Proposal to Sequence the First Reptilian Genome: the Green Anole Lizard, *Anolis carolinensis*

Ad hoc Reptilian Genomics Working Group:

Jonathan Losos, Washington University
Ed Braun, University of Florida
Dan Brown, University of Florida
Sandy Clifton, Washington University
Scott Edwards, Harvard University
Jeremy Gibson-Brown, Washington University
Travis Glenn, Savannah River Ecology Laboratory
Lou Guillette, University of Florida
Dorrie Main, Clemson University
Pat Minx, Washington University
William Modi, NIH/NCI
Michael Pfrender, Utah State University
David Pollock, Louisiana State University
David Ray, Louisiana State University
Andrew Shedlock, Harvard University
Wes Warren, Washington University

July 11, 2005

1. The case for sequencing genomes from Reptilia

a) Introduction and Justification: Research on the comparative genomics of mammals is now in high gear with the completion of complete genome sequences for several species and many additional species receiving high priority for lower coverage genomic sequencing (http://www.genome.gov/10001852). By contrast, the comparative genomics of Reptilia–the sister group of mammals, which includes birds and non-avian reptiles[1] (Fig. 1)—lags far behind. Although the recently completed chicken genome has made a significant contribution, there is nonetheless an urgent need to build genomic resources for the reptiles, the only major vertebrate lineage for which there is still no complete genome sequence. Only through studying the sister group of mammals will the idiosyncrasies of mammalian genome evolution become apparent. Furthermore, a number of reptile lineages serve as important models for developmental biology, neurobiology, physiology, endocrinology and other fields.

This proposal, written on behalf of the large international community of researchers using reptiles as models for biological and evolutionary inquiry, proposes the complete sequencing of a genome of an *Anolis* lizard (*Anolis carolinensis*; 6X coverage; genome size 2.2 Gb) as well as low coverage of a genome of an American Alligator (2.5 Gb). These resources will provide a critical base from which to proceed with comparative genomics among vertebrates.

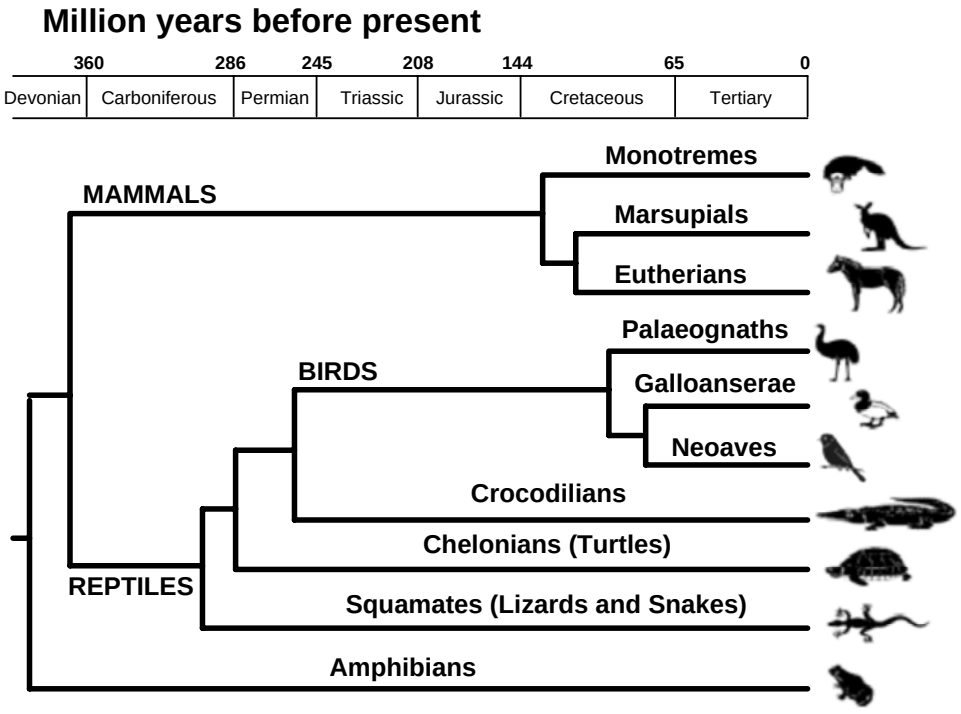

Figure 1. Consensus phylogeny of tetrapod vertebrates based on whole mitochondrial genomes, nuclear DNA, and fossil data (E. Braun, unpubl.). Note that the major groups of reptiles are substantially more divergent from each other than are the clades of mammals (moreover, most extant mammals fall within the Eutheria and have been diverging for an even shorter period of time, estimated to be 50-100 mya). Drawing adapted from http://www.biol.lu.se/cellorgbiol/phylogeny/# with permission.

Whereas mammals comprise about 4300 species in total, their sister group contains ~17,000 species, with reptiles comprising about 7,000 of these. Reptiles display a remarkable range of life histories and reproductive modes, including oviparity, ovoviviparity and viviparity, as well as both genetic and temperature-dependent sex determination. Research on the genomes of reptiles, therefore, promises to uncover a diverse set of biological

[1] For convenience, we will use the term “reptiles” to refer to the traditional, yet paraphyletic group of ‘non-avian’ reptiles.

phenomena, more diverse in many respects than that revealed by genomic comparisons among mammals.

The Reptilia is an ancient group that diverged from the evolutionary line that led to mammals more than 300 million years ago. Surviving members of the Reptilia occur in three groups (Fig. 1): the Archosauria contains crocodilians and birds, whose most recent common ancestor lived ~ 250 mya; the Lepidosauria, which contains the Squamata (lizards and snakes) and the tuatara (a lizard-like reptile found only in New Zealand); and turtles. Fossil and molecular data indicate that these groups have been evolving separately for more than 280 million years (by contrast, although the ancestors of mammals first appeared more than 250 mya ago, most types of modern mammals have diverged in only the last 50-100 million years).

b) Importance of Reptilia for comparative genomics and for annotating the human genome: Reptilia is the sister group of Mammalia. The major lineages of Reptilia represent the best possible outgroups to understand the evolution of mammalian genomes. Not only would reptile genomic data contribute toward annotating the human genome and better understanding the chicken genome, but it would also assist in rooting the 15 or so mammalian genomes currently being sequenced, fill in a gap on the evolutionary tree of vertebrates, aid in identifying conserved regulatory regions and facilitate understanding mechanisms of gene duplication in the evolution of multigene families.

Reptiles provide critical additional lineages for understanding genome evolution in vertebrates, particularly in amniotes (i.e., vertebrates with an amniotic egg—reptiles, birds, and mammals; Waltari & Edwards 2002). To understand genome evolution in amniotes, comparative genomicists currently can only compare the human and other mammalian genomes with the chicken genome. This narrowly focused comparison is inadequate for illuminating the evolutionary origins and history of amniote genomes because it omits the many lineages of reptiles that arose since the lines leading to birds and mammals diverged more than 300 mya. For example, we still do not know whether the large, repeat-rich genomes of mammals or the smaller, repeat-poor genomes of birds reflect the ancestral state for amniotes. A reptile genome would, therefore, add a critical link to facilitate comparisons between the human and chicken genomes. Genomic information from a reptile as distant/divergent as possible from the chicken would provide the largest amount of information for such comparisons. In addition, a reptile phylogenetically close to birds would be especially useful in leveraging information available from the chicken and would serve as the best possible outgroup for all work within birds, among which there are many important model species.

c) Rationale for our approach: Based on the above information, it is clear that at least one reptile should be sequenced. The question is: which one? The *ad hoc* Reptilian Genomics Working Group met in April 2005 to determine which reptile species would best serve the diverse scientific communities that would benefit from the information. A variety of species were discussed. The group concluded that the first priority was to choose a species that provides the greatest increase in evolutionary information. Because an archosaur (the chicken) has already been sequenced, either a squamate or a turtle would be preferable to another archosaur (i.e., a crocodilian). Based on NHGRI criteria, a squamate was clearly ranked above a turtle.

Compelling arguments were made for both a lizard (green anole) and a snake (garter snake). Following the meeting, web pages explaining relevant background information were constructed with an on-line interface for voting and feedback at www.reptilegenome.com. An extensive e-mail campaign was used to make scientists aware of this new web site. Information was gathered to determine which species best met NHGRI criteria and would be used most.

Based on feedback obtained from the web site, e-mail, and presentations at scientific meetings (details given below under "demand for the new sequence data"), it is clear that the Green Anole has the most background information, best meets NHGRI criteria, and would be of use to the largest community of scientists. For this reason, we propose 6x coverage sequencing of the green anole.

However, it is equally clear that at least partial genomic information from three additional reptiles (a crocodilian, a snake and a turtle) is needed critically. We therefore propose to obtain full genome information from the Green Anole and to develop resources and strategies to obtain the most critical information from the other species in the most cost effective manner possible. Initially, development activities should focus on a crocodilian, the American alligator, because it has the most extensive genomic information and resources currently available for any reptile, as well as being best positioned to make use of information available from the chicken. For this reason, we propose low coverage sequencing of the American alligator.

d) Species to be sequenced: We propose the North American green anole lizard, *Anolis carolinensis*, as the squamate species to be sequenced at 6x coverage. *Anolis* is a model system both for laboratory-based studies of organismal function and for field-based studies of ecology and evolution. Indeed, in terms of the breadth of our knowledge of its biology and natural history, the lizard genus *Anolis* is among the best known of vertebrates, or indeed of any kind of animal. The reason is that because of their size, abundance, and hardiness, anoles have been used for a wide variety of biological studies for more than four decades (see reviews in Crews 1980; Crews & Greenberg 1981; Losos 1994; Wade 1999; Godwin & Crews 2002; Baxter 2003; Lovern et al. 2004b). The first paper on these animals was published more than 120 years ago (Monks 1881), and there are many more publications cited in the Web of Science and PubMed for *Anolis* than for any other reptile genus. Moreover, because of their extraordinary biological diversity, species within the genus exhibit a wide variety of ecological adaptations, making them suitable subjects for many types of studies.

Throughout the 20th century, *A. carolinensis* was "the lizard" for comparative studies in fields like physiology, neurology, and behavior. For example, a recent paper was entitled "The green anole (*Anolis carolinensis*): a reptilian model for laboratory studies of reproductive morphology and behavior" (Lovern et al. 2004). Such studies have been conducted at all levels of biological organization—molecular, cellular, neurological, endocrine—and the interplay between these systems is well-understood. More recent and advanced molecular approaches to systems biology have continued to use anoles widely.

In addition, beginning in the 1960's, anoles became a model system for studies of behavior and ecology. This happened for several reasons. First, anoles are extremely abundant and easily studied in the field. Second, they are very hardy in the laboratory. Third, they display an incredible extent of biodiversity. With nearly 400 described species, and many new species being described regularly, *Anolis* is the 2nd largest genus of tetrapod vertebrates. Moreover, independent evolutionary diversification on Caribbean islands has produced "replicate evolutionary radiations" which, despite their evolutionary independence, are nearly identical in the range of different adaptive types produced (Williams 1983; Losos et al. 1998). This almost unique situation has led to an enormous amount of evolutionary research. Lastly, *Anolis* lizard populations are readily manipulated in the field, which has made *Anolis* a model system for experimental studies, including experimental studies of evolutionary change (e.g., Calsbeek & Smith 2003; Losos et al. 1997, 2003). For these reasons, *Anolis* is a widely-used system for integrative studies of ecology, behavior, and evolution (reviewed in Losos 1994; Roughgarden

1995); general and advanced textbooks are full of examples based on anole studies.

Similarly, the American alligator, *Alligator mississipiensis*, is the best-studied crocodilian. Information on the broad array of studies of alligator biology is presented in the next section.

2. NHGRI Criterion A:
"Specific biological/biomedical rationales for the utility of new sequence data"

Anolis carolinensis is an excellent model system for investigating mechanisms involved in the regulation of numerous factors important to human biology and health. Several features make the green anole an ideal laboratory animal:

1) The green anole has a well-studied brain (e.g., a stereotaxic atlas exists [Greenberg 1982]) with an easily recognizable neuroanatomy that exhibits clear homology with human brain regions of interest. Further, the anole brain is "split" in that, unlike mammals, the lizard brain has very limited projections from one side to the other, allowing use of the contralateral side as an internal control for experimental manipulations.

This split brain function has been used to develop a model that can be applied to the study of focal seizures. Systemic administration of kainate, a glutamate receptor agonist, will produce seizures in anoles just as in mammals, and with similar regional cortico-limbic activation, as detected with 2-deoxyglucose (2-DG) and *c-fos* (Riley et al. in prep.). This and other epileptogenic drugs can be applied locally to a spot on one hemisphere to make a focal seizure, just as in mammals. However, in mammals, the extensive corpus callosum always gives rise to extensive "mirror foci" seizures contralaterally, preventing the use of the other side of the brain as a control. Similar reasoning applies to brain injury. Anoles survive local hemispheric brain injury very well. By using the contralateral brain as a control, the anole system has the potential for understanding neurorehabilitation.

The green anole has been used successfully as a model for the tic-like repetitive behaviors seen in human obsessive-compulsive disorder (OCD) and Tourette's syndrome. A homologous cortico/limbic-basal ganglia-thalamic system is involved in *Anolis* displays (Baxter et al. 2001). Moreover, 5-HT functions in anoles in a manner similar to that seen in humans with OCD both in terms of the circuits activated by 5-HT drugs that affect OCD and in symptomatic responses seen to such administrations (Clark & Baxter 2000; Baxter et al. 2001). Further, compared to rodents, *Anolis* possesses 5-HT and dopamine (DA) receptors that demonstrate a pharmacological binding profile much more like that found in primates; for this reason, anoles may be a model of choice for drug studies relevant to these human illnesses (Clark & Baxter 2000; Clark et al. 2000; Baxter et al. 2001). With regard to the similarity in dopamine receptors, clearly the potential exists to use *Anolis* in drug models of Parkinson's and related diseases. For example, a type of drug-induced Parkinson's disorder usually studied in primates, can also be studied by systemic drug administration in anoles (Greenberg et al. 1989); this could be better exploited by lesioning one hemisphere via local injection and leaving the other as the control. Such experiments have been done with monkeys, but the lack of a split brain has led to problems in the interpretation of results.

A variety of other aspects of anole neurobiology and behavior have potentially important implications. For example, in *Anolis* the parietal eye and pineal grand are relatively well-developed and easy to manipulate: the parietal eye can be patched or anesthetized with topical lidocaine. The role of the parietal eye in *Anolis* circadian rhythm regulation is complex (Underwood & Calaban 1987); moreover, melatonin receptors are dense in the left habenula, but are absent in the right habenula (Wiechmann & Wirsig-Wiechmann 1992). Given the great importance of circadian rhythms in human major and bipolar depression and the role of limbic

structures like the habenula in mood disorders, this is another area in which *Anolis* research is likely to lead to important discoveries relevant to human illnesses.

2) Regional changes in neurotransmitters occur in association with dominance and territorial behavior (Summers et al. 1998, 2003a; Baxter 2001; Baxter et al. 2001). The simple and easily-studied neural circuits, which are critical for the display of courtship and copulation, also are affected by these behaviors. Importantly, like the brain, these structures are bilaterally symmetrical (Jones et al. 1983b) and few connections exist between the two sides of the nervous system; even function of the two copulatory organs, the hemipenes, is independently regulated (Ruiz & Wade 2002; Holmes & Wade 2004a). This feature allows experimental control not possible in any other vertebrate, such that one can unilaterally manipulate a muscle or area of the nervous system and use the contralateral side as an internal control.

3) Years of work on the green anole have also detailed the role of steroid hormones, as well as their metabolizing enzymes and receptors, in the display of reproductive behaviors (Crews 1973, 1975; Crews et al. 1974; Morrell et al. 1979; Tokarz & Crews 1980; Jones et al. 1983a; Greenberg & Crews 1990; Wade 1997; Winkler & Wade 1998; Rosen & Wade 2000, 2001; Lovern & Wade 2001, 2003; O'Bryant & Wade 2002; Rosen et al. 2002). The same gonadal hormones that facilitate aggressive and reproductive behaviors in other vertebrate species activate these behaviors in anoles.

4) Anoles share with mammals many of the neurochemical and endocrine responses that are used as models for social stress, aggression and depression (Summers et al. 2005a). Even as a model for depression, anoles have some advantages over the mammalian (even primate) models for two reasons: 1) the social system of anoles is relatively simple (Greenberg 1977), and basic responses can be examined, unencumbered by influences of the complex behavioral repertoires that in mammals are supported by neurocircuitry interwoven with that of the basic circuitry of stress responsiveness (Summers et al. 2005b). 2) Similarly, unlike in mammals, responses of anoles are not influenced by a highly developed neocortex (Greenberg 1982). How the function of the anole brain under conditions of social stress and dominance is different from that of other animal models for human major depression is now being studied with *in situ* hybridization studies of selected *Anolis* 5-HT genes, generated through homology screening against other species. Genome sequence data would allow this work—especially development of a gene-expression chip array—to proceed much more quickly.

5) Like human females, but very few other vertebrates, green anoles ovulate one egg at a time, alternating between the ovaries, with concomitant alternating changes in hypothalamic monoamines (Jones et al. 1983a, 1997; Desan et al. 1992). Understanding the molecular events that influence or are influenced by hormones to control all of these functions is clearly the next step in this research, but sequence information is critical.

6) Anoles communicate primarily through visual signals (Korzan et al. 2000, 2002) and have extremely sophisticated color vision; the genes for some anole opsins have already been sequenced. They provide an excellent opportunity to advance understanding of the mechanisms involved in the production, reception and perception of these signals on a basic level. In addition, work in this area is particularly relevant to humans, who utilize visual signals extensively; by contrast, communication in many other animals (e.g. rodents) relies heavily on chemical cues and thus is less relevant to humans. This feature of anoles also has practical advantages because the relevant information that the lizard receives can easily be controlled; it is far more difficult to monitor chemical signals in an experimental environment.

7) Anoles are particularly well suited for studying the role of hormones in development and in adult nervous system plasticity (Meyer et al. 2004), topics of great current interest. Because these animals lay eggs, rather than producing live young, one can study the mechanisms regulating development with relative independence from maternal influences. One can readily introduce substances of interest, in many cases simply by topically applying them to the egg (Holmes & Wade in press). Recent work has documented remarkable differences in the timing of sexual differentiation and in the degree of ontogenetic morphological change in both neuromuscular systems and more peripheral tissues associated with courtship and copulation (O'Bryant & Wade 1999; Holmes & Wade 2004b; Lovern et al. 2004a). Understanding the molecular mechanisms interacting with steroid hormones to produce these selective responses at various life stages should aid in elucidating potential therapies for damage to or degeneration of neuromuscular systems. It can also inform us about the mechanisms through which endocrine disrupting compounds may affect the nervous and reproductive systems of vertebrates.

8) The great species diversity of anoles provides the possibility that comparisons of closely related species can identify the particular genes involved in particular functions or structures. For example, *A. carolinensis* and *A. sagrei* differ in the way dominant males react to subordinates. This behavioral difference may result from a difference between the species in 5-HT_{1A} expression in the serotonin producing cells in the brainstem (Baxter & Riley in prep.).

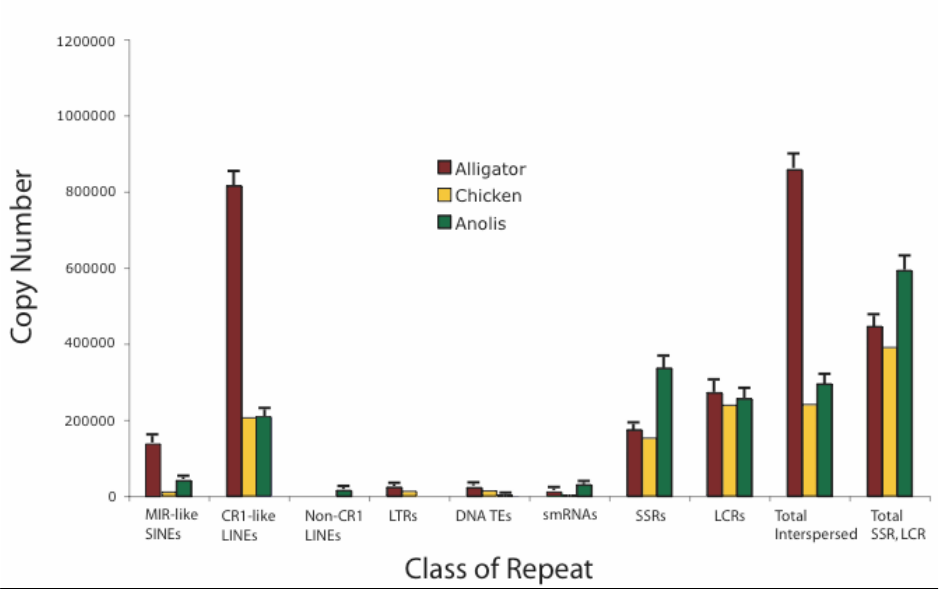

Figure 2. Estimated copy number per genome of repetitive elements for three reptilian species based on RepeatMasker (Smit et al. 2004) scanning of ~ 2.5 Mb and 1.9 Mb non-overlapping paired end sequence obtained from *Alligator mississipiensis* and *Anolis smaragdinus*. Error bars are 95% confidence intervals for the entire genome. Copy numbers for chicken are taken from published whole genome results (Intl. Chicken Genome Consortium 2004). Copy numbers of repeats were estimated using the formula:
Mean ± SD = $(repeats \pm \sqrt{repeats})$(genome size in bp x 1/sequence data in bp)
To tally repeat counts, we used output tables of RepeatMasker to determine the number of identical elements detected in both queries against the chicken and human database, and then subtracted this number from the combined total for each class of repeat. SINE, Short Interspersed Element; LINE, Long Interspersed Element; MIR, Mammalian Interspersed Repeat; CR1, Chicken Repeat 1; LTR, Long Terminal Repeat Retrotransposon; TE, Transposable Element; SmRNA, Small RNA; SSR, Simple Sequence Repeat; LCR, Low Complexity Repeat.

9) Characterizing genome structure in reptilians will help close the present information gap in the study of comparative vertebrate genomics, and *Anolis* provides an important window on the non-avian genomic condition presently invisible from only mammal-chicken comparisons. A preliminary survey of genome structure in multi-megabase

sequences from *Alligator mississippiensis* and *Anolis smaragdinus* (until recently considered a subspecies of *A. carolinensis*) suggests that the repetitive landscape of reptiles is far more diverse than would be expected from consideration of only the avian genome, and likely contains a large diversity of lineage-specific repetitive elements (Fig. 2). G+C content is also apparently elevated relative to the chicken and human genomes (Shedlock, unpubl.). A complete genome assembly of *Anolis* would uniquely provide more robust inference of the molecular pathways by which amniote genomes diversified, whereas phylogenetic methods using available genomic data are insufficient for this purpose. In addition, the chromosome number for nearly 100 species of *Anolis* is available (http://www.scienze.univpm.it/professori/chromorep.pdf). Chromosome numbers in this group range from 2N = 26-46 (Gorman & Atkins 1969; Webster et al. 1972) with a wide range of configurations of sex chromosomes. This range of chromosomal diversity makes *Anolis* an ideal system for investigating the evolution of genome organization.

Research on the American alligator also would provide many applications to advance human health and welfare, which we briefly summarize here. Alligators have been used to study physiology and blood chemistry (Coulson & Hernandez 1983); antiviral and antimicrobial activity of blood proteins (Mayeaux & Winston 1998; Roche et al. 2002; Merchant et al. 2005); microbial induction of immune response (Brown et al. 2001); the effects of environmental contaminants as endocrine disruptors (Guillette et al. 2000); temperature dependent sex determination (Lance 1997; Western et al. 2000); and the development of the heart (Crossley & Altimiras 2005), scales and skin (Alibardi 2004; Alibardi & Thompson, 2000, 2001; Alibardi & Sawyer 2002; Wu et al. 2004), and feathers (Sawyer et al. 2000, 2003, 2005a,b). Alligators harbor West Nile virus (Miller et al. 2003; Klenk et al 2004; Jacobson et al. 2005a,b; Merchant et al. 2005), Mycoplasma (Helmick et al. 2002; Brown 2002), and other disease vectors that endanger human health (Debyser & Zwart 1991). Large numbers of eggs and animals are available for research (e.g., ~ 500,000 alligators are on farms in Louisiana alone).

The chromosome number of most crocodilians has long been known (Cohen & Gans 1970) and complete mtDNA sequences have been obtained (Janke & Arnason 1997; Mindell et al. 1999). Genetic variation within and among American alligator populations has been investigated using many techniques (reviewed by Dessauer et al. 2001; Davis et al. 2001; Glenn et al. 2001). Genomic resources recently developed include a BAC library (http://evogen.jgi.doe.gov/second_levels/BACs/Our_libraries.html; see also http://www.benaroyaresearch.org/investigators/amemiya_chris/libraries.htm) with ~ 1900 pairs of clone-end reads (Edwards et al. unpubl.), full sequences of targeted BACs (Green et al. unpubl.; http://www.nisc.nih.gov/open_page.html?/projects/comp_seq.html), thousands of expressed sequenced tag sequences from liver and testis libraries (Guillette et al., unpubl.), and characterization of repetitive elements from BAC end reads (Shedlock et al., submitted).

3. NHGRI Criteria B:
"Strategic issues in acquiring new sequence data"

1) Demand for the new sequence data— In the first two weeks of input at www.reptilegenome.com, 105 researchers commented upon the prioritization of reptiles for sequencing and voted on which squamate species should be sequenced. Most researchers conveyed the sentiment that having genomic information from any reptile would be a huge benefit, and only one of the 105 respondents disagreed with the general outline for reptile genomics proposed on the website. Sixty-four voted for the green anole as the top priority and 31 voted for garter snake. The ratio of 2:1 generally reflects the disparity in the number of researchers working on these model systems and the number of papers published. A partial list

of researchers who voted for *Anolis* and provided useful comments is given in Appendix 1. It should be noted that most who voted for garter snake as the top priority will still benefit greatly from the *Anolis* sequence data and that additional researchers who will make use of the *Anolis* genome sequence are still being identified.

Figure 3. Ease of animal husbandry in a captive colony of *Anolis carolinensis*; (A) Standard animal facility equipment (mouse cages and racks); (B) Each contains reproductive lizards, as well as perching sticks and a potted plant for egg laying; (C) An adult male anole; (D) Incubation of hundreds of eggs in Petri dishes at 30^0 C in a humidified incubator; (E) A single egg incubated in moist vermiculite (roasted mica), a sterile soil substitute.

2) Suitability of the organism for experimentation

Anoles are candidates to become a model vertebrate genetic system like the mouse. Their body plan is representative of a 'typical' tetrapod: anoles have four legs, a post-anal tail, and a regionally differentiated vertebral column (cervical, thoracic, lumbar, sacral, tail regions distinct) as in mammals, including mouse and human. If a mouse is a “typical mammal,” an anole is a “typical reptile.”

Lizards can be reared in captivity in standard mouse or rat cages, readily available in any conventional animal facility, obviating the need for substantial capital investment to establish breeding colonies. Because captive breeding is straightforward, inbred lines, mutant strains and controlled genetic crosses can be developed. In addition, maintaining *Anolis* lizard colonies entails low husbandry costs, ~ 1/5th that of a mouse due to their low rate of excrement production (no urine) and cheap diet (wingless *Drosophila* or crickets). Moreover, anoles do not exhibit parental care, which makes them easy to manipulate without social, nutritional or other influences that can confound experiments.

3) Rationale for the complete sequence of the organism—As discussed above, reptile genome sequence will be invaluable for many reasons.

4) The cost of sequencing and the state of readiness of the organism’s DNA for sequencing— Overall direct sequencing costs are estimated at ~$19 million. Details of the sequencing plan are summarized in Table 1. Animals are available to make all necessary libraries.

5) Are there other sources of funding available or being sought for this project? No

<u>Sequencing Plan</u>

Our strategy for sequencing the 2.2 Gb genome of the green anole genome will require a map-assisted whole genome shotgun (WGS) approach. This will consist of a BAC-based physical mapping component targeted at 15x physical clone coverage, a 6-fold WGS component consisting of both small and large insert clones and a directed pre-finishing component. The physical map, along with paired end sequences from fosmids and the mapped BAC clones, will

provide a framework by which the genome sequence can be accurately assembled and ordered. In addition, we, in collaboration with others, will confirm the anchoring of a significant number of large sequence assembly supercontigs to the green anole chromosomes using Fluorescent In Situ Hybridisation (FISH).

Genomic DNA will be prepared from selected whole green anole animals. Populations with low genetic diversity are known (arrangements have already been made to procure specimens from eastern Texas [Webster et al. 1972; Wade et al. 1983]) and inbred lines are in development. We will investigate SNPs in candidate strains (see sequencing plan) using the most appropriate strain. WUGSC will assay the heterozygosity rate of these samples by PCR-based resequencing of 100 random loci. Our analysis will indicate if the proposed sample is adequate for whole genome sequencing. Based on previous experience with several organisms, we expect heterozygosity rates of better than 1 SNP in every 800 bp to be a threshold that should not adversely affect WGS sequence assembly. We will construct whole genome shotgun libraries of ~ 4 kb inserts in the plasmid vector pSMART and a large insert library of ~ 40 kb inserts in the fosmid vector pCC-fos1 from a single identified DNA source. Sequence read pairs will be generated from these libraries to produce ~6X coverage of the *A. carolinensis* genome. Isolation of plasmid DNA from individual BAC clones is according to the manufacturer's recommendations (Brinkman Instruments), while fosmids and small insert plasmids will be isolated by established alkaline lysis protocols. End sequences of BAC plasmids will be obtained using previously established methods with minor modifications (Kelley et al. 1999). Quality assurance of sequence traces is maintained with sequencing analysis software (Applied Biosystems). The resulting sequence traces are transferred into a database and processed using customized perl scripts, Autobacend. All trace files will be submitted to the NCBI trace archive (ftp://ftp.ncbi.nih.gov/pub/TraceDB/). Sequence assembly of the genome will be accomplished using the program PCAP (Ding et al. 2004).

EST Project

Because very little is known about codon usage, gene structure and organization in the green anole genome, we propose to include a relatively small EST sequencing program as a component of this project. Several cDNA libraries will be constructed from a number of frozen tissues (e.g. brain, heart), and the resulting clones sequenced from the 5' ends only. We propose to produce 100,000 ESTs from these libraries (Table 1). This will provide a critical resource for sequence analysis and annotation. Of note, a search of dbEST revealed ~5,000 entries for *Anolis sagrei*.

Table 1. Proposed whole genome sequencing of the *Anolis carolinensis* genome.

Description	Quantity	Coverage
4 kb plasmids	21M	(6x)
40 kb fosmids	1.6M	(0.3x)
BAC end reads	0.1M	(0.02X)
BAC fingerprints	0.2M	(15X physical coverage)
ESTs	0.1M	NA

Physical map construction

A 15X BAC library will be constructed by Dr. Pieter de Jong. All large insert libraries will be constructed from the same genome that is utilized for the WGS sequencing efforts. These BAC and fosmid libraries will be available for distribution according to the standard practices of CHORI (http://bacpac.chori.org). The restriction enzyme digestion and capillary size separation of large insert clone fragments from green anole BAC libraries will be completed as described (Luo et al. 2003). Fingerprints are assessed for probability of overlap using the FPC v8 software (Soderland et al. 2000).

Directed pre-finish
To improve on the quality and continuity of the 6X genome sequence, we will perform a subsequent round of computer-directed sequence improvement, "pre-finishing," in which oligonucleotides are algorithmically selected to extend sequence contigs into gap and other low coverage regions. BAC and fosmid clones would serve as the templates for the necessary sequencing reactions. The methods, computational tools and laboratory pipelines for automated finishing are already in place at the Washington University Genome Sequencing Center (WUGSC). We would expect that with ~ 6.3-fold sequence coverage from the plasmids and large insert clones, a directed read would be required at 8kb average intervals.

Genome annotation
Analysis and annotation of the *Anolis carolinensis* genome will be accomplished in a manner similar to our work on the chicken genome. We will collaborate with the green anole community to ensure that the resulting annotation is consistent with the requirements of their respective research needs. A preliminary gene set will be built using either protein similarity, sequence conservation between green anole and chicken or a hybrid approach employing protein similarity, sequence conservation and *ab initio* gene predictors. This preliminary gene set will then be corrected based on ESTs and mRNA sequences and provided to the research community for further annotation and curation. As soon as an assembly of WGS reads provides sufficiently long contigs, testing will begin on the best approach to making a gene set. Genwise (http://www.ebi.ac.uk/Wise2/documentation.html) and FGENESH+ (http://softberry.com/berry.phtml?topic=products) results will be compared for genes based on protein similarity. Twinscan (http://genes.cs.wustl.edu/) and FGENESH-2 (http://softberry.com/berry.phtml?topic=products) will be compared for genes based on sequence conservation between two related genomes. The resulting gene set will be compared to EST and cDNA sequences and discrepancies corrected using a modification of Eannot (Ding et al. 2004), a software tool developed at the WUGSC for the analysis of human chromosomes 2 and 4.

Data availability
Nightly downloads of shotgun reads to the NCBI Trace Archive will be performed to provide the community of users with rapid access to most of the *A. carolinensis* genome sequence. Well-documented genome browsers will be utilized to display all pertinent sequence information. We are committed to minimally displaying the *A. carolinensis* genome on these three browsers: Ensembl, University of California-Santa Cruz and the National Center for Biotechnolgy Information (NCBI).

Conclusion

For these reasons, we propose obtaining the complete sequence for the green anole as the first reptilian genome to be so characterized. We also recommend sequencing the American alligator at low coverage to facilitate comparisons between the anole, the chicken, and mammals. Additional ancillary activities that would greatly enhance our understanding and the usefulness of reptilian genomes include (1) EST projects for alligator, garter snake and a turtle, and (2) BAC mapping/fingerprints of the existing alligator BAC library. To complete the data needed critically for reptiles, our working group expects to develop subsequent proposals for low coverage genomic sequencing and BAC mapping of the garter snake and a turtle.

Appendix 1

Partial list of scientists who provided comments in support of genome sequencing for *Anolis carolinensis*:

Helen Arthur (University of Newcastle)
Lewis R. Baxter, Jr. (University of Florida College of Medicine)
Matt Brandley (University of California, Berkeley)
Marguerite Butler (University of Tennessee)
Todd Campbell (University of Tampa)
Charles J. Cole (American Museum of Natural History)
William E. Cooper (Indiana University-Purdue University)
David Crews (University of Texas at Austin)
Gary W. Ferguson (Texas Christian University)
Leo J. Fleishman (Union College)
Richard E. Glor (University of California, Davis)
John Hancock (MRC Mammalian Genetics Unit)
Blair Hedges (Penn State)
Paul E. Hertz (Barnard College)
Raymond B. Huey (University of Washington)
Peter L. Hurd (University of Alberta)
Michele A. Johnson (Washington University)
Rosemary Knapp (University of Oklahoma)
Jason Kolbe (Washington University)
Charles Linkem (University of California Berkeley)
Matthew B. Lovern (Oklahoma State University)
Luke Mahler (Washington University)
Anita Malhotra (University of Wales, Bangor)
Emilia P. Martins (Indiana University)
Gregory C. Mayer (University of Wisconsin-Parkside)
Jim McGuire (University of California, Berkeley)
Jane Melville (Museum Victoria, Melbourne)
Mark Merchant (McNeese State University)
Craig Moritz (University of California, Berkeley)
Randall Morrison (McDaniel College)
Kenji Murata (University of California, Davis)
Kirsten Nicholson (Washington University)
Kimberly Orrell (Virginia Polytechnic Institute and State University)
Susan L. Perkins (American Museum of Natural History)
Steven Poe (University of New Mexico)
Patrick Reeves (USDA-ARS-National Center for Genetic Resources Preservation)
Erica Bree Rosenblum (University of California, Berkeley)
Thomas Sanger (Washington University)
Chuck Schaffer (University of North Florida)
Christopher J. Schneider (Boston University)
Thomas W. Schoener (University of California, Davis)
James A Schulte II (Smithsonian Institution)

Jack W. Sites, Jr. (Brigham Young University)
Judy Stamps (University of California Davis)
Cliff H. Summers (University of South Dakota)
Roger S Thorpe (University of Wales, Bangor)
Thomas Turner (University of California, Davis)
Juli Wade (Michigan State University)
David B. Wake (University of California, Berkeley)
Sarah Wise (University of Colorado)
Walter Wilczynski (University of Texas at Austin)

Literature Cited

Alibardi, L. 2004. Dermo-epidermal interactions in reptilian scales: Speculations on the evolution of scales, feathers, and hairs *J. Exp. Zool. (Mol. Evol. Dev.)* 302B:365-383.

Alibardi, L., and M.B. Thompson. 2000. Scale morphogenesis and ultrastructure of dermis during embryonic development in the alligator (*Alligator mississippiensis*, Crocodilia, Reptilia). *Acta Zoologica* 81:325-338.

Alibardi, L., and M.B. Thompson. 2001. Fine structure of the developing epidermis in the embryo of the American alligator (*Alligator mississippiensis*, Crocodilia, Reptilia). *J. Anat* 198:265-282.

Alibardi, L., and R.H. Sawyer. 2002. Immunocytochemical analysis of Beta keratins in the epidermis of Chelonians, Lepidosaurians and Archosaurians. *J. Exp. Zool.* 293:27-38.

Baxter, L.R. 2001. Brain mediation of *Anolis* social dominance displays: III. Differential forebrain ^{3}H-Sumatriptan binding in dominant vs. submissive ,males. *Brain, Behavior and Evolution,* 57:202-213.

Baxter, L.R. 2003. Basal ganglia systems in ritualistic social displays: reptiles and humans; Function and Illness. *Physiol Behav.* 79:451-460.

Baxter, L.R., R.F. Ackermann, E.C. Clark and J.E. Baxter. 2001. Brain mediation of *Anolis* social dominance displays: I. Differential basal ganglia activation. *Brain, Behavior and Evolution* 57:169-184.

Baxter, L.R., E.C. Clark, R.F. Ackermann, G. Lacan and W.P. Melega. 2001. Brain mediation of *Anolis* social dominance displays: II. Differential forebrain serotonin turnover, and effects of specific 5-HT Receptor Agonists. *Brain, Behavior and Evolution* 57:185-201.

Brown, D.R., M.F. Nogueira, T.R. Schoeb, K.A. Vliet, R.A. Bennett, G.W. Pye, and E.R. Jacobson. 2001. Pathology of experimental mycoplasmosis in American alligators. *J. Wildl. Diseases* 37:671-679.

Calsbeek, R. and Smith, T.B. 2003 Ocean currents mediate gene flow in island lizards *Nature* 426:552-555.

Clark, E.C. and L.R. Baxter. 2000. Mammal-like striatal functions in *Anolis*. I. Distribution of serotonin receptor subtypes, and absence of striosome and matrix organization. *Brain, Behavior and Evolution* 56:235-248.

Clark, E.C., L.R. Baxter, L.S. Dure, R.F. Ackermann, G.F. Kemp and S.E. Bachus. 2000. Mammal-like striatal functions in *Anolis*. II. Distribution of dopamine D-1 & D-2 receptors, and a laminar pattern of basal ganglia sub-systems. *Brain, Behavior and Evolution* 56:249-258.

Cohen, M.M., and C. Gans. 1970. The chromosomes of the order Crocodilia. *Cytogenetics* 9:81-105.

Coulson, R.A., and T. Hernandez. 1983. *Alligator Metabolism: Studies on Chemical Reactions* in Vivo. Oxford:Pergamon Press.

Crews, D., 1973. Behavioral correlates to gonadal state in the lizard, *Anolis carolinensis*. *Horm. Behav.* 4:307-313.

Crews, D., 1975. Effects of different components of male courtship behaviour on environmentally induced ovarian recrudescence and mating preferences in the lizard, *Anolis Carolinensis*. *Anim. Behav.* 23:349-356.

Crews, D., 1980. Interrelationships among ecological, behavioral, and neuroendocrine processes in the reproductive cycle of *Anolis carolinensis* and other reptiles. In: J.S. Rosenblatt, R.A. Hinde, C.G. Beer, M.-C. Busnel (Eds.), *Advances in the Study of Behavior*. Academic Press, New York, pp. 1-74.

Crews, D., and N. Greenberg. 1981. Function and causation of social signals in lizards. *Amer. Zool*. 21:273-294.

Crews, D., J.S. Rosenblatt, and D.S. Lehrman. 1974. Effects of unseasonal environmental regime, group presence, group composition and males' physiological state on ovarian recrudescence in the lizard, *Anolis carolinensis*. *Endocrinology* 94:541-547.

Crossley, D.A. amd J. Altimiras. 2005. Cardiovascular development in embryos of the American alligator Alligator mississippiensis: effects of chronic and acute hypoxia. *J. Exp. Biol.* 208:31-39.

Davis, LM., T.C. Glenn, D.C. Strickland, L.J. Guillette, Jr., R.M. Elsey, W.E. Rhodes, H.C. Dessauer, and R.H. Sawyer. 2002. Microsatellite DNA analyses support an east-west phylogeographic split of American alligator populations. *J. Exp. Zool. (Mol. Dev. Evol.)* 294:352-372.

Debyser, I.W.J. and P. Zwart. 1991. Review of the most important diseases in Crocodylia which possibly interfere with human health. *Vlaams Diergeneeskundig Tijdschrift* 60:164-169.

Desan, P. H., K.H. Lopez, H.B. Austin, and R.E. Jones. 1992. Asymmetric metabolism of hypothalamic catecholamines alternates with side of ovulation in a lizard (*Anolis carolinensis*). *J. Exp. Zool.* 262:105-112.

Dessauer H.C., TC. Glenn, and LD. Densmore. 2002. Studies on the molecular evolution of the crocodilia: footprints in the sands of time. *J. Exp. Zool. (Mol. Dev. Evol.)* 294:302-311.

Ding, L., A. Sabo, N. Berkowicz, R.R. Meyer, Y. Shotland, M.R. Johnson, K.H. Pepin, R.K. Wilson, and J. Spieth. 2004. EAnnot: a genome annotation tool using experimental evidence. *Genome Res*. 12:2503-2509.

Glenn, T.C., JL. Staton, A. Vu, LM. Davis, JR. Alvardo Bremer, WH. Rhodes, IL. Brisbin Jr., and RH. Sawyer. 2002. Low mitochondrial DNA variation among American alligators and a novel non-coding region in crocodilians. *J. Exp. Zool. (Mol. Dev. Evol.)* 394:312-324.

Godwin, J., and D. Crews. 2002. Hormones, Brain, and Behavior in Reptiles. In: D.W. Pfaff, A.P. Arnold, A.M. Etgen, S.E. Fahrbach, R.T. Rubin (Eds.), *Hormones, Brain and Behavior*. Academic Press, New York, pp. 545-585.

Gorman, G.C., and L. Atkins. 1969. The zoogeography of Lesser Antillean Anolis lizards: an analysis based on chromosomes and lactic dehydrogenases. *Bull. Mus. Comp. Zool.* 138:53-80.

Greenberg, N. 1977. A neuroethological study of display behavior in the lizard, *Anolis carolinensis* (Reptilia, Lacertilia, Iguanidae). *Am. Zool.* 17:191-201.

Greenberg, N., 1982. A forebrain atlas and sterotaxic technique for the lizard, *Anolis carolinensis*. *J. Morph.* 174:217-236.

Greenberg, N., and D. Crews. 1990. Endocrine and behavioral responses to aggression and social dominance in the green anole lizard, *Anolis carolinensis*. *General and Comparative Endocrinology* 77:246-255.

Greenberg, N., G.M. Burghardt, D. Crews, E. Font, R.E. Jones, G. Vaughan. 1989. Reptile models for biomedical research. Pp. 289-308 in A.D. Woodhead, Ed, *Nonmammalian Animal Models for Biomedical Research*. CRC Press: Boca Raton.

Guillette, L.J., D.A. Crain, M.P. Gunderson, S.A.E. Kools, M.R. Milnes, E.F. Orlando, A.A. Rooney, and A.R. Woodward. 2000. Alligators and endocrine disrupting contaminants: A current perspective. *Amer. Zool.* 40:438-452.

Guillette, L.J. and T. Iguchi. 2003. Contaminant-induced endocrine and reproductive alterations in reptiles. *Pure and Applied Chem.* 75:2275-2286.

Holmes, M.M., and J. Wade. 2004a. Characterization of projections from a sexually dimorphic motor nucleus in the spinal cord of adult green anoles. *J. Comp Neurol.* 471:180-187.

Holmes, M.M., and J. Wade. 2004b. Seasonal plasticity in the copulatory neuromuscular system of green anole lizards: A role for testosterone in muscle but not motoneuron morphology. *J. Neurobiol.* 60:1-11.

Holmes, M.M. and J. Wade. 2005. Sexual differentiation of the copulatory neuromuscular system in green anoles (*Anolis carolinensis*): Normal ontogeny and manipulation of steroid hormones. *J. Comp. Neurol*. In press.

Huang, X., J. Wang, S. Aluru, S.P. Yang, and L. Hillier. 2003. PCAP: a whole-genome assembly program. *Genome Res.* 9:2164-70.

International Chicken Genome Consortium. 2004. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature* 432:695-716.

Jacobson, E.R., A.J. Johnson, J.A. Hernandez, S. J. Tucker, A. P. Dupuis, II, R. Stevens, D. Carbonneau, and L. Stark. 2005. West Nile virus infection in farmed American alligators (*Alligator mississippiensis*) in Florida. *J. Wildl. Diseases* 41:96-106.

Janke, A., and U. Arnason. 1997. The complete mitochondrial genome of *Alligator mississippiensis* and the separation between recent archosauria (birds and crocodiles). *Mol. Biol. Evol.* 14:1266-1272.

Jones, R. E., L.J. Guillette, Jr., C.H. Summers, R.R. Tokarz, and D. Crews. 1983a. The relationship among ovarian condition, steroid hormones, and estrous behavior in *Anolis carolinensis*. *J. Exp. Zool.* 227:145-154.

Jones, R. E., K.H. Lopez, T.A. Maldonado, T.R. Summers, C.H. Summers, C.R. Propper, and J.D. Woodling. 1997. Unilateral ovariectomy influences hypothalamic monoamine asymmetries in a lizard (*Anolis*) that exhibits alternation of ovulation. *Gen. Comp Endocrinol.* 108:306-315.

Jones, R. E., C.H. Summers, H.B. Austin, H.M. Smith, and T.T. Gleeson. 1983b. Ovarian, oviductal, and adrenal vascular connections in female lizards (genus *Anolis*). *Anat. Rec.* 206:247-255.

Kelley, J.M., C.E. Fields, M.B. Craven, D. Bocskai, U. Kim, S.D. Rounsley and M.D. Adams. 1999. High throughput direct end sequencing of BAC clones. *Nucleic Acids Res.* 27:1539–1546.

Korzan, W. J., T.R. Summers, P.J. Ronan, and C.H. Summers. 2000. Visible sympathetic activity as a social signal in *Anolis carolinensis*: Changes in aggression and plasma catecholamines. *Horm. Behav.* 38:193-199.

Korzan, W. J., T.R. Summers, and C.H. Summers. 2002. Manipulation of visual sympathetic sign stimulus modifies social status and plasma catecholamines. *Gen. Comp Endocrinol.* 128:153-161.

Lance, V.A. 1997. Sex determination in reptiles: an update. *Amer. Zool.* 37: 504-513.

Losos, J.B. 1994. Integrative approaches to evolutionary ecology: *Anolis* lizards as model systems. *Annual Review of Ecology and Systematics* 25:467-493.

Losos, J.B., T.R. Jackman, A. Larson, K. de Queiroz, and L. Rodríguez-Schettino. 1998. Historical contingency and determinism in replicated adaptive radiations of island lizards. *Science* 279:2115-2118.

Losos, J.B., T.W. Schoener, and D.A. Spiller. 2004. Predator-induced behaviour shifts and natural selection in field-experimental lizard populations. *Nature* 432:505-508.

Losos, J.B., K.I. Warheit, and T.W. Schoener. 1997. Adaptive differentiation following experimental island colonization in *Anolis* lizards. *Nature* 387:70-73.

Lovern, M.B., M.M. Holmes, C.O. Fuller, and J. Wade. 2004a. Effects of testosterone on the development of neuromuscular systems and their target tissues involved in courtship and copulation in green anoles (*Anolis carolinensis*). Horm. Behav. 45:295-305.

Lovern, M.B., M.M. Holmes, and J. Wade. 2004b. The green anole (*Anolis carolinensis*): A reptilian model for laboratory studies of reproductive morphology and behavior. *ILAR Journal* 45:54-64.

Lovern, M.B., and J. Wade. 2001. Maternal plasma and egg yolk testosterone concentrations during embryonic development in green anoles (*Anolis carolinensis*). *Gen. Comp. Endocrinol.* 124:226-235.

Lovern, M.B., Wade, J., 2003. Sex steroids in green anoles (*Anolis carolinensis*): Uncoupled maternal plasma and yolking follicle concentrations, potential embryonic steroidogenesis, and evolutionary implications. Gen. Comp. Endocrinol. 134, 109-113.

Luo, M.C., C. Thomas, F.M. You, J. Hsiao, S. Ouyang, C.R. Buell, M. Malandro, P.E. McGuire, O.D. Anderson, and J. Dvorak. 2003. High-throughput fingerprinting of bacterial artificial chromosomes using the SnaPshot labeling kit and sizing of restriction fragments by capillary electrophoresis. *Genomics* 82:378-389.

Mayeaux, M.H., and G.W. Winston. 1998. Antibiotic effects on cytochromes P450 content and mixed-function oxygenase (MFO) activities in the American alligator, *Alligator mississippiensis*. *J. Vet Pharm. Ther*. 21:274-281.

Merchant, M.E., M. Pallansch, R.L. Paulman, J.B. Wells, A. Nalca, and R. Ptak. 2005. Antiviral activity of serum from the American alligator (*Alligator mississippiensis*). *Antiviral Research* 66:35-38.

Mindell, D.P., M.D. Sorenson, D.E. Dimcheff, M. Hasegawa, J.C. Ast and T. Yuri. 1999. Interordinal relationships of birds and other reptiles based on whole mitochondrial genomes. *Syst. Biol.* 48:138-152.

Monks, S.P. 1881. A partial biography of the green lizard. *Am. Nat.* 15:96-99.

Morrell, J.I., D. Crews, A. Ballin, A. Morgentaler, and D.W. Pfaff. 1979. ^{3}H-estradiol, ^{3}H-testosterone and ^{3}H-dihydrotestosterone localization in the brain of the lizard *Anolis carolinensis*: An autoradiographic study. *J. Comp. Neurol*. 188:201-224.

Meyer, W. N., J. Keifer, W.J. Korzan, and C.H. Summers. 2004. Social stress and corticosterone regionally upregulate limbic N-methyl-D-aspartate receptor (NR) subunit type NR_{2A} and NR_{2B} in the lizard *Anolis carolinensis*. *Neuroscience* 128: 675-684.

O'Bryant, E.L., and J. Wade. 1999. Sexual dimorphisms in a neuromuscular system regulating courtship in the green anole lizard: Effects of season and androgen treatment. *J. Neurobiol*. 40:202-213.

O'Bryant, E.L., and J. Wade. 2002. Seasonal and sexual dimorphisms in the green anole forebrain. *Horm. Behav*. 41:384-395.

Riley, J, R.F. Ackermann, and L.R. Baxter. in prep. An *Anolis*-specific c-fos insitu hibridization probe and patterns of c-fos and 2-DG activation during drug-induced seizure in lizard.

Roche, C., K. Cabello, B. Young, M.E. Merchant, D. Thibodeaux, R. Van Gossen, J. Prudhomme, and R.M. Elsey. 2002. Antibacterial properties and complement activity of serum in the American Alligator (Alligator mississippiensis). Pp.178-186 In *Proceedings of the 16th Working Meeting of the Crocodile Specialist Group, Gainesville, FL*. World Conservation Union, Gland, Switzerland.

Rosen, G.J., E.L. O'Bryant, J. Matthews, T. Zacharewski, and J. Wade. 2002. Distribution of androgen receptor mRNA expression and immunoreactivity in the brain of the green anole lizard. *J. Neuroendocrinol*. 14:19-28.

Rosen, G.J., and J. Wade. 2000. The role of 5a-reductase in sexual behaviors of the green anole lizard. *Physiol. Behav*. 69:487-498.

Rosen, G.J., and J. Wade. 2001. Androgen metabolism in the brain of the green anole lizard (*Anolis carolinensis*): Effects of sex and season. *Gen. Comp. Endocrinol*. 122:40-47.

Ruiz, C.C., and J. Wade. 2002. Sexual dimorphisms in a copulatory neuromuscular system in the green anole lizard. *J. Comp. Neurol*. 443:289-297.

Roughgarden, J., *Anolis Lizards of the Caribbean: Ecology, Evolution, and Plate Tectonics.* Oxford University Press, 200 pp.

Sawyer, R.H., T.C. Glenn, J.O. French, B. Mays, R.B. Shames, G.L. Barnes, Jr., W.E. Rhodes, and Y. Ishikawa. 2000. The expression of Beta keratins in the epidermal appendages of reptiles and birds. *Amer. Zool.* 40:530-539.

Sawyer, R.H., T.C. Glenn, J.O. French, and L.W. Knapp. 2005a. Developing antibodies to synthetic peptides based on comparative DNA sequencing of multigene families. *Methods in Enzymology* 395:636-652.

Sawyer, R.H., L Rogers, L. Washington, T.C. Glenn and L.W. Knapp. 2005b. The evolutionary origin of the feather epidermis. *Developmental Dynamics* 232:256-267.

Sawyer, R.H., B.A. Salvatore, T.F. Potylicki, J.O. French, T.C. Glenn, and L.W. Knapp. 2003. Origin of feathers: Feather beta ( ) keratins are expressed in discrete epidermal cell populations of embryonic scutate scales. *J. Exp. Zool (Mol. Dev. Evol.)* 295B:12-24.

Shedlock, A. M., C. W. Botka, S. Zhao, J. Shetty,T. Zhang, J. S. Liu, P. J. Deschavanne, and S. V. Edwards. Submitted. Comparative genomics of non-avian reptiles.

Smit, A.F.A., R. Hubley, and P. Green. 2004. *RepeatMasker Open*, vers. 3.0.5. Available at http://www. repeatmasker.org.

Soderlund, C., S. Humphray, A. Dunham, and L. French. 2000 Contigs built with fingerprints, markers and FPCV4.7. *Genome Research* 10:1772-1787.

Summers, C. H., G.L. Forster, W.J. Korzan, M.J. Watt, E.T. Larson, O. Overli, E. Hoglund, P.J. Ronan, T.R. Summers, K.J. Renner, and N. Greenberg, N. 2005a. Dynamics and mechanics of social rank reversal. *J Comp Physiol A Neuroethol. Sens. Neural Behav. Physiol* 191:241-252.

Summers, C. H., E.T. Larson, P.J. Ronan, P.M. Hofmann, A.J. Emerson, and K.J. Renner. 2000. Serotonergic responses to corticosterone and testosterone in the limbic system. *Gen. Comp Endocrinol.* 117:151-159.

Summers, C. H., E.T. Larson, T.R. Summers, K.J. Renner, and N. Greenberg. 1998. Regional and temporal separation of serotonergic activity mediating social stress. *Neuroscience* 87:489-496.

Summers, C.H., T.R. Summers, M.C. Moore, W.J. Korzan, S.K. Woodley, P.J. Ronan, E. Höglund, M.J. Watt, and N. Greenberg. 2003a. Temporal patterns of limbic monoamine and plasma corticosterone response during social stress. *Neuroscience* 116:553-563.

Summers, C. H., M.J. Watt, T.J. Ling, G.L. Forster, R.E. Carpenter, W.J. Korzan, J.L Lukkes, and Ø. Øverli. 2005b. Glucocorticoid interaction with aggression in non-mammalian vertebrates: reciprocal action. *Eur. J. Pharmacol.* in press.

Summers, T. R., J.M. Matter, J.M. McKay, P.J. Ronan, E.T. Larson, K.J. Renner and C.H. Summers. 2003b. Rapid glucocorticoid stimulation and GABAergic inhibition of hippocampal serotonergic response: *in vivo* dialysis in the lizard *Anolis carolinensis*. *Horm. Behav* 43:245-253.

Tokarz, R.R., and D. Crews. 1980. Induction of sexual receptivity in the female lizard, Anolis carolinensis: Effects of estrogen and the antiestrogen CI-628. *Hormones and Behavior* 14:33-45.

Underwood, H., and M. Calaban M. 1987. Pineal melatonin rhythms in the lizard *Anolis carolinensis*: II. Photoreceptive inputs. *J. Biol. Rhythms* 2:195-206.

Wade, J., 1997. Androgen metabolism in the brain of the green anole lizard (*Anolis carolinensis*). *Gen. Comp. Endocrinol*. 106:127-137.

Wade, J., 1999. Sexual dimorphisms in avian and reptilian coursthip: Two systems that do not play by mammalian rules. *Brain Behav. Evol*. 54:15-27.

Wade, J.K., A.C. Echternacht, and G.F. McCracken. 1983. Genetic variation and similarity in Anolis carolinensis (Sauria: Iguanidae). *Copeia* 1983:522-529.

Waltari, E, and S.V. Edwards. 2002. Evolutionary dynamics of intron size, genome size, and physiological correlates in archosaurs. *Amer. Nat.* 160:539-552.

Webster, T. P., W. P. Hall, and E. E. Williams. 1972. Fission in the evolution of a lizard karyotype. *Science* 177:611-613.

Webster, T.P., R.K. Selander, and S.Y. Yang. 1972. Genetic variability in the *Anolis* lizards of Bimini. *Evolution* 26:523-535.

Wiechmann, A.F., and C.R. Wirsig-Wiechmann. 1992. Asymmetric distribution of melatonin receptors in the brain of the lizard *Anolis carolinensis*. *Brain Res*. 593: 281-286.

Winkler, S.W., and J. Wade. 1998. Aromatase activity and regulation of sexual behaviors in the green anole lizard. *Phys. Behav.* 64:723-731.

Williams, E.E. 1983. Ecomorphs, faunas, island size, and diverse end points in island radiations of *Anolis*. Pp. 326-370 *in* R.B. Huey, E.R. Pianka and T.W. Schoener, eds. *Lizard Ecology: Studies of a Model Organism.* Harvard Univ. Press, Cambridge, MA.

Wu P., L.H. Hou, M. Plikus, M. Hughes, J. Scehnet, S. Suksaweang, R.B. Widelitz, T.X. Jiang, and C.M. Chuong. 2004. Evo-Devo of amniote integuments and appendages. *Intl. J. Dev. Biol.* 48:249-270.