Genome Evolution in Reptilia, the Sister Group of Mammals

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Abstract

The genomes of birds and nonavian reptiles (Reptilia) are critical for understanding genome evolution in mammals and amniotes generally. Despite decades of study at the chromosomal and single-gene levels, and the evidence for great diversity in genome size, karyotype, and sex chromosome diversity, reptile genomes are virtually unknown in the comparative genomics era. The recent sequencing of the chicken and zebra finch genomes, in conjunction with genome scans and the online publication of the *Anolis* lizard genome, has begun to clarify the events leading from an ancestral amniote genome—predicted to be large and to possess a diverse repeat landscape on par with mammals and a birdlike sex chromosome system—to the small and highly streamlined genomes of birds. Reptilia exhibit a wide range of evolutionary rates of different subgenomes and, from isochores to mitochondrial DNA, provide a critical contrast to the genomic paradigms established in mammals.

INTRODUCTION

It may seem odd for a review on genome evolution in reptiles to appear in a journal devoted largely to human and mammalian genomics. However, if the rise of comparative genomics in the last 15 years has taught us anything, it is that no species or lineage can be studied genomically in isolation from related lineages. Human genomics has clearly benefited from the broader view afforded by genomic comparisons with other mammals, and the draft sequences of some 32 eutherian, marsupial, and monotreme mammals have shed important light on the origin of primate and human genes and genomes, rates of evolution, dynamics of retroelements, and many other topics. In this review, we hope to put mammalian and human genome evolution in yet broader perspective by comparison with the sister group of mammals, the Reptilia.

The Reptilia consist of birds and so-called nonavian reptiles, the latter being those members of the Reptilia that are not birds. It may seem unbalanced to compare mammals, with their approximately 5,200 extant species, with a clade that not only contains two evolutionarily, physiologically, and structurally divergent lineages, the birds and nonavian reptiles, but that now consists of ~17,000 species (~9,800 for birds and ~7,500 for nonavian reptiles). Yet comparison of sister groups (groups that are closest relatives to one another) is a cornerstone of inference in evolutionary biology. Sister groups by definition are of similar age, since they both diverged from the same common ancestor, the amniote ancestor. Amniotes share a series of extra-embryonic membranes that protect and nourish the embryo, and the clade Amniota consists of the amniote ancestor and all its descendants. Technically, the term Reptilia denotes birds and nonavian reptiles, but for ease of reading we will frequently relax terminology, using the term reptile to refer to nonavian reptiles. Our review will focus primarily on the least-studied amniote group, the reptiles, since at least the dim outlines of genome evolution in birds have become clearer and have been reviewed since the sequencing of the chicken genome in 2003 (50, 51, 58, 75).

In the last five years, genomics of Reptilia has come to the fore as a critical counterbalance to the studies of human and mammalian genomics. Increased interest has been paid to reptile genomes, as evidenced by newly sequenced genomes from Reptilia, including two avian [chicken (*Gallus gallus*; 80) and zebra finch (*Taeniopygia guttata*; 190)] and one nonavian [green anole (*Anolis carolinensis*)] genomes in the databases; by the establishment of significant genomic resources for some reptile groups; and by the awareness that genomic diversity in Reptilia is likely to be substantially greater than even the considerable diversity in mammals. This new awareness has been signaled by recent symposia and research compendia devoted to reptile genomics in journals such as *Integrative and Comparative Biology* and *Cytogenetic and Genome Research* (90, 138). Several initiatives of the National Human Genome Research Institute, such as *Evolution of the Human Proteome* (**http://www.genome.gov/25521740**), have specifically called for the sequencing of nonavian reptile genomes [such as the painted turtle (*Chrysemys picta*), an ongoing project led by the Washington University Genome Center] in an effort to provide better phylogenetic resolution and enhanced accuracy of reconstructing ancestral states within vertebrates. Both birds and nonavian reptiles figure prominently in a new genome initiative, Genome 10K, that proposes to sequence the genomes of 10,000 vertebrate species (76).

In this review we will describe elements found throughout the genome including retroelements [i.e., long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs)], simple-sequence repeats, and isochores. We will also outline chromosomal organization of reptilian genomes, including mitochondrial sequences and the presence or absence of microchromosomes and sex chromosomes. We will also address phylogenetic inferences on genomic characteristics of extinct reptiles. Lastly, we will provide a brief summary of current resources

for reptilian genomics, including a genome project for the green anole, the first project of its kind for a nonavian reptile. We hope our review will further increase interest in reptile genomics and foster a broader view of the evolutionary context of mammalian and human genomics.

DIVERSITY OF EXTINCT AND EXTANT REPTILES

What we would recognize colloquially as the first reptiles date back to the Late Carboniferous Period over 300 million years ago (MYA) (17, 40, 178, 184). Trackways from New Brunswick, Canada, have been discovered in sediment formed by seasonally dry river channels dating to the lower Pennsylvanian Epoch (55). This evidence suggests that early amniotes were living in seasonally water-stressed environments, highlighting the adaptive advantage of the amniotic egg, which affords protection from desiccation and allows eggs to be laid on land. Tens of millions of years after the colonization of land by amphibians during the Middle Devonian (~395 MYA), two major divisions appear in the fossil record: the reptiles proper (also called sauropsids) and the synapsids, which are represented today only by mammals (16). The earliest reptiles, e.g., the lizardlike *Hylonomus lyelli* (26), and the earliest synapsids, e.g., *Protoclepsydrops haplous* (156), date to roughly 312 MYA. There is debate about the phylogenetic placement of these early lineages and their influence on estimating divergence times for amniotes, mammals (synapsids), and reptiles (111, 157). The consensus view is that sauropsids and synapsids diverged somewhere between 312 and 330 MYA (78).

Both groups of amniotes diversified through the Permian Period (299 to 251 MYA). A massive extinction at the end of the Paleozoic Era (the Permo-Triassic extinction) marks the beginning of the Mesozoic Era, roughly 251 MYA. During the Mesozoic, the synapsid lineage diversified into new forms, e.g., the therapsids, and sometime in the Jurassic, early mammals emerged, evolving into ecomorphotypes convergent with later mammalian forms (114). Meanwhile, reptiles became the dominant terrestrial vertebrate fauna during the Mesozoic. Members of this lineage further diversified on land (e.g., rhynchosaurs, squamates, crocodylomorphs, and dinosaurs), back into the ocean (e.g., turtles, ichthyosaurs, and plesiosaurs), and into the air (pterosaurs and birds).

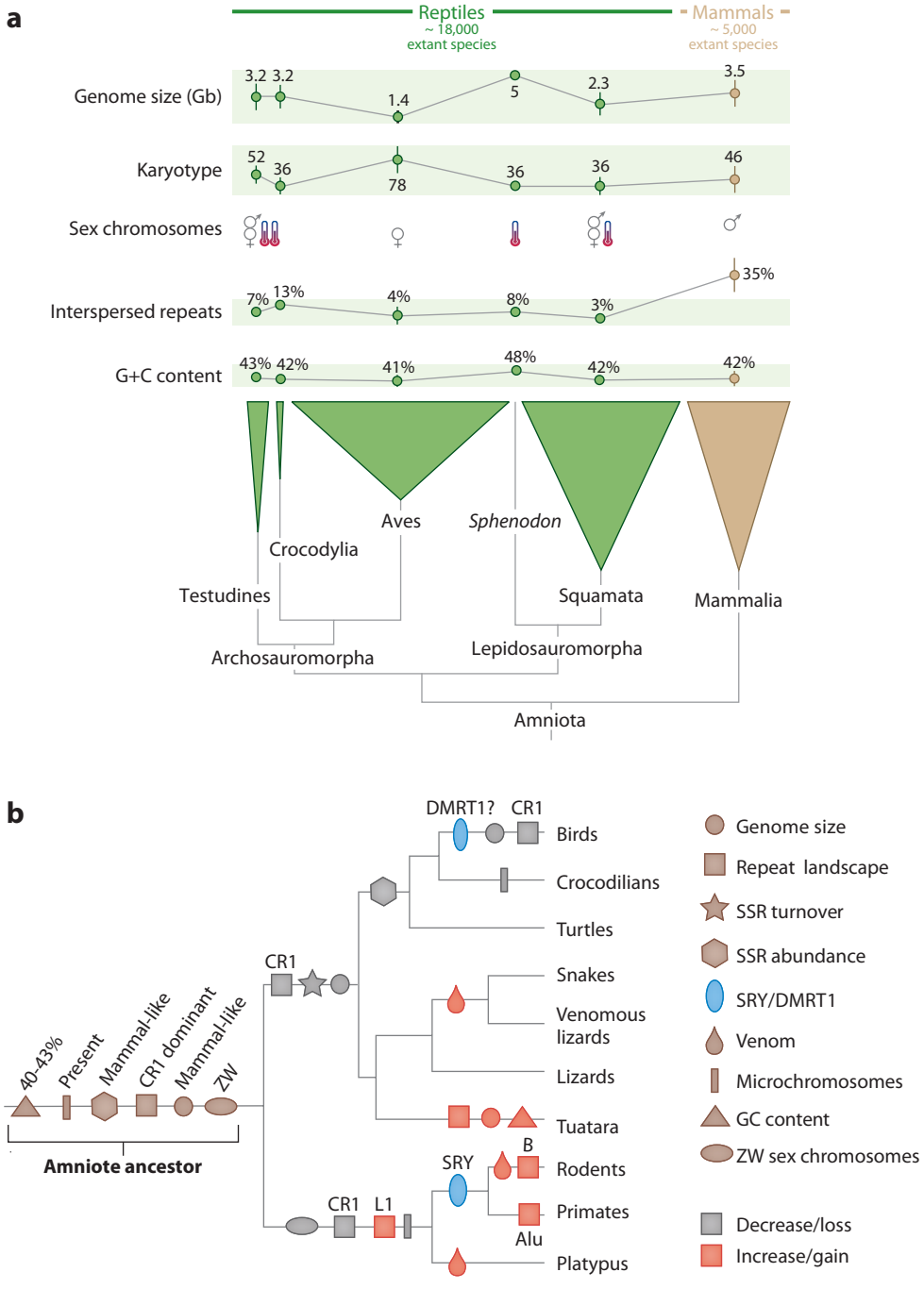
Despite the mass extinction in which the large dinosaurs perished at the Cretaceous–Paleogene (K-Pg, or K-T) boundary, extant Reptilia are still far more speciose than mammals, with over three times as many species. Reptilia rival the morphological diversity of mammals, with morphotypes ranging from legless snakes, to turtles that develop their pelvic and pectoral girdles inside their ribcage, to birds with their feathered integuments. Reptilia displays an extraordinary range of life history and behavioral traits, e.g., reproductive mode (egg-laying and placental live birth) (142, 168, 169), brain size and intelligence (93), and metabolic rate (4, 187, 188). In addition to exhibiting substantial diversity in their own right, the genomes of reptiles, as we discuss below, are essential for contextualizing genome biology of mammals and for characterizing the breadth of genomic structure and adaptation more broadly among amniotes.

DIVERSITY OF REPTILE GENOMES AND SUBGENOMES

Genome Size and Chromosome Number

In general, as befitting their diversity, reptiles exhibit substantial genomic variation across different organizational levels (**Figure 1*a*,*b***). Genome size is variable in both mammals and reptiles, with the genomes of birds unusually small and less variable than other amniotes (70, 177), especially those of hummingbirds (Trochilidae), which have the smallest genomes among birds (73). Presumably due to a lower abundance of interspersed repetitive elements (165, 166), the genomes of reptiles are generally smaller than those of mammals, which have

an average phylogenetically corrected genome size of 3.37 pg ± 0.04 pg (74). Reptile genome sizes exhibit both low averages and variances for groups such as agamids (mean = 1.9 pg, range = 1.4–2.5) and birds (mean = 1.4 pg, range = 0.97–2.2) and high averages and variances for groups such as emydid turtles (mean = 2.8 pg, range = 1.8–4.2).



Genome size has been studied in detail with respect to tempo and mode across the phylogenetic tree of Reptilia, as has the evolutionary correlation of genome size with other genomic traits. The pattern of genome size evolution in Reptilia appears to follow a proportional model, insofar as larger genomes are found to evolve in size at faster rates than smaller genomes (136). Another quantitative phylogenetic study (186) showed that the variation in genome size within each of birds, reptiles, and mammals was less than expected for a simple Brownian motion model (the null hypothesis in most comparative models), suggesting constraints on genome size imposed in each lineage. Both mammals and birds displayed coefficients of variation in genome size that were far smaller than those predicted by a simple Brownian motion model, suggesting strong constraints and stabilizing selection on this trait. However, in a later study, Brownian motion was found to fit well for genome size and karyotype data in nonavian reptiles specifically (143).

Phylogenetically controlled tests have found that genome size and numbers of micro- and macrochromosomes are not evolutionarily correlated, a pattern also found in several plant and animal groups (143). Using comparative phylogenetic approaches to reveal patterns of tempo and mode underlying traits on phylogenetic trees (such as the punctuated equilibrium model, in which most evolutionary change in a trait is associated with speciation events, or nodes in phylogenetic trees), genome size in reptiles has been found to evolve in a continuous, gradual fashion with time (149). For example, the reduced genome sizes of birds appear not to be the result of a rapid shift but rather were initially reduced in therapod dinosaurs and then further reduced in the common ancestor of extant birds. Karyotypic evolution has undergone a disproportionate amount of change on longer and early branches in the reptile tree (139, 143). Thus karyotypic change appears not to be confined to speciation events and achieved much of its current diversity during the early diversification of Reptilia.

An inverse mechanistic link between metabolic rate and genome (as well as intron) size was established early on through comparisons between chicken and human, a link that was generally upheld in subsequent phylogenetic comparisons (82, 83, 186). Hughes and Hughes (83) first proposed that flight itself, with its consequences for increased metabolic rate, drove cell size down in avian ancestors, due to the advantages of small cells for increased metabolic flux. However, additional research quickly showed that the decrease in genome size from an amniote ancestor likely took place well before the origin of flight (see Paleogenomics section, below).

Figure 1

(*a*) Sparkline plots of amniote genomic diversity. The width of clades on the tree is proportional to species diversity (~300 species in Testudines, 23 species of crocodilians, ~9800 species of birds, 3 species of *Sphenodon*, and ~7500 species of squamates). Genome size in gigabases (Gb) is reported as the clade-wide average. Karyotype is reported as the average number of chromosomes, including micro, macro, and sex chromosomes. For sex chromosomes, female heterogamety is denoted by the female symbol, male heterogamety by the male symbol, and temperature-dependent sex determination by the thermometer symbol. Interspersed repeats and GC content are reported as a percentage of the total genome (these estimates are based on a relatively small sample size for reptiles: 1 turtle, 1 crocodilian, 4 birds, *Sphenodon*, and 1 squamate). Bars behind the data points are standard deviation and the light green background marks the data range in reptiles. (*b*) Major trends of genome evolution among amniotes and character reconstruction of ancestral states along branches and the base of the amniote tree. Simple-sequence repeats are abbreviated as SSRs. *Sry* and *Dmrt1* are mammalian and avian sex-determining genes, respectively. *Dmrt1* is indicated with a question mark because the avian sex-determining function of *Dmrt1* may have arisen in birds or in a nonavian ancestor. This tree includes a recent report of convergently evolved venom proteins in the North American shrew, *Blarina brevicauda* (3), and other mammals. Data were collated from References 143, 166, 178, 191.

The current consensus is that, while unknown factors other than flight initiated the decrease in genome size in avian ancestors, flight may have contributed to the decrease (145). An alternative view is that genome size variation is a nonadaptive consequence of variation in population size, with small populations allowing slightly deleterious variation, such as retroelements, to accumulate and cause increases in genome size and population expansions allowing more efficient retroelement removal (115). One scenario suggests that amniote retroelements trace their ancestry to 65 MYA ago at the K-Pg boundary, and that many mammalian retroelements trace their ancestry to before the K-Pg boundary, and that population sizes crashed due to the environmental chaos brought on by the asteroid impact and were followed by population expansions that in turn improved the efficiency of removal of proliferating retroelements (159). While appealing, this scenario relies on a number of methodological assumptions and, furthermore, does not address variation in genome size and retroelement diversity among different amniote lineages as a result of the asteroid impact.

By contrast, chromosomal variation is far greater in Reptilia than in mammals, owing to the presence of microchromosomes in both birds and nonavian reptiles (137). Microchromosomes are present in nearly every avian lineage, although in reduced numbers among some diurnal raptors (56), as well as numerous nonavian reptile lineages, such as agamid lizards, soft-shelled turtles, and rat snakes (53, 100, 123). Although the platypus has some very small chromosomes, these are typically not referred to as microchromosomes because the distribution of chromosome lengths is more gradual than in birds (191). Paradoxically, microchromosomes are absent from the genomes of crocodilians, the closest living relatives of birds (87). They are structured differently than macrochromosomes, possessing a higher G+C content (9, 10, 24) and containing a higher density of genes than do macrochromosomes (58, 75, 80). Recombination rates, for birds at least, are high, up to five times higher than those seen in mammalian macrochromosomes (11, 47, 89, 160, 161). This may be related to smaller average chromosome length in birds compared to mammals, or may represent methodological differences in estimating recombination rate (83). Although prior to the sequencing of the chicken genome, some analyses suggested that intron size in chickens and mammals is indistinguishable (182), with the greater resolution of whole genomes, birds do seem to possess smaller introns than do mammals. In reptiles and, intriguingly, the platypus, the distribution of predicted intron lengths is similar to that in chicken (143, 186). The total gene count in chicken is lower than the gene count in placental mammals (80), although the extent to which these differences occur throughout Reptilia is unknown.

A genetic linkage map for the saltwater crocodile (*Crocodylus porosus*), the first map of its kind for a nonavian reptile, showed longer genome-wide recombination map lengths in females than in males (125). Because crocodiles most likely lack sex chromosomes, this result confirms that sex-differential recombination rates do not depend on the presence of sex chromosomes, a phenomenon proposed in some evolutionary models to be a result of sexual antagonism (119) (see Sex Chromosome Evolution section below). Clearly, we have only scratched the surface of genome size and structural variation in nonavian reptiles (143, 166, 205).

Retroelements

Retroelements are among the most prominent and ubiquitous components of amniote genomes and have provided an important source of mutation and novel genomic variation (103, 196). Two major classes of functionally and evolutionarily related retroelements include long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs). Approximately 45% of the human genome is composed of LINEs or their related SINEs, which in humans include the

well-known Alu-element repeat family (41). LINEs proliferate and integrate throughout genomes. SINEs differ from LINEs in having an internal promoter requiring a different polymerase. Whereas LINEs are autonomous in their ability to copy and paste themselves around the genome from a parent to target loci, SINEs depend on the reverse transcriptase of partner LINE elements for their mobility, as shown originally by the conserved 3′ flanking sequence with LINEs elsewhere in the genome (135).

With their streamlined genomes, typically less than half the size of the human genome, birds have a depauperate diversity of retroelements compared to mammals (80, 186). Early studies of reassociation kinetics indicated that only 15–20% of avian genomes were composed of repetitive elements (52, 163). The dominant retroelement family in the chicken genome, and perhaps in birds generally, is the CR1 family of LINE elements. Although about 200,000 CR1s have been annotated in the chicken genome assembly [constituting ~80% of dispersed repeats or ~9.4% of the total chicken chromosomal DNA (80)], there appear to be almost no intact full-length (~4.5 kb) active CR1s remaining, even after an intensive sequencing survey of the highly repetitive fraction of the chicken genome (199). The CR1 family is now known to fall into eight subfamilies, or major monophyletic clusters (179, 199), and displays a clustered distribution within and among chromosomes of gamebirds, ducks, perching birds, and hawks (36). Whereas the phylogeny and high frequency of truncation of CR1 elements suggest that these elements are not actively proliferating in the chicken genome, other novel elements recently discovered in the chicken genome, such as the DNA transposon *Gallubop*, show a shallow, bushy phylogeny among copies, implying ongoing or recent proliferation (199). However, none of these patterns have been quantified in a phylogenetic framework, nor have they been investigated in a systematic way across taxa. The discovery of novel subfamilies of CR1s in penguins and their allies (192), and the abundance of CR1s apparent in songbird cosmid clones (48, 67, 79) and other database sequences, suggests that active diversification of derived CR1s is likely occurring in several avian lineages. However, the zebra finch genome exhibits a very inactive and low-diversity CR1 landscape (190).

Some of the earliest insights into the structure of nonavian reptile genomes were based on surveys of repeats using PCR approaches and screening of genomic libraries, as well as serendipitous discovery of repeated elements (98, 202). In contrast to the avian genome, nonavian reptiles display a more diverse and active suite of SINE and LINE subfamilies. Shedlock et al. (166) surveyed several megabases of bacterial artificial chromosome (BAC)-end sequence from an American alligator (*Alligator mississippiensis*), a painted turtle (C. *picta*), and (with plasmid-end sequence) a Bahamian green anole lizard (*Anolis smaragdinus*). This study found that CR1s remained the most common LINE element in reptile genomes but were more abundant than in chicken and displayed more evidence for active or recent proliferation. The study also suggested that some lineages of CR1 in the chicken genome have ancient roots that likely originated in the amniote ancestor, consistent with findings in other vertebrates, including amphibians, fish, and even lungfish (98, 165, 171).

The retroelements of the green anole (*Anolis*) have begun to receive attention since the release of the online genome sequence and have raised the possibility of horizontal gene transfer in vertebrates. Novick et al. (134) mined the *Anolis* genome bioinformatically and conducted phylogenetic analyses of diverse non–long terminal repeat (LTR) LINEs. They identified five active families of LINEs, including L1 and CR1 (the most abundant element in mammals and reptiles, respectively), as well as L2. In general, *Anolis* LINEs exhibit a higher diversity but lower abundance than LINEs in mammalian genomes. Based on sequence divergences among elements, Novick et al. (134) demonstrated a lower than expected fraction of older elements in the *Anolis* genome,

suggesting the possibility of high turnover and purging of deleterious elements due to natural selection. Moreover, the exceptionally low level of sequence divergence between some *Anolis* elements and those of other species, particularly mammals, raises the spectre of horizontal gene transfer (HGT) as a common mechanism for amniote repeats (134, 152). This applies in particular to miniature inverted transposable elements (MITEs), which have undergone extensive proliferation in *Anolis*, some plants and bats, and the RTE-BovB LINEs (148, 197). HGT is also implicated in the presence of some SINEs and LINEs in snake genomes (153).

A comparison of retroelement diversity in birds, nonavian reptiles, and mammals (**Figure 2**) suggests that CR1 was prevalent in the genome of the amniote ancestor and L1s were not. The only study to quantitatively reconstruct retroelement phylodiversity (166) estimated ~260,000 CR1s in the amniote ancestor, with a gain of ~180,000 in the lineage leading to birds and crocodilians, followed by a rapid loss in the lineage leading to birds. At the same time, the lineage leading to eutherian mammals experienced a substantial loss of ~200,000 CR1 elements, and a simultaneous expansion of L1s to their present diversity. This pattern is consistent with a model in which ancestral bottlenecks provide opportunities for retroelement regime changes and competition between different retroelement families for genomic space (115, 116). This competition might arise because of the reduction in the efficiency of removing mildly deleterious mutations and the increased importance of drift in small populations. However, these studies used databases consisting of previously characterized retroelements and so may have missed novel classes not yet characterized.

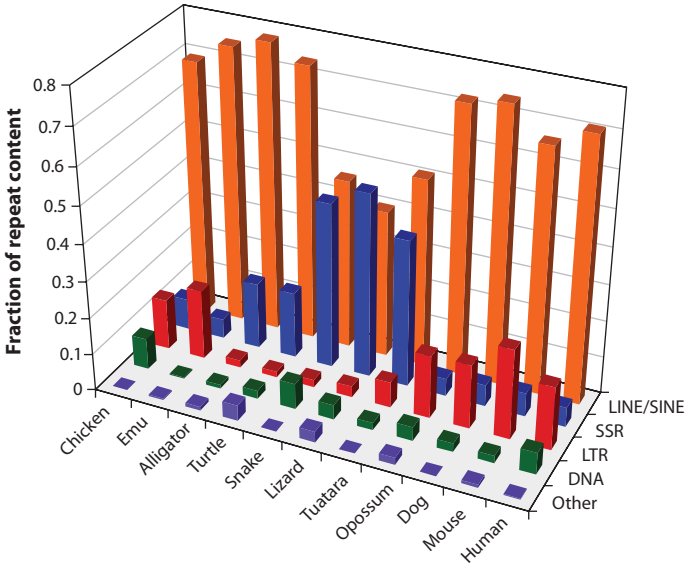


Figure 2

The proportion of major classes of elements comprising the repetitive fraction of genomes for species representing major clades of amniotes, based on published genome assembly information and reptilian bacterial artificial chromosome (BAC) library interrogations (updated from 165; A.M. Shedlock, C. Chapus, S.V. Edward, unpublished manuscript). Abbreviations: SINE, short interspersed nuclear element; LINE, long interspersed nuclear element; SSR, simple sequence repeat; LTR, long terminal repeat retrotransposon; DNA, DNA transposon.

Simple-Sequence Repeats (Microsatellites)

Since the first genomic studies in chickens, it has been known that birds possess fewer and shorter simple-sequence repeats (SSRs) than do mammals (154). In their BAC-end sequencing survey, Shedlock et al. (166) found that microsatellites in alligator and turtle, as in chicken, were shorter than in mammals. The microsatellite landscape of *Anolis* was surprisingly similar to the human landscape, with SSRs noticeably smaller than in mouse but substantially larger on average than in birds and other reptiles. They also discovered a unique set of 50-bp tandemly repeated sequences in turtle, alligator, and anole that, bizarrely, shared no structural or sequence similarity except for length. Rates of SSR turnover appear to be 10–25 times higher in mammals than in nonavian reptiles as judged by changes in SSR frequency between species (166). In a recent next-generation sequencing study, Castoe et al. (27) detected 14,612 SSRs among 128,773 reads from a 454 FLX sequence survey of squamate genomes, establishing this approach as a quick and useful method for SSR discovery in nonmodel species.

The *Anolis* genome sequence (see below), although an excellent resource, represents just the tip of the iceberg in our understanding of retroelement and SSR diversity in squamates and other reptiles. For example, preliminary studies show that unique lineages such as tuatara (*Sphenodon*) possess a high degree of lineage-specific evolution not found in other clades (A.M. Shedlock, C. Chapus, S.V. Edward, unpublished manuscript). Genome sequences from additional and diverse reptiles will no doubt improve the picture.

Isochores

Isochores are large genomic tracts (>100 kb) with relatively homogenous, biased base composition. They are a prominent feature in mammalian and avian genomes but are largely absent in fish and amphibians (20). When they exist, GC-rich isochores positively correlate with several key genomic features, including recombination rate (62), gene density (130), epigenetic modifications (88), intron length (43), and replication timing (193). These relationships underscore the importance of isochores as functional genomic elements. When and how they arose in amniotes remain unclear: Did they arise independently in endotherms (mammals and birds), or in the amniote common ancestor? Are they maintained by a thermal selection regime in mammals and birds (19, 20), or are they a by-product of ongoing amniote-specific, AT→GC recombination-driven biased gene conversion (42, 63, 65, 112)? Do isochores follow similar evolutionary trajectories in divergent lineages [such as the continuous shifting of isochores as seen in mammals (15, 44)], or do isochores change in a lineage-specific manner? Tackling these questions will require knowledge of the phylogenetic distribution of isochores in reptiles.

Tangential evidence allows characterization of isochores in reptiles. For example, CsCl fractionation of reptile genomes has revealed extensive variation of GC composition in snakes, turtles, and crocodilians (84), and correlations of high GC values at third-codon positions between mammals and reptiles imply some isochore structuring in turtles and crocodiles (32, 33, 57, 85). However, there are as yet no direct measurements of GC content along chromosomes similar to those available for mammals and birds (34, 35, 201). Obtaining these measures is critical, particularly given the recent criticism of using third-codon GC content as a proxy for isochore presence (49). Furthermore, preliminary scaffold scans of the *Anolis* genome sequence offer little evidence for isochore structure similar to that seen in mammals and chicken (M. K. Fujita, unpublished data).

The causes and correlates of variation in recombination rate across the genome pose a major question in human and mammalian genetics. Insight into this question can be provided by examination of reptile genomes. Mechanistic links between GC content and recombination rates have been proposed by Duret and Galtier (64) as an important driver of variation in recombination rate across the genome. This link results from the biased production of T→C substitutions during repair of the double-strand breaks that initiate recombination events, as well as during gene conversion. This link could explain many features of reptilian and mammalian genomes, such as the relationship between the high-GC content of small chromosomes, including microchromosomes, and their small size. Duret and Galtier's (64) model has support from a number of empirical and theoretical directions. In reptiles, it can be tested by examining GC contents of genomes with and without microchromosomes, the prediction being microchromosome-rich genomes should have higher GC contents. A comparison of various mammalian, avian, and nonavian reptile genomes suggests, however, a more complex relationship. There does indeed appear to be an association between the presence of microchromosomes and high-GC content in several species (**Figure 3**). However, crocodilians, as exemplified by the American alligator, deviate from this pattern in showing high GC in the absence of microchromosomes. It is likely that both historical contingency in GC content as well as the recombination-mediated evolution

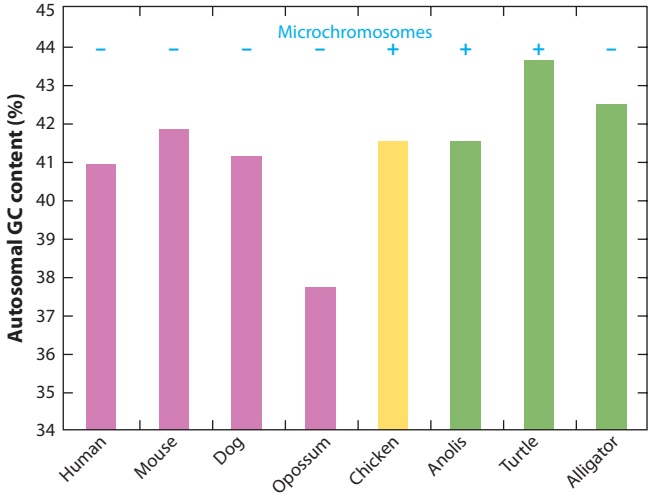


Figure 3

Test of the biased gene conversion model of GC content through comparison of autosomal GC content and presence or absence of microchromosomes in various amniote genomes. %GC data from nonavian reptiles [American alligator (*Alligator mississippiensis*), painted turtle (*Chrysemys picta*), and green anole (*Anolis smaragdinus*)] are from reference 166. %GC for the other species is from the original papers of the draft genomes of the respective mammalian species indicated. The presence (+) or absence (–) of microchromosomes is shown for the species indicated. Notice the American alligator lacks microchromosomes yet has a relatively high genome-wide GC content.

of isochores and high-GC content envisioned by Duret and Galtier (58) play a role in molding GC content in amniotes. It may also be that the presence of microchromosomes is too coarse a proxy for recombination rate, diluting its link with GC content.

In humans, base composition is a significant correlate with many diseases and cancers that result from deletions and translocations. AT-rich regions, for example, are prone to deletion break points, and GC-rich regions are prone to translocation break points (1). This pattern extends to other mammals, e.g., explaining both the rearranged karyotype and the GC composition variation in dogs (195). Continued research on the evolution of isochores in reptiles will help identify ubiquitous processes affecting genome structure as well as lineage-specific processes, with the immediate applicability to understanding the origin of phenotypic variation, including disease in humans.

Mitogenomics

Mitochondrial genomes contain a conserved set of genes, including 13 protein-coding genes, 22 tRNA, 2 rRNA, and 1 control region that regulates DNA replication and RNA transcription (21). The arrangement of these genes along the mitochondrial circular chromosome is also strongly conserved within Metazoa, although the discovery of several alternative gene orders implies that mitochondrial genomes are more labile than previously thought. In contrast to mammalian mitochondria, which tend to follow the typical and presumably ancestral gene order [notable exceptions include two marsupials, the wallaroo (*Macropus robustus*; 91) and the opossum (*Didelphis virginiana;* 92)], several reptile lineages exhibit alternative mitochondrial gene arrangements. One feature that has evolved independently—often undergoing concerted evolution—in several reptile lineages is the duplicated control region that characterizes the mitochondrial genomes in "advanced" snakes, Australasian agamid lizards, monitor lizards, turtles, and birds (2, 45, 105, 108, 128, 150). Structural rearrangements have occurred with other genes as well, most prominently involving tRNA genes as observed in crocodilians (91, 107), chameleons (106), amphisbaenids (117), and the tuatara (158).

The most accepted mechanism for mitochondrial genome rearrangement is the duplication-random loss model, whereby a large portion of the mitochondrial genome tandemly duplicates and random loss of one copy of each of the duplicated genes results in a different gene order (21, 129). Studies of reptile mitochondria have allowed researchers to identify mechanisms responsible for the original duplication and discover the transient gene-degeneration step of the duplication-random loss model. For example, in parthenogenetic (asexual) whiptail lizards (*Aspidoscelis*), large, tandem mitochondrial duplications likely arose due to faulty light-strand DNA synthesis initiation as a result of misidentification of the origin of light-strand synthesis (O_L, typically

a stem-loop structure located ~2/3 the length of the genome from the control region) (174). Alternatively, Fujita et al. (61) invoked slipped-strand error in DNA replication to explain mitochondrial duplications in parthenogenetic Australian geckos (*Heteronotia binoei*); this occurs when the elongating light strand dissociates and the leading edge reanneals at a downstream location, causing a portion of the genome to replicate twice, resulting in a duplication. Interestingly, two lineages of *Heteronotia* that share the same tandem duplications show different patterns of non-functionalization that would eventually lead to alternative gene orders, demonstrating that duplication and random loss can occur at very short timescales (61). Alternative and variable mitochondrial gene orders also occur in birds (128), but their rarity in both birds and mammals highlights the opportunities that reptiles provide in understanding the processes behind mitochondrial genome evolution. These mechanisms can provide insight into the origin and establishment of several mitochondrial diseases caused by mitochondrial gene rearrangements in humans, including mitochondrial myopathies, lethal childhood pancyopenia, and Kearns–Sayre syndrome (185).

Perhaps because of its tremendous importance in evolutionary genetics, mitochondrial DNA has developed a benign reputation for evolving neutrally, or at least nearly neutrally (12). However, recent discoveries indicate that mitochondrial genomes are anything but neutral (e.g., 14), and variation in protein-coding regions may even have contributed to phenotypic evolution such as thermal adaptation in humans (162). One example of mitochondrial adaptive evolution in reptiles occurred early in the evolution of snakes, when substantial amino acid changes occurred in functionally important sites in every mitochondrial protein, especially in Cytochrome c oxidase subunit I, perhaps coinciding with the advent of their multiple phenotypic and physiological innovations (e.g., metabolic efficiency, lung reduction, venom evolution, etc.; 28). Furthermore, these rapid changes occurred independently (convergent evolution) in agamid lizards (27). Although the underlying cause of such convergence is unknown, the pattern is compelling, urging further investigations in other groups to identify the prevalence of adaptive mitochondrial genome evolution.

Sex Chromosome Evolution

Nonavian reptiles exhibit a remarkable diversity of sex-determining mechanisms categorized broadly as either temperature-dependent sex determination (TSD), in which the sex of an individual is primarily directed by incubation temperature, or genotypic sex determination (GSD), in which sex is directed by chromosomal inheritance at conception (23). Among nonavian reptiles, all crocodilians and tuataras, some turtles, and some lizards exhibit TSD and all snakes, some turtles, and some lizards exhibit GSD (**Figure 4**). Reptiles with TSD lack sex chromosomes (23), whereas sex chromosomes have been found in some but not all GSD reptiles (155). Reptilian sex chromosomes can be characterized as either female heterogamety, in which males and females carry two Z sex chromosomes or one Z and one W sex chromosome, respectively. In female heterogametic reptiles, the W sex chromosome appears to be a degenerated copy of the Z sex chromosome. Likewise, in male heterogametic reptiles, in which males and females carry one X and one Y chromosome or two X sex chromosomes, respectively, the Y chromosome appears to be a degenerated copy of the X sex chromosome (54).

The variability of sex-determining mechanisms and organizations of sex chromosomes suggest that, among reptiles, these traits change repeatedly and reversibly (141). All birds and snakes exhibit female heterogamety, but comparative cytogenetic maps demonstrate no sequence similarity between the Z and W sex chromosomes of chickens and those of the Japanese four-striped rat snake (*Elaphe quadrivirgata*) (102, 124). This suggests that the sex chromosomes of chickens and other birds

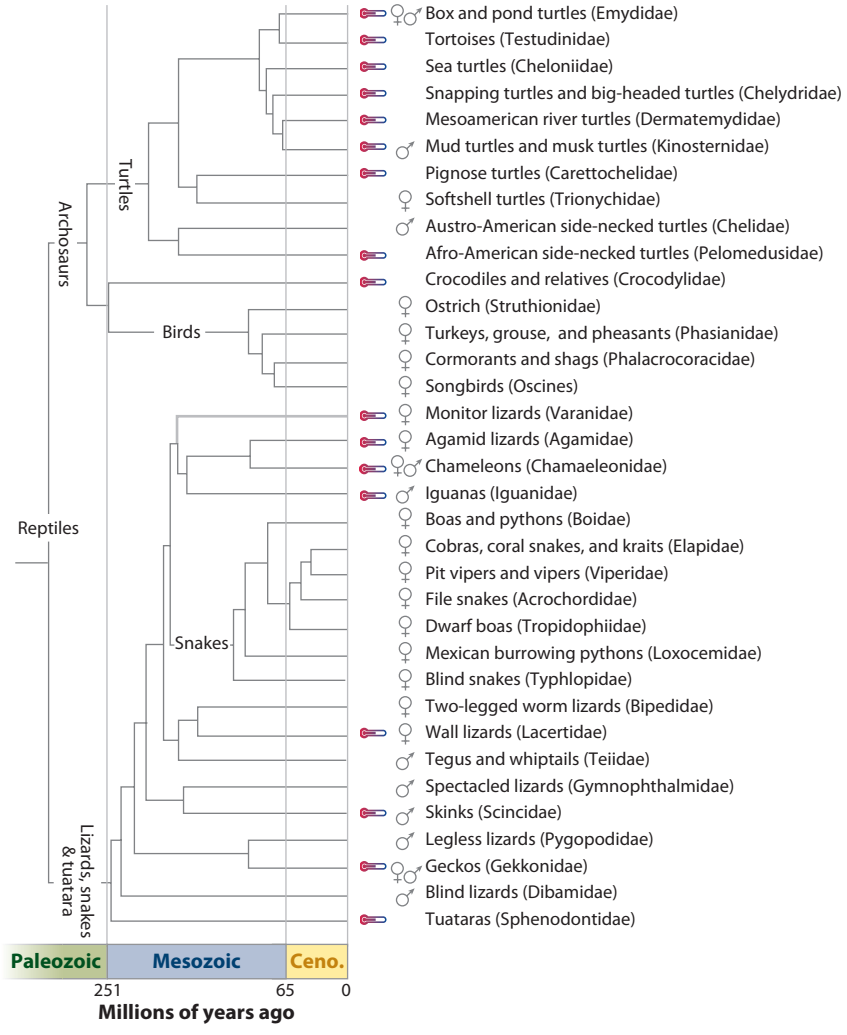


Figure 4

Sex-determining mechanisms across families within Reptilia. A thermometer indicates that species within the family exhibit temperature-dependent sex determination. Male and female symbols indicate that species within the family exhibit genotypic sex determination in the form of either male or female heterogamety, respectively. This sampling of reptilian families is not exhaustive but demonstrates the variability of sex determining mechanisms found in the clade.

evolved from a different pair of ancestral autosomes than the sex chromosomes of Japanese four-striped rat snakes and other squamates.

Sex chromosomes, in general, are believed to have arisen as a result of development or translocation of a sex-determining gene on a pair of ancestral autosomes (68). Sexually antagonistic loci confer a benefit to one sex but a detriment to the other sex (5, 30). Once an autosome becomes the seat of a sex-determining gene and perhaps other sexually antagonistic loci, recombination breaks down in the neighborhood of that locus. Reduced recombination leads to the accumulation of deleterious mutations that are subsequently purged, leading to degeneration of one of a pair of nascent sex chromosomes. This results in either male or female heterogamety depending

on which sex carries the degenerate sex chromosome. *Sry* and *Dmrt1* are sex-determining genes in mammals and birds, respectively. In mammals like human (*Homo sapiens*), the presence of *Sry* on a Y sex chromosome initiates male development by interacting with *Sox9* to masculinize bipotential embryonic gonads (170). Although they share no domains, *Dmrt1* serves a similar function as *Sry* by interacting with *Sox9* in chicken (172, but see 109). However, unlike the male-dominant *Sry*, it is thought that *Dmrt1* acts in a dose-dependent manner because it is on Z sex chromosomes and absent from the W.

Chickens that possess two Z sex chromosomes and therefore two functional copies of *Dmrt1* are male. Chickens with only one functional copy of *Dmrt1* are female. In GSD nonavian reptiles, *Sry* is absent and *Dmrt1* is present but has not been reported in sex-differential doses, suggesting it does not determine sex in nonavian reptiles as it does in chickens. Matsubara et al. (123) mapped *Dmrt1* to autosomes in the Japanese four-striped rat snake (*E. quadrivirgata*), habu (a snake) (*Protobothrops flavoviridis*), and Burmese python (*Python molurus bivittatus*). *Dmrt1* and five other linked markers (*ACO1/IREBP*, *RPS6*, *CHD1*, *GHR*, and *ATP5A1*) from the chicken Z chromosome mapped to both Z and W sex chromosomes in the gecko lizard (*Gekko hokouensis*), suggesting a common origin of this species' sex chromosomes and those of birds (101), although the presence of *Dmrt1* on both Z and W sex chromosomes also suggests that it does not determine sex in the gecko lizard. Further, the XY sex chromosomes of platypus (*Ornithorhynchus anatinus*) have strong homology with the ZW sex chromosomes of birds but not the XY sex chromosomes of therian mammals (180). The wide distribution and frequent homology of ZW sex chromosomes and ancestral reconstructions have led to the conclusion that the common amniote ancestor was most likely GSD with a ZW sex chromosome organization (141, 173, 194).

Molecular cytogenetics has proved extremely powerful in suggesting homologies and evolutionary trends in reptile sex chromosomes (204). However, the number of species that have been mapped in this way is still small, and phylogenetic analysis of ancestral states of reptile sex chromosomes can often suggest different scenarios. For example, despite the synteny of six markers on the sex chromosomes of gecko lizards and birds, parsimony and likelihood estimates describe independent origins of the Z and W sex chromosomes of Squamata, including geckos, and Aves, including chicken (141). In short, the linkage group may have been conserved for more than 300 million years, but the sex-determining function of the chromosomes bearing those markers has been gained or lost at least once between birds and squamates, including snakes and gecko lizards. At present, molecular targets affected by either temperature or gene dosage have not yet been recognized in any nonavian reptiles.

The characterization of sex chromosomes across Reptilia is still grossly incomplete. Many reptiles have been described as exhibiting GSD only because their offspring sex ratios cannot be altered experimentally by varying incubation conditions. Surprisingly, in many GSD reptiles, sex chromosomes have not been described. This may be the case because some GSD reptiles possess very young, undifferentiated sex chromosomes or because cytogenetic techniques are not sufficiently sensitive to detect them. Two GSD reptiles, bearded dragons (*Pogona vitticeps*) and Macquarie turtles (*Emydura macquarii*), have recently been described as possessing heteromorphic sex chromosomes (53, 122). In bearded dragons, the heterogametic sex is female and the sex chromosomes are also microchromosomes, further complicating their detection (53). In Macquarie turtles, the heterogametic sex is male and the heteromorphy of the sex chromosomes is slight, detectable only by comparative genomic hybridization and GTG-banding (122). More sensitive methods for detection of reptilian sex chromosomes will depend on increased genomic resources like bacterial artificial chromosome libraries and genome projects for comparative studies informed by phylogenetic inference (90).

Sex determination has played a vital role in the evolution of reptiles. For example, extinct marine reptiles such as keichousaurs and icthyosaurs were able to colonize pelagic environments, in part because they were viviparous (25), and throughout Reptilia, viviparity has evolved in a correlated manner with GSD. Few closely related squamates [including southern water skinks (*Eulamprus tympanum*), yellow-bellied water skinks (*Eulamprus heatwolei*), and possibly snow skinks (*Niveoscincus ocellatus*)] are known to exhibit both viviparity and TSD. All other viviparous, nonavian reptiles studied thus far exhibit GSD, a situation that likely enabled viviparity by freeing mothers from the burden of maintaining bimodal internal temperatures to produce sons and daughters, as would be required by TSD (142). For this reason, it has been concluded that GSD enabled viviparity, thereby enabling pelagic existence of extinct marine reptiles. Recent studies have also implicated the presence or absence of heteromorphic sex chromosomes in the evolution of sexual dimorphism (118), local and global dosage compensation (120), and the accumulation of sexual antagonisms (121). To date, these studies have focused on sex chromosomes of mammals, birds, and invertebrates. With the advent of novel genomic resources, nonavian reptiles have recently become a new frontier in the study of sex chromosome evolution.

THE VENOME

Venome is a term coined to represent the genes and transcripts that comprise venom and venom-delivery systems (59). Transcriptional analyses have informed studies of venom system evolution and cataloged the contents of venom from several squamate reptiles (lizards and snakes). Nine toxin-type transcripts were sequenced from venom gland cDNA libraries of snakes, iguanians, and anguimorphs (60). A Bayesian phylogenetic analysis of these transcripts resulted in a monophyly of each toxin type, suggesting a single origin of venom systems occurring about 200 MYA (60). Also, expressed sequence tags (ESTs) from venom glands have been used to identify venom components from three snake families: Viperidae, Colubridae, and Elapidae (31, 94–97, 110, 113, 183). Identification of venom components (i.e., metalloproteinases, C-type lectins, serine proteases, three-finger toxins, phospholipases, and natriuretic peptide precursors) and venom-related genes has not only revealed toxins associated with hemorrhaging and coagulopathy but has also provided clinical information for the preparation of species-specific antivenins (110, 113). Similar genes are active in the venom system of platypus, but the genes appear to have been co-opted convergently from the same gene family as venom-related genes of squamate reptiles (191, 198).

PALEOGENOMICS AND RATES OF EVOLUTION

Understanding organismal biology and evolution among reptiles and mammals is greatly improved by harnessing the variation in form captured by the fossil record, as poor as it may be sometimes. Trace fossils, exceptional preservation, and ecological and physiological correlates provide additional evidence and insights into the behavior, function, and physiology of amniote biology and evolution. Moreover, many evolutionary patterns, e.g., the early diversification in traits that later become fixed in extant lineages, become clear only when evidence from the fossil record is considered. The goal of paleogenomics is to use fossil evidence to address questions concerning the evolution of the genome and to reconstruct the genome biology of extinct species. Unfortunately, the ability to leverage the fossil record to better understand genome biology is limited. There are two broadly defined avenues to investigate the genetics and genomics of extinct species: comparative phylogenetic studies and direct sequencing. Each has its strengths and weaknesses.

Phylogeny can be harnessed to improve precision and accuracy when predicting values for characters within species (66). Such phylogenetically informed predictions provide a powerful tool to investigate the genomic biology of extinct species as well as macroevolutionary trends in genome evolution (142, 145). For example, genome size and cell size are correlated across eukaryotes (71, 72) for a variety of tissue types among plants and animals, although there is no simple relationship between genome size, nucleus volume, and cell volume (131). Nevertheless, relationships between the genome and cellular correlates, e.g., cell size, can be used to infer genome characteristics in extinct organisms (181). For example, Organ et al. (145) used Bayesian statistical inference to estimate the genome sizes of extinct dinosaurs and birds by using osteocyte lacunae size as a proxy for genome size. The finding in this study that nonavian dinosaurs possessed genomes as small as those of modern birds directly rejected the hypothesis (83) that flight was initially responsible for driving down genome size in ancestral avian lineages. This study also suggested that the depauperate retroelement landscape of modern birds was likely inherited from their dinosaur (saurischian) ancestors (140, 145). Although only gross descriptors of the genome can be analyzed in this way, important insights into the tempo and mode of genome evolution have been made.

While comparative phylogenetic methods offer important insights into genome evolution, their scope is limited. Ideally, we want to obtain whole-genome sequence data from extinct species. Once the subject of much debate (77, 146, 147), whole-genome sequencing of recently extinct species has proved an increasingly realistic goal with the release of large sequence data sets from Pleistocene cave bears (*Ursus spelaeus*) (132), the moa (*Dinornis*) (86), and the whole nuclear genome of the mammoth (*Mammuthus primigenius*) (126) and Neanderthal (*H. neanderthalensis*) (69). As of this writing, whole-genome sequencing of extinct amniotes has focused solely on mammals. The big drawback of whole-genome paleogenomics is the ever present possibility of sequence contamination and the fact that only recently extinct species, perhaps no earlier than 500,000 years ago, can be sequenced with rigor and confidence (77). For example, the mammoths sequenced by Miller et al. (126) date to 20,000 years ago, and the Neanderthal sequence being examined dates to 38,000 years ago.

Although nucleic acids may not remain preserved for millions of years, there is some evidence that at least some types of proteins are preserved and that phylogenetic information can be retrieved from such material (8, 144). For example, fragments of Collagen $\alpha 1(1)$ and $\alpha 2(1)$ protein were sequenced from the fossilized bones of a 160,000- to 600,000-year-old mastodon (*Mammut americanum*) and a 68-million-year-old *Tyrannosaurus rex* using mass spectrometry (6). Although controversial (6, 7, 22, 151), reanalysis of the mass spectrometry data supports the original study (18). The results from phylogenetic analysis of the mastodon and *T. rex* sequences matched the phylogenetic predictions based on over a century of morphological analysis insofar as *T. rex* clustered closely with a chicken sequence and the mastodon clustered with sequence from an extant elephant. These results suggest that preserved biomolecules may have utility for resolving the placement of fossils whose phylogenetic position based on morphology is ambiguous (144).

Although a number of studies have explored the relative rates of point substitution and other trends in mammals, birds, and reptiles, very little work has taken place thus far in the era of genomics. An important hypothesis that emerged from the pregenomic era of sequence comparisons was that rates of amino acid substitution in birds should be slower than in mammals because of the higher body temperature of birds, resulting in a narrower neutral space for avian proteins (127). This hypothesis received some empirical support from ribosomal genes and six protein coding genes. Stephen et al. (176) performed a large-scale analysis of ultraconserved elements (UCEs) in

alignments of multiple vertebrate genomes and found that UCEs were better conserved among amniotes than in fish to a degree that suggested a slowdown in rate of UCE evolution in amniotes. Reptile multigene families such as the major histocompatibility complex show homology with mammals but possess divergent gene lineages that track the antiquity of species such as the tuatara (203). Using unaligned sequence comparisons of several reptiles, birds, and mammals, Shedlock et al. (166) suggested that rates of oligonucleotide turnover in mammal genomes were higher than in birds and nonavian reptiles, suggesting a slowdown in Reptilia relative to mammals. Another study mapped nearly 12,000 paired BAC-end sequences from emu, American alligator, and painted turtle to the chicken genome and suggested that conservation of microsynteny was rare at this level of resolution, notwithstanding the reduced rate (22–49% of reads) at which random genomic sequences will reliably map across >200 million years of evolution (29). A slowdown in rates of evolution in the amniote ancestor and in Reptilia relative to mammals are two hypotheses that can serve as jumping-off points for future genome-wide studies of evolutionary rate.

THE ANOLIS GENOME

As the first genome from a nonavian reptile, the *Anolis* genome promises to put the genomics of amniotes in high gear, and indeed already has. A draft sequence has been available since early 2007, and the first papers utilizing this resource began to appear in 2008. A literature search on the ISI Web of Science using the term "*Anolis* AND genome" yielded 28 citations, 16 of which were published after October 2008 and appear to have directly queried the *Anolis* draft genome. Naturally, these papers have skimmed the evolutionary cream off the low hanging fruit—highly repeated transposable elements and multigene families involved with sensing the environment and lizard-specific adaptations, such as olfactory receptor genes (175), retrotransposons (104, 133), DNA transposons (134), immunoglobulin genes (13, 39, 200), keratin genes and adhesion to surfaces via setae of the foot (39, 46), neurobiological gene families (38), globin genes (81), and protocadherins (95). With two relevant papers in 2008, eight in 2009, and five as of this writing (May 2010), all indications are that analysis of the *Anolis* genome is in the exponential growth phase and has already yielded a number of important secrets. Some of the best examples of horizontal gene transfer involving vertebrates, usually involving transposons, now come from the *Anolis* genome. These analyses have found unusually close relationships between the DNA transposons of various verterbrate and invertebrate groups, including *Anolis*, primates and other mammals, and planarians and suggest that, of those groups investigated thus far, horizontal gene transfer occurs more frequently among these than among other groups (134). These analyses reveal little about mechanisms of transfer between species but promise to accelerate research in this area.

A number of recent papers, including those published on data independent of the *Anolis* genome, suggest an intriguing similarity between the genomes of *Anolis* and those of mammals, despite the fact that birds and *Anolis* share a more recent common ancestor. The kappa and lambda immunoglobulin light chain genes of *Anolis* are both organized similarly to mammals, whereas the chicken light chain genes have a simpler organization (200). There are two type I and four type II keratin genes in the *Anolis* genome, a larger number than in chickens, and these genes show structural and organizational similarity to those of mammals (46). Surveying the family across the amniotes, Eckhart et al. (46) conclude that the keratins composing mammalian hair were co-opted from a rich repertoire present in *Anolis* and presumably the amniote ancestor, including cysteine-rich alpha-keratins previously thought to be confined to mammals. By contrast, several gene families, such as globins, as well as retroelements, reveal high rates of turnover and lineage specificity in analyses involving *Anolis* (81, 133), and the protocadherin gene

cluster of *Anolis* shares ancestral genes with, e.g., the coelocanth, that are currently not found in mammals (95). Thus, although many of the initial mammal-*Anolis* similarities emanating from the *Anolis* genome suggest a large, repeat-rich genome of the amniote ancestor, we predict that the *Anolis* genome will exhibit a patchwork pattern combining both primitive and derived characteristics.

GENOME RESOURCES FOR NONAVIAN REPTILES

In recent years, a number of significant genomic and bioinformatics resources have been established. Below we list some of the more prominent reagents and sources of information on reptile genomics as of this writing.

- BAC libraries. In addition to BAC libraries from several birds (reviewed in 48), several BAC libraries from nonavian reptiles are available: painted turtle (*Chrysemys picta*), tuatara (*Sphenodon punctatus*), American alligator (*Alligator mississippiensis*), saltwater crocodile (*Crocodylus porosus*; 164), garter snake (*Thamnophis sirtalis*), and gila monster (*Heloderma suspectum*; see 37). These libraries have produced a number of insights into reptile genomics (164, 167, 189, 204) and are an ongoing resource for examination of sex chromosomes and multigene families.
- Genomic sequencing surveys. The National Center for Biotechnology Information (NCBI) trace archive contains sequences including expressed sequence tags (ESTs) from squamate venom glands (see The Venome section above) as well as tissues from green anole, American alligator, and painted turtle. These are useful resources for gene discovery and sequence comparisons (32, 33). For example, ESTs from American alligator have yielded sequences of the proto-oncogenes *c-Jun* and *DJ-1* (99). The transcript accumulation of these genes will inform their role in sex determination among reptiles that differ in their sex-determining mechanisms (99).
- Web sites. **http://www.reptilegenome.org**: helped organize community input that led to the eventual sequencing of the *Anolis carolinensis* genome; **http://www.genome.gov/25521740**: describes evolution of the human proteome; **http://www.snakegenomics.org**: brings together researchers whose diverse interests will benefit from enhanced genomics of snakes.

CONCLUSIONS

The promise of reptile genomics stands on a number of grounds: the intrinsic nature of reptile genomes and mechanisms, the need for greater taxonomic coverage across the phylogenetic tree for amniotes, and the insights that will be gained through comparisons between birds, reptiles, and mammals. Phylogeny plays a larger role than just assisting in the assembly of genomes. Phylogenetic comparisons can facilitate the identification of genes and other functional genomic elements, the estimation of rates of nucleotide substitution and rates of indels, and the piecing together of the complex series of events, including chromosomal translocations and copy number variations, that have resulted in large changes in genome size and architecture. But not all genomes are equally informative for reconstructing ancestral states, especially when current taxon sampling is meager. While greater taxon sampling of major lineages of mammals has clarified a variety of trends in mammalian genome evolution, such as the evolution of sex chromosomes (191), the promise of reptile genomics lies in the even greater diversity of reptiles as compared to mammals, and in the increased definition of ancestral states and the range of genomic possibilities explored through the course of amniote evolution.

This review marks a prelude to what will surely be an explosion of reptile genomics in the next few years. Several new genomes of birds and nonavian reptiles are being contemplated and planned by various research groups,

and the *Anolis* genome has barely yielded its secrets. Thus, most of the results that we review here stem from the pregenome era of reptile genomics. We are still ignorant of many of the types of variation in reptiles that have risen to the forefront of human genomics, such as copy number variation and gene expression. Even with present resources, we are poised to answer many important questions that have been broached in mammalian genomics. The next five years will undoubtedly witness an explosion of comparative projects that synthesize over 300 million years of amniote genome evolution.

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Errata

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