The transposable element profile of the anolis genome

How a lizard can provide insights into the evolution of vertebrate genome size and structure

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Abbreviations: TE, transposable element; LTR, long terminal repeat; LINE, long interspersed nuclear element; SINE, short interspersed element

The recent sequencing of the lizard genome provides a unique opportunity to examine the evolution of vertebrate genomes in a phylogenetic context. The lizard genome contains an extraordinary diversity of active transposable elements that far exceeds the diversity reported in extant mammals and birds. Retrotransposons and DNA transposons are represented by multiple active families, contributing to the very diverse repetitive landscape of the lizard. Surprisingly, ancient transposon copies are relatively rare suggesting that the transposon copy number is tightly controlled in lizard. This bias in favor of young copies results from the joint effect of purifying selection acting on novel insertions and a high rate of DNA loss. Recent analyses have revealed that the repetitive landscape of reptiles differ drastically from other extant amniotes by their diversity but also by the dynamics of amplification of their transposons. Thus, from the point of view of mobile elements, reptile genomes show more similarity to fish and amphibians than to other amniotes.

Introduction

The original justification of the Human Genome Project was its obvious application to the genetics of medicine—to shed light on the origin and function of genes as well as the underlying variation that affects human disease.^{1,2} More recently, there has been an explosion of genomic data available for many other species,³⁻¹³ providing a robust taxonomic sampling of evolutionary groups of vertebrates. Investigators can access the NCBI and Ensembl databases and find 39 and 37 placental mammal genomes, respectively, in addition to two metatherians.^{14,15} Browsers such as the UCSC Genome Browser¹⁶ (at <http://genome.ucsc.edu>) now contain the assemblies of two birds, a reptile, an amphibian, five

teleost fish and a cyclostome. The field of comparative genomics, concerned with the forces generating and maintaining biological variation, is thus today well equipped for addressing longstanding questions in evolution.

In 2007, the first draft of the green anole genome, *Anolis carolinensis*, was completed by the Broad Institute and made publicly available. *A. carolinensis* (Metazoa; Chordata; Vertebrata; Sauropsida; Lepidosauria; Squamata; Iguania; Dactyloidea¹⁷) is a small lizard found in the Southeastern United States. It was an obvious candidate for a genome-sequencing project that would target a reptile. *Anolis* was “the” lizard represented in comparative vertebrate studies during the 20th century, and has been a laboratory model contributing to the fields of neuroscience, behavioral psychology and developmental biology. With over 400 species described in the New World, *Anolis* is the most speciose amniote genus and is arguably the premiere vertebrate model for the study of adaptive radiation and morphological evolution (reviewed in ref. 18). A remarkable variety of *Anolis* “ecomorphs” living on Caribbean islands has attracted the attention of evolutionary biologists for decades, and their research has provided insights into the mechanisms driving species diversification. Now available as a valuable genomic model, *Anolis* has much to offer our understanding of vertebrate genome evolution.

Among the genomic features that show the most variation among vertebrate taxa is the abundance and diversity of transposable elements (TEs). In fact, with the exception of autopolyploidy, the abundance of TEs is the major determinant of genome size differences among animals.¹⁹ At one extreme, mammalian genomes contain extremely large numbers of TEs, which can account for as much as 50% of their size.^{1,4} These genomes harbor a low diversity of mobile elements as they are dominated by the *L1* retrotransposon and its non-autonomous counterparts (SINEs). At the other end of the spectrum, some teleostean fish genomes are considerably smaller than mammalian genomes yet contain a comparatively more diverse TE profile, comprised of families usually represented by small numbers (<100) of very similar copies.²⁰⁻²²

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Figure 1. Phylogenetic relationships among vertebrates.

The transition from the relatively small but diverse genomes of teleostean fish to the large, *L1*-dominated genomes of mammals is one of the most important, yet poorly understood, transitions in the evolution of vertebrates. Mammals and reptiles (or sauropsids, which include snakes, lizards, turtles, crocodilians and birds) represent the monophyletic group Amniota, which branched off from other amphibian-like tetrapods and radiated into terrestrial niches more than 300MYA (Fig. 1). The tempo and mode of this history is well documented with transitional forms in the fossil record.²³ The genomic evolution associated with this history is less understood since we must infer ancestral states using sequence data from terminal taxa, and from this point of view, *Anolis* helps fill an important phylogenetic gap.

A phylogenomic analysis of *L1* across deuterostomes completed before the release of the *Anolis* genome revealed that *L1* was most likely very diverse in the tetrapod and amniote ancestors, as it is found in extant amphibians. As amniotes diversified, there may have been complete stochastic loss of *L1* in certain lineages (for instance, all crocodilians and birds), with retention of *L1* diversity in some sauropsids such as lizards and snakes, along with a loss of diversity and explosion of copy number in the ancestor of mammals.²⁴ This analysis properly placed the evolution of a transposable element in the context of the evolution of its host across time. We believe it is important to review recent insights into the repetitive landscape of the *Anolis* genome, and discuss how they may impact our knowledge of vertebrate genome evolution.

The Transposable Element Profile of the Green Anole

The *Anolis* genome (and possibly squamate genomes in general, as we will discuss later) is characterized by an extraordinary diversity of mobile elements that far exceeds the transposon diversity found in other extant amniotes. Even more surprising

is the fact that the majority of these families have recently been active or is still active. Thus the anole genome is exposed to a tremendous level of TE activity, unparalleled among extant amniotes and possibly among vertebrates. A large number of active families of class I elements (i.e., elements that require an RNA intermediate for their mobilization or retrotransposons) were recovered from the anole genome.²⁵⁻²⁷ *Anolis* retrotransposons are extremely diverse and representative of the two main categories of these elements—those with Long-Terminal Repeats (LTRs) and those that lack LTRs—were found to be active. Within the non-LTR retrotransposons alone, 43 distinct families, belonging to five clades (*L1*, *L2*, *RTE*, *CR1* and *R4*), show sign of recent activity.²⁶ For instance, in *Anolis* the *L1* clade is represented by 20 active families, the origins of which predate the split between reptiles and mammals. Each *L1* family is represented by a small number (<100) of very similar copies, reminiscent of the diversity reported in the zebrafish genome^{21,28} but drastically more diverse than in modern mammals.²⁹ A recent study of the newly discovered *Ingi* clade of non-LTR retrotransposons found these elements in the *Anolis* genome, suggesting there may be more of these kinds of TEs to be discovered in this genome.³⁰ The biochemical machinery encoded by non-LTR retrotransposons can act on other transcripts and is responsible for the amplification of several SINE families. In particular, two SINEs have amplified to extremely large copy number:²⁷ Sauria SINE which is mobilized by an RTE retrotransposon (>200,000 copies) and *Anolis* SINE2 which is mobilized by a LINE-2 element (~140,000 copies). LTR-retrotransposons are also very diverse in the *Anolis* genome, as representatives of the four major groups of LTR elements (Metaviridae, Pseudoviridae, BEL/Pao and Retroviridae) have been identified^{25,27} and appear to be represented by multiple, highly conserved copies, suggestive of their recent activity.^{25,27} Interestingly, it was observed in a recent study of BEL/Pao that *Anolis* contains the highest copy number (397) of these elements across metazoans.³¹

The Anolis genome also contains a large diversity of active class II transposons (i.e., elements that do not use an RNA-intermediate for their mobilization). At least seven autonomous families, representing 3 recognized super-families of DNA transposons (*hAT*, *Mariner* and *Helitron*), show recent signs of activity in the Anolis genome,³² whereas three other types of transposons are either extinct (*Chaparev* super-family) or found in very small copy number (*PIF/Harbinger* and *Polinton/Maverick*). These families have generated a plethora of non-autonomous families that outnumber their autonomous progenitors by two orders of magnitude. The most diverse DNA transposons in Anolis are members of the *hAT* superfamily, which is represented by 5 autonomous and 32 non-autonomous families. This diversity results from the ability of autonomous and non-autonomous *hAT* elements to exchange genetic information. The exchange of sequence and domain, possibly by recombination, yielded a number of chimerical elements that have retained their mobility and yielded highly prolific families. For instance, multiple recombination events between non-autonomous *hobo* elements have generated at least 11 chimerical families that have amplified to several hundred copies.

Another interesting feature of DNA transposons is their ability to incorporate other transposable elements in their sequence.^{27,32} Some autonomous *hobo* elements carry up to five transposon fragments and mobilize these elements with their own sequence. This can significantly contribute to the increase in copy number of some elements. For instance, a Sauria SINE has amplified to very high copy number due to the amplification of an *Helitron* element it is embedded in.²⁷

Until recently, most sequenced amniote genomes were found to lack active DNA transposons and it was believed that this was due to the difficulty for horizontal transfer in amniote germ line. However, the Anolis genome contains a number of families that show an extremely high level of similarity with elements found in distant species (e.g., bats, opossums, flatworm) that can only be explained by horizontal transmission. At least five families have been laterally transferred into Anolis and these families are responsible for the amplification of ~15,000 copies.^{33,34} Interestingly, the five families for which horizontal transfer was demonstrated have invaded the Anolis genome at different points in time, suggesting that horizontal transfer of DNA transposons is probably occurring much more frequently than previously thought.³⁴ Although the exact mechanism used by transposons to invade the germ line remains unclear, it was proposed that the lateral transfer of transposons was facilitated by host-parasite interactions.³⁵

The Impact of Transposons on the Lizard Genome

The extreme TE diversity in Anolis does not translate into a much larger genome size than in other amniotes, although these genomes host a reduced number of active families. In fact, TE families in Anolis tend to be relatively young and ancient (i.e., divergent) families are very rare. Thus it seems that TEs are in some ways purged from the lizard genome. A first explanation comes from the analysis of the *L1* clade. *L1*

copies are extremely similar to each other and divergent (i.e., ancient) *L1* copies are virtually absent from the anole genome.²⁶ This profile is similar to the one reported in fish and insects and is consistent with a model in which the insertion of new elements is counteracted by the removal of deleterious copies by purifying selection. This suggests that *L1* activity could have a high fitness cost in Anolis, possibly because of a higher rate of ectopic recombination.²⁶

However, the turn-over model provides only a partial explanation for the relatively young age of mobile elements because other TE families, in particular DNA transposons and shorter retrotransposons, do reach fixation and accumulate to large numbers. Another explanation comes from the examination of these somewhat older families, such as the *RTE-BovB* family that used to be prolific in Anolis but recently became extinct. When *RTE* copies are compared to each other, they exhibit a much larger number of deletions than elements of similar age in mammals, suggesting that the rate of DNA loss is much higher in Anolis,²⁶ and possibly in other reptiles,³⁶ than in mammals. Together with a low rate of fixation of insertions, a rapid decay of fixed elements in anole could explain the relative paucity of ancient elements in this genome.

Although the vast majority of TE insertions in Anolis are likely to be either deleterious or neutral, it is plausible that some of the abundant genetic variation introduced by transposons can be advantageous and co-opted by the host. For instance, DNA transposons of the *PIF/Harbinger* super family show level of conservation consistent with their exaptation by the host.³² Another observation suggesting the recruitment of TEs by the host for its own benefit comes from the observation that, in lizards, transposons tend to accumulate near developmental genes in general, and within *Hox* clusters in particular.³⁷ Typically, vertebrate *Hox* clusters are extremely compact and almost transposon free. In contrast, *Hox* clusters in anoles contain a large number of transposons. As transposons are restricted to the *Hox* clusters and are not as abundant in their flanking regions, it is likely that a large number of insertions were retained in *Hox* clusters because they provided an adaptive advantage. This raises the intriguing possibility that transposons have played a significant role in the diversification of a morphologically diverse genus such as Anolis, and possibly could explain the large diversity of form and shape in reptiles.³⁸

What have We Learned from the Anole Genome?

With a phylogenetic perspective, we can consider the pattern of genome evolution in vertebrates, including amniotes. Teleostean fish contain an astonishing diversity of both class I and II elements. The lone amphibian that has been fully sequenced is *Xenopus*, and it contains a similar diversity of both TE classes with high copy numbers that contribute to nearly a third of the genome. The repetitive landscape of the Anolis genome suggests that non-avian reptile genomes are more similar to fish and amphibians than to mammals and birds. Therefore, the most parsimonious model of genome evolution in vertebrates is one in which the ancestral amniote harbored a large diversity of class

I and II elements, followed by substantial losses of transposon diversity in birds and eutherian mammals.

The completion of the *Anolis* genome emphasizes the diversity of the repetitive landscape among amniote genomes and indicates that amniote lineages have evolved drastically different strategies to cope with their intra-genomic parasites. Mammals have experienced a considerable reduction in transposon diversity yet they have much larger genomes than many other vertebrates due to the amplification of L1 retrotransposons. Population genetics and genomics studies have shown that the majority of L1 elements behave as neutral alleles and accumulate readily in the genome of their mammalian host. This does not mean that L1 activity is fully neutral. In humans, a fitness cost related to the length of L1 elements has been demonstrated,³⁹⁻⁴¹ yet it is insufficient to prevent the fixation of most elements, hence the extremely large number of copies in mammals. In contrast, the copy number of TE in *Anolis* appears to be much more regulated. Some families of elements fail to reach fixation and are probably subject to a high rate of turn-over due to a negative impact on their host genome. Like in mammals, longer elements seem to have a stronger deleterious effect than shorter ones suggesting that their negative effect could result from their ability to mediate deleterious chromosomal rearrangements through ectopic recombination.^{26,32} However, the high rate of turnover of these elements, compared to their fixation in mammals, suggest that retrotransposons impose a much heavier genetic load on *Anolis* than on humans. This raises the intriguing possibility that the rate of ectopic recombination is much lower in mammals than in *Anolis*. Such a difference in the rate of ectopic recombination was previously proposed to account for the different dynamics of amplification of TEs in mammals, insects and fish.^{28,42}

A large number of transposons do reach fixation in *Anolis*, yet ancient TE families are disproportionately rare in this genome. This again is in sharp contrast with mammalian genomes that have accumulated extremely large number of TE copies since the origin of mammals so that these genomes are littered with ancient, long-extinct families. This difference could be due to differences in the regulation of DNA content among amniotes.

We have shown that transposons decay faster in *Anolis* than in humans suggesting that the rate of DNA loss could be far greater in reptiles than in mammals. If confirmed, this could be yet another drastic difference in the way some vertebrate genomes evolve.

The *Anolis* genome represents a single reptilian species, and one can predict that other reptilian genomes will bring further insights into the genomic evolution of vertebrates since the Mesozoic Era, as the divergence times of extant reptile lineages are on average much deeper than those of mammals.²³ *Anolis* is a member of the suborder Iguania and forms a monophyletic group with snakes (suborder Serpentes) called Toxicofera that arose almost 200 million years ago.^{43,44} A recent comparison of the repetitive landscapes of two snake genomes, python (*Python molurus bivittatus*) and copperhead (*Agkistrodon contortrix*), has revealed interesting patterns in squamate genome evolution.⁴⁵ While both snakes contain a similar diversity of elements reminiscent of the situation in *Anolis*, it appears that TE copy number in the python is much more restricted than in the copperhead (comprising 21% of the genome versus 45%, respectively) even though both genomes are of similar size. The high copy number in the copperhead is probably mostly due to recent TE expansion, as evidenced by the low pairwise divergence between elements as well as the discovery of a large proportion of TE-related transcripts.

Combined with our overview of *Anolis*, the observations in these snakes are consistent with our phylogenomic hypothesis of a TE-diverse ancestral sauropsid genome. The differences in activity and copy number between toxicoferan squamates indicate that the dynamics of amplification of TEs may differ greatly within this group. Such differences could be due to variations in metabolic demands, demographic history or regulation of transposition by the host. Understanding the role of these different factors in determining the proliferation or loss of TE diversity and abundance within reptilian lineages will require further analyses, both at the molecular level and at the population level. Lessons learned through comparative analysis of more reptiles will prove to be essential elements in the still unfolding story of the vertebrate genome.

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