Reptile genomes open the frontier for comparative analysis of amniote development and regeneration

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ABSTRACT  Developmental genetic studies of vertebrates have focused primarily on zebrafish, frog and mouse models, which have clear application to medicine and well-developed genomic resources. In contrast, reptiles represent the most diverse amniote group, but have only recently begun to gather the attention of genome sequencing efforts. Extant reptilian groups last shared a common ancestor ~280 million years ago and include lepidosaurs, turtles and crocodilians. This phylogenetic diversity is reflected in great morphological and behavioral diversity capturing the attention of biologists interested in mechanisms regulating developmental processes such as somitogenesis and spinal patterning, regeneration, the evolution of “snake-like” morphology, the formation of the unique turtle shell, and the convergent evolution of the four-chambered heart shared by mammals and archosaurs. The complete genome of the first non-avian reptile, the green anole lizard, was published in 2011 and has provided insights into the origin and evolution of amniotes. Since then, the genomes of multiple snakes, turtles, and crocodilians have also been completed. Here we will review the current diversity of available reptile genomes, with an emphasis on their evolutionary relationships, and will highlight how these genomes have and will continue to facilitate research in developmental and regenerative biology.

KEY WORDS: reptile, genomics, gene expression, somitogenesis, regeneration

Introduction

A major goal of developmental genomics is to understand the genetic mechanisms underlying vertebrate patterning and differentiation. The most successful and diverse group of modern land-adapted vertebrates are amniotes, and they display a wide array of forms, from parakeets to people to pythons. This diversity comes with the opportunity to learn about shared and divergent pathways regulating development during the evolution of the vertebrate body plan. Much has been learned, through comparisons of amniote (chicken and mouse) and anamniote (zebrafish and *Xenopus*) developmental and genomic models, about molecular mechanisms that underlie important pathways. The human genome project brought with it the initial promise that we would one day understand the true origins of human genes and genetic disorders (Lander et al., 2001) and the more recent advent of next-generation sequencing technologies has yielded assembled genome representatives for most mammalian orders (Chinwalla et al., 2002; Lindblad-Toh et al., 2005; Liu et al., 2009; Mikkelsen et al., 2007; Wade et al., 2009; Warren et al., 2008; Zhang et al., 2013) in addition to 28 avian genomes (Table 1; Zhang et al., 2014). These sequences are freely available to the scientific community as the foundation for developmental studies. For instance, one could easily navigate to the University of California, Santa Cruz (UCSC) Genome Browser (available at http://genome.ucsc.edu/) and access the complete genomes of 48 mammals and five of the aforementioned birds. Next-generation sequencing has facilitated genomic studies of non-traditional model organisms at a cheaper cost, and the genomes of many more vertebrate species are being sequenced, contributing to the Genome 10K project (Genome 10K Community of Scientists, 2009). Of the more than 30,000 living amniote species, almost 10,000 are reptiles, yet there have been relatively few genomic resources available for non-avian reptiles until only very recently. This is despite the fact that non-avian reptiles contain far more diversity than mammals and birds in many aspects of development and physiology. Here we will review the phylogenetic diversity of currently available reptile genomes, and discuss how they have contributed to the knowledge of vertebrate developmental biology. Also, we will review current and potential avenues of research that are shedding light on comparative studies...
of regenerative capacity. Here, we will use the term “reptiles” in the historical sense to include the following: Testudines, or all living turtles; Crocodylia, or alligators, caimans, gharials, and crocodiles; and Lepidosauria, or all lizards, snakes, amphisbaenians (or all squamates) and the tuatara. For each of these groups, we will review their unique sets of adaptations and phenotypes, and how genome-sequencing efforts have facilitated work in that area.

The traditional class “Reptilia” refers to the living ectothermic amniotes. Although they share with amphibians several aspects of lifestyle, behavior, ecology and a whole field of study known as herpetology, as amniotes reptiles are more closely related to mammals and birds. After radiating into terrestrial environments during the Carboniferous around 320-310 million years ago (Donoghue, Benton, 2007; Pyron, 2010) (Fig. 1), amniotes split into two recognized groups based on cranial morphology: synapsids and sauropsids (Benton, 2005). The synapsids include all mammals as well as many extinct lineages of “mammal-like reptiles” from the Permian and Mesozoic Eras (i.e., pelycosaurs, therapsids and cynodonts). Synapsids reached the peak of their diversity in the Permian, and most lineages disappeared at the Permian-Triassic boundary extinction event; the remaining extant synapsids constitute class “Mammalia”. While their ancestors were similar in many respects to modern reptiles, modern mammals differ a great deal from reptiles in several important traits such as endothermy, mammary glands, fur, and the eutherian placenta.

The second amniote group is the sauropsids, which includes all living reptiles and birds, and originated 250-280 million years ago (Fig. 1). Sauropsids survived the Permian-Triassic extinction and diversified to dominate terrestrial and marine environments throughout the Mesozoic Era. The evolutionary history of sauropsids includes a Lepidosauria branch and a branch containing the order Testudines and the Archosauria, which includes birds and crocodiles. While the surviving modern reptiles constitute “saurian” reptiles, birds evolved from dinosaurs (Gauthier 1986; Brusatte et al., 2010) and so are also sauropsids, albeit with a set of highly derived set of “non-reptilian” constraints such as endothermy, feathers, and flight. A recent integration of developmental and paleontological evidence has clarified patterns of loss, fusion and re-evolution of wrist features that were integral to the early evolution of flight in bird-like dinosaurs and their avian descendants (Botelho et al., 2014). When focusing on the “reptilian” sauropsids, the times to the most recent common ancestors of Testudines, Archosauria, and Lepidosauria are on average much older (~240 million years) than those between the modern placental mammalian orders (~100 million years) and even between placental mammals and monotremes (~200 million years ago) (Donoghue, Benton, 2007; Pyron, 2010), making the sequence of divergence between the sauropid orders – and thus the ancestral states of various developmental milestones and divergent phenotypes – a controversial subject. One major area of disagreement has been the placement of turtles on phylogenetic trees. Turtles have a unique body plan, the most obvious trait being the shell, as well as a lack of temporal fenestrae in the turtle skull, differing greatly from the skulls of synapsids and other sauropsids that do contain fenestrae. Earlier studies based on anatomy and paleontology placed turtles in the sister lineage to all other amniotes (“Parareptilia”) (Benton, 2005). Some more recent genetic work has suggested that turtles form a clade with lepidosaurs (Lyson et al., 2012). However, the majority of genomic evidence supports a turtle-archosaur clade (Crawford et al., 2012; Crawford et al., 2014; Shaffer et al., 2013; Wang et al., 2013) and it is likely that the loss of temporal fenestrae was
a signature trait in the early evolution of turtles (Kuratani et al., 2011) and we adopt this approach in this review (Fig. 1).

**Genomic resources for Reptiles**

**Complete genomes for Lepidosauria**

The first reptile to have a complete genome sequence was a lepidosaur, the green anole lizard (Anolis carolinensis), which was made available by the Broad Institute in 2007 and published in 2011 (Table 1; Alföldi et al., 2011). It was mainly chosen to bridge the phylogenetic gap between chicken and human for comparative genomic studies in order to understand the origin of human genes (Janes et al., 2010), and its initial analysis yielded important insights to the evolution of amniote genomes (Alföldi et al., 2011). For instance, very few chromosomal rearrangements have occurred since A. carolinensis diverged from chicken ~280 million years ago, and there is a high degree of synteny conservation. In addition, the lack of isochromes in the green anole genome suggested for the first time that GC content may be less integral to genomic integrity that previously thought (Fujita et al., 2011).

Since the release of the green anole genome, the genomes of two other lepidosaurs have been made available. The first was the Burmese python (Python molurus bivittatus), which was published in 2013 (Castoe et al., 2011), and therefore have associated with changes in organ size and metabolism due to the

**Complete genomes for Testudines**

The first published turtle genome was that of the green sea turtle (Chelonia mydas), which was sequenced to better understand the regulatory components and evolutionary origins of the complex venom system (Vonk et al., 2013), while the first analysis of the speckled rattlesnake (Crotalus mitchelli) draft genome focused on multiple episodes of endogenous viral element integration (Gilbert et al., 2014).

**Complete genomes for Crocodilia**

While they traditionally have been placed in the class "Reptilia", crocodilians are archosaurian reptiles that share common ancestry within modern birds (Brusat et al., 2010), and therefore have the most promise for understanding genomic and developmental

### Table 1

SAUROPSIDS, INCLUDING REPTILES AND BIRDS, WITH AVAILABLE COMPLETE GENOME SEQUENCES

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name</th>
<th>Order</th>
<th>Family</th>
<th>Year reported</th>
<th>DOI</th>
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transcriptome resources for Reptiles

While the complete sequencing of a reference genome will certainly facilitate studies of genes and their expression and can shed light on developmental processes, the de novo sequencing and assembly of transcriptomes by way of next-generation sequencing technologies (i.e., RNA-Seq) in the absence of a reference genome has also been useful (Gibbons et al., 2009). To date, several transcriptome resources have been developed for reptiles lacking a complete genome, including the western terrestrial garter snake (Thamnophis elegans) (Schwartz et al., 2010) and the common chameleon (Chamaeleo chamaeleon) (Bar-Yaacov et al., 2013). Complete brain transcriptomes have been generated for the Nile crocodile (Crocodylus niloticus), the corn snake (Pantherophis guttatus), the bearded dragon (Pogona vitticeps) and the red-eared slider turtle (Trachemys scripta) (Tzika et al., 2011) (available at www.reptilian-transcriptomes.org), and the vomeronasal organ transcriptome has been generated for the corn snake (Brykczyńska et al., 2013).

A particularly valuable resource will be the transcriptome of the tuatara (Sphenodon punctatus) (Miller et al., 2012), which is a non-squamate lepidosaur (Fig. 1). Sphenodon is the surviving genus of the order Rhynchocephalia, which had a global distribution until the late Cretaceous (65-80 million years ago) (Apesteguía, Novas, 2003). The range of Sphenodon today is limited to a few small islands in New Zealand. Until there is a complete genome, the tuatara transcriptome will facilitate future research in various avenues of genomic evolution and conservation of reptiles. As complete genomes are now available for all major groups of reptiles (lepidosaurs, turtles and archosaurs), these transcriptomes can be easily mapped to their nearest relatives (Sphenodon to Anolis, for example), and can help shed light on the diversity and numbers of reptilian transcripts and how they differ from current model amniotes representing mammals and birds.

Evolution of gene families in reptiles

Based on prediction and homology alone, the green anole genome was initially reported to contain 17,472 protein-coding genes that were largely predicted through ab initio efforts (Alföldi et al., 2011). A subsequent transcriptome-based annotation increased the gene number to 22,962 (Eckalbar et al., 2013), which is comparable to other amniotes. In comparison, 25,385 genes were annotated in the python genome (Castoe et al., 2013), although only 68% of these contained a protein domain. 21,796 protein-coding genes were found in the painted turtle genome (Shaffer et al., 2013), and ~22,200 were reported in the Chinese alligator (Wan et al., 2013). The initial prediction for the chicken genome was that it contained between 20,000 and 23,000 protein coding genes (International Chicken Genome Sequencing Consortium, 2004). The mouse genome (GRCm38, accessed from www.ensmbl.org) contains 22,592 protein coding genes, and the number of protein coding genes found in the human genome by the ENCODE project was 20,687 (The ENCODE Project Consortium, 2012), although recent work shows that this number for humans could be reduced to less than 20,000 (Ezkurdia et al., 2014). This suggests that the total number of expected genes in any amniote genome should be in the range of 20,000 genes. Nonetheless, gene evolution across amniotes has been dynamic with considerable gene family loss and/or expansion since the time of divergence between the living vertebrates. For instance, the green anole lizard genome contained 3,994 protein coding genes with one-to-one orthologues in human, mouse, dog, opossum, platypus, chicken, zebra finch and pufferfish (Alföldi et al., 2011), which is considerably less than the total number of predicted genes for each of these genomes and suggests a high degree of gene duplication and loss during the evolution of these lineages.

The differences in various gene family expansions between reptiles and mammals are substantial, and have been linked to particular adaptations that are unique to each lineage. For instance, 11 opsin gene families were present in the green anole lizard genome as well as several species of invertebrate, fish and frog, but are absent in mammals (Alföldi et al., 2011) and this was related to the superior color vision in lizards when compared to most mammals. In addition, the green anole lizard genome featured significant duplications and expansion of several egg protein gene families, with an elevated rate of molecular evolution that indicates episodic positive selection and bouts of adaptation. Vivipary, or the birth of live young, evolved early and often during the diversification of squamates (115 times versus 140 in all vertebrates) (Pyron, Burbink, 2014), with frequent reversions to ovipary which would require many changes in egg-laying at the molecular level during saurispsid evolution. Indeed, out of the 276 protein-encoding genes expressed in the eggs of A. carolinensis, only 50 orthologues were confirmed in chicken, suggesting high turnover. Significant expansion of olfactory receptor families were found in the soft shell turtle genome, including 1,137 intact and possibly functional genes which is an amount similar to what is found in most mammals (Wang et al., 2013). Other examples of reptile-specific and functionally-related gene family expansion are the venom proteins in snakes, as revealed by comparisons between the python and king cobra genomes (Castoe et al., 2011; Vonk et al., 2013), and the contrasting evolutionary patterns of vomeronasal receptor repertoires that were observed between mammals and reptiles (Brykczyńska et al., 2013).

Genome vs. transcriptome based expression studies in reptiles

Though genomes are continually being released, there are many species of interest for which a genome is not available. Mapping
RNA-Seq reads to the reference genome of the same species remains the “gold standard” for gene expression studies (Fig. 2A) (Guttman et al., 2010; Trapnell et al., 2012); however, for those species without an available genome, one option for analysis is de novo transcriptome assembly (Fig. 2B). There are many tools available for de novo transcriptome assembly and differential expression analysis of these transcriptomes (Davidson, Oshlack, 2014; Grabherr et al., 2011; Haas et al., 2013; Robertson et al., 2010; Schulz et al., 2012) In cases where the reference genome is of low quality, i.e., with misassemblies and large genomic deletions, genes of interest that are absent in the genome assembly can be present in the transcriptome (Park et al., 2014). Another possible approach is mapping assembled transcripts to a closely related reference genome, which has been utilized with non-human primates, across the mammalian clade, and the zebra finch and human genomes (Fig. 2C) (Benjamin et al., 2014; Hornett, Wheat, 2012; Vijay et al., 2012). In silico mapping to distant reference genomes with up to 15% sequence divergence outperformed mapping to de novo transcriptome assemblies, generally recovering more of the transcriptome and reducing the number of mismappings from poorly annotated genes (Vijay et al., 2012). Another study found that mapping to divergent species within 100 million years apart represented more genes than mapping to the transcriptome alone, with similar results to those derived from high quality genomes (Hornett, Wheat, 2012).

Examples of developmental studies using reptilian genome resources

Evolution of genetic pathways regulating somitogenesis in reptiles

There are a number of morphologically divergent features observed in reptiles that are not seen in mammals, particularly in the vertebral column. First, there is an underlying genetic diversity in the regulatory networks that shape vertebral segments that has been revealed through comparative studies adding reptiles in the analysis (Eckalbar et al., 2012; Gomez et al., 2008). Second, there is greater diversity of vertebral segment number and allocation along the body axis (reviewed in Keye & Smith, 2014; Kusumi et al., 2013; Richardson et al., 1998). Unlike mammals, which are generally constrained to having only seven cervical vertebrae, reptiles display a great diversity of vertebral segment number expansions.

Among tetrapods, there are differences in vertebral morphology and development between the amniotes and amphibians. Since many amphibians have both aquatic and terrestrial life stages, there is development of both a larval spine as seen in tadpoles and subsequent axial reorganization in metamorphosis to adult morphology (Handrigan, Wassersug, 2007; Trueb, Hanken, 1992). In contrast, amniote tetrapods completely form their vertebrae during embryogenesis (reviewed in Rawls, Fisher, 2010). Since the mouse, chick and Xenopus frog are developmental model systems, their vertebral development has been well characterized (Burke et al., 1995; Christ et al., 2000; Gossler, Tam, 2002; Ročková, Roček, 2005; Trueb, Hanken, 1992). Molecular studies of axial development have been reported in different species of squamates (Cohn, Tickle, 1999; Eckalbar et al., 2012; Gomez et al., 2008). The evolution of spinal diversity derives from changes in developmental mechanisms controlling the size of vertebral elements, segment number and distribution (lumbar, sacral, caudal, etc.), and embryonic timing (reviewed in Gomez, Pourquie, 2009).

The formation of axial segments, or somites, is regulated by genetic networks regulated by the Notch, Wnt, and FGF pathways collectively called the ‘segmentation clock’ (reviewed in Kusumi et al., 2013). Most of what we understand about the segmentation clock has been restricted to studies in four model systems (mouse, chicken, frog, and zebrafish) with the following conserved features (EM, O, 2008; Holley, 2007; Krol et al., 2011; Sparrow, 2008): i) Posterior gradients of FGF8, WNT3a, and hairy/enhancer of split

Fig. 2. Read mapping methods for differential expression analysis of RNA-Seq data. (A) With a complete reference genome (shown in black), it is advantageous to map RNA-Seq reads (shown in red) to the genome and then perform differential expression testing. (B) Without a complete reference genome, it is possible to first assemble a reference transcriptome de novo (shown in blue) and then map RNA-Seq reads to the transcriptome contigs. (C) A third method is to assemble a reference transcriptome de novo, map RNA-Seq reads to the reference transcriptome, and then BLAST reference transcriptome contigs to a closely related complete genome. This is followed by normalization of read abundance and counts and differential expression testing. This third approach makes it possible to combine multiple transcriptome contigs that may represent the same gene and to use robust annotations from a related species, allowing for more accurate differential expression testing than method B.
to mammals that can regenerate whole structures, and the green anole has a reference genome and robust annotation (Alfoldi et al., 2011; Eckalbar et al., 2013), allowing for transcriptome-wide studies of molecular pathways and mechanisms involved in lizard tail regeneration.

Though the regenerating tail has a different structure than the original tail, it is an impressive example of regeneration of cartilage, de novo muscle groups, skin, vasculature, and neural ependymal cells (Fisher et al., 2012; Gilbert et al., 2013; Hutchins et al., 2014; McClean & Vickaryous, 2011; Ritzman et al., 2012). While blastema formation is fairly well characterized during limb and fin regeneration in amphibians and teleost fish, lizards follow a different mechanism of regeneration. Blastema formation is traditionally characterized by dedifferentiation of tissue, proliferating cells focused at the tip of the regenerating appendage, and the absence of a vascular bed (Iten & Bryant, 1973; Mescher, 1996; Peadon & Singer, 1966; Singer, 1974; Smith & Wolpert, 1975). However, there is no evidence of dedifferentiation in the lizard (Cox, 1969; Fisher et al., 2012; Hughes & New, 1959; Hutchins et al., 2014; Simpson, 1965). Additionally, in the leopard gecko (Eublepharis macularius) and green anole (A. carolinensis), proliferating cells are present throughout the regenerating tail, and the distal tip is highly vascular (Hutchins et al., 2014; McClean, Vickaryous, 2011).

Though there is a lack of evidence for blastema formation in regenerative squamates, studies of the molecular basis of tail regeneration have shown many shared pathways with other vertebrates (Hutchins et al., 2014). There are hundreds of genes that are differentially expressed along the proximal-distal axis of the regenerating tail, including those related to wound healing, musculoskeletal development, hormonal response, embryonic morphogenesis, and the Wnt and MAPK/FGF signaling pathways. The Wnt pathway in particular has been identified as a key regulator of regeneration in the salamander limb blastema (Knapp et al., 2013; Wu et al., 2013) and mouse digit tip (Takeo et al., 2013). It is possible that all vertebrates have inherited the innate genetic and regulatory repository associated with regeneration. What is unclear is why some lineages, such as mammals, have lost the ability to regenerate in the adult stage despite conserving the genes involved in regrowth. Unlike amniote models zebrafish or salamander, lizards can provide information on amniote-specific pathways and patterns necessary for regeneration.

**Carapace and plastron formation and tooth loss in turtles**

The shell is a novel phenotype that unites all turtles, and comprises of a set of highly derived morphologies which combine to create a bony shield on both the dorsal (known as the carapace) and ventral sides (known as the plastron) of the animal. While fossil turtles are well known due to the fact that their hard and bony shells fossilize quite readily, the very early and rapid appearance of a complete shell in turtle evolutionary history has contributed to a relative lack of transitional forms in the fossil record. The oldest known turtle, Odonotochelys, (Li et al., 2008) was found in 220 million year old deposits in China and has a complete plastron and an under-developed carapace. This pattern matches the emergence of the turtle shell during embryonic development, which diverges significantly from the more conserved ancestral amniote condition (Gilbert, 2001) The painted turtle genome revealed significant gene family expansions in beta-keratins which play an important role in the formation of the shell, and mRNAs extracted from Pseudemys

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**Regeneration in lizards**

Regeneration of appendages occurs throughout vertebrates, though the extent of regeneration varies throughout taxa (Bely, Nyberg, 2010). Amphibians and teleost fish are spectacular examples of limb and tail regeneration (Stocum, Cameron, 2011). Many lizards are capable of tail regeneration following tail amputation and/or autotomy, and tail regeneration in alligators has been reported in the field (Han et al., 2005). Birds and mammals have limited regenerative capacity in comparison, though some neonatal and juvenile mammals can regenerate digit tips, and African spiny mice can autotomize and regenerate skin (Han et al., 2008). As amniotes, lizards are the most closely related organisms (HES and HER) proteins and rostral gradient of retinoic acid in the unsegmented paraxial mesoderm, ii) cyclical expression of genes in the Notch, Wnt, and FGF pathways, iii) the mesp2 gene that integrates segmentation network gene information at the determination front. In the segmentation clock, information from gradients of gene expression within the presomitic mesoderm (PSM) is integrated with the expression of genes that are cyclically transcribed in that tissue. Somite boundaries are determined based on the periodic interaction of the cycling genes and these gradients. These four model organisms of focus for previous studies of somitogenesis do not capture the full diversity of vertebrates. Analysis of somitogenesis in the green anole lizard and the American alligator identified convergence in cycling expression (lunatic fringe in both mouse and chicken, but not in anole or alligator) and conservation of genes expressed in gradients in the presomitic mesoderm in both squamates and anamniotes (hes6 in green anole, Xenopus, and zebrafish) (Eckalbar et al., 2012).

Axial identity and boundaries of Hox gene expression are also set during somitogenesis (Alexander et al., 2009; Zákány et al., 2001). Mutations in the Notch pathway effector Rbpj were shown to disrupt the dynamic expression of Hoxd1 and Hoxd3, and, in transgenic mice with dominant negative alleles ofDll1 that have reduced Notch signaling in the PSM, there are homeotic vertebral transformations and subtle changes of Hox gene expression (Cordes et al., 2004). In homozygous Lfng null mutants and in transgenic animals overexpressing Lfng, vertebral identities were altered, numbers of segments in the cervical and thoracic regions were reduced, and expression of Hoxb6 was shifted rostrally. Altogether, these findings confirm that the segmentation process is coupled to the determination of axial identity through Notch pathway regulation of Hox expression.

Snakes are some of the most striking examples of both increased number of vertebral segments combined with loss of limbs (Caldwell, 2003; Gans, 1975; Greer, 1987; Greer, 1991; Lande, 1978). The emergence of a “snake-like” morphology is estimated to have arisen independently at least twenty-five times in the squamates (Brandley et al., 2008; Wiens et al., 2006). Molecular studies of the corn snake identified that generation of over 300 vertebral segments was associated with both increased rate of the segmentation clock rate together with increased formation of presomitic mesoderm in the tailbud (Gomez et al., 2008). There was also an expansion in expression of thoracic Hox genes in the python (Cohn & Tickle, 1999). With the whole genome sequencing of additional squamates, we will better understand whether common or divergent genetic regulatory changes are driving the repeated evolution of “snake-like” morphology.
nelson/shell precursor cells revealed independent patterns of beta-keratin involvement in turtle shells and bird feathers (Shaffer et al., 2013). Cross-species gene expression profiling between chicken and softshell turtle embryos suggest a conserved vertebrate phylotypic period, followed by significant turtle-specific repatterning of 233 genes whose gene ontology categories include ossification and extracellular matrix regulation, as well as crucial roles of 212 microRNAs and a co-option of the Wnt signaling pathway in the development of the carapacial ridge (Wang et al., 2013). Another key trait of turtles that differs from other reptiles is tooth loss, which has been associated with extensive generation of pseudogenes, including degradation of tooth-specific genes such as enamelin (ENAM), which contains multiple stop codons and non-conserved sequence (Shaffer et al., 2013). The availability of genomic resources for turtles will continue to shed light on the development of the characteristic traits of this enigmatic group.

Development and evolution of the archosauromorph heart

The septation of the heart tube to form a four-chambered heart arose independently in mammals and in archosauromorph reptiles. The emergence of this developmental septation process represents a well-known case of evolutionary convergence (Farmer, 1999). While it makes sense that the metabolic demands of flight would lead to cardiac septation in birds, modern crocodilians have a much more ectothermic “reptilian” lifestyle, and the four-chambered heart is likely a vestigial trait that was ancestral to highly active and likely endothermic stem archosaurs. A study of non-crocodilian reptiles (the turtle T. scripta and the green anole A. carolinensis) that focused on gene expression in developing ventricles showed that turtles and lizards initially form a ventriculosem chamber which homogeneously expresses the Tbx5 transcription factor, while in chicken and mouse Tbx5 expression is restricted to a left ventricle and excluded from the prospective right ventricle (Koshiba-Takeuchi et al., 2009). Transgenic ectopic expression of Tbx5 in the prospective right ventricular region of mice led to loss of the ventricular septum, and changes in genetic regulation of Tbx5 are thought to have arisen independently in the avian and mammalian lineages.

How will further reptile genomes advance comparative developmental studies?

Next-generation sequencing technologies promise to increase the number of reptilian genomes – adding to the currently available four lepidosaurs, three turtles, and four crocodilians – to allow the research community to address many unresolved questions using comparative methods. While it has been shown that the mapping of transcripts to a moderately distantly related reference genome could prove useful, highly divergent genes, which can be of great interest, are underrepresented in analyses without an available reference genome. Using only transcriptomes, it is difficult to study cis-regulatory elements, copy number variation, transposable elements, and noncoding RNAs, which can be important regulators of gene expression. The continuing availability of reptile genomes will provide more resources for comparative gene expression studies. For instance, squamates are sorely unrepresented as there are currently four available complete genomes out of >9,400 species. Squamates as a group contain many convergent phenotypes, such as leglessness, and their genomes would be a prime resource for understanding the development of axial and appendicular morphologies. Given the ever-increasing pace of ease and affordability of genome sequencing projects in the next-generation sequencing era, it is likely that the current gaps in phylogenetic sampling across reptiles will begin to get bridged, and a true appreciation for the diversity of forms across amniotes will emerge.

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