

The emergence of squamates as model systems for integrative biology

Thomas J. Sanger

Harvard University, Department of Organismic and Evolutionary Biology, Cambridge, MA 02138, USA,

Address for Correspondence (email: tsanger@oeb.harvard.edu)

Most of the major vertebrate clades have representative model species with vast molecular and laboratory resources. Evolutionary developmental biology (evo-devo) has progressed on the backs of these model systems using their tools for comparative studies among relatively closely related species. Despite a rich history of ecological, behavioral, physiological, and evolutionary study of squamates (lizards and snakes; Sites et al. 2011), this group has yet to realize a well-developed model species. The details of squamate development and the unique qualities of squamate genomes have been largely unexplored. With the advent of more accessible molecular tools, the lower costs of sequencing, and the publication of the genomes of the green anole (*Anolis carolinensis*) and Burmese python (*Python molurus bivittatus*), this is changing rapidly (Alföldi et al. 2011; Castoe et al. 2011). One of the hallmark objectives of evo-devo is to understand the developmental and molecular bases of morphological diversity. The goal of this article is to introduce some of the key features of squamate diversity, highlight some of the recent advances in squamate development, and outline ongoing efforts to develop *Anolis* lizards as a model clade for integrative research.

SQUAMATE DIVERSITY AND EVO-DEVO

There are approximately 9100 described squamate species and there is growing consensus regarding the phylogenetic relationships of the major squamate clades (Wiens et al. 2010). In many of these clades, morphological, physiological, and ecological diversity underlies a wide array of life-history strategies (reviewed in Sites et al. 2011). Evo-devo biologists are just starting to explore this diversity but have successfully laid the foundation for many exciting new research avenues.

A striking morphological trend among squamates is the independent evolution of elongate, limb-reduced body plans no fewer than 25 times (Wiens et al. 2006). Snakes are only one of many lineages that have converged on this body plan. Cohn and Tickle (1999) showed that the loss of snake forelimbs is correlated with a shift in the expression of anterior *Hox* genes. Axial elongation in snakes is due to a relatively fast segmentation clock (Gomez et al. 2008), whereas a simplified *Hox* code underlies the increased number of thoracic

and caudal vertebrae (Di-Poï et al. 2010). It remains unknown whether these same mechanisms have been independently employed by other squamates to converge upon a similar body plan.

Limb reduction typically proceeds from the most distal elements to those more proximal. Digits, therefore, are likely some of the first elements to be lost during the transition to a snake-like body form and many lineages have independently reduced their digits, although through unique sequences (Shapiro et al. 2007). Modification of Sonic hedgehog expression has been implicated in the gradual loss of digits in the skink genus *Hemiergis* (Shapiro et al. 2003), but again, this mechanism has not been examined in other lineages. Exploring trends of digit reduction, body elongation, and limb loss at different phylogenetic scales among squamates may yield novel insights into the biological nature of parallel evolution and shared developmental constraints.

One of the most iconic features of squamate diversity is the snake fang, the specialized tooth used to deliver venom to prey or pursuer. The developmental and evolutionary origins of snake dentition have been a topic of much debate. Vonk et al. (2008) suggest that anterior and posterior maxillary teeth became developmentally decoupled at the origin of Elapidae and Viperidae, allowing for the evolutionary flexibility to evolve fangs. There also appears to be significant variation in other aspects of squamate tooth development programs. For example, many species possess palatal teeth in addition to those around the margin of the mouth and the unique properties of palatal tooth development have only recently been appreciated (Handrigan and Richman 2011; Richman and Handrigan 2011). Many squamates also undergo constant tooth replacement making them a potential model of direct biomedical significance (Handrigan and Richman 2010a, b; Handrigan et al. 2010).

ANOLIS AS A MODEL CLADE FOR INTEGRATIVE RESEARCH

It is now becoming increasingly common for exciting conceptual breakthroughs to come from studies at the interface of the biological disciplines, from those studies dissecting the developmental bases of morphological variation

in the appropriate ecological and evolutionary contexts. *Anolis* lizards, or anoles, are an oft-used model for studies of ecology and evolutionary biology (Losos 2009) and of increasing value in studies of physiology and neurobiology (Wade 2012). Evo-devo research on *Anolis* lizards is, therefore, primed to become a significant front for future research synthesizing information across biological and evolutionary scales.

Anoles diversified rapidly throughout the Caribbean and independently evolved suites of nearly identical habitat specialists (ecomorphs) on each island of the Greater Antilles (Losos 2009). The ecological mechanisms (e.g., natural selection) underlying ecomorph convergence have been the subject of much research but only recently have biologists begun investigating the developmental bases of this convergence. Sanger et al. (2012a) showed that limb length convergence, the primary morphological correlate with habitat-specific performance, occurred through similar developmental transitions. The skull of anoles is also morphologically diverse but follows a unique pattern of ecomorphological convergence compared to the postcranium (Sanger et al. 2012b). During craniofacial evolution, the anole skull not only converged in shape but also in patterns of modularity suggesting that integration among different anatomical units does not limit morphological diversification. Further investigation of the molecular bases of morphological evolution in *Anolis*—in the appropriate phylogenetic and ecological contexts—may yield a seamless understanding of phenotypic diversification across biological levels.

The phylogenetic position of anoles (and squamates more generally) also allows for rigorous comparisons among the amniote clades, orienting observations commonly performed between the chicken and mouse. For example, Eckalbar et al. (2012) recently described the evolution of vertebrate somitogenesis by incorporating expression data from *A. carolinensis* and *Alligator mississippiensis* into their developmental studies of axial elongation. Their analysis revealed that each lineage had both shared and derived features of this seemingly ancient developmental program. Further comparisons between *Anolis*, chicken, and mouse (and others) will elucidate the developmental peculiarities of amniote development with greater resolution than previous comparisons among the classic model species alone.

Rather than developing a model species in isolation from its close relatives, *Anolis* is being developed as a model clade. A time-calibrated phylogeny of over 100 species is already available alongside a wealth of ecological data (Losos 2009; Alföldi et al. 2011). Genomic and laboratory resources are now being developed by the community to address long-standing questions about the mechanisms of morphological divergence and convergence among anoles specifically, and amniotes more generally. The *Anolis* Gene Nomenclature Committee (AGNC) was formed to facilitate communication among researchers and disseminate resources such as

sequence information and laboratory protocols. The AGNC recently published guidelines on the annotation and identification of genomic data from *A. carolinensis* and its relatives (Kusumi et al. 2011). Additional transcriptome and genome sequencing efforts are now being considered. Guidelines for the preservation and curation of digital, molecular, and histological materials are being discussed with the museum community. To learn more about these efforts visit <http://www.anoleannals.org> (anole research blog), <http://www.anolisgenome.org> (sequencing and genome updates), or <http://www.lizardbase.org> (genomics and geographic mapping portals).

The pieces are in place to address fundamental questions in evolutionary biology by combining studies of ultimate and proximate causation. *Anolis* lizards, in particular, offer the opportunity to combine data from disparate biological disciplines—ecology, evolution, development, physiology, and neurobiology—into a comprehensive understanding of phenotypic evolution. The communal efforts of the *Anolis* community will expedite the utility of this genus rapidly making it a novel model system for integrative research.

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