# Squamate Reptile Genomics and Evolution

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# Squamate Reptile Genomics and Evolution

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## Abstract

Squamates exhibit some of the most extreme and fascinating biological adaptations among vertebrates, including the production of a wide diversity of venom toxins. The rapid accumulation of genomic information from squamate reptiles is generating important new context and insights into the biology, the regulation and diversity of venom toxins, and the evolutionary processes that have generated this diversity. It is an exciting time as we discover what the unique aspects of the squamate genome can tell us about the molecular basis of such interesting and diverse phenotypes and explain how the extreme adaptations of squamate biology arose. This chapter reviews what is known about major patterns and evolutionary trends in squamate genomes and discusses how some of these features may relate to the evolution and development of unique features of squamate biology and physiology on the whole, including the evolution and regulation of venom toxins. It also discusses current challenges and obstacles in understanding squamate genome size, diversity, and evolution, and specific issues related to assembling and studying regions of squamate genomes that contain the genes and regulatory regions for venom toxins. Evidence is presented for a relatively constant genome size across squamates even though there have been major shifts in genomic structure and evolutionary processes. Some genomic structural features seem relatively unique to squamates and may have played roles in the evolution of venom toxins.

## Introduction

### Overview of Squamate Reptiles

Squamates, including lizards and snakes, are a diverse lineage of reptiles that are unique from other branches on the reptilian tree of life. This radiation represents a particularly interesting history in the evolution of vertebrates. Beginning with a limited number of ancestral reptiles in the mid-Triassic, present-day Squamata comprise over 9,000 species (Reptile Database; www.reptile-database.org) inhabiting a large diversity of habitats globally, making it one of the most important and speciose vertebrate radiations. Novel adaptations to a wide spectrum of habitats and ecological roles have led to an even wider array of behaviors, phenotypes, and life history traits that are unique to squamates. The current influx of information about squamate genome structure, content, and diversity holds great potential to enlighten us about the unique and diverse adaptations possessed by these species, including the evolution and regulation of diverse venom toxins.

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The Squamata diversification began nearly 250 million years ago (MYA), and divergence times among amniotes and within squamates have been estimated previously (Hedges et al. 2006; Castoe et al. 2009b). The order Squamata is comprised of the suborders Iguania (comprised of exclusively lizards) and Scleroglossa (which includes the remaining lizards, amphisbaenians, and snakes). The Squamata diverged from its sister group, the Sphenodontia (tuataras) ∼240 MYA. These lineages split from other reptilian groups (birds, crocodiles, and turtles) ∼275 MYA. Within the Squamata, the suborder Scleroglossa is further subdivided into infraorders Amphisbaenia, Anguimorpha, Gekkota, Scincomorpha, and Serpentes. The Gekkota diverged other Scleroglossan lineages ∼200 MYA. The Scincomorpha diverged from the clade containing Amphisbaenia, Anguimorpha, and Serpentes greater than 180 MYA. The divergence time for the split between Amphisbaenia and the Anguimorpha-Serpentes clade is estimated at ∼179 MYA. Finally, the divergence time between Anguimorpha and Serpentes is ∼175 MYA.

The intrinsic medical relevance of venom has made it a focus of fascination and study for hundreds of years. Recently, additional interest in squamates and their toxins has motivated whole new fields of inquiry: the potential for a role for venom toxins in medicine, understanding the evolutionary origins of these toxins from presumably nontoxic ancestral proteins, understanding how these toxins are regulated at multiple levels and through ontogeny, and their variation among species, populations, and individuals (Calvete 2010; Casewell et al. 2012). The precise definition of a "venom" or "toxin" has not, however, been clear or consistent in the literature. For example, some practical definitions of "venoms" appear to include all proteins expressed in venom or other oral glands in squamates, regardless of their biological activity or toxicity. A greater understanding of the evolutionary relationships among putative venom toxins and their origins will provide important clues to guide this debate. Furthermore, the relationship between changes in sequence, structure, and function is expected from a greater understanding of squamate genomics, and this will reshape our conceptual understanding of venoms and their origins.

## Importance of Genomic Resources for Squamates

Prior to discussing genomes and genomics, it is worthwhile to briefly discuss what a "complete genome" means in a practical sense, and why different "complete genomes" might differ tremendously in their information content. A "complete genome" represents a hypothetical reconstruction of the genome based on combining information from multiple types of sequence reads. Vertebrate genomes are normally sequenced from a single representative individual of the species of interest. Often the larger, more repetitive, and more heterozygous a genome is, the harder it is to reassemble in silico. Among "complete genomes," there may exist a large range of completeness and accuracy. In practice, "complete genomes" are comprised of thousands of "scaffolds" or "scaffolded contigs," which represent the largest genomic chunks that could be put together into single reconstructed pieces.

In addition, biologically motivated questions require genome annotation (identification of repeat elements, genes, and untranslated regions). Annotation can be based on predicted similarity to previously studied species or on empirical data from the same organism. Transcriptome analysis is a particularly important empirical method used to evaluate the total set of transcripts produced by a species and estimate transcript and splice forms produced by genes (for a given tissue surveyed). The number and diversity of tissue types surveyed for transcriptome studies may have a large impact on the quality of these empirical annotations. Ultimately, these considerations mean that different complete and annotated genomes will be of different quality and thus different utilities. It is therefore important that when available data is surveyed, these factors relating to information content and quality of genome assemblies and annotations are taken into account.

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For several decades, the vast majority of what was known about squamate genes and proteins was focused on venom proteins and the transcripts that encode them. The majority of snake gene sequences in Genbank, for example, are from venom gland cDNA sequencing. Studies of venom gland transcriptomes have, however, lacked context due to the lack of transcriptomes from other tissues and from other squamate reptiles. Similarly, studies of venom genes focused on inferring patterns of selection in squamate venom proteins, and on inferring the genetic and ontological origins of venom genes in squamates, have suffered from a lack of knowledge of the full complement of genes in squamate species and the expression patterns of these genes across many different tissue types. This lack of context with which to understand and interpret the origins, relationships, and patterns observed in venom toxins has limited comparative analyses and limited our understanding of the evolution of venom toxins. Dramatic increases in computer power and decreasing costs have improved the feasibility of large-scale genome projects, and numerous full-scale squamate genome projects have recently begun to emerge.

## Available Squamate Genomes

In recent years, numerous squamate genome projects have been proposed and initiated. Several have now been completed, and many more are expected in the near future. The Carolina anole lizard (*Anolis carolinensis*) genome project provided the first complete and annotated squamate genome that is now freely available. Through comparisons with avian and mammalian genomes, the *Anolis* genome yielded insights into the evolution of amniotes and into differences between the genome birds and mammals and that of *Anolis*, and likely other squamates (Alfoldi et al. 2011). Major findings include a high degree of similarity between *Anolis* and avian microchromosome structure, but with the caveat that the *Anolis* microchromosomes show a greater degree of repeat content than was found in mammalian and avian genomes (Alfoldi et al. 2011). Another peculiarity was the finding that *Anolis* lacked GC-biased isochores (or long segments of similar GC-content that differ across the genome), in contrast to mammals and birds.

In addition to the *Anolis* lizard genome, several snake genome projects have been completed. A high-quality draft genome of the Burmese python (*Python molurus bivittatus*), complete with annotations, has recently been assembled and released (Castoe et al. 2013). Transcriptomic resources have also been made available and are being developed further (Castoe et al. 2011c). This genome project was largely motivated by the importance of this species in studies of the molecular basis of extreme physiological and phenotypic traits, including the ability for some snakes (such as the python) to undergo tremendous fluctuations in metabolism after eating massive prey items. This nonvenomous snake genome is also expected to aid in understanding the evolutionary origins of venom toxins in other snake lineages. The genome for the venomous king cobra (*Ophiophagus hannah*) is also currently available and provides insights into the molecular basis for the evolution of the sophisticated snake venom system (Vonk et al. 2013). Numerous other squamates have been targeted for genome sequencing through the efforts of the Genome 10 K community (G10KCOS) and the Beijing Genomics Institute (BGI) and by various consortia or individual laboratories. Currently available information about many of these target species is available via the Genome 10 K website (http://genome10k.soe.ucsc.edu), and a summary of all known projects is provided in Table 1.

Genomes for the nonvenomous garter snake (*Thamnophis sirtalis*; Castoe et al. 2011b), the Texas blind snake (*Leptotyphlops dulcis*), the venomous prairie rattlesnake (*Crotalus viridis*), and the timber rattlesnake (*Crotalus horridus*) are currently being sequenced. The genome of the Boa constrictor (*Boa constrictor*) is complete and available but lacks any annotation (Bradnam et al. 2013; www.assemblathon.org). The addition of multiple snake genomes to the *Anolis* genome

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**Table 1** Status of current squamate reptile genome projects as of July 2013 (Data were gathered and adapted from GenBank (NCBI) the Genome 10 K public lists (G10K), literature, and personal communications)

| Species | Family | Common name | Status | Source |
|---|---|---|---|---|
| **Lizards** | | | | |
| *Podarcis muralis* | *Lacertidae* | Wall lizard | In progress | G10K |
| *Shinisaurus crocodilurus* | *Shinisauridae* | Chinese crocodile lizard | Completed | G10K |
| *Pogona vitticeps* | *Agamidae* | Bearded dragon | Completed | G10K |
| *Ophisaurus harti* | *Anguidae* | Chinese glass lizard | In progress | G10K |
| *Eublepharis macularius* | *Geckonidae* | Leopard gecko | In progress | G10K |
| *Heloderma suspectum* | *Helodermatidae* | Gila monster | Proposed | G10K |
| *Anolis apletophallus* | *Polychrotidae* | Slender anole | In progress | G10K |
| *Anolis carolinensis* | *Polychrotidae* | Green anole | Published | NCBI |
| *Aspidoscelis tigris* | *Teiidae* | Western whiptail | Proposed | G10K |
| *Varanus komodoensis* | *Varanidae* | Komodo dragon | Proposed | G10K |
| **Snakes** | | | | |
| *Boa constrictor* | *Boidae* | Boa constrictor | Completed | G10K |
| *Thamnophis sirtalis* | *Colubridae* | Garter snake | In progress | Castoe et al. (2011b) |
| *Ophiophagus hannah* | *Elapidae* | King cobra | Completed | Vonk et al. (2013) |
| *Leptotyphlops dulcis* | *Leptotyphlopidae* | Texas blind snake | In progress | Castoe, personal communication |
| *Python molurus* | *Pythonidae* | Burmese python | Completed | Castoe et al. (2013) |
| *Crotalus horridus* | *Viperidae* | Timber rattlesnake | In progress | Sanders, personal communication |
| *Crotalus viridis* | *Viperidae* | Prairie rattlesnake | In progress | Castoe, personal communication |

is expected to yield new and valuable insight into the evolution of amniote and squamate genomes and provide much needed "omic" context to existing information on venom proteins, genes, and transcriptomes. In addition to new snake genomes, there are multiple lizard genomes, including individuals of *Heloderma*, *Pogona*, and *Varanus* – all lizard members of the clade "Toxicofera," which also includes snakes and is proposed to have evolved venoms on its ancestral lineage (Fry et al. 2006). These and other lizard genomes are expected to provide tremendous and much needed evolutionary and comparative context for understanding the origins of venoms in squamates, the number of times venoms may have evolved, and from what genetic and ontological sources.

## Squamate Genome Size

Genome size is an important metric for inferring large-scale changes across genomes, for estimating the effort required to sequence and assemble a genome, and for identifying what characteristics of interest might be related to changes in genome size. Indeed, repetitive element content, organism longevity, metabolic rate, and development rate have all been proposed to correlate with genome size (Gregory 2001). Though there does not appear to be a correlation between genome size and organism complexity, genome size does have an impact on cellular physiology, nuclear volume, and overall cell size (Gregory 2005). Squamate genome sizes have been estimated using three main methods: Feulgen density (FD), static cytometry (SFC), and flow cytometry (FCM). Estimates of genome size based on the full collection available from all these methods suggest that squamate genome size is relatively variable. The current method of choice, flow cytometry, is likely the most accurate technique to estimate genome size (Leutwiler et al. 1984; Hedley et al. 1985), although all previous summaries (and analyses) of genome size have incorporated all three estimates despite their

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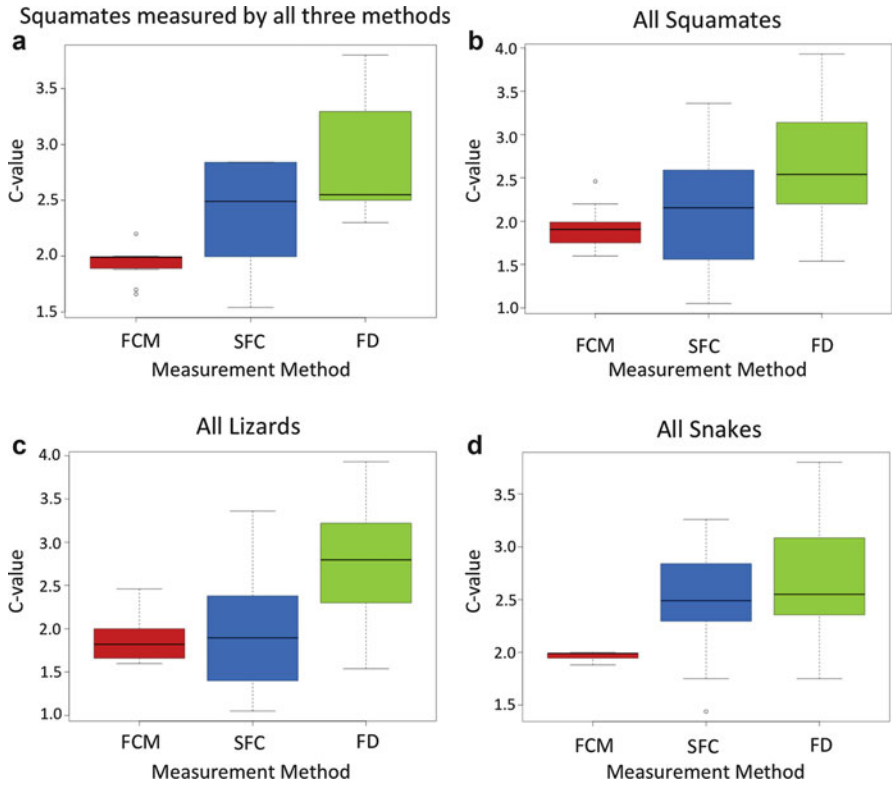


**Fig. 1** Box-plot comparisons of genome size estimates based on three different methods. For all panels, methods are abbreviated: *FCM* flow cytometry, *SFC* static flow cytometry, *FD* Feulgen density. (**a**) Genome size estimates for all lizard and snake species that have been measured by all three methods. (**b**) Genome size estimates for all snake and lizard species that have been measured by at least one of the three methods. (**c**) Genome size estimates for lizard species that have been measured by at least one of the three methods. (**d**) Genome size estimates for snake species that have been measured by at least one of the three methods (Data based on the Animal Genome Size Database (Gregory 2013))

differences in accuracy and precision. Genome size estimates using each of the three techniques for all squamates with data currently available in the Animal Genome Database (Gregory 2013) are summarized in Fig. 1, separated by technique. These estimates (Fig. 1a) show multiple forms of bias across methods. Squamate genome size estimates from Feulgen density and static cytometry are bigger and have a much higher variance than other measurements. These two techniques are thus less precise and possibly less accurate than flow cytometry (Fig. 1a). Thus, previous perspectives of high variance in genome size among squamate reptiles based on these estimates may be artifactual, due to the methodological inconsistency of the techniques used. There is a strong argument for careful interpretation of genome size estimates made by methods other than flow cytometry methods.

The average squamate haploid genome size estimate based on flow cytometry is 1.9 Gbp ($n$ = 90, range = 1.3–3.0 Gbp; Fig. 2b). This average is intermediate in size between birds (1.4 Gbp) and mammals (3.5 Gbp) and is also smaller than other non-avian reptiles (3.2 Gbp in Testudines and Crocodilia and 5.0 Gbp in *Sphenodon* (Janes et al. 2010b)). The average lizard genome size based on flow cytometry is also 1.9 Gbp ($n$ = 58, range = 1.3–2.8 Gbp; Fig. 2c). The average snake genome size based on flow cytometry is also 1.9 Gbp ($n$ = 32, range = 1.5–3.0 Gbp; Fig. 2d). Previous work on the pattern of genome size evolution found that the Reptilia have experienced continuous gradual evolutionary change in genome size with no rapid shifts in genome size since the early reptile radiation (Organ et al. 2008). Other research, however, has found that larger genomes evolve in size

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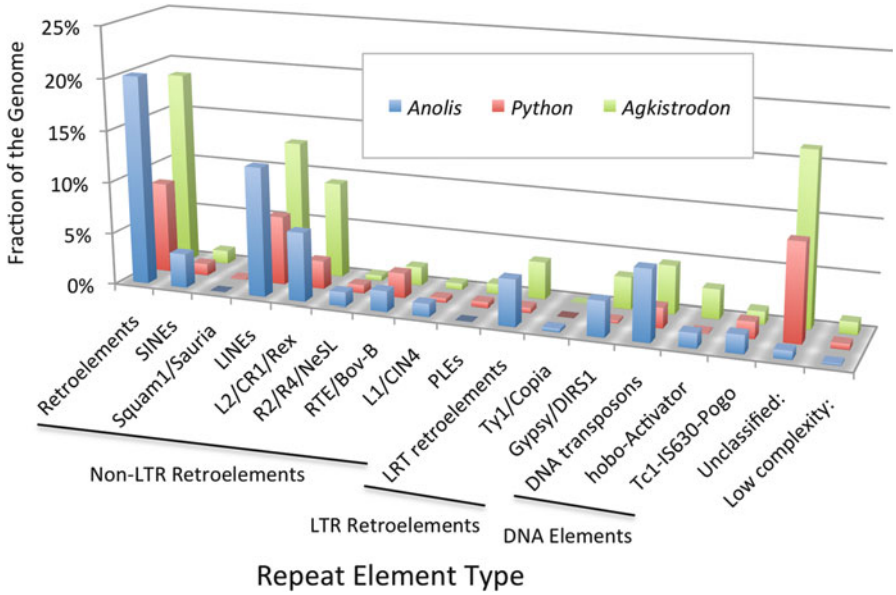


**Fig. 2** Comparison of genome repeat content between *Anolis carolinensis*, *Python molurus bivittatus*, and *Agkistrodon contortrix*. Various repeat element families and their overall classification are shown on the *horizontal axis*. The *vertical axis* indicates the proportion of the total genome constituted by a repetitive element (Data based on Castoe et al. (2011a) and analysis of the repeat-masked complete *Anolis* genome from the UCSC Genome Browser)

at faster rates than smaller genomes in reptiles (Oliver et al. 2007). These previous studies all have used data from all three methods of genome size estimation above, and it is unclear if or how this may have impacted their conclusions.

## Squamate Genome Structure

### The Mitochondrial Genome

Genomics typically invokes reference to the nuclear genome, although the mitochondrial genomes of squamate reptiles have been studied most thoroughly to date. This smaller organellar genome typically contains 13 protein-coding genes central to mitochondrial oxidative metabolism function, the ribosomal and tRNAs to accomplish translation of these proteins, and a control region that functions in mitochondrial genome replication and transcription. Snake mitochondrial genomes have been of particular interest because they evolved a number of characteristics that are unlike most vertebrates. With the exception of the scolecophidian snakes (blind snakes and their relatives), which are the most ancestral extant group of snakes, all snakes appear to have a duplicated mitochondrial control region, and both control region sequences are maintained at nearly identical sequences by an unknown mechanism of concerted evolution (Kumazawa et al. 1996; Jiang et al. 2007). Molecular evolutionary evidence suggests these control regions are both likely to act as origins of genome replication (and probably also as promoters for RNA synthesis) (Jiang et al. 2007; Castoe et al. 2009b). It has been hypothesized that these duplicate control regions might function in the rapid metabolic upregulation in some snakes, which is associated with feeding (Jiang et al. 2007; Castoe et al. 2009b). In addition to large-scale genome structure, studies have shown that snake mitochondrial proteins have experienced an extreme adaptive event that included unprecedented coevolutionary change along with a great excess of radical amino acid replacements. These findings imply that snake oxidative metabolism might function uniquely among vertebrates,

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due to the large number of unique and radical changes observed in these snake proteins (Castoe et al. 2008). Further evidence for the largest known episode of convergent molecular evolution having occurred between the proteins of snakes and acrodont lizards implies strong convergent patterns of selection (Castoe et al. 2009a). The uniqueness of squamate mitochondrial genome structure and protein evolution raises many questions about the scope of adaptation in the nuclear genomes of squamates and if there might have been evolutionary interactions between extreme metabolic adaptation and the evolution of venom systems in squamate reptiles.

## Nuclear Chromosomal Structure

Chromosomal variation is far greater in reptiles than in mammals, mainly due to the presence of microchromosomes (Olmo 2005). Microchromosomes are structurally and functionally similar to macrochromosomes but are roughly half the size of macrochromosomes on average (Rodionov 1996). They are two to three times more gene dense than macrochromosomes (Smith et al. 2000), and avian microchromosomes appear to have a higher recombination rate than macrochromosomes (Rodionov et al. 1992). Compared with macrochromosomes, nucleotide content in microchromosomes tends to be GC rich and contains higher frequencies of CpG dimers, and these microchromosomes are also relatively depauperate in repetitive elements (Hillier et al. 2004).

On average, squamates have 36.6 chromosomes (range = 27–51 chromosomes) divided roughly into one half macrochromosomes (average = 18, range = 12–35) and one half microchromosomes (average = 18.9, range = 2.1–24) (Olmo and Signorino 2013). Snakes appear to have relatively highly conserved karyotypes, with the most common diploid number being $2n = 36$. Karyotypes of snakes typically consist of eight pairs of macrochromosomes and 10 pairs of microchromosomes (Matsubara et al. 2006; Srikulnath et al. 2009). Lizards, in contrast, have large variations in chromosome number and morphology. In lizards, one of two main karyotypes tends to be observed in a given species: either a mixture of macrochromosomes and microchromosomes or few or no microchromosomes (Srikulnath et al. 2009). No phylogenetically controlled correlation exists between haploid genome size and the number of microchromosomes, macrochromosomes, and total chromosomes (Organ et al. 2008), so it is difficult to make any inferences about relationships between chromosome number and genome size.

## Sex Chromosomes

Sex determination in squamates results from one of two mechanisms, both of which are scattered across various squamate lineages. One mechanism, which is more common in non-squamate reptiles (e.g., turtles and crocodilians), is temperature-dependent sex determination (TSD), in which the sex of offspring is governed by incubation temperature. The more common sex-determination mechanism in squamates is genetic sex determination (GSD), where chromosomal inheritance dictates sex. In most squamates GSD follows a Z/W sex chromosome system. Among squamates, snakes are straightforward in this respect, and all exhibit a sex chromosome system with female heterogamety (ZW), which is the general trend across the squamate tree of life (Janes et al. 2009). Analyses of snake sex chromosomes have revealed increased differentiation in a phylogenetic gradient from pythons to colubroid snakes (Matsubara et al. 2006). In contrast, lizards can have either heterogametic males or females, possess X/Y or Z/W sex chromosome systems, and sometimes exhibit TSD. This diversity of sex-determining mechanisms makes squamates an ideal system for understanding sex determination (Ezaz et al. 2005).

The Z/W sex-determination system parallels the better-known X/Y sex-determination system in that the W sex chromosome is often a degenerated copy of the Z sex chromosome just as the Y sex chromosome is often a degenerated copy of the X sex chromosome. Sex-determining genes have

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been resolved for mammals (*Sry*) and birds (*Dmrt1*), but not for squamates. *Dmrt1* has been mapped to autosomal chromosomes in four snake species (Matsubara et al. 2006) and is therefore not the sex-determining factor for snakes. Additionally, *Dmrt1* from the chicken Z chromosome has been mapped to both Z and W sex chromosomes in *Gekko hokouensis*, and analyses of the *Anolis* genome further indicate that *Dmrt1* is unlikely to be the sex-determination gene (Alfoldi et al. 2011). Complicating the search for a sex-determination locus in squamates is the finding that sex chromosomes are not homologous between reptile groups, which are consistent with sex chromosomes evolving many times independently in reptiles (Ezaz et al. 2009).

## Genomic GC-Isochore Structure

GC isochores are large tracts of genomic DNA with internally relatively homogeneous base composition that varies over large chromosomal scales. GC-rich isochores positively correlate with many important genomic features, including recombination rate, gene density, epigenetic modifications, intron length, and replication timing, implying their importance as functional genomic elements (Janes et al. 2010b). The *Anolis carolinensis* genome was found to lack GC-rich isochores, which was an unexpected result (Alfoldi et al. 2011). Recent analyses of the Burmese python and king cobra genomes indicate a higher degree of GC-isochore structure than *Anolis* (Castoe et al. 2013). These findings may suggest that snakes have re-evolution GC isochore since their divergence from *Anolis* or that GC isochore was lost in an ancestor of *Anolis*.

## Ultraconserved Regions

Ultraconserved elements (UCEs), or small stretches of the genome that are conserved across distantly related vertebrates, have become popular for inferring the phylogenetic relationships among vertebrate organisms (McCormack et al. 2012); Crawford et al. 2012 discovered a dramatically increased substitution rate in UCEs in the squamate lineage, and particularly in snakes. Squamates, therefore, appear to show a shift in conserved regulatory genomic regions that have otherwise remained relatively static in other amniote lineages. Other research on long, conserved noncoding sequences (LCNSs), another class of highly conserved genomic elements, found that a higher percentage of these sequences is conserved in reptiles, which may reflect differing roles and constraints in gene regulation in the reptile lineage (Janes et al. 2009, 2010a). Future studies on squamate genomes may provide additional insights into the evolutionary patterns of conserved genomic elements and the functional consequences of changes in such conserved genomic regions in squamates.

## Transposable Element Diversity

Although our current knowledge of vertebrate genome structure and diversity is strongly slanted towards mammals, new sequence-based information on reptilian genome structure and content is just beginning to emerge (Shedlock et al. 2007; Kordis 2009; Novick et al. 2009; Piskurek et al. 2009; Castoe et al. 2011d, 2013). Like most vertebrates, large portions of squamate genomes are comprised of repeat elements, and based on the small numbers of examples known, squamate genomes appear to contain a highly diverse repertoire of repeat element types (Shedlock et al. 2007; Castoe et al. 2011d, 2013). In contrast to the genomes of mammals and birds, most (non-avian) reptile genomes are comprised of a particularly diverse repertoire of different types of transposable elements (TEs) and multiple apparently active TE types, subtypes, and families (Fig. 2). Whereas mammal and bird genomes often have undergone recent expansion of one or a small number of TEs, such as L1 LINEs and Alu SINEs in humans, reptilian genomes examined have experienced recent (and presumably ongoing) activity and expansion of multiple TE types; this is particularly true of the

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squamate reptiles studied to date (Castoe et al. 2013). Based on preliminary genomic analyses of the lizard *Anolis*, trends in the squamate lineage include an increase in simple sequence repeat (SSR) content, the dominance of CR1 LINE retroelements, and a high overall diversity of retroelements (Shedlock et al. 2007; Novick et al. 2009; Piskurek et al. 2009).

Genomic sample sequencing and analysis of unassembled random genomic sequences from two snake species (*Python molurus bivittatus* and *Agkistrodon contortrix*) determined that among the snakes, the relative abundance of different repeat elements varies widely, while genome size and repeat element diversity do not (Fig. 2). Sample sequencing from ten total snake genomes indicates that repeat content varies widely, while the diversity of repeat elements stays fairly consistent (Castoe et al. 2013). It is also notable that major differences in repeat element content between snakes is based on the difference in abundance of most repeat element classes rather than expansion or contraction of one or a few repeat element groups (Castoe et al. 2011d, 2013). Two groups of non-LTR retrotransposons, CR1 LINEs and Bov-B LINEs, appear to be particularly abundant and active in snake genomes (Castoe et al. 2013). There are also probably several classes of abundant SINEs in snakes, but they have not been identified and are either novel or too divergent to be recognized by RepBase libraries and therefore are likely included in the set of "unclassified" repeats (Fig. 2). It is notable that previous studies have overestimated the abundance of Bov-B LINEs in snakes and lizards (Walsh et al. 2013) due to an incorrect annotation of a hybrid Bov-B/CR1 LINE (as a Bov-B LINE) reference sequence in RepBase (Castoe et al. 2011d). Current information on the transposable element landscapes of squamates suggests that there appears to be major shifts in abundance and presumably activity of multiple transposable element families, and a greater sampling of species is necessary to understand at what temporal scale and at which nodes in the squamate tree such shifts may have occurred.

## Horizontal Transfer of Transposable Elements

Knowledge of the presence and absence of transposable element types across vertebrate lineages remains fragmentary due to the limited sampling of vertebrate genomes; this is especially the case for squamate reptiles. Despite this, different types of elements in squamate genomes, including LINEs (Kordis and Gubensek 1997, 1998, 1999), SINEs (Piskurek and Okada 2007; Piskurek et al. 2009), and DNA transposons (Gilbert et al. 2008; Pace et al. 2008), may owe their origins to horizontal transfer. Multiple studies have inferred horizontal transfer of Bov-B LINE retrotransposons between mammals and snakes or squamate reptiles to explain the enigmatic distribution of these elements across amniote vertebrates (Kordis and Gubensek 1997, 1998). Based on phylogenetic analysis of Bov-B sequences from available vertebrate genomes and the sampled genomes of the python and copperhead, multiple episodes of horizontal transfer of Bov-B LINEs to or from squamate reptiles appear to have also occurred (Castoe et al. 2011d; Fig. 3). In Fig. 3, horizontal transfer is implicated because sequences of Bov-B from squamates are extremely closely related to similar sequences from mammals. Multiple transfers are indicated by the result that two clades of snakes do not form a clade exclusive of lizards, implying multiple independent transfers to ancestral lineages of snakes and/or squamates. Similarly, space invader (SPIN) elements, a type of hAT DNA transposon, are also inferred as having been independently horizontally transferred into the genomes of multiple tetrapod lineages within the last 15–46 million years (My), including into multiple lineages of squamates (Gilbert et al. 2008; Pace et al. 2008; Novick et al. 2009; Castoe et al. 2011d). Gilbert et al. (2012) determined that at least 13 independent episodes of SPIN element horizontal transfer events took place within Squamata within the last 50 My on at least three different continents. Evidence suggests that these transfers may have been mediated by parasites (Gilbert et al. 2010; Walsh et al. 2013).

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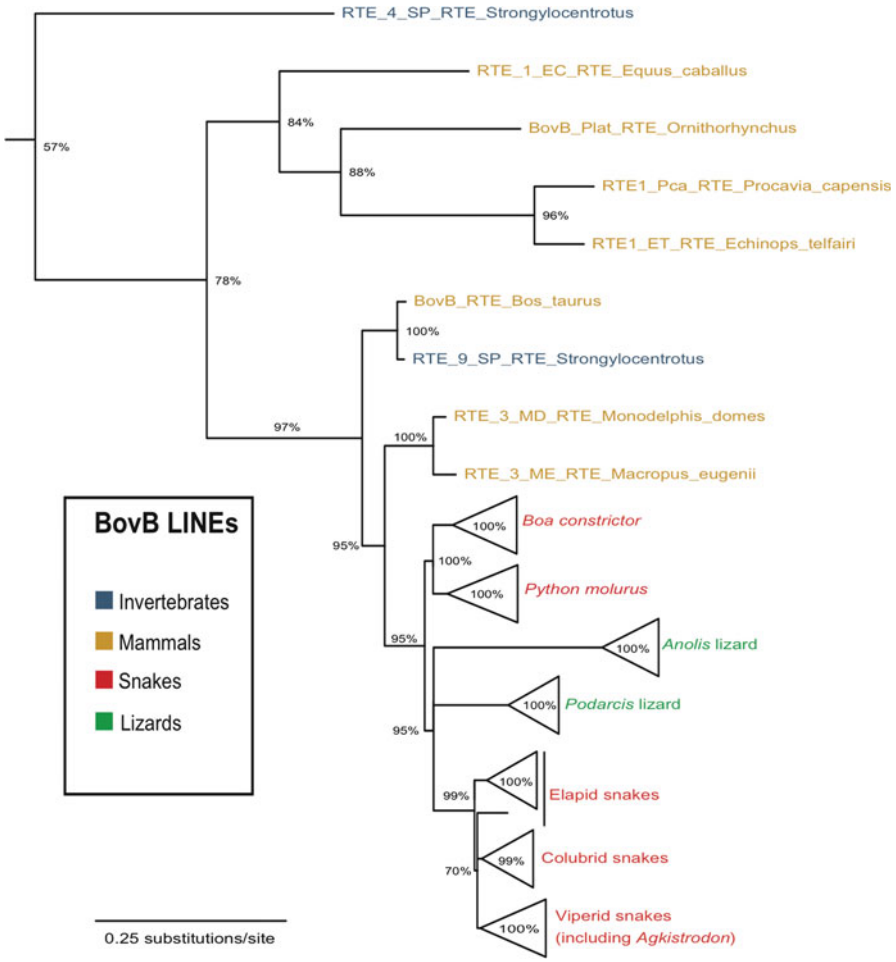


**Fig. 3** Bayesian phylogenetic tree comparing the relationships of Bov-B LINEs in a variety of vertebrate and invertebrate lineages. Lineage names are color coded based on taxonomy (see key), and posterior probabilities for nodal support are indicated. The topology suggests multiple horizontal transfers between deeply diverged vertebrate and invertebrate lineages (Figure adapted from Castoe et al. (2011d), supplemental figure S8)

## Microsatellite Seeding by Transposable Elements

It has been shown that transposable elements may occasionally contain microsatellite or simple sequence repeats (SSRs) on their tails and are therefore capable of seeding novel microsatellite loci on large scales throughout the genome. Snake genomes are the most extreme example of this in vertebrates (Castoe et al. 2011d). Analysis of two snake genome samples indicated a conspicuous increase in the genomic SSR and low complexity content, apparently indicating a secondary increase in SSR evolution and turnover in snakes (Castoe et al. 2011d). It is notable that this change must have occurred subsequent to the slowdown in SSR evolution and turnover earlier in the reptilian lineage (Shedlock et al. 2007). Snake1 (L3) CR1 LINEs appear to increase in frequency in snakes (Fig. 2), and also seed microsatellites, because the 3-prime tail of these elements contains a microsatellite repeat (Castoe et al. 2011d, 2013). These LINEs tend to contain one of two SSR repeat sequences, both of which are related in sequence (Fig. 3). The impact of such SSR seeding is extreme and obvious in the genome of the copperhead (*Agkistrodon*), in which Snake1 CR1 LINEs have become relatively abundant compared to python (Fig. 3). Specifically, a majority of all SSRs in the copperhead are one of three closely related sequences (AGA, AGAT, or AGATA; Fig. 3). Sequence sampling of ten total snake genomes indicates that these microsatellite-seeding Snake1

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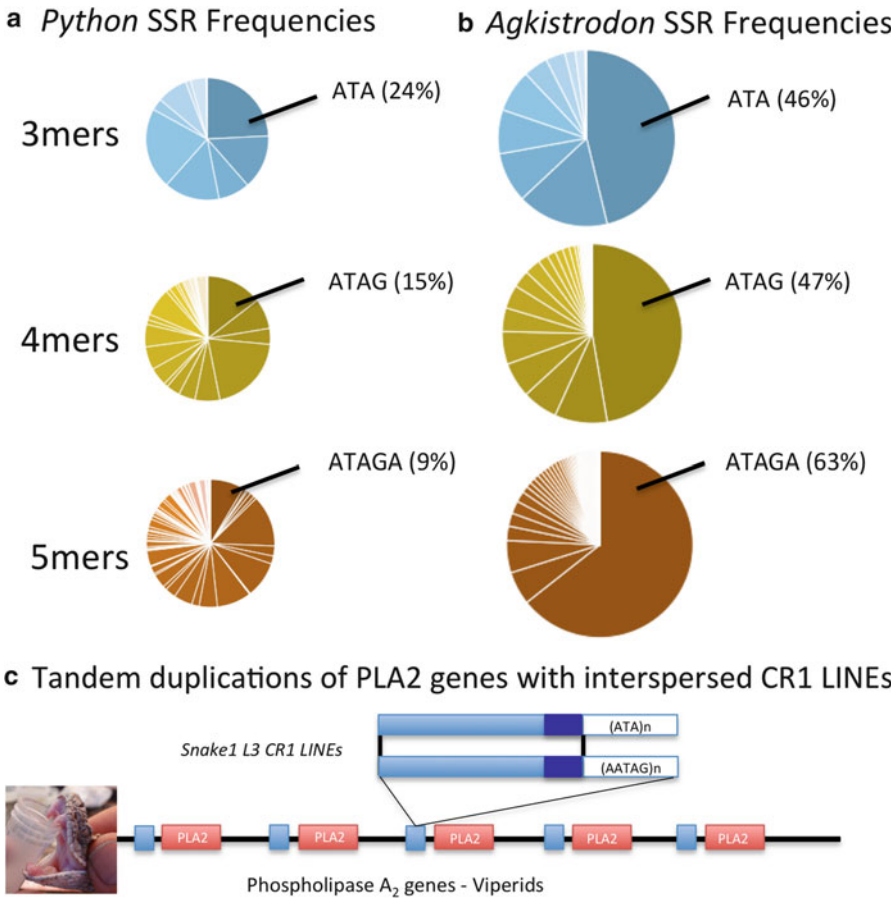


**Fig. 4** The estimated proportions of simple sequence repeat (SSR) content within *Python molurus bivittatus* and *Agkistrodon contortrix* genomes based on random unassembled genomic sequence data and a plausible connection between the taxonomic bias in SSR content and venom evolution. (**a**) 3, 4, and 5mer SSR estimates for *Python* only. (**b**) 3, 4, and 5mer SSR estimates for *Agkistrodon* only. (**c**) Hypothetical representation of phospholipase A2 (PLA2) venom genes in viperids, which are interspaced by Snake1 L3 CR1 LINEs whose associated SSR tails have putatively led to altered recombination and thus the tandem duplication motif that is common in venom genes (Figures based on data from Castoe et al. (2011a), and PLA2 gene cluster sequence reported by Ikeda et al. (2010))

CR1 LINEs have expanded extensively in colubrid snakes, providing further details supporting a trend seen in the comparison between python and copperhead (Castoe et al. 2013).

Microsatellites may alter genome recombination structure and rates and, together with other repeat elements (e.g., CR1 LINEs), may facilitate unequal crossing over events that lead to tandem duplication of segments of the genome. From what is currently known about snake genome structure, it appears that most venom genes (Casewell et al. 2012) are derived from other nontoxic gene families that experienced gene duplication. Interestingly, the current model for the evolution of venom toxins (at least in snakes) includes the tandem duplication of genes (Ikeda et al. 2010). Snake1 CR1 LINEs are also notable because, from what is currently known of snake genomes, they occur at high frequency throughout phospholipase venom genes in viperid snakes (Ikeda et al. 2010; Fig. 4c), in numerous other venom genes in viperids and elapids (Castoe et al. 2011d), and in Hox gene clusters of colubrid snakes (Di-Poi et al. 2010). Therefore, transposable elements and seeding of microsatellites may have contributed to the genomic context that facilitated the evolution and radiation of venom loci in snakes.

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# Genomics of Squamate Venom Toxins

## Genetic and Genomic Structure of Squamate Venom Toxins

The ability to leverage emerging high-throughput technologies for genomic, transcriptomic, and proteomic analysis continues to improve our understanding of the squamate lineage and the evolution of squamate venoms. Developing a deeper knowledge of toxin gene structure, and the genomic context in which toxin genes exist and in which they have evolved, is central for understanding the evolutionary origins and regulation of these genes. Most of what is currently known about squamate venom genes, however, provides little genomic context because it is based on cDNAs of venom gland transcripts, thus providing information only about the transcribed exons and UTRs. Because there are multiple opportunities for regulation of gene expression and protein activity after transcription (e.g., siRNA, miRNA, translation efficiency, posttranslational modification, etc.), there remain many gaps in our knowledge in relating mRNA transcript levels directly to levels of functional toxins in venoms. We expect that with the availability of venomous snake reference genomes like that of the king cobra (Vonk et al. 2013), we will be better equipped to fill these gaps in our understanding.

Venom genes have been shown to often occur in duplicated tandem arrays (Ikeda et al. 2010), and the evolution of venoms is thought to involve the duplication of nontoxic physiological protein-coding genes that are subfunctionalized or neofunctionalized to become venom toxins (Casewell et al. 2012; Vonk et al. 2013). Additionally, alternative splicing may provide further variation in functional venom proteins, increasing the number of protein products per locus, as has been shown in *Vipera lebetina* (Siigur et al. 2001). This typical structure of a venom gene locus can make it difficult to accurately translate information from transcriptome data. Based on transcriptome sequences, for example, it might be difficult to discern the difference between different alleles at the same locus, alternative splice forms from the same locus, or different recently duplicated loci. Therefore, in the absence of reference genomes for squamate reptiles, there is some ambiguity in translating venom protein diversity to transcript diversity and ultimately to inferences of venom locus diversity in the genome. Such inferences are made more difficult in studies where the aim is to use transcriptome or proteome data to analyze genetic variation in venom loci across individuals and populations because allelic variation among individuals may further complicate this mapping to genomic loci. The recent release of a draft genome sequence for the king cobra will help to fill this void, although this draft genome estimate was not able to completely assemble venom gene regions. This resource has, however, already provided important support for the tandem duplication model of the gene duplication and neofunctionalization for venom locus evolution and indicates ontological or developmental links between the venom gland and the pancreas based on similarities in small RNA expression (Vonk et al. 2013).

Despite substantial progress in forging connections between the genome, venom genes, their transcripts, and venom proteins and their effects, there are still substantial advances to be made with the availability of genomic resources for squamates. One critical and fundamental step forward would be the availability of well-assembled and annotated genomes for multiple venomous squamates to provide multiple complete genome references in which venom genes, along with their genomic context, can be directly linked to venom gene transcripts and venom proteins. Additionally, many other important questions regarding the genetic and ontological origins of venom toxins require additional genomic and transcriptomic resources for squamates to fully address, including the following: (1) are venom loci exclusively expressed in the venom glands or some forms expressed elsewhere in the organism? (2) Are there specific sequences that are identifiable that target venom genes for transcription only in the venom glands? (3) What were the expression

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patterns and biological functions of ancestral venom genes prior to their recruitment as venoms? (4) Is there evidence that certain sequences, such as simple sequence repeats or transposable elements, have played a central role in facilitating duplication and diversification of venom gene loci?

### Challenges Facing Genome Assembly of Squamate Genomes and Venom Gene Regions

Our ability to confidently study the genomic context of venom genes is limited by our ability to not only collect genomic information, but further by the ability to accurately reconstruct the regions of the genome in which venom genes occur. If the prevailing view that most venom genes in squamates have undergone duplication is correct, assembling these regions of the genome is difficult. Moreover, in cases where this duplication occurred via tandem duplication (Ikeda et al. 2010), de novo genome assembly of these regions of the genome will be particularly difficult. Venom genes are also known to contain relatively high allelic variation, increasing the likelihood for heterozygosity at venom loci, which is known to make genome assembly more difficult. The evidence that these tandem duplicate copies may also be interspersed with highly repetitive transposable elements and other repeats further complicates genome assembly. These factors collectively make venom-related regions of the genome difficult to confidently reconstruct in de novo genome assemblies, particularly with current sequencing strategies that employ short sequence reads. As a result, it is expected that some of the most difficult regions of squamate genomes to assemble will be those that are of the greatest interest and value for studying venom, and even "complete genomes" may provide limited and fragmentary information about the genetic structure and genomic context of venom genes. As sequencing technologies continue to evolve, there is hope that hybrid sequencing approaches that combine multiple different types of reads (including perhaps low quality but very long sequences) may help in accurately assembling these critical regions of squamate genomes.

## Conclusion and Future Directions

Squamates represent an extensive and ancient component of vertebrate evolutionary history and biodiversity, yet their genomic diversity has been remarkably poorly studied in comparison to mammals and birds. Multiple aspects of their extreme biology, including the evolution of a great diversity of toxic venoms, argue strongly for the importance of establishing genomic resources for squamates to illuminate key connections between genotypes and key phenotypes of interest. Emerging evidence implies that squamates have a relatively consistent genome size across species, yet may have marked difference in genomic repetitive content, making them excellent models for understanding relationships between genome size and repeat content. Squamates also are of biological interest because they represent an ideal comparative system for studying mechanisms of sex determination in vertebrates.

The sequencing and annotation of complete vertebrate genomes are increasingly feasible and affordable. An emerging central goal of toxinological research is to develop a seamless understanding of the connection between the genome and venom toxins, incorporating gene regulation and the forces that act to modulate transcription and translation of venom genes. This is, of course, complicated by difficulties discussed above, including problems assembling tandem venom gene arrays in the genome, differentiating alleles, isoforms, and loci from transcriptomic data. Recent evidence also strongly implicates a role for small RNA in the modulation of ontogenetic and other shifts in venom composition (Calvete 2010). Furthermore, although no studies to date have

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identified such effects, it is reasonable that there may be epigenetic regulatory effects that additionally modulate expression of venoms. Among vertebrates, snakes in particular possess a tremendous number of unique or extreme phenotypes. A greater understanding of the molecular and genomic basis of these phenotypes holds exciting potential to increase broad understanding of the function and functional flexibility of the vertebrate genome and to illuminate the mechanisms by which such unique phenotypes can be evolutionary created from the raw material of the common vertebrate genome plan.

As more squamate genome and gene expression data become available, the toxinological community might consider a careful reevaluation of the precise language used for putative toxins upon discovery and acceptable criteria to be used to identify genes as "venom toxins." This also requires better organism-wide context of where else various genes are expressed and what genomic content is associated with those genes. Such studies would also have good potential for forging new links between the structure and evolutionary processes of squamate genomes, and how these might have shaped the evolution of venoms and other extreme phenotypes of squamates. Furthermore, understanding the ancestral state of venom gene orthologs in nonvenomous and venomous squamate species would provide novel insight into what processes and genomic features, at what times during squamate evolution, initiated and fostered the evolution of squamate venoms.

It is motivating that, although we know relatively little about squamate genomes currently, the details about squamate genomes that we do know tell a compelling story about the uniqueness and relatively extreme features of squamate genomes compared to other lineages of vertebrates and suggest an exciting future of discovery as more squamate genome information becomes available. This chapter has outlined multiple arguments motivating additional squamate genome sequence information as central to exposing the details of some of the most intriguing biological features known in vertebrates, including the evolution and function of deadly venom toxins and other extreme aspects of squamate biology. As a new generation of genome sequencing technology becomes more established and inexpensive, this data will likely begin to become available, making the coming years exciting for squamate biologists, toxinologists, vertebrate evolutionary biologists, and genome scientists.

## Cross-References


- ► Accelerated Evolution of Toxin Genes
- ► Adaptive Evolution and Neofunctionalization of Snake Venom Phospholipase A(2) Genes
- ► Animal Toxin Transcriptomics
- ► Evolution of Snakes and Venoms
- ► High-Throughput Transcriptomics
- ► Shotgun Approaches for Venom Analysis
- ► Snake Venom Peptidomics
- ► Snake Venom Proteopeptidomics: What Lies Behind the Curtain
- ► Toxin Evolution
- ► Venoms of Colubrids

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# References

Alfoldi J, Di Palma F, Grabherr M, et al. The genome of the green anole lizard and a comparative analysis with birds and mammals. Nature. 2011;477:587–91.

Bradnam KR, Fass JN, Alexandrov A, Baranay P, Bechner M, Birol I, Boisvert S, Chaptman JA, et al. Assemblathon 2: evaluating de novo methods of genome assembly in three vertebrate species. GigaScience. 2013;2:10.

Calvete JJ. Antivenomics and venom phenotyping: a marriage of convenience to address the performance and range of clinical use of antivenoms. Toxicon. 2010;56:1284–91.

Casewell NR, Wuster W, Wagstaff SC, Renjifo C, Richardson MK, Vonk FJ, Harrison RA. The origin and evolution of metalloproteinases in the venom of snakes. Toxicon. 2012;60:119.

Castoe TA, Jiang ZJ, Gu W, Wang ZO, Pollock DD. Adaptive evolution and functional redesign of core metabolic proteins in snakes. PLoS One. 2008;3:e2201.

Castoe TA, de Koning APJ, Kim H-M, Gu W, Noonan BP, Naylor G, Jiang ZJ, Parkinson CL, Pollock DD. Evidence for an ancient adaptive episode of convergent molecular evolution. Proc Natl Acad Sci. 2009a;106:8986–91.

Castoe TA, Gu W, De Koning APJ, Daza JM, Jiang ZJ, Parkinson CL, Pollock DD. Dynamic nucleotide mutation gradients and control region usage in squamate reptile mitochondrial genomes. Cytogenet Genome Res. 2009b;127:112–27.

Castoe T, de Koning A, Hall K, et al. Sequencing the genome of the Burmese python (*Python molurus bivittatus*) as a model for studying extreme adaptations in snakes. Genome Biol. 2011a;12:1–8.

Castoe TA, Bronikowski AM, Brodie ED, Edwards SV, Pfrender ME, Shapiro MD, Pollock DD, Warren WC. A proposal to sequence the genome of a garter snake (*Thamnophis sirtalis*). Stand Genomic Sci. 2011b;4:257–70.

Castoe TA, Fox SE, De Koning APJ, Poole AW, Daza JM, Smith EN, Mockler TC, Secor SM, Pollock DD. A multi-organ transcriptome resource for the Burmese python (*Python molurus bivittatus*). BMC Res Notes. 2011c;4.

Castoe TA, Hall KT, Mboulas MLG, et al. Discovery of highly divergent repeat landscapes in snake genomes using high-throughput sequencing. Genome Biol Evol. 2011d;3:641–53.

Castoe TA, de Koning AP, Hall KT, et al. The Burmese python genome reveals the molecular basis for extreme adaptation in snakes. Proc Natl Acad Sci U S A. 2013;110:20645–50.

Crawford NG, Faircloth BC, McCormack JE, Brumfield RT, Winker K, Glenn TC. More than 1000 ultraconserved elements provide evidence that turtles are the sister group of archosaurs. Biol Lett. 2012;8:783–6.

Di-Poi N, Montoya-Burgos JI, Miller H, Pourquie O, Milinkovitch MC, Duboule D. Changes in Hox genes' structure and function during the evolution of the squamate body plan. Nature. 2010;464:99–103.

Ezaz T, Quinn A, Miura I, Sarre S, Georges A, Marshall Graves J. The dragon lizard *Pogona vitticeps* has ZZ/ZW micro-sex chromosomes. Chromosome Res. 2005;13:763–76.

Ezaz T, Warre SD, O'Meally D, Marshall Graves JA, Georges A. Sex chromosome evolution in lizards: independent origins and rapid transitions. Cytogenet Genome Res. 2009;127:249–60.

Fry BG, Vidal N, Norman JA, et al. Early evolution of the venom system in lizards and snakes. Nature. 2006;439:584–8.

Gilbert C, Pace JK, Waters PD. Target site analysis of RTE1_LA and its AfroSINE partner in the elephant genome. Gene. 2008;425:1–8.

Gilbert C, Schaack S, Pace Ii JK, Brindley PJ, Feschotte C. A role for host-parasite interactions in the horizontal transfer of transposons across phyla. Nature. 2010;464:1347–50.
Gilbert C, Hernandez SS, Flores-Benabib J, Smith EN, Feschotte C. Rampant horizontal transfer of SPIN transposons in squamate reptiles. Mol Biol Evol. 2012;29:503–15.
Gregory TR. Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. Biol Rev. 2001;76:65–101.
Gregory TR. Genome size evolution in animals. In: Gregory TR, editor. The evolution of the genome. Boston: Elsevier Academic Press; 2005. p. 4–71.
Gregory TR. Animal genome size database. 2013. http://www.genomesize.com
Hedges SB, Dudley J, Kumar S. TimeTree: a public knowledge-base of divergence times among organisms. Bioinformatics. 2006;22:2971–2.
Hedley DW, Friedlander ML, Taylor IW. Application of DNA flow cytometry to paraffin-embedded archival material for the study of aneuploidy and its clinical significance. Cytometry. 1985;6:327–33.
Hillier LW, Miller W, Birney E, et al. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. Nature. 2004;432:695–716.
Ikeda N, Chijiwa T, Matsubara K, Oda-Ueda N, Hattori S, Matsuda Y, Ohno M. Unique structural characteristics and evolution of a cluster of venom phospholipase A(2) isozyme genes of *Protobothrops flavoviridis* snake. Gene. 2010;461:15–25.
Janes DE, Organ CL, Edward SV. Variability in sex-determining mechanisms influences genome complexity in Reptilia. Cytogenet Genome Res. 2009;127:242–8.
Janes DE, Chapus C, Gondo Y, Clayton DF, Sinha S, Blatti CA, Organ CL, Fujita MK, Balakrishnan CN, Edward SV. Reptiles and mammals have differentially retained long conserved noncoding sequences from the Amniote ancester. Genome Biol Evol. 2010a;3:102–13.
Janes DE, Organ CL, Fujita MK, Shedlock AM, Edwards SV. Genome evolution in Reptilia, the sister group of mammals. Annu Rev Genomics Hum Genet. 2010b;11(11):239–64.
Jiang ZJ, Castoe TA, Austin CC, Burbrink FT, Herron MD, McGuire JA, Parkinson CL, Pollock DD. Comparative mitochondrial genomics of snakes: extraordinary substitution rate dynamics and functionality of the duplicate control region. BMC Evol Biol. 2007;7:123.
Kordis D. Transposable elements in reptilian and avian (sauropsida) genomes. Cytogenet Genome Res. 2009;127:94–111.
Kordis D, Gubensek F. Bov-B long interspersed repeated DNA (LINE) sequences are present in Vipera ammodytes phospholipase A(2) genes and in genomes of Viperidae snakes. Eur J Biochem. 1997;246:772–9.
Kordis D, Gubensek F. Unusual horizontal transfer of a long interspersed nuclear element between distant vertebrate classes. Proc Natl Acad Sci U S A. 1998;95:10704–9.
Kordiš D, Gubenšek F. Molecular evolution of Bov-B LINEs in vertebrates. Gene. 1999;238:171–8.
Kumazawa Y, Ota H, Nishida M, Ozawa T. Gene rearrangements in snake mitochondrial genomes: highly concerted evolution of control-region-like sequences duplicated and inserted into a tRNA gene cluster. Mol Biol Evol. 1996;13:1242–54.
Leutwiler LS, Hough-Evans BR, Meyerowitz EM. The DNA of *Arabidopsis thaliana*. Mol Gen Genet MGG. 1984;194:15–23.
Matsubara K, Tarui H, Toriba M, Yamada K, Nishida-Umehara C, Agata K, Matsuda Y. Evidence for different origin of sex chromosomes in snakes, birds, and mammals and step-wise differentiation of snake sex chromosomes. Proc Natl Acad Sci. 2006;103:18190–5.

DOI 10.1007/978-94-007-6649-5_34-2


McCormack JE, Faircloth BC, Crawford NG, Gowaty PA, Brumfield RT, Glenn TC. Ultraconserved elements are novel phylogenomic markers that resolve placental mammal phylogeny when combined with species-tree analysis. Genome Res. 2012;22:746–54.

Novick PA, Basta H, Floumanhaft M, McClure MA, Boissinot S. The evolutionary dynamics of autonomous non-LTR retrotransposons in the lizard *Anolis carolinensis* shows more similarity to fish than mammals. Mol Biol Evol. 2009;26:1811–22.

Oliver MJ, Petrov D, Ackerly D, Falkowski P, Schofield OM. The mode and tempo of genome size evolution in eukaryotes. Genome Res. 2007;17:594–601.

Olmo E. Rate of chromosome changes and speciation in reptiles. Genetica. 2005;125:185–203.

Olmo E, Signorino GG. Chromorep: a reptile chromosomes database. http://chromoprep.univpm.it/ (2013).

Organ CL, Moreno RG, Edwards SV. Three tiers of genome evolution in reptiles. Integr Comp Biol. 2008;48:494–504.

Pace JK, Gilbert C, Clark MS, Feschotte C. Repeated horizontal transfer of a DNA transposon in mammals and other tetrapods. Proc Natl Acad Sci. 2008;105:17023–8.

Piskurek O, Okada N. Poxviruses as possible vectors for horizontal transfer of retroposons from reptiles to mammals. Proc Natl Acad Sci U S A. 2007;104:12046–51.

Piskurek O, Nishihara H, Okada N. The evolution of two partner LINE/SINE families and a full-length chromodomain-containing Ty3/Gypsy LTR element in the first reptilian genome of *Anolis carolinensis*. Gene. 2009;441:111–8.

Rodionov AV. Micro versus macro: a review of structure and functions of avian micro- and macrochromosomes. Russ J Genet. 1996;32:597–608.

Rodionov AV, Myakoshina YA, Chelysheva LA, Solovei IV, Gaginskaya ER. Chiasmata on lampbrush chromosomes of *Gallus gallus* domesticus: a cytogenetic study of recombination frequency and linkage group lengths. Russ J Genet. 1992;28:53–63.

Shedlock AM, Botka CW, Zhao SY, Shetty J, Zhang TT, Liu JS, Deschavanne PJ, Edward SV. Phylogenomics of nonavian reptiles and the structure of the ancestral amniote genorne. Proc Natl Acad Sci U S A. 2007;104:2767–72.

Siigur E, Aaspõllu A, Siigur J. Sequence diversity of *Vipera lebetina* snake venom gland serine proteinase homologs – result of alternative-splicing or genome alteration. Gene. 2001;263:199–203.

Smith J, Bruley CK, Paton IR, et al. Differences in gene density on chicken macrochromosomes and microchromosomes. Anim Genet. 2000;31:96–103.

Srikulnath K, Nishida C, Matsubara K, Uno Y, Thongpan A, Suputtitada S, Apisitwanich S, Matsuda Y. Karyotypic evolution in squamate reptiles: comparative gene mapping revealed highly conserved linkage homology between the butterfly lizard (*Leiolepis reevesii rubritaeniata*, Agamidae, Lacertilia) and the Japanese four-striped rat snake (*Elaphe quadrivirgata*, Colubridae, Serpentes). Chromosome Res. 2009;17:975–86.

Vonk FJ, Casewell NR, Henkel CV, et al. The king cobra genome reveals dynamic gene evolution and adaptation in the snake venom system. Proc Natl Acad Sci U S A. 2013;110:20651–6.

Walsh AM, Kortschak RD, Gardner MG, Bertozzi T, Adelson DL. Widespread horizontal transfer of retrotransposons. Proc Natl Acad Sci. 2013;110:1012–6.

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# Index Terms: