Trends in the evolution of reptilian chromosomes

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Synopsis
Reptiles are a karyologically heterogeneous group, where some orders and suborders exhibit characteristics similar to those of anamniotes and others share similarities with homeotherms. The class also shows different evolutionary trends, for instance in genome and chromosome size and composition. The turtle DNA base composition is similar to that of mammals, whereas that of lizards and snakes is more similar to that of anamniotes. The major karyological differences between turtles and squamates are the size and composition of the genome and the rate at which chromosomes change. Turtles have larger and more variable genome sizes, and a greater amount of middle repetitive DNA that differs even among related species. In lizards and snakes size of the genome are smaller, single-copy DNA is constant within each suborder, and differences in repetitive DNA involve fractions that become increasingly heterogeneous with widening phylogenetic distance. With regard to variation in karyotype morphology, turtles and crocodiles show low variability in chromosome number, morphology, and G-banding pattern. Greater variability is found among squamates, which have a similar degree of karyotypic change—as do some mammals, such as carnivores and bats—and in which there are also differences among congeneric species. An interesting relationship has been highlighted in the entire class Reptilia between rates of change in chromosomes, number of living species, and rate of extinction. However, different situations obtain in turtles and crocodiles on the one hand, and squamates on the other. In the former, the rate of change in chromosomes is lower and the various evolutionary steps do not seem to have entailed marked chromosomal variation, whereas squamates have a higher rate of change in chromosomes clearly related to the number of living species, and chromosomal variation seems to have played an important role in the evolution of several taxa. The different evolutionary trends in chromosomes observed between turtles and crocodiles on the one hand and squamates on the other might depend on their different patterns of G-banding.

Introduction
The classic phylogeny of living reptiles based on morphology of temporal fenestrae identifies three distinct lineages, crocodiles, lepidosaurs, and turtles, for which turtles would be basal to the others. Also based on recent studies of nuclear and mitochondrial genes the classification of reptiles has undergone a number of important changes. According to these changes turtles would be a sister group of crocodiles and birds, while squamates are placed at the base of the tree (Zardoya and Meyer 1998; Hedges and Poling 1999; Mannen and Lis 1999; Cao et al. 2000; Hugall et al. 2007).

Genomic and karyological analysis also has the potential to provide interesting contributions to reptilian phylogeny.

Reptiles are a karyologically heterogeneous group; some orders and suborders exhibit characteristics similar to those of anamniotes and others share similarities with homeotherms. Different trends in the evolution of the size and composition of genome and chromosomes are also noted in this class.

Base composition
Bernardi and coworkers have shown that the Eukaryote genome is made up of discrete units with homogeneous base composition, or isochores (Bernardi 2004).

Endothermic vertebrates exhibit a higher guanine–cytosine (GC) concentration and an asymmetric isochore distribution (i.e., DNA fractions with a higher GC percentage than the modal value are more frequent than those with a lower GC percentage compared with the modal value) due to accumulation of isochores with a higher concentration of GC. Ectothermic vertebrates have a lower GC concentration than do endothermic ones, as well as a symmetric distribution of isochores around the modal value, and higher cytosine methylation levels (Bernardi 2004).
Also with regard to GC concentration and the levels of cytosine methylation, reptiles are heterogeneous, with some species showing similarities to endotherms, others to ectotherms and others still exhibiting intermediate characteristics (Varriale and Bernardi 2006).

Turtles have the highest GC percentage of all reptiles (Vinogradov 1998; Olmo et al. 2002) and an asymmetric base composition distribution that is comparable to that of mammals (Hughes et al. 2002; Bernardi 2004), whereas in lizards, the GC percentage is lower (Vinogradov 1998; Olmo et al. 2002) and the symmetric distribution of isochores is similar to that found in amphibians (Hughes et al. 2002; Bernardi 2004). In snakes the asymmetry actually ranges from the values observed in amphibians to those of humans and birds (Hughes et al. 2002; Bernardi 2004).

The level of cytosine methylation in turtles and crocodiles is also close to that of mammals; in turtles it rises with the increase in GC percentage, whereas in snakes and lizards it covers the whole range from fishes to mammals, and is not affected by the increase in GC percentage (Varriale and Bernardi 2006).

In vertebrates, the GC percentage is directly related to body temperature, suggesting that it may be linked to adaptation to environmental temperatures (Jabbari et al. 2003; Bernardi 2004, 2007).

In reptiles this correlation could be important. In this regard, it is interesting to note that some desert lizards, which have a higher GC percentage, tolerate higher critical and lethal temperatures than those tolerated by some desert snakes, which have a lower GC percentage. In this connection the hypothesis has been advanced that a higher GC level could help DNA withstand the destabilizing effect of temperature and genetic damage, which could derive from an increased metabolic rate correlated with increased production of free radicals (Olmo 2003).

Differences between different reptilian lineages

A characteristic feature of reptilian chromosomal evolution is the different evolutionary trend of karyological parameters such as size and composition of the genome and the rate of change in chromosomes in the various orders and suborders.

Turtles and scaly reptiles differ in genomic size and composition. The former exhibit a higher and more variable DNA content, with differences among congeneric species (like Testudo graeca, Testudo carbonaria, and Testudo hermanni) (Olmo et al. 1985; Olmo 1986). Information on the crocodile genome size is very limited; however this order seems to have similar values to those observed in turtles (Olmo 1986). Genomic size is smaller and more consistent in lizards, and smallest in snakes (Olmo 1986). Such differences depend mainly on a different tendency of turtles and crocodiles to preserve and accumulate repetitive DNAs (Olmo et al. 1985). In lizards and snakes, the percentage of single-copy DNA is constant within each suborder, and differences in size of the genome are chiefly related to a diverse repetitive DNA content. The increase in genome size depends almost wholly on the increase in repetitive DNA. Analysis of the variation of the different DNA fractions based on phylogenetic relationships evidences that in squamates differences among congeneric species depend almost wholly on highly repetitive DNA, those between genera of the same suborder also involve middle repetitive DNA, while only the differences in genome size between lizards and snakes also depend on different amounts of single-copy DNA (Olmo et al. 1985).

In turtles the picture is more complex, and differences are apparent both in single-copy and repetitive DNA amount, even in closely related species. The genomic size increment depends on a similar increase in all single-copy and repetitive fractions, and differences in homogeneous and heterogeneous fractions are also seen in species of the same genus (Olmo et al. 1985). In the genomic composition of turtles- crocodiles and lepidosaurs such differences could involve specific repetitive sequences. Indeed, certain simple sequence repeats (SSR) have been seen to be much more frequent in the lizard Anolis than in alligators and turtles (Shedlock et al. 2007).

It has been hypothesized that deletion events limiting the accumulation of functionally dispensable DNA fractions occurred more frequently in squamates (Olmo et al. 1985). A similar greater evolutionary dynamics has also been observed in coding sequences. Indeed Hughes and Mouchiroud (2001) found that several squamate coding sequences evolved at a faster rate than those of turtles, crocodiles, and birds.

With regard to variations in karyotypic morphology, turtles show low variability in the number, morphology, and G-banding pattern of chromosomes. Indeed, most turtles have bimodal karyotypes, i.e., constituted of macrochromosomes and microchromosomes (Bickham 1984; Olmo 1986); the latter might represent an ancestral karyological character in various vertebrates (Olmo 1986; Morescalchi 1991; Burt, 2002). All macrochromosomes of species belonging to the different families of cryptodiran turtles exhibit nearly identical G-banding patterns.
Few changes in chromosomes have occurred during the evolution of these reptiles (Bickham 1981, 1984), and the morphology and G-banding pattern of chromosome one have remained unchanged for over 200 million years (Bickham 1981).

A similar situation is seen in crocodiles, in which the karyotypes of all extant species descend from a single basic model. These reptiles do not have microchromosomes, but the G-banding of macrochromosomes is also very conservative in this order, as shown by a comparison of the chromosomes of two species of the genus Crocodylus and of the Caiman, Caiman crocodilus (King et al. 1986). The karyotypes of these two species—albeit differing in diploid number and in the number of biarmed and uniarmed chromosomes, due to Robertsonian rearrangements—can be ascribed to a single model, yielding an identical pattern of G-banding (King et al. 1986).

Greater variability prevails among squamates, which share similar levels of karyotypic changes with some mammals, such as carnivores and bats (Coyne 1984). Different karyotype models are observed in sphenodontids, lizards, and snakes (Olmo 1986). Interspecific and intraspecific variations of autosomes or sex chromosomes related to different types of Robertsonian and non-Robertsonian mutations are relatively frequent (Bickham 1984; Olmo 1986; Olmo et al. 2002). Moreover, differences in G-banding are also noted in species of the same genus and a tendency to reduction in the number of microchromosomes has been noted in several families (Olmo et al. 2002).

An interesting relationship can be observed between the rate of change in chromosomes, number of living species, and extinction rate. In the entire reptile class, the rate of chromosomal change is directly related to the number of living species and inversely related to the extinction rate (Olmo 2005). However, also in this case different situations obtain in turtles and crocodiles on the one hand and in lizards and snakes on the other (Olmo 2005).

In turtles, the number of chromosomal changes per million years is very small and is unrelated to the number of living species. Moreover, the various evolutionary steps do not appear to have involved marked chromosomal variation. The same situation is observed in crocodiles (Table 1).

In lizards, a high rate of change in chromosomes shows a direct and significant correlation with living species both using the average values of each infraorder and the average value of each family. Moreover, the evolution of most infraorders, of several families and also of some genera has been accompanied by considerable chromosomal variation (Table 1).

A similar situation obtains in snakes, notwithstanding a lower rate of change in chromosomes and the fact that marked chromosomal variability is found only in Caenophidia, which are the more evolved snake infraorder (Table 1).

These data suggest that chromosomal variability may play a role in speciation, particularly in lizards.

Table 1 Mean values of the rate of change in chromosomes per million years, in the different taxonomic levels in reptiles (from Olmo 2005)

<table>
<thead>
<tr>
<th>Taxon chromosome changing rate</th>
<th>Infraorder or Family</th>
<th>Order</th>
<th>Suborder</th>
<th>Family</th>
<th>Genus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testudines Cryptodira</td>
<td></td>
<td>2.34</td>
<td>0.489</td>
<td>0.006</td>
<td>0.006</td>
</tr>
<tr>
<td>Testudinoidea</td>
<td></td>
<td>2.34</td>
<td>0.489</td>
<td>0.116</td>
<td>0.056</td>
</tr>
<tr>
<td>Trionychoida</td>
<td></td>
<td>2.34</td>
<td>0.489</td>
<td>0.392</td>
<td>0.098</td>
</tr>
<tr>
<td>Pleurodira</td>
<td></td>
<td>2.34</td>
<td>1.85</td>
<td>0.924</td>
<td>0.197</td>
</tr>
<tr>
<td>Crocodylia Alligatorida</td>
<td></td>
<td>0.387</td>
<td>0.32</td>
<td>0.083</td>
<td></td>
</tr>
<tr>
<td>Crocodylida</td>
<td></td>
<td>0.387</td>
<td>0.098</td>
<td>0.038</td>
<td></td>
</tr>
<tr>
<td>Gavialida</td>
<td></td>
<td>0.387</td>
<td>0.013</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>Squamata Lacertilia Amphibiaenia</td>
<td></td>
<td>25.595</td>
<td>22.379</td>
<td>0.217</td>
<td>0.111</td>
</tr>
<tr>
<td>Anguimorpha</td>
<td></td>
<td>25.595</td>
<td>22.379</td>
<td>0.538</td>
<td>0.136</td>
</tr>
<tr>
<td>Gekkota</td>
<td></td>
<td>25.595</td>
<td>22.379</td>
<td>2.101</td>
<td>2.0695</td>
</tr>
<tr>
<td>Iguania</td>
<td></td>
<td>25.595</td>
<td>22.379</td>
<td>5.321</td>
<td>1.887</td>
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<tr>
<td>Serpentes Caenophidia</td>
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<td>25.595</td>
<td>3.18</td>
<td>3.132</td>
<td>0.8145</td>
</tr>
<tr>
<td>Henophidia</td>
<td></td>
<td>25.595</td>
<td>3.18</td>
<td>0.065</td>
<td>0.026</td>
</tr>
<tr>
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<td></td>
<td>25.595</td>
<td>3.18</td>
<td>0.097</td>
<td>0.0485</td>
</tr>
</tbody>
</table>
A correlation between rates of chromosome evolution and speciation has been observed in several eukaryotes, including some reptile groups (Bernardi 1993; King 1993; Britton-Davidian 2001), and some authors believe chromosomal rearrangements to play a large role in speciation (Rieseberg 2001; Navarro and Barton 2003; Marques-Bonnet and Navarro 2005). Moreover, it has been noted in some mammals that genes found in highly rearranged regions show greater divergence among species, and rates of chromosomal rearrangements and of genic divergence are related (Marques-Bonet and Navarro 2005), although this pattern seems to be limited to a small number of species (Marques-Bonet et al. 2007).

Cases of lizard populations or species characterized by changes in number and morphology of chromosomes are common, as demonstrated by the rapid chromosome diversification of chromosomes of the species and populations of the gekkonid Gehyra (King 1984); by the species and subspecies of Pyrenean lizards, distinguished both by number of chromosomes and by the presence and morphology of sex chromosomes (Odierna et al. 1996); and by the extraordinary chromosomal diversification found in dozens of populations and species of the genus Sceloporus (Hall and Selander 1973; Sites 1983). The case of the so-called Lacerta-kulzeri complex is particularly significant. Several reproductively isolated populations have been described in this complex of lizards; they are characterized by a narrower genetic distance compared with the subspecific level, but show significantly different morphology of the sex chromosomes and different heterochromatin content in autosomes and sex chromosomes (in den Bosch et al. 2003).

### Causes of different levels of chromosomal variability in the various reptilian lineages

As regards the possible causes of the different levels of chromosomal variability seen in different reptilian lineages, some correlations between level of chromosome changes and some compositional and structural features of the genome need to be considered. Among these, the inverse relation between the frequency of meiotic chiasmata and percentage of repetitive DNA and between the rate of change of chromosomes and the size of the genome in reptiles (Table 2 and Fig. 1) seen in various vertebrates (Olmo et al. 1989) are of some interest. However, although such correlations are fairly clear, it is not easy to establish which are the causes and which the consequences.

Other karyological features that could influence the rate of change in chromosomes are size of the chromosomes and the pattern of G-banding. In yeast, humans and birds it has been noted that meiotic recombination is enhanced by decreasing chromosomal size (Kaback et al. 1992; Kaback 1996; Hughes and Piontkivka 2005); in this regard, lizards and snakes have on average smaller chromosomes.
than do turtles and crocodiles. It has also been observed that the recombination events underlying chromosomal rearrangements are distributed neither randomly nor uniformly on chromosomes (Bernardi 2004; Marques-Bonet and Navarro 2005), but are prevalently located on R bands—which are richer in GC and display a greater density of genes—and in transition areas between G and R bands (Bernardi 1993, 1995, 2004). In addition, in the yeast Saccharomyces cerevisiae recombination hotspots are often associated with GC-rich DNA regions (Gerton et al. 2000).

These observations suggest that the level of recombination is influenced by the presence of DNA sequences with a specific composition and by the frequency of GC-rich (corresponding to R bands) or GC-poor (corresponding to G bands) regions on each chromosome.

In this respect, different situations and evolutionary trends are seen in turtles (and partly also in crocodiles) and in squamates.

The genome of turtles is organized into large, discrete, and separate regions with relatively homogeneous composition and different gene density. As mentioned above, the karyotypes of most turtles are constituted of macrochromosomes and microchromosomes (Bickham 1984; Olmo 1986); the latter are often euchromatic with few G bands (Sites et al. 1979; Bickham et al. 1980, 1983); in the turtle Pelodiscus sinensis, it has recently been demonstrated that macrochromosomes have a lower GC content and gene density, whereas most GC-rich genomic fractions, and 50% of genes, are found in microchromosomes, although they contain only 23% of total DNA (Kuraku et al. 2006). Moreover in turtles, the macrochromosomes are characterized by broad G-positive bands and a small number of R bands (Bickham 1984; Olmo 1986), hence by a limited number of transitional areas.

The picture is less definite among crocodiles, which do not have microchromosomes, but their pattern of G-banding is nearly identical to that of turtles (King et al. 1986; Olmo et al. 2002).

Among squamates, even though several lizard and snake species have euchromatic microchromosomes they exhibit a greater tendency toward reduction via translocation to macrochromosomes compared with turtles (Mengden 1982, 1986; Olmo 1986; Odierna et al. 1987). Two areas with presumably different compositions, i.e., those of microchromosomes and of macrochromosomes—which in turtles are separate—in squamates tend to mix, a fact that may have changed the G-banding pattern of macrochromosomes (Stock et al. 1974). Indeed the G-banding pattern of squamate chromosomes is different from that of turtles-crocodiles and more similar to that of homeothermal vertebrates, with highly interspersed G and R bands, hence a larger number of transitional areas. This has led to formation of more compositionally heterogeneous chromosomes that are thus more prone to rearrangement.

Despite the limited information on the composition and structure of reptilian chromosomes, these observations suggest that genome and chromosome size, presence of distinct micro- and macrochromosomes, and chromosome banding patterns are interrelated, and that the different rates of change in chromosomes observed in the different reptile lineages depend on complex interactions between these features, although it is difficult to establish an exact sequence of causes and effects.

As noted above, chromosome size and G-banding pattern seem to have a direct influence on the level of recombination and, consequently, on the rate of chromosome mutation (Olmo 2005). Logically, therefore, a karyotype like that of turtles, rich in microchromosomes, having larger macrochromosomes, and large G bands should be more conservative than that of squamates; it is harder to understand the reason for the change in banding pattern noted in the different reptile lineages and the cause-effect relation, if any, between genome size and composition and banding pattern.

One of the most complex questions to unravel is whether one or more mechanisms underlie the variations in genome size and banding pattern. The data collected so far confirm that genome size variations depend on different amounts of repeated sequences, particularly of transposons and retrotransposons (Vinogradov 2004; Gregory 2005; Kidwell 2005), which are preferentially located in the intervening nongenic regions. It has also been observed that larger genomes have longer introns and a greater distance between genes (Vinogradov 2004; Gregory 2005). However, there is still no single hypothesis on the mechanisms responsible for the increases and reductions in these sequences. The situation found in reptiles could be explained by the model proposed by Hartl and Petrov (Hartl 2000; Petrov 2002), whereby different organisms would have different spectra of spontaneous insertions and deletions of non-coding DNA (indels), whose fixation would be ruled primarily by random genetic drift (Hartl 2000). Larger genomes, longer introns, and wider intergenic distances would be found in the species where insertions prevail (Vinogradov 1999; Petrov 2002; Gregory 2005). According to this model, the larger genomes and the greater
amount of heterogeneous repetitive DNA found in chelonians would result from a lower rate of DNA loss and a greater frequency of nongenic DNA insertions; squamates, which have smaller genomes and differ especially in the amount of highly repetitive DNA, would have a higher rate of deletions. The model of Hartl and Petrov could also account for the increased size of G bands. These bands are known to contain generally gene-poorer isochores (Mouchiroud et al. 1991), where longer introns and larger interspersed DNA repeats are found (Vinogradov 1999); this situation provides a larger number of non-deletious insertion sites for insertions of non-coding sequences. According to the model, insertions would prevail on deletions in turtles, leading to increased genome size, chromosome size, and larger G bands. As mentioned above, a banding pattern characterized by large G bands would have enabled a low level of recombination and a low rate of change in chromosomes, which in turn would have favored accumulation of non-coding DNA in G bands and consequently a further increase in the size of genomes and chromosomes, hence a further reduction in the level of recombination and in the rate of chromosome change.

Deletions would prevail on insertions in squamates, limiting the genome and G band increment. Moreover, progressive integration of microchromosomes into macrochromosomes would have favored an increase in R band number. Such karyotypes would have led to an increase in recombination levels and a high rate of chromosome change, which in turn would have brought about a reduction in the size of genomes and chromosomes. Such reduction would have caused a further increase in the recombination level and a consequent increase in the rate of chromosome change.

However, a different balance of insertions and deletions would not be the sole mechanism controlling genome size, given that the relations between genome size and morphological and functional parameters, e.g., cell size and metabolic rate, also play a significant role in adaptive processes. In reptiles, as in other vertebrates, an inverse relation has been noted between genome size, cell size and metabolic rate (Olmo 2003), and the hypothesis has been advanced that a certain genome size is required to achieve a given metabolic level (Gregory 2005). Squamates have smaller genomes and cells compared with turtles and crocodiles that would have allowed them to reach higher metabolic levels, resulting in greater adaptability to more variable environments. The smaller genome size of squamates could thus also depend on the selective pressure exerted on metabolic rate and cell size.

Conclusions

Although data on the genome composition and karyotype morphology of reptiles are fairly abundant, they still fail to provide sufficient knowledge of the molecular organization of the genome of these vertebrates, or to support definitive hypotheses on the different evolutionary trends that can be detected in the lineages of this class. However, they do allow some preliminary conclusions to be drawn and working hypotheses to be made.

The composition and structure of the genome and chromosomes have evolved differently in turtles (and possibly also in crocodiles) on the one hand and in squamates on the other. The former are characterized by larger genomes richer in non-coding DNA, by karyotypes where microchromosomes are conserved and by macrochromosomes where G-banding shows large G bands and narrower R bands. In squamates, the genomes are smaller and less rich in repetitive sequences; G bands are smaller and there is a tendency to microchromosome reduction due to translocation onto macrochromosomes.

Genome compositional differences and different chromosome morphologies would have resulted in different rate of change in chromosomes, which would have played a significant role in the evolution of the different reptile taxa.

The variations in genome size, amount of non-coding repetitive DNAs and chromosome composition, and organization (G-banding and microchromosome conservation) would be interrelated and would depend on a single mechanism.

The available information on genome composition; on the presence of specific sequences such as transposons and retrotransposons and their possible role in the level of recombination and chromosome mutations; on any differences in gene distribution on micro- and macro-chromosomes; on potential compositional differences in the microchromosomes of turtles and of squamates, which could account for the greater conservativeness of these chromosomes in turtles; and on the composition of G and R bands is still scarce for reptiles.

Although several data still need to be collected, reptiles clearly are an interesting model for the study of genome evolution, both in terms of the different trends that can be seen in turtles-crocodiles and in squamates, and because of the basal position of reptiles among terrestrial vertebrates. This suggests that a greater understanding of the genome of the
class can shed light on the cytological and molecular evolution of amniotes.

References


