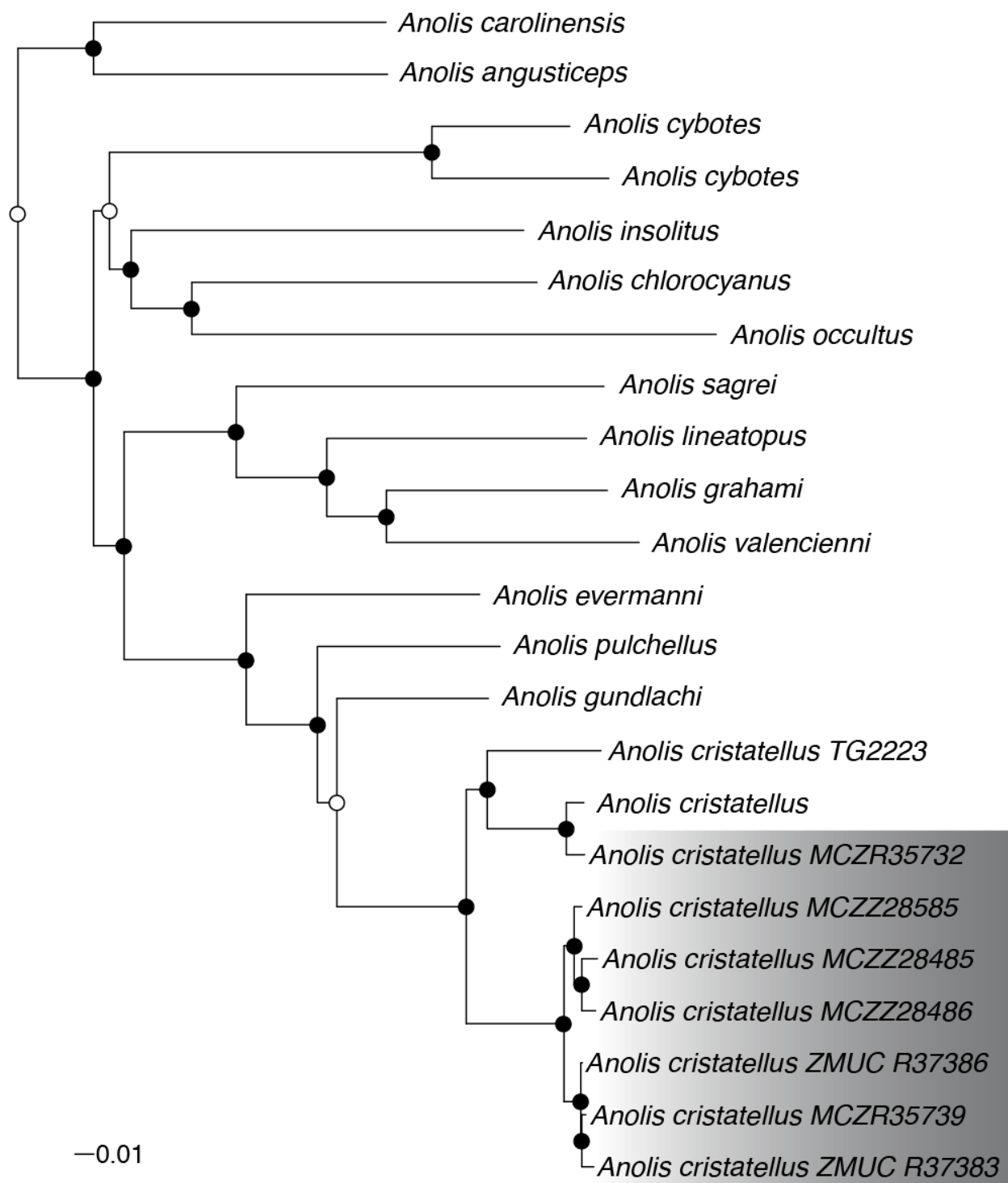
Figure 7. Phylogeny estimated from mitogenome sequences with RaxML. Black circles at nodes indicate a bootstrap percentage greater than 70. Seven archival samples are highlighted. These samples and the modern sample TG2223 are from this study; other samples from GenBank or generated in Gamble lab.
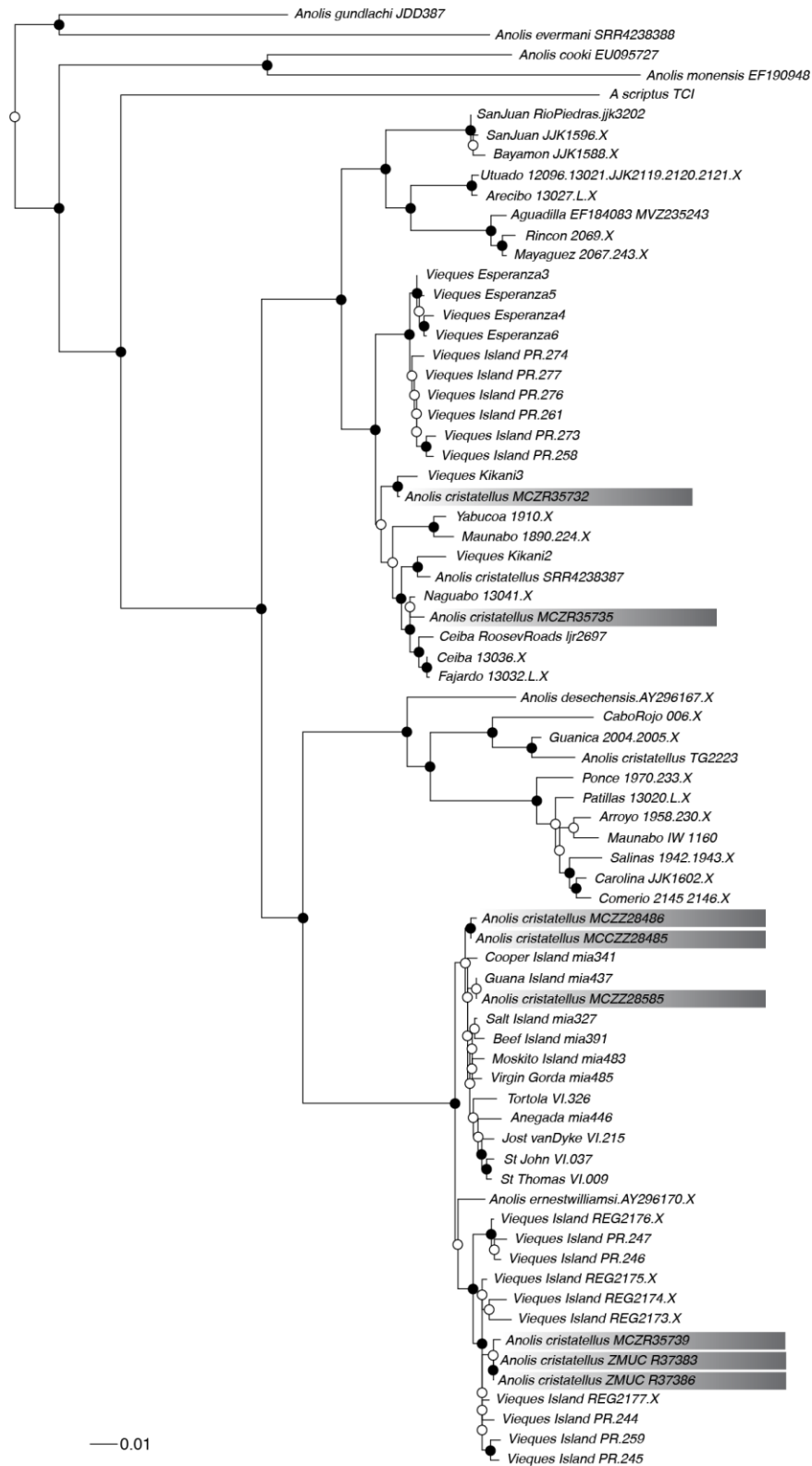
Figure 8. Phylogeny estimated from ND2 sequences with RaxML. Black circles at nodes indicate a bootstrap percentage greater than 70. Eight archival samples are highlighted. These samples and the modern sample TG2223 from this study; other samples mostly from Reynolds et al. (2017).

Also note that Grant's specimens are divided between the two divergent clades identified by Reynolds et al. (2017)—"PR East" and "Virgin Islands"; both of Riise's specimens are in the "Virgin Islands" clade. And finally, our modern specimen, from Boqueron, Puerto Rico, falls within a clade of other southwestern Puerto Rico specimens. All these results are as would be expected. Together, they confirm the localization of the samples to geographically sensible parts of the tree.

Conclusion

So, archival DNA in anoles has passed the Eddington test—it produces results that are entirely reasonable given what we already know, based on well-confirmed estimates of phylogeny. We thus conclude that extraction and analysis of archival DNA is a promising method for investigations of *Anolis roosevelti.* The results for *roosevelti*, unlike those for *cristatellus*, for which we already had well-confirmed expectations, will be novel and interesting. And we also conclude that archival DNA has promise for investigations of the genomes and phylogeny of anoles in general.

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Anoles not found

Anoles are an evolutionarily successful group, with 400 or so species distributed throughout the warm temperate and tropical regions of the New World, where they occupy a diverse array of ecological niches, often with high local abundance and species richness (Losos, 2009). Another aspect of anoles' success is what Ernest Williams referred to as "anoles out of place": anoles that have been introduced and become established in areas outside their native ranges, often spreading out from points of introduction to become widespread and conspicuous elements in their new homes (Losos et al., 1993). These "anoles out of place" have flourished in the New World on various islands (e.g., Bermuda: Wingate, 1965; Losos, 1996) and mainlands (e.g., Florida: Meshaka et al., 2004; Stroud, 2014; Krysko et al., 2019), and even in distant parts of the world, such as Hawaii (Shaw and Breese, 1951; McKeown, 1996), across the Pacific (Mayer and Lazell, 1992; Michaelides et al., 2017), and subtropical Japan (Ota et al., 1995, 2004; Toda et al., 2010).

It has long been recognized that introduced and invasive species, while often being of conservation concern, nonetheless present favorable materials for the study of ecological and evolutionary phenomena (Elton, 1958; Simberloff, 2013). By inducing 'experiments' in community composition, they allow us to examine the dynamics and trajectory of species' presence and abundance in a community disturbed perhaps far from an equilibrium condition. They allow us to see not only ecological responses, but also any evolutionary responses that may occur as a result of changes in the species' "conditions of existence" (as Darwin put it), as well as other genetic phenomena attendant upon the arrival and establishment of a colonizing species. Anole introductions have already provided useful insights into these phenomena, and hold the promise of providing much more (Losos, 2009).

But, as Simberloff (1988) has emphasized, we need to know the history of not just the successes, but also the failures, if we are to understand the phenomena of colonization. So, here, we report upon two anole introductions that are "out of place", but which have, perhaps only temporarily, failed to spread further into what seems to be accessible, and acceptable, habitat. The recording of successes or failures to expand, based on recurrent surveys, allows us to follow

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the time course of an invader's spread, and thus gain a better understanding of the ecological and evolutionary phenomena attendant to range expansion (Losos, 1996).

I. *Anolis cristatellus* along Carretera Sarapiquí (Hwy. 4), Provincia Heredia, Costa Rica

Anolis cristatellus, the crested anole of Puerto Rico and the Virgin Islands, was designated a "minor colonizer" by Williams (1969)— in addition to its' home island bank, it, or a close relation, has colonized several other banks (Mona, Monito, Desecheo near Puerto Rico, and several more in the Turks and Caicos and the southern Bahamas: Brandley and de Queiroz, 2004; Powell and Henderson, 2012). There have also been anthropogenic introductions, one of the earliest being to Limon, Costa Rica in 1970 (J.M. Savage, pers. comm.), from whence they have spread inland to Turrialba, and down the coast to Cahuita (Savage, 2002; Mayer, 2010).

In January, 2011, one of us explored eastern Costa Rica to determine the if *A. cristatellus* had spread further, and located them in Bribri, south of Cahuita, and Siquirres, on the highway northeast of Turrialba; they were not found at Guapiles, west of Siquirres (Losos, 2011a, 2011b). From Siquirres, mid- to low-lands, and a good highway, extend west through Guapiles, and then north into Prov. Heredia, making this a likely avenue for further spread. In March, 2012, a further survey was made, traveling from Puerto Viejo de Sarapiquí, Heredia, back south and east to Guapiles, stopping periodically to seek *cristatellus* in likely habitats, but none were found (Losos, 2012).

In March, 2014, a third survey was rewarded with success (Losos, 2014). *Cristatellus* were found at Guapiles, and at Rancho Robertos, a restaurant on Carretera Sarapiquí (Hwy. 4), at the intersection (*cruce*) of that highway with Carretera Braulio Carillo (Hwy. 32). It is not clear whether this was a spread by lizards on the ground from Siquirres (37 km to the east of Guapiles), or by jump dispersal via vehicles. There is a bus terminal across Hwy. 4 from Rancho Robertos, and much traffic moves through the *cruce*.

We report here on surveys in 2017 and 2018, along Hwy. 4 and at Estación Biologica La Selva. We did not find any *cristatellus*, except at Rancho Robertos, where they appear to be thriving.

2-3 January 2017

JBL arrived at Hotel Hacienda Sueño Azul, located about 3-4 km by road west of Hwy. 4 (about 1 km straight line) on 31 December 2016. On 2 January 2018 he was joined by GCM. After a brief joint examination of the hotel grounds, we departed at about 1300 h in a hired vehicle, and drove out to Hwy 4, then turned south toward Rancho Robertos. We had our driver stop at promising places along the way, usually a combination of a business establishment or other disturbed area with the presence of suitable vegetation. At each site, we searched for *A. cristatellus*, noting any other lizards seen. The weather was overcast as we began, but was sunny

by the end of the day. After stopping at Rancho Robertos, we turned back north, stopping at other promising places. At 1552 h, we arrived at Servicentro Puerto Viejo in Puerto Viejo de Sarapiquí, just north of the bridge over the Río Sarapiquí. We walked from there to the intersection of Hwy. 4 with Hwy. 505, just west of downtown Puerto Viejo, including a foray into a residential neighborhood east of Hwy.4 and south of the intersection, departing at 1624 h for Estación Biologica La Selva.

Figure 1. Adult male *Anolis cristatellus* at Rancho Robertos, Carretera Sarapiquí, Prov. Heredia, Costa Rica, 2 January 2017.

The results of this first day's survey are in Table 1. *A. cristatellus* were found only at Rancho Robertos, the same as in 2014 (Fig. 1). At Rancho Robertos, four adult males, 2 adult females (including a mating pair), and one juvenile were seen. It was sunny by the time we reached Rancho Robertos. The other locations, based on our experience with *cristatellus* in many parts of its range, seemed suitable. Lizards, including other anoles, were found at every location, indicating that the failure to find *cristatellus* did not stem from conditions being wholly unsuited for lizards in general.

On the morning of 3 January, from 0830 to 1200 h, we looked for *A. cristatellus* on the grounds of La Selva, beginning with the Annex (east of the Río Puerto Viejo), then crossing the river to the clearing around the cabins and laboratory buildings (these first two areas being the likeliest for finding them), then the River Station, and finally the Arboretum. Lizards of several species were seen (*Anolis limifrons, Iguana iguana, Basiliscus vittatus, B. plumifrons,* and

Ameiva festiva), but no *cristatellus*. OTS staff told us that no unusual lizards had been reported.

Table 1. Results of a survey for *Anolis cristatellus* along Carretera Sarapiquí (Hwy. 4), Provincia Heredia, Costa Rica, on 2 January 2017. (Note that the locations are ordered by distance from Rancho Robertos, not the order in which they were visited.)

Km north of Rancho Robertos	Location	Lizard species
0	Rancho Robertos	*Anolis cristatellus, Basiliscus vittatus*
1	Fuerza Publica building, just north of Rio Sucio	*Gonatodes albogularis*
2	Turnoff to La Isla de Israel	*Anolis limifrons*
5	Soda de Campesina	*Basiliscus vittatus*
7	Bus stop and side road	*Anolis biporcatus, Basiliscus sp.*
10	Sun Sun Hotel	*Gonatodes albogularis*
11	Turnoff to Hotel Hacienda Sueno Azul/Horquetas	*Anolis limifrons*
12	Vivero Herpa-nursery and forest	*Anolis limifrons, Gonatodes albogularis*
21	Peruto Viejo, from Servicentro Puerto Viejo (just north of Rio Sarapiqui) and neighborhood east of highway, north to intersection of Hwy. 4 and Hwy. 505	geckos

In the afternoon, starting at 1323 h, GCM walked from the Annex out to Hwy. 4, then north along Hwy. 4 to Puerto Viejo. The habitat along this part of the highway was not promising, the road edge being grassy with no human development. In Puerto Viejo, he turned east onto Hwy. 505, into, around, and through downtown Puerto Viejo, to the far east end of town, coming to a small bridge, and searching on the far side, and then returning the same way, departing by taxi for La Selva at 1553 h. These parts of Puerto Viejo provided much apparently suitable habitat (Fig. 2), but no *cristatellus* were found; other lizards, all in Puerto Viejo, were *Basiliscus vittatus, Gonatodes albogularis*, and *Ameiva* sp. It was important to search Puerto Viejo, because trucks and other vehicles stop much more frequently in town than at other places along the highway, and, if jump dispersal by vehicle occurs, Puerto Viejo is the nearest place to Rancho Robertos to which such dispersal would be expected.

Figure 2. Apparently suitable urban habitat in park east of soccer pitch, Puerto Viejo de Sarapiquí, Prov. Heredia, Costa Rica.

In addition to the more intense searches detailed above, more casual observations were made by JBL on 31 December 2016-1 January at Hotel Hacienda Sueño Azul, and on the afternoon of 3 January, and 4-6 January at La Selva; while GCM did so at La Selva on the morning of 4 January.

15-17 May 2018

On 15 May, GCM, along with a group of students, stopped at Rancho Robertos for lunch, and *Anolis cristatellus* was common. Seven individuals, of both sexes, again including a mating pair, were seen (Fig. 3). Arriving at La Selva the same day, the group stayed till midday on the 17th. Time was spent in the Annex, the River Station, the laboratory clearing, and in the forests. Much more time was spent in good forest habitat, which is *not* expected to be prime habitat for *cristatellus*, than during the 2017 visit. However, a watchful eye was kept for all lizards, including *cristatellus*, and a couple of hours were spent in the built-up areas specifically looking for them. But while many other species were seen (*Anolis limifrons, A. humilis, A. capito, Basiliscus vittatus, B. plumifrons, Corytophanes cristatus, Iguana iguana, Hemidactylus* sp., *Thecadactylus rapicauda, Gonatodes albogularis, Lepidophyma flavimaculatum*, and *Ameiva*

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festiva,), no *cristatellus* were. OTS staff again stated there were no reports of unusual lizards.

Figure 23. Mating pair of *Anolis cristatellus* at Rancho Robertos, Carretera Sarapiquí, Prov. Heredia, Costa Rica, 15 May 2018.

Summary and conclusion

Fig. 4 shows the locations visited during 2017 (all marked locations) and 2018 (only Rancho Robertos and La Selva). *Anolis cristatellus* were first seen at Rancho Robertos in 2014, and at only this location in 2017 and 2018. In 2017, we did not find them at numerous locations along the highway north of Rancho Robertos, nor in Puerto Viejo, a likely locus for jump dispersal by vehicle. They were not found at La Selva in either year. Although the evidence is negative, it seems that *cristatellus* has *not* spread north of Rancho Robertos.
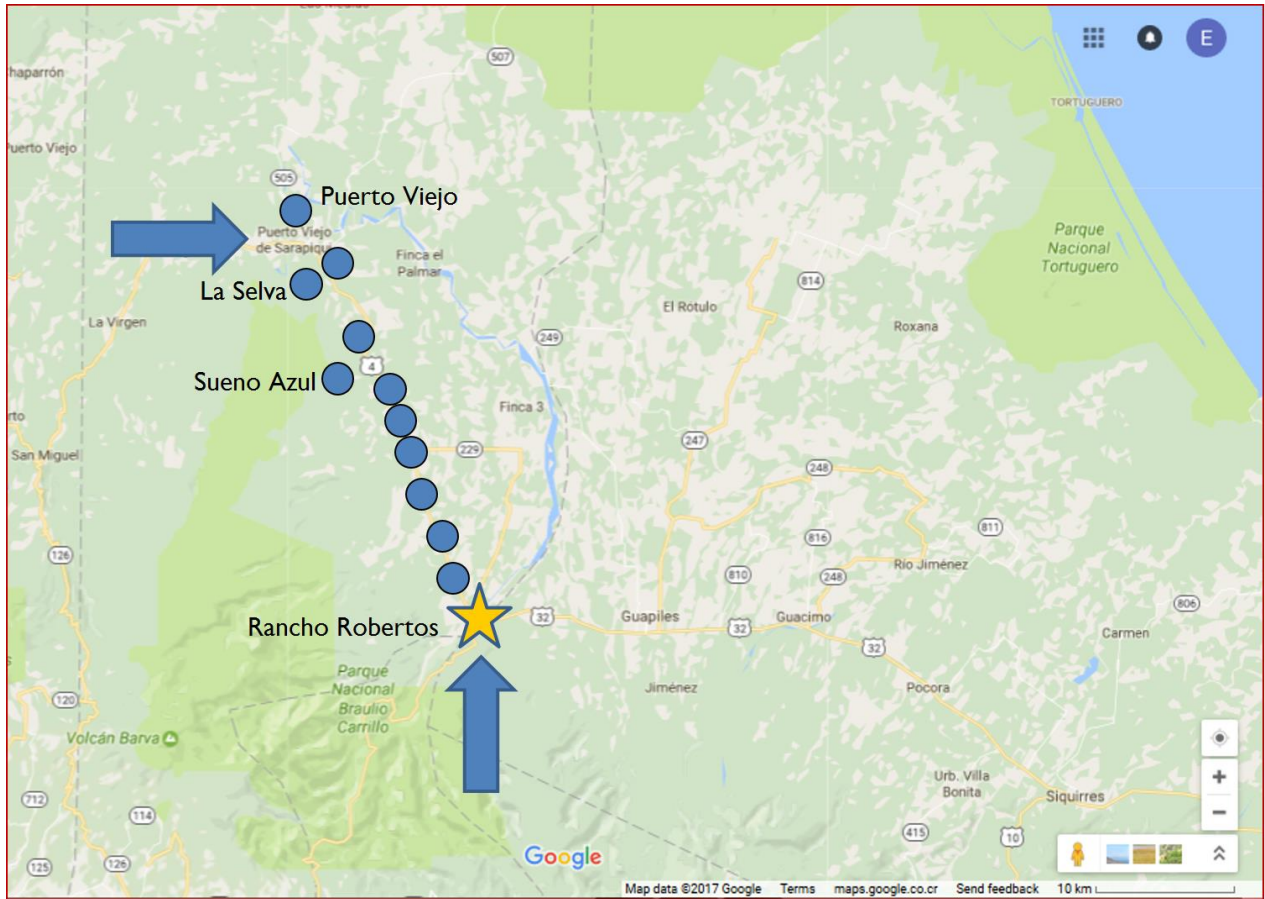
Figure 4. Sites surveyed in Prov. Heredia, Costa Rica in 2017 and 2018. Arrows: southern (Rancho Robertos) and northern (Puerto Viejo) termini of the surveyed area. Circles: *Anolis cristatellus* not found. Star: *Anolis cristatellus* present.

II. *Anolis carolinensis* on Okinawa, Japan

Anoles of the *carolinensis* group, "the most successful of all anole colonists", have an extensive natural range in the West Indies and adjacent North America (Williams, 1969). They have also been successfully introduced, especially on islands in the Pacific (Mayer and Lazell, 1992; Michaelides et al., 2017). They were first found on Okinawa in 1994, in the capital, Naha City (Ota et al., 1995). Ota et al. (2005) report that they had not been found at Shuri, the original site in Naha, in 2004, but that they had been collected at another site in the city, Kanagusuku, and suggested that a nearby site, Oroku, should also be inspected. In 2017, Ota (pers. comm.) reported that they occurred around Naha Airport, and the nearby Japanese Self-Defense forces base.

From May 17-20, and May 25-June 2, 2017, GCM visited Okinawa, staying in Chatan, and traveling to several other places on the island, all the while being alert for the presence of anoles. No anoles were found.

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In Chatan, GCM regularly visited the area from Sunabe Baba Park south along the Sunabe Seawall to the boat basin at Minato, and then south of the basin to Chatan Park and Araha Beach. This was a coastal urban area with parks of varying sizes, and presented what seemed to be suitable habitat (Fig. 5), based on urban habitats used by *carolinensis* in New Orleans.

Figure 5. Apparently suitable urban habitat in Miyagi, Chatan, Okinawa, Japan.

Outside of Chatan, single visits were made to each of the following locations, each of which had at least some areas of potentially suitable habitat: Katsuren Castle, the remains of a 15th century castle; Shuri Castle, a reconstructed castle in Naha City, with surrounding gardens—it was raining when visited; Ryukyu Mura Village, a reconstructed native Okinawan village; Maeda Beach, a coastal locality with littoral shrublands; Zampa (or Zanpa) Point, another coastal locality, with more stunted littoral vegetation; Ocean Expo Park, an extensive coastal park containing botanical gardens and a reconstructed native Okinawan village; and Tumaigushiku Beach, a coastal locality with littoral woodland—it was raining when visited. Brief visits to other places on the island, such as shopping areas in Naha, and inspection of shrubs at the Naha Airport, were also unavailing.

Adding to the absence of evidence for anoles spreading north from their original

detection point in Naha City, Lt. Caroline N. Mayer, USN, who resided in Chatan from August 22, 2015, till September 20, 2017, and visited many other parts of the island, never saw any anoles. Lt. Mayer was well familiar with *carolinensis* from having lived in Mississippi and Florida

The only lizards observed by GCM were individuals of *Hemidactylus* sp. in a restaurant along the Sunabe Seawall and during a brief visit to Kadena, and an unidentified gecko seen at Ocean Expo Park. In Chatan, geckos were also frequently heard calling, and lizard droppings were found on leaves, so geckos may be reasonably abundant. Lt. Mayer also reported only geckos. Feral cats (Fig. 6) were extraordinarily abundant on Okinawa, and it is possible that the high density of cats has a negative effect on lizard populations (Marra and Santella, 2016). This would be especially so for anoles, as opposed to geckos, whose secretive and nocturnal habits might offer some protection. The late Robert Sutton, of Marshall's Pen, Jamaica, once told GCM that a cat had done in the *Anolis grahami* that had formerly frequented his garden.

Figure 6. Feral cat in Sunabe Baba Park, Okinawa, 28 May 2017.

Summary and conclusion

Many hours in apparently appropriate habitat in Chatan, and briefer visits to seven other sites on Okinawa, revealed no introduced green anoles, *Anolis carolinensis* (Fig. 7). In over two

years' residence in Chatan, C.N. Mayer, an observer well familiar with the species, also found none. *Anolis carolinensis* seems not to have spread north from its original foothold in Naha City.
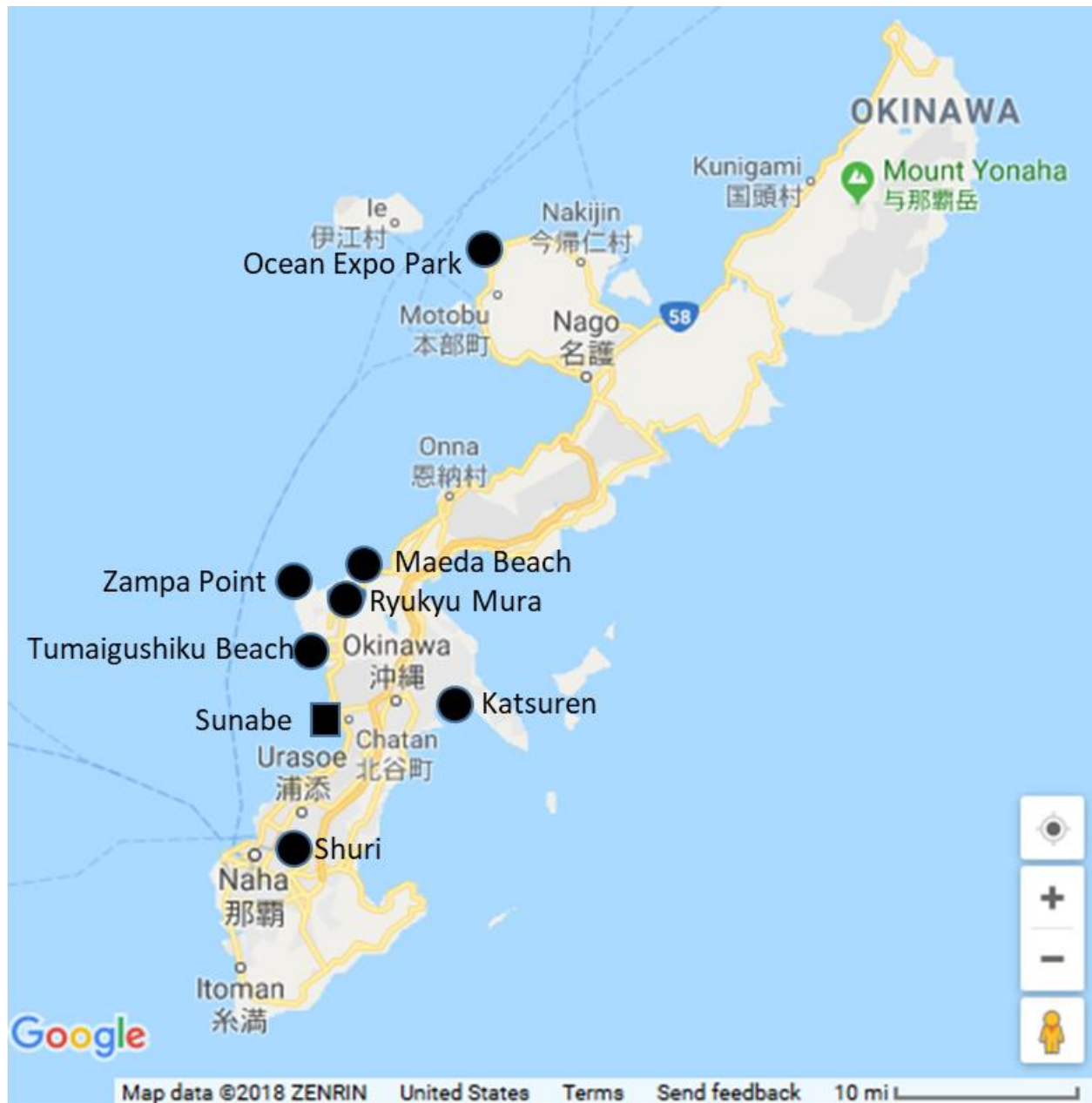

Figure 7. Locations on Okinawa where *Anolis carolinensis* was not found. Circles: localities visited once. Square: the area from Sunabe Baba Park to Araha Beach that was visited frequently.

Hidetoshi Ota kindly provided information about *Anolis carolinensis* on Okinawa, C.N. Mayer kindly shared her observations (or lack thereof) for the same island, and J.M. Savage kindly discussed the history of the Costa Rican introduction of *Anolis cristatellus*.

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The winds of stability: A south Florida residential *Anolis* assemblage over time

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Beginning in 2002, I took what became a long-term interest in the community dynamics of the herpetofauna of a recently constructed gated development in Miramar, a town which is located just north of the Miami-Dade County line in Broward County, Florida. In a paper that summarized survey findings during 2007–2007 (Meshaka et al. 2008), we found high numbers of several exotic herpetofauna among others. We also found a dearth of native species and few of the individuals that we did see. Unlike the days of old, not so long ago, when developments were constructed over intact or slightly-altered habitat, the Nautica site exemplified a new and advantageous stage in synanthropic exotic species colonization dynamics in southern Florida, whereby severe disturbance existed prior to development of the residential community. Therefore, unlike in earlier developments, exotic species were already present when development began. With a foot in the door so to speak and with incidental human-mediated transport, some of these species could succeed. Conversely, most native reptiles and all native amphibians were gone by the time Nautica opened which indicated that the native species diversity (= species richness + species evenness) was already compromised on the site prior to its development. Well, that was the state of affairs 11 years ago. In the giant urban landscape of Miramar, interspersed with blacktop and canals, how the herpetofauna responded over the long-term to the initial end-Permian-like human meteor that reshaped the herpetofaunal community is a matter for different paper. For the anoline assemblage comprised of three species, I find the disparate trends conformed to deterministic pressures that have varied only in degree during 2002–early-2018.

With 148 standardized 2.0 mi. walks during March 2002–March 2008 and in every month except February and August, *Anolis carolinensis* never had a chance (Figure 1). 2006 was the year of *A. carolinensis*. With eight of the 24 observations recorded during 25 March–1 April on Bismark Palms and *Tabebuia*. Among months, eight individuals were recorded in March concomitant with courtship. No other month exceeded three individuals. With respect to habitat structure, *A. carolinensis* should have succeed at Nautica. I do not know the extent to which it is susceptible to predation by native species, such as residential *Coluber constrictor*, Northern Blue Jays, and Northern Mockingbirds, or to exotic species such as *Basiliscus vittatus* (Figure 2) and feral cats; however, *A. sagrei* (Figure 3) is well known to negatively impact *A. carolinensis*

(Campbell, 1999; Echternacht, 1999; Vincent, 1999), and *A. equestris* (Figure 4) is certainly a predatory danger to it. Interspecific overlap in time, ambient temperatures associated with activity, and perch height provides little margin for *A. carolinensis* to avoid direct contact with either of the other two anoles. In that regard, March is as good a time as any to encounter *A. sagrei* (Figure 5a) and *A. equestris* is becoming more active for the season (Figure 5b). Thermal preference of *A. carolinensis* overlaps more so with that of *A. sagrei* than with *A. equestris* (Figure 6a-c). On the other hand, perch height preference was more similar between *A. carolinensis* and *A. equestris* than either was to that of *A. sagrei* (Meshaka et al. 2008).

Anolis equestris, with 285 observations, and *A. sagrei*, with 7832 observations, are still there, and for hopefully non-counterintuitive reasons have remained successful. With a flush of young-of the year, numbers of observations are highest for *A. sagrei* towards the end of the wet season (Figure 5a). Doubtless, this occurs with *A. equestris*, but its penchant for wet season activity (Figure 5b) and crypsis of hatchlings obscure this event. Consequently, I compared numbers observed during April–July when both species are active and breeding. With189 observations to examine, I found that *A. equestris* experienced no significant ups and downs in the course of this study (single factor ANOVA; $p = 0.89$) (Figure 7a). *Anolis sagrei*, on the other hand, experienced significant changes in summer relative abundance over the years (single factor ANOVA; $F = 42.698$, $p < 0.000$) (Figure 7b). Despite hurricane damage in August 2011 and September 2017 (Figure 9a-c), November–March relative abundances (5572 observations) remained high (single factor ANOVA; $F = 90.77$, $p < 0.000$) (Figure 7c).

As I discovered with several West Indian colonizing species (Meshaka, 1993) and particularly with *Osteopilus septentrionalis* (Meshaka 2001), very obviously, if you are to be subjected to hurricanes in your evolution, you either 1. Go extinct, survive them, or 3. *exploit them*. The answer for the species I followed was option 3. Neither *A. equestris* nor *A. sagrei* is any sort of climax forest species. I maintain that they are hurricane-adapted species. Periodic hurricanes, just like periodic fire in pyrogenic communities, opens up forest to provide the species with much needed sunlight without loss of all vertical structure. It effectively maintains a goldilocks optimum between sun and shade and other advantages. For *A. sagrei*, debris became habitat. For *A. equestris*, loss of vertical structure is loss of home and reflected in a lower number of observations in 2012. However, remaining trees refoliated quickly, an important bottleneck to their survival. No new trees were planted but because either individuals were more easily seen or from subsequent use of other trees, numbers did not change (two-tailed t-test; $p = 0.71$) between periods before (mean = 4.85 individuals) and after the 2011 hurricane (mean = 4.39 individuals). Thus, *A. sagrei* took immediate advantage of the storm, whereas *A. equestris*, suffered a short-term loss for long-term stability: Having lost some trees it was spared eventual shading out of its habitat, a result in conflict to its thermal needs.

I suppose *A. equestris* in its range of tolerance could occur in an infrequently disturbed hammock but it would not thrive. Nothing novel in this big lizard needing big trees for its body size and for shade, but its thermal demands require open canopy, even more so for *A. sagrei* among its prey, not mature forest. To their advantage, like that of *O. septentionalis*, hurricanes maintain, and human development aesthetics create and maintain, swaths of artificial analogues of structurally ideal habitat in urban southern Florida. To that end, persistence of *A. carolinensis* at Nautica with its congeners is impressive, whereas, in light of evolutionary history, that of *A. equestris and A. sagrei* is to be expected.

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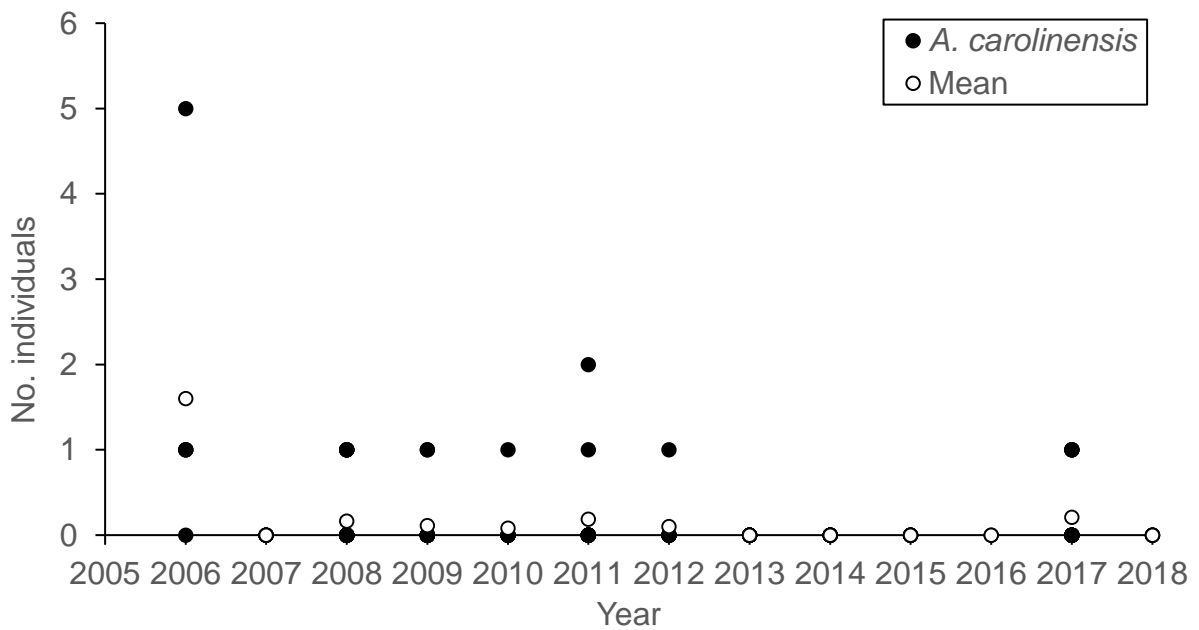
Figure 1. Relative abundance of *Anolis carolinensis* over time at a residential site in Miramar, Broward County, Florida, during March 2006–March 2018.

Figure 2. *Basiliscus vittatus*, a lizard-eater, is observed primarily at opposite ends of my study site in Miramar, Broward County, Florida. Photograph by W.E. Meshaka, Jr.

Figure 3. *Anolis sagrei*. Variable in pattern and very abundant at my study site in Miramar, Broward County, Florida. Photograph by W.E. Meshaka, Jr.
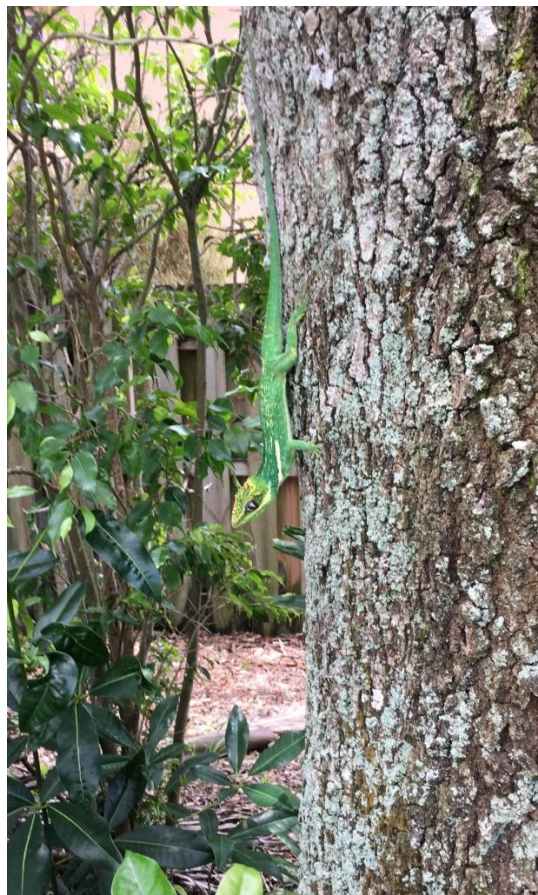
Figure 4. *Anolis equestris*. The anoline *T. rex* of the trees, an abundant anole at my study site in Miramar, Broward County, Florida. Photograph by W.E. Meshaka, Jr.
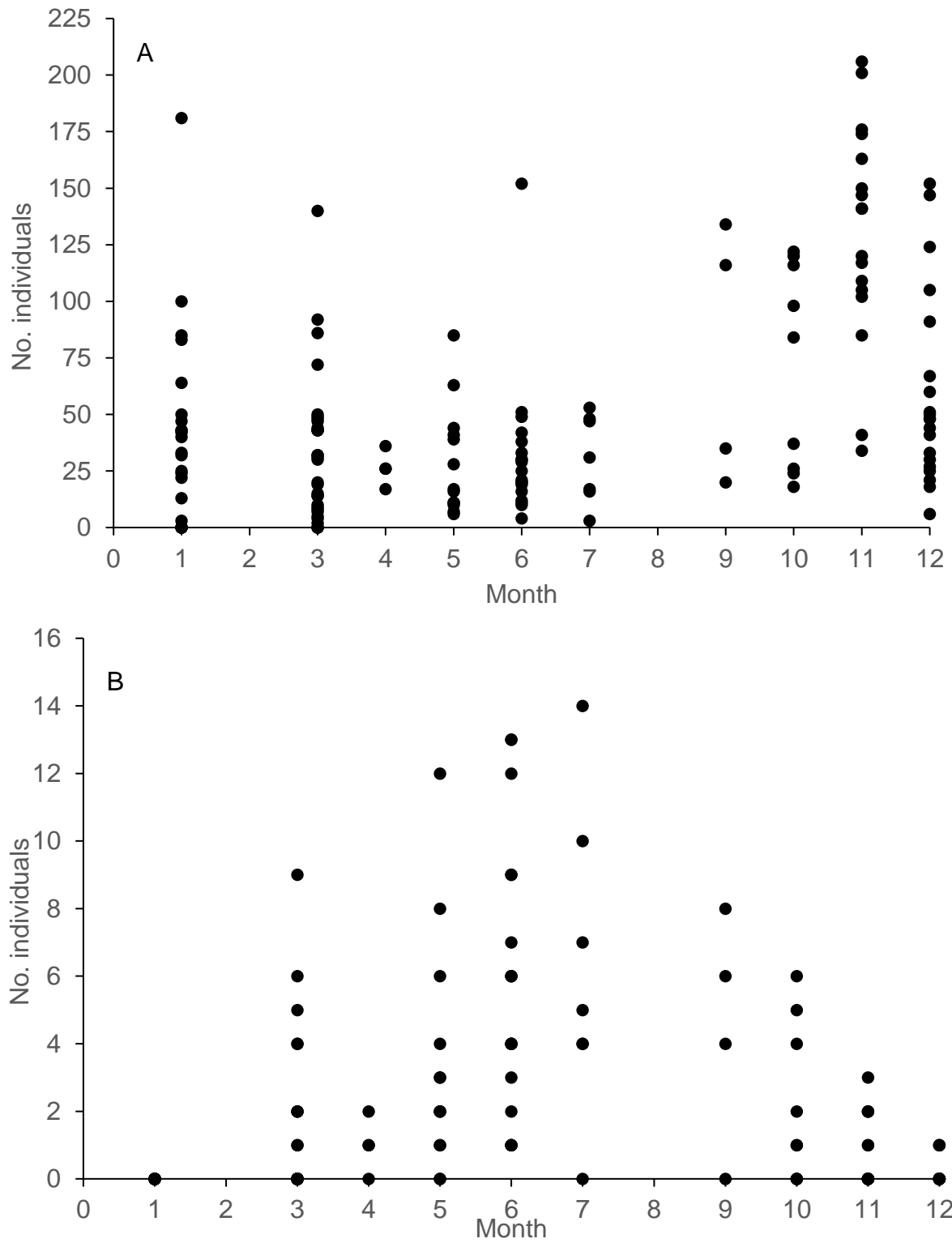
Figure 5. Relative abundance of *Anolis sagrei* (A) and *A. equestris* (B) over time at a residential site in Miramar, Broward County, Florida, during March 2006–March 2018.
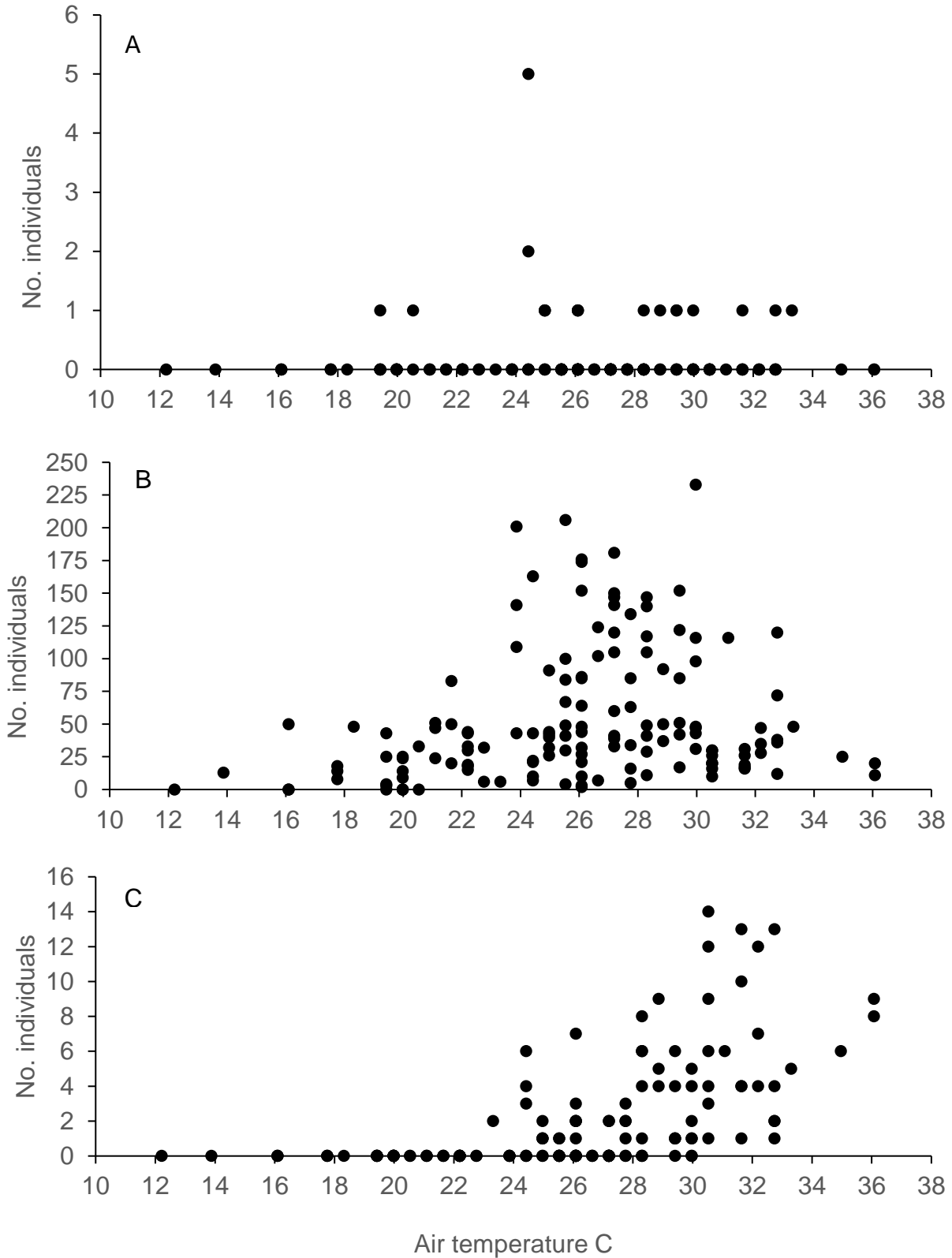
Figure 6. Numbers of *Anolis carolinensis* (A), *A. sagrei* (B), and *A. eqeustris* (C) in relation to ambient temperature at a residential site in Miramar, Broward County, Florida, during March 2006–March 2018.
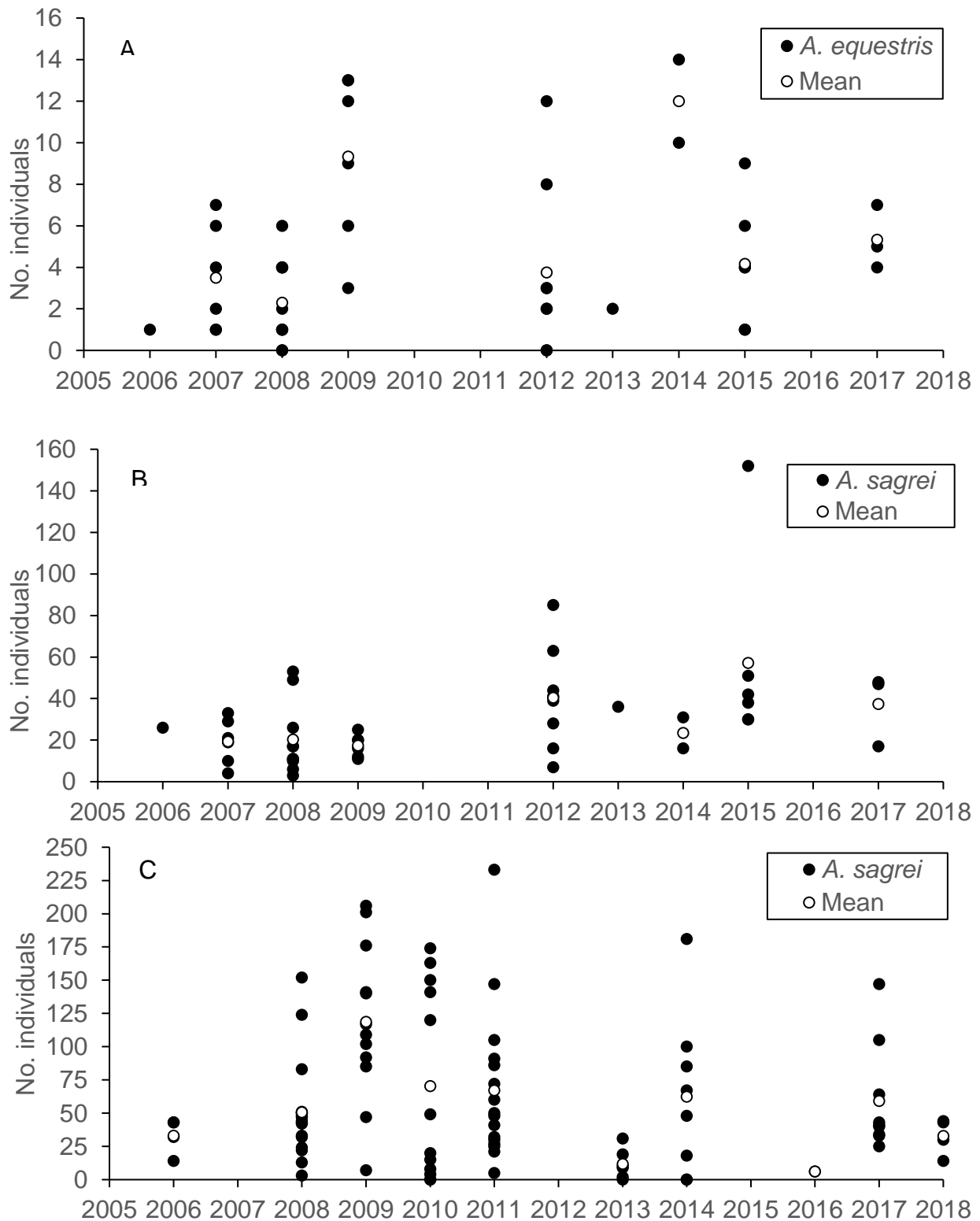
Figure 7. Relative abundance of *Anolis equestris* (A) and *A. sagrei* (B) over time at a residential site in Miramar, Broward County, Florida, during April–July of 2006– 2017 and *A. sagrei* (C) during November–March of 2006–2018.

Figure 8. The Nautica study site, Broward County, Florida. The photographs were taken on 15 October 2017 (A, B, and C) and 2 November 2017 (D) by W. E. Meshaka, Jr., not long after a hurricane in September. Note the *Iguana iguana*, a species seldom seen at the site, using a fallen branch to bask (C).
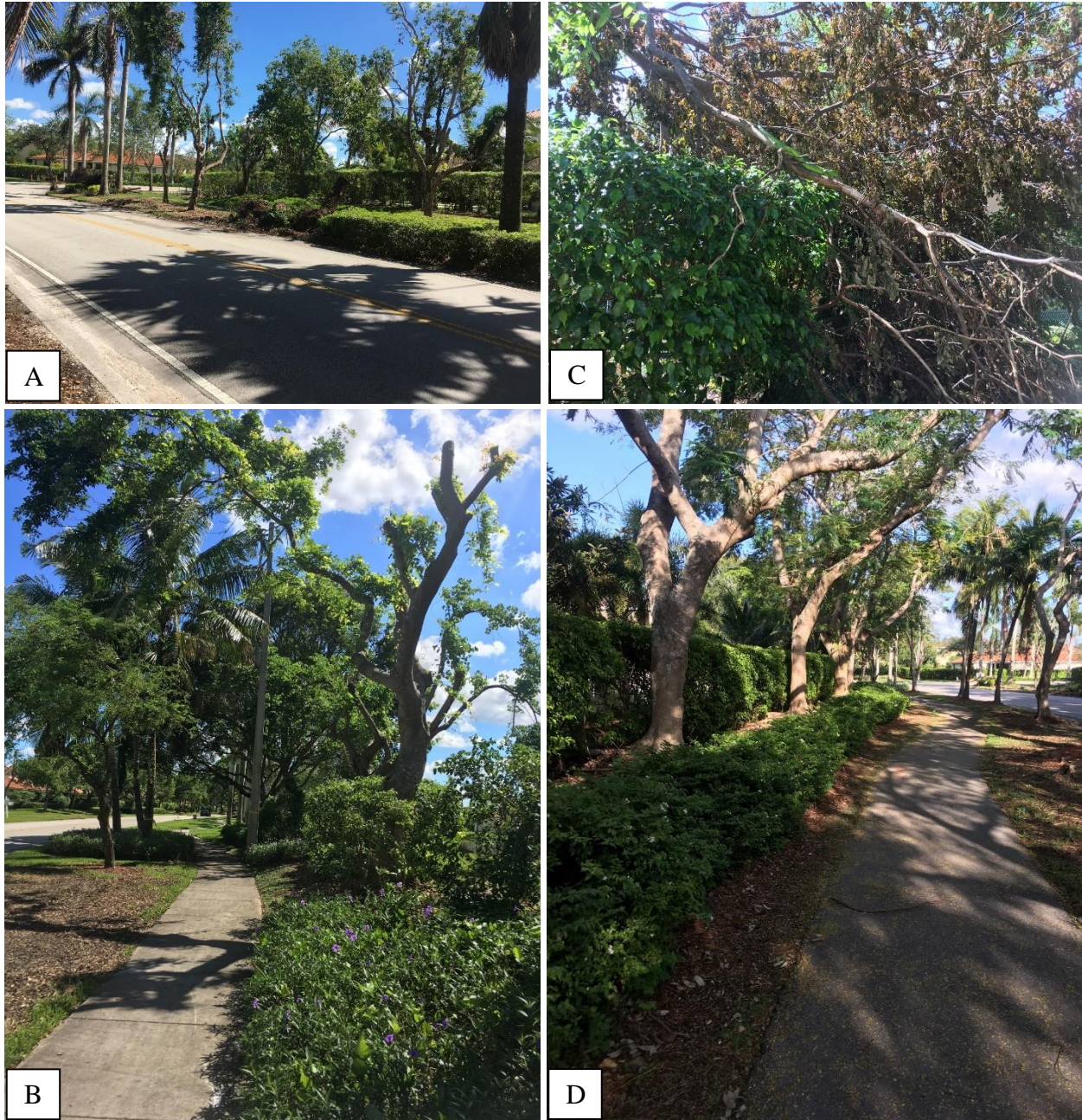
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What are the ecological costs and benefits to northern geographic expansion by a successful anole?

Dispersal brings with it varying weights of costs and benefits. We study relative measures in the process of colonization. I wonder what and how heavy are the costs and benefits of northern expansion by species X having continuous gene exchange southward vs. an isolated colony, a young colony vs. an older colony? *Anolis sagrei* ranges farther north in the United States than any other of the exotic anoles. Aspects of its ecology have been explored in southern Florida, where it is an old resident, but no comprehensive life history study exists for this species anywhere in Florida. Evolutionary processes are rapid in this group and faster yet to a population subjected to human-mediated dispersal so often initiated as unpredictably as the roll of several dice all at once. But then what happens?

So, I wonder: What is gained and what is curtailed or lost in the ecology of a successful colonizing species as it expands northward? It is doubtlessly successful in parts of northern Florida but why? I suggest a profitable study of measuring the ecological responses to geographic expansion of *A. sagrei* from the wet/dry cycle of southern Florida to the hot summer/cold winter pattern of northern central Florida. Thinking out loud here, I would count and collect individuals from a city/urban heat island and from a natural or relatively natural site lacking in human development each in extreme southern Florida and in northern Florida. Testable predictions exist with respect to diet, age and body size at sexual maturity, survivorship, and fecundity. Such a dataset would provide the information necessary to, within the umbrella of understanding why it is successful, identify and measure the benefits and the costs that shift in association with northern expansion. An idea to accept, reject, modify.

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A call for more long-term studies of plasticity in anoles

Reptiles are important models for studying phenotypic plasticity because they are quite sensitive to environmental conditions experienced during development, and naturally experience a broad range of environmental conditions during this time. There are a number of interesting biological traits of reptiles that make them great models for research on phenotypic plasticity. For example, temperature-dependent sex determination, where incubation temperature irreversibly determines sex during development, is a fascinating polyphenism that is widespread among reptiles (Warner 2011). Additionally, the sensitivity of developing embryos to environmental factors (like temperature or hydric conditions) has been implicated as a primary force behind the evolution of various maternal reproductive strategies including viviparity or nest-site choice (Shine and Thompson 2006; Mitchell et al. 2013). Accordingly, there exists a rich literature documenting the effects of embryonic environments on the phenotypes and survival of reptiles during early life.

Figure 1. *Anolis sagrei* hatchling for use in a plasticity experiment. Because of their abundance, ease of husbandry, short lifespan, and ease of recapture in the field, anoles are well-suited for long-term studies of plasticity.

A major shortcoming of this literature is that the vast majority of studies terminate shortly after hatching. That is to say, our understanding of phenotypic plasticity in reptiles is biased towards phenotypes apparent in early life. Yet we rarely know if these phenotypes are persistent or transient, or if conditions experienced during development have delayed effects, or effects on reproductive traits. Furthermore, terminating plasticity studies during early-life stages can sometimes even mislead. This is the case with Warner and Shine's work on temperature-dependent sex determination in Jacky Dragons. Reasonable interpretations of preliminary results suggested one thing (Warner and Shine 2005), where the long-term version of the same experiment suggested something fundamentally different (Warner and Shine 2008).

Together with coauthors Fred Janzen and Dan Warner, I have recently published a review that discusses the shortcomings of terminating plasticity studies during early life, and highlights the important contributions that have come from the relatively few long-term studies in existence (Mitchell et al. 2018). We call for experiments that specifically look at the effects of embryonic environments on adult phenotypes, and offer a number of approaches to address this problem. I expect anoles will be an outstanding model for such experiments.

There are a number of anole species that are very tractable models for experiments addressing the influence of embryonic conditions on adult phenotypes, reproduction, and survival. *Anolis sagrei*, for example, readily breeds in captivity, is highly fecund, and reaches reproductive maturity in a matter of months. Anoles are tractable for detailed assays on reproduction in the laboratory, and raising anoles from egg to adult in the lab is entirely feasible under reasonable timelines. Though it is no small task, it is very possible to incubate hundreds of anole eggs under different conditions, mark the hatchlings, and then release them into the field in a place where migration is not possible (like a small island). Periodically resampling that island can give insights into the effects of incubation conditions on adult phenotypes and survival under natural conditions (e.g. Pearson & Warner, 2018). I encourage anyone interested in plasticity to consider using anoles as models, and anyone interested in anoles to consider using them for long-term studies of plasticity.

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Predicting the invasion dynamics of anoles (and other lizards) using ecological niche modeling

Abstract

Ecological niche models are commonly used to predict areas of environmental suitability for non-native species. Depending on these models to enact appropriate management plans assumes that they are accurate, however most niche model studies do not provide appropriate validation. South Florida hosts the world's largest and most globally diverse non-native lizard community, providing a unique opportunity to evaluate the predictive ability of niche models by comparing model predictions to observed patterns of dispersal, abundance, and physiology in established non-native populations. Using Maxent, we developed niche models for 30 non-native lizard species established within Miami-Dade County, FL, including all 8 established non-native *Anolis* species, using native range data to project suitability in the invaded range. We then compared projections to data available on distribution, as well as empirically collected data on abundance and physiology (upper and lower thermal tolerances). Maxent performed well in predicting general invasion patterns of non-native species across geographic space, however performed poorly in predicting the relative invasion success of each species. Additionally, comparisons between predicted and observed thermal tolerances showed that most of the models overpredicted the range of suitable thermal habitat for each species. Overall, the niche models accurately predicted geographic hotspots for these species to occur but could not predict relative invasion success of each species individually. These results suggest that other factors, such as time since introduction, dispersal ability, biotic interactions, adaptation, and source populations may also influence the relative success of non-native species after they become established.

Introduction

Given widespread human-induced global change, one pertinent result is a significant increase in the dispersal and establishment of non-native species (Hoffman et al., 2010, Hobbs et al. 2013, Helmus et al. 2014). Non-native species can often impact native species negatively, meaning they may pose an important conservation risk. It is important for conservation

practitioners to know where non-native species will spread to once they have established to effectively mitigate potential, or observed, negative impacts on native species. Ecological niche models (ENMs) are important tools in predicting the range dynamics and dispersal patterns of invasive species (Ficetola et al. 2007; Jeschke and Strayer 2008; Rödder et al. 2008). However, the accuracy of these predictive models is rarely tested, which has profound effects on how well the models are to be trusted when making conservation and management decisions.

Climate matching between native and invasive ranges has been observed to have a strong influence on establishment success of non-native species (Bomford et al. 2009; van Wilgen et al. 2009) and is shown to be one of the most important predictors of species distributions (Thullier et al. 2004; Algar et al. 2013). However, there are many other factors that can also influence invasion dynamics besides the climatic niche. For example, biotic interactions (Araújo and Luoto 2007), dispersal limitation (Algar et al. 2013), life history traits (Allen et al. 2017), topographic heterogeneity (Liu et al., 2014), and propagule pressure (van Wilgen and Richardson, 2012; Strubbe et al., 2015) may all be substantially influential. There is thus an insistent need for studies that validate niche model predictions, which can most thoroughly be achieved by contrasting model predictions with independently collected field data from the same geographic areas being projected to. However, due to logistic constraints, few studies have carried out this approach (Costa et al. 2010; Searcy and Shaffer, 2014; West et al. 2016), and even fewer on systems with multiple non-native species. Here we present one of the most extensive field validations of the ability of ENMs to predict the dispersal and range dynamics of invasive species by utilizing the world's largest community of non-native lizards, which is found in Miami, Florida. Our objective was to test ENM accuracy in predicting both where these non-native lizards are most likely to occur across geographic space and their relative success within a given geographic area. This was accomplished by comparing mean habitat suitability predicted by the ENMs built for the 30 non-native lizard species established in Miami-Dade County to field data determining observed geographic spread and patterns of relative abundance. We also examined the validity of the niche model predictions by comparing the predicted thermal limits to observed physiological thermal limits measured for non-native lizards residing in South Florida.

Methods
All niche modeling was performed using Maxent (Phillips et al. 2006), one of the most popular ENM algorithms due to its strong predictive abilities compared to other ENMs (Elith et al. 2006), especially in cases with low sample size (Pearson et al. 2007; Wisz et al. 2008), and which has been documented as a useful tool when predicting into novel climatic conditions such as those in non-native ranges (Elith et al. 2010, Strubbe et al. 2015). Maxent is a presence-only method, which uses species' occurrence data and environmental variables at those occurrences to predict the species distribution across environmental and geographic space. We built niche

models for each of the 30 non-native lizard species established in Miami-Dade County, and for one native species, *Anolis carolinensis*, building the models with native range data and projecting them into Florida. All models were implemented using the 'DISMO' package (Hijmans et al. 2017) in R version 3.3.2 (R Core Team 2017). We obtained native range localities for each species from the Global Biodiversity Information Facility (https://www.gbif.org) and VertNet databases (https://www.vertnet.org) and removed outliers (geo-referencing errors or invasive range localities) by making comparisons to native range maps. The climate variables used were the 19 Bioclim variables at ~1-km^2 resolution downloaded from the WorldClim database (Hijmans et al. 2005), which represent different combinations of temperature and precipitation that are biologically important and most often used in Maxent modeling (Booth et al. 2014). For inquiries on detailed modeling methods contact the corresponding author (Caitlin C. Mothes).

We used a wide array of datasets to evaluate the observed relative abundance and geographic spread of Miami-Dade County's 30 established exotic lizard species. One dataset consisted of herpetofaunal field surveys conducted by members of the Searcy Lab in 30 parks spread throughout Miami-Dade County (S. L. Clements, unpublished data). A second dataset we used was the number of Florida counties each species has been recorded in (Krysko, Enge, et al., 2011). Third, we used the GBIF database to calculate the number of known localities in both Florida and Miami-Dade County for each species. Finally, we used the Krysko, Burgess, et al. (2011) dataset, which assigns each species a ranking from 1-5 based on how abundant and widespread its established populations are in Florida. We then used multiple linear regression to analyze how well Maxent models predicted each of these observed measures of relative invasion spread and abundance. For the surveys conducted by the Searcy lab and the number of GBIF localities in Miami-Dade County, the predictor variable we considered was mean habitat suitability predicted by Maxent across Miami-Dade County. For the other success metrics, we considered mean habitat suitability across all of Florida as the predictor. For all analyses, the year in which each species was first introduced to Florida was used as a covariate, since current abundance/incidence of each species will be a combination of its ability to invade and the amount of time it has had to do so. All these analyses assess Maxent's ability to predict relative invasion success within a given geographic extent (either Miami-Dade County or all of Florida). To assess Maxent's ability to predict hotspots for non-native lizard invasion across the state, we averaged the predicted habitat suitability across Florida for all 30 non-native lizard species, and then calculated the mean predicted suitability for each of Florida's 67 counties. We then calculated the total number of records for these 30 species in each county (using the GBIF data) and created a linear model relating the number of records to the mean predicted suitability, using county area as a covariate.

As another method of testing the accuracy of the niche models, we measured the thermal limits of individuals caught in the Miami area to compare with the model's response curves,

which plot predicted suitability against each individual environmental variable. We used response curves for Bioclim variable Bio5 (maximum temperature of the warmest month) to determine the predicted maximum thermal limit and Bio6 (minimum temperature of the coldest month) to determine the predicted minimum thermal limit. We considered the predicted thermal limit as the temperature at which the response curve reached its minimum suitability value, and then compared this temperature to the 95% confidence interval of the observed thermal limit and recorded whether the predicted limit fell above, below, or within the 95% CI of the observed limit.

Individuals were collected from ten species, with an average sample size of eight individuals per species. The majority of the lizards were captured at Fairchild Tropical Botanic Gardens in Miami FL, while *Ameiva ameiva* were captured at Evelyn Greer Park (Pinecrest FL) and *Anolis chlorocyanus* and *A. cybotes* were collected in Parkland FL. The physiological traits measured were critical thermal maximum (CT_{max}) and critical thermal minimum (CT_{min}). These thermal limits were measured as the temperature at which an individual lost the ability to right itself, signifying ecological death as such an impairment would be lethal if sustained in the wild (Huey and Stevenson 1979). Thermal tolerance data was collected between Fall 2016 and Spring 2018, utilizing non-lethal methods (as in Gunderson and Leal 2012). Individuals were first acclimated to room temperature, with starting body temperature averaging 25.6° C for both tests. To calculate CT_{max}, individuals were placed in a large cardboard box with a 150 W incandescent lightbulb suspended 1 m above the lizard. To prevent individuals from taking shelter from the heat lamp, a noose was tied around the waist and staked to the bottom of the box. The noose was made long enough to allow individuals some movement to lower stress levels. A thermocouple thermometer was placed in the cloaca and secured with a small piece of surgical tape to monitor the rise in body temperature. Once the body temperature reached 36°C, we flipped the individual on its back at 1°C increments, pinching the thigh of the lizard to induce a righting response. When the individual was no longer able to right itself, the body temperature was recorded as that individual's CT_{max}. Similar methods were used to calculate CT_{min} by placing individuals in a Tupperware within a larger cooler of ice to gradually decrease body temperature, and flipping them on their backs starting at 14° C.

Results

Averaging the predicted habitat suitability across all 30 models projected onto Florida, we see a strong correlation between the predicted distribution of these non-native lizards and their observed abundance (Habitat suitability: $P < 0.001$; County area: $P < 0.001$; $R^2 = 0.60$; Figure 1). However, when looking at relative invasion success within a given geographic extent, Maxent does a poor job predicting which non-native species are most abundant or widespread. We used mean predicted habitat suitability for each species to rank the predicted invasion success in both Florida and Miami-Dade County. We compared these predicted values to actual invasion success based on four different datasets. For the Miami-Dade park survey data, we did

not find any relationship between mean predicted suitability in Miami-Dade County and either total abundances (Habitat suitability: $P = 0.76$; Year of introduction: $P = 0.04$) or number of parks in which a species occurred (Habitat suitability: $P = 0.77$; Year of introduction: $P = 0.06$).

At the statewide scale, the number of counties each species has been recorded in was not related to the ranking of mean predicted suitability in Florida (Habitat suitability: $P = 0.62$; Year of introduction: $P = 0.001$). Using the museum records from GBIF, we did not find any relationship between mean predicted habitat suitability and number of recorded localities in either Florida (Habitat suitability: $P = 0.7$, Year of introduction: $P = 0.001$) or Miami-Dade County (Habitat suitability: $P = 0.97$, Year of introduction: $P = 0.005$). Using the establishment rankings from Krysko et al. (2011) we also did not find any correlation with mean predicted habitat suitability across Florida (Habitat suitability: $P = 0.71$; Year of introduction: $P = 0.03$).
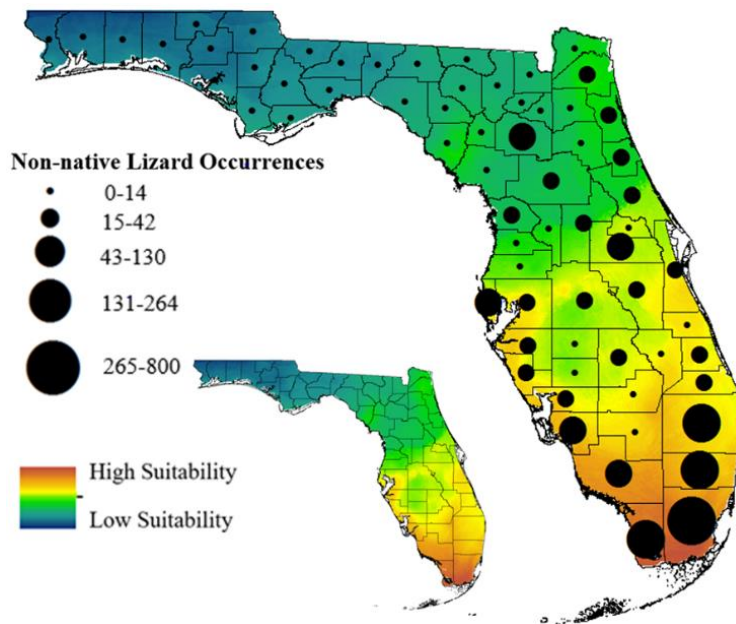

Figure 1. Predicted habitat suitability averaged across all 30 non-native lizard species established in Miami-Dade County. Black circles represent the number of recorded non-native lizard localities within each Florida county.

We summarized the relationship between the predicted and observed thermal limits into four categories (Table 1). Namely, predicted thermal limits either fell below, within, or above the 95% CI of the observed thermal limit based on the physiological data, or were classified as 'NA' if the variable did not contribute to the niche model of the species in question (i.e., the response curve was flat). Looking at the relationship between observed and predicted CT_{max} based on the response curves for Bio5, we see that for the majority (7 out of 10 species), the relationship could not be determined because Bio5 did not play a role in generating the niche model for that species. This suggests that few of the lizard species we modeled are up against their maximum thermal limit. For the comparison between observed and predicted CT_{min}, the majority (6 out of 10 species) showed the predicted thermal limit below the observed thermal limit. This means that Maxent is predicting suitable regions with temperatures colder than these non-native lizards can persist in based on their physiology, unless they find some other means of dealing with these colder climates (see Discussion).

Table 1. Summary of the relationship between Maxent's predicted thermal limits and the observed thermal limits based on the measured physiological data.

Relationship of Predicted to Observed Thermal Limits	CT_{min}	CT_{max}
Below	6	2
Match	0	1
Above	1	0
N/A*	3	7
Total Species	10	10

*No constraints based on this variable are included in the species' niche model, and thus the response curve is flat (i.e., there is no indication of the species being up against this thermal limit).

What about the anoles?

Figure 2 shows the predicted habitat suitability maps for eight *Anolis* species established within south Florida. The *Anolis* species with the highest predicted suitability across Florida was the Hispaniolan big-headed anole (*Anolis cybotes*), followed closely by the Cuban brown anole (*A. sagrei*), and the lowest was the Hispaniolan green anole (*A. chlorocyanus*). When predicting within only Miami-Dade County, the Hispaniolan bark anole (*A. distichus)* had the highest predicted environmental suitability, followed closely by *A. sagrei*, with *A. chlorocyanus* again showing the lowest predicted suitability. As with the entire non-native lizard community, we did not find any relationships between the observed and predicted invasion success when looking at just the *Anolis* group. When conducting analyses, we removed the Cuban green anole (*A. porcatus*) due to the difficulty in differentiating the species correctly from the American green anole (*A. carolinensis*; Camposano 2011). For the thermal tolerances, we collected data on five out of the eight non-native anoles, and the native *A. carolinensis* (along with four other non-native species; Table 2). When we compared our observed critical thermal minimum to the predicted minimum temperatures, models for *A. cybotes* and *A. sagrei* did not detect minimum temperature as an important variable in determining their distributions. However, *A. cristatellus* had an observed thermal minimum that was below the predicted limit, and *A. carolinensis*, *A. chlorocyanus*, and *A. distichus* all had observed thermal minimums that were above the predicted limit. Maximum temperature was not a significant contributor in predicting the species distribution for any species, with the exception of *A. carolinensis* which had an observed CT_{max} that was warmer than the predicted maximum temperature.
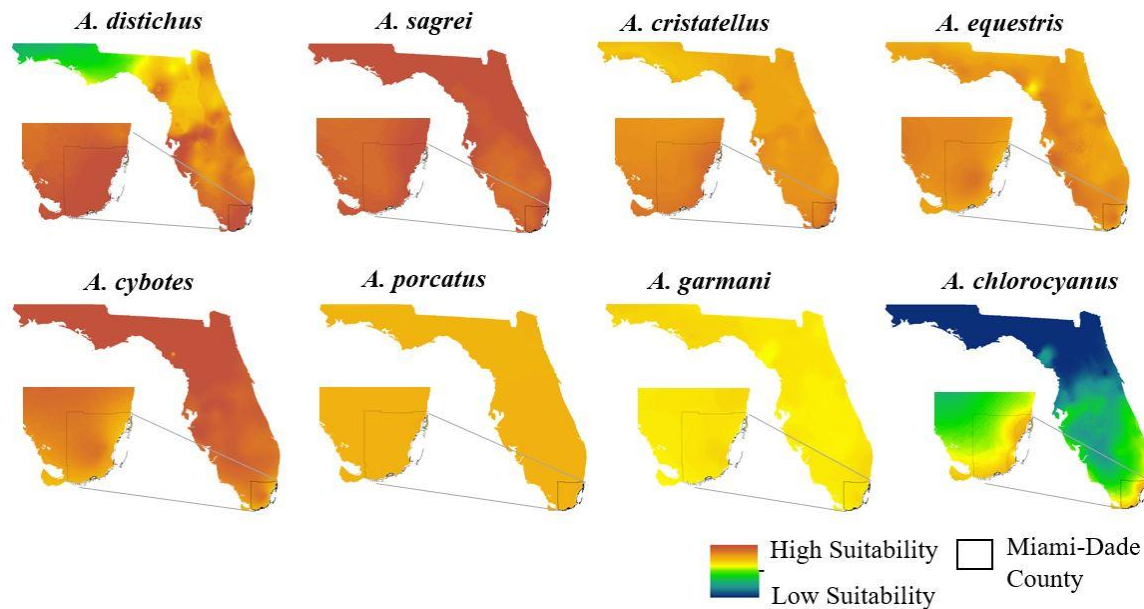
Figure 2. Maxent's predicted habitat suitability for all established non-native *Anolis* species in Florida.

Our results show that the niche models performed quite well at predicting the hotspots across Florida where non-native lizards are most likely to occur (Figure 1). However, within this non-native lizard community the models were not able to predict relative invasion success of individual species in terms of their total abundance or geographic spread. Overall, Maxent accurately predicted regions of suitable climate supporting establishment of these lizards, but other factors not included in niche model calculations may be impacting a species' ability to multiply and spread after colonization. Previous studies have also found that ecological niche models are accurate in predicting establishment success (Bomford et al., 2009; van Wilgen et al., 2009), but not subsequent spread (Gallardo et al. 2013; Liu et al., 2014).

While none of our metrics of invasion success across the entire group of non-native lizards exhibited a correlation with predicted habitat suitability, almost all of them showed a strong relationship with year of introduction. This indicates that a species' observed invasion success is largely determined by the amount of time it has had to establish, reproduce, and disperse, such that species introduced longer ago will generally be both more abundant and more widespread. This agrees with other studies that have identified time since introduction as a main driver of invasion success among both coastal marine invertebrates (Byers et al. 2015) and woody trees (Pyšek et al. 2009). This may provide evidence that priority effects are particularly important in the establishment of non-native species, and the subsequent development of community structure and organization. Further research on the general importance of priority effects in the assembly patterns of *Anolis* communities would be valuable (see Stroud 2018).

Table 2. Sample size, mean, and 95% confidence intervals for each thermal limit measured from individuals collected in South Florida.

Species	CT$_{max}$ (°C)			CT$_{min}$ (°C)		
	N	Mean	[95% CI]	N	Mean	[95% CI]
Agama agama	6	45.10	[44.29, 45.91]	6	9.77	[9.01, 10.52]
Ameiva ameiva	6	44.67	[43.64, 45.69]	5	12.24	[11.34, 13.16]
Anolis carolinensis	11	42.96	[42.38, 43.54]	12	9.75	[8.90, 10.60]
Anolis chlorcyanus	6	39.12	[38.45, 39.79]	6	9.18	[8.75, 9.62]
Anolis cristatellus	10	39.10	[38.53, 39.67]	10	8.04	[7.45, 8.62]
Anolis cybotes	8	38.76	[37.69, 39.84]	8	9.54	[8.50, 10.57]
Anolis distichus	10	39.76	[39.15, 40.38]	11	9.60	[8.60, 10.6]
Anolis sagrei	10	42.13	[41.37, 42.89]	11	9.05	[8.46, 9.64]
Basiliscus vittatus	11	41.43	[40.37, 42.49]	10	11.29	[10.66, 11.92]
Hemidactylus mabouia	6	40.38	[38.79, 41.97]	6	8.57	[7.57, 9.56]

What determines invasion success? And other insights from* Anolis *lizards

When we investigate patterns among only non-native *Anolis* in Florida, we can gain important insights into additional factors that may be impacting the invasion success of these lizards post-establishment. One factor may be biotic interactions. Many studies have shown how interspecific interactions between native/non-native and non-native/non-native *Anolis* species has impacted the community structure of these lizards in the non-native range (Losos et al. 1993; Losos 2009; Stuart et al. 2014; Kolbe et al. 2016). Therefore, these negative relationships at the micro-scale may reflect patterns observed at the macro-scale.

The capability of these species to disperse through Miami's urban landscape may also impact how abundant and widespread they are in this non-native range. The native habitat in the Miami area is highly fragmented (reduced to <2% of its original extent; Bradley and Martin 2012) and dominated by an urban matrix, providing novel challenges that many of these species may not have dealt with in their native ranges. For example, *A. cristatellus* is largely constrained to forest

habitat and appears incapable of unaided dispersal across open habitats and impervious urban surfaces, causing it to have a low dispersal rate compared to other non-native *Anolis* species (Kolbe et al., 2016).

Adaption to the non-native range is another aspect of invasion success that is not accounted for in Maxent models. One might expect that rapid adaptation may be unlikely in non-native populations due to bottleneck effects and subsequent low genetic diversity, but a study of the eight non-native *Anolis* species in Miami showed that the majority of them come from multiple source populations, suggesting this is a common trend for non-native lizards (Kolbe et al., 2007). Subsequent admixture between these source populations increases genetic diversity and the possibility for rapid phenotypic shifts, such as the rapid shift in thermal tolerance observed in *A. cristatellus* (Leal and Gunderson 2012; Kolbe et al. 2012). There is also evidence of adaptation to the non-native range in *A. sagrei*, which shows significant physiological variation along the latitudinal gradient of Florida, with the northernmost populations experiencing and subsequentially tolerating colder temperatures (i.e. exhibiting a lower critical thermal minima; Kolbe et al. 2014).

Another factor that may affect comparisons between the empirical data and the niche models is the source populations that these non-native lizards originated from. We generated our Maxent models based on the entire native range, but the source populations may constitute only a small subset of that range. If there is local adaption to climate, then these source populations will not encompass the total climatic tolerance found in the native range and will determine how individuals respond to the habitat of the non-native region. For example, *A. cristatellus* has two populations in Miami-Dade County that originated from climatically different areas of Puerto Rico, and therefore have shown differential responses to Florida climate (Kolbe et al. 2012). Many of these species' native range populations are distributed across a variety of altitudes and temperatures, but the source populations may be primarily coastal, low altitude populations, and therefore may not be representative of the entire range of populations used to train the model. This may explain why the majority of our response curve comparisons showed the niche models predicting that species could persist at colder temperatures than indicated by the observed physiological traits of the non-native populations.

Conclusions
The niche models performed well at their originally intended function: predicting the distribution of species across geographic space. The predictive ability of ENMs has been repeatedly supported across native ranges (Elith et al., 2006; Costa et al., 2010; Searcy & Shaffer, 2014) and for individual non-native species (Ficetola et al., 2007), but this was the first time it had been documented across such a broad suite of non-native taxa (30 non-native lizard species, including 8 introduced *Anolis*). Where Maxent failed was its ability to predict relative

invasion success within the pool of established species, which complicates its use in prioritizing management actions within this non-native community. Reasons for the discrepancies we see are likely due the confounding influences of length of time since introduction, interspecific variation in ecology and dispersal capability, interspecific interactions, and founder effects of non-native populations. Future studies will need to investigate which of these factors best determine relative success within this diverse assemblage of non-native species, as such novel ecosystems are expected to increase in frequency around the world (Hobbs et al., 2013).

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Do male-male interactions drive changes in dewlap size?

Male dewlap size varies dramatically among and within anole species (Nicholson et al. 2007; Vanhooydonck et al. 2009). Within species, population-level differences in dewlap size have been documented and are thought to be driven by habitat differences (Ng et al. 2013a), predator absence/presence and variation in sexual size dimorphism (Vanhooydonck et al. 2009). However, dewlap size also varies substantially at the individual level, and is considered to be an honest signal of bite force (e.g. Vanhooydonck et al. 2005; Lailvaux and Irschick 2007; Henningsen and Irschick 2011) and fighting ability (Lailvaux and Irschick 2007). Furthermore, dewlap size can also vary within the lifespan of an individual, particularly with the change of seasons (Irschick et al. 2006; Lailvaux et al. 2015), which may be associated with changes in testosterone levels (Cox et al. 2009) and the frequency at which dewlaps are displayed (Lailvaux et al. 2015) during the breeding versus non-breeding seasons. Such seeming plasticity suggests that dewlap size is not a heritable trait and may be influenced by social interactions. For example, if increased or decreased dewlap displays are driven by differing testosterone levels (Tokarz 1987; Winkler and Wade 1998; Tokarz et al. 2002), and this drives changes in dewlap size (Lailvaux et al. 2015), males that more frequently undertake dominant or aggressive behaviors towards other males may have larger dewlaps than subordinate males. Here, we conduct laboratory experiments with *Anolis distichus*, a species that has been observed to exhibit a large range of intraspecific male dewlap sizes (Fig. 1), to test (i) whether dewlap size has a heritable component, and (ii) whether social interactions with other males influence dewlap size changes.
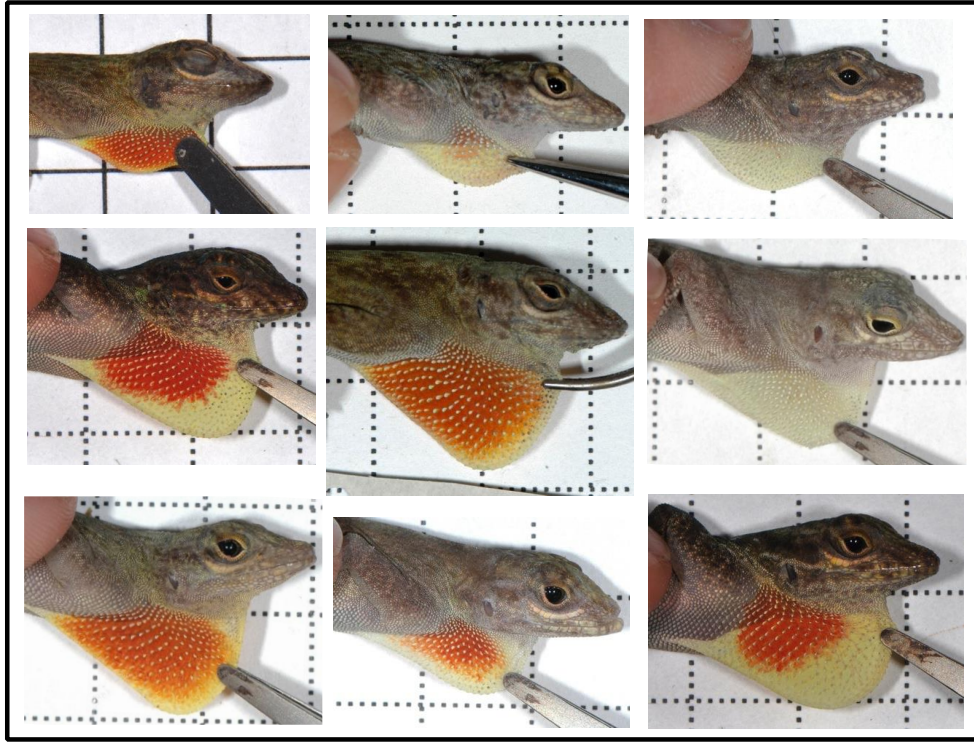
Figure 1. Dewlap size variation in *Anolis distichus* from the Dominican Republic.

Does Dewlap Size Have a Heritable Component?

Methods

We assessed the extent to which dewlap size is heritable by breeding wild-caught *Anolis distichus* from the Dominican Republic in a laboratory environment and comparing the dewlap sizes of 12-month-old laboratory-raised male offspring to their father. Breeding, husbandry and paternity analysis followed Ng et al. (2013b). We measured the dewlap size of each male by taking a high-resolution digital photograph with a Nikon D90 of the lizards positioned on their left side and their dewlaps fully extended with forceps. A ruler was included in each photograph. We quantified dewlap size using ImageJ v1.45 (Abramoff et al. 2004), with each dewlap measured twice, averaged, then ln-transformed. We controlled for body size by measuring the snout-vent length (SVL) of each individual twice using calipers, ln-transforming the average measurement, and then regressing ln(dewlap size) with ln(SVL). The resulting residuals for all offspring from the same father were averaged prior to analyses.

Results

With a total of 13 fathers and 28 offspring, we did not find a strong association between the dewlap size of fathers and their offspring ($r^2 = 0.01$, p-value = 0.309) (Fig. 2). These results

suggest that dewlap size has low heritability and that dewlap size differences are likely due to environmental effects.
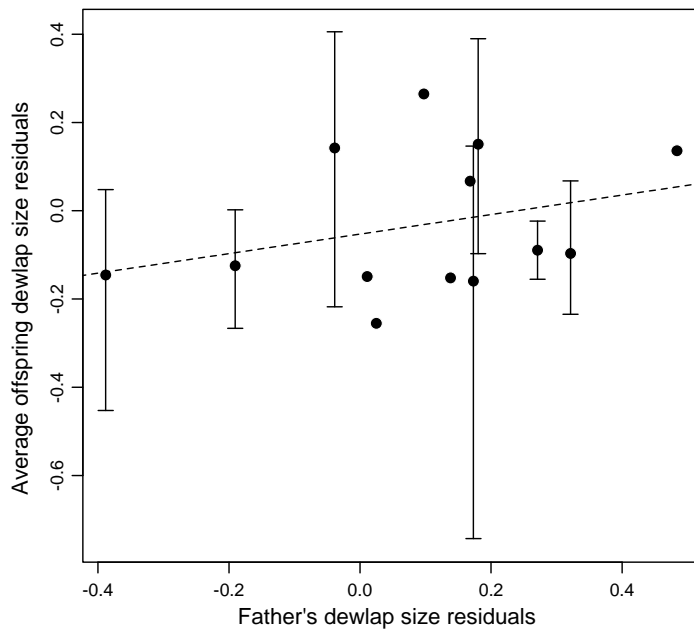

Figure 2. The relationship between the dewlap size of fathers and their offspring is not significant ($r^2 = 0.01$, p-value = 0.309), suggesting that dewlap size has low heritability. The bars represent the range of offspring dewlap sizes for each father.

Is Dewlap Size Influenced By Social Interactions?

Methods

Given the low heritability of dewlap size, we examined whether social interactions with other males influenced dewlap size changes. In October 2012, we filled 20 custom-built plexiglass cages (29.6cm L, 10.3cm W, 35.7cm H) with three laboratory-raised *A. distichus* adult males (>8 month old) of similar dewlap size (average difference between largest and smallest: $5.28mm^2$ [range: 0.21–23.64 mm^2]) and similar SVL (average difference between largest and smallest: 3.50 mm [range: 1.01–5.67 mm]) (herein referred to as 'experimental cages'). To reduce non-independence due to shared inheritance or effects from previous social interactions, we ensured that none of the experimental cages contained siblings or individuals that had previously shared a cage. We also housed 8 additional laboratory-raised *A. distichus* adult males alone to serve as controls (dewlap size: 31.40–119.93 mm^2). Other adult males or females were not visible from any of the cages during the length of the experiment. Each cage contained the same materials: a thin layer of organic potting mix, two sterilized wooden dowel rods arranged as an angled "X", and an artificial sprig of four ivy leaves. Animal husbandry followed Ng et al. (2013b). We then re-measured dewlap size and SVL of each male after one and two months. All measurements of dewlap size were conducted using methods described in the previous section, while SVL was quantified by placing each lizard beside a ruler on transparent plexiglass, photographing the ventral surface of the lizard from below, and quantifying measurements using ImageJ.

Results

After one month, we found that dewlap size increased for at least one male in all but one experimental cage. Of the males that exhibited the largest increase in dewlap size within each cage, dewlap size increased an average of 41.81% (range: 2.88–122.59%). In 15 of the 20 experimental cages, dewlap size decreased for at least one male. Of the males that exhibited the largest reduction in dewlap size within each cage, dewlap size decreased an average of 20.67% (range: 4.66–38.82%). Dewlap size also changed for the control males, whereby half of the males exhibited an increase in dewlap size while the other half exhibited a decrease in size (range: -10.87–43.05%). After categorizing each of the three males within the same experimental cage into having the largest, smallest or medium-sized dewlap, we found that these changes in dewlap size among these groups were significantly different (ANOVA: $p \ll 0.01$) (Fig. 3). The change in dewlap size exhibited by the largest-dewlapped males was significantly different from the control males (Tukey's posthoc test: $p < 0.05$), but the changes in medium-sized and smallest-dewlapped males were not (Tukey's posthoc test: $p > 0.05$). These same qualitative results were found when controlling for changes in SVL. However, when we conducted 1000 random assignments of individuals to cages to compare our results to a null distribution, we also found a significant dewlap size difference between the largest-dewlapped males and the control males 48.5% of the time.

After two months, dewlap size further increased for 26 of the 60 males in the experimental cages (average additional increase: 18.69%, range: 0.63–55.45%), while dewlap size further decreased for 11 males (average additional decrease: 12.58%, range: 0.81-30.69%). We, however, did not observe as large a range of dewlap size changes between the first and second month (range= -40.02–70.81%), as during the first month (range: -38.82–122.59%). For the control males, dewlap size further increased for half of the males (range: 14.24-35.14%) and further decreased for one male (8.73%). When reassigning relative dewlap size categories, we found that, like after the first month, the change in dewlap sizes among the three categories after two months were significantly different from each other (ANOVA: $p \ll 0.01$) (Fig. 3). In contrast to the first month, the change in the smallest dewlapped-males was significantly different from the control males (Tukey's posthoc test: $p < 0.01$), rather than the largest dewlapped-males. However, this dewlap size difference between the smallest-dewlapped males and the control males was also significant for 37.3% of 1000 randomized cage assignments.
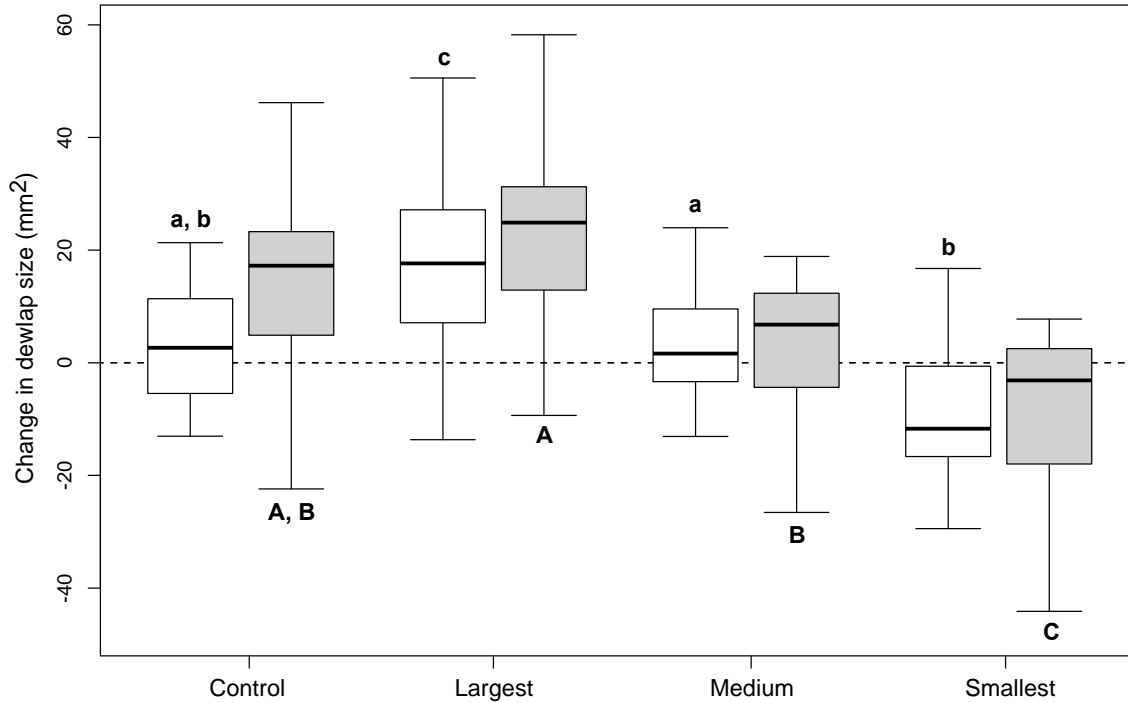
Figure 3. Boxplot showing the change in dewlap size of males after one month (white boxplots) and after two months (grey boxplots). Males within the same cage were categorized as having the "largest", "smallest" or "medium"-sized dewlap each month. The different letters above and below the boxplots indicate significantly different groups as assessed using Tukey's posthoc test.

Comparing the first month with the second month, we found that assignments of individuals to each dewlap size category ('largest', 'medium', 'smallest') did not change for seven of the experimental cages (Fig. 4a), but did for 13. Within five of the 13 cages, the smallest and medium-sized dewlapped individuals swapped categories during the second month while the male assigned as having the largest dewlap remained the same (Fig. 4b). In three cages, the largest and medium-sized dewlapped males swapped categories during the second month (Fig. 4c), and in three other cages, the individual with the smallest dewlap in the first month became the largest-dewlapped individual in the second month, while the individual assigned as the largest in the first month remained relatively larger than the individual assigned as middle-sized (Fig. 4d). In the last two cages, the largest-dewlapped male at one month became the smallest-dewlapped male during the second month while the medium-sized became the largest (Fig. 4e).
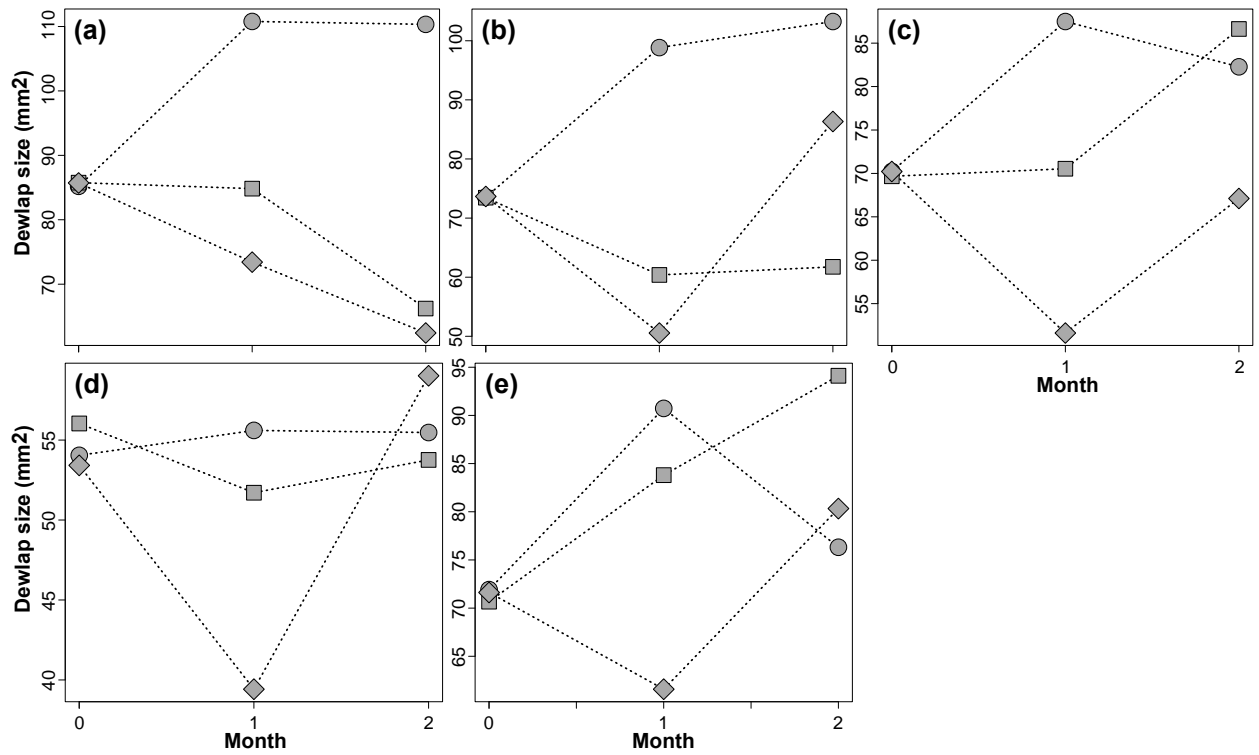
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Figure 4. Dewlap size change through time for three males (different shapes) housed within the same cage. These five cages represent examples of the patterns observed of relative dewlap size change among the three males.

Conclusions

Our study showed that the size of male dewlaps not only has low heritability but can dramatically change over the course of just one month in both males with opportunities for male-male interactions, as well as those lacking opportunities for any social interactions. Given that males display their dewlap not only as part of stereotyped species-specific display repertoires, but also during other contexts (Jenssen 1977), this may explain the dewlap size changes observed in males housed alone. Despite solo males also exhibiting changes in dewlap size, we found that dewlap size changed even more (increased or decreased) when males were able to interact with other (Fig. 3), although this pattern was no different from randomized individuals. These results further support previous suggestions that dewlap size is plastic (Irschick et al. 2006; Lailvaux et al. 2015), and suggest that male-male interactions may not be the sole driver of dewlap size change. Future studies quantifying both dewlap size changes as well as the behaviors of males interacting with both males and females are clearly needed to further investigate whether social interactions help explain the dramatic diversity of dewlap sizes observed in naturally-occurring populations.

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Natural History Note: *Anolis sagrei* foraging on a patch of obvious prey

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The uneven spatial distribution of food resources has led to a variety of strategies to optimize foraging behavior (Stephens and Krebs 1986). Theory predicts that an individual should continue to forage at a locality with abundant prey as long as there is a net energetic gain (MacArthur and Pianka 1966). Numerous factors determine prey abundance, but certain patches may attract and concentrate prey thus attracting foraging predators. Examples include lions hunting herbivorous mammals that congregate at water holes (Davidson et al. 2012), lizards feeding on insects attracted to vegetation (Durtsche 1995), and a variety of species that feed on the explosive emergence of winged termites (Dial and Vaughan 1987; Bauer et al. 1989). Here we report an *Anolis sagrei* foraging on flies attracted to canine feces.

We visited Deering Estate, in Palmetto Bay, Miami-Dade County, Florida on 19 March, 2018 around 2:00 PM where we observed an adult female *A. sagrei* near a pile of feces. The feces were in an open area, covered in leaf litter, and approximately 3 meters from the nearest tree trunk. Deposition likely occurred less than 12 hours previously as the pile was still moist. The feces appeared to be from a domestic dog or coyote. The anole moved near and on the feces (Fig 1A) for the 20 minutes of observation. Numerous flies (Muscidae) and yellowjackets (Vespidae) were attracted to the feces (Fig1B) and the anole made several attempts to catch the flies, although none of the observed attempts were successful.

Amphibians and reptiles foraging on congregating insects is well documented, including numerous examples of lizards feeding on insects attracted to night lights (Perry and Fisher 2006) and citations therein. However, associations of amphibians and reptiles eating insects attracted to feces are far less common. We could find only four examples: skinks (*Emoia*) eating flies congregated by an abundance of cattle dung (McCoid et al. 1995); salamanders (*Ambystoma*) eating insects attracted to prairie dog pellets (Kolbe et al. 2002); various frogs and lizards eating insects attracted to the abundance of feces in tortoise (*Gopherus*) burrows (Lips 1991); and *Ameiva exsul* eating insect larva from dried dog feces (Perez-Rivera and Molina-Opio 2008). While examples in the literature are sparse, we suspect this behavior may be fairly widespread and we encourage herpetologists to be alert for it — and to watch where they step.

Figure 1. A. *Anolis sagrei* perched on canine feces in Miami-Dade County, Florida, USA. B. *Anolis sagrei* foraging near canine feces. Arrows indicate position of anole relative to a fly perched on the feces.

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Satellite cells demonstrate expanded musculoskeletal potential

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In response to predation, anole lizards will autotomize their tails and will regenerate new vasculature, cartilage, muscle with tendinous attachments, nerves, and skin. Data from us, and others, demonstrated that regeneration of tissues in tails post-autotomy is a stem cell mediated process. We had previously identified a cell type in lizard muscle that expressed Pax7 and would fuse into multinucleated myofibers in vitro, like mammalian satellite cells, the muscle stem cell population. In order to determine the identity of these cells, and understand how they compare to their mammalian counterparts, we carried out a transcriptomic analysis using XGSA (**C**ross species **G**ene **S**et **A**nalysis), a tool specifically designed to overcome the challenges of comparing gene expression between different species. The transcriptome of our anole muscle derived cells was compared to dozens of mouse and human transcriptome data sets from ENCODE. The analysis revealed that our cells are most similar to mouse and human satellite cells than any other cell or tissue type examined, indicating they are indeed anole satellite cells.

A closer look at individual genes revealed that both mouse and anole satellite cells upregulate muscle-specific genes necessary for development, maintenance, and repair of muscle. However, anole satellite cells also upregulated genes involved in chondrogenesis, indicating perhaps that they have expanded musculoskeletal plasticity, and given the right context, can become both muscle and cartilage. We next determined whether the satellite cells derived from anole lizards demonstrated increased musculoskeletal plasticity. To assess this, PAX7 positive satellite cells satellite cells were cultured in a 3D micromass format, which is known to favor chondrogenesis. Micromasses were incubated with either growth medium or chondrogenic differentiation medium, without exogenous morphogens. Anole satellite cells in micromass culture formed nodule-like structures consistent with chondrogenic differentiation, while the mouse satellite cell micromasses differentiated into myotubes, and did not form nodules. The chondrogenic nature of the nodules was confirmed via positive immunostaining for collagen 2a1, a cartilage specific collagen, and Alcian blue which stains the glycosaminoglycan rich matrix of cartilage blue. The mouse micromasses did not stain positive for collagen 2a1 or aggrecan, nor did they exhibit distinct Alcian blue staining. Subsequent analysis of the gene expression by RT-QPCR demonstrated that lizard satellite cells up-regulated genes involved in chondrogenesis, *bmp2* and *sox9*, and cartilage specific extracellular matrix genes, collagen2a1 (*col2a1*) and aggrecan (*acan*). Thus, the PAX7 positive satellite cells from lizard can become cartilage without the need for exogenous morphogen, indicating that changes in the regulation of genes for myogenesis and chondrogenesis likely contributes to the regenerative ability of these animals.
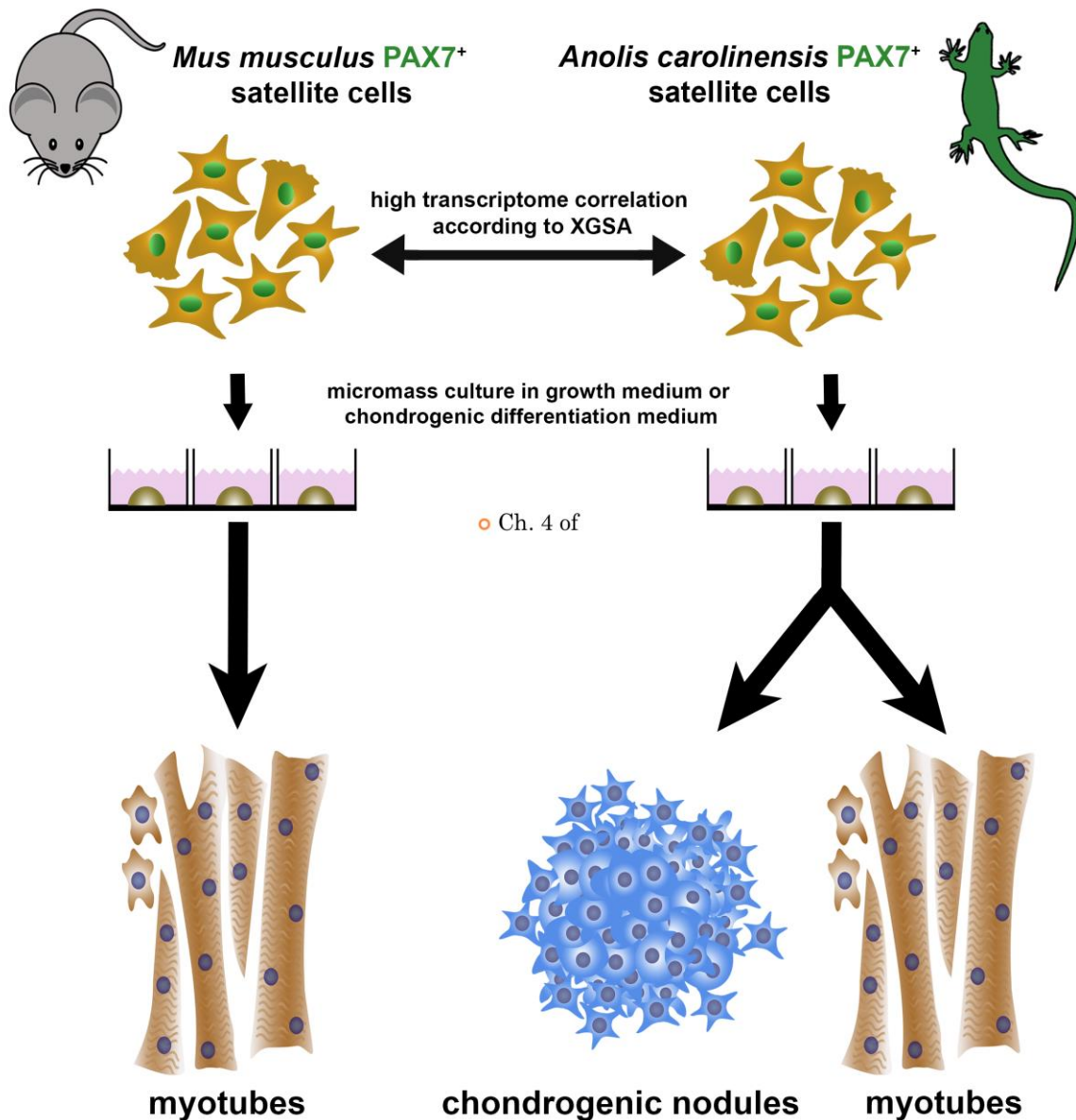
Illustration From: Palade et al., 2018 *Developmental Biology*

The greater degree of plasticity in lizard satellite cells provides a clue to understanding how de novo generation of muscles and cartilage occurs in tail regeneration. We plan to examine the expression of other musculoskeletal regulatory genes that are differentially expressed based on the XGSA analyses. We want to understand the process and the mechanisms by which the lizard cells are able to adopt one pathway over another. Research into the function and regulation of mammalian genes and pathways has thrived due to a rich toolbox of techniques and resources. Commercially available antibodies and arrays, as well as genomic manipulation of animal models have enabled ground-breaking discoveries. However, the same resources are lacking in

this animal model. We are currently trying to establish a robust anole satellite cell transfection system, which would allow us to silence, over-express, or otherwise alter genes of interest, in order to better understand the molecular pathways underpinning the enhanced plasticity of anole satellite cells. Further inquiry into the nature of the process and the mechanisms by which the lizard cells are able to adopt one pathway over another will not only shed light on the complex process of lizard tail regeneration, but also provide a means to understand how to reprogram mammalian satellite cells to produce new muscles with tendons and cartilage, which is important for the use of these cells in regeneration.

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Using mainland anole genomes to understand habitat shifts through time

Studies of *Anolis* lizards in the Caribbean have contributed tremendously to our understanding of the processes of adaptive radiation and convergent evolution (Losos, 2009). However, anoles have also become an important system for studies of historical biogeography in the mainland. Over the last six years, my collaborators and I have used genome-wide data from these lizards to learn about the history of South American habitats. By uncovering how environmental change affects habitat distribution through time, anoles have shed light on the origin of diverse tropical biotas and informed projections of species responses to anthropogenic global change (Prates et al. 2016a).

Figure 1. An undescribed montane species of Anolis from Brazil. Close evolutionary relationships among this cold-tolerant anole and species from distant mountains suggest that, around five millions of years ago, the intervening lowland environments were very different from today's (Prates et al., in review). Top: Female. Bottom: Male.

Nearly 50 years ago, anoles inspired one of the most influential ideas in biogeography: the theory of Pleistocene rainforest refugia. Based on patterns of morphological similarity among taxa in the *Anolis chrysolepis* species group, Vanzolini and Williams (1970) hypothesized that recurrent rainforest fragmentation led to population isolation and differentiation. The idea that glacial-interglacial climatic cycles promoted speciation in forest organisms was independently proposed by Haffer (1969) based on birds. In recent years, however, this theory has largely been rejected, and anoles have played a role in this as well. Based on rates of molecular evolution coupled to fossil calibrations, my collaborators and I found that Amazonian anole species diverged millions of years earlier than the temporal framework implied in the theory of Pleistocene refugia (Prates et al. 2015).

This is not to say that climate change through time was not important for the establishment of current biodiversity patterns in South America. In northeastern Brazil, xeric Caatinga shrublands now

separate Amazonia and the coastal Atlantic Forest. However, several species occur disjunctively in both regions, suggesting that the two forests were connected in the past. Among these species are *Anolis punctatus* and *Anolis ortonii* and the bush anole *Polychrus marmoratus*. To infer the timing and spatial location of past forest connections, my collaborators and I investigated the history of these three lizards based on a multi-locus DNA dataset. The results indicated that the three species synchronously colonized the northern Atlantic Forest from eastern Amazonia in the mid-Pleistocene, supporting that climate-driven faunal interchange was essential to the assembly of regional biotas (Prates et al., 2016b).

Anoles have also been used to test historical hypotheses proposed by Earth scientists. Chemical records from caves suggest that precipitation patterns have changed over the last thousands of years in South America, with some regions receiving more rain than others. To examine how this dynamic has affected rainforests, my collaborators and I inferred the demographic history of *A. ortonii*, *A. punctatus*, and *P. marmoratus* from thousands of DNA markers (Prates et al., 2016a). By comparing the genetic data with data simulated under alternative historical scenarios, we found support for population expansions or contractions within the time frame of proposed precipitation fluctuations. However, the three species showed discordant demographic trends across regions. It is possible that differences in phenotype and ecology, such as body size and tolerance to forest edges, have attenuated or exacerbated the impact of habitat shifts on each of these lizard species (Prates et al., 2016a).

Evolutionary studies in mainland anoles have also provided valuable insights into landscape evolution at deeper time scales. Building upon previous genetic studies (Ayala-Varela et al., 2014; Castañeda and de Queiroz, 2011; Poe et al., 2015), my collaborators and I have investigated phylogenetic relationships in the *Dactyloa* clade of *Anolis*. We found close relationships among narrowly distributed species associated with montane systems separated by thousands of kilometers, namely *Anolis nasofrontalis* and *Anolis pseudotigrinus* from southern Atlantic Forest, *Anolis dissimilis* from the Andean foothills and adjacent western Amazonia, *Anolis neblininus* from a Guiana Shield *tepui* mountain, and *Anolis calimae* from the Andes. This result suggests that the ancestors of these species occupied the intervening lowlands during colder times in the Miocene (Prates et al., 2017).

Mainland anoles have also helped us to understand how species colonize and adapt to novel habitats in South America. My collaborators and I found that wide-ranged species like *A. ortonii* and *A. punctatus* expanded from warm and wet settings in Amazonia into the colder and drier Atlantic Forest (Prates et al., 2016b). To test whether species occurrence in varied climates is linked to adaptation, we examined whether genome-wide allele frequencies are associated with geographic variation in temperature and precipitation (Prates et al., 2018). We found that genes involved in energy metabolism, immunity, and development are associated with climate gradients in *A. punctatus*, supporting a scenario of local adaptation. However, no candidate loci

were inferred in *A. ortonii*. Constraints from population structure and history, such as levels of gene flow, do not seem to explain these discrepant results between species. Instead, this discrepancy may stem from differences in climatic space occupancy over the range of each species (Prates et al., 2018).

These examples illustrate how recent studies of *Anolis* have contributed to our understanding of habitat history in South America. However, this work has also improved our knowledge of anole diversity and evolution. For instance, molecular studies revealed that the rostral proboscises of *Anolis phyllorhinus* and *Anolis proboscis* evolved independently (Prates et al., 2015, 2017); that *Anolis philopunctatus* and *A. punctatus* show no genetic divergence despite having distinct dewlaps (Prates et al., 2015); and that a twig anole-like morphology evolved (or was lost) repeatedly in montane anoles (Prates et al., 2017). Field inventories led to the rediscovery of *A. nasofrontalis* and *A. pseudotigrinus*, undetected for over 40 years (Prates et al., 2017); to the first record of the *tepui* anole *Anolis neblininus* in Brazil; and to the discovery of a new montane species (Prates et al., in review; Fig. 1). Lastly, this work reported the exotic *Anolis sagrei* and *Anolis porcatus* in Brazil, with a genetic study of *A. porcatus* suggesting a Floridian or western Cuban source of introduction (Prates et al., 2016c; Oliveira et al., 2017).

Molecular studies of mainland anole lizards have already contributed a great deal to our understanding of how habitats have changed during the last millions of years. These studies have expanded or opened new research avenues about how species colonize and adapt to novel habitats and how populations respond to environmental change through time. Future investigations of these topics will benefit from an increasing availability of genomic resources for anoles (Tollis et al., 2018) and from complementary sampling efforts and interactions between research groups working in South America, Central America, and the Caribbean.

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Establishment of genome editing methods in *Anolis sagrei*

Studies of gene function in anoles and other squamate reptiles have lagged dramatically behind other amniote groups due to a lack of genome editing and transgenic methods. As a consequence, investigations of gene function have almost completely excluded this diverse and highly successful group of animals. The establishment of gene editing technologies in *Anolis* would enable functional investigations of the genetic basis of phenotypic diversity and convergent evolution in the *Anolis* genus. The ability to make targeted mutations in anoles would also more broadly open studies of gene function in squamate evolution, behavior, physiology, and development. Therefore, we are attempting to establish genome editing technologies in anoles with the goal of producing genetically modified strains of *Anolis*.

Given the successful use of the CRISPR/cas system to generate targeted mutations in many vertebrate species, we have opted to employ this technology in our efforts to produce genetic alterations in anoles. With a relatively modest amount of effort, CRISPR has been successfully used in a variety of mammals, birds, amphibians, and fish. However, decades of germ cell and embryo manipulation work in mouse, chicken, Xenopus, and zebrafish laid the groundwork for the successful use of CRISPR in these species and their kin. In comparison, efforts to culture or manipulate squamate embryos and germ cells have been very limited. The most common approach for CRISPR mediated gene editing in vertebrates is to directly inject fertilized eggs or early stage embryos with CRISPR reagents (i.e., Cas9 protein and one or more guide RNAs designed to target loci of interest). In vertebrates with external fertilization, accessing and injecting early stage embryos can be relatively straightforward. In animals with internal fertilization, alternative approaches are required. For instance, in mice and other mammals fertilized eggs can be either be isolated from the oviducts of females shortly after mating or generated through *in vitro* fertilization. After microinjection of CRISPR reagents, the injected embryos are transferred to a host female, where they can implant and develop. These approaches are not currently feasible in any squamate. Instead, we have developed an alternative approach; instead of injecting fertilized oocytes, we have opted to microinject unfertilized oocytes within the ovaries of adult female anoles (Fig. 1).
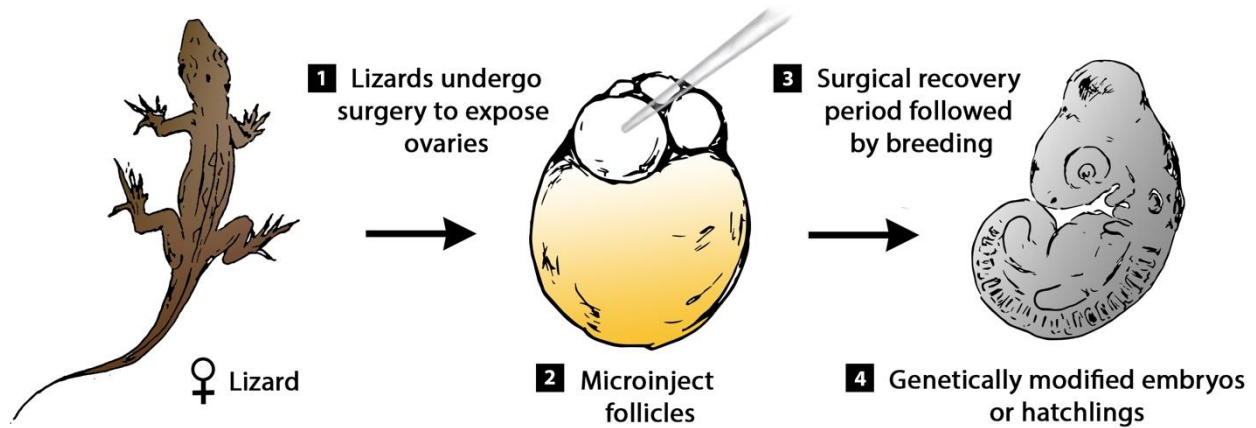
Figure 1. Strategy for the production of genetically modified anoles.

Through a series of pilot studies in *Anolis sagrei*, we have established a surgical procedure that enables us to microinject CRISPR/cas components into maturing, unfertilized oocytes located within the ovaries of adult females. We find that female fertility is maintained after the microinjection procedure, indicating that microinjected oocytes can be fertilized and produce viable animals. Using our microinjection method, we have begun microinjecting CRISPR/cas reagents into oocytes to create targeted mutations in pigmentation genes, and we are now screening the resulting embryos and hatchlings for CRISPR/cas induced mutations. We anticipate the genome editing methods that we develop in *A. sagrei* will be transferable to other members of the *Anolis* genus and will provide a roadmap for the establishment of these technologies in other squamate groups. Based on our initial results, we expect to have exciting news to report to the *Anolis* research community very soon.

Anolis genome editing workshops

We are pleased to announce that we will be running two genome editing workshops for the *Anolis* community. These workshops will be funded through a technology development grant that we recently obtained through the NSF EDGE program. The workshops will cover the anesthesia, surgical, microinjection, and screening methods used to create genetically modified anoles using CRISPR/cas genome editing. Both workshops will run for one week and will be hosted at the University of Georgia in Athens, GA. Tentative dates for the workshops are June 2020 and June 2021. Funds are available to subsidize travel and lodging for workshop participants. As our workshop plans develop, we will post additional details on Anole Annals.

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Can we detect differences in the rate of discrete character evolution between clades of anoles?

Phylogenetic comparative biology consists of the activity of drawing inferences about the evolutionary process from a pattern of observations for species combined with an estimate of the phylogeny. For nearly thirty years phylogenetic comparative methods have been used to great effect to study the evolution of lizards in the genus *Anolis*. In this short article, which is based on my presentation at the *Anolis Symposium VII* of 2018 in Miami, Florida, I'll first describe a new phylogenetic method designed to test the hypothesis that the rate (or process) of evolution of a discretely valued phenotypic character has changed in one or more places on the phylogeny. I'll then apply the method to investigate the possibility that the rate of dewlap color evolution and/or the rate of caudal vertebrae number evolution differ between mainland and island lineages of anoles. Finally, I'll discuss some caveats attached to this case study in particular.

Introduction

Phylogenetic comparative analysis represents the general task of using the phylogeny to make inferences about the evolutionary process or past. Over the past several decades phylogenetic comparative methods have steadily grown in their importance and now assume a relatively central role in evolutionary research. The majority of phylogenetic comparative methods combine a phylogenetic tree with phenotypic trait data for the constituent species of that tree with the aim of using the two to better understand the evolution of the trait or traits on the tree (and sometimes, though less often, the evolution of the phylogeny in the context of one or more traits).

Phylogenetic comparative methods have played a significant role in the history of evolutionary research on *Anolis*. In fact, I would argue that even the simple observation that the ecomorphs of different islands are (usually) not particularly closely related – a fact that forms the basis of an enormous fraction of evolutionary research on the group – depends intrinsically on the phylogeny and is thus an (informal) result of phylogenetic comparative analysis. Furthermore, some of the earliest adopters of modern phylogenetic comparative methods have been researchers studying anoles. For instance, Losos (1990) was among the first empirical

publications to employ both the squared-change parsimony method of ancestral state reconstruction (Huey & Bennett 1987) and Felsenstein's phylogenetic independent contrasts method (Felsenstein 1985).

Herein, I will describe a new method to analyze the evolution of a discretely valued character on the tree. This method is a modest generalization of the typical Mk model for studying discrete trait evolution on phylogenies, but in which the evolutionary process can exhibit different transition rates between states in different parts of the tree – specifically in cases in which those clades or edges have specified *a priori* by the user. I will then proceed to use the method to analyze character evolution for two different discrete traits in *Anolis* lizards: dominant dewlap color and total number of caudal vertebrae.

Model and methods

Details of the model

Much like virtually all modern methods for studying the evolution of discretely valued character states on phylogenies, the model of this study is a flavor of the so-called Mk-model of Lewis (2001). The Mk-model is so-named because it describes a continuous-time discrete-state Markov chain with a total of k possible states. (Thus an Mk-model with two states is sometimes called an M2-model; a model with three states an M3-model; and so on.) Under this model a set of non-negative real numbers $(q_{i,j})$ give the instantaneous transition rates between states i and j for all $i \neq j$.

In the simplest case, we could imagine an M2 process in which $q_{0,1} = q_{1,0}$: that is to say, the rates of transition from state 0 to 1 and from state 1 to 0 are equal. In this scenario, the probability of beginning in state 0 and ending in state 1 after time t can be written as:

$$P(1|0) = 1 - e^{-q_{0,1}t}.$$

Whereas the probability of starting and ending in the same state is merely one minus this, or:

$$P(0|0) = e^{-q_{0,1}t}.$$

These quantities are obtained by integrating an exponential distribution with shape parameter $q_{0,1}$ from $0 \to t$ and $t \to \infty$, respectively.

Why do we integrate an exponential distribution to obtain the cumulative probabilities that a change has or has not occurred? Well, the exponential distribution is what is sometimes called a waiting-times distribution. That is to say, it is a distribution of times that we must wait

for an event to occur, when that event occurs randomly and with a constant probability. To use a relative straightforward analogy, let's imagine that we are taking the MBTA Green Line (a subway line) in the city of Boston. We can furthermore imagine that trains are leaving the Lechmere station (the end of the line) at random, but with a constant rate – that is to say, with a constant probability for any infinitesimally small time period. If the leaving rate averages one train every fifteen minutes, then the average time we have to wait for a train at Park St. is 15 minutes – but the distribution of waiting times will be exponential with a shape parameter, λ, determined by the leaving rate. When we go to evaluate our model, be it a model about the arrival of subway trains or about discrete character changes on the tree, we need to be able to compute the probability that after some time an event (the train arrival, or a change in our discrete character's state) has occurred or has not occurred. To obtain these probabilities, we merely integrate the exponential distribution of waiting times under the model. The reason we compute an integral in this case is because the integral from 0 through the current time t gives us the cumulative probability that we are no longer waiting by time t, and thus that the event has occurred. The integral from t through ∞ (or, often more simply, one minus the previous integral) is the probability that we are still waiting because the event has not yet occurred. This is nothing new, and it is straightforward to extend this from a process with two states to one with an arbitrary number, the details of which are not necessary to describe here.

The additional extension of the model in this study is one in which we allow the rate (or process) of discrete character evolution to itself change over time according to an *a priori* hypothesis. To return to our MBTA Green Line analogy, let's imagine that if instead of trains departing Lechmere station randomly at a constant rate of one in every 15 minutes, that instead from 7:00am to 9:00am every morning trains depart randomly at the higher rate of one every 10 minutes, while during the rest of day they leave (randomly) at their regular rate. Let's imagine we arrive at the Park St. station at exactly 7:15am and the transit time for a train between Lechmere and Park is always precisely 20 minutes. Now the probability of a train arriving to Park St. by some arbitrary future time, say, by 7:25am, is a function of two separate probabilities: the probability that a train arrives by 7:20am (having thus left Lechmere between 6:55am and 7:00am when the leaving rate was one train every 15 minutes) and the probability that (given that this has not occurred) a train arrives between 7:20am and 7:25am (meaning that it left Lechmere between 7:00am and 7:05am when the leaving rate was once every ten minutes).

Here instead of modeling train arrivals, I have modeled morphological changes for a discrete character on the tree, but the principal is precisely the same. Just as we know that the (random) Green Line leaving rate changes between 6:59 and 7:01am, we assume *a priori* that the rate of change in the character is different along particular, pre-specified branches of the tree than it is on others. Then, to compute the probability of obtaining the data that we have indeed observed, we must merely accumulate these different probabilities of change across all the branches of the tree.

Note that this model is best suited for conditions in which we have good reason to hypothesize *a priori* that the rate of discrete character evolution has changed between specific clades or branches of the tree. For instance, a hypothesis that the rate of discrete character evolution changes after colonization of a new area (in which colonization occurs only one or a small number of times and thus can be reconstructed unambiguously on the phylogeny), or in which the rate of evolution changes following the evolution of a key trait (in which the trait evolves only once or on a very small number of occasions), would be circumstances in which the model could be well put to use. It should not, on the other hand, be used under circumstances in which we hypothesize that the state of one discrete character affects the rate of evolution in a second, but in which the evolutionary history of the first trait is totally unknown. In this case it would be much more appropriate to integrate over uncertainty in the evolution of both traits – as would be done (for instance) using the well-known existing method of Pagel (1994). The model of this article is instead best viewed as an exact discrete character analog of the insightful continuous character method developed by O'Meara et al. (2006). It is implemented in my R package *phytools* which depends in turn on the important core phylogenetics package *ape* as well as (naturally) on R itself (Revell 2012; Paradis et al. 2004; R Core Team 2018).

Empirical examples

For the purposes of my presentation at the *Anolis Symposium VII*, and for publication in this *Anolis Newsletter*, I fit the model to two different empirical cases. In both cases, I examined the rate of evolution of my discrete character in mainland vs. island lineages of anoles. Since the number of transitions from mainland to island (and *vice versa*) is relatively few, I decided that these could essentially be treated as having occurred in known locations in the tree. In particular, I assumed that the global ancestral node of the anole tree was present on the continental mainland, that occupancy of the Caribbean islands from mainland lineages (or *vice versa*) occurred via colonization, and then I proceeded to place colonization events precisely halfway along the edge leading to each clade in which descendants were present in the islands. (As in previous studies, I also reconstructed one island to mainland colonization event, and within this clade a further secondary colonization of islands. See Figure 1.) The mainland/island history that I assumed for the purposes of this analysis is given in Figure 1.

Using this mainland/island history as basis for all subsequent inferences I next analyzed dewlap color evolution. The data for this analysis were kindly provided to me by T. Ingram. My (perhaps dubious) logic in comparing the rate of anole dewlap color evolution between island and continental faunas was the following. Though not supported by any particular quantitative datum, I supposed that it could be reasonably assumed that lizard communities of the Caribbean islands contained, on average, more syntopic anole species than do their mainland equivalents. If so, then the strength of divergent natural selection on the dewlap color to avoid mismating with non-conspecifics should be higher in the Caribbean than on the mainland, resulting in a higher

rate of dewlap color evolution on islands than in continental anole lineages.

Dewlaps come in many colors and color-combinations, but for the purposes of this study dewlap color was coded by placing the dominant color of each species' dewlap as being closest to one of the following five states: black, pink, red, white, or yellow (Figure 1). Note that so doing resulted in what I would consider some fairly peculiar dominant color classifications. For instance, to my (imperfect) eye the Puerto Rican species *Anolis cristatellus* and *A. gundlachi* have fairly similarly colored dewlaps. However, presumably because the orange of its dewlap is relatively dull, the species *A. gundlachi* was classified as having a dominant color of 'brown,' which was considered to be closest in color to 'black' in the reduced color set – though I know of no herpetologist who would say that *A. gundlachi* has a black dewlap! By contrast, *A. cristatellus*, whose dewlap is orange with a hint of green in the center, is coded as 'yellow,' perhaps because orange is not a color that was coded in the reduced set (Figure 1). Nonetheless, in my mind it makes little sense that these so similar dewlaps would be coded as distinct on such a crude color scale. Unfortunately, given the size of the dataset, it is not possible for me review
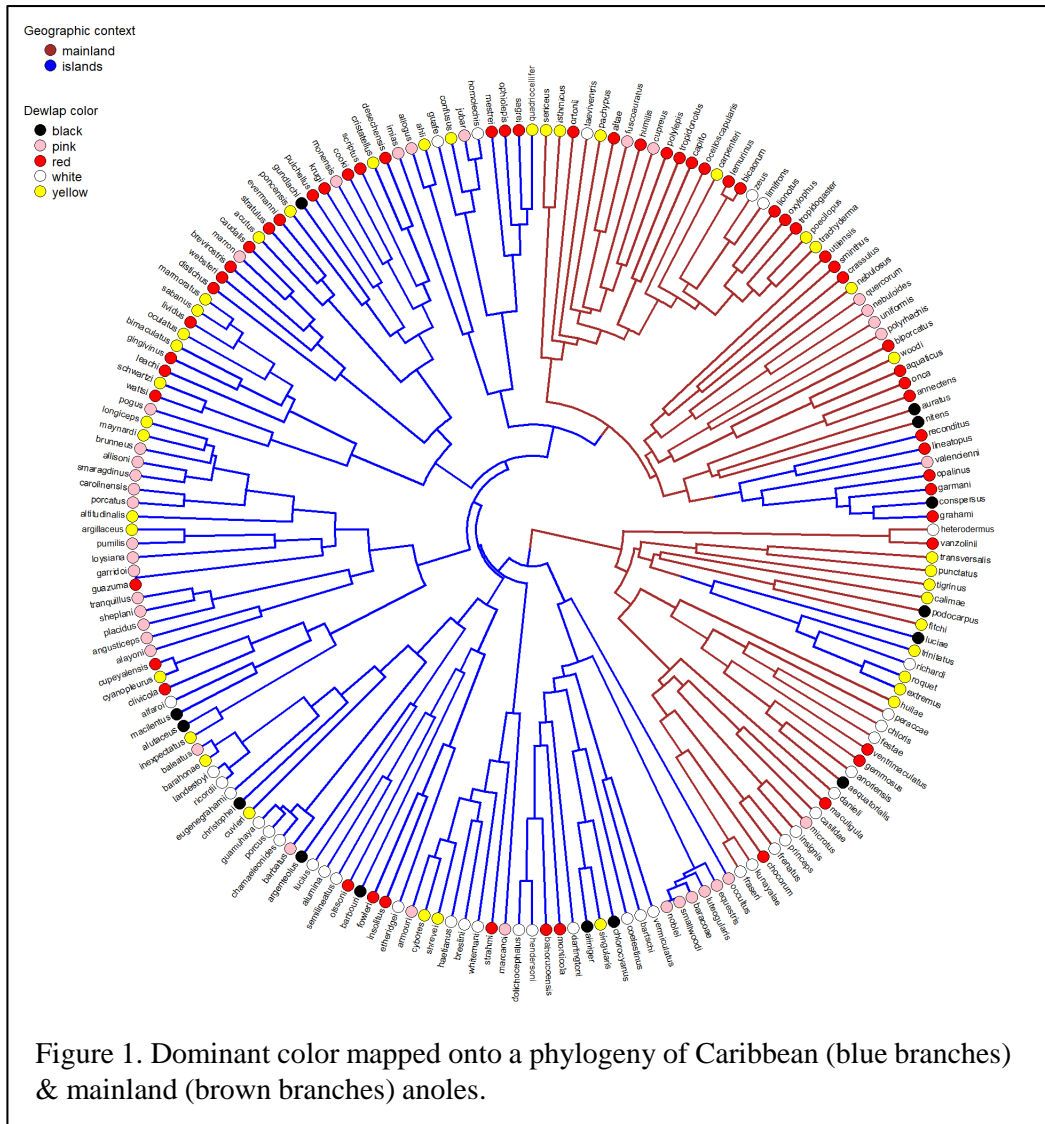

Figure 1. Dominant color mapped onto a phylogeny of Caribbean (blue branches) & mainland (brown branches) anoles.

all color classifications and re-code them (and, as I'm partially colorblind, my classifications would be quite unreliable, besides).

Using these data, afore-alluded warts and all, I then proceeded to fit a series of six models to the data and tree. These six models consisted of an evolutionary process in which: transitions occurred at the same rate between all pairs of states (ER); transitions occurred at the same backward and forward rate between each pair of states, but could occur at different rates between different state pairs (SYM); and transitions occurred at different rates between each pair of states (ARD). I fit each of these models either allowing for different rates between mainland and island lineages (-M) or forcing them to have the same rates of change between character states (-S), thus resulting in the six models in total (ER-S, ER-M, SYM-S, SYM-M, ARD-S, and ARD-M). Results from this analysis are given in Table 1. In general, although in all multi-rate models the average transition rate between states was higher on islands than in mainland lineages – penalizing for the number of parameters to be estimated, the best-fitting model was clearly a model in which both mainland and island fauna dewlap dominant color evolved under the same set of rates of transition between states (SYM-S; Table 1).

Table 1. Mean transition rates, log-likelihoods, number of fitted parameters, and AIC for the six fitted models of dewlap dominant color evolution described in the main text. The best supported model (SYM-S) is highlighted in red text.

Model	$\overline{q}_{islands}$	$\overline{q}_{mainland}$	$\log(L)$	k	AIC
ER-S	9.284	9.284	-296.1	1	594.3
ER-M	9.296	0.007	-281.6	2	567.2
SYM-S	0.011	0.011	-259.5	10	538.9
SYM-M	0.014	0.005	-254.3	20	548.5
ARD-S	0.010	0.010	-252.9	20	545.8
ARD-M	0.008	0.014	-246.7	40	573.4

In addition to this character, I also analyzed island and mainland caudal vertebrae number evolution using data that were kindly provided to me by L. Mahler. These data were obtained by simply counting the number of vertebrae from the pelvic girdle to the tip of the tail in a specimen in which the tail was previously deemed to be completely intact (Figure 2). My logic in comparing the rate of anole caudal vertebrae evolution between island and mainland lineages is simply that conventional wisdom suggests that Caribbean anoles are more ecologically and morphologically varied than are their mainland congeners. The tail is an appendage that can play an important role in locomotion, particularly in an arboreal setting. Consequently, it seemed reasonable to imagine that it might be under stronger divergent selection in the Caribbean, where anoles fill a broader diversity of ecological roles, than it is in

continental clades.

Given that the number of caudal vertebrae varies on quite a broad range (from 34 through 55 in these data), one might intuitively assume that the number of parameters to estimate in this model would be impossibly large. In fact, if we make some relatively reasonable simplifying assumptions (keeping in mind that all models are, by definition, intended to be simplifications of reality) the dimensionality of the problem can be quite reasonable, even though the state-space is   big. Specifically, I decided to treat the acquisition and loss of caudal vertebrae as an ordered process – in which gain and loss were free to proceed with different tempos, but in which changes in the same direction between any pair of adjacent states should occur with the same rate (Figure 3). Once again, though I found that the estimated rate of character evolution in the best-fitting model was higher in island than in mainland anole lineages, the best-supported model (accounting for parameterization) was, as before, the ordered, single-rate model (Table 2).
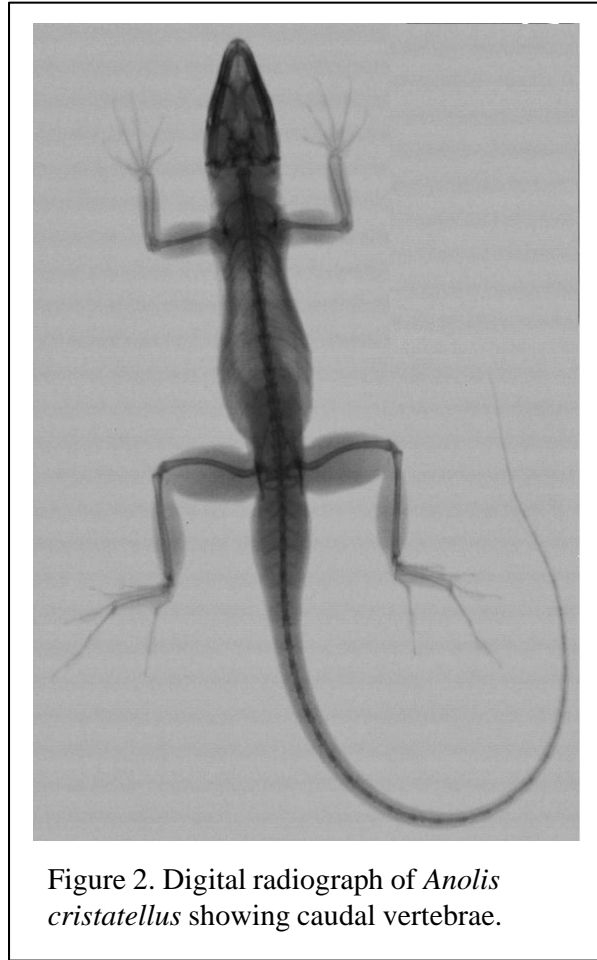

Figure 2. Digital radiograph of *Anolis cristatellus* showing caudal vertebrae.

Table 2. Rate of caudal vertebrae loss & gain on the mainland [M] and islands [I], log-likelihoods, number of fitted parameters, and AIC for the two fitted models caudal vertebrae number evolution described in the main text. As in Table 1, the best-supported model (ordered-single) is highlighted.

Model	q_{loss}	q_{gain}	log(L)	k	AIC
ordered-single	0.218	0.130	-303.4	2	610.9
ordered-multiple	0.224 [I] 0.187 [M]	0.133 [I] 0.115 [M]	-303.1	4	614.3

Conclusion

Herein I describe a new model for discrete character evolution that I intend to present in much greater detail with a formal publication elsewhere. The method is one in which the rate of evolution for a discretely-valued character state is allowed to differ between different pre-specified branches or clades of a phylogenetic tree. For the purposes of presenting this model at the wonderful *Anolis Symposium VII* in Miami, Florida, I applied the method to two different empirical datasets. These datasets were for dominant color of the dewlap and number of caudal vertebrae in (obviously) the tail. Aside for convenience and availability of the data, the biological premises on which I justified these tests were as follows. First, my grossly-simplified impression is that (on average) the Caribbean islands tend to be typified by more syntopic *Anolis* in any particular ecological community. If true, I thought, then perhaps the dewlap should be under divergent natural selection to change more rapidly in island fauna to avoid mismating mistakes with related taxa. Second, Caribbean anoles are well-known for having diversified more extensively in their morphology and ecology than have their mainland cousins. Since the tail can play an important ecomorphological role in

Figure 3. Fitted multi-rate symmetric ordered transition model for the evolution of caudal vertebrae number in mainland and Caribbean anole lineages.

some lizards, it seemed reasonable to expect that the number of caudal vertebrae might be under greater pressure to diversify among island vs. continental anoles. In fact, though in both cases the parameter values of the best-fitting model differed one from the other in the expected direction (that is, with both the mean rate of transition in dominant dewlap color and the rate of gain or
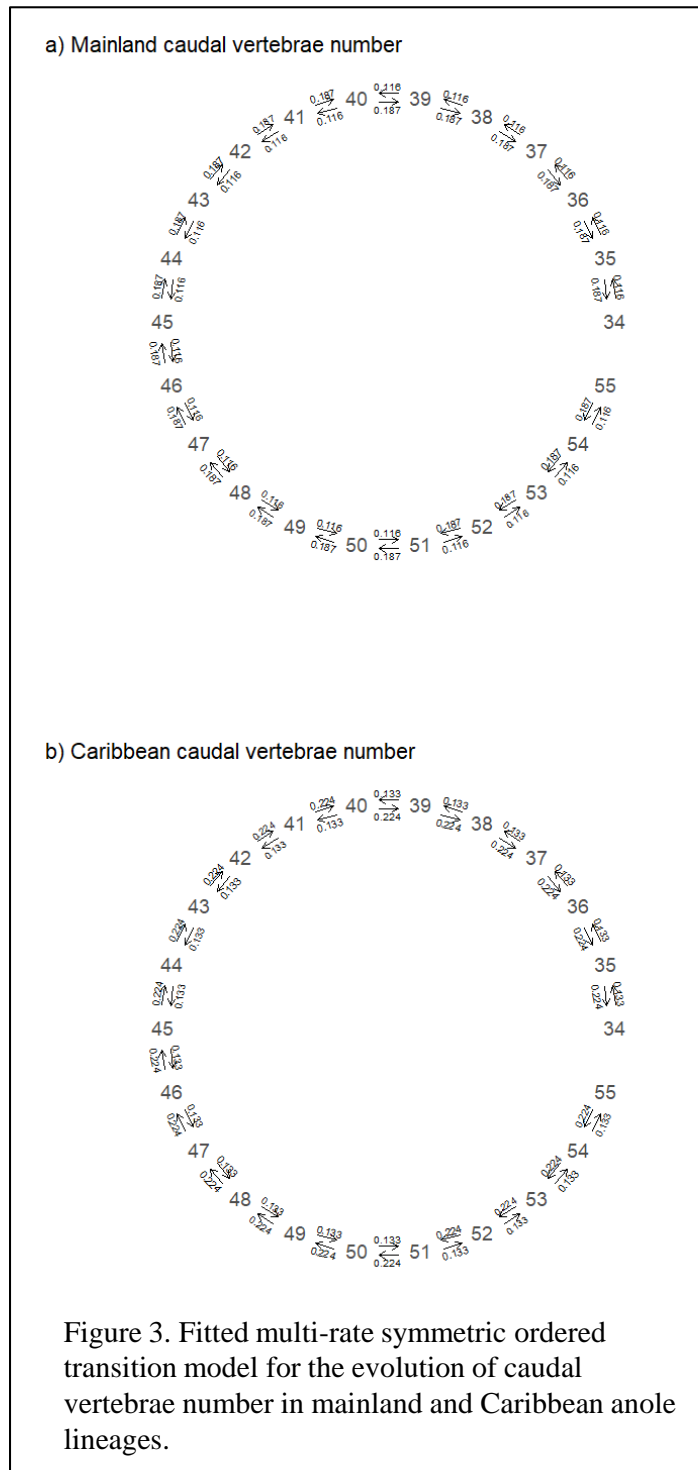
loss of caudal vertebrae higher in island than in mainland lineages; Table 1 and 2), information theoretic model selection criteria do not suggest that these more parameter-rich models are well-justified compared to a single-rate model for each character.

Even if they agree with the mechanistic basis of my hypotheses, readers familiar with the evolutionary biology of anoles might note that the soundness of my simplifying assumptions seem dubious. For instance, with regard to the assumption that islands tend to feature more syntopic anoles than do mainland ecosystems, an astute anole biologist would probably point out that the Caribbean has many islands with but one or two species, and furthermore that the mainland includes anole faunas with multiple syntopic species. I agree wholeheartedly that this critique could explain my non-result herein. It's possible that in future I might attempt to obtain more refined data on syntopy and repeat the analyses undertaken here.

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Role of a sweet-toothed anole (*Anolis conspersus*) in orchid pollination

Abstract

Fruit and nectar feeding is characteristic of a large number of island lizards leading to pollination and seed dispersal mutualisms and the potential for lizard-driven evolutionary change in island plants. Oceanic islands, in particular, are recognized as potent sources of pollinator novelty. Unusually, the Cayman Islands endemic orchid, *Myrmecophila thomsoniana*, is pollinated by deceiving cetoniid flower chafer beetles to penetrate under the column, thereby extracting and depositing pollinia. The flowers are non-rewarding other than to produce some nectar on the exterior surfaces of the sepals and ovary which is collected by ants and *Anolis conspersus*. Direct observation of flower visitors shows that Blue-throated Anoles may visit orchid inflorescences between 0.4 – 1.5 times per hour and lick nectar up to 0.8 times per hour. Observed anole influences on pollination are threefold. Anoles jumping into and climbing within the flowers can disrupt beetles from approaching and entering flowers or cause them to fly after pollinia extraction. Rarely an anole may extract pollinia itself. Depending on the timing, the

F ig. 1 Male *Anolis conspersus* licking extrafloral nectaries at base of tepals of Myrmecophila thomsoniana on 8 June 2016 at 16:17, in the Ponciana genet, Lower Valley, Grand Cayman.

anoles can thus affect fitness by decreasing pollination opportunities or increasing outcrossing among genets.

Introduction

Oceanic islands are recognized as potent sources of pollinator novelty (Mayer *et al*. 2015). An outstanding example is Reunion Island's *Angraecum cadetii*, whose main pollinator, a raspy cricket, represented an entire new Order of orchid pollinator when discovered (Micheneau *et al*. 2010). It is also sometimes pollinated by a nectar-feeding day gecko (Bėgue *et al*. 2014). In the Caribbean, anoles are well-known for fruit and nectar feeding (e.g. Losos, 2009; Losos, 2012; Ríos-Lopez, *et al.* 2016). West Indian anoles are even partial to banana sap (Norval and Mao 2013). It has been argued that such behaviours may lead to pollination and seed dispersal mutualisms, and the potential for lizard-driven evolutionary change in island plants (Olesen and Valido 2003).

For Anolis Symposium VII I described some of the data from ongoing research that show how nectivory by the Grand Cayman Blue-throated Anole, *Anolis conspersus,* could play a role, via both positive and negative disruption of pollinator behaviour, in the evolution of *Myrmecophila thomsoniana* var *thomsoniana*, a Grand Cayman, endemic, epiphytic orchid. *A. conspersus* is also a Grand Cayman endemic. Both plant and reptile have evolved in the 2-3 million years since the low-lying carbonate island last emerged from the sea.

Like all orchids, the male and female reproductive parts of the *M. thomsoniana* flower are fused into a column. The stigma is separated from the anther by a rostellum which prevents autogamy. *M. thomsoniana* is however self-compatible if pollinia are transferred to the stigma by external forces. Isolated observations of anoles visiting the extrafloral nectaries were first reported by Echternacht *et al*. (2000).

Methods

In 2015 and 2016, orchid genets within three phorophytes were monitored daily, in detail, for pollinators from a central location within a 35 by 50 m plot in a mid-island location in Lower Valley, where A. c. conspersus is the relevant subspecies present. I used intensive direct observation paired with time-lapse video to conclusively identify and record behaviours of pollinators and other visitors to the flowers. Anole observations focused on a genet in a Ponciana tree which provides the core of a home range of a male *A. conspersus*. This genet produces up to ten inflorescences in the flowering season, between May and early July. Additionally a minimum of one still image and one 30 second video were captured every five minutes on two Bushnell NatureView HD Max field cameras fitted with f460mm close-up lenses and set up on other inflorescences within the site.

Pollinators

Unusually, the principal pollinator is a flower chafer beetle, *Gymnetis lanius* (Coleoptera: Cetoniinae) (Rose-Smyth, in press). *G. lanius* is limited in distribution to Grand Cayman and Jamaica (Ratcliffe, in press). There are only a handful of orchids known to be pollinated by cetoniids: in South Africa, Asia and Argentina (Singer and Cocucci 1997; Johnson *et al.* 2007; Pedersen *et al.* 2013; Peter and Johnson 2014; Arakaki *et al.* 2016). Additionally, I discovered the Asian Mango flower beetle, *Protaetia fusca* (Coleoptera: Cetoniinae), a first record for this adventive species in Cayman, and an equally effective pollinator of *M. thomsoniana*. Although both species of beetle crawl over the extrafloral nectaries they do not appear to be obviously attracted to them and can spend long periods crawling back and forth on the pedicels and stems and around the outside of the corollas, never visibly feeding. *G. lanius* actually feeds on the flowers of a wide variety of trees and shrubs, most having dense white-flowered inflorescences.

The exact mechanism by which the beetles are deceived to enter the nectarless lip is not yet clear. During the first leg of pollination all eight pollinia are extracted from the anther and glued to the visitor's body. Deposition of pollinia is variable; as few as one, or all eight may be delivered to the stigma.

In 2016, a sample of 19 inflorescences on 5 orchid genets, produced 316 flowers, of which 31% experienced pollinia extraction only, a further 11% experienced pollinia extraction and deposition, and fruit set was 9% (Rose-Smyth, in press). The effective pollinators were the two beetles and to a lesser extent, feral honeybees. Additionally, the Bananaquit, *Coereba flaveola,* and on a single occasion, *A. conspersus,* contributed to extractions-only of pollinia.

Anole behaviour

During the 4-6 weeks that *M. thomsoniana* is in bloom anoles with home ranges encompassing the orchid visit the nectaries at the base of the flower (Fig. 1) and also at the tips of unopened buds, as do a number of ant species. Anoles may creep up the stems, run, and leap from the tree limbs into the flowers. Females sometimes perch, effectively camouflaged, on the brown, persistent, prior year stems. Importantly, anoles do not dislodge the pollinia-containing anther when climbing on the flowers.

In both 2015 and 2016 the Ponciana tree had a resident male and two-three female/sub-adults. In 2015 the orchid was observed for a total of 57.3 hours over 11 days in June, from as early as 7:20 and up to 18:00. Anole events were observed within the flower clumps 60 times (Fig. 2). Visits equated to between 0.4 – 1.5 times per hour and nectar-licking visits occurred up to 0.8 times per hour. Events ranged in duration from almost instantaneous jumps on and off, to periods of several minutes foraging within the flowers. Forty-four were events where either there was no nectar licking or I could not see the anole's mouth clearly; 16 included confirmed nectaring, often at multiple flowers. Female/sub adults seemed more active than the male even taking into account that they outnumbered the male at least 2:1. Lastly, three of the events

occurred when beetles were present; one caused the beetle to fly, the other two anole interventions did not overtly affect the beetle's behaviour (Fig. 2, starred).
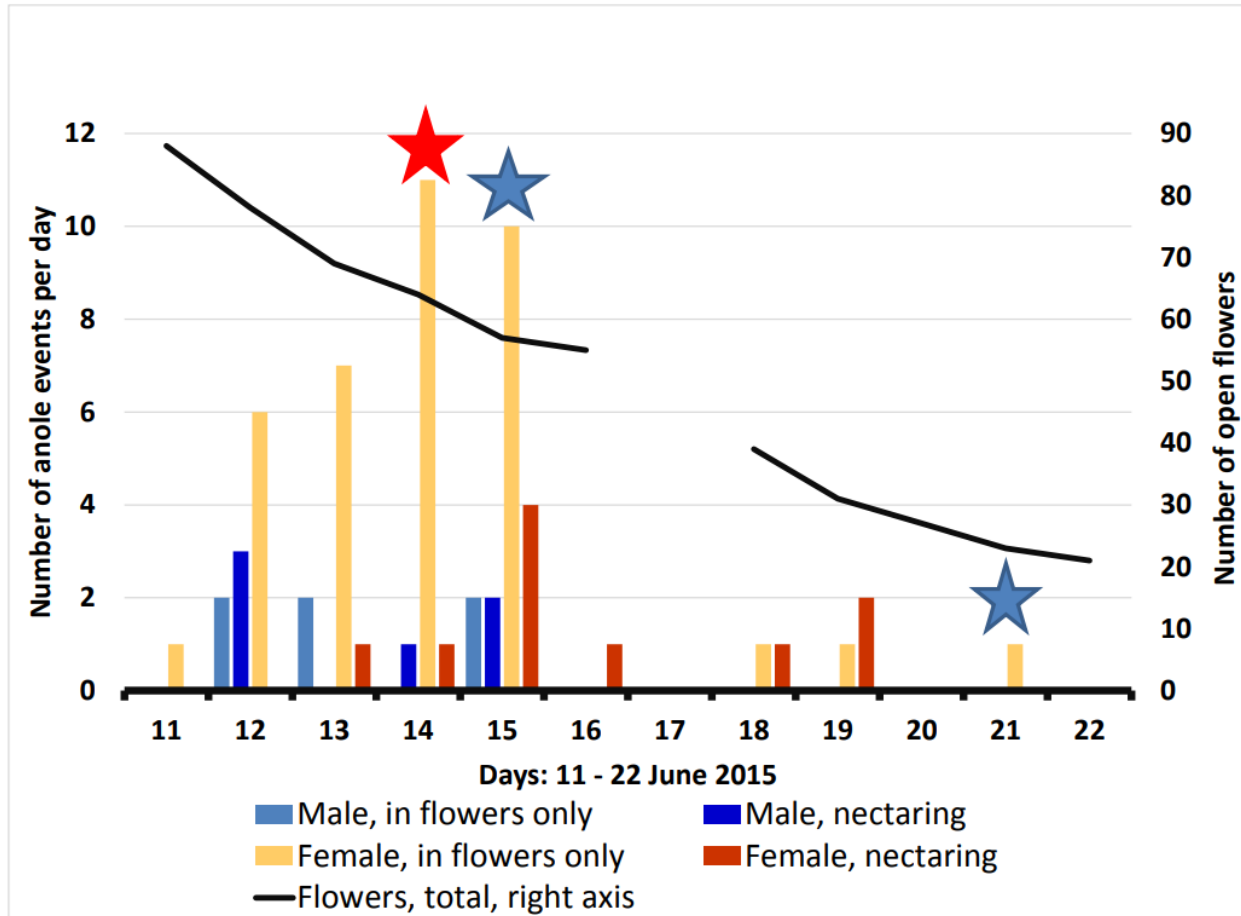

Fig. 2 *Anolis conspersus* activity in the Ponciana genet, Lower Valley, Grand Cayman from 11th to 22nd June 2015. Left axis = number of anole events per day, total n = 60. Right axis = number of open flowers. No data were collected on 17th June. Blue stars = anole activity on flowers in presence of beetle pollinator having no apparent effect on the beetle; red star = anole activity causing a *Protaetia fusca* beetle with pollinia load to fly away before entering any other flower.

As well as consuming nectar from flowers anoles predate the ants that also visit the nectaries. (See supplemental data video on YouTube: https://youtu.be/rvVHkj7UQz4). Although licking the extrafloral nectaries was never observed to involve the anole putting its head inside the labellum of the flower, when pursuing ants, an anole could be led to do so by an ant running into the tubular cavity of the lip. Based on these behavioural factors, I conclude that a single example of pollinia extraction by a female anole in 2016 (Fig. 3) was most likely the result of the anole snatching at an ant. In this instance the anole did not go to perform pollination. The only

pollinia deposition in the entire genet after she acquired the pollinia was directly observed to be by a *Protaetia* beetle.

Fig. 3 Female *Anolis conspersus* in the flowers of *Myrmecophila thomsoniana* in the Ponciana genet, Lower Valley, Grand Cayman on 8 June 2016 at 17:05. During the course of the day she was observed in the flowers and without pollinia between 09:24 and 09:32; the first confirmed sighting with eight pollinia attached to her head occurred at 15:41 and last at 17:56, shortly before data recording ceased at 18:00 and approximately one hour before sunset. She had lost, or groomed off, all but two of the eight pollinia by the next morning and was observed later in the day with only a yellow smudge remaining.

Discussion

Anolis conspersus has a role in a pollination network that includes an orchid, two beetles, the honeybee, a flower-visiting bird and ants. By extracting pollinia, the behaviour of the anole is confirmed to, albeit rarely, contribute to floral male reproductive fitness and could, conceivably, evolve towards more effective pollinator status. Floral deception is considered to be the ancestral state in orchids and has been shown to have evolved to nectar rewards at least nine times in the large South African genus, *Disa* (Johnson *et al.* 2013). *Myrmecophila thomsoniana* has

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apparently undergone one pollinator shift already. The nearest Central America congener of *M. thomsoniana* is pollinated by solitary bees (Parra-Tabla and Vargas 2007).

Currently, *A. conspersus* affects the consistent pollinators both positively and negatively in terms of floral reproductive success. Depending on whether it disturbs beetles before or after pollinia extraction, anole activity can lead to decreased pollination opportunities, or potential for increased outcrossing among genets, respectively. Further, the pollinia extraction, deposition, and natural fruit set rates observed in 2016 are consistent with a degree of pollinator limitation, thus increasing the relative impact of other visitors on pollination success. Importantly, even rare events that promote outcrossing can be significant in an orchid that can produce tens of thousands of seeds in a single fruit.

My future aims include: (a) integrate and analyse all of the 2015 and 2016 data; (b) gather additional quantitative data at the primary site with expanded camera capabilities; and (c) extend the project to assess whether there are any differences in *Anolis conspersus lewisi* behaviour in the eastern districts of Grand Cayman, where the potentially invasive Mango flower beetle is not yet established and pollination rates appear to be lower than at the mid-Island site.

I am pleased to report that the addition of the second field site is paying dividends already. On 15 June 2018 a second occurrence of an anole with pollinia on her head (three) was observed there, within 3 m of the only pollination events of that, or the prior two days, on adjacent inflorescences. These were one extraction-only and one extraction/deposition of four pollinia which implies, at minimum two pollinators and are not inconsistent with the anole causing the pollination. However, actual pollination by *Anolis conspersus* remains to be definitively confirmed.

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Using introduced anoles as natural experiments in ecology and evolution

My dissertation research attempted to test several fundamental ecological and evolutionary hypotheses using communities of introduced non-native *Anolis* lizards. The idea to use non-native species as 'natural experiments' in ecology and evolution certainly isn't new, in fact I can't even claim any anole-specific originality to the idea[4]. However, to my surprise, few people had so far jumped on board this train so far in Miami, FL, where I had found myself enrolled in graduate school at Florida International University. I had arrived in south Florida having accepted a PhD position in the lab of Ken Feeley – a specialist in studying how tropical plants are responding to climate change in the Peruvian Andes. The plan was for me to investigate whether the patterns Ken had uncovered in the Andes – that plant distributions were shifting upslope in response to contemporary climate change – extended to the cold-blooded (and therefore [presumably] similarly thermally sensitive) herpetological diversity. Yet, I had found myself already in a lush subtropical metropolis surrounded by lizards. I soon learned that the vast majority of this peculiar fauna were Caribbean *Anolis*, and the more I watched and read about anoles, the more they fascinated me[5].

An adult male Cuban brown anole (*Anolis sagrei*) in Miami, FL.

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[4] See the last paragraph of Chapter 11 in Jonathan's book (Losos 2009), "Finally, introduced species provide unparalleled opportunities to study ecological interactions and their evolutionary effects [in *Anolis*]", as well as all of the other [introduced] anolologists who's shoulders I have stood on.

[5] I had always come from a lizard/reptile research background; I had (partially) tricked my way in to Ken's lab under the guise of being a forest ecologist (having completed by Master's research on forestry plantations, albeit only in the context of looking for snakes within them). On reflection, there was little chance that Miami's diverse exotic lizard community wasn't going to steal my attention. Also, I learned early on that the Andes get cold. That wasn't appealing to me.

Like most anole researchers, I soon became interested in the over-arching and broad questions concerning the origins and maintenance of such diversity. Specifically, I was interested in four main topics; (i) what triggers adaptive radiations, (ii) once a clade starts to radiate, how do phenotypically-similar species coexist, (iii) how does this influence broader patterns of community assembly, and (iv) what are the ecological, behavioral, and morphological consequences of coexistence?

Much of our understanding about the mechanisms that have generated anole diversity has relied on inferring process from pattern. In some senses, this is unavoidable; evolutionary biology is classically historical in nature – one must collect evidence in the present to test hypotheses about the past (Cleland 2001, Mayr 2004). For the most part, the picture we have of anoles is already an end product; adaptive radiation has happened, and we are left to study only those species which have stood the test of time. However, what generates this diversity? How do interactions in the early stages of radiation shape patterns of diversification? How do species coexist if they have not yet diverged in phenotype? These are all fundamental yet difficult questions surrounding the (notoriously elusive) early stages of adaptive radiation. In the absence of identifying a natural scenario in which early stages of divergence could be occurring among closely-related species (these situations are often cryptic and difficult to identify[6]), observing the processes which drive early stages of divergence would be much easier with a time machine.

However, there are contemporary alternatives, which I will take this opportunity to discuss. But first, to understand how to study these processes we must first pick apart the various stages of adaptive radiation and identify the assumptions that underlie them. Here, I loosely follow the classic model of adaptive radiation as first put forward by Simpson (1953) and then developed further by Schluter (2000)[7]:

1. An ancestral species finds itself in a resource-rich environment.
2. Speciation occurs; (reproductively-isolated) species coexist and communities assemble.
3. Resources are partitioned to minimize (costly) interspecific interactions[8].
4. Species adapt to each respective 'niche'.

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[6] Although see Rich Glor, Julienne Ng, Anthony Geneva, and Dan MacGuigan's (and associated colleagues!) excellent work investigating patterns of divergence in the *distichus* complex.

[7] But which I, like many anole students, discovered by way of Losos (2009, p.206-7).

[8] Classically, interspecific exploitative competition for resources is the interaction expected to drive this process (and the most common approach taken in anoles), and so a depletion of resources leading to resource limitation would be expected priori to partitioning. However, the degree to which interference competition, for example agonistic interactions, can drive the same patterns deserves more research attention (in adaptive radiations in general, and in anoles specifically).

I will discuss each stage of this model of adaptive radiation, attempt to explain how my research has picked apart at (small) pieces of the story, and highlight opportunities which I think deserve further study.

1. *Ecological opportunity*

The initial stage of adaptive radiation in which a species finds itself with new access to competitor-free resources is usually referred to as *ecological opportunity*. Classically, ecological opportunity, like adaptive radiations themselves, is often thought about in the context of islands. For example, an ecological opportunity may be presented following the colonization of an island depauperate in competitors. Famous case studies of island radiations include Darwin's eponymous finches in the Galapagos, or the lobeliads of the Hawaiian archipelago. However, this may also span to other island-like scenarios, such as the colonization of land-locked lakes (as in the African Rift Lake cichlids or the Sulawesi silversides). There are other ways in which an ecological opportunity may be experienced: following a mass extinction (for example, the explosive radiation of mammals following the extinction of the archosaurs and other non-avian dinosaurs), the appearance of new resources (such as new habitats which developed during the uplift of the Andes), or key innovations[9] (like the evolution of the pharyngeal jaw of cichlids and the explosive diversification in trophic morphology which followed; Fig 1). As the start of my dissertation, I reviewed the relationship between ecological opportunity and adaptive radiation (Stroud & Losos 2016), although not explicitly within the context of anoles.

Luke Mahler's work had previously found macroevolutionary support for the role of ecological opportunity – as defined by rates of diversification decreasing through time (i.e. ecological opportunity was highest at the start of the radiation because trait diversification was fastest, but then decreased through time as that trait-space was filled) – in the adaptive radiations of anoles (Mahler et al. 2010). However, we know very little about how ecological opportunity works mechanistically. One way to think about ecological opportunity would be to visualize a species gaining access to a new adaptive landscape which is comprised of many unoccupied peaks (each representing a distinct ecomorphological phenotype). Through time, colonization of those peaks, with selection carving out the valleys separating them, will produce an adaptive radiation – each species in the radiation will find itself stranded on an independent adaptive peak. However, what the shape of (multi-species) adaptive landscapes actually look like, much less how natural selection acts to shape them, remains poorly understood[10]. This is true at both the

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[9] Some suggest that the evolution of toepads in anoles are one such key innovation, providing access to the arboreal realm with far greater performance than any other competitors (see Losos 2009 p.332-5 for a nice summary)

[10] A further piece to this puzzle is that, so far, estimates of fitness surfaces at the microevolutionary scale in anoles has relied on survival fitness rather than reproductive fitness. A clearer evaluation of how well these two estimates reflect true biological fitness in anoles would be valuable.

micro- and macro-evolutionary scale. Following Luke's work, little progress has been made on the role of ecological opportunity in anole radiations; a more detailed macroevolutionary understanding of how the landscape itself evolves (if it is considered to not be static through time) would help to further bridge the pattern-process divide.
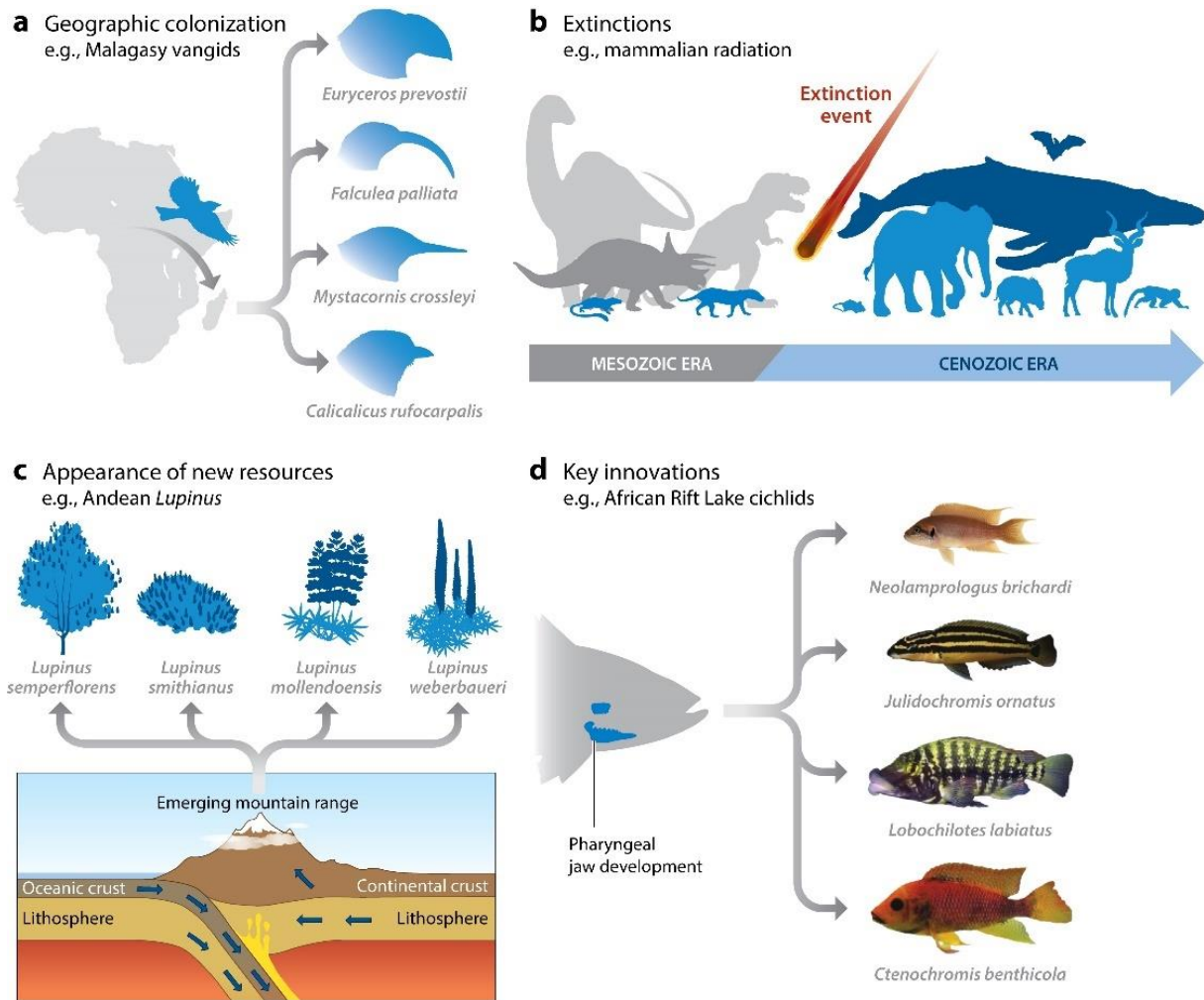

Fig 1. The various ways in which an ancestral species (or clade) may experience an ecological opportunity (from Stroud & Losos 2016, which also includes a much more informative figure legend; used with permission of *Annual Reviews*).

(1.b *Ecological release*)

Following the discovery of an ecological opportunity and free from the shackles of previous biotic constraints, an ancestral species may be expected to take full advantage of the breadth of this new and exciting resource spectrum. As the diversity in resource use of the ancestor expands, this will present as an increase in total niche width. This process of niche

expansion is known as *ecological release*. This hypothesis is of particular importance to adaptive radiations as it provides the mechanistic basis on which disruptive selection can drive within-species divergence[11]. If assortative mating occurs within these diverging phenotypes, then reproductive isolation may evolve, and lead to sympatric speciation[12]. So far, there has been very little evidence for sympatric speciation having occurred in anoles. Two lines of evidence support this; (i) a lack of gene flow (and regions of sympatry) among sister species in the Greater Antilles[13], and (ii) the two-species islands found in the Lesser Antilles would appear a likely place for it to have occurred, yet all species pairs are not closely related and are the result of independent colonization events.

The reason I discuss sympatric speciation (despite previous studies providing relatively little support for it in anoles), is that there also exists scant support for something often considered an important precursor – *ecological release* – in the anole literature[14]. If we are to fully understand whether sympatric speciation occurred in anoles (or, even, if it was *likely* to have occurred), then a better understanding of ecological release would be valuable. The current prevailing view of anole radiations is that they were largely driven by bouts of allopatric speciation with phenotypic divergence occurring on secondary contact (e.g. through character displacement) or local adaptation in allopatry. Whether ecological release existed, exists, or would be predicted to exist in anoles remains unclear and deserves further investigation[15]. Introduced species could provide a unique opportunity to study this at the ecological level; a handful of successful invaders (e.g. *A. sagrei, A. carolinensis, A. distichus,* and *A. cristatellus*) are now found in a range of different ecological communities. These communities are often comprised of many different species and so may represent a biotic gradient with which to test for the presence of ecological release through quantifications of resource use and niche breadth[16]. Alternatively, the comprehensive ecomorphological assessment of multiple island populations of

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[11] A nuance to this is that the population must have high within-population variation for disruptive selection to occur; ongoing research with Sean Giery aims to understand this pattern in a widespread focal species (*Anolis sagrei*), while work with Ambika Kamath and Michele Johnson aims to elucidate patterns of within-population variation (now fashionably referred to as 'individual specialization') across anole species and ecomorphs.

[12] This is just one mode of sympatric speciation and a highly simplified synopsis at that – see Nosil (2012) for much more detail!

[13] Ongoing work by Guinevere Wogan and Ian Wang is attempting to uncover ancient hybridization in the Puerto Rican clade, which may yet reveal new insights.

[14] Only Lister (1976) has so far provided convincing empirical support for ecological release in anoles, which stems from his ecological assessments of *A. sagrei* in various natural communities of different compositions; the so called "chronosequence" method.

[15] Ecological release may occur in the allopatric speciation model of adaptive radiation, as often favored in anoles, but it isn't thought of as a necessity in the same way as under as sympatric model of adaptive radiation.

[16] See Sean Giery's contribution in this issue which presents some of our research testing the ecological release hypothesis in *Anolis sagrei*.

the brown anole (*A. sagrei*), spanning its entire natural distribution and spearheaded by Graham Reynolds and Anthony Geneva (among others), may provide the same chronosequential comparison.

If support for ecological release is found in anoles, then the ensuing conversation about its evolutionary implications will be interesting. Presumably, if accepting that sympatric speciation is an unlikely outcome, a broader niche – in concert with high within-population variation – could pre-adapt a species for novel interspecific interactions. For example, if phenotypes already exist in a population which would be favored under novel selection regimes (for example, if interacting strongly with a novel congener), then coexistence may be achieved from rapid phenotypic shifts, side-stepping the alternative; competitive exclusion. In these ways ecological release of two species in allopatry could accentuate (and possibly accelerate) character displacement on contact, driving the rapid diversification patterns observed in anole radiations. However, if niche expansion through ecological release is driven by increased generalization of individuals (i.e. the opposite of individual specialization), then this adaptive power is presumably lower. Therefore, it is important to not only understand the basic pattern of ecological release (niche expansion), but also the underlying structure of it (degree of within-population variation; "*individual specialization*"). I aim to establish future research projects to address some of these questions.

2. *Speciation, species coexistence, and community assembly*
There is no avoiding that speciation is an integral component of adaptive radiation. However, I am not going to discuss (in more detail) the various phenomena through which speciation can take place. I will, however, take this opportunity to briefly highlight that we still know very little about assortative mating patterns in anoles; this would be a rich opportunity for future research given its apparent importance in evolutionary radiations and to the process of speciation.

Instead, I will focus this section more on the other topics at hand which comprised the majority of my dissertation research; *species coexistence* (and the phenotypic consequences of it) and *community assembly*. While these can (generally) mean the same thing depending on the scale in which they are discussed, I will refer here to *species coexistence* as investigating the coexistence mechanisms of a focal pair of species, while *community assembly* as co-occurrence patterns of more than two species. I conducted separate studies on these two phenomena during my dissertation research. To study patterns of community assembly, I travelled to the island of Bermuda, while I conducted a detailed investigation on coexistence of phenotypically similar species on two trunk-ground ecomorphs in Miami, Florida.

Bermuda has a rich and well-documented history of anole introductions spanning the past century[17]. In 1905, Graham's anoles (*A. grahami*) were purposefully introduced from Jamaica as a biological control of crop-destroying scale insects (*Carulaspos minima*) (Wingate 1965). Despite the quick establishment, high population density, and rapid expansion of *A. grahami* in Bermuda, the scale insect population did not appear to suffer. Upon analysis of the stomach contents of a selection of *A. grahami*, it was discovered that these lizards rarely – *if ever* – ate scale insects...this was the first stage of a calamitous cascade of biological invasions on Bermuda. *Anolis grahami* quickly became so abundant that in the 1950s it was deemed that their population now needed control. And so, in 1957, Great kiskadee flycatchers (*Pitangus sulphuratus*) were introduced from the Jamaican realm of *A. grahami* to control the lizard populations. As you may have predicted, in a classic case of conservation <u>mis</u>-management, kiskadees also rarely, if ever, ate *A. grahami* (Fig 2). Both species flourished and are now found across the entirety of the island.

Fig 2. A Greater Kiskadee flycatcher (*Pitangus sulphuratus*) not eating an anole.

In the 1940's two additional anoles were introduced, albeit this time unintentionally[18]: first, the Antiguan anole (*A. leachii*; known locally as "the Warwick lizard") was observed in Central Bermuda, and second, the Barbadian anole (*A. extremus*) was recorded from Sandy's Parish in north-west Bermuda. Losos (1996) conducted an update in 1991 of the distributions of each species since the last comprehensive survey 30 or so years prior (Wingate 1965). Losos (1996) observed that both *A. leachii* and *A. extremus* had dispersed towards each other, and were

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[17] And these introductions were not limited only to anoles; Bermuda is also the only place in the world (to my knowledge) where someone has attempted to introduce Galapagos marine iguanas. That credit falls to the Bermudian naturalist Louis L. Mowbray, who thought it was a good idea in 1933. Unfortunately, due to the absence of its main marine food source, none survived. His exploits with other Galapagian fauna were more successful: Mowbray was the first person to successful breed Galapagos penguins and giant tortoises in captivity, some of the latter are still housed at the Bermuda Zoo.

[18] Or at least, no evidence has surfaced so far suggesting that it was intentional, although it seems most likely.

tantalizingly close to meeting at contact zone, but had not yet done so (within 250m!). Sean Giery and I returned in 2014 and 2015 to provide the third update in this series to discover what had happened during the next 30 years[19].

We discovered that range expansion at the contact zone of *A. leachii* and *A. extremus* had been asymmetrical; *A. leachii* had invaded the range of *A. extremus*, but this was not reciprocated (Fig 3). This was a curious result, and so we set about attempting to understand the ecological and behavioral mechanisms which may have driven this pattern.
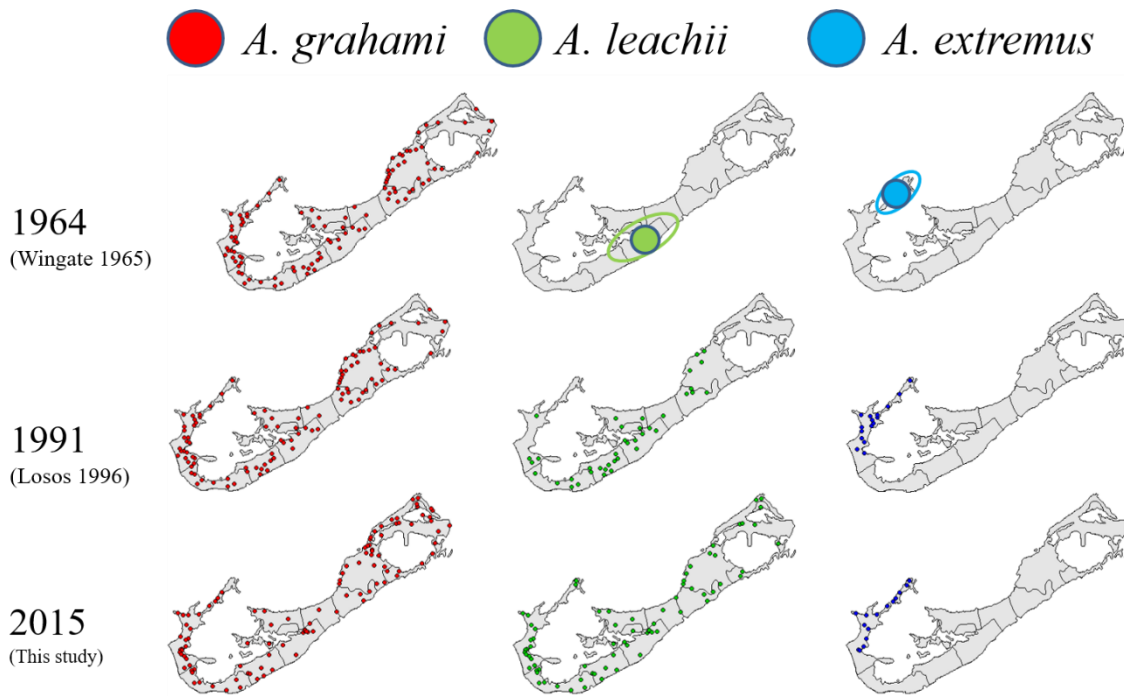

Fig 3. The range dynamics of introduced anoles on Bermuda. 1964 highlights the site of introduction (large dot) and the estimated range (ellipse); at this time *A. grahami* was already found across the island. In the ensuing years, Losos (1996) record range expansion in both *A. leachii* and *A. extremus* although the ranges had not yet met. We returned to see that range expansion at this contact zone had been asymmetrical.

Through detailed assessments of the ecology of each species, we saw that *A. leachii* and *A. extremus* were extremely ecologically similar when existing in allopatry in Bermuda (in other words, when in a community with only *A. grahami*, which is ubiquitous). They overlapped

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[19] It would be unfair to say that we were the only people aware of this; Joe Macedonia had been working in Bermuda for a number of years and was also interested in documenting the range dynamics (Macedonia et al. 2016). Joe was exceptionally helpful, welcoming, and supportive of my research studies in Bermuda.

significantly in all of the major resource axes: perch height, perch diameter, and diet[20]. However, when *A. leachii* invaded communities of *A. extremus* and *A. grahami*, it shifted dramatically (and significantly) to higher perches (and into a region of ecological space under-used by both resident species). Conversely, we observed that *A. extremus* does not change any aspect of its ecology in any community it's found in on Bermuda. These patterns provide support for two things; (i) the role of priority effects in community assembly, and (ii) that niche shifts may alter the outcome of priority effects.

We suggest that priority effects through niche incumbency is displayed by both *A. leachii* and *A. extremus*. In other words, once either spaces occupied a given niche space, it was rendered unavailable to an ecologically-similar invader. However, we observed that ecological character displacement (i.e. niche shift to increased arboreality) allowed *A. leachii* to bypass these priority effects, and therefore influence patterns of coexistence and community assembly (Fig 4; Stroud et al. 2019 [hopefully]). These points form the majority of the formal discussion that resulted from this study, but I will now take the opportunity to discuss the next obvious question from this pattern: why does *A. leachii* shift and *A. extremus* doesn't? I don't have any particularly robust answers, and so here comes some speculation.
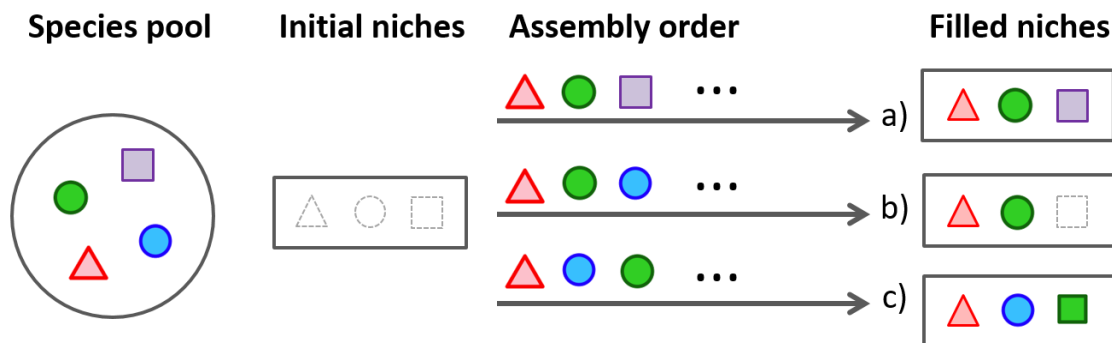

Fig 4. Conceptual representation of mechanisms through which different ecological communities can be formed from the same species pool: i) All species occupy independent niches; ii) An incumbent species blocks an ecologically similar species from joining the community through priority effects by niche pre-emption; iii) Priority effects blocks access to an ecologically-similar species, but niche shifts (i.e. ecological character displacement) facilitates species coexistence and community assembly as each species occupies independent portions of ecological space. Symbol colours indicate different species. Symbol shapes denote the general ecological niche which that species occupies. Open symbols represent vacant niches.

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[20] We should note that we didn't quantatively assess the thermal ecology of all species, instead our coarse qualitative analysis suggested they were similar in that respect too – the lack of more detailed analysis is simply because I hadn't yet garnered enough research funds to buy the equipment necessary to do so.

Perhaps we can draw clues to the differences between species in their degree of ecological lability from their evolutionary history. Those species originating from more diverse communities may have experienced a greater diversity of biotic interactions throughout their recent evolutionary history, and therefore be pre-adapted to mediate biotic interaction to facilitate coexistence[21]. In this example, as *A. extremus* has been isolated on Barbados for ~6my it would, therefore, be presumably less labile than *A. leachii* (which occurs on Antigua and Barbuda with *A. wattsi*), and far less than *A. grahami* (which is from the more speciose Jamaican community; Fig 5)[22]. This unequal degree of ecological lability (think of it as the extent to which a species can be ecologically 'flexible') between species may explain broad patterns in ecological community assembly dynamics and community diversity, and could be an interesting hypothesis to explain non-random macroevolutionary patterns, such as phylogenetic tree imbalance and a clustered community phylogenetic structure.
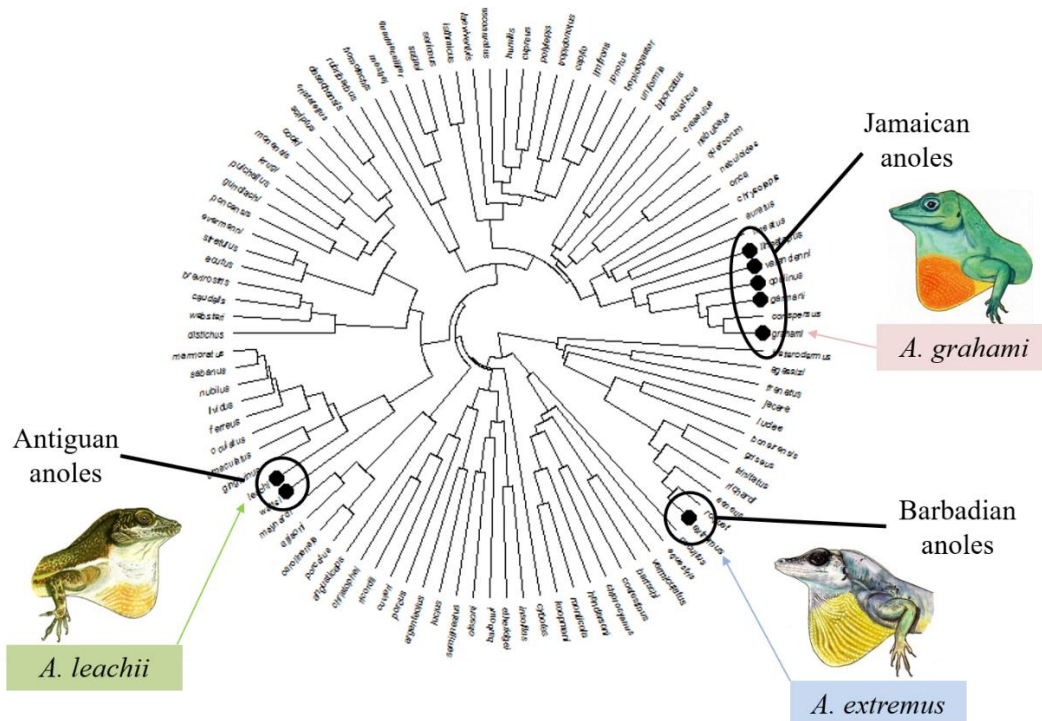

Fig 5. The phylogenetic distribution of each introduced anole in Bermuda, grouped alongside the species with which they co-occur in their native distributions. Anole illustrations are used with permission from Schwartz & Henderson (1985).

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[21] Although the acute readers among you (if anyone has made it this far) will notice this opposes what I had earlier suggested when discussing ecological release.

[22] I should note that this idea has not been well-received at all during the peer review process (as one might expect when throwing an idea out there with little [some journal Editor's may have argued "*no*"…] support). Consequently, as this may otherwise never see the light of day, I think it's a curious hypothesis to float to this forum.

Aside from the unsupported perspectives, this research highlighted two things that deserves further attention in anoles. First, we still don't really understand the role of priority effects in anoles (either ecological or evolutionary). Twenty-five years ago, Losos et al. (1993) investigated how priority effects may influence the outcome of anole invasions. Although this study didn't explicitly refer to priority effects *per se*, it was an investigation into the how niche incumbency might influence the success of contemporary anole invasions. Since then there have been many more anole invasions into a much greater diversity of incumbent communities. This study deserves revisiting[23], and presents an exciting opportunity for further investigation in a burgeoning research area (see Fukami 2015). Secondly, a more comprehensive understanding of character displacement is needed, but especially in how character displacement may operate at range edges or as an ongoing process (perhaps facilitator) during range expansion and invasions. As noted by Losos (2009), and supported (with unashamed bias) by me, south Florida offers great possibilities for doing so[24]. I will now briefly discuss a detailed case study of character displacement from there.

(2.b *Character displacement*)

If we think back to the early stages of adaptive radiation immediately following speciation, those nascent species are expected to be reproductively isolated but may not have diverged in any other aspect of their phenotype (as would be expected if, for example, the two species were allopatric but occupied similar habitats). Upon secondary contact, those species would interact strongly due to the phenotypic similarity, leading to either competitive exclusion or divergence (i.e. character displacement). Through repeated bouts of this process, an adaptive radiation of extraordinary ecomorphological disparity might form. Unfortunately, opportunities to study novel contact zones of phenotypically-similar but reproductively isolated species are rare in the natural world of anoles. The most wonderful experiment to test these hypotheses would be to throw together two species of the same ecomorph and see what happens. However, for all sorts of ethical reasons, this approach is often unreasonable and unattainable.

However, introduced species offer scenarios analogous to these experiments, and I stumbled upon one in Miami (Fig 6). In the late 1970's, the Puerto Rican crested anole (*A. cristatellus*) was introduced to the Pinecrest region in south Miami, which was already home to

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[23] For example, it was suggested that the failure of some trunk-crown-type anoles (*A. ferreus* and *A. extremus*) to establish in Miami was due to priority effects imposed by *A. carolinensis*. Since then, two new trunk-crowns have invaded and become established (*A. chlorocyanus* and *A. allisoni*), suggesting this hypothesis may not be well supported.

[24] The presence of 3 trunk-ground species (*A. sagrei, A. cristatellus, A. cybotes*), 3 trunk-crown species (*A. carolinensis, A. chlorocyanus, A. allisoni*…4 if you include *A. porcatus*), and 2 crown-giants (*A. equestris* and *A. garmani*) provide ample and exciting opportunities for doing so.

several established non-native anoles. However, the introduction of *A. cristatellus* was different. For the first time, a second species of one ecomorph class was entering the community – the Cuban brown anole (*A. sagrei*) had already been present there for many decades. Each of these species, having never coexisted previously and deeply separated in evolutionary time, were members of the trunk-ground ecomorph class. Again, I wasn't particularly original in choosing to study the interaction between these two species – Salzburg (1984) had provided a nice study of the coexistence patterns during its formative years, and Losin (2012) had followed this up with some fabulously detailed behavioural studies. This system was not just unique in that both species occurred sympatrically, but that there also exists allopatric sites within Miami; this presented a wonderful framework with which to conduct a comparative study of character displacement.
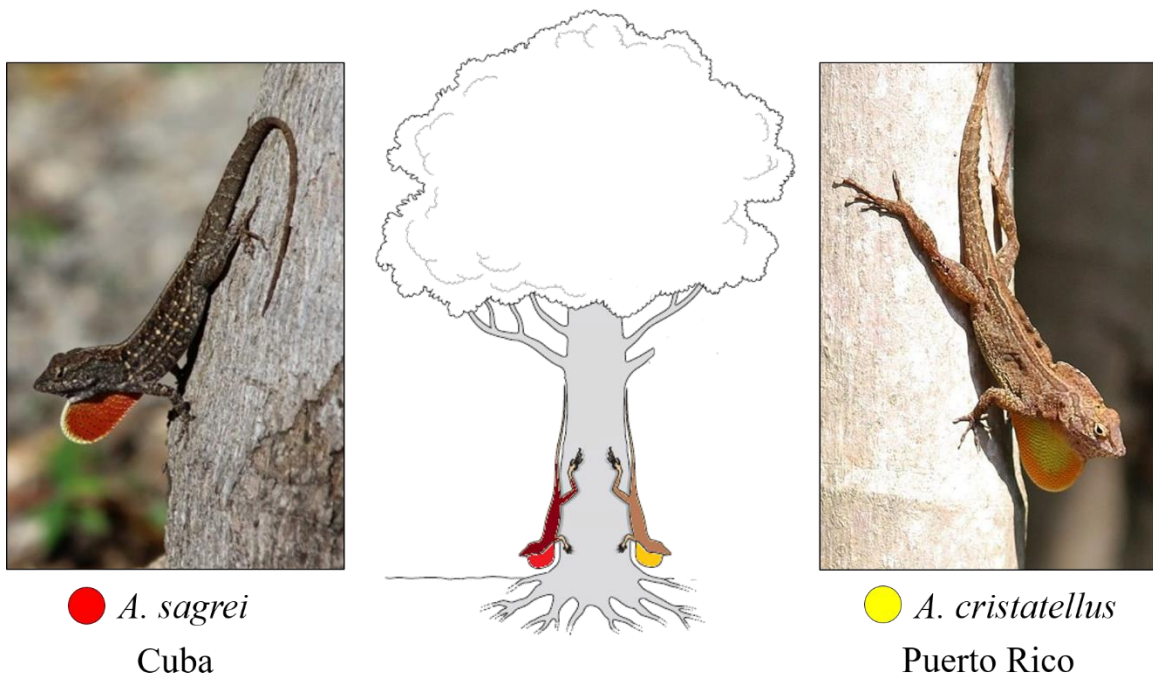

Fig 6. Two introduced Trunk-Ground anoles established in Miami, FL; the Cuban brown anole (*A. sagrei*; left) and the Puerto Rican crested anole (*A. cristatellus*; right).

The presence of ecological character displacement in sympatric communities of *A. sagrei* and *A. cristatellus* in Miami is clear and consistent from very simple data collection on perch use; *A. cristatellus* perches higher (increases in arboreality) and *A. sagrei* perches lower (increases in terrestriality), whereas in allopatry they occupy similar perch heights. Perch height is a common axis along which species partition the environment in anole communities, and it has been repeatedly seen to also occur when previously-allopatric species come into contact[25].

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[25] Yoel Stuart's work on the effect of *A. sagrei* invasion on the ecology of native *A. carolinensis* in Florida is probably the most famous recent example of this (Stuart et al. 2014), but there are also many others.

However, whether this divergence in perch height had shifted the selection regimes that each species encountered, such that it has led to morphological shifts, remained to be seen.

My comparative assessments of morphology showed that both species also had consistent differences between allopatric and sympatric populations. An increase in terrestriality of *Anolis sagrei* led to morphological changes as expected by the form-function relationship in anoles; sympatric populations had fewer toepad lamellae (suggesting relaxed selection on clinging force) and longer limbs (suggesting directional selection for faster sprint speed on broad surfaces, such as the ground). However, I observed no complementary differences in *A. cristatellus* as predicted under this relationship; an observed increase in arboreality did not lead to larger toepads or toepads with more lamellae (as one might expect if an increase in arboreality lead to directional selection for greater clinging force). Instead, the only aspect of the morphology of *A. cristatellus* that showed any significant differences was head size; populations sympatric with *A. sagrei* had significantly smaller heads than those without. Intuitively, head morphology can often be driven by diet. And so, we conducted an extensive and exhaustive assessment of stomach contents of *A. cristatellus* from allopatric and sympatric communities. These investigations revealed no difference in the type or size of prey, the two axes of diet which might lead to differences in head size (for example, larger or harder prey items might need larger heads to managed them). In all cases in Miami, both *A. sagrei* and *A. cristatellus* are generalist invertivores and show little variation among populations[26].

Head shape and size is not only an important predictor of trophic ecology, but many studies (in anoles as well as other lizards) highlight its importance in the light of sexual selection. Larger heads generally bite harder[27] and biting hard can be an important determinant of the outcome of agonistic interactions. Presumably there are fitness consequences associated with winning or losing those interactions. Perhaps a change in the (intraspecific) social landscape of *A. cristatellus* when sympatric with *A. sagrei*[28] has led to a shifting regime of sexual selection, which may explain differences in head size.

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[26] Although we did observe that the source of prey in *A. sagrei* changes slightly; *A. sagrei* eat more ground-dwelling arthropods when sympatric with *A. cristatellus* (presumably as a result of it being on the ground more…).

[27] We conducted performance assessments of bite force of *A. cristatellus* in Miami which support that this is true for this case study specifically.

[28] I can't claim originality for this idea either – Sandy Echternacht presented a beautiful perspective in the *Anolis* Newsletter V (p.23) proposing how the availability of territories for *A. carolinensis* might change due to the presence of *A. sagrei*. This small figure had a profound impact on how I thought about interspecific interactions (and the consequences of ecological divergence).

Fig 7. The progression of an aggressive male-male social interaction between Puerto Rican crested anoles (*A. cristatellus*). Increased head size, which corresponds with increased bite force – an important predictor of success in aggressive combat, might be favored in populations with high levels of intraspecific social interactions.

Fig 8. Bite marks from a conspecific male are apparent on the shoulder/dorsum and forelimbs of this male crested anole (*A. cristatellus*). The agonistic interaction which led to these marks was observed (so their source are known), with the lizard easily noosed as it lay stunned on the ground having been usurped.

To investigate this hypothesis I approached it from two angles; ethological observations of natural behavior (which I am eternally indebted to Sarin 'Putter' Tiatragula for spending long, buggy days helping out with) and analysis of the social networks of marked individuals (an approach I developed with Rob Heathcote). In short, data collected from these two approaches highlighted that when *A. cristatellus* are sympatric with *A. sagrei* they; (i) move between trees in their environment significantly less, (ii) have relatively fewer conspecific interactions with other *A. cristatellus*, and (iii) low conspecific interactions was driven by high interspecific interactions with *A. sagrei*.

Instead, as an indirect effect of increased arboreality, concomitant with decreases in population size and relative abundance, *A. cristatellus* become (intraspecifically) socially-isolated. This has relaxed selection on sexually-important traits associated with intraspecific interactions, specifically bite force, and led to a decrease in head size in sympatry. I suggested that simple ecological character displacement, such as vertical partitioning of habitat as observed here, can lead to phenotypic divergence much more complex than anticipated, and therefore may be responsible for a greater volume of observed phenotypic variation than previously recognized.

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A classic model of character displacement might expect ecological divergence to occur along one resource axis, with concomitant divergence in traits associated with the acquisition of that resource. For example, a divergence in seed size in finches might lead one to expect selection for large bills in the species that shifts to large seeds and small bills in the species that eats small seeds. In anole terms, divergence in perch diameter might lead one to expect selection for longer limbs in the species that shifts to broader perches and smaller limbs in the species that shifts to thinner perches; this is a classic [symmetrical] model of character displacement.

Here, I suggest that apparently simple ecological divergence along one resource axis (perch height) can profoundly impact the new selection regimes facing each species. Following this more complex model, resource partitioning in structural habitat (such as perch use) may drive phenotypic diversification far quicker than previously appreciated.

Conservation implications of introduced species

It is important to be aware that non-native species, whilst occasionally providing exciting – if unintended – opportunities for eco-evolutionary studies, can also negatively interact with native species and pose a conservation threat. It would be irresponsible of any ecologist or evolutionary biologist using introduced species as a model system to not acknowledge this. As a result of my research investigating the range dynamics and assembly patterns of anoles on Bermuda, Sean Giery and I also discovered two independent populations of brown anoles (*A. sagrei*; Stroud et al. 2017)[29]. Bermuda has only one endemic lizard, the Critically Endangered Bermuda skink (*Plestiodon* [*Eumeces*] *longirostris*) (Wingate 1965, Davenport et al. 2001; Fig. 9), which are terrestrial, leaf-litter specialists, and are similar in size and other aspects of their ecology to *A. sagrei*. At present, the populations of *A. sagrei* that we identified are still locally distributed and confined to urban areas where Bermuda skinks are

Fig 9. The Critically Endangered Bermuda skink (*Plestiodon longirostris*), endemic to the Bermuda archipelago and one of the rarest lizards in the world with a total global population of ca. 3,500 individuals. Photographed on Nonsuch Island, Bermuda. Not an anole, but a nice lizard nonetheless.

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[29] I used "discovered" very loosely here – we provided the first official record. Joe Macedonia must take credit for this discovery having posted pictures of Bermudian *A. sagrei* on *Anole Annals*, which led us to seek them out.

not present[30].

We were awarded a small grant from the Bermuda Zoological Society to assess the potential ecological impact that *A. sagrei* might have on Bermuda skinks were they to invade known populations. We conducted detailed assessments of habitat use, diet, population size, and morphology, and concluded (due to high overlap with skinks in all) that *A. sagrei* likely pose a significant conservation threat to Bermuda skinks via ecological resource competition. These findings strongly highlight that continuing to monitor the distribution and ecology of *A. sagrei* on Bermuda should be considered an important aspect of Bermuda skink conservation management. As anoles continue to spread far and wide around the world, I expect this situation to continue to become increasingly more common; this study might provide a framework which others can adopt.

Conclusions

If you have got this far, thank you for sticking with it – those discussions presented an overview of my dissertation research, as well as various topics of current or future research interests (alongside some general commentary on anole ecology and evolution). Broadly, I add to the body of evidence in anole research that character displacement can both facilitate coexistence and drive phenotypic change, therefore strengthening the importance of the process in explaining patterns of ecology and evolution.

I conclude with two points from my introduction to anole biology over the past few years: (i) the utility of anoles as a model system for testing broader hypotheses in ecology and evolution is more powerful now than ever before, in no small part due to the foundational work that so many of you have dedicated your research careers towards, and (ii) there is still an awful lot that we don't know about anoles!

If any of the topics I have highlighted here interest you then please feel free to reach out and get in contact. I would be excited to discuss some of these ideas further, and I am always looking for new research collaborations!

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[30] Incidentally we also described the first *verified record of an American green anole (*A. carolinensis*) on Bermuda, although we presume this was only a single specimen (Stroud et al. 2016). *Verified because there is a record of *A. carolinensis* on Bermuda from an expedition to Bermuda by the American Zoologist G. Brown Goode in 1867 (then erroneously labeled "*A. principalis*"), but no specimen is available for analysis. Either way, there is no evidence that a population did (or now does) exist on Bermuda.

Fig 10. A Jamaican anole (*A. grahami*) displaying at a Bermuda skink (*P. longirostris*) on Nonsuch Island; one of the last large populations of Bermuda skinks in the world. This, of course, has nothing to do with what I have just written, but I thought it's a unique opportunity to point out some distant relatives communicating.

Future directions

In 2018, I started a postdoctoral position in the Losos Lab. The majority of my time will be spent understanding how patterns of natural selection in anole communities facilitates coexistence. This stems from a project in Miami that I have been conducting for multiple years on the introduced species there (although one that I didn't discuss in this contribution). I will attempt to extend this project to include natural communities across the replicated adaptive radiations of the Greater Antilles (specifically; Jamaica, Dominican Republic, and the Bahamas). This project aims to understand the nature of natural selection in anole communities through space and time and will hopefully give some insights into the structure and topography of fitness landscapes in anole communities.

Acknowledgements

Thank you to all of the anole scientists that I have had the pleasure to have crossed paths with so far. You're all talented scientists and great people, and I am thrilled to have stumbled into such an exciting research community. I also want to take this opportunity to thank everyone that was able to attend the 2018 *Anolis* Symposium; your support made the event such a success!

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Thoughts on the ecology and evolution of anoles; insights from 5 years of meandering strolls

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"*When an observer is fortunate enough to see and record behavior significant in the natural history of a species, his observations should be published. The advocates of biometrical methods need to recognize that some types of behavior are not readily quantified because they are so rarely observed. Even a single observation may constitute a valuable contribution, and may be a break-through in understanding the species' ecology. There are many kinds of anecdotes, and the fact that some are trivial is a poor excuse for condemning all narrative statements or accounts in scientific writing.*"
Fitch, H.S. 1987. The sin of anecdotal writing. *Herpetological Review* 18 (4): 68

While conducting my dissertation research in Miami I found myself in a situation not afforded to all graduate students, especially those that choose to study tropical lizards; I was able to live and walk among a rich and diverse community of my study organisms every day. This fortune wasn't frivolous – I found myself indirectly familiarizing myself with anole behaviors, subconsciously tracking activity times, and catching the occasional glimpse of a bizarre interaction, which all added towards my education of anole biology. Any student entering the world of anoles, in whichever of the countless sub-disciplines this remarkable model system now spans, would benefit from this same opportunity. I echo Michele Johnson's thoughts in her contribution to this *Newsletter* that it is still vitally important to better understand anole behavior. Those of you that are the head of your own research labs – encourage your students to spend some time on field trips watching lizards under no obligations or pressure to complete a project or collect data. And, as a call to you students, regardless of your research interests – sit and watch lizards. Learn to take informal field notes and record observations of behavior, ecology, physiological, or morphology, especially those that appear atypical, however seemingly small and uninteresting! *Anole Annals* provides a wonderful outlet for sharing these insights with the research community, as does the *Natural History Notes* section of journals such as *Herpetological Review*.

On that note, here I present some ideas, perspectives, and hypotheses that have crossed my mind over the past few years from some of my wanderings through south Florida – many of which I have little (or no) actual data to support them, but have been the subject of my musings nonetheless.

Character displacement in the crown and the evolution of frugivory

The tree canopies of the Greater Antillean islands are broadly inhabited by three classes of anole ecomorph; Trunk-Crowns, Crown-Giants, and Twigs. Twigs are fairly obscure and unique in their perch use, morphology, and behavior compared to other ecomorphs, and so it is the two former classes that I will focus these thoughts on. Trunk-Crown and Crown-Giant species often appear to overlap in perch use and activity patterns[31], however there is a very obvious axis through which these ecomorphs differ dramatically; body size. Here I will present a hypothesis outlining how this difference in body size between the two ecomorphs may have originally been driven by partitioning in the size of prey items, which was then reinforced by the prey items which fell within the respective size classes as divergence ensued.

Fig 1. A freshly noosed adult knight anole exhibiting the gaping behavior typical for this species upon capture. However, this time the gape comes with a present; a freshly ingested palm fruit. (Fairchild Gardens, Miami FL)

Over the past few years, I have become increasingly interested in the dietary relationships of anoles, leading to several research projects with trophic ecologist Sean Giery. Originally, we had two primary questions of interest; (i) do replicated patterns of ecomorph community organization (e.g. in perch use) extend to diet, and (ii) how does diet vary within species and between populations (Sean has written at length on this in his contribution to this *Newsletter*).

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[31] Of course, this could just be an artifact of it being difficult to study canopy anoles, nevertheless lots of independently collected data generally point towards this being true.

When we first started discussing these topics, Sean surprised me with one of his early findings of anole diets. In a study of an anole community in North Miami, FL (Florida International University, Biscayne Bay Campus; Giery et al. 2013), one of the main items which Sean found in the stomachs of Cuban knight anoles (*A. equestris*) – a large and established crown-giant ecomorph in Florida – was various types of fruit. In fact, Sean found that 50% of all items found in the stomachs of 24 (!) individual *A. equestris* was fruit (Fig 1).

This wasn't what I had naturally expected. From my readings of the classic anole literature, I was under the assumption that the trophic ecology of crown-giants was to be quite different. Various authors have written about the predator-prey relationship between crown-giants and all other ecomorphs, some even suggesting a role for it in the evolution of the ecomorph community structure. And, in *A. equestris* at least, they certainly do eat other anoles. In Miami, we have been keeping track of each time we observe an *A. equestris* chowing down on an unlucky anole[32] (Fig 2). So, perhaps Sean's findings were idiosyncratic to that study site and not representative of the general ecology of crown-giants? So, we set about sampling knight anoles from other communities. To our surprise, we found exactly the same result. Similar to Sean's findings in north Miami, we discovered that ~60% of all stomach items in 10 adult *A. equestris* from

Fig 2. Knight anoles do definitely eat other anoles, but do they do it any more frequently than other species? Here I found a young *A. equestris* ingesting an adult bark anole (*A. distichus*). This was during an attempted mark-recapture project where I had managed to find and mark an astounding…6 bark anoles in my study plot; this lizard had eaten 17% of my bark anole population in one go.

Fairchild Gardens in south Miami were fruit[33]. We were a little stumped. On delving into the

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[32] No surprises here – they eat all of them; *A. sagrei* (multiple pers. obs.), *A. cristatellus* (Ljustina & Stroud 2016), *A. distichus* (Stroud 2013), and even several instances of cannibalism (pers. obs. – Winter Beckles also posted a series of great photos on *Anole Annals* of a cannibalism event he observed in south Miami). Thawley et al. (2017) also observed *A. equestris* eating a house gecko (*Hemidactylus* sp.), while Dalrymple et al. (1980, and references therein) report on them feasting on nestling birds and tree frogs.

[33] The majority of the fruit from Giery et al. (2013) were from fig trees (*Ficus* sp.); conversely, we found the Fairchild population to eat a lot of palm fruits (*Roystonea* sp., among others). This

literature, we were surprised to find more of the same; Brach (1976) recorded fruit comprising ~50% of stomach items, while Dalrymple (1980[34]) observed ~30% (both studies were also from Miami populations).

Our immediate questions pointed towards the ecological importance of this behavior; if *A. equestris* are eating lots of fruits, is it possible that they have a role as seed dispersers? Kirsten Nicholson's excellent work at the nearby campus of the University of Miami (Nicholson & Richards 011[35]) provided us with data on home range size to think this could be a possibility. Nicholson & Richards (2011) discovered that *A. equestris* have average home ranges of ~0.06ha, which would provide ample distance for an ingested seed to move far enough away from a parent tree to avoid parent-offspring competition (i.e. a radius of approx. 14m from a given tree, improving the density/distance dependent mortality relationship as predicted by the Janzen-Connell hypothesis). However, this was all still hypothetical – although we had found lots of fruits in the stomachs of knight anoles, we hadn't yet established if those seeds, once passed, are viable. And so we set about testing this hypothesis.

After collecting several knight anoles, we patiently sat and waited for stomach contents to be passed and discover if seeds were among them. After a few unsuccessful individuals, we managed to retrieve our first seeds passed from a wild caught and naturally foraging knight anole. After examination, these turned out to be from the fruit of the royal palm (*Roystonea regia*), which we frequently found knight anoles in Fairchild Gardens inhabiting. We duly took the seeds, planted them, and waited (again, patiently) to see if they would germinate, neither of us really believing that anoles might actually disperse the seeds of…palm trees. Yet, they sprouted! Who knew crown-giants might play a role as seed dispersers? And of palm trees! To our knowledge, this provided the first empirical evidence supporting the hypothesis of any *Anolis* acting as viable seed dispersers[36] - you can read more about this study in Giery et al. (2017). However, our discovery of widespread and consistent frugivory of *A. equestris* throughout Florida lead me to think about how this might have driven the evolution of large body size in crown-giants.

Fruits are generally large (especially from the perspective of most anoles) and often have a small surface-volume ratio. Therefore, a large intestinal tract is generally needed to consistently digest them (King 1996), as well as to actually pass the seeds themselves. Similarly,

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is probably driven by the composition of the trees at each site, but it's variability also suggests that it is a widespread and flexible component of the ecology of *A. equestris*.

[34] Coincidentally, this study was also conducted at Fairchild Gardens, nearly 40 years before ours.

[35] Data were first presented in the *Anolis* Newsletter V (p. 95-98).

[36] Although frugivory has been recorded in many species and certainly isn't limited to crown-giants (see Herrel et al. 2004 for a much more comprehensive review and discussion).

Table 1. Diet of coexisting Crown-Giant (*A. equestris*) and Trunk-Crown (*A. carolinensis*) anoles; data collected from Fairchild Tropical Botanic Gardens, Miami FL. Values represent proportion of prey items.

Ave. size (mm^3)	Prey item (Taxa)	Crown-Giant *A. equestris*	Trunk-Crown *A. carolinensis*
3143.400	Gastropoda: *Snails*	0.40	-
1202.320	*Fruit*	0.33	0.03
426.506	Lepidoptera: *Adult*	0.07	0.06
167.422	Lepidoptera: *Caterpillar*	0.07	0.13
139.995	Homoptera: *True bugs*	-	0.07
48.939	Hymenoptera: *Bees and Wasps*	0.07	0.11
21.480	Diptera: *Flies*	-	0.14
14.130	Squamata: *Lizards*	0.07	-
3.022	Coleoptera: *Beetles*	-	0.04
2.201	Hymenoptera: *Ants*	-	0.14
1.143	Psocoptera: *Bark lice*	-	0.07
1.042	Arachnida: *Spiders*	-	0.14
0.461	Thysanoptera: *Thrips*	-	0.03
0.196	Arachnida: *Pseudoscorpions*	-	0.01
0.003	Arachnida: *Mites*	-	0.01
	Ave. size prey item (mm^3)	1416.17	42.69

anoles – those which are to be eaten by another hungry anole, that is – are comparatively larger than most arthropod prey and so presumably a larger body size (of the predator) would benefit both ingestion and digestion. Perhaps size differences between trunk-crown and crown-giant anoles were first driven by small divergences in prey size, with the prey items which fell into those classes accelerating divergence in body size. Our dietary analysis of trunk-crown anoles in Florida (*A. carolinensis*) revealed that they generally consume prey items 33x smaller than crown-giants (*A. equestris*), and of a completely different composition (see Table 1 below). As larger bodies better process and digest large prey items, perhaps inital divergence in the diet of crown anoles could have driven character displacement in body size, accelerated by an increasing capacity for frugivory (and, to a lesser extent, predation[37]) in crown-giants. Although

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[37] I choose to highlight frugivory rather than predation because I think this is the most likely *driver*. Nearly all other anoles will also eat other anoles (both conspecifics and heterospecifics), and so that trait is often present across species and ecomorphs. In our studies we have noticed that the consumption of entire fruits, however, is largely constrained to the crown-giants – although other species will forage on fruit, I have most often seen them taking bites from fruit flesh, rather than attempting to consume it whole (seed included).

whether (and how) this occurred depends on the perspective of the ancestral phenotype of Greater Antillean *Anolis*. In other words, if the ancestor was similar to a crown-giant, then perhaps frugivory is a conserved trait, with trunk-crown anoles instead diverging to exploit a niche of smaller prey items (most evidence points to this not being the case).

So what happens when two crown-giants co-occur? I have no idea – as I mentioned before, I find canopy anoles tough to study well[38]. But for anyone interested in tackling the question, All America Park in South Miami may provide the opportunity. Here two crown-giants exist in very close proximity; *A. equestris* and the Jamaican crown-giant *A. garmani* (Fig 3), although the population size of the latter is small and sensitive to periodic collecting by members of the pet trade.

Fig 3. Habitat overlap of two Crown-Giants, the Cuban knight anole (*A. equestris*) and the Jamaican giant anole (*A. garmani*), in South Miami, FL. These two species have probably been sympatric at this site for ~40 years, but their coexistence and interactions have not yet received much research attention. (Photo: March 2014)

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[38] For this same reason, I think it is also difficult to get at the behavioral and ecological mechanisms underlying coexistence in trunk-crowns, especially those newly coexisting pairs which provide particularly exciting opportunities, for example *A. carolinensis*, *A. chlorocyanus*, and *A. allisoni* in Florida.

On diel activity patterns and interspecific interactions

The hallmark of most ecological studies of anoles since the development of the ecomorph model revolves around perch use. Population level patterns in this aspect of anole ecology can be linked to population level patterns in morphology, providing insights into our populations are adapted to different environments (i.e. under the form-function relationship). However, how consistent is perch use within a population? How does habitat use change throughout the day?

As anole communities appear to be largely structured by partitioning of perches, variation in perch use could have profound impacts on how interspecific interactions are understood within a community. For example, although direct behavioral interactions are fairly rare between sympatric *A. sagrei* and *A. distichus* in Miami, FL[39] (*A. sagrei* perch low, while *A. distichus* generally perch higher), there are periods within the day where perch use is highly overlapping (Fig 4; shaded area) versus highly divergent.

The common view that these two species only marginally interact – population level patterns of mean perch use is often consistently significantly different, and they also generally eat different things[40] – could just be a factor of when sampling took place. If perch data from Fig 4 were collected from 1100-1300h (grey shading) instead of 1300-1500h, for example, perhaps conclusions would be very different. The extent to which perch use is fluid vs. static throughout a day is unclear (at all scales – individuals, populations, and species), and deserves more research attention.

The evolution of the nocturnal niche: who is better adapted?

Anoles and geckos have both come to exploit one of the many new anthropogenic niches which exist in human settlements; the night light niche[41]. The illuminating presence of lights at night in urban areas provides the opportunity for lizards to extend activity periods, particularly for foraging (Fig 5). Many ecological, physiological, and evolutionary questions immediately jump out: Are night light foragers exposed to a whole new community of prey species? Are the same individuals active during both the day *and* night? If so, do lizards get tired? Or, are there individuals who are nocturnal specialists? What are the consequences of anole-gecko interactions? Are night light foragers *adapting* to this new niche?

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[39] I base this on not having seen it very often – I more frequently observe *A. sagrei* in confrontations with *A. cristatellus* (both Trunk-Ground anoles), while *A. distichus* (Trunk) and *A. carolinensis* (Trunk-Crown) seem to have a particular penchant for annoying each other. Of course, this isn't saying it doesn't happen.

[40] In Miami, we have found that *A. distichus* eat primarily ants as they stream up and down tree trunks and branches (as in other diet studies of *A. distichus*; e.g. Schoener 1968) while *A. sagrei* is much more of a broad generalist of various leaf-litter invertebrates.

[41]Often now referred to as the 'ALAN' niche ("*Artificial Light At Night*"); Jason Kolbe's lab (and driven by Chris Thawley's recent postdoctoral work) are providing a comprehensive assessment of the impact of ALAN on anoles, but several researchers have been interested by this quirky behavior (e.g. see Henderson & Powell 2001 and Perry et al. 2008, among others).
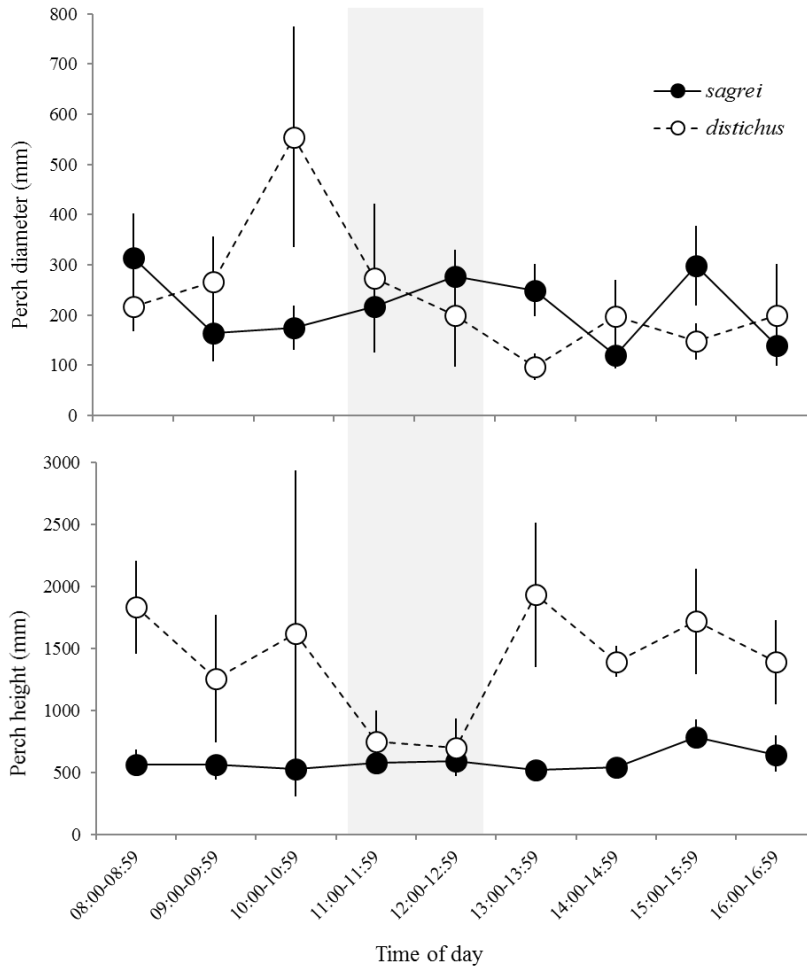
Fig 4. Perch use of Cuban brown anoles (*A. sagrei*; Trunk-Ground ecomorph) and Hispaniolan bark anoles (*A. distichus*; Trunk ecomorph) throughout a continuous sampling session. These data are from only 1 day…because after I finished I promised myself that I would never do this type of sampling ever again (it didn't help that I had the bright idea to do this during a typical 100F Summer day in Miami FL). Error bars indicate +/- 1. S.E.

I will offer an alternative question: who is already better adapted? Anoles are diurnal, geckos are nocturnal. Subsequently, one would presume, each has developed visual apparatus best suited to their respective periods of highest activity[42]; during the day and during the night. These two time periods are at polar ends of the light spectrum.

So when anoles and geckos collect at lights during the night, who is best suited to take advantage of the ensuing barrage of flies, moths, and other inverts? The species which can best observe insects arriving from outside the spotlight, but may be subsequently blinded by the light

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[42] Anoles can be seen in the crepuscular period and occasionally at night, but it's not a general trend. Much like geckos may be seen basking during the day, but it isn't when they are most active.

while scuttling in to forage (nocturnal geckos), or the species which can see less efficiently when outside the light but at an advantage inside (diurnal anoles). Whether there is partitioning within this niche, for example in foraging times or prey items, is also so far unclear. Lots of research opportunities for future anole biologists in the Anthropocene!

Hawaii might offer a comparative test – there, geckos which are adapted to diurnality (aptly named day geckos [*Phelsuma* sp.]) can also be commonly observed gathering and foraging under lights at night (Seifan et al. 2010), often alongside nocturnal geckos (most commonly also *Hemidactylus* sp.). American green anoles (*A. carolinensis*) and Cuban brown anoles (*A. sagrei*) are also present and relatively widespread on Hawaii, with some scattered records of *A. equestris*. Communities of coexisting *Phelsuma* and *Anolis* also exist in the Florida Keys, so another possibility for a study site may also be found there.

Fig 5. A [diurnal] knight anole (*A. equestris*) shares a light at night with [nocturnal] house geckos (*Hemidactylus mabouia*). Photo taken at Fairchild Gardens, Miami, FL (from Stroud & Giery 2013).

Don't dismiss territoriality yet! Seasonal shifts as an adaptive strategy?

As many of you may have been aware, the world of anole mating systems has recently exploded! Anoles have long been thought to display typical mating behaviors and strategies associated with polygyny. In its simplest and strictest terms, the classic model posits that males defend spatial territories to ensure exclusive access to mating opportunities of females within them. Male-male aggressive interactions, which can be casually observed throughout the anole reproductive season, are often used as support for this claim of resource defense (whether that resource be space, females, or both). However, it has long been recognized that multiple males can share space, so a strict notion of male spatial segregation appears unlikely. Since the advent of molecular analyses allowing for the identification of parentage, evidence for multiple paternity throughout 'polygynous' and 'territorial' animals has been growing (Uller & Olsson 2008), including in anoles (Calsbeek et al. 2007). So, if multiple paternity is common, what does that mean for the mating systems that underlie this pattern? Assumedly they are not then strictly polygynous? So why are anoles aggressive? What roles do females have in anole mating

systems? Do females choose males? Do males choose females? The nature of territoriality in anoles – and whether it exists at all – is currently a hot topic in anole biology.

Recently, from an extensive and detailed study of a population of brown anoles (*A. sagrei*) in northern Florida, Ambika Kamath presented evidence linking patterns of space use to reproduction (Kamath & Losos 2018a). Specifically, Ambika noted that during the course of a breeding season females frequently encountered and mated with multiple males, which had a substantial influence on the paternity of their offspring (up to 81% of mothers bore offspring sired by >1 male; Kamath & Losos 2018a). Ambika's thesis was that the concept of anoles operating in a traditional model of polgynous territoriality needed a rethink (Kamath & Losos 2017), which led to a healthy discussion in the literature (Bush & Simberloff 2018 and Stamps, 2018 both wrotes comments on the debate, including a reciprocal response from Kamath & Losos 2018b), as well as many hearty conversations among the non-peer-reviewed world of anole biologists. I encourage everyone to read these papers.

Here, I will suggest an alternative hypothesis in this debate. And I must be clear that this represents nothing more than an untested hypothesis for those studying mating systems – I have no data to support it, these thoughts simply stemming from casually observing lizards throughout the course of a year and therefore spanning both reproductive and non-reproductive seasons. Specifically, I propose that territoriality may be fluid within the breeding season, and that shifts through time from classically polygynous behaviors associated with territoriality, such as mate guarding and defending of space[43], to a relaxation of these behaviors and increased dispersal, may be a viable adaptive strategy that can be evolutionarily stable[44].

I find the maintenance of strictly polygynous territories in anoles unlikely on two counts; (i) it's incredibly costly to maintain a territory (here I use territory to mean the defense of a spatial area with exclusive access to the females that fall within it), and (ii) not all anoles have the same phenotype (i.e. lizards have different personalities[45]). It is important here to note that

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[43] Although these are two different things with different underlying predictions. If they occur at the same time then it's reliant on an underlying assumption that females don't move, which in itself may or may not be correct. If not, then one may not happen due to the other (i.e. if females move and mate guarding occurs then spatial defense must not, and vice versa).
[44] This is similar to the point made by Bush & Simberloff (2018) that the definition of territoriality doesn't explicitly include details about the time period for which a territory may be maintained.
[45] I recognize this is loaded and controversial to some, but I do not see it that way at all. Also, I think that this is true of both sexes alike, not just males. Although I won't talk about this too much here, the extent to which inter-individual variation (i.e. personalities) in social and sexual behavior influence mating strategies deserves more attention. This ties in with the idea of 'territory-holders' vs. 'sub-ordinates' vs. 'floaters' as different male phenotypes, although

most lizards, even those that fall within the tropics, often exhibit temporal cycles of reproduction within a given year. While the structure or duration of these cycles may not always be consistent among species (or even among populations of the same species), for the sake of this perspective I will treat anole reproduction with a simple unimodal model of activity which I am most familiar with observing in south Florida; lizards begin courting in the Spring, copulate in the late Spring through to early Fall, and cease reproductive activities through the Winter. This is consistent with the reproductive behavior I have observed, as well as being supported by temporal patterns of egg production (see Josh Hall's contribution in this newsletter about our ongoing research on this topic).

Fig 6. An adult male Puerto Rican crested anole (*A. cristatellus*) perches close to a female in early Spring as the breeding season commences. Photo taken on 14th Feb 2017.

Could a mating system exist whereby males change mating strategies as the breeding season progresses? In this scenario, males may be classically territorial in the early stages of the breeding season, in other words demonstrate behaviors consistent with being philopatric, spatially defensive, and with high levels of mate guarding, but this then decreases as the season continues. Whether these three behaviors occur independently or in concert is unclear but testable. In this system, males which establish a 'territory' at the start of the season would therefore guarantee sole access to a female or group of females. In turn, this would mean that those males are highly likely to sire the first series of clutches from those females (especially if it represents their first reproductive season), and, by virtue of sperm storage, may also sire future clutches even if/when females mate with other males. In this way, if a male has already guaranteed exclusive mating with one (or a small number) of females, then at some stage – perhaps at the onset of egg-laying – it would be beneficial for the male to relax costly behaviors associated with territoriality and attempt to mate with other females in the population. In this way, male anoles may switch from a conservative (high territoriality) to a diversified (high promiscuity) tactic of bet hedging in mating.

This hypothesis comes with a couple of caveats, some I'm sure that I have missed. Firstly, this model assumes that females don't move. Although female anoles do often have significantly smaller home ranges than males, it's unclear if this is a fair assumption. I know

whether these represent distinct behavioral categories or are more likely points along a behavioral continuum remains unclear.

from my own research that the longest surviving females in my survival study on a population of brown anoles are those which are highly philopatric (the ones which don't survive might also be, so the relationship isn't clear just by that observation alone). Secondly, this also assumes that females will copulate with whichever males they share space; the concept of the 'passive' female. Again, this is unclear and deserves more attention. Thirdly, it would be important to determine the difference in time between when anoles start exhibiting copulatory behavior and when egg laying starts (and how consistent this is between populations). It is possible to test all of these caveats in a well-designed study.
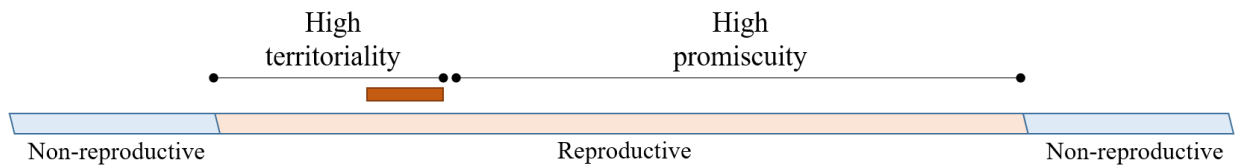
Fig 7. An alternate perspective on the social cycle of reproduction in anoles. If females are collected in the period during (or immediately after) the short dark orange section, representing when egg laying starts in the population, would the ensuing clutches be more likely the result of fertilization from a single male compared to collection at the end of the reproductive season? In other words, is mating with multiple males consistent throughout the entire reproductive season or is there a temporal pattern from one to many?

These ideas stem from casualy noticing that early on in the commencement of the breeding season (here I'll call it the 'courtship phase') mature males can nearly always be found within a very short distance of a mature female, although copulations generally don't yet occur (e.g. Fig 6). I have noticed this for *A. sagrei* and *A. cristatellus* (both Trunk-Ground) and *A. equestris* (Crown-Giant). This behavior dissipates as the breeding season continues. Perhaps this happens for two reasons; (i) as I previously mentioned, maintaining exclusive breeding rights to a female (or females) becomes increasingly more difficult and time consuming (i.e. more costly), and (ii) males which may have been immature at the start of the season develop rapidly through the Summer, bringing with it an increase in male-male sexual competition as the reproductive season progresses. In this model, territoriality may play an important role in anole mating systems and in explaining selection for agonistic behaviors, but the temporally static nature of territoriality should not be one of the assumptions.

Lastly, and kind of related but also kind of not, what is the significance of female aggression? Anyone that has sat and watched anoles for extended periods of time will note that while male-male interactions can be dramatic and showy, females can be equally as quick to aggressively confront a conspecific (I have witnessed females attacking both other females and

adult males!). Ellee Cook's current doctoral research is tackling this topic and promises to provide novel insights into female aggression and associated interactions.

My real impetus for writing this piece on territoriality and mating systems isn't to contribute anything of particular substance, but instead to keep the conversation going among anole researchers. This is an exceptionally exciting phase of research into the social and sexual lives of anoles, and one which I hope continues! The accessibility of newer and more advanced technology aimed at mapping fine-scale movement of individuals could provide an interesting opportunity in this field.

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Ongoing research on the ecology and behavior of *Anolis aquaticus*

Anolis aquaticus is perhaps the most water loving of the semi-aquatic anoles, with a tiny range in southwestern Costa Rica and a sliver of western Panama. For the past few years, I have been fortunate to spend each summer studying this unusual species with the help of a group of minority undergraduate researchers. As I write, we are wrapping up another successful season at Las Cruces Biological Station in Costa Rica. This year, in collaboration with Bree Putman (UCLA), my group and I tackled several questions to get a better idea of *aquaticus*'s basic ecology and behavioral biology.

Figure 24 – Adult male *Anolis aquaticus* (left) on a streamside wall and (right) underwater.

Anolis aquaticus lives at the very edges of premontane and lowland streams and swims readily and often to escape threats. A few highlights of our observations this summer demonstrate that this quirky anole can easily tolerate extended dives of at least 15 minutes, eat aquatic insects possibly while underwater, and may use a diving mask-like bubble to "breathe" when submerged. In addition to nascent projects on these specialized aquatic adaptations, our work this summer included measuring *aquaticus* home ranges and social interactions, identifying intersexual differences in boldness, and testing how deforestation affects the thermal biology of *aquaticus* populations, among other topics. Some early results and project highlights below:

Thermal tolerance – *A. aquaticus* is a thermoconformer and is most active at low daytime body temperatures in the field (~19 – 22 °C). We explored how *aquaticus* fared under short-term high temperature events, which may be experienced by *aquaticus* populations intermittently as the

climate warms. We were particularly interested in how *aquaticus* thermal tolerance related to the land-use history of a population's habitat. We decided to test whether populations living at sites with histories of deforestation have adapted to better tolerate heat stress. Anole populations from sites that were historically deforested for pasture (but are currently in the process of reforestation) should experience higher temperatures due to reduced canopy cover at those sites. As a first step toward testing this larger idea, we focused our study on *aquaticus* from three sites, one in primary forest, another in secondary forest, and the third in an abandoned but partially reforested pasture. We 1) deployed operative temperature models of *aquaticus* to record the range of temperatures experienced by individuals in these populations and 2) measured anole body temperatures in the field to confirm that, yes, *aquaticus* do experience higher body temperatures in the abandoned pasture than in the secondary forest, and those in the secondary forest experience higher body temperatures than those in the primary forest (with a difference of about 2 °C between pasture and secondary, and secondary and primary). Our next finding surprised us. We brought anoles into the lab to test their critical thermal maxima (CT_{max}),

predicting that we'd find that those from the warmest populations would be able to "take the heat" most successfully. We found the opposite: anoles from the primary forest tolerated short-term temperature stress the best, with the highest CT_{max} (30.5 °C), followed by those from the secondary forest (30 °C), and lastly those from the abandoned pasture (29.5 °C). Though our study was admittedly preliminary, with only one site per type, it's suggestive that populations with a legacy of exposure to anthropogenic stress might in turn fare more poorly to high-temperature stress. Or in other words: living in the heat doesn't mean you can stand it.

Figure 2 – Example of the high degree of overlap in *Anolis aquaticus* home ranges; females in pink, males in blue, juvenile in white. Individual IDs listed for anoles without enough points for home range calculation. Bottom scale bar in 10 m increments.

Home range – We conducted home range studies for three *aquaticus* populations as the first step in a multi-year effort to better understand the social structure of this species. More than 450 observations and 150 individuals were identified as part of this study, from which we were able to build 27 individual home ranges with an average size of 22 m². Our primary observations so far suggest a high degree (average of 62%, with a max near 100%) of overlap among lizards of all sexes and age class combinations. We've rarely observed any male or female territorial behavior in the
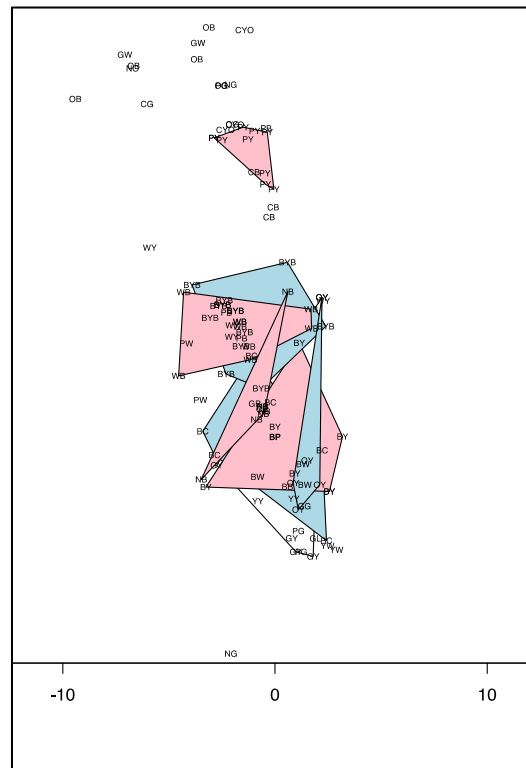
years we've studied this species, which complements the home range findings and seems to suggest that *aquaticus* individuals are fairly tolerant of their conspecifics in these populations. We also found no sex differences in territory size, though larger lizards of both sexes had larger home ranges and utilized higher, more exposed microhabitats. We plan to expand the home range study for the next two years to include additional sites, as site type (i.e., streams within primary forest, secondary forest, and abandoned pasture) appears to affect multiple home range and social overlap parameters.

Diet – In collaboration with Justin Montemarano (Armstrong State), we explored *aquaticus* gut contents to get a better idea of this species' diet. Although it's been speculated that *aquaticus* consumes aquatic prey, no studies of this species' diet have been conducted. In addition to an abundance of non-aquatic prey, one of our more interesting finds included a naucorid, a freshwater insect we've almost exclusively observed underwater. Also of interest were a pseudoscorpion, a crab, and an egg!

Boldness and Activity – In our continuing quest to understand individual and sex differences in this species, we're focusing on *aquaticus* personality. Recently, we identified that *aquaticus* male sexual signals relate directly to boldness, with dewlap size negatively relating to scanning (vigilance) behavior. This summer, we took a step back to begin exploring the wider picture of how sex affects boldness and activity. Our laboratory trials suggest that males exhibit bolder behavior, whereas females have higher levels of activity. Relating this back to our field observations, with males on exposed perches and females spending a good deal of time foraging, helps us generate a clearer idea of sex differences in natural systems.
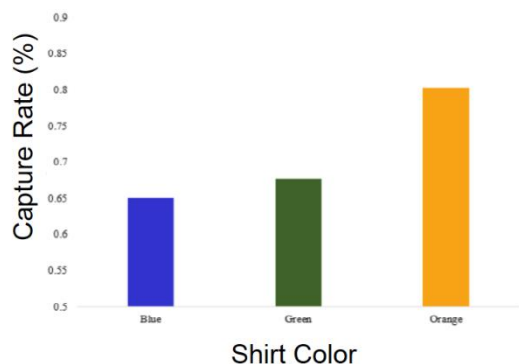

Figure 3 - (Left) Researcher shirt color influences capture rate of water anoles (*Anolis aquaticus*). Right: Part of the team (L. Swierk, D. Lopera, M. Delfin, left to right) wearing the team shirt color of the day. (Photo by S. Walter).

Researcher impact on anoles – We all know that we inevitably change what we study. One student in the group decided to explore just how the observer effect plays out in our field

research on anoles. Her slant was to identify how clothing color affected our anole sighting and capture success, with the hypothesis that wearing a color displayed in *aquaticus* sexual signals (orange) would result in reduced anole disturbance and increased capture rates than would wearing a cryptic color (green) or a novel color (blue). As you can see, she was on to something!

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Beating the Heat: nest characteristics of anoles across suburban and forest habitats in South Miami

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One would have to try very hard to walk down the suburban areas of Miami and not notice the abundance and diversity of anoles! Because these conspicuous lizards are literally everywhere, people rarely stop and observe their charismatic behaviors. But those that do take notice readily see that their social lives are highly complex. Indeed, these lizards are constantly patrolling territories, communicating with each other, and darting at various prey. But one aspect of their biology that is much less conspicuous is their nesting behavior. Even biologists that have devoted countless hours studying their behaviors know very little about where females lay eggs, what microhabitats they prefer, and how those nesting behaviors impact embryonic development.

Anoles occupy diverse environments from dense tropical forests to small arid islands, which poses challenges to nesting females. In addition, as more natural areas are converted into urban areas like in Miami, some species of anoles have found themselves in heavily modified habitats. Urbanized areas are hotter than the forest due to less canopy cover, more impervious surfaces, and heat retaining materials (e.g. concrete). For oviparous ectotherms that lack parental care like anoles, eggs are left to the mercy of prevailing environments (because they cannot move away to find better spots). Hotter temperatures in urban areas due to the urban heat island effect presents a challenge for developing embryos. Yet, anoles are very common in South Miami, with crested anoles (*Anolis cristatellus*) and brown anoles (*Anolis sagrei*) among the two most common species. While previous studies have shown that these species can withstand putative nest temperatures in urban areas (Tiatragul et al. 2017), we still lack data from real nest sites in the wild. Apart from a description of crested anole communal nesting behavior by Stan Rand (1967), no formal study has been conducted to describe the nest sites of crested or brown anoles.

As part of research for a MS degree at Auburn University (for Sarin Tiatragul), we designed an ambitious study that involved searching for crested anole nests in a suburban area ("Red Road" along Snapper Creek and Pinecrest Neighborhood) and a nearby forest (Matheson Hammock Preserve) in South Miami during the peak breeding season (between June and

August). With assistance from an undergraduate colleague (Nathanial Pavlik, University of New Mexico), we randomly sampled plots (1m^2) at both sites in search of eggs (Fig. 1).

Figure 1. A random 1m^2 quadrat laid on the ground to demarcate where to search for nests. Microenvironment variable data were collected from each plot.

We recorded microenvironmental variables (shade cover, distance to closest tree, temperature, and substrate moisture) for every plot, whether it contained an egg or not. We then compared the microenvironment variables between plots that contained eggs (n=22 suburban; 36 forest) to those that did not have an egg (n=29 suburban; 20 forest) as a way to quantify the microhabitat that females choose for nesting. The location of each egg is considered a nest since anoles lay single egg clutches (Fig. 2).

Our data indicated that plots in the suburban site were approximately 13% less shaded than the forest. Plots with nests are usually found close to trees, perhaps indicating that anoles do not venture far to lay eggs on the ground. Mean nest temperatures in the suburban site was 28.4°C compared to 26.8°C in the forest. While maximum temperature reached by a nest in the suburban area was 39.5°C compared to 33.0°C in the forest, there was no difference between the minimum temperature. Temperatures that reach as high as some sites in the suburban area have previously been shown to reduce hatching success in anoles (Hall & Warner 2018; Sanger et al. 2018). Substrate moisture in the suburban site is about 3.4% drier than the forest, possibly due to its negative correlation with temperature and canopy openness (see table 1).

Figure 2. An anole egg found in the suburban site.

Some plots had multiple eggs in close proximity to each other (<50cm apart), particularly in the suburban habitat; this "clustering" of eggs may represent communal nesting, or may be explained by females exhibiting nest-site fidelity, or by different females using preferred microhabitat cues that are limited in suburban areas. These alternative explanations for "nest clustering" warrant further investigation. Furthermore, it is important to note that we only searched the ground for nests. There are multiple accounts (Sexton et al. 1964; Rand 1967; Andrews 1982) of eggs being found above ground (e.g., tree holes) or in areas that might have been excluded by our search protocols. We encourage those who find eggs to document their observations and report it to the community via the Anole Annals blog (or any other appropriate medium).

Table 1. Selected quantitative descriptions of nests between two sites in South Miami.

	Forest				Suburban			
	Mean	Max.	Min.	Var.	Mean.	Max.	Min.	Var.
Canopy openness (%)	9.45	16.42	4.25	11.55	22.77	45.40	5.94	121.70
Distance to closest tree (m)	0.37	0.95	0.00	0.06	1.02	3.66	0.00	0.85
Tree Size (m)	0.26	2.44	0.01	0.15	1.17	3.58	0.02	1.32
Temperature (°C)	26.8	33.0	22.0	0.9	28.4	39.5	22.0	2.4
Substrate moisture (%)	10.0	31.4	2.0	15.0	6.8	26.5	0.2	15.9

This work provides a rare quantitative assessment of anole nesting habitat. Although we do not know the species for each egg found in this study, we suspect that most are from *Anolis cristatellus* due to its relatively high abundance at the field sites compared to other anoles. Generating data on nesting behavior and egg microhabitats in the wild is challenging due to a variety of reasons (e.g., relatively small size/speed of anoles, lack of conspicuous digging marks on the ground as seen in other reptiles). This is probably why very few studies have focused on this topic, and why ours is one of the first to quantify nest sites in the wild. We hope the data we present here will be useful in designing ecologically relevant experiments, which is important considering the roles anoles have played as model organisms in ecology and evolution. Future studies from our group (Warner Laboratory at Auburn University) will focus on the consequences of maternally-selected nest sites compared to random areas in urban habitats. Using programmable incubators, we are currently incubating eggs under temperature regimes that mimic natural nest fluctuations and those in urban areas not chosen by female anoles. If the maternally-selected nest conditions yield high hatching success of eggs (relative to those exposed to conditions that females do not choose), maternal nesting behavior may be a major factor that help embryos beat the city heat and facilitate urban establishment of these lizards.

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Sterility in odd-looking *Anolis mestrei* (Dactyloidae) living in sympatry with trunk-ground anoles

Intrinsic reproductive isolation (independent of ecological context) is believed to be complete among most species of *Anolis*, with premating reproductive isolation considered the main isolating force (Losos 2009). There are only a few examples in this genus where premating reproductive isolation has failed and almost none where interspecific hybrids are viable (Losos 2009). During my masters thesis, I identified a potential example of postmating reproductive isolation in anoles in nature by showing that putative hybrid males between two species of Cuban trunk-ground anoles are largely or completely incapable of producing sperm. If ongoing genetic work confirms the hybrid status of these individuals, this case would represent the first evidence in nature for strong postmating reproductive isolation in anoles due to hybrid male sterility.
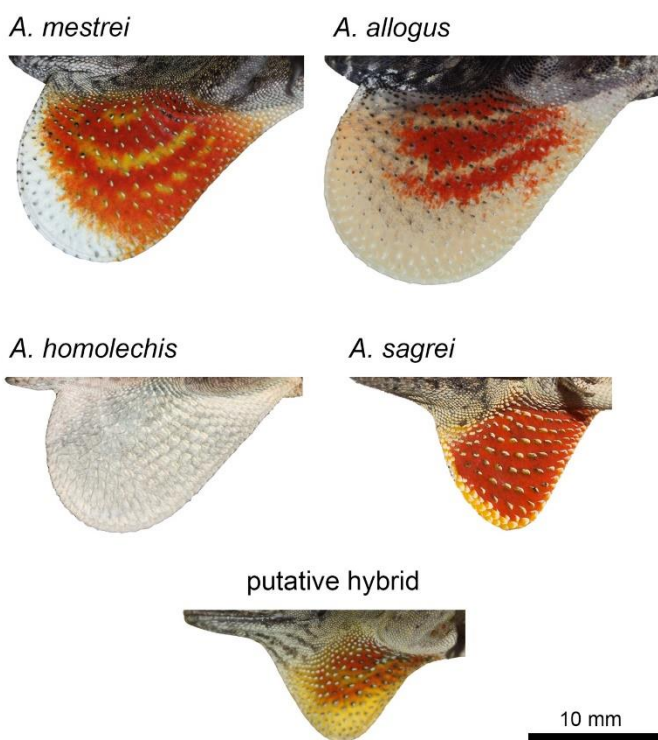
While studying interspecific ecological segregation among trunk-ground anoles in western Cuba, I detected odd-looking representatives of *A. mestrei* in areas where it co-occurs with three other trunk-ground anoles that also belong to the sagrei species group (*A. allogus*, *A. homolechis*, and *A. sagrei*) (Fig. 1). Sympatry is not uncommon between species of the same ecomorph in Cuba, but it is particularly noteworthy among trunk-ground anoles because assemblages of up to five trunk-ground species are not uncommon among the karstic hills of Western Cuba known as *mogotes* (Rodríguez-Schettino et al. 2013).
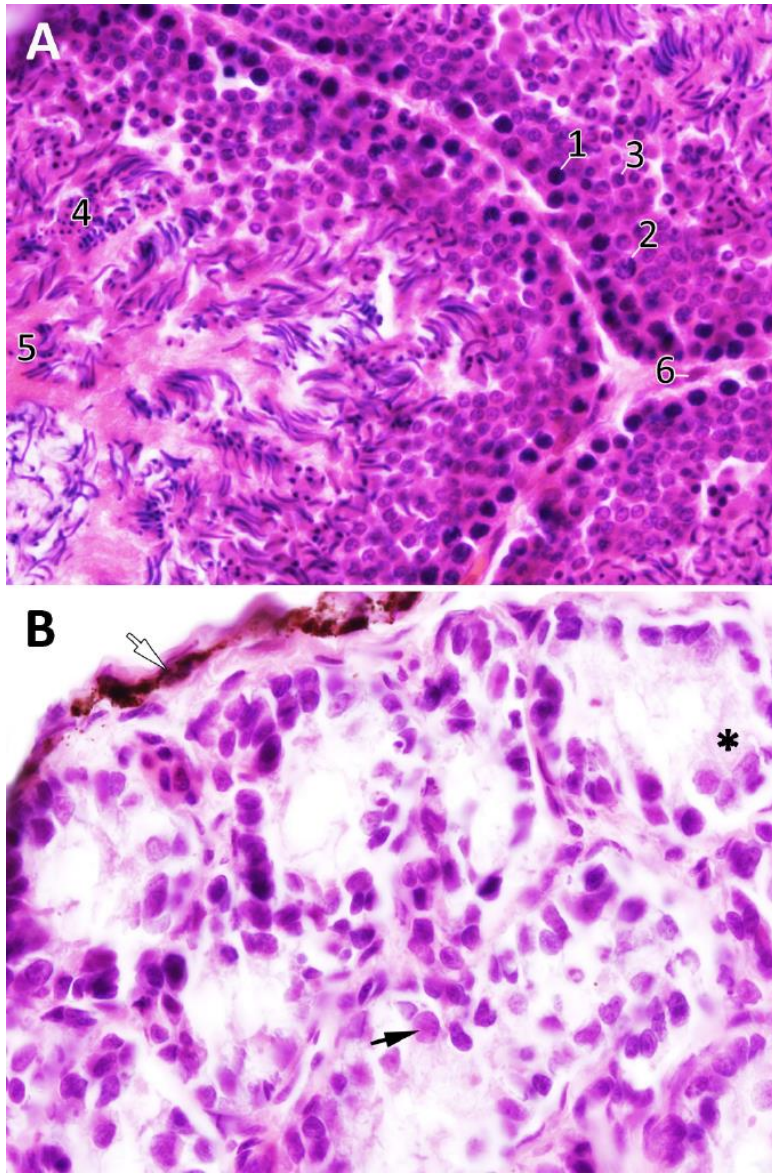

Figure 1. Dewlps of sympatric Cuban anoles in western Cuba alongside the delwpa of a putative hybrid of *A. mestrei* with another *Anolis* species.

Figure 2. Cross-sections of testicles of anoles of the trunk-ground anoles from the Sierra de Canalete, Pinar del Río, Cuba. (A) Anolis allogus: (1) spermatogonium, (2) primary spermatocyte, (3) secondary spermatocyte, (4) sperm cells, (5) lumen of the seminifer ous tubule with secretions from the Sertoli cells, and (6) interstitial cells. (B) Putative hybrid: the black arrow marks a spermatogonium, and the asterisk marks secretions within the seminiferous tubule and the white arrow marks the tunica albuginea. Magnification 1000x. Note differences including (1) disorganization of structures, (2) much smaller size of seminiferous tubules, and (3) lower production of cells and particularly of sperm cells in the putative hybrid individual.

The previously mentioned species segregate by structural habitat (e.g., trunks vs. rocks) and microclimate (e.g., shady forest vs. open sun) (Ruibal 1961, Rodríguez-Schettino and Coy-Otero 1999), but this segregation is incomplete. For example, within the mogotes, *Anolis allogus*, *A. homolechis*, and *A. mestrei* can all be found in a single forest patch. Where they co-exist, I found several males that were not easily identified as belonging to any of these three species due to their relatively small size, small dewlap with an orange or red base and a yellowish marginal band instead the white that is typical in *A. mestrei*, and small testicles.

I hypothesized that these males with unusual phenotypes were interspecific hybrids that would have diminished reproductive fitness. To measure reproductive fitness, I first examined the germinal epithelium of the odd-looking *A. mestrei* individuals and classified it as normal or abnormal by comparing its structure with that of sympatric trunk-ground anoles (*A. mestrei*, *A. allogus*, *A. homolechis* and *A. sagrei*). Second, I counted the different cell types in a standardized section of the seminiferous tubules and epididymis and compared the different trunk-ground species with

the odd-looking *A. mestrei*. To test the hypothesis, I acquired 34 adult males, including six odd-looking and six normal *A. mestrei*, seven *A. allogus*, eight *A. homolechis*, and six *A. sagrei* from the area of sympatry. I obtained all samples between June and August in 2009 and 2011, at the peak of Caribbean anoles reproductive activity (Licht and Gorman, 1970).

I found that all of the putative hybrids had small gonads, an abnormal germinal epithelium and lower cell counts. The abnormal germinal epithelium was structurally chaotic and had spermatogonia only (Fig. 2), the basal-most cell type directly related to reproduction in males (Torres in prep.). To my knowledge there are no male fertility studies in anoles, but the traits diagnosed in these individuals are associated with male sterility in other vertebrates (e.g., Dixson et al. 2004, Good et al. 2008). If what it is occurring in this situation is interspecific hybridization, this system would provide an opportunity to study the genetics of postmating reproductive isolation in *Anolis* through the application of genomic and/or transcriptomic tools. I am currently using molecular genetic methods to test the hybrid status of these unusual male individuals.

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Are anole appetites altering ambient ant assemblages?

Many island-dwelling *Anolis* regularly consume large numbers of ants, including the terribly invasive and highly endearing brown anole, *Anolis sagrei*. Over the course of my dissertation research into the food web effects of invading *A. sagrei* in Florida (Turnbough 2016), I grew increasingly interested in the causes and consequences of such ant predation. Below I briefly explore each of these topics, highlight some relevant findings from my work in Florida, and outline plans for further research on *Anolis*–ant interactions.

Why do anoles eat so many ants?

Given that ants often possess chemical defenses or venom-armed stings, why do anoles eat them? Further, why does this interaction appear to occur most frequently in island habitats? Several factors likely influence patterns of *Anolis* ant consumption, including anole body size and microhabitat use, optimal nutrition intake strategies, differences in ant palatability, relative and absolute ant abundances, and total availability of arthropod prey; I focus here primarily on ant palatability and prey community characteristics.

Many of the ants consumed by anoles may be highly palatable prey. Not all ant defense strategies and mechanisms are likely to be equally effective in deterring anole predation, and some might be quite ineffective. *Pheidole* ants, for instance, rely almost exclusively on physical force and the enlarged mandibles of their soldier caste for offensive and defensive interactions, which is probably a fairly useless strategy against *Anolis* predators that are orders of magnitude larger in size (Fig. 1). Prey choice experiments indicate that *Pheidole* are indeed palatable prey for anoles (Vogel and von Brockhuzen–Holzer 1984), and the limited data that are available suggest that *Pheidole* may comprise the vast majority of ants consumed by at least one species— *A. sagrei* (Norval et al. 2011, Giery et al. 2013; see also Stroud et al. 2017). By contrast, the majority of ants consumed by *A. distichus* may be relatively unpalatable (e.g., *Camponotus* spp.), though it seems unlikely that this would be the general case among anoles as *A. distichus* appears to specialize to a degree on ants (Schoener 1968, Cullen and Powell 1994, Cast et al. 2000, Giery et al. 2013). Because ant genera are rarely reported in published anole diets (and further because their palatability has rarely been assessed), whether there are broad patterns in the palatability of *Anolis* ant prey remains unknown.

The frequency at which anoles feed on ants, palatable or otherwise, is likely to be partially determined by the general availability of larger, more preferred prey. Anoles often

preferentially select larger arthropod prey, and when the availability of such prey is limited, anoles may compensate by consuming greater numbers of smaller arthropods (Sexton et al. 1972, Andrews 1979, Floyd and Jenssen 1983). Since ants typically comprise a large fraction of the small arthropods available to anoles, a dietary shift towards greater numbers of smaller prey is likely to increase ant consumption, especially if palatable ants are available. However, even relatively unpalatable ants may be consumed if palatable prey abundances are sufficiently low. For example, Stamps et al. (1981) found that juvenile *A. aeneus* consumed more ants, including "forms noted for their defenses (e.g., *Solenopsis*)", during the dry season, when total prey abundances were diminished to the extent that they constrained anole growth rates (Stamps and Tanaka 1981). Higher levels of ant predation have also been observed during the dry season for *A. cupreus* and immature *A. opalinus* (Fleming and Hooker 1975, Floyd and Jenssen 1983), but in these and most other *Anolis* dietary studies, prey availability was not assessed.
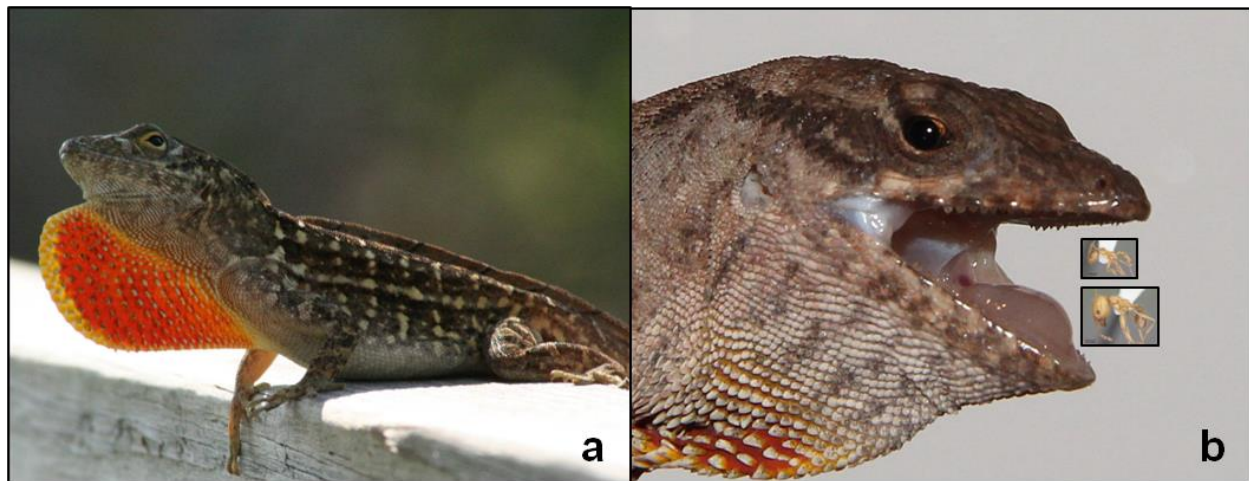

Figure 1 Male brown anoles (*Anolis sagrei*) demonstrating (a) territorial display behavior and (b) the futility of *Pheidole* mandibular defense against anole predation. *Pheidole* worker (top ant) and soldier (bottom ant) image sizes are scaled to the size of the anole.

Andrews (1979) may have been the first to draw attention to the fact that West Indian anoles tend to consume far more ants than their counterparts on the Central and South American mainland, at least in tropical lowland environments. Her comparisons of *Anolis* populations and arthropod communities in similar habitats (cacao plantations) on Grenada and the Costa Rican mainland led her to postulate a mechanism for the phenomenon, namely that higher population densities (due to a lack of predators) and lower large arthropod prey availability cause island anoles to be food limited, and as a result they consume greater numbers of smaller, non-preferred prey, including ants (Andrews 1979). Under this hypothesis, less dense mainland anoles are generally predator limited and have the "option" of consuming relatively abundant large, high-value prey. Although Andrews provided strong supporting evidence for this mechanism, she did not assess the degree to which island and mainland ant faunas were similar (or dissimilar) in

composition. Thus it is possible that island–mainland differences in the relative or absolute abundances of palatable ants may be at least partly responsible for the observed dichotomy in ant consumption frequency (though I have not yet searched the literature to evaluate the likelihood of such a possibility). To my knowledge, ant predation frequencies of island and mainland *Anolis* have not been compared in any other published study.

Further insight into the mechanisms governing ant consumption patterns might be gleaned from within-species, across-locality dietary comparisons, particularly for widely introduced *Anolis* species. Across-locality dietary data are currently most available for *A. sagrei*, and they appear to support an island–mainland dichotomy in ant consumption levels (Fig. 2). Before exploring these data further, it is important to note the relative crudeness of the comparisons: the source studies vary in sample size ($n = 5–502$), collection effort and protocol (e.g., number of collection days, months, and years), sex ratio (adult males only versus a mix of adult males and females), and habitat (from natural and densely vegetated to open and highly disturbed).

The available *A. sagrei* dietary data permit a test of Andrews' (1979) ant predation hypothesis, insofar as landmass area negatively correlates with anole density and corresponding food limitation. Larger islands tend to have more species of anole predators, which may correspond to increased anole mortality rates, lower *Anolis* densities, and reduced anole food limitation (Schoener and Schoener 1978, Lister 1981, Schoener and Schoener 1982, Moermond 1983, Waide and Reagan 1983; but see Wright et al. 1984). As expected under Andrews' hypothesis and a landmass area–food limitation relationship, the proportional representation of ants in *A. sagrei* diets tends to decrease with increasing island or landmass area (Fig. 3a). This pattern is driven by a tendency for *A. sagrei* to consume more ants on smaller islands (Fig. 3b), as the mean number of non-ant prey does not appear to be associated with landmass area (Fig. 3c). The apparent negative association between landmass area and total prey number (Fig. 3d) therefore reflects ant predation levels. Quite interestingly, the number of non-ant prey consumed tended to be lower (and thus the proportional representation of ants in the diet higher) where *A. sagrei* has been introduced. This difference may be even stronger than it appears, as all five of the data points that incorporated dietary data from both male and female anoles came from studies in *A. sagrei*'s invaded range, and adult female *A. sagrei* consumed more non-ant prey on average than did adult males in studies permitting the comparison (Schoener 1968, Campbell 2000). Speculation into possible causes for the difference would probably be premature at this point, given that the source studies (1) vary greatly in design and habitat setting and (2) mostly lack measures of prey size or volume that could be important for deriving or excluding hypotheses. Perhaps the publication of additional *A. sagrei* dietary data in the near future will facilitate a more rigorous comparison of ant predation patterns.
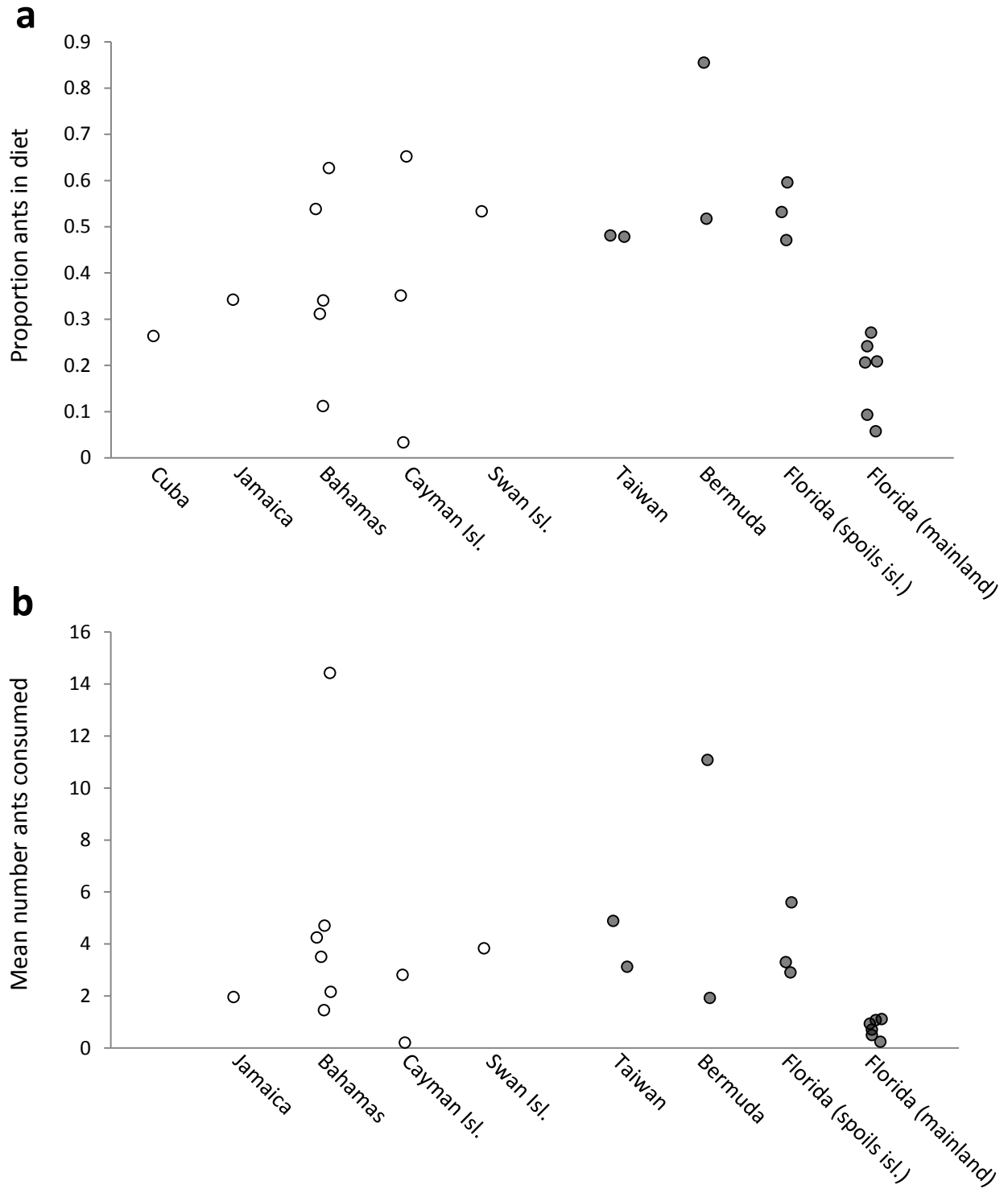
Figure 2 (a) Proportion and (b) mean number of ants in *Anolis sagrei* diets (gut content analyses) for sampled populations in the lizard's native (open circles) and invaded (shaded circles) ranges. Data extracted from Schoener 1968, Lister 1976, Berovides-Álvarez and Sampedro-Marín 1980*, Campbell 2000, Huang et al. 2008*, Wright 2009 (corrected data tables, pers. comm.), Norval et al. 2010*, Giery et al. 2013*, and Stroud et al. 2017*; enclosure experiment data were excluded. Where possible, data were extracted for adult males only; asterisks (*) denote studies yielding data derived from adults of both sexes. Empty stomachs and non-prey items (e.g., plant debris) were excluded from proportion calculations.
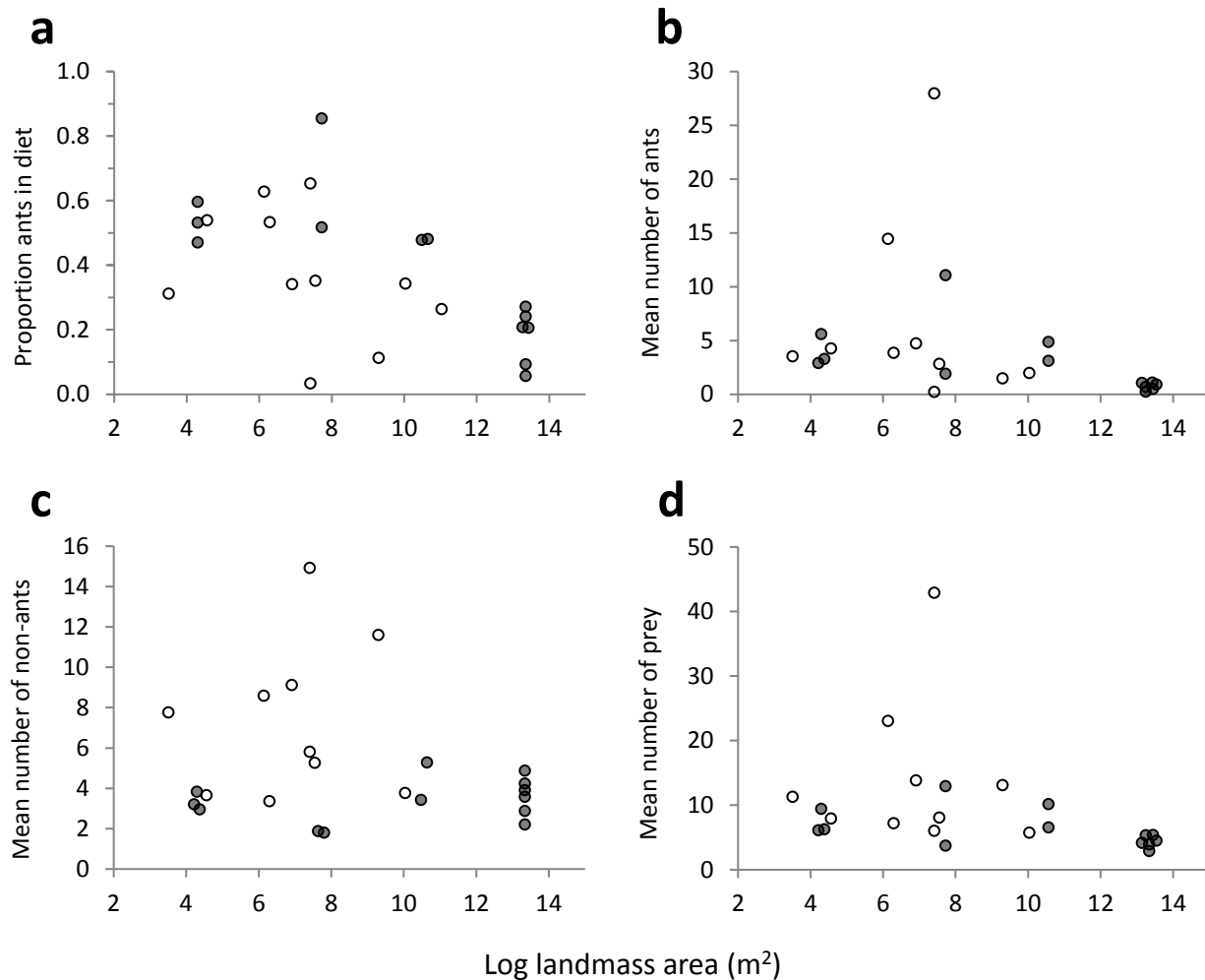
Figure 3 Log_{10}-transformed island or landmass area versus the (a) proportion of ants and mean numbers of consumed (b) ants, (c) non-ants, and (d) prey in *Anolis sagrei* gut content studies. Open circles = native range, closed circles = invaded range. Data as described in Fig. 2. Landmass area for mainland Florida was taken to be that of the North American continent. A small amount of log area scatter was added to overlapping data points in order to increase their visibility.

Can anoles affect ant assemblages?

If anoles differentially prey on some subset of the ant species in an assemblage, for example the most palatable species, then it stands to reason that they could affect the composition of that assemblage. Interspecific competition is a hallmark of ant community dynamics, and ants disadvantaged by greater vulnerability to anole predation may be outcompeted or displaced by other ant species that do not suffer this additional source of worker mortality. Assuming differential susceptibility to anole predation among ants, factors likely to increase anole impacts on ant assemblages include higher *Anolis* densities, lower abundances of

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non-ant preferred anole prey (e.g., large arthropods), and a stronger competitive milieu amongst ants.

To date, potential anole effects on ant assemblages have been reported in only one study. Huang et al. (2008) found that introduced *A. sagrei* reduced the abundance of dominant *Pheidole* species by 45% in experimental enclosures located within a Taiwanese palm plantation. Although the authors could not rule out the possibility that *Pheidole* were simply avoiding enclosures with anoles (which could also lead to their decline via reduced access to food resources), their results are consistent with predation-induced losses, especially considering that *A. sagrei* appear to selectively prey on *Pheidole* ants (see references above and Turnbough 2016 p. 177). My dissertation research supplies additional evidence that *A. sagrei* may affect the composition of syntopic ant assemblages.

Covariation in ant assemblage composition and *A. sagrei* abundance on spoils islands

One of the more interesting and surprising findings of my work on *A. sagrei* food web effects in Florida was the degree to which spoils island ant faunas covaried with the abundance of invading *A. sagrei*. Brown anole abundance significantly explained 15.6–21.8% of the variation in the composition of ground-active ant assemblages in open (xeric) and forested island habitats, and it remained a significant predictor for xeric ant assemblages even after entire sets of environmental and spatial covariables were forced into the model (Fig. 4).

For ground-active ants, the primary species-level associations driving the overall assemblage-level covariation patterns were negative associations between the abundances of *A. sagrei* and large *Pheidole* species (*P. dentata* and *P.morrisi*) and positive associations between *A. sagrei* abundance and the abundances of *Solenopsis invicta* (red imported fire ant, RIFA) and *Brachymyrmex* ants (Turnbough 2016). Negative associations between *A. sagrei* and large *Pheidole* abundances were best explained as the direct effects of *A. sagrei* predation: brown anoles appear to preferentially select *Pheidole* ants as prey, and (literature-based) back-of-the-envelope sorts of calculations suggest that mature *Pheidole* colonies could lose 22–39% of their worker populations to *A. sagrei* predation on a monthly basis (Turnbough 2016, pp.65, 174–181). Positive associations between *A. sagrei* and RIFA abundances appear to have been rooted in contingency: brown anoles and RIFA both tended to invade, through different mechanisms, those islands that were closest to boat launches (Turnbough 2016, pp. 164–167). It is also possible that *A. sagrei*-induced reductions in *Pheidole* densities contributed to the *A. sagrei*–RIFA association via a reduction in ant-based biotic resistance to RIFA colonization (Turnbough 2016, pp. 181–183). Although *A. sagrei* could plausibly indirectly affect the abundance of *Brachymyrmex* ants by directly altering the abundance of their predators or competitors in ant or spider assemblages, I found no evidence that such a mechanistic pathway was operating to produce the observed positive association between *A. sagrei* and *Brachymyrmex* abundances (Turnbough 2016, p. 190).

I expect to gain a better understanding of—and stronger evidence for—*A. sagrei* effects on spoils island ant faunas from two field experiments that I have completed but not yet analyzed, each involving population-level manipulation of anoles on small islands.

Further research

Over the next few years I plan to further investigate the impacts of invading *A. sagrei* on resident ant faunas in the southeastern US. Initially this will probably involve a combination of (1) feeding trial experiments to assess the palatability of various ant species, (2) gut content studies to determine which ant species brown anoles are consuming, and (3) ant assemblage sampling to establish the availability of potential ant prey and assess whether the ant faunas of invaded and uninvaded sites differ in predictable ways.

A closing plea

The vast majority of published anole diets resolve prey only to the ordinal level, but that level of taxonomic resolution is simply too coarse to provide much useful ecological information for many orders (e.g., Coleoptera, Hemiptera, and Araneae). As a worker experienced in attempts to relate the patterns of anole trophic interactions to their consequences (Turnbough 2016), I implore researchers conducting gut content studies to resolve and report prey down to the level of family for at least the most ecologically diverse orders. Although resolving to family will entail some additional time and effort, the return in ecologically useful information should be large relative to the amount of extra effort expended. Resolution of prey to genus or even species would of course also yield valuable ecological information, but in most cases such an endeavor would likely involve a large amount of additional effort for a relatively small gain in useful information. Exceptions to this family-level rule of thumb should be made, when possible, for ecologically diverse families that tend to be highly represented in anole diets (e.g., Formicidae) and genera or species that are easily identified (e.g., *Gryllus* crickets and *Argiope* spiders). It is not necessary to resolve all prey down to the same taxonomic level, and electronic supplements provide an excellent medium for disseminating taxonomically expanded dietary data. You might be surprised by the ways your data can be helpful to other workers. For example, I doubt Giery et al. (2013) could have anticipated that the high-resolution gut content data they published (via electronic supplement) would provide the key information I needed to infer that *A. sagrei* preferentially prey on *Pheidole* ants, and therefore that this interaction was likely the direct cause for the near absence of *Pheidole* on islands where *A. sagrei* were abundant (Turnbough 2016). I hope that greater taxonomic resolution in anole gut content studies will foster many more instances of unforeseen benefits in years to come.
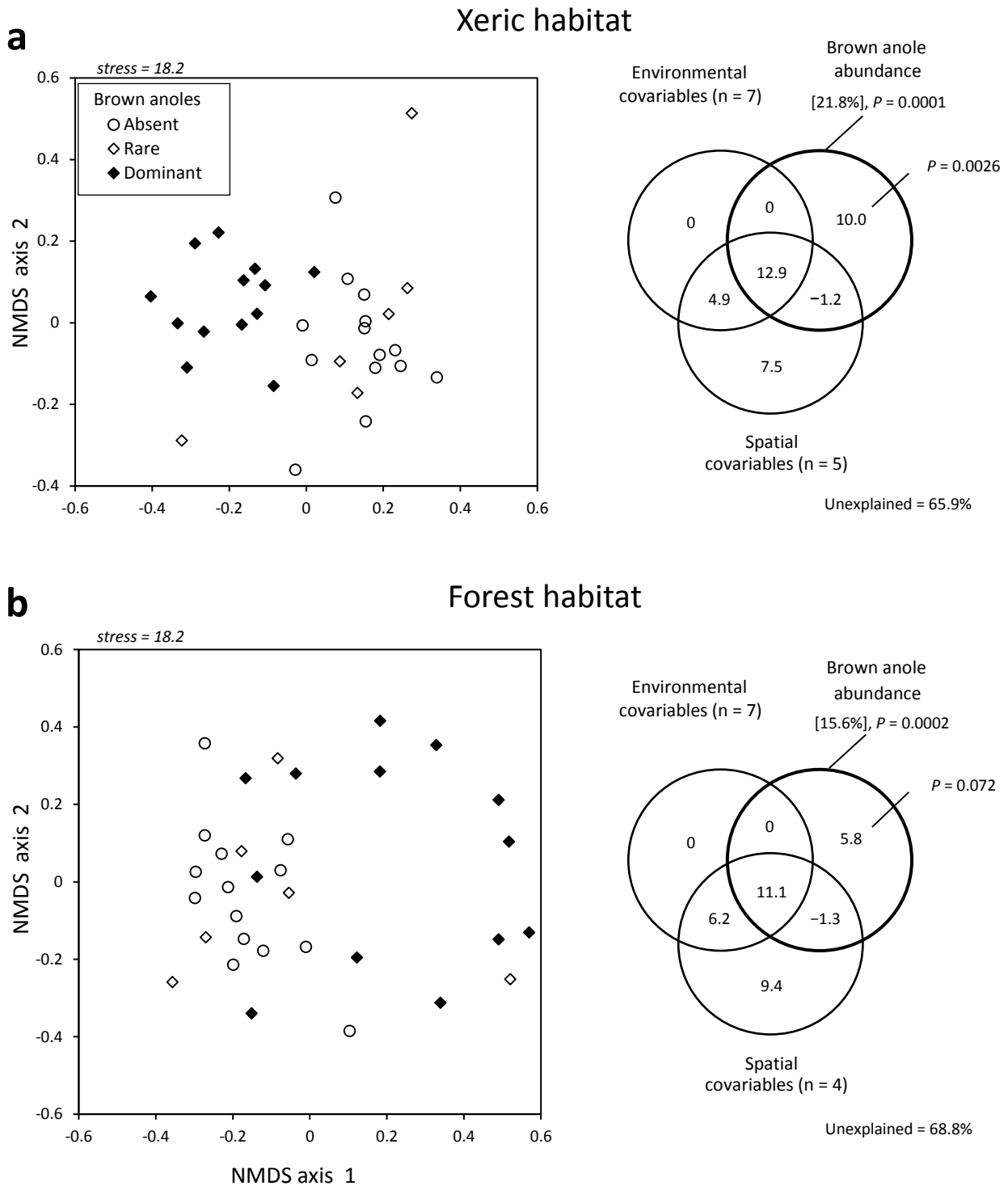
Figure 4 Non-metric multidimensional scaling (NMDS) plots and partitioned variation fractions (% explained, 100*adjusted-R^2) for ground-active ant assemblage dissimilarities in (a) xeric and (b) forested island habitats. Data preparation and analyses as in Turnbough (2016). Invasion class assignments in NMDS plots are purely heuristic; log-transformed brown anole abundance was used in all analyses. Venn diagrams partition explained variation in ant assemblage composition (Bray–Curtis dissimilarities) into fractions jointly explained and fractions uniquely explained by brown anole abundance and environmental and spatial covariable sets. P-values derived from permutation tests.

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Inter-specific predation between two eco-morphologically similar *Anolis* lizards

Cuban brown anoles (*Anolis sagrei*) were first introduced to Miami in the 1950s (Kolbe et al. 2005). Since their initial establishment they have dispersed rapidly and are now present throughout south Florida (Kolbe et al. 2007). Puerto Rican crested anoles (*A. cristatellus*) were first introduced to the Pinecrest/South Miami neighborhood in south Florida in the 1970s (Salzburg 1984), on the Snapper Creek canal on Red Road in Pinecrest, approximately 1km from the Fairchild Tropical Botanic Gardens. Since introduction, *A. cristatellus* have spread radially from this point of initial establishment throughout Miami-Dade county (Kolbe et al. 2016).

Trunk-ground ecomorphs, of which *A. cristatellus* and *A. sagrei* are both categorised, are generalist insectivores, mainly consuming leaf-litter arthoropods (Schoener 1968, Giery et al. 2013). However, intra-specific predation (i.e. cannibalism) of smaller individuals has been observed in both species (*A. sagrei*, Cates et al. 2014, JTS *pers. obs.*; *A. cristatellus*, Campbell et al. 2018), which suggests the consumption of vertebrates occurs, although possibly only opportunistically during seasonal periods when appropriate sized prey are available (e.g. during the emergence of hatchlings in mid-late Summer).

Here, we report the predation of an adult female Cuban brown anole (*A. sagrei*) by an adult male Puerto Rican crested anole (*A. cristatellus*) in the Fairchild Tropical Botanic Gardens, Miami FL USA (25.677°N, 80.276°W). On May 14th, 2018, at 1300h, an adult male *A. cristatellus* was caught by KW using a telescopic fishing pole (Cabelas Inc). Upon inspection, we noticed a prey item half-ingested in the mouth of the *A. cristatellus*. Following a gentle stomach massage, the prey item was fully regurgitated and determined to be a female *A. sagrei*. The prey item had been ingested head first. The female *A. sagrei* was an adult of reproductive size (i.e. >35mm svl; JC Lee 1989) and measured 42mm svl, while the male *A. cristatellus* measured 63mm svl (see Fig 1. for a size comparison photo with scale bar).

We then compared the size of the predated *A. sagrei* to a large data set of body sizes (snout-vent lengths)of *A. sagrei* within the community located in Fairchild Tropical Botanic Gardens (Fig 2). The comparison revealed that the female *A. sagrei* was not abnormally small, but rather only slightly smaller than the median body size. The size of the male *A. cristatellus* was similarly compared to a data set of body sizes (snout-vent length) of male *A. cristatellus* within the community (Fig 3). The captured *A. cristatellus* was also slightly below the median svl. Neither the *A. cristatellus* or *A. sagrei* were unusual in size. Therefore, predation upon other *A. sagrei* by *A. cristatellus* may be possible.

Figure 1. Size comparison of male *A. cristatellus* (top: larger lizard, 63mm svl) and ingested female *A. sagrei* (bottom: smaller lizard, 42mm svl). Digital calipers set to 50mm included for scale.

It was previously presumed that the relationship between adult *A. cristatellus* and *A. sagrei* in Miami FL was only agonistic and competitive (Salzburg 1984, Kolbe et al. 2016, Stroud 2018). However, this observation establishes the existence of a predatory relationship between *A. cristatellus* and *A. sagrei* that had not been previously observed. Predation is a much more powerful biotic interaction in driving changes in behavior and ecology, with the frequency of predation events not needed to be high for a behavioral response to be elicited by prey species. The extent to which this relationship is symmetrical (i.e. do large male *A. sagrei* predate small female *A. cristatellus*) is unclear and deserves further study.

Although more observations are needed to determine the relative frequency of such a predation event, it is possible that the uncovering of this relationship between *A. cristatellus* and *A. sagrei* could be important in explaining observed ecological shifts of *A. sagrei* when coexisting with *A. cristatellus* (Salzburg 1984, Kolbe et al. 2016, Stroud 2018). Specifically, when sympatric with *A. cristatellus* in Miami FL, *A. sagrei* perch lower and are more frequently

found on the ground than when allopatric (Stroud 2018). As A. cristatellus continues to disperse throughout Miami, new sympatric communities of *A. cristatellus* and *A. sagrei* are forming where previously only *A. sagrei* occurred. These novel communities may be especially important in understanding how rapid the nature of this intraguild relationship is, or if it only presents after prolonged sympatry. Similarly, further effort should be afforded to understanding the full extent of intraguild predation between all members of sympatric anoles (in both natural and non-native communities). Although studies of anole diet and trophic ecology were pioneered in the 1960's and 1970's (e.g. Schoener 1967, 1968, Roughgarden 1972, 1974, Lister 1976), little development has been made to understand the ecological and evolutionary importance of diet in anoles past just recording diet contents. This topic is considered particularly valuable to future anole research.
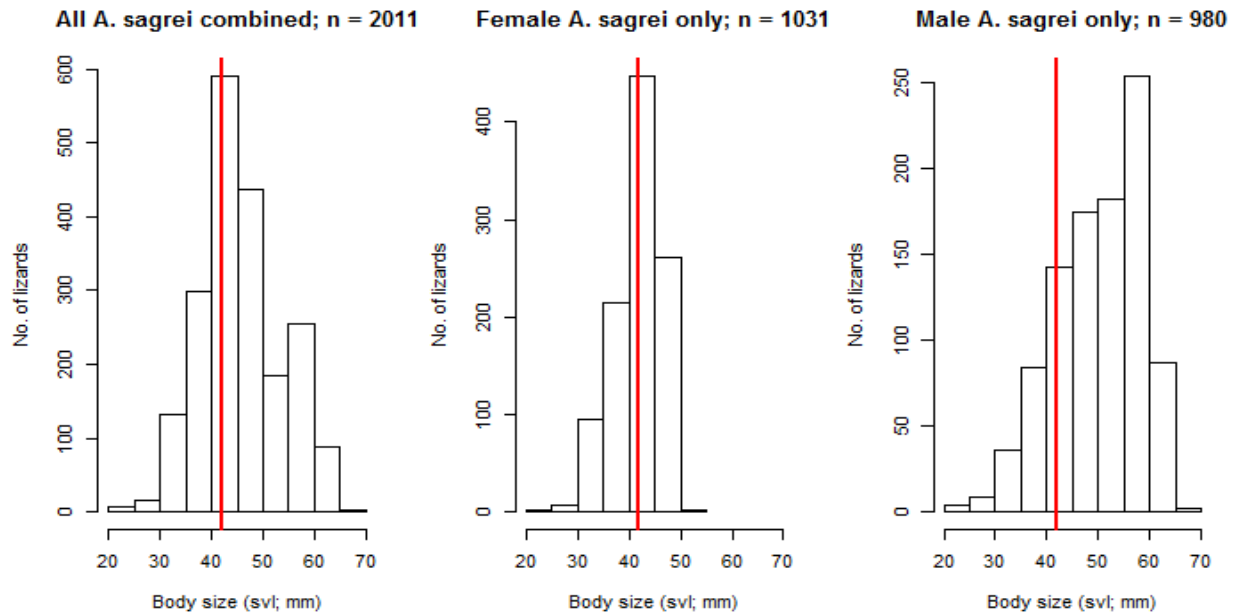

Figure 2. Size comparison between the ingested *A. sagrei* (red line) and other *A. sagrei* present within the same community (left: all individuals combined; middle: females only; right: males only). The histograms represent the distribution of body sizes for each class, red line indicates the size (svl) of the predated female *A. sagrei* in this observation. 30.48% of all *A. sagrei* are equal or smaller than the predated female, however 41.51% of all females are smaller or equal compared to only 18.88% of males. Those percentages indicate the proportion of individuals which are assumed to be vulnerable to an equal sized predatory *A. cristatellus*.
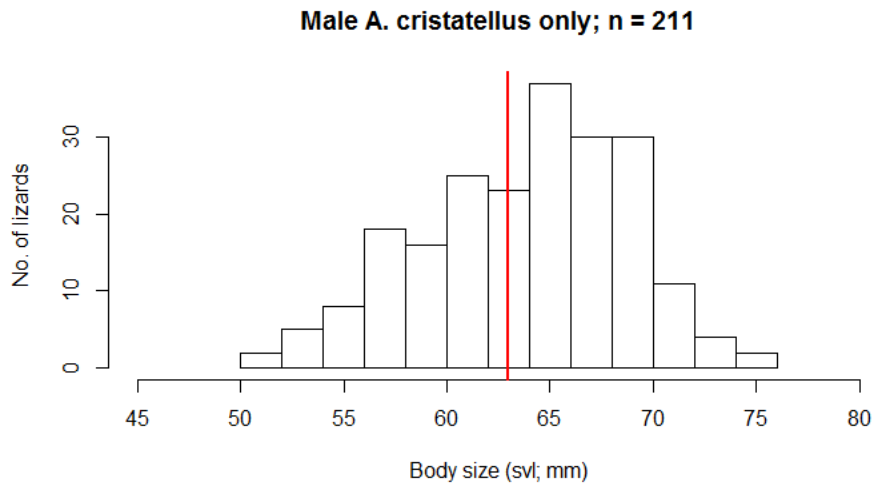
Male A. cristatellus only; n = 211

Figure 3. Size comparison of captured male *A. cristatellus* (red line) among all mature male *A. cristatellus* sampled in Fairchild Tropical Botanic Gardens. This histogram shows the distribution of sizes of mature *A. cristatellus* males and highlights that the individual from this observations was typical. This is restricted only to adult males, i.e. those >50mm svl. 60.19% of *A. cristatellus* males are larger than the male in this observation (and therefore it is likely that even larger *A. sagrei* may be predated than the female from this observation).

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Urban habitats: A natural experiment perfect for anoles

Introduction

Many readers of this newsletter (and many ecologists and evolutionary biologists in general) would not consider urban areas to be perfect places to study wildlife. Urban environments have long been dismissed as "less than" more pristine forest environments and removed from the "natural" world. But urbanization is drastically transforming the natural world with significant consequences for wildlife, and this change is expected to intensify in coming years (United Nations, 2015). Given this, we must try to understand how species are responding to anthropogenic changes. Surprisingly, this topic has only recently gained attention, although anoles are quickly proving to be an ideal system for investigating ecological and evolutionary questions in urban environments.

My research is motivated by a desire to understand how animals respond to anthropogenic change. While completing my master's research on urban turtle ecology, I was surprised to find that turtles were abundant in urban areas. I wondered how they might be adapting to urban life, but realized I needed to find a group of organisms that gave me better chances of detecting contemporary adaptive change in order to answer evolutionary questions. Anoles fit the bill perfectly. With their evolutionary history of specializing to climatic and structural habitat and evidence of rapid contemporary adaptation to environmental change, it seemed likely that they might also respond to structural and climatic differences of urban environments. In addition, the extensive literature linking habitat use, morphology, performance, and fitness in
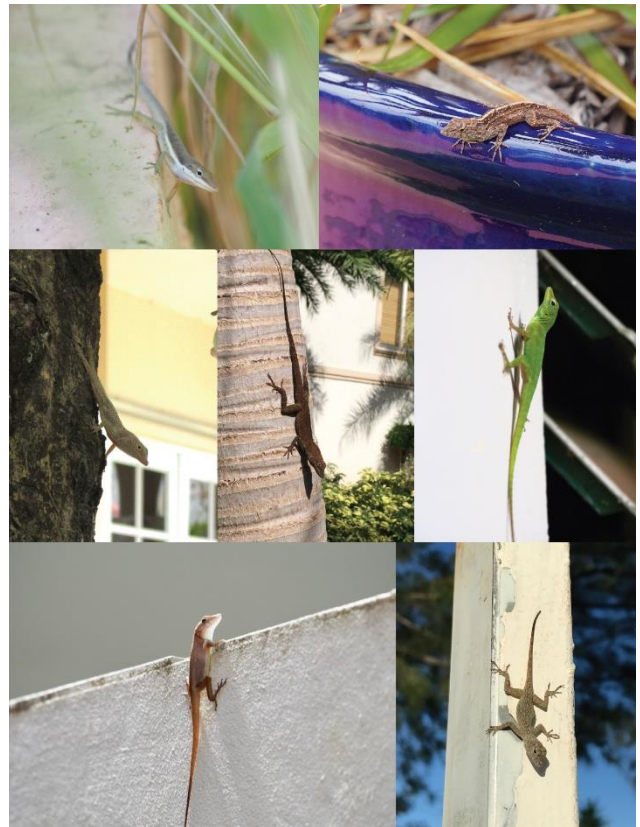
Many anoles are found in urban areas where they use anthropogenic resources to differing extents. Top: *A. pulchellus, A. sagrei*; Middle: *A. stratulus, A. scriptus, A. evermanni*; Bottom: *A. cristatellus, A. distichus*.

natural environments allowed me to develop hypotheses of potential urban adaptive shifts and gave me a context for interpretation.

Although urban environments differ from "natural" forests in many ways, the same biological principles are valid in both. Urban environments thus present a unique opportunity to investigate ecological and evolutionary responses to environmental variation. Urban habitats are often more open than forests with heterogeneous landscape and clustered resources. From an anole's perspective, this means a lot of open space dominated by anthropogenic structures. These structures, like buildings and fences, tend to be less complex (unbranching), stiffer, more steeply inclined, and smoother than naturally occurring substrates (trees and vegetation). Urban environments are also typically much hotter and drier than nearby forested areas. Despite these stark environmental differences, many anole species are found in urban areas where they exploit unique anthropogenic structural habitat. In doing so, they are subject to different selective pressures related to functional morphology and physiology, which can lead to evolutionary change. Moreover, because urban environments are drastically modified in similar ways worldwide they represent a large-scale replicated ecosystem with replicated variations. Urbanization thus creates an unparalleled "natural experiment" to test ideas of contemporary adaptation and evolution.

The field of urban evolution hardly existed when I began my doctoral research in 2011 and there was only a handful of studies on urban anoles. In the last *Anolis* newsletter (2010), the word "urban" only appeared 5 times in total, and not as a critical detail of the research presented. A few studies at the time hinted at the potential of urban anoles as a study system. Anoles clearly use anthropogenic habitat: Perry et al. (2008) listed 17 species of anoles known to forage at artificial night lights and Henderson and Powell (2001) estimated that 23% of West Indian anole species used buildings as perches at least occasionally. Two studies even considered morphological differences related to habitat use in disturbed environments: *A. carolinensis* on the Tulane University campus (Irschick et al., 2005) and *A. sagrei* in human-dominated habitat in the Bahamas (Marnocha et al., 2011). Yet much remained unknown about urban anoles.

Urban *A. cristatellus* have relatively larger toepads with more lamellae compared to forest lizards. Left image in each pair is urban, right images forest (scaled to size).

Key Findings

In my doctoral research, I explored how anoles use urban environments and how their habitat use influences morphology, physiology, and fitness. Here I provide an overview of some key findings related to habitat use and morphology. I found that anthropogenic

structures create a novel niche space within the urban environment that some species, such as *A. cristatellus*, exploit (Winchell et al., 2018a). In expanding their niche space to incorporate buildings, fences, and other anthropogenic structures, these lizards are subject to novel selection pressures that may lead to adaptive phenotypic change. Based on trait-environment relationships and the extensive use of anthropogenic perches (which are typically broader and smoother than trees and vegetation), we predicted phenotypic shifts in limb length and lamellae number. Indeed, we found urban lizards had relatively longer limbs and more lamellae than nearby forest lizards; a trend that was replicated across all three urban-forest pairs sampled and maintained in common-garden rearing (Winchell et al., 2016).

To connect the dots between habitat use, morphology, and fitness, I then examined locomotor performance of lizards on natural (bark) and anthropogenic (painted concrete, unpainted metal) substrates (Winchell et al., 2018b). In doing so we found that urban lizards ran faster than forest lizards, but that performance differences were mainly driven by track inclination and not surface type. We also found that lizards with typical urban morphology sprinted the fastest, particularly on challenging anthropogenic substrates. Our findings suggest that natural selection is likely acting primarily on sprint speed on less inclined substrates (e.g., the ground) to favor longer limbs, and secondarily favoring larger toepads with more lamellae to counteract the negative effects of long forelimbs on smooth steeply inclined substrates like buildings. While this study provides some insight into the mechanism of natural selection in shaping urban phenotypes, a significant goal in this study system (and in urban evolutionary studies more generally) remains to quantify natural selection in the wild, a goal that is particularly challenging in urban environments for many reasons.

Future Directions

I remain interested in studying the ecology and evolution of urban anoles. Since starting this work, the nascent field of urban evolution has exploded and garnered a fair amount of interest from the scientific community and general public. The anole community is catching on too, as evidenced not only by my research but of several others as well (in particular, the Kolbe and Warner labs, who shared the NSF grant that funded a large portions of my research). Although we now know significantly more about anoles in urban environments than we did 8 years ago, there remains a significant amount of work and seemingly endless questions to answer in the urban anole system. One of the main goals for my future and ongoing research is to understand how widespread the phenomenon of morphological and physiological adaptation is in urban anoles and how (and why) species vary in their adaptive responses.

Urban environments present an unprecedented opportunity for anole biologists. In studying anoles in this context, we gain insight into fundamental ecological and evolutionary questions. For example, why do some (but not all) species adapt to rapid environmental change? How does behavior affect the strength and direction of adaptive responses? How does natural

selection shape phenotypes in novel environments? What role does plasticity play in adaptation? How predictable are adaptive responses? Moreover, understanding which species adaptively respond to urbanization and what environmental factors promote stable urban populations will help inform conservation decisions in our rapidly urbanizing world. We are entering an exciting new era of anole biology and I look forward to seeing anoles become a model system for urban evolutionary studies, which they certainly have the potential to be.

Acknowledgements

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Some thoughts on the use of experimental enclosures for studying anoles

At the seventh Anolis Symposium, I presented ongoing work using experimental enclosures to study interactions between introduced anoles and day geckos in Hawaii. In discussions with folks after my talk it became clear that many anole biologists were unfamiliar with the enclosure design—it's a classic! The enclosure design was formally described in a 3-page paper entitled "A technique for enclosing *Anolis* lizard populations under field conditions" by Pacala, Rummel & Roughgarden (1983). They used this clever design to manipulate factors such as species composition and habitat availability to study anoles in the Lesser Antilles (e.g., Pacala and Roughgarden 1984, Rummel and Roughgarden 1985). The key feature of Pacala et al.'s design is that the enclosures are open-topped, which allows aerial prey to enter and efficiently encloses tall vegetation to provide habitat for anoles. Similar enclosures have been used to study ecotypic variation in *Anolis oculatus* (e.g., Mahotra and Thorpe 1993, Thorpe et al. 2005), and the effects of anoles on food webs in the Bahamas (e.g., Spiller and Schoener 1988, 1994, 2001). My goal with this essay is to share some background about this key tool for anole biologists, and give some pragmatic advice for those that might consider building enclosures themselves. Dave Spiller was kind enough to chat with me for this piece, and the advice that follows is based on both of our experiences.

Figure 1. Aerial view of enclosures used by Dave Spiller and Tom Schoener on Staniel Cay, Exuma, Bahamas. Photo by Dave Spiller, 1990.

Study design considerations

The two main options for experimentally manipulating anole populations and communities are enclosures and small islands. While both have their pros and cons, the major study design difference between them is that enclosures allow for much better control of spatial variation among units. For example, in the Staniel Cay enclosures (Fig. 1), all of the replicates are within tens of meters of each other. Compare this to a recent experiment that Dave and I and our collaborators Jonah Piovia-Scott, Louie Yang, and Tom Schoener did in the Bahamas, where we manipulated the frequency and magnitude of seaweed subsidies as well as the presence of lizards on 32 small islands that spanned several kilometers. The Staniel enclosures are much more spatially homogenous than the small islands in terms of things like vegetation, prey availability, and exposure. Indeed, in the subsidy experiment, despite carefully choosing similar islands, measuring covariates to capture biologically relevant differences among islands, and blocking islands, we still see signatures of island identity that may impede our ability to estimate treatment effects. In my Hawaii enclosures (Fig. 2), the units are extremely homogeneous spatially because I planted the habitat from scratch in an empty field.

Figure 2. Drone image of enclosures used by Amber Wright, Oahu, Hawaii. Photo taken in 2016, 6 months after initial construction. Enclosures are 10 x 10 m.

The key advantage of small islands is that they are less artificial than enclosures—lizards naturally occur and persist on them. Cage effects are a real concern when using enclosures. Dave found this to be most striking when he deployed bird netting to exclude avian predators (pro tip from Dave: put an inverted bowl on the top of the posts that hold the netting up so that the bird net can slide around freely). He found an unintended cage effect whereby the bird netting reduced wind. It turned out that wind was a major structuring force: by reducing wind speed, the bird netting also increased the temperature and affected the growth form of vegetation in the enclosures. Worse, the spiders that they were studying started building their webs on the netting itself! These effects became more pronounced over time. While Dave and Tom published ~10 papers from the Staniel enclosures, they never published the bird study because of these cage effects. In my dissertation, I used a closed-top enclosure design in an experiment to compare

brown anole fitness in urban vs. forested habitats in Hawaii (Wright 2009). In this case the habitat quality within enclosures was insufficient to support the density of lizards I stocked with, and I saw strong cage effects: the largest male in each enclosure maintained or gained weight over the study, while the other males lost weight over time, regardless of habitat treatment. Serious thought should go into cage controls, but they may not always be possible. In my current experiment, the whole point of the enclosures is to create particular lizard species assemblages under specific resource availability. The obvious cage control would be to monitor free-ranging assemblages, but due to the haphazard nature of introductions and subsequent spread (and likely also species interactions), not all of the lizard combinations occur in the field that are necessary for detecting competitive effects. In other words, "natural" cage controls don't exist.

Any experimental design is going to be comprised of a series of trade-offs, with decisions depending on what question(s) you are trying to address. In my current study, I chose enclosures because a) small islands are not available (we do have some offshore islets in Hawaii, but just a handful and they are mostly protected seabird habitat), and b) controlling/manipulating habitat availability (i.e., spatial heterogeneity) is critical for answering the questions I am interested in. A major benefit of using enclosures for my current study is that they are easily accessible. I built my enclosures at a university agricultural research station near campus, which makes it very easy to collect data, include students in research, and host visits for outreach. When we are working on small islands in the Bahamas, every day is a logistical challenge in terms of access. Some islands we can only get to at high tide, or when the sea is calm, or in a certain order, so we are constantly juggling how to most efficiently use our relatively limited time in the field. This would largely be the case even if I had small islands to study locally. On the other hand, building and maintaining enclosures is not trivial. I recall as a grad student Dave telling me something to the effect of, you only do an enclosure experiment once, and indeed he has gone on to do several small island studies but never built another set of enclosures!

Overview of enclosure design

The Pacala et al. enclosure design is beautifully illustrated in their Figure 1. The essential aspects are that the bottom edge of the fencing material is buried underground, and a plastic lip is placed on top of the fence line (Fig. 3). The plastic lip helps keep lizards in (or out) because it is difficult for lizards to cling upside-down. A soft gasket material is used between the plastic lip and the top of the fence to help fill any gaps. Vegetation is cleared within two meters of the fence line on either side to prevent lizards from jumping into or out of the enclosures from above.

Figure 3. Hawaii enclosures, installing top plastic in progress. The wooden frame is in place (2x2" uprights, 1x2" top frame), and the mesh is stapled to the frame on top and buried on the bottom. The black gasket material (1/4" thick neoprene) can be seen draping off to the left. The top of the enclosure is a sandwich of 1x2" wood, gasket, plastic, 1x2" wood, all screwed together. The plastic lip overhangs 20 cm on either side of the fence.

Materials

I built my Hawaii enclosures in an abandoned agricultural field with tons of help spread out over a whole summer. We had power tools, heavy equipment, and the luxury of big box hardware stores minutes away. I cannot imagine how tough it was for Pacala et al. to build those first enclosures; they had to clear forest and troubleshoot with whatever tools and supplies were available. For the Bahamas enclosures, Dave had to buy materials in Florida and then (over) load them into a 4-seater prop airplane flown by John Chamberlin. Once, the plane was so heavy they weren't even sure if they would be able to take off. Because the enclosures were right above the beach, the hardware cloth got corroded by salt spray and had to be replaced every year!

The elements are indeed the enemy of enclosures—at least if they're long-term, and if you're going to bother building them they might as well be. In my Hawaii site, the main issue has been UV radiation, which of course never occurred to me until it was too late. I initially used regular 1/8" thick polypropylene plastic for the top lip. This lasted less than two years before becoming incredibly brittle, cracking in place and snapping on contact. Dave similarly had to replace his plastic top at least once. Eventually, I had the bright idea to replace all of the top plastic with material used for building greenhouses (Solexx, item #GSR-160). Its structure is like corrugated cardboard, but it's made out of UV-protected plastic. You can get it in super long rolls (so no need for overlapping pieces as in Fig. 1 of Pacala et al. and my Fig. 3, see also Fig. 5), it cuts easily by sliding with regular scissors or a blade, it's flexible, and it's easy to drill through. It is supposed to last 10 years.

Instead of hardware cloth, I used a UV-treated plastic mesh from industrialnetting.com (item #OV6200). This is much easier to handle than metal hardware cloth, and has held up admirably over the last three years. It is easier to damage than hardware cloth, but it is not hard to mend. We sew rips using UV-resistant thread made for sewing sail cloth (sailrite.com), and cover the mends with 1.5" wide strips of velcro (the mesh is sandwiched between the velcro). In my dissertation enclosures, I used 1/4" mesh because the cages were fully enclosed (i.e., not open-topped) and I was worried about not enough prey getting in if the mesh was smaller. To my dismay, it turned out that adult female *A. sagrei* could fit through the holes (Fig. 4). The current enclosures have 1/8" mesh, which I suspect that very fresh *Anolis* hatchlings could escape through, though we recover many juveniles in the enclosures, perhaps because they are not inclined to disperse immediately.
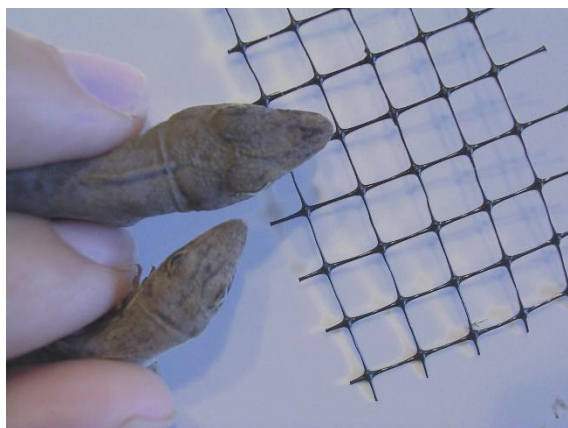

Figure 4. *Anolis sagrei* head size relative to 1/4" mesh. Top is a male and bottom is a female. Adult females (as pictured) were able to fit through this mesh size.

The role of the plastic lip is to prevent lizards from escaping—it's hard to cling upside-down. To further discourage escape, I coat the underside of the plastic with fluon (sold by BioQuip as Insect-A-Slip). It's like Teflon in a suspension, so you're making the plastic lip more like nonstick cookware. If you look it up, you'll mainly see people using it on the sides of ant colony tanks to prevent escape. After trying various application methods, we now dilute it 50-50 with water and use a small household spray bottle to mist it onto the plastic (wear goggles and a mask). You want a thin, even layer. Unfortunately, the efficacy diminishes over time in humid conditions like the tropics. We wipe it off and reapply monthly when lizards are in enclosures. When it's fresh, you can stick a gecko to it upside down, but as soon as they take a step they fall off. Anoles fare even more poorly.

We have documented some escapes, with marked individuals caught outside of the enclosures or even in new, different enclosures occasionally. We nicknamed one female day gecko Houdini because at least twice during the pilot study we found her in an adjacent plot, moved her back to her home plot, and found her again in the adjacent plot. We have had very little incursion from non-experimental animals, probably because the surrounding habitat (agricultural fields) is not great for lizards. Our animals are VIE tagged, so we know when we have an interloper. Dave

had to remove animals regularly from his enclosures to maintain treatments, as he built his enclosures around resident green and brown anoles. On a few occasions, he observed adult lizards jump up from the side of the enclosures to the edge of the plastic lip. Pacala et al. (1983) specifically talk about the efficacy of the enclosure design for keeping different species in and out.

For the gasket material, I used 1/4" thick sheets of neoprene ordered by the roll from Amazon that we cut into strips using rotary cutters and a straight edge. We used ShoeGoo to tack the neoprene strips to the 1x2" wooden frame, and later caulked any gaps. The neoprene gets a bit compressed and brittle over time, but not enough to worry about.

Most of my materials were not available locally, and below-ground treated wood was no exception. I was too impatient to wait for a special order by boat, so we used above-ground outdoor treated 2x2"s for the upright posts and coated the bottom third with copper-based paint to discourage microbes (Fig. 5). These have not shown any sign of rotting after three years in fairly wet conditions (the enclosures are on the wet, windward side of the island). After we suffered some wind damage (see Challenges), we reinforced the wooden uprights by adding metal T-posts at the corners and the two middle uprights of each wall. We pounded the T-posts in and zip-tied them to the wooden posts. We haven't had any damage since putting in the T-posts, but then again we haven't had any major storms yet. People often suggest that we build the whole structure out of T-posts, but I haven't figured out a solution for how you would attach the top plastic lip if you did that. Right now, we have 1x2"s across the top of the fence posts, and to this we staple the top edge of the mesh and attach the plastic lip on top (Fig. 3). If the uprights were metal you would still need some kind of top frame, but you couldn't just screw the frame to the posts like we have done. I've seen plastic caps for the tops of T-posts which maybe you could secure a frame to.

Building
Just a couple comments on building and some pictures of the process (Fig. 5). My enclosures definitely look like they were built by a bunch of biologists. Our motto during building was, "it doesn't have to be pretty, it just has to keep lizards in." The ground at our site was uneven enough that the mountain sides of the plots are lower than the ocean sides of the plots (this is how we give directions in Hawaii). We used an 8-foot level to keep the top frame flat (2-foot levels perched on top of the wood frame did not cut it). To sink our 2x2" wooden posts, we pre-dug holes using a 1.5" auger bit sold for planting flower bulbs and an electric drill plugged into a generator. Battery powered drills weren't strong enough, and renting a gas-powered post-hole auger seemed like overkill. We also brought a miter saw to the field and ran it off the generator to trim the posts before pounding once we knew the height we needed. Sinking the posts was definitely the most labor intensive part. Thankfully, the field station staff dug the trenches and the holes for planting the trees using farm equipment.

Figure 5. Clockwise from top left, with people identified left to right. Bob Thomson and Robyn Screen checking whether the heights of the upright posts are level. Anthony Barley, Rachel Goodman, and Laci Gerhart-Barley attaching the top frame (the bottom of third of the posts are darkened by copper paint). Robyn Screen and Laci Gerhart-Barley stapling mesh to the frame. You can see the berm of dirt that will be used to refill the trench. Stevie Kennedy-Gold and Marlin Dart attaching the top plastic (Solexx, note how it is one continuous sheet).

Getting in and out

One of the first things people always ask when they see the enclosures is how we get in and out because there are no doors. Doors definitely would have exceeded our building skills, and probably would have resulted in more ways for lizards to escape. The thin wood pieces we used have warped over time (Fig. 6), so a door would definitely have gaps around it by now. Dave got in and out of his enclosures by, in his words, "vaulting", sometimes doing the jumps 120 times a day! He was able to do this because his enclosures were only 1 meter high. We use a pair of ladders that we carry around from plot to plot (Fig. 6). I got the lightest ladders available locally, they weigh about 15 lbs and are made by Gorilla. We cut the top plastic frame off with a hacksaw so we can step right through. In my dissertation enclosures, I made a door by cutting an L-shaped slit in the mesh and hot-gluing Velcro flaps on to keep it shut. This worked fine as a

short-term solution (I only ran these enclosures for 6 weeks, and didn't enter them often). I experimented a bit with installing tent-door zippers on my current enclosures as a more long-term solution, but worried that this would put too much wear and tear on the mesh panels.

Figure 6. Using ladders to exit enclosures. You can see the tall grass to the left of the enclosure, and that the top frame has warped and curved a bit over time. Crossing is Bam Auetumrongsawat, holding the ladder steady is Stevie Kennedy-Gold.

Challenges

Building the enclosures was a massive amount of work, but also kind of fun in retrospect. The much bigger beast has been maintenance. While Dave had to put a lot of work into initially clearing vegetation around the fence line, he just had to maintain that clearing over time as the enclosed vegetation was mature and did not change much from year to year. I didn't have to deal with initially clearing vegetation, but because this is such a productive site the vegetation is constantly growing. This adds temporal variation to the study, though we carefully measure all of the vegetation before every replicate following Schoener 1974 so we can account for this change. On a day to day basis the bigger issue is that we have to spend a lot of time on landscaping to maintain the plots.

We have to mow the whole set up inside and outside of enclosures every 2-3 weeks year-round, regardless of whether the enclosures are generating data or not. If we don't mow regularly the grass will be neck-high in a month (see Fig. 6). Grass will also grow through the holes in the mesh and break it, so we regularly weed the fence line by hand. Because there are no doors, any mowing gear has to be lifted up and over. For the first year, we just used hand tools, sickles and a push mower. This was gentle on the plots but incredibly labor intensive, so we switched to string trimmers. This is easier and faster but the trimmers send up projectiles that can rip the

mesh. We added a strip of UV-protected plastic sheeting along the base of the enclosure walls to help prevent these rips. We also use a battery powered lawnmower, the lightest I could find (a Ryobi 15"). One set of batteries cost as much as the mower—wait for Father's Day sales. People always ask if the mowing bothers or hurts the lizards. The lizards are all originally collected from an urban area, so they have seen and heard mowing before. We also thoroughly disturb each plot before mowing. We have a grid of flags placed every meter in the plots that we use to estimate an X,Y coordinate for every individual sighting so we can track spatial use (Fig. 7). Before mowing we have to pull every flag, and this likely spooks any lizards out of the way. The lizards are rarely just hanging out in the grass anyway; they are mostly perched up in the vegetation out of harm's way. As an aside, picking and replacing the flags takes as long as mowing each plot.

Another unexpected plant-based issue has been the bananas. I initially planted a single dwarf apple banana in each plot (Fig. 7). Little did I know that banana trees fruit once and then die and you have to cut the tree down or it will eventually fall (potentially taking out a side wall), and that they continually send up new sprouts from the base. I went from that one tree in each plot to over a dozen mature trees at any given time per plot within months. On the plus side this created a lot of habitat. The drawback is that we are constantly having to trim the trees. This was all tolerable until some of the plots got infected by banana bunchy top virus—a well-known scourge to local banana farmers. I had gone in thinking of the plants primarily as lizard habitat. I worried about things like, what plants did the lizards use that would be easy to get and provide a range of perch heights, diameters, and textures? I was not thinking of the plants as living things with their own needs and enemies. The problem with the bunchy top is that it stunts the growth of the trees. Because the infection was not uniform across all plots, this lead to increased spatial variation among the plots—the infected plots have not had their canopies replaced by new sprouts at the same rate as uninfected plots, and are now more sparse and open. I will probably cut down all the banana and replace them with something else for the next experiment (after I research potential pathogens).

Other challenges have been various destructive natural and man-made forces. Fortunately, the enclosures are relatively modular and easy to repair, and the disturbances so far have all occurred in the intervals between replicates when lizards were not in enclosures. A freak wind storm destroyed 20% of the side panels (Fig. 8). In the days after the windstorm, some feral pigs wandered into the now-open plots and rooted around. The day after we fixed all of the fences, we found several pig-sized holes punched through the mesh. The farm crew put up an electric fence for us after that and we haven't had any trouble since. On the man-made side, we've had multiple equipment thefts, to the point that we no longer store anything on site. We've also had vandals slit the mesh for seemingly no reason. Once they slit the mesh and stole two plants out of one plot. The plants they stole, ti plants, are probably the most common landscaping plants in Hawaii and I couldn't have paid more than $3 a piece for them originally. The damage

to the experiment, however, could have been priceless. I was so irritated I made a police report and everything. The officer was a total pro and didn't bat an eye at my story. At one point his cell phone rang and his ringtone was the Hawaii Five-O theme song at full blast!

Figure 7.

Bottom: Initial planting. A single banana plant about 5 feet tall was planted in each plot. This single plant (unexpectedly) gave rise to a whole stand in each plot.

Right: Stevie Kennedy-Gold inside of a plot after 3 years of growth. The white and yellow 1-meter flag grid can also be seen in this photo.

The open-topped design lets the vegetation grow tall and allows aerial prey to come in, but it also means the enclosures are open to bird predation. We have seen birds take our experimental animals on three occasions. One time it was cattle egrets; they just grab a lizard and then it's straight down the hatch. Twice it was mynah birds. The mynahs land in the enclosure in groups of two or three. When they get a lizard, they fly out of the enclosure and then land nearby and bash the lizard repeatedly against the ground. Now, given the thousands of person-hours we have spent out there watching lizards, this is not a lot of observations. But we think it happens more than we see it because we often see birds in the enclosures, and we have documented injuries that were likely caused by birds such as tail loss, bite marks, and what appears to be bruising (Fig. 9). But only by being out there every day do we have a chance of even documenting these things, underscoring how hard it is to observe predation directly. I am planning on adding bird exclusion to my next set of experiments to determine the relative importance of competition vs. predation in structuring the novel assemblage of anoles and day geckoes in Hawaii.

Figure 8. Wind damage. This is how we found the enclosures one morning. Hawaii's tradewinds usually come from the northeast. During this event the winds changed directions, and came from the southwest. As the wind rushed down the steep face of the Ko'olau Mountains (seen in the background) we got gusts near 50 mph. I think the plastic lip helped catch the wind, lifting and snapping the posts. The mesh was mostly intact though.

Figure 9. Female *A. sagrei* with possible predation injury. She was placed in the plot on 10/12/17, and was observed on several days leading up to the top photo, including two days prior (10/22/17) where she looked normal. The large dark patch was first noted on 10/24/17, was largely cleared up by 11/01/17 (bottom photo), and eventually went away completely. The fact that it "healed" makes me think it was a bruise, and my best guess is that a bird grabbed her. I'm not sure why the midline is not discolored.

Conclusion and ongoing work

Overall, the decision to build enclosures should not be taken lightly; it's a large commitment of time, money, and personnel. That being said, using enclosures has definitely been worth the effort in our ongoing experiment to determine whether anoles and day geckoes are competing in Hawaii, what the mechanisms of competition are, and ultimately, the prospects for long-term coexistence in this unique community. In collaboration with Stevie Kennedy-Gold, Carla Piantoni, and Tim Higham, we are manipulating the lizard species assemblage using all combinations of *A. carolinensis, A. sagrei,* and *Phelsuma laticauda*. We are measuring several

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responses, such as fitness, behavior, and resource use and availability along all three key niche axes for anoles (structural, thermal, and diet). At the broadest level, we are asking how general is the *Anolis* resource partitioning story? Can this framework be used to predict what will happen when anoles are confronted with a novel player, the ecologically convergent day geckoes? Testing these predictions and determining the mechanisms underlying the species interactions can only be accomplished by using manipulative experiments. I am grateful to the pioneering work of previous anole biologists in developing and implementing enclosures, and I hope this contribution will be helpful for anyone considering building enclosures.

Acknowledgements

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Identifying molecular and cellular mechanisms of tail regeneration in anoles

Lizards are the most closely related vertebrates to humans that are able to regenerate a complex, multi-tissue appendage such as the tail. The high degree of homology in genes between humans and the green anole, *Anolis carolinensis*, permit comparisons of the signaling pathways activated during regeneration. The regenerative process in the green anole can be divided into three phases. In the initial phase, from 0 to 15 days, there is formation of a wound epithelium but not much outgrowth. In the second phase, starting approximately 15 days there is more overt growth, proliferation, and patterning of differentiating tissue. In the third phase, starting approximately 60 days, there are clearly defined and differentiated tissues, but development and maturation of the peripheral nervous system and neuromuscular junctions continue.

We have been examining the regenerative process at the molecular level. Building on our genome annotation of the green anole (Eckalbar et al., *BMC Genomics*, 2013), we used RNA-Seq to identify differentially expressed genes (total and microRNAs) during the peak of tail outgrowth (25 days post autotomy). Transcriptomic analysis revealed 326

Figure 1. Two female green anoles with regenerated tails. Photo credit Joel Robertson.

differentially expressed genes regulating wound and immune response, hormonal regulation, and musculoskeletal development (Hutchins et al., *PLoS ONE*, 2014). Comparative genomic analysis with other regeneration model organisms identified common patterns of activation of the canonical Wnt and Wnt5-calcium signaling pathways. MicroRNA analysis identified both novel
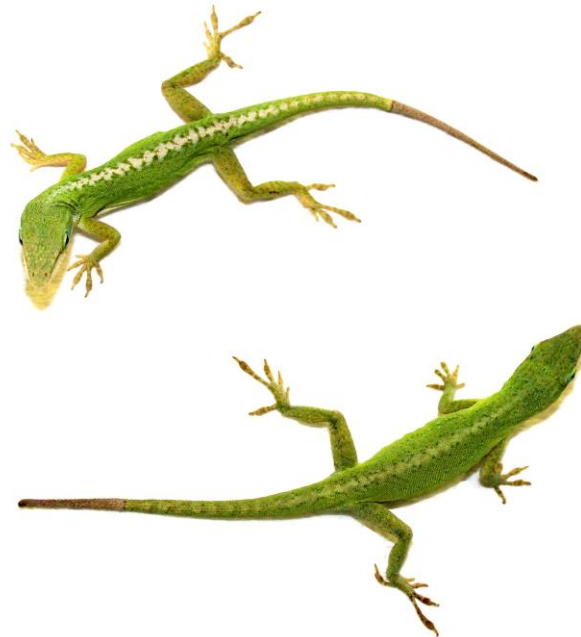
sequences to the anole as well as orthologues of microRNAs involved in stem cell regulation in other organisms (Hutchins et al., *BMC Genomics*, 2016). We are currently analyzing the differentially expressed genes at the earliest stages of regeneration (0 to 15 days), to understand the activation of this remarkable process.

We have previously described that the organization of the regenerated *A. carolinensis* tail is a functional but not an anatomical replacement of the original tail (Fisher et al., *Anat Rec*, 2012; Ritzman et al., *Anat Rec*, 2012). The regenerated tail is radially symmetric and lacks segmentation along the anterior-posterior axis. Articulated bony vertebrae are replaced by cartilage that surrounds the regenerated spinal cord, which is composed of ependymal cells and deficient in grey matter. Moreover, *de novo* muscle is no longer organized into distinct quadrants and lack clear interdigitation. Despite these differences, the regenerated tail has peripheral sensorimotor function, and we are examining the process by which the key structures are reformed.

We analyzed the process of tail regeneration from 15 to 250 days post autotomy using immunofluorescence with markers for motor axons, myelin from Schwann cells, and neuromuscular junctions (Tokuyama & Xu et al., *Dev Biol,* 2018). During earlier stages in regeneration, the density of axons and NMJs in muscle

Figure 2. Distribution of neuromuscular junctions in a single muscle group of original and regenerated tails (120 and 250 days). Red TUJ1 marker, axons; green BTX marker, neuromuscular junctions; blue DAPI stain, cell nuclei. From Tokuyama & Xu et al., 2018.

(Figure 2) are higher but then are pruned and reduced in numbers, which recapitulates embryonic development of the neuromuscular junctions. We are currently extending these studies to analyze the molecular interactions between motor axons, ependyma and Schwann cells, and emerging muscle groups during initial stages of *de novo* neuromuscular regeneration. In addition, we are working to characterize the development of *de novo* proprioceptive muscle spindle apparatus and sensory neurons during *de novo* peripheral nerve regeneration. Identifying the genetic pathways
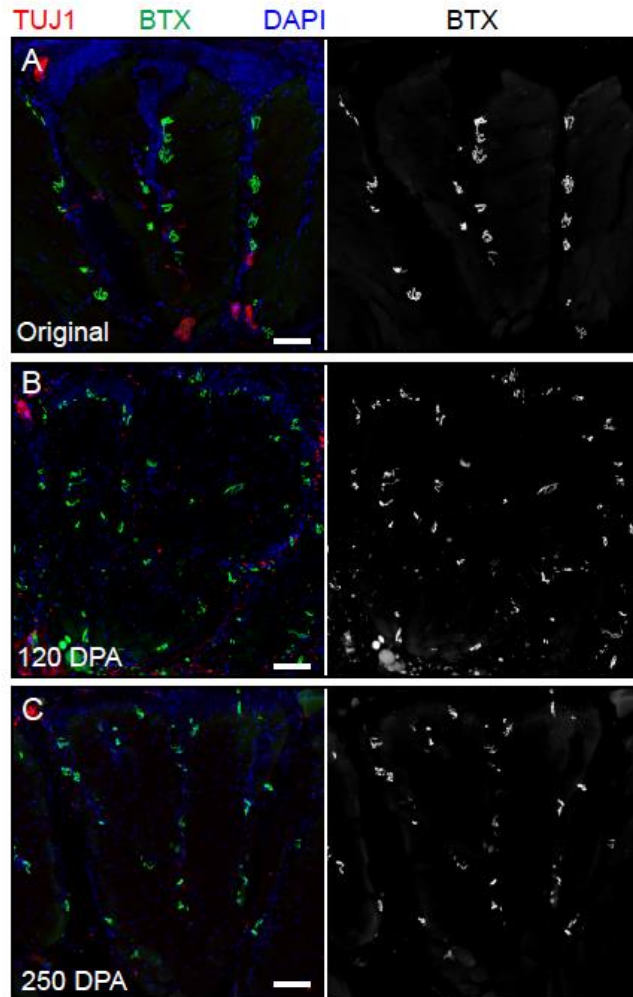
for activation of neuromuscular and peripheral nerve regeneration would greatly improve motor function and quality of life of individuals who have lost function due to injury or treatments such as chemotherapy.

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Oral presentations at the VII *Anolis* Symposium

Saturday, 17th March 2018

James T. Stroud, *Florida International University*
    Introduction and welcome.

Michele A. Johnson, *Trinity University*
    Physiological mechanisms underlying behavioral convergence in Caribbean anoles.

Tony Gamble, *Marquette University*
    Anolis sex chromosomes, past, present, and future.

Rosario Castañeda, *Universidad Icesi*
    When did anoles diverge? An analysis of multiple dating strategies.

Colin Donihue, *Harvard University*
    Hurricane-induced adaptive shifts in the morphology of an island lizard.

Leo J. Fleishman, *Union College*
    Why are there so many yellow dewlaps?

Graham Reynolds, *University of North Carolina Asheville*
    Genetic and morphometric diversification in the brown anole suggest early pathways of anole colonization and evolution in the Caribbean.

Nathalie Feiner, *Lund University*
    Transposable elements, Hox gene clusters and genome evolution– How special are *Anolis* lizards?

Thomas J. Sanger, *Loyola University Chicago*
    The mechanisms of thermal stress induced craniofacial malformation in lizards developmental biology.

Sozos N. Michaelides, *University of Rhode Island*
    Invasion history of four *Anolis* lizard species introduced to Bermuda.

Kristin M. Winchell, *University of Massachusetts Boston*
    Performance consequences of urban morphological shifts.

Kenro Kusumi, *Arizona State University*
    Comparative genomics reveals accelerated evolution in conserved pathways during *Anolis* diversification.

Sean T. Giery, *University of Connecticut*
    Some thoughts on the trophic ecology of *Anolis* lizards.

D. Luke Mahler, *University of Toronto*
> Land use and the restructuring of anole communities across an elevational gradient.

Ivan Prates, *Smithsonian Museum of Natural History*
> Genomic signatures of adaptation associated with a history of range expansions in South American anoles.

Oriol Lapiedra, *Harvard University / CREAF*
> Predator-induced natural selection in behavior.

Caitlin C. Mothes, *University of Miami*
> Using South Florida's exotic lizard community to evaluate the use of ecological niche models in predicting biotic invasions.

Neil Losin, *Day's Edge Productions*
> The Lizard's Tale and Anole Annals v2.0: An enhanced platform for *Anolis* outreach.

Sunday, 18th March 2018

Douglas B. Menke, *University of Georgia*
> Genome editing methods for the production of genetically modified anoles.

Sarin Tiatragul, *Auburn University*
> A shady way to beat the Miami heat.

Joanna O. Palade, *Arizona State University*
> *Anolis carolinensis* satellite cells have expanded musculoskeletal potential.

Gregory C. Mayer, *University of Wisconsin*
> Using archival DNA to elucidate anole phylogeny.

Liam J. Revell, *Universidad del Rosario and UMass Boston*
> Can we detect differences in the rate of discrete character evolution between clades of anoles?

Amber N. Wright, *University of Hawaii*
> Predicting the outcome of species interactions in a novel species assemblage: *Anolis* vs. *Phelsuma* in Hawaii.

Andrew C. Battles, *University of Rhode Island*
> The other Miami Heat: Urban areas alter thermal biology and influence persistence and spread of two invasive *Anolis* species.

Nathan W. Turnbough, *Independent Researcher*
> Covariation in arthropod community composition and dominant anole identity on dredge

spoils islands in Florida.

Cindy Xu, *Arizona State University*
> Tail regeneration in anole lizards: Insights from comparative genomic analysis and reformation of the peripheral motor nervous system.

Michael L. Logan, *Smithsonian Tropical Research Institute*
> Using experimental islands to explore evolutionary dynamics under climate change.

Christine Rose-Smyth, *Verdant Isle Orchids*
> Role of a sweet-toothed anole in orchid pollination.

Christopher J. Thawley, *University of Rhode Island*
> Let there be light: Widespread use of human-produced light at night by anoles and its consequences.

Sean Doody, *University of South Florida, St. Petersburg*
> Environmentally cued hatching in anoles.

Winter A. Beckles, *University of Miami*
> Signal divergence and habitat partitioning among non-native bark anoles in South Florida.

Stephanie L. Clements, *University of Miami*
> Non-native species dominate herpetofaunal community composition in both native and non-native habitat patches in Miami-Dade County.

Zachary A. Chejanovski, *University of Rhode Island*
> Predators influence prey body size variation in an urban landscape.

Joshua M. Hall, *Auburn University*
> Does season-dependent reproductive value of offspring drive the evolution of life-history traits in *Anolis* lizards?

Jonathan B. Losos, *Washington University in St. Louis*
> Concluding remarks.