

New resources inform study of genome size, content, and organization in nonavian reptiles

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Synopsis Genomic resources for studies of nonavian reptiles have recently improved and will reach a new level of access once the genomes of the painted turtle (*Chrysemys picta*) and the green anole (*Anolis carolinensis*) have been published. Eleven speakers gathered for a symposium on reptilian genomics and evolutionary genetics at the 2008 meeting of the Society for Integrative and Comparative Biology in San Antonio, Texas. Presentations described results of reptilian genetic studies concerning molecular evolution, chromosomal evolution, genomic architecture, population dynamics, endocrinology and endocrine disruption, and the evolution of developmental mechanisms. The presented studies took advantage of the recent generation of genetic and genomic tools and resources. Novel findings demonstrated the positive impact made by the improved availability of resources like genome annotations and bacterial artificial chromosomes (BACs). The symposium was timely and important because it provided a vehicle for the dissemination of novel findings that advance the field. Moreover, this meeting fostered the synergistic interaction of the participants as a group, which is anticipated to encourage the funding and creation of further resources such as additional BAC libraries and genomic projects. Novel data have already been collected and studies like those presented in this symposium promise to shape and improve our understanding of overall amniote evolution. Additional reptilian taxa such as the American alligator (*Alligator mississippiensis*), tuatara (*Sphenodon punctatus*), and garter snake (*Thamnophis sirtalis*) should be the foci of future genomic projects. We hope that the following articles in this volume will help promote these efforts by describing the conclusions and the potential that the improvement of genomic resources for nonavian reptiles can continue having in this important area of integrative and comparative biology.

Introduction

The symposium for reptilian genomics and evolutionary genetics at the 2008 annual meeting of the Society for Integrative and Comparative Biology hosted the presentation of studies of evolutionary genetics and genomic evolution whose comprehensive scope was enabled through the increased availability of reptilian genomic resources. The development of bacterial artificial chromosome (BAC), cDNA and expressed sequence tag libraries, microarray technology, real-time quantitative reverse transcriptase-polymerase chain reaction, and gene silencing (e.g., RNAi) among other techniques and resources, are fostering studies of comparative functional and evolutionary genomics and genetics in reptiles (McGaugh et al. 2007). The symposium included presentations of research on gene mapping and expression, and studies of gene, genomic, and chromosomal evolution that characterize the

evolution and diversity of sex-determining mechanisms at multiple levels of organization. For instance, the advent of modern genomic tools is enabling discoveries of the functional mechanics and evolution of sex-determining mechanisms in reptiles, paralleling advances obtained from studies of non-reptilian model organisms. Indeed, genomic and genetic studies in reptiles have shed further light on the evolution of sex chromosomes (Schartl 2004; Ezaz et al. 2005, 2006), the evolution and regulation of the gene networks underlying sex determination and differentiation (Western et al. 2000; Gabriel et al. 2001; Maldonado et al. 2002; Schmahl et al. 2003; Takada et al. 2004; Yao et al. 2004; Wang et al. 2006; Valenzuela et al. 2006; Ramsey and Crews 2007; Rhen et al. 2007; Shoemaker et al. 2007; Valenzuela and Shikano 2007; Valenzuela 2008), and the relative importance of genetic and environmental factors in shaping the development and evolution of secondary sexual traits and sexual dimorphism

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(Crews et al. 2006; John-Alder et al. 2007). As an introduction to the symposium, we describe 3 frontiers of reptilian genomics that define the focus of the symposium and the following articles in this volume. These frontiers concern (i) genome size and content, (ii) genome compartmentalization (organization into microchromosomes versus macrochromosomes, and autosomes versus sex chromosomes), and (iii) gene function and evolutionary potential under climatic change (e.g., sex determination).

Frontiers of reptilian genomics

Genomic architecture and sequencing

As body size is to organismal biology and ecology, genome size is to genomics. Consequently, genome size, whether in weight (picograms), base pairs, or any other measure, is a basic parameter for describing and understanding the genome biology of a species. Because the number of vertebrate genes is relatively consistent across clades, genome size reflects the increase or decrease of the density of repetitive elements, shifts in intron size, and changes in other noncoding DNA (Kazazian 2004). The global average genome size for reptiles is about 1.98 gb haploid with a SD of 0.73 (Table 1), reflecting the diversity of genome size, and hence underlying architecture, in reptiles. Interestingly, with the exception of the tuatara, the 2 groups with the most extreme means (crocodilians with an average genome size of ~3 gb and birds with an average genome size of ~1.4 gb) are sister taxa. Birds provide a baseline for comparison of nonavian reptilian genomes because they are known to have smaller introns than do mammals (Waltari and Edwards 2002), as well as a characteristic repetitive landscape (Shedlock et al. 2007). The significance of disparities in genome size, intron size, and repetitive element content are, as yet, unknown, although they pose interesting questions about the tempo and mode of genome contraction and expansion, and of sequence evolution.

Aside from repetitive DNA, virtually nothing is known about reptilian genomes at the sequence level. Future publications from the *Anolis* genome data will certainly remedy this situation, but a detailed picture will only arise with the sequencing of whole genomes of more reptiles, thereby capturing the diversity of reptilian genomes. Characteristics such as gene number, gene length, exon and intron size, as well as single nucleotide polymorphisms (SNPs) will provide essential details for elucidating the forces

that have shaped amniote genomes from deep time to recent population divergences.

Genome compartmentalization (sex chromosome and microchromosome organization)

Greater diversity of sex chromosomes is exhibited by reptiles than either by birds or mammals (Solari 1994). The ZZ/ZW system of sex chromosomes that is typical of birds and the XX/XY system typical of mammals are both found in some chelonians and lepidosaurs, while all crocodilians and rhynchocephalians and some chelonians and lepidosaurs are environmentally sex determined (Olmo 1986). Avian and mammalian sex chromosomes appear to have arisen from different pairs of ancestral autosomes as little or no homology has been detected between avian ZZ/ZW systems and mammalian XX/XY systems (Matsubara et al. 2006; Smith and Voss 2007). Both of these systems likely arose from autosomes that attained a sex-determining mutation and later accumulated sexually antagonistic mutations that caused a block to recombination (Charlesworth et al. 2005; van Doorn and Kirkpatrick 2007). Studies on avian chromosomes have described, among other phenomena, conservation of chromosomal content among chickens, pigeons, and passerines (Derjusheva et al. 2004) and between chickens and mallard ducks (Fillon et al. 2007); lower diversity in sex chromosomes than in autosomes in chickens (Sundstrom et al. 2004); greater recombination and divergence in microchromosomes than in macrochromosomes of chickens and turkeys (Rodionov et al. 1992; Burt 2002; Axelsson et al. 2005); and chromosomal rearrangements between chickens and vultures (Nanda et al. 2006). By exploring syntenic and recombination patterns within and between sex chromosomes and autosomes in reptiles, researchers should be able to identify one or more pairs of ancestral reptilian autosomes that gave rise to the avian and mammalian sex chromosomes. Further, synteny studies will describe possible shared ancestry between reptilian sex chromosomes (ZZ/ZW or XX/XY) and their avian or mammalian counterparts. To date, studies of reptilian synteny suggest a lack of homology between reptilian sex chromosomes and either avian or mammalian sex chromosomes (Matsuda et al. 2005; Matsubara et al. 2006; Kawai et al. 2007). This further reinforces the idea of multiple conversions of autosomes to sex chromosomes upon attaining a sex-determining gene. Alternatively, the recent tracing of avian and mammalian sex chromosomes to a single autosomal pair in amphibians supports the counter

Table 1 Summary of the variability in haploid genome size in reptiles (Sauropsida)

Taxa	N	Mean	SD	SEM	Low 95%	Up 95%	Min	Max
Rhynchocephalia								
Sphenodontidae	1	5.00	–	–	–	–	5	5
Squamata								
Anguidae	12	2.16	0.63	0.18	1.76	2.56	1.54	3.8
Varanidae	10	2.07	0.39	0.12	1.80	2.35	1.19	2.43
Gekkonidae	26	2.56	0.44	0.09	2.39	2.74	1.86	3.43
Pygopodidae	1	2.51	–	–	–	–	2.51	2.51
Agamidae	27	1.90	0.35	0.07	1.77	2.04	1.37	2.52
Chamaeleonidae	6	2.46	0.68	0.28	1.75	3.17	1.99	3.79
Corytophanidae	2	2.06	0.02	0.02	1.86	2.25	2.04	2.07
Crotaphytidae	1	2.38	–	–	–	–	2.38	2.38
Iguanidae	3	2.45	0.57	0.33	1.02	3.88	1.8	2.89
Phrynosomatidae	4	2.38	0.25	0.13	1.98	2.78	2.14	2.73
Polychrotidae	13	2.13	0.42	0.12	1.87	2.38	1.44	3.06
Amphisbaenidae	1	1.32	–	–	–	–	1.32	1.32
Bipedidae	2	2.01	0.13	0.09	0.87	3.15	1.92	2.1
Lacertidae	35	1.90	0.49	0.08	1.73	2.07	1.12	2.94
Teiidae	11	2.03	0.60	0.18	1.62	2.43	1.32	3.05
Trogonophidae	1	1.65	–	–	–	–	1.65	1.65
Cordylidae	24	3.11	0.66	0.14	2.83	3.38	1.93	3.93
Scincidae	35	1.75	0.46	0.08	1.60	1.91	1.05	3.2
Boidae	12	1.99	0.57	0.17	1.63	2.36	1.4	3.15
Colubridae	67	2.16	0.49	0.06	2.04	2.28	1.43	3.8
Elapidae	9	2.06	0.39	0.13	1.76	2.36	1.54	2.66
Hydrophiidae	3	2.95	0.67	0.39	1.29	4.60	2.4	3.69
Tropiduridae	3	2.13	0.10	0.06	1.89	2.37	2.05	2.24
Typhlopidae	3	2.28	0.61	0.35	0.76	3.79	1.89	2.98
Viperidae	21	2.06	0.49	0.11	1.83	2.28	1.3	3.06
Squamata averages	332	2.16	0.59	0.03	2.10	2.22	1.05	3.93
Testudines								
Cheloniidae	1	2.64	–	–	–	–	2.64	2.64
Chelydridae	5	2.64	0.14	0.06	2.47	2.81	2.49	2.81
Emydidae	32	2.76	0.62	0.11	2.53	2.98	1.82	4.18
Kinosternidae	3	2.70	0.08	0.05	2.50	2.91	2.65	2.8
Pelomedusidae	3	2.09	0.27	0.15	1.43	2.75	1.79	2.3
Testudinidae	16	3.23	0.87	0.22	2.76	3.70	1.89	5.44
Trionychidae	3	3.32	1.08	0.62	0.64	6.01	2.7	4.57
Testudines averages	63	2.86	0.71	0.09	2.68	3.04	1.79	5.44
Archosauria								
Alligatoridae	6	2.93	0.51	0.21	2.39	3.47	2.49	3.91
Crocodylidae	3	3.21	0.64	0.37	1.62	4.80	2.84	3.95
Aves	276	1.44	0.17	0.01	1.42	1.46	0.97	2.16
Archosauria averages	285	1.49	0.34	0.02	1.45	1.53	0.97	3.95
Sauropsida averages	681	1.95	0.68	0.03	1.90	2.00	0.97	5.44

Data, from the Animal Genome Size Database (Gregory 2007), reported in picograms.

hypothesis that both systems share a common ancestral pair of autosomes (Smith and Voss 2007). If true, an explanation for the transitions between sex chromosome systems is still needed and warrants further research.

Microchromosomes pose a similar question of convergence or shared ancestry because of their presence in reptiles and birds. Microchromosomes, at least in birds, are especially gene-dense compared to mammalian chromosomes (Burt 2002). Many reptiles, such as the anole (*Anolis carolinensis*) and painted turtle (*Chrysemys picta*), have a significant number of microchromosomes (Burt et al. 1999) but these are notably absent in crocodylians (Olmo 1986). The number of independent fissions and fusions that likely generated the different patterns of microchromosomes among reptiles could be estimated by fluorescent mapping of bacterial artificial chromosomes (Matsuda et al. 2005) bearing known microchromosomal and macrochromosomal loci, thereby allowing researchers to retrace the events that led to present-day chromosomal constitutions across reptiles, birds, and mammals.

Gene function and evolutionary potential under climatic change

Sex determination provides a good example of the influence of genomic resources in helping solve enduring questions in functional and evolutionary biology. The annotation of reptilian genomes allows comparisons of different sex-determining mechanisms among closely related vertebrates. Most vertebrates possess genotypic sex determination (GSD) with sex chromosomes and male-heterogamety (XY), or female-heterogamety (ZW), but other systems are known (Bull 1983; Valenzuela and Lance 2004). Some vertebrates lack sex chromosomes or any consistent genetic differences between the sexes. In such taxa, sex is determined after fertilization by incubation temperature (TSD) (Bull 1983; Solari, 1994; Valenzuela et al. 2003). Among vertebrates, TSD is unknown in snakes, birds, and mammals, infrequent in fishes (although new reports are increasing), but very common in reptiles; it occurs in all crocodylians and in the tuatara, is prevalent in turtles, and is infrequent in lizards (Valenzuela and Lance 2004). However, the absolute prevalence of TSD and GSD systems among reptiles (as well as vertebrates in general), is still uncertain due to incomplete taxonomic sampling. Likewise, the evolutionary significance of sex-determining mechanisms in vertebrates remains obscure (Shine 1999; Valenzuela and Lance 2004), partly because we lack

full understanding of the functional mechanics of TSD at the molecular level and how it differs from GSD systems in closely related taxa. Additionally, several recent studies have identified environmental influences on GSD (Goth and Booth 2005; Helle et al. 2008; Radder et al. 2008) and genotypic influences on TSD (Janes and Wayne 2006; Quinn et al. 2007), underscoring the significance of intermediate mechanisms in the continuum between strict GSD and pure TSD (Valenzuela et al. 2003).

Developmental biology and the use of genomic tools are rapidly filling this gap by helping provide the necessary functional and evolutionary explanations to this outstanding diversity. For instance, gonadogenesis requires a delicate balance between short- and long-range signaling molecules during the developmental cascade that leads to ovarian or testicular formation (Kim and Capel 2006). These events involve a gene regulatory network composed of numerous elements common to all vertebrates (Valenzuela and Lance 2004), but whose initial triggers vary between and within GSD and TSD systems. Genetic and genomic research will allow the full characterization of the composition and regulation of such a network and its initial trigger, which remains incomplete.

Sex determination affects the evolution of multiple traits and phenomena, such as sex allocation, sex ratio, sexual dimorphism, sexual selection, sexual conflict, chromosomal and genomic evolution, and ultimately, speciation and extinction (Berry and Shine 1980; Rice 1984; Lindholm and Breden 2002; Edwards et al. 2005). Therefore, an incomplete knowledge of sex determination also hinders our ability to understand the evolution of these associated processes. The consequences of this gap are particularly acute for reptiles given that they exhibit the highest prevalence of TSD known so far.

Deciphering the proximate and ultimate dynamics of sex determination is also essential for conservation of reptilian taxa. In particular, many TSD species are endangered (such as turtles and crocodylians), and drastically biased sex ratios induced by predicted climatic change and exposure to environmental hormones and endocrine disruptors may significantly increase their probabilities of extinction (Girondot et al. 2004; Guillette and Edwards, 2005; Janes et al. 2007). Therefore, there is a pressing need to understand TSD mechanisms in order to evaluate the effects of conservation practices that involve nest transplants and artificial incubation programs that affect sex ratios in the wild, and to implement appropriate management practices for endangered

species. Without such knowledge, one cannot assess TSD's ecological significance and its evolutionary implications in the face of climatic change. But the challenge of global warming extends beyond TSD species. Temperature has significant effects on mortality, morphology, growth, energy reserves, physiology, behavior, performance, and reproductive success of both TSD and GSD species, sometimes in a sex-specific manner (Valenzuela and Lance 2004) such that TSD sex ratios are only one of the many traits likely to be affected by changing climate. The accelerated understanding of the functional mechanics of these many characters gained by the use of modern genomic and genetic tools at our disposal may prove essential to predict and evaluate the short-term effects and the ultimate potential and direction of evolutionary change that may accrue as a consequence of global warming.

Summary

The impending publications of *Chrysemys picta* and *Anolis carolinensis* genomes promise to dramatically change methodologies and interpretations of vertebrate genomic studies. For example, the distribution and evolution of avian genomic characteristics within Diapsida are unknown in part because of the lack of critically needed genomic information from non-avian reptiles. Also, comparative genomics provide a wealth of data from which to infer molecular clocks used to date divergence times, a fundamental problem within evolutionary biology. There is much activity in developing interdisciplinary approaches that combine fossil and molecular data (Near and Sanderson 2004). However, the gaping hole in our knowledge of reptilian genomics makes a solid estimation of these phenomena within amniotes difficult. These data are needed, for example, for discovering correlated evolution among genes and genomes with environmental changes in deep time (Hedges and Kumar 2002). A related area of active research is concerned with whether or not animal genomes have been shaped primarily through selection or through neutral forces, such as drift and mutation (Lynch and Force 2000; Lynch 2007). One of the more exciting aspects of the emerging wealth of data on reptilian genomes is the ability to ask how and why the amniote genome evolved to its present state of constraints and diversity.

Genomic projects for a broad array of amniotes are currently ongoing. For the reasons presented in this introduction and by the speakers of this symposium, we argue that nonavian reptilian genomes provide particularly exciting raw material for

testing hypotheses about overall amniote evolutionary history. Furthermore, we also hypothesize that it is likely many adaptations as well as neutral changes in chromosome dynamics, sex determining systems, and genome size and molecular evolution that led to the diversification of birds and mammals will be discovered to have been reptilian in origin.

Manuscripts presented in this volume describe novel research concerning genomic architecture and functional genomics of reptiles. The symposium participants present their studies of reptilian genomic architecture (Chojnowski, Olmo, and Organ), genes and mechanisms (Bermudez, Glenn, Janes, Kohno, and Valenzuela), and overall patterns in evolution (Janes, Organ, and Schneider). Chojnowski describes isochore structure among turtles, other reptiles, birds, and amphibians, and Olmo and Organ describe genome size and compartmentalization, karyology, and sequence evolution among reptiles. Janes reports the discovery of a novel gonad-developing gene common to, at least, mammals and avian reptiles. Glenn and Valenzuela describe genes associated with generation of β -keratins and reptilian sex determination, respectively. Bermudez and Kohno report findings on thyroid-dependent reproductive endocrinology and steroid receptor evolution among nonavian reptiles. Lastly, Janes and Organ employ comparative methods to estimate ancestral sex-determining mechanisms and Schneider describes the implementation of the green anole genome to study speciation and adaptive radiation. Among other accomplishments, we hope the results and research plans generated by this symposium encourage the sequencing of additional reptilian genomes in the near future.

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collaborators in the field of reptilian genomics. Our conference discussions and the manuscripts presented in this volume will provide a foundation for several experiments to be conducted both before and after the publication of the genome of the green anole (*Anolis carolinensis*). The publication of additional nonavian reptilian genomes like that of the painted turtle (*Chrysemys picta*) and the American alligator (*Alligator mississippiensis*), will allow us to reconvene for a symposium in several years to describe the resulting advances in our field.

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