Anolis Symposium VII

March 17-18, 2018 · Miami, FL
Fairchild Tropical Botanic Gardens

Program of Oral and Poster Presentations

Saturday, 17 March 2018

Physiological mechanisms underlying behavioral convergence in Caribbean anoles

09:30 **Michele A. Johnson** **Trinity University**

Abstract: All animal movements result from muscular contractions, and variation in muscle physiology can influence the behaviors supported by a muscle. In particular, differences in muscle fiber size, fiber type composition, neuromuscular junctions, or sensitivity to androgens can result in variation in a muscle's generation of force and/or its frequency of behavioral use. Over the past few years, my lab has examined 30 species of Caribbean *Anolis* lizards, quantifying field behavior and measuring these physiological traits in the lab, to determine whether the same combinations of physiological traits underlie similar behavioral patterns, or whether different combinations of these traits can result in similar behavior. Our work focuses on two muscles: the ceratohyoid, the muscle that controls dewlap extension, and the retractor penis magnus, one of the two muscles involved in hemipenis movement. Overall, our results suggest that the sizes of muscle fibers and the neuromuscular junctions of those fibers, as well as muscle fiber type composition, are more strongly associated with the size of the structure moved by the muscle (dewlap or hemipenis) than the frequency or duration of muscular contraction. Together, these analyses represent the largest comparative study of muscle physiology to date, and suggest that the evolution of social behavior across the genus is not constrained by these muscular traits.

Anolis sex chromosomes, past, present, and future

09:45 **Tony Gamble** **Marquette University**

Abstract: Squamates generally, and *Anolis* specifically, are ideal models for studying sex chromosome evolution and maintenance. Anolis karyotypes in 1966 provided the first evidence of XX/XY sex chromosomes in a reptile. Subsequent research identified sex chromosome scaffolds in the *Anolis* carolinensis genome, shown that all *Anolis* species share the same XX/XY sex chromosome system, and found evidence of a complex dosage compensating mechanism. I will review previous and current research on *Anolis* sex chromosomes and discuss possible topics for future research.

When did anoles diverge? An analysis of multiple dating strategies

10:00 **Rosario Castañeda** **Universidad Icesi**

Additional Authors: Cristian Román-Palacios, Jose Tavera

Abstract: Most of the divergence estimations for *Anolis* lizards have dated the crown group between 38–70 Ma. A single study has recovered a significantly older age, 87 Ma, for the same node. These two sets of estimations imply very different biogeographical scenarios, most notably for Caribbean species. We used three calibration sources to infer the most probable divergence timing for anole lizards: 1) a mitochondrial rate for ND2 gene (Rate); 2) the fossil *Anolis dominicanus* (F1); and 3) a group of fossils assigned to the Priscagamines, Iguanines and Isodontosaurus clades (F2). We analyzed 4700 bases of mitochondrial ND2 and COI, and nuclear RAG1 genes of 68 anole species. We ran seven different analyses in BEAST, each incorporating a different dating strategy: a calibration source alone (rate, F1, F2), or combined (rate+F1, rate+F2, F1+F2, rate+F1+F2). We selected the best hypothesis based on likelihood scores and Bayes Factors. We also explored if date estimations varied between deep and shallow nodes by plotting the standard deviation of mean ages between time-calibrated phylogenies across time. Last, we compared across resulting chronograms the congruence between the estimated divergence date of *Anolis agassizi* (an endemic species from the Pacific island of Malpelo) and the island geology. The preferred estimation, the F1+F2 strategy, suggests that anoles diverged 72 Ma (71–73 Ma), with the crown group established around 58 Ma (51–66 Ma). Dispersal is therefore supported as the major driver in the biogeography of the group, and in Caribbean lineages in particular. Differences in age estimations between calibration strategies gradually decreased towards younger nodes, regardless of the position of the constrained node, with younger nodes (< 14 Ma) being largely congruent among strategies (standard deviation < 0.8). All age estimations inferred that *Anolis agassizi* diverged from its closest relative, *A. insignis*, before the emergence of Malpelo island (preferred age estimation: 26 Ma (95% Highest Probability Density = 19–32); Malpelo's emergence: 16–17 Ma). We also found that rate-based estimations pulled dates toward younger ages, and that the F2 calibration source (which dated

the Pleurodonta crown clade) had a heavy influence on the estimation of deep nodes, but not of shallow ones.

Hurricane-induced adaptive shifts in the morphology of an island lizard

10:15 **Colin Donihue** **Harvard University**

Additional Authors: Anthony Herrel, Anne-Claire Fabre, Ambika Kamath, Anthony J. Geneva, Thomas W. Schoener, Jason J. Kolbe, and Jonathan B. Losos

Abstract: Hurricanes are catastrophically destructive. Beyond their toll in human life and livelihoods, hurricanes have massive and often long-lasting effects on ecological systems. Despite many examples of mass mortality events following hurricanes, hurricane-induced natural selection has never been demonstrated. Immediately after we finished a survey of *Anolis scriptus*, a common, small-bodied lizard found throughout the Turks and Caicos archipelago, our study populations were battered by Hurricanes Irma and Maria. Shortly thereafter, we revisited the populations to determine whether morphological traits related to clinging capacity had shifted in the intervening six weeks and found that surviving lizards differed in limb length and toepad size. Our results, the first to employ an immediate before-after comparison to test this question, demonstrate that hurricanes can induce phenotypic change in a population, and strongly implicate natural selection as the cause. In the decades ahead, as extreme climate events are predicted to become more intense and prevalent, our understanding of evolutionary dynamics needs to incorporate the impact of these severe selective episodes.

Why are there so many yellow dewlaps?

10:30 **Leo J. Fleishman** **Union College**

Additional Authors: Manuel Leal

Abstract: In a review of dewlap colors of 140 anoline species, Nicholson et al. (2007) found that yellow is the most common dewlap color, followed by orange and red. Behavioral studies have shown that dewlap visibility is directly related to the absolute value of dewlap-versus-background luminance (= brightness) contrast plus dewlap-versus-background chromatic contrast. We studied 17 species and found that yellow dewlaps are more common in darker habitats, while red and orange are more common in brightly lit habitats. We tested three hypotheses. (1) We asked whether yellow dewlaps provide higher luminance contrast in darker habitats. This does occur in some habitats, but in most habitats luminance contrast is the same for red and yellow dewlaps. (2) We asked whether differences in spectral quality among habitats result in higher chromatic contrasts for yellow dewlaps in some habitats and for red in others. We found, however, that in all habitats, red dewlaps created the highest chromatic contrast. Finally we hypothesized that yellow dewlaps are more visible in darker habitats due to an interaction between dewlap total intensity (higher reflectance plus transmittance) and chromatic contrast. We speculated that under low light conditions, photon noise limits the effectiveness of darker, red dewlaps, and favors yellow dewlaps, which reflect and transmit more total light. We carried out a behavioral experiment to test the visibility of red or yellow stimuli viewed against a green background in low and high light conditions. The red stimulus was more visible in high light, while yellow was more visible in low light. The results support the hypothesis that yellow dewlaps are more common in low light because they reflect and transmit a higher rate of photon flux, and create higher chromatic contrast with the background (than red) in low light habitats.

Genetic and Morphometric Diversification in the Brown Anole Suggest Early Pathways of Anole Colonization and Evolution in the Caribbean

11:30 **Graham Reynolds** **University of North Carolina Asheville**

Additional Authors: Jason J. Kolbe, Richard E. Glor, Marta López-Darias, Alexis S. Harrison, Kevin De Queiroz, Jonathan B. Losos

Abstract: Some of the most important insights into the ecological and evolutionary processes of diversification and speciation have come from studies of island adaptive radiations, yet few studies examine how these radiations might have begun. The Brown Anole (*Anolis sagrei*) is a candidate for understanding the origins

of the Caribbean *Anolis* adaptive radiation—an investigation of the morphological and genetic variation in this widespread species might provide insight into how a colonizing anole begins to undergo allopatric diversification. To investigate the extent of diversification across *A. sagrei*, the most widespread species of anole, we conducted genetic and morphological analyses for representative populations across the entire native range of the species, encompassing 95 sampling locations. We generated mtDNA sequence data for 295 haplotypes and genomic data (31,702 SNPS) for 69 representative individuals, which we analyzed using phylogenetic and population genetic methods. We generated a morphometric dataset including 10 linear and meristic measurements obtained from radiographs and photographic scans from each of 558 representative specimens. We find that *A. sagrei* originated in the early Pliocene, with the deepest divergence occurring between western and eastern Cuba. Lineages from these two regions subsequently colonized a large portion of the northern Caribbean, with some evidence for multiple colonization of regions such as the Bahamas, and a native lineage in Jamaica. We find that at the broadest scale, morphological diversification does reflect evolutionary history, though this relationship is somewhat more clouded within sub-groups. Taken together, our results show a complex history of ancient and recent Cuban diaspora yielding morphological homoplasy regardless of phylogenetic contingency. It is likely that the processes driving divergence and diversification in *A. sagrei* are operating at smaller, intra-island scales, providing insight into the original diversification of colonist anoles at the beginning of the radiation. Our study furthermore provides an evolutionary framework for the many studies of this increasingly important species in ecological and evolutionary research.

Transposable elements, Hox gene clusters and genome evolution – How special are Anolis lizards?

11:45 **Nathalie Feiner** **Lund University**

Abstract: Transposable elements (TEs) have long been considered to be 'junk' DNA, or even harmful genomic parasites. Recently, a more creative role for TEs in evolution has been put forward emphasizing their capacity to rewire the gene regulatory landscape, or to accelerate the speciation process by increasing genomic incompatibilities between incipient species. And indeed, genomes of iconic examples of rapid speciation (*Heliconius* butterflies, cichlid fishes or *Anolis* lizards) have been discovered to be unusually rich in TEs. Despite this circumstantial evidence, we lack a formal test of the role of TEs in diversification. Here I present a systematic, genome-wide comparison of TEs across squamates (lizards and snakes) and assess their correlation with patterns of diversification. I will also make a case for TEs having an impact on the expression of the phenotype; the developmentally crucial Hox gene clusters of *Anolis* lizards have been invaded by TEs, and the otherwise highly conserved expression patterns of several Hox genes show divergence from this paradigm in *Anolis* embryos. My talk will provide insights into the role of TEs in evolution and development, and I will critically assess in how far genomic factors might play a role in evolutionary diversification.

The Mechanisms of Thermal Stress Induced Craniofacial Malformation in Lizards

12:00 **Thomas J. Sanger** **Loyola University Chicago**

Additional Authors: Dryden Lachance, Laura Harding, Judith Kyrkos, Beata Czesny, Christian Mata, James T. Stroud

Abstract: Terrestrial ectothermic reptiles are under increasing risk of global warming. Embryonic life is particularly sensitive to thermal stress, although few studies have examined the tissue- and stage-specific effects of thermal stress in reptiles. We have examined the production of developmental malformations in the burgeoning embryological model system *Anolis sagrei*. Examination of field incubation conditions indicates that this species is developing on the cusp of its thermal limits during the peak summer months. We have discovered a narrow window around ovipositon that is sensitive to thermal stress and the concomitant induction of developmental malformations. Craniofacial malformations represent the most abundant class of malformation observed. These malformations range from a foreshortened or narrow face to embryos with complete loss of the neural crest derived facial skeleton. We have shown that these malformations are correlated with localized cell death in the developing forebrain during a narrow window of craniofacial patterning. Using a concentration gradient of a small inhibitors applied directly to the developing egg we have reproduced the craniofacial phenotypes observed in the thermally stressed embryos, verifying our candidate mechanism of induction. Our results raise greater concern over the potential for climate change to disrupt the reproduction and survival of terrestrial ectotherms.

Invasion history of four Anolis lizard species introduced to Bermuda

12:15 **Sozos N. Michaelides** **University of Rhode Island**

Additional Authors: James T. Stroud, Jason J. Kolbe

Abstract: Islands are the recipients of numerous invasive species, challenging island biogeography theory and often with negative impact on the native biodiversity. More than 20 anole species have been introduced via human activities beyond their native range resulting in many established populations worldwide. Bermuda, a small, isolated oceanic island located in the western North Atlantic, had only one endemic terrestrial vertebrate. However, since the 1900s, four *Anolis* lizards have been introduced; *Anolis grahami* (native to Jamaica), *Anolis extremus* (native to Barbados), *Anolis leachii* (native to Antigua and Barbuda) and *Anolis sagrei* (native to Cuba and the Bahamas). Here, we used mtDNA data in a phylogeographic framework to elucidate their origin, genetic diversity and identify patterns associated with their successful introduction. We found evidence of both single and multiple introductions with subsequent admixture in certain populations. In comparison to the native range, genetic variation (haplotype diversity) was lower in the introduced range for the majority of species. We discuss these results in relation to the different introduction histories for each anole species and the potential consequences of the spread of these species across the island.

Performance Consequences of Urban Morphological Shifts

14:00 **Kristin M. Winchell** **University of Massachusetts Boston**

Additional Authors: Inbar Maayan, Jason R. Fredette, Liam J. Revell

Abstract: Urban environments pose novel locomotory challenges for urban-tolerant species. We investigated consequences for *Anolis cristatellus* using manmade structures in urban areas by comparing locomotor performance of urban and forest lizards on tracks differing in angle of inclination and substrate type. We found that using manmade substrates comes at a cost: lizards ran substantially slower and lost traction on metal and painted concrete tracks compared to bark. We also found that morphological differences between urban and forest lizards were correlated with performance differences. Overall urban lizards had longer limbs, wider bodies, larger toepads, and more lamellae (relative to body size) than forest lizards; all traits correlated with faster sprint speed. Notably, urban lizards ran faster on both manmade and bark surfaces. Positive trait-performance relationships were most apparent on gradually inclined tracks, with significant negative relationships between limb lengths and sprint speed on steeply inclined tracks. These results suggest that there is not a trade-off in performance when using manmade versus natural substrates and that natural selection is acting most strongly on locomotor performance across flat surfaces such as the open ground. In contrast, forest lizards face competing demands of arboreal lifestyles where longer limbs are disadvantageous. Our findings provide a mechanism for natural selection to shape the phenotypic shifts we have observed in urban populations in this and previous studies.

Comparative Genomics Reveals Accelerated Evolution in Conserved Pathways during Anolis Diversification

14:15 **Kenro Kusumi** **Arizona State University**

Additional Authors: Marc Tollis*, Elizabeth D. Hutchins*, Jessica Stapley, Shawn M. Rupp, Inbar Maayan, Eris Lasku, Dale F. DeNardo, S. Tonia Hsieh, Oris Sanjur, Melissa A. Wilson Sayres, Rebecca E. Fisher (*Authors contributed equally)

Abstract: The *Anolis* adaptive radiation is a striking example of morphological diversification in vertebrates. Towards uncovering the genomic bases of these adaptations, we sequenced and assembled the draft genomes of three anole species - *Anolis frenatus* (Central American giant anole), *A. auratus* (grass anole) and *A. apletophallus* (slender anole)- for comparison with the available reference genome of the green anole, *A. carolinensis*. Comparative analyses revealed a rapid background rate of molecular evolution consistent with a model of punctuated equilibrium, and strong purifying selection on functional genomic elements in anoles. We found evidence for accelerated evolution in genes involved in behavior, sensory perception, and reproduction, as well as in genes regulating limb bud development and hindlimb specification. Morphometric analyses of anole forelimbs and hindlimbs corroborated these findings. We detected signatures of positive

selection across several genes related to the development and regulation of the forebrain, hormones, and the dewlap, suggesting molecular changes underlying behavioral adaptations known to reinforce species boundaries were a key component in the diversification in the *Anolis* genus. Specimens collected from an additional 9 species in the *Anolis* radiation (*A. biporcatus*, *A. capito*, *A. cryptolimifrons*, *A. humilis*, *A. lemurinus*, *A. pentaprion*, *A. poecilopus*, *A. tropidogaster*, and *A. vittigerus*) are a resource for further morphometric and genomic analysis.

Some thoughts on the trophic ecology of Anolis lizards

14:30 **Sean Giery** **University of Connecticut**

Abstract: Understanding what *Anolis* lizards eat has been the subject of intensive investigation for almost a century. Detailed assessments of *Anolis* diet has fundamentally shaped our current understanding of interspecific interactions and niche theory. One main finding of these early studies was that interspecific competition over food (and habitat) drives resource partitioning among species – a coexistence mechanism underpinning most (all) evolutionary and ecological theory from adaptive radiation to community assembly. Subsequent assessments of *Anolis* natural history have yielded taxonomically detailed diet data for nearly 60 species of ecologically diverse anoles from Bermuda to Brazil. Interestingly, these later studies largely conclude that many, if not most, *Anolis* species are dietary generalists – a finding seemingly at odds with the aforementioned work on niche partitioning. The goal of my talk is to refocus contemporary research on the dietary axis of niche partitioning in *Anolis* lizards in order to help resolve this apparent discrepancy. First, I will highlight some examples of evolved trophic specialization in *Anolis* lizards. Second, I will discuss initial results that illustrate patterns of trophic variation within and among populations of the brown anole (*A. sagrei*). Overall, I hope to convey a rather general message - much remains to be learned regarding the evolutionary ecology of trophic ecology in *Anolis* lizards. By combining published and unpublished data on the trophic ecology of *Anolis* lizards I will identify outstanding questions and new approaches to address them.

Land use and the restructuring of anole communities across an elevational gradient

14:45 **D. Luke Mahler** **University of Toronto**

Genomic signatures of adaptation associated with a history of range expansions in South American anoles

15:45 **Ivan Prates** **Smithsonian's National Museum of Natural History**

Additional Authors: Anna Penna, Miguel T. Rodrigues, Ana C. Carnaval

Abstract: Environmental variation across geographic space constrains the physiological performance of organisms and therefore limits species ranges, suggesting that expansions into new environments rely on adaptation. On the other hand, many experimental studies indicate physiological conservatism within species over wide environmental gradients. To assess whether adaptation underscores range expansions across habitats, we undertook a comparative study of *Anolis ortonii* and *Anolis punctatus*, which occur in Amazonia and the Atlantic Forest but not in the intervening open settings. To assess former range expansions within each species, we infer genetic structure and population history, estimating effective population sizes and gene flow — forces that could oppose local adaptation. We then apply genome-environment association analyses to test whether allele frequencies at thousands of loci are linked with temperature and precipitation gradients. Results for both species indicate large effective population sizes, mid-Pleistocene divergences across Amazonia and the Atlantic Forest, and limited post-divergence gene flow. We find 33 candidate loci in *A. punctatus*, seven of which map to genes involved with energy metabolism, water homeostasis, immunity, development, and cell signaling. By contrast, no candidate loci were found in *A. ortonii*. Distinct species signatures of adaptation do not appear to stem from constraints related to population structure and history. Instead, they are consistent with analyses of climatic space occupancy, which suggest that *A. punctatus* has expanded into colder, meridional coastal regions, presumably through adaptation. By contrast, *A. ortonii* is restricted to Atlantic Forest sites that are climatically more similar to this species' source in Amazonia, pointing to physiological conservatism.

Predator-induced natural selection in behavior

16:00 **Oriol Lapiedra** **Harvard University, CREAF**

Additional Authors: Thomas Schoener, Manuel Leal, Jonathan Losos, Jason Kolbe

Abstract: Evolutionary biologists have long debated the evolutionary role of animal behavior. This question has acquired even more importance in the current context of human induced rapid environmental change; since behavior largely determines how animals interact with their environment, it could be crucial to drive success of animal populations confronted with new selective pressures. Several authors have recently suggested that individuals that consistently differ in their behavior may have different chances to cope with rapid environmental challenges. However, the ecological and evolutionary implications of this variation remain unclear due to the lack of manipulative field studies of natural selection. In a field experiment explicitly designed to assess the ecological and evolutionary consequences of individual variation in behavior, we first documented consistent inter-individual variation in ecologically relevant risk-taking behavior in *Anolis sagrei* lizards. Then, we translocated them onto a replicated set of small, experimental islands in the Bahamas. On these islands, we manipulated the presence or absence of a well-known ground predator, *Leiocephalus carinatus*. Our study revealed that natural selection acted on risk-taking behavior. Selection differed between experimental treatments: on islands with the ground predator, *Anolis* lizards with a tendency to avoid the ground—as measured in laboratory behavioral assays—survived better, whereas on islands without the predator, lizards that were quick to begin exploring survived better. We also found that selection in behavior can occur in parallel with selection in morphological traits; but in the presence of predators selection was relatively more important on behavior than on morphology. Our results imply that selection can operate strongly on behavioral variation under different selective regimes and that the selective benefit of behavioral traits is context-dependent.

Using South Florida's exotic lizard community to evaluate the use of ecological niche models in predicting biotic invasions

16:15 **Caitlin C. Mothes** **University of Miami**

Additional Authors: James T. Stroud, Stephanie L. Clements, Christopher A. Searcy

Abstract: Ecological niche models are often used by conservation and management practitioners to predict the potential for non-native species to invade new geographic regions. Depending on these models to enact appropriate management plans assumes their accuracy, but most niche model studies do not provide any validation for their model outputs. South Florida hosts the world's most diverse non-native lizard community, providing a unique opportunity to evaluate model accuracy by comparing niche model predictions to field data collected from established non-native populations. Using Maxent, we developed niche models for 30 lizard species that are non-native and established within Miami-Dade County. We then compared model predictions of relative invasion success to multiple sources of field data representing abundance, geographic spread, and thermal tolerances. Maxent performed well in predicting across geographic space where these species were most likely to persist in Florida. For measures of relative invasion success, Maxent was accurate in its predictions among the *Anolis* lizards, but was not capable of detecting the relative success within the entire pool of non-native lizards. Additional comparisons between predicted and observed thermal tolerances showed that most of the models overpredicted the range of suitable thermal habitat. Our results suggest that factors other than climate, such as dispersal capability, biotic interactions, and genetics of non-native populations, may be determining the success of lizards in this novel habitat, causing niche shifts between the native and non-native ranges of these species.

The Lizard's Tale and Anole Annals v2.0: An enhanced platform for Anolis outreach

16:30 **Neil Losin** **Day's Edge Productions**

Additional Authors: Nathan Dappen, Ian Scott, Jonathan Losos

Abstract: Anole Annals (www.anoleannals.org) is an online platform used by the *Anolis* research community to share research methods and findings, natural history observations, and general anole trivia, both with

fellow researchers and the general public. In 2016, with support from the John Templeton Foundation, we started a project with two primary objectives: 1) to produce a series of seven broadcast-quality short films about *Anolis* biology; and 2) to redesign the Anole Annals website, incorporating these films as a core component of the visitor experience.Both objectives will be completed in the summer of 2018. In this presentation, I will premiere the first episode of the web series (The Lizard's Tale, Episode 1: Meet the Anoles, runtime 6 minutes) and showcase a few key features of the all-new Anole Annals website. I will also share some of the results of our ongoing effort to reach non-scientist audiences outside the small but loyal readership of Anole Annals. These efforts include: Laws of the Lizard, a 1-hour broadcast documentary about *Anolis* lizards on the Smithsonian Channel, bilingual classroom educational films produced for the Howard Hughes Medical Institute, and a short film produced in collaboration with the California Academy of Sciences.When the new Anole Annals launches later this year, we encourage all members of the *Anolis* research community to contribute to the site via blog posts (e.g. field updates, summaries of new peer-reviewed publications, and natural history observations) as well as researcher profiles, species profiles, and science explainers. We hope that the new Anole Annals v2.0 will continue to be a useful tool for collaboration among *Anolis* researchers, as well as a valuable platform for public engagement.

Sunday, 18 March 2018

Genome editing methods for the production of genetically modified anoles

09:30 **Douglas B. Menke** **University of Georgia**

Additional Authors: Ashley M. Rasys, James D. Lauderdale

Abstract: 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. Therefore, we are attempting to establish genome editing technologies in anoles with the goal of producing genetically modified lizards. Given the successful use of the CRISPR/cas system to generate targeted mutations in many other species, we have opted to employ this technology in our efforts. The biggest obstacle for the performance of genome editing in anoles is delivery of the genome editing reagents into oocytes or early stage embryos. Through a series of pilot studies in *Anolis sagrei*, we have established a surgical procedure that enables us to microinject solutions into maturing 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 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.

A shady way to beat the Miami heat

09:45 **Sarin Tiatragul** **Auburn University**

Additional Authors: Joshua M. Hall, Danial A. Warner

Abstract: One would have to try very hard to walk down the suburban areas of Miami and not notice innumerable anoles (Brown, Crested, Green, and Bark)! But have you wondered how all these anoles started their lives as embryos, and how they battled against the unforgiving urban thermal incubation environment so they could bob their heads relentlessly at you? In this presentation, we take you on a journey, telling the story of how two students with spoons scoured and scorched the soil along the streets to uncover where these city-slickers lay their eggs. While their luck sometimes led them astray from plots where lizards are using, they had a great deal of triumph and found heaps of eggs. Using modern temperature sensors and a good bit of old-fashion labor, they characterized the conditions of each nest to unveil the conditions that embryos experience during their development in the field. Ultimately, they learned female anoles in the city may have more choices in nesting microenvironments than their forest counterparts.

Anolis carolinensis satellite cells have expanded musculoskeletal potential

10:00 **Joanna O. Palade** **Arizona State University**

Additional Authors: Djordje Djordjevic, Elizabeth D. Hutchins, Rajani M. George, John A. Cornelius, Alan Rawls, Joshua W. K. Ho, Kenro Kusumi, Jeanne Wilson-Rawls

Abstract: Lizards are evolutionarily the closest vertebrates to humans that demonstrate the ability to regenerate entire appendages containing cartilage, muscle, skin, and nervous tissue. We previously isolated PAX7-positive cells from muscle of the green anole lizard -*Anolis carolinensis*- that can differentiate into multinucleated myotubes. We generated a transcriptome from proliferating lizard PAX7-positive cells and compared them to transcriptomes from mouse human tissues from the ENCODE project using XGSA, a statistical method for cross-species gene set analysis. These analyses determined that the lizard progenitor cell transcriptome was most similar to mammalian satellite cells. Further examination of specific GO categories of genes demonstrated that among genes with the highest level of expression in lizard satellite cells were an increased number of genetic regulators of chondrogenesis. In micromass culture, lizard PAX7-positive cells formed Alcian blue and collagen 2a1 positive nodules, without the addition of exogenous morphogens, unlike their mouse counterparts. Subsequent quantitative RT-PCR confirmed upregulation of expression of

chondrogenic regulatory genes and cartilage specific structural genes in lizard cells. Taken together, these data suggest that tail regeneration in lizards involves significant alterations in gene regulation with expanded musculoskeletal potency.

Using archival DNA to elucidate anole phylogeny

10:15 Gregory C. Mayer University of Wisconsin-Parkside

Additional Authors: Tony Gamble

Abstract: Archival DNA—DNA extracted from specimens that were not preserved with the intent of preserving the specimens' DNA—has proven to be a valuable source of data for the study of extinct species and populations. For anoles, although some poorly known species have not been recently collected, the only species widely acknowledged to have gone extinct in historical times is *Anolis roosevelti* of the eastern islands of the Puerto Rican Bank, where it is known to have occurred on Vieques, Culebra, St. John, and Tortola. Before new experimental methods can be accepted, they must be validated by showing that new results comport with well-confirmed earlier findings. 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. The latter consideration is clearly of concern with *Anolis roosevelti*, of which only six extant specimens, collected by A.H. Riise in the 1860s and Chapman Grant in the 1930s, are known. Thus, preliminary to study of *roosevelti*, we have attempted the extraction of archival DNA from specimens of *Anolis cristatellus* collected by Riise (3 specimens) and Grant (5 specimens) approximately coincident in time and place with their specimens of roosevelti. In addition, we studied 4 more recently collected fluid-preserved specimens. We have obtained partial to good mtDNA assemblies for 5 of the 8 historical specimens, and all of the more recent ones. Phylogenetic analyses of the mitogenomes and ND2 confirm that all successfully analyzed specimens occur in reasonable places in the tree. We thus conclude that extraction and analysis of archival DNA is a promising method for investigations of the genome and phylogeny of *Anolis roosevelti*, and of anoles in general.

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

10:30 Liam J. Revell Universidad del Rosario, UMass Boston

Abstract: Phylogenetic comparative biology consists of the activity of drawing inferences about the evolutionary process from a pattern of observations for species. For nearly thirty years phylogenetic comparative methods have been used to great effect to study the evolution of lizards in the genus *Anolis*. Herein, I 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 then apply this method to investigate the possibility that the rate of dewlap color and pattern evolution differs between mainland and island anole clades. Finally, I discuss some caveats and limitations of this approach for modeling heterogeneity in the rate of discrete character evolution on reconstructed trees in general, as well applied particularly to this case study.

Predicting the outcome of species interactions in a novel species assemblage: Anolis vs. Phelsuma in Hawaii

11:30 Amber N. Wright University of Hawaii

Additional Authors: Stevie Kennedy-Gold, Carla Piantoni, Timothy E. Higham, Robyn M. Screen

Abstract: A key paradigm in ecology and evolution is that functional traits determine resource use, thereby setting limits on distribution and abundance. This relationship between phenotype, performance, and ecology is potentially powerful: if we can mechanistically link functional traits to the outcome of species interactions, we can generate testable predictions about species coexistence in different environments. Many decades of work on anoles have contributed to identifying functional traits related to resource use along three axes that structure anole communities: structural habitat, thermal microhabitat, and diet. We are testing whether this framework can be used to make predictions regarding coexistence in a novel assemblage of *Anolis* and *Phelsuma* (day geckoes). The arboreal, diurnal, insectivorous lizard guild in Hawaii is dominated by *Anolis carolinensis*, *A. sagrei*, and *P. laticauda* (all introduced). To date, we have found that the three species all overlap considerably in body size and thermal preference in the lab–traits related to diet and thermal mi-

crohabitat, respectively–making competition likely. For structural habitat, *A. carolinensis* and *P. laticauda* overlap in clinging ability on smooth substrates, while *A. carolinensis* and *A. sagrei* overlap in clinging ability on rough substrates. These results suggest that *A. carolinensis* may be particularly disadvantaged because it is the only species that overlaps with both of the others. Using ongoing competition experiments in enclosures that vary species composition while controlling for lizard density and resource availability, we are measuring 1) fitness correlates to determine the relative strength of intra- vs. interspecific competition and therefore the potential for long-term coexistence, 2) resource availability and individual resource use along all three axes to document resource partitioning, 3) and behavior to document interference and time budgets. Preliminary results show a similar interaction between the two anoles as seen elsewhere in their range, and that *A. carolinensis* increases use of rough substrates in the presence of *P. laticauda*.

The other Miami Heat: Urban areas alter thermal biology and influence persistence and spread of two invasive Anolis species.

11:45 **Andrew C. Battles** **University of Rhode Island**

Additional Authors: Jason J. Kolbe

Abstract: For ectotherms, higher average temperatures in urban areas (the urban heat island effect) and the dramatic changes to the spatial configuration of microhabitat may pose significant challenges to thermoregulation and thus alter temperature-based activity. To evaluate whether these changes to the thermal quality of available habitat may affect the persistence and dispersal of ectothermic species introduced to an urban area, we took canopy photographs and measured operative temperature (Te), the body temperature of a non-thermoregulating lizard, using copper lizard models in random locations in four urban and four natural sites in Miami-Dade County, FL, USA. While these models were recording Te, we captured lizards of two introduced species (*Anolis cristatellus* and *Anolis sagrei*) and recorded internal body temperature (Tb), between 0700 hrs and 1800 hrs. We found that urban areas had more open canopies compared to natural areas, which led to higher Te in urban sites than in natural habitats. We also found that lizards actively thermoregulated, maintaining Tb higher than Te in all sites. From thermal preference trials in a thermal gradient, we found that Tb of *A. cristatellus* were lower than those of *A. sagrei*. Urban sites may lower thermoregulatory costs for both species, but we found only *A. sagrei* Tb more often within their preferred temperature range in urban compared to natural habitat. Furthermore, based on available Te within each species' preferred temperature range, urban sites with only *A. sagrei* appear less-suitable to *A. cristatellus*, and vise 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. For *A. cristatellus*, dispersal may depend on human activity or connectivity of preferred habitats.

Covariation in arthropod community composition and dominant anole identity on dredge spoils islands in Florida

12:00 **Nathan W. Turnbough** **Independent scholar**

Abstract: In Florida, invading brown anoles (*Anolis sagrei*) frequently displace native green anoles (*A. carolinensis*) from structurally-simple habitats, which alters the identity of the dominant anole operating in the food web. I assessed arthropod community responses to this shift in dominant anole species by sampling ground and foliage-dwelling arthropods on a series of invaded and uninvaded dredge spoils islands along Florida's Intracoastal Waterway. Brown anole abundance, a proxy variable for dominant anole status, significantly explained 9.4–17.9% of the variation in the composition of these communities, and it remained a significant predictor even after full suites of environmental covariables were incorporated into the models. Further, several interesting covariation patterns emerged from within-community analyses of arthropod response groups, particularly ant and spider assemblages.

Tail Regeneration in Anole Lizards: Insights from Comparative Genomic Analysis and Reformation of the Peripheral Motor Nervous System

12:15 **Cindy Xu** **Arizona State University**

Additional Authors: Minami A. Tokuyama, Elizabeth D. Hutchins, Rebecca E. Fisher, Jeanne Wilson-Rawls, Jason M. Newbern, Kenro Kusumi

Abstract: Many vertebrates display the ability to regenerate appendages such as the tail, but lizards further evolved the ability for autotomy, or self-amputation, as a predator response. There are a number of questions about how lizards regulate this process, including the source of cells for regeneration, the patterning and differentiation of tissues, and the functional integration with the nervous and circulatory systems. To address these questions, we have used two approaches.First, we have examined the regenerative process at the molecular level. Building on our genome annotation of the green anole, *Anolis carolinensis*, we analyzed the total RNA and microRNA transcriptomes during tail regeneration during maximal growth at 25 days post autotomy (dpa). Transcriptomic analysis revealed 326 differentially expressed genes, of which 302 have clear human orthologues, regulating wound and immune response, hormonal regulation, and musculoskeletal development. Comparative genomic analysis with regeneration in other vertebrate models identified common patterns of activation of the canonical Wnt and Wnt5-calcium signaling pathways. MicroRNA sequencing of lizard regenerating tail and associated tissues revealed both novel and known microRNAs involved in stem cell regulation. We are currently carrying out transcriptomic analysis of the initial steps in the regenerative process, from 0 to 10 dpa.Second, we have examined the reformation of the neuromuscular system in the regrowing tail at the cellular level. The regenerated tail is a replacement of the original, and reformation of the central and peripheral nervous systems are essential for its function. We used specific antibody markers by immunofluorescence to examine the regeneration of peripheral nerves and neuromuscular junctions (NMJs). Interestingly, the density of axons and NMJs in muscle was higher in the regenerated tail and the morphological maturation recapitulates normal development.Given the conservation of gene regulatory networks between anole lizards and humans, findings from these studies can help to direct the development of future regenerative medical therapies.

Using experimental islands to explore evolutionary dynamics under climate change

14:00 **Michael L. Logan** **Smithsonian Tropical Research Institute**

Abstract: In recent years, large-scale field-experiments have revolutionized the field of evolutionary biology, while developments in high-throughput DNA sequencing have done the same for the field of genetics. These tools have rarely been combined to investigate evolutionary dynamics under climate change. In this talk, I will introduce two large-scale transplant experiments currently underway in Panama and The Bahamas. We have transplanted thousands of *Anolis* lizards to small islands that differ dramatically in their thermal environments, providing a source of selection on thermal physiology. We are combining estimates of selection with genome scans to track the evolution of genotypes and phenotypes over time. With this system we can ask a number of questions, including: What genes underlie thermal adaptation and do they evolve convergently in populations undergoing similar selection pressures? Can populations evolve fast enough to compensate for warming? Is rapid evolution constrained by phenotypic plasticity, fitness trade-offs, or genetic architecture? How does gene flow alter evolution under climate change?

Role of a sweet-toothed anole in orchid pollination

14:15 **Christine Rose-Smyth** **Verdant Isle Orchids**

Abstract: Fruit and nectar feeding is characteristic of a large number of islands 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 non-rewarding 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 do however 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 anoles can thus affect fitness by decreasing pollination opportunities or increasing outcrossing among genets.

Let There Be Light: Widespread Use of Human-Produced Light at Night by Anoles and Its Consequences

14:30 Christopher J. Thawley **University of Rhode Island**

Additional Authors: Jason J. Kolbe

Abstract: As anthropogenic global change, including urban expansion, continues to increase, artificial light at night (ALAN) affects many species worldwide. Contemporary research shows that ALAN has impacts on the survival, physiology, and reproduction of many taxa, yet we lack a clear understanding of how ALAN affects reptiles. Some taxa, including lizards such as geckos, exploit the "night-light niche", an opportunity for extended activity or augmented resources in habitats subject to ALAN. Anoles are adapted to specific photic environments and considered to be strongly diurnal. However, many anole species thrive in human-altered environments where ALAN is prevalent. We compiled reports from the literature showing that use of the night-light niche created by ALAN is widespread in anoles, being documented in ¿20 species. Anoles from multiple ecomorphs and all major geographic areas were reported to use this niche. A full two-thirds of anoles observed to use ALAN are species which have become invasive, suggesting a potential link between tolerance of ALAN or ability to exploit it and invasiveness in this group. Our research with brown anoles (*Anolis sagrei*) shows that exposure to ALAN may induce onset of reproduction and increase reproductive output, suggesting that urban tolerant anoles may be able to benefit from ALAN. As urban areas expand, artificial lighting is likely to increasingly affect anoles and create ecological opportunities for these species to exploit the novel night-light niche.

Environmentally Cued Hatching in Anoles

14:45 Sean Doody **USF St. Petersburg**

Abstract: Environmentally cued hatching, whereby embryos hatch at different times based on changing risks, is widespread and possibly common in animals, but its prevalence in reptiles is unknown. One type is early hatching, in which embryos assess risks (e.g., predation) based on external cues (e.g., vibrations) and choose their own birthday to reduce those risks. Early hatching may be common in lizards, but a number of biases has conspired to preclude determination of its taxonomic distribution. Recently, researchers have revealed evidence for early hatching in three anole species, suggesting that the behavior is common or even ubiquitous in the group. I discuss the evidence and a way forward for revealing the taxonomic distribution, evolutionary significance, ecological relevance and developmental mechanisms behind early hatching in anoles, and thus lizards, reptiles and animals.

Signal divergence and habitat partitioning among non-native bark anoles in South Florida

15:45 Winter A. Beckles **University of Miami**

Additional Authors: J. Albert C. Uy

Abstract: Despite decades of research, explaining why our planet contains a stunning array of diversity rather than just a few species remains a major challenge. Part of the difficulty in understanding the processes that maintain diversity is the difficulty of observing evolution operating in the wild. To this end, rapid changes in the environment can function as natural experiments, providing opportunities to observe the mechanisms of evolution as populations respond to habitat perturbations. We here leverage an extreme weather event as an experiment to determine how populations of the bark anole *Anolis distichus* respond to rapid changes in the environment. The bark anole has been introduced to Florida from the Caribbean on multiple occasions over the past century, and we have taken advantage of these introductions to explore how populations adapt to the fragmented landscape of South Florida. We previously found that the color of male dewlaps, which are throat fans extended during courtship displays to females, matches the lighting conditions of their habitat, suggesting that males have adapted to their new environment. With Hurricane Irma, the environment changed overnight, resulting in the relationship between dewlap color and habitat lighting to

disappear. By investigating how the relationship between habitat light and bark anole signal design continues to change over time, this work provides unique experimental insights into the mechanisms of evolution.

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

16:00 **Stephanie L. Clements** **University of Miami**

Additional Authors: Christopher A. Searcy

Abstract: South Florida is the global hotspot for the introduction of non-native reptile and amphibian species. Many non-native species that arrive in Florida persist and establish populations, while many native species are now seldom seen. Native species may be adapted to the specific and rare habitats of South Florida such as the Pine Rocklands, which have been reduced to 2% of their original extent. We conducted herpetofauna surveys in 15 pairs of native/non-native parks to determine whether native herpetofauna are persisting in areas where native habitat is preserved. Less than 10% of the individuals that we recorded were native, and we found no difference in the relative abundance or richness of native species between native and non-native parks. *Anolis* species (one native and four non-native) comprised 86% of the observed individuals. Community analyses indicate that only 9% of the total variance in herpetofaunal community composition is between native and non-native parks. Even this difference is driven by non-native species, with brahminy blind snake (Ramphotyphlops braminus), the most widely introduced species of snake in the world, being the best indicator of native habitat patches, and two anoles from the Caribbean (*Anolis sagrei* and *A. equestris*) being the best indicator of non-native habitat. These results demonstrate that non-native herpetofauna dominate both non-native and native habitat patches in Miami-Dade County. This could be the result of non-native species outcompeting native species even within native habitats, or could be due to a continuous influx of non-native species into native habitat from the surrounding urban matrix.

Predators influence prey body size variation in an urban landscape

16:15 **Zachary A. Chejanovski** **University of Rhode Island**

Additional Authors: Jason J. Kolbe

Abstract: Human-induced environmental change is currently impacting animal populations on a global scale. For example, the rise and spread of urban areas has drastically altered the environmental conditions experienced by organisms inhabiting cities. These novel conditions may promote change in key phenotypic traits of urban wildlife in order to maximize fitness. Conversely, certain traits may remain unchanged if they are constrained (e.g., through genetic correlations with other traits) or already optimized under these new conditions. Previous work in southeast Florida has shown that brown anoles (*Anolis sagrei*) from urban environments are larger (i.e. snout-vent length) compared to conspecifics from nearby natural habitats. Additionally, this work has also revealed a positive relationship between anole body size and the abundance of its predator (i.e. the curly-tailed lizard, Leiocephalus carinatus). However, the mechanism(s) producing these patterns remains unknown. To address this, we presented tethered brown anoles of varying sizes (i.e. SVL) to curly-tailed lizards in the field to assess whether predator attack behavior depends on prey size. We also conducted a common garden experiment to determine whether body size differences among anoles from habitats with and without predators are genetically based. Indeed, smaller anoles were attacked at shorter latencies compared to larger ones. Under common conditions, male hatchlings from habitats with predators grew faster compared to males from habitats without predators, but for females we found no differences in growth rate. Overall, our data suggest that predators may be important agents of natural selection in urban habitats.

Does season-dependent reproductive value of offspring drive the evolution of life-history traits in Anolis lizards?

16:30 **Joshua M. Hall** **Auburn University**

Additional Authors: Timothy S. Mitchell, Sarin Tiatragul, Philip R. Pearson, and Daniel A. Warner

Abstract: An important component of the reproductive strategy of many animals involves a seasonal shift in reproductive traits (i.e., clutch size, egg quality). Such shifts typically occur because environmental factors that influence reproduction (i.e., climate or food availability) change throughout the year; thus, organisms must change their reproduction as the environment changes to maximize fitness. Anole lizards are unique among squamates because they lay multiple, successive, single-egg clutches throughout the entire breeding season. As such, a single female can lay eggs approximately once per week for the greater portion of a year. Therefore, these lizards can serve as appropriate models to understand how seasonal changes in the environment interact with a reproductive strategy to maximize fitness. We synthesize the results of multiple lab and field experiments to 1) demonstrate how aspects of anole reproduction change seasonally and 2) discuss how these seasonal shifts may influence fitness. Our preliminary lab results demonstrate that key reproductive traits like egg size, egg quality, and hatchling size can increase through the reproductive season but field experiments suggest survival probability of hatchlings decreases across the same period. Further analyses will quantify variation in other reproductive traits, such as inter-clutch interval, total reproductive effort, and the relationship between this variation and seasonal changes in adult body size. Anoles have often served as model organisms for studies of ecology and evolution. By learning about seasonal shifts in reproduction and their influence on fitness, we will both increase our knowledge of anole natural history and enhance our general understanding of life history theory.

Posters

Are semi-aquatic anoles convergent?

Christopher K. Boccia **University of Toronto**

Additional Authors: D. Luke Mahler

Abstract: Six independent anole lineages (2 Caribbean, 4 mainland) have evolved to occupy semi-aquatic habitats. Theory predicts that these anoles should converge along ecomorphological axes. Previous authors have suggested that there is an absence of convergence in semi-aquatic anoles based on morphology relevant to arboreal living; however, a multidimensional approach is needed for this problem. We compared the morphology, colour/pattern, behaviour, ecology, and performance of semi-aquatic anoles to closely related terrestrial anole species to determine if semi-aquatic anoles have converged.This poster will focus on swimming performance and a novel behaviour we have termed 'rebreathing,' wherein a semi-aquatic anole sequentially exhales and re-inspires a bubble while diving.

Growing apart: characterizing the development of sexual dimorphism

Bonnie K. Kircher **University of Florida**

Additional Authors: Martin J. Cohn

Abstract: Species vary widely in their pattern and magnitude of sexual dimorphism, yet the proximate mechanisms that regulate these differences remain poorly understood. Sexually dimorphic characters present special challenges to our understanding of anatomical evolution because the sexes share the majority of their genomes yet can diverge in size, shape, and anatomical characters during development. Lizards in the genus *Anolis* (anoles) are an often-used model for evolutionary studies of sexual dimorphism and are also an emerging model for comparative developmental analyses. An example of a sexually dimorphic trait in *Anolis* is the dewlap, a colorful throat fan that is usually larger in males than in females and is used frequently by males (but infrequently by females) during courtship and aggression. Located on the throat and extending down the belly, the dewlap is supported by the second ceratobranchial (C2) cartilage in the hyoid system. We investigated the developmental basis of the sexually dimorphic dewlap apparatus in anoles and identified differences in C2 development between males and females that suggest sex-specific regulation of early skeletogenesis.

Natural selection on thermal reaction norms of lizard embryos

Phillip R. Pearson **Auburn University**

Additional Authors: Daniel A. Warner

Abstract: Theory predicts that natural selection will favor plasticity in spatially or temporally heterogeneous environments that vary in predictable ways. Like most traits, the potential for plasticity to evolve depends on the degree of heritability and strength of selection. To gain insight into evolution of plasticity, we assessed among-family variation in developmental plasticity for a variety of phenotypes in the brown anole (*Anolis sagrei*), and then evaluated the strength of selection on family-level reaction norms. Eggs from wild-caught females were incubated under thermal environments that mimicked temperature regimes characteristic of the early vs late reproductive season. Offspring were uniquely marked, measured for their size and locomotor performance, and then released onto an island near the collection site of their mothers. Recapture efforts were performed before and after the first winter season to quantify offspring survival and natural selection on phenotypes, as well as on family-level developmental plasticity. Incubation duration was significantly shortened by the late-season temperatures, and this pattern not differ among family groups. For morphological and performance traits, however, late season thermal conditions generally produced larger and faster offspring than early season regimes, but the strength and direction of the effects varied substantially among family groups. This family-level variation in reaction norms suggest that plasticity observed here might have an additive genetic basis. In addition, this variation in reaction norms provides an opportunity

for selection to operate on plasticity. Next, we will quantify the strength and form of selection on reaction norms to better understand how embryo responses to developmental environments are shaped by this process.

The effects of egg aggregation on offspring survival and phenotype

Jenna E. Pruett **Auburn University**

Additional Authors: Renata Brandt, Joshua M. Hall, Daniel A. Warner

Abstract: Oviparous reptiles exhibit diverse reproductive strategies; some species produce large clutches while others lay a single egg at a time. The brown anole, *Anolis sagrei*, lays single egg clutches across an extended nesting season, and eggs are often found individually or near other eggs. Nesting strategies have the potential to affect offspring. Indeed, communal nesting behavior has been found to enhance offspring body size and sprint speed in another lizard species. In this experiment, we set out to determine the effects of egg aggregation on hatching success and phenotypes in *Anolis sagrei*. Eggs were incubated at three treatments of one, four, and nine eggs per dish. We found that eggs incubated in aggregations gained significantly less mass than those incubated singly. Furthermore, eggs incubated in the center of the nine-egg aggregate gained significantly less mass during incubation than those in other positions in the aggregation. Finally, egg aggregation had a negative effect on egg survival. Overall, these results suggest that egg aggregation in this species has the potential to negatively affect offspring.