A Proposal to Sequence the Genome of a Garter Snake (Thamnophis sirtalis)

Citation
Castoe, Todd A., Anne M. Bronikowski, Edmund D. Brodie, Scott V. Edwards, Michael E. Pfrender, Michael D. Shapiro, David D. Pollock, and Wesley C. Warren. 2011. A proposal to sequence the genome of a garter snake (Thamnophis sirtalis). Standards in Genomic Sciences 4(2): 257–270.

Published Version
doi:10.4056/sigs.1664145

Permanent link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:10436327

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

Share Your Story
The Harvard community has made this article openly available. Please share how this access benefits you. Submit a story.

Accessibility
A proposal to sequence the genome of a garter snake (Thamnophis sirtalis)

Todd A. Castoe1*, Anne M. Bronikowski2, Edmund D. Brodie III3, Scott V. Edwards4, Michael E. Pfrender5, Michael D. Shapiro6, David D. Pollock1, Wesley C. Warren7

1Department of Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, CO
2Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA
3Department of Biology, University of Virginia, Charlottesville, VA
4Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA
5Department of Biological Sciences, University of Notre Dame, IN
6Department of Biology, University of Utah, Salt Lake City, UT
7Genome Sequencing Center, Washington University School of Medicine, St. Louis, MO

*Corresponding Author: Todd A. Castoe, todd.castoe@ucdenver.edu

Here we develop an argument in support of sequencing a garter snake (Thamnophis sirtalis) genome, and outline a plan to accomplish this. This snake is a common, widespread, nonvenomous North American species that has served as a model for diverse studies in evolutionary biology, physiology, genomics, behavior and coevolution. The anole lizard is currently the only genome sequence available for a non-avian reptile. Thus, the garter snake at this time would be the first available snake genome sequence and as such would provide much needed comparative representation of non-avian reptilian genomes, and would also allow critical new insights for vertebrate comparative genomic studies. We outline the major areas of discovery that the availability of the garter snake genome would enable, and describe a plan for whole-genome sequencing.

Introduction

We propose to sequence the 1.91 Gb genome of a garter snake (Thamnophis sirtalis, Figure 1), a common, widespread, nonvenomous North American snake that has served as a model for diverse studies in evolutionary biology, physiology, genomics, behavior and coevolution. Comparative genomic studies in vertebrates are now well underway, and recent months have seen the publication of high-quality genomes of mammals based on de-novo assembly of short-read next-generation sequencing platforms [1]. As of February 2011, the NCBI database and Ensembl contain 51 vertebrate chordate genomes. Among amniotes (which include mammals, birds and non-avian reptiles) only three birds (chicken, turkey, and zebra finch) and one non-avian reptile (a lizard, Anolis carolinensis) are represented. Thus, there is high taxonomic imbalance among the currently sequenced amniote genomes, meaning that detailed comparative analyses with reasonably diverse taxonomic sampling can only be performed within the mammals. Additional non-mammalian amniote genomes are still required to fully leverage the comparative potential of the impressive set of mammalian genomes sequenced or in progress.

We propose to sequence the garter snake as the next non-mammalian genome because of its key phylogenetic position and because it has been an important research focus for many disciplines, including physiology, evolutionary genetics, morphology, ecology, comparative genomics and life history evolution. In addition to providing much-needed additional taxonomic coverage of the tree-of-life for non-mammalian amniotes and vertebrates generally, a garter snake genome would provide crucial insight into many areas of biology, including: 1) the genetic basis of limblessness and axial patterning, 2) the genetic basis of highly variable coloration and integumentary patterning, 3)
A proposal to sequence the genome of a garter snake

the genetic basis of physiological and metabolic adaptation, 4) adaptation to toxin resistance, 5) birth-death evolution of large multigene families, 6) venom gene evolution, 7) genome structure in terrestrial ectotherms, 8) genetic basis of axial patterning, and 9) genome evolution in Reptilia, the sister group of Mammalia. We outline below the areas of discovery that would be accelerated by a garter snake genome, and include a list of researchers who support this proposal (Appendix) to establish the level of general scientific interest in the garter snake genome.

The garter snake as a model system in evolutionary biology, ecology and physiology

Garter snakes are the most studied snake model system in the areas of ecology, evolution, behavior, and physiology. Not only is the breadth of work considerable, but the seminal nature of work in behavior genetics, development of personality, toxin resistance, pheromonal communication, and reproductive physiology places the genus *Thamnophis* among the major vertebrate models for organismal biology. Garter snakes have provided their most significant contributions as a model to investigate biological questions in the context of natural populations in the wild.

Evolutionary diversification

Garter snakes have historically been the most important snake group for examining speciation and differentiation of ecologically important phenotypes. Some of the original work describing the concept of ring species and the “artenkreis” problem of population differentiation emerged from work on western *Thamnophis* species including *T. sirtalis* [2-4]. Since that time, robust phylogenies have been developed at the species and population level [5-7]. These studies indicate at least occasional hybridization between species [8], suggesting incomplete reproductive isolation and the potential for introgression leading to local adaptation.

Population and genetic differentiation in a range of behavioral and morphological traits have been modeled as evolving through quantitative genetic processes. Research on prey preferences, color pattern polymorphism, skeletal morphology, life history profiles, and antipredator behaviors of *Thamnophis* all stand as primary empirical examples of selective and genetic processes that lead to population level differences in traits [9-14]. Ecological studies of diet differences, thermal biology, homing, and hibernation physiology and behavior show similar patterns of trait differentiation influenced by environmental contexts [13,15-18].
Evolutionary developmental biology and axial patterning

Snakes feature several radical departures from the ancestral amniote body plan, perhaps most notably the absence of limbs and a pre-sacral vertebral column composed of highly similar vertebrae. Remarkably, the absence of forelimbs and the homomorphy of vertebrae might be developmentally and genetically linked. The expression of certain Hox genes that typically mark the boundaries of different vertebral regions are expanded in snakes. This expansion might have a dual role in the evolution of the snake body plan because it eliminates the expression boundaries that typically correlate with the site of forelimb outgrowth, and it results in similar Hox expression patterns in nearly all pre-sacral trunk segments [23]. Expansions and shifts in Hox expression domains are closely associated with shifts in vertebral identity [24,25], and the homomorphic vertebrae of snakes are likely due, to broadly overlapping domains of Hox expression that are typically more restricted in vertebrates with regionalized axial skeletons [23]. A whole genome assembly for the garter snake would allow unprecedented access to both the coding and well-characterized regulatory domains of the Hox clusters, thereby enabling both comparative genomic and experimental genetic studies of these important regions. The sheer number of vertebrae in snakes represents another classic departure from other amniotes, a change that is due to an acceleration of the embryonic segmentation clock [26]. A complete snake genome would provide an opportunity to examine the conserved regulatory network that determines the pace of this clock, thereby allowing novel insights into a basic feature of segmented embryos.

Gene expression assays and experimental embryology have implicated the Sonic Hedgehog (Shh) and Fibroblast Growth Factor (Fgf) pathways in the reduction of snake hindlimbs [23]. Studies of cetaceans show the involvement of these pathways in hindlimb reduction in mammals as well [27]. While it is clear that Shh and Fgfs contribute to the developmental basis of limb loss, we still lack information about the genetic changes that control this change. Limb loss occurs in many vertebrate lineages, and strongest genetic evidence for causal mutations comes from the stickleback fish [28]. In this case, the underlying mutations occur in the hindlimb-specific transcription factor Pitx1, not in the Shh or Fgf pathways; morphological evidence suggests that changes in similar genes might occur in other species as well [29]. Thus, the availability of the garter snake genome could help identify additional molecular correlates of limb loss, and test for modifications in genes and regulatory elements already known to play a role in limb outgrowth and patterning.

Reproductive physiology, sexual behavior, and seasonality

Garter snakes are famous for their communal hibernacula and the mass mating balls that accompany spring emergence in northern populations. This habit has lead to in-depth studies of the environmental and endocrine controls of reproductive physiology and mating behavior. Both sexes have disassociated courtship behavior and hormonal cycles, with courtship occurring independent of seasonal changes in testosterone or estradiol [30-34]. Instead, rising temperature apparently stimulates sexual activity even in females not in reproductive condition, though this responsiveness is counteracted by pinealectomy [35,36]. Mating results in spikes of estradiol in females, which are surprisingly correlated with a decline in sexual receptivity. The relationships between brain anatomy, endocrine profiles and reproductive cycles in T. sirtalis are better understood than for any other reptile except perhaps Anolis lizards [17,31,36-40].

Exercise physiology and functional morphology

Because of their unique morphology, snakes, and Thamnophis in particular, have been popular subjects for exploring the functional morphology and physiology of locomotion [41,42]. Speed and endurance are known to be selected upon in natural populations, and complementary studies have linked variation in performance with muscle physiology, skeletal morphology, enzyme activities and metabolism [9,43-46]. Quantitative genetic approaches have demonstrated heritable variation in many of these traits, as well as important covariances among both organismal performance variables and underlying mechanisms.
Chemical communication and pheromones
The sensory world of garter snakes is dominated by chemical signals. Pheromones are the primary means of species and sex recognition [47]. Males covered in skin or skin lipids extracted from females are courted by other males. Attractiveness of non-volatile skin lipids is influenced by seasonality and temperature [48]. Some males produce female-like attraction pheromones naturally [49,50]. These “shemales” are courted by other males in large mating balls and apparently gain some thermal benefit through the heat produced by courting males [51].

The neurophysiology of chemosensation in garter snakes serves as the major model of chemical signal transduction in the nasal sensory system [52,53]. The vomeronasal morphology and abilities of garter snakes are fully developed at birth, and behavioral assays including tongue flick responses and trailing behaviors have been critical in evaluating the results of experimental degradation and ablation [52,54].

Arms Races and toxin resistance
The coevolutionary dynamics of arms races between predators and prey have been revealed primarily through investigations of geographic variation in tetrodotoxin resistance in Thamnophis sirtalis. Thamnophis feeds on amphibians, including newts of the genus Taricha, that possess the neurotoxin tetrodotoxin (TTX) as a defense. Populations of T. sirtalis vary in resistance, both organismal and physiological, in a pattern that suggests the form of arms-race dynamics in natural populations [55-57]. In some cases, populations of predators have escaped from the arms race by evolving extreme levels of resistance to TTX. TTX resistance has been linked to specific amino acid substitutions in the NaV1.4 gene that encodes voltage gated sodium channels in skeletal muscle [58-60]. The availability of the genome of the garter snake would enable tremendous opportunity for genome-scale analyses of coevolutionary interactions between toxins and toxin-resistant genes in Thamnophis. For example, the availability of a garter snake genome would facilitate identification of genes potentially related to TTX resistance, thereby enabling efficient screening of putatively relevant toxin-resistance genes from garter snake species and populations with differential sensitivity to TTX.

Development of behavior and personality
Garter snakes serve as one of the few non-mammalian (and only reptilian) model species for studies of behavior development. Examining aggressive displays and feeding preferences, researchers have shown consistent individual personalities, and followed development of those personalities over ontogeny [61-64]. Experience with predators and prey, threats, and visual and chemical stimuli all modify individual behavior. Population and species differences in “personality” have been linked to ecological contexts including food availability and risk [65-67].

Genome characteristics of snakes
Snake genomes are often smaller than mammalian genomes, ranging from ~1.3 Gbp to 3.8 Gbp, with an average of 2.08 Gbp [68]. The most recent estimate for the genome size of the Garter Snake (Thamnophis sirtalis) suggests it is in the middle of this range at 1.91 gigabases (Gbp) [69], making it less than 2/3 the size of the human genome. All snakes are thought to have ZW genetic sex determination, and their sex chromosomes reveal increased differentiation in a phylogenetic sex determination from the morphologically “primitive” boids to the more “advanced” colubrid, elapid and viperid snakes [70]. In comparison with other tetrapod groups, chromosome number in snakes tends to be highly conserved; most species possess ~36 chromosomes, with ~16 macrosomes and ~20 microsomes [71].

Although our current knowledge of vertebrate genome structure and diversity is strongly slanted towards mammals, new information on reptilian genomes is just starting to become available [72-76]. In contrast to the genomes of mammals and birds, most (non-avian) reptile genomes are comprised of a particularly diverse repertoire of transposable elements (TEs). Whereas mammal and bird genomes often have undergone recent expansion of one or a small number of TEs, such as L1 LINES and Alu SINES in humans, reptilian genomes examined have experienced recent (and presumably ongoing) activity and expansion of multiple TE types; this is particularly true of the only squamate reptile genome sequenced to date. Based on preliminary genomic analyses of the lizard Anolis, trends in the squamate lineage include an increase in simple sequence repeat (SSR) content, the dominance of CR1 LINE retroelements, and a high overall diversity of retroelements [72,74,75].
Figure 2. Preliminary estimate of the repeat content of the *Thamnophis sirtalis* genome compared to the *Anolis* lizard genome. Estimates based on RepeatMasker analyses using the RepBase tetrapoda repeat library. Snake data is based on 454 random survey-sequencing of 49 Mbp for *Thamnophis sirtalis*; *Anolis* based on analyses of four *Anolis* Scaffolds totaling 55 Mbp.

Recent data (Castoe and Pollock, unpublished) from a small number of snake lineages based on low coverage sample sequencing of 454 shotgun libraries (30-60 Mbp/species) also provides insight into repeat element dynamics within the snake lineage. Here we provide some preliminary estimates based on analyses of the *Anolis* genome in comparison to 49 Mbp of randomly sampled genomic data (from 454 shotgun sequencing) of the *T. sirtalis* genome. These data suggest that both CR1 LINEs, as well as RTE/Bov-B LINEs appear relatively abundant and active in the garter snake genome (Figure 2). Because there are no reptile-specific repeat element libraries in RepBase, the RepeatMasker identification of elements (based on using the tetrapoda repeat library in RepBase) presented here is likely a substantial underestimate of repeat content, and is expected to identify only repeat elements in reptiles with sequence similarity to those in other sequenced vertebrate genomes with complete repeat libraries. Although few SINE elements were detected based on RepeatMasker analyses (Figure 2), there are probably several classes of abundant SINEs in the garter snake genome, but they have not been identified and are either novel or too divergent to be recognized by RepBase libraries. There is also a moderate increase in the SSR and low complexity content detected in the garter snake genome (Figure 2), apparently indicating a secondary increase in SSR evolution and turnover in snakes; note that this change must have occurred subsequent to the slowdown in SSR evolution and turnover earlier in the reptilian lineage [72]. While not yet completed for the garter snake, preliminary de novo sets of repeat elements were identified and classified from the Burmese Python (*Python molurus*) and the Copperhead (*Agkistrodon contortrix*) [76]. These snake-specific element libraries, together with de novo analyses from the *Thamnophis* sample sequencing set analyzed here, will provide an excellent preliminary database of snake-specific repeat element sequences for annotating the garter snake genome. Notably, this database will be ready far in advance of the annotation phase of this project.

http://standardsingenomics.org
A proposal to sequence the genome of a garter snake

Ongoing studies of garter snake ecological & evolutionary genomics
Studying extreme adaptation and convergent evolution in snake proteins

The evolutionary origin of snakes involved extensive morphological and physiological adaptations to a subterranean lifestyle, including limb loss, the functional loss of one lung, trunk and organ elongation. They also evolved a suite of radical adaptations to consume extremely large prey relative to their body size, including the evolution of diverse venom proteins [77,78], and the ability to drastically remodel their organs and physiology [79,80] while enduring metabolic and oxygen consumption rate fluctuations that are among the most extreme known in vertebrates [80]. Previous research has shown that snake aerobic metabolism is mechanistically and functionally unique among vertebrates due to tremendous bursts of adaptive evolution that have radically altered the functions of numerous metabolic proteins [81-83]. A complete garter snake genome will allow evaluation of hypotheses of accelerated evolution, positive selection, and molecular convergence across the breadth of snake proteins. The work of Castoe and colleagues [81,82] strongly suggests that other snake proteins, in addition to the metabolic genes already studied, are likely to show evidence of extreme and rapid evolution. These patterns are also likely to provide important insight into major adaptations that have accompanied the highly dynamic and extreme metabolism and physiology of snakes. The identification of other components of snake genomes that demonstrate such coordinated adaptive phenomena would provide critical insight into the coevolution and function of vertebrate metabolism, physiology, development, and ecology, with the potential for identifying new links between molecular evolution and functional change in vertebrates.

Impact of transposable elements on snake genome evolution

Our understanding of the presence and absence of different transposable element types across vertebrate lineages remains fragmentary due to the limited sampling of vertebrate diversity, although many different types of elements, including LINEs [84-86], SINEs [75,87], and DNA transposons [76,88,89] may owe their origins to horizontal transfer. In the snakes, a number of different elements currently fit this hypothesis. This includes SPIN DNA transposons that appear to have recently invaded a number of vertebrate lineages, including Anolis, long after the split between Anolis and snakes ~170MYA [76,89]. SPIN element sequences have been found in Agkistrodon and Thamnophis, but not in Python, suggesting a possible horizontal transfer into the common ancestral lineage of the garter snakes and vipers (Castoe and Pollock, unpublished). Additionally, an apparent poxivirus-mediated transfer of a SINE element from snakes to rodents (via parasitizing the reverse transcriptase of a Bov-B LINE) has been shown, demonstrating that viruses may mediate such horizontal transfer events [87]. The most interesting case of apparent horizontal transfer of transposable elements is the Bov-B LINEs in snakes [84-86]. This is because Bov-B LINEs, together with CR1 LINEs, appear to have played a role in the evolution of snake venom and expansion of venom gene families [76]. Greater genomic resources for snakes will provide important information to evaluate and understand the modes, frequency, and potential functional consequences that horizontal transfer of genetic material has played in snake genomes, and in vertebrate genomes in general.

Genomic resources for garter snakes

BAC Library and tissue availability

A high-quality, high density BAC library has been made for the garter snake (Thamnophis sirtalis). This library is available for use by the scientific community [90] via the Joint Genome Institute. In addition to this library, there is excellent access to a great deal of additional garter snake tissues (from laboratory and natural populations) from particular labs that work heavily on the species (e.g., Brodie and Bronikowski), and from major research collections (e.g., Harvard, UC Berkeley, Smithsonian, American Museum). Furthermore, a genome sequence of a snake species will greatly increase the value of existing genetic resources for reptiles in research collections and museums.

Online and unpublished sequences

Molecular resources for reptiles are severely limiting, particularly for snakes. Very recently, however, several cDNA-based and genomic shotgun sequencing-based resources for garter snakes, and other snake species, have become available or are expected to be released in 2011. We outline these below.
The most relevant resource is a public website hosted by the Bronikowski laboratory [91]. At its core, this site contains a dataset of 1.24 million 454 FLX and Titanium reads from *T. elegans* from multiple organs and sexes [92]. This is the first large-scale, multi-organ transcriptome for an ectothermic reptile, and is the most comprehensive set of EST sequences publicly available for an individual non-avian reptile species. These reads have been assembled into 96,379 contigs, and 25% of these contigs were assigned an ID based on homology when compared to NCBI-NR, HomoloGene, UniGene (Chicken), and the draft *Anolis* lizard draft genome (AnoCar1.0). This data has additionally enabled identification of a substantial amount of allelic diversity, including 133,713 SNPs and 53,943 INDELS in 28,901 contigs (30%). This resource will assist studies on gene expression, comparative genomics, and facilitate the study of evolutionarily important traits at the molecular level, in addition to assisting in assembling gene model predictions for the garter snake genome.

There is also a relatively small amount of *T.sirtalis* genome sequence available (~49 Mbp; NCBI Sequence Read Archive accession: SRA029935) from 454 shotgun library sequencing. These, and similar data from ~10 additional snake species, will be made available online via the snakegenomics.org website [93] and accessioned at NCBI. This data will provide early access to a sampling of sequences from snake genomes that will enable identification and characterization of snake repeat elements far in advance of the garter snake genome, speeding annotation and assembly progress of the genome. Additional comparative cDNA data (454 and Illumina) for a diversity of other snakes including multiple blind snakes, the Burmese python, and venemous copperhead will be made available via snakegenomics.org [93] and accessioned at NCBI; these should further assist annotation of the garter snake genome, and be useful for comparative analyses.

**Method for genome sequencing**

**Sequencing**

The whole genome shotgun strategy provides an efficient method for producing a draft genome sequence, a process whereby each genome is a unique case that requires assembly parameter optimization to achieve the highest possible contiguity with few mis-ordering events. This method produces “unfinished” assemblies that require post-assembly manipulation, such as merging contigs and breaking erroneous scaffolds. Our strategy with the garter snake genome is to employ Illumina HiSeq sequencing of paired reads with increasing insert size. A similar strategy was recently used to assemble the human and mouse genomes [94].We are planning on collecting a total of 100× coverage of the genome overall, including 40× coverage from short length (200-300 bp) shotgun libraries, 40× coverage from 3kb paired reads, 5× coverage of 8kb paired reads, and <1× of 40kb paired reads.

**Genome assembly**

Perhaps most critical to our success will be in developing methods for integration of the assembly information with all other ancillary data resources available and our attention to detail at every step in the process. The ALLPATHS-LG will be utilized to assemble all read types using an iterative process [94]. The ALLPATHS-LG software resides on four 300 GB 10,000 RPM SAS hard drives, with eight 2.9GHz Quad-Core AMD Opteron Model 8389 processors, 512KB L1 Cache (32 processor cores total) and 512 GB of memory (consisting of 32 16 GB DDR2-667 ECC DIMM).

Most short-read assemblers rely on the de Bruijn graphical structures, a directed graph that represents homogenous overlap between sequences (see review [95]). In brief, genome assembly will involve four principal steps that progress from forming contigs from raw sequence reads, to connecting contigs into scaffolds using paired-end sequence of large fragments, to gap filling and finally error correction. A base of smaller contigs will serve as anchor points for an iterative adding of longer range insert sizes serving to build scaffold length. Gaps that exist in the scaffolds can be filled in most cases by the use of all reads. We expect longer read lengths from the third generation instrument of Pacific Biosciences to be used as needed to improve scaffold size expansion and filling of gaps within.

Although we expect a shorter contig size than the traditional Sanger based assemblies we believe these contig lengths will be sufficient for gene predictions and post-assembly alignment based analysis. From the recent human whole genome study contig (assembled *de novo*) and scaffold N50 values of 24kb and 11Mb, respectively, were achieved [94]. Moreover, high assembly accuracy
A proposal to sequence the genome of a garter snake

was obtained with the number of ambiguous bases at 0.08%. Since the garter snake genome is considerably smaller than either of these mammalian genomes and contains fewer predicted repeats, we expect assembly contiguity to be sufficient for accurate gene predictions.

Genome assembly annotation
Despite improvements in assembly algorithms, assembling genomes from millions of small sequence reads in automatic fashion is susceptible to producing errors. We will assess the accuracy of the assembled garter snake genome using several methods, these include read chaff rate, read depth of coverage, average quality values per contig, discordant read pairs, gene footprint coverage (as assessed by cDNA contigs) and comparative alignments to the most closely related species. In this proposal we also will take advantage of mapped cDNA contigs from various garter snake tissues (10 tissue types will be selected) to improve assembly contiguity and accuracy, strengthening the genic component of this assembly. Each of these metrics reveals something unique about the assembly and defines overall the strengths and weaknesses of an assembly. During our manual review of the assembly errors these are corrected when possible.

We will screen the genome assembly for contamination, and then submit the de-contaminated sequence to the WGS division of Genbank for an independent contamination analysis. A Genbank analysis typically will reveal small amounts of additional contamination due to BLAST parameter differences and the use of updated databases that are removed followed by resubmission to Genbank. The final assembly will be uploaded to multiple online databases and genome browsers, including Ensembl [96], the University of California Santa Cruz [97] and NCBI for public queries.

Gene annotation
First-pass gene prediction will use a modified Ensembl pipeline [98], for evidence-supported gene model building and model merging. Uniprot protein sequences from several species will be used sequentially as seeds for coding sequence prediction. In addition, cDNA sequences from the garter snake will be aligned and used to find genes and add UTR information. The consortium will select 10 diverse tissues for Illumina RNA sequencing. A portion of the Ensembl mandate is to work directly with genome sequencing projects, and use custom-curated data sets (such as EST sequences and specific Uniprot data sets) to enable annotation. Should other groups provide gene sets with independent gene prediction algorithms, the Ensembl group can easily merge these gene predictions into a unique set of predicted genes. We have successfully followed this paradigm for the many other species.

Promise of garter snakes for ecological and comparative genomics
Promise as a model system in physiology
Reptiles possess many adaptations related to mortality selection that suggest their usefulness in studies that link morphological and physiological evolution. Snakes in particular, have evolved venom, limblessness, extended metabolic shutdown– including both hibernation and estivation, starvation resistance, heat tolerance and hypoxia resistance. Furthermore, although species possess species-specific lifespans, little physiological decline occurs with advancing age in snakes until near the end of their lifespans. Thus, some snakes may indeed exhibit the phenomenon known as negligible senescence [99] – the lack of age-related deterioration. Furthermore, many snake species have indeterminate growth and fecundity such that costs of reproduction– at least with respect to the lifespan – are not apparent [100]. The oldest individuals in natural populations are often the most fecund and robust [101]. Therefore, in many snakes, strong selection against late-age deleterious mutations may exist, thereby leading to increased longevity and longer reproductive life spans.

Snakes (and other reptiles) are also a model for the trade-off between life span and reproduction [102] because they have evolved plastic responses to external stresses and, putatively, plastic modulation of cell signaling pathways. Ectothermic reptiles have different physiological and cellular responses to environmental and metabolic stress, relative to endotherms. This may be driven by the reptilian ability to regulate metabolic function by behaviorally modulating their body temperature, which results in lower energy requirements than birds and mammals that must use their metabolism to maintain higher body temperatures. Many reptilian adaptations to environmental stress are known to activate molecu-
lar pathways linked to mechanistic theories of aging – e.g., the free radical theory of aging (and its derivations) – which provides a priori predictions of outcomes for stress-response experiments [99].

There are only a handful of species for which the in-depth understanding of life-history, physiology, behavior, and quantitative genetics allows for the examination, and elucidation, of molecular pathways, and linking of these pathways across amniotes by leveraging comparative genomics. The garter snake is one such species, and will yield insights into the evolution of stress response. This is a particularly exciting venture as it is recently apparent that the molecular mechanisms underlying the complex traits of life history, stress response, and metabolism are controlled by evolutionarily conserved, and equally complex, molecular networks [103].

The importance of the garter snake for comparative genomics and annotating the human genome

To understand genome diversity and evolution in amniotes, it is currently possible to compare only the human and other mammalian genomes with a small number of avian genomes, and a single lizard genome (Anolis). This narrowly focused comparison is largely inadequate for illuminating the evolutionary origins and history of amniote genomes because it omits the many lineages of reptiles that arose since birds and mammals diverged more than 300MYA. It is therefore nearly impossible to identify a trait that distinguishes mammals from other amniotes, and what is merely a trait specific to birds. A well-rounded understanding of vertebrate genome evolution and diversity, therefore, must include comparative data for more lineages spanning the diversity of reptiles, and vertebrates in general. Thus, in addition to providing a long-sought window into the genetic underpinnings of variation and unique traits in snakes, the garter snake genome also will be a critical comparative resource for vertebrate genomics in general.

Snakes represent a major ~170-million-year-old lineage on the branch of the vertebrate tree of life for which very little genomic information is currently available. As such, understanding the content of snake genomes will contribute broadly to an understanding of vertebrate genomics. Reptilia is the sister group of Mammalia, and 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 and finch genomes, but it would also assist in rooting the many 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.

Conclusion

A garter snake genome would enable numerous avenues of research in basic physiology, ecology, evolutionary biology and comparative genomics. It would provide important insights into the evolution of limblessness, antitoxin resistance, ectothermy and extreme physiology and metabolism. It would provide a genome for a critically important lineage of amniotes and would improve the accuracy of reconstruction of the ancestral vertebrate genome, a major goal that will ultimately improve our understanding of the human genome. Finally, with its relatively small size and novel landscape of transposable elements, a garter snake genome would provide new insights into the diversity of repeated elements and their roles in evolution.

References

1. Li R, Fan W, Tian G, Zhu H, He L, Cai J, Huang Q, Cai Q, Li B, Bai Y, et al. The sequence and de novo assembly of the giant panda genome. Nature 2010; 463:311-317. PubMed doi:10.1038/nature08696

2. Fitch HS. A biogeographical study of the ordinoides artenkreis of garter snakes (genus Thamnophis). Univ Calif Publ Zool 1940; 44:1-150.

3. Fox W. The relationships of the garter snake Thamnophis ordinoides. Copeia 1948:113-120 doi:10.2307/1438415.

4. Fox W. Relationships among the garter snakes of the Thamnophis elegansrassenkreis. Univ Calif Publ Zool 1951; 50:485-529.

5. de Queiroz A, Lawson R, Lemos-Espinal JA. Phylogenetics relationships of North American garter snakes (Thamnophis) based on four mitochondrial
A proposal to sequence the genome of a garter snake

genes: How much DNA sequence is enough? Mol Phylogenet Evol 2002; 22:315-329. PubMed doi:10.1006/mpev.2001.1074

6. Janzen FJ, Krenz JG, Haselcorn TS, Brodie ED, Jr., Brodie ED, III. Molecular phylogeography of common garter snakes (Thamnophis sirtalis) in Western North America: Implications for regional forces. Mol Ecol 2002; 11:1739-1751. PubMed doi:10.1046/j.1365-294X.2002.01571.x

7. Alfaro ME, Arnold SJ. Molecular systematics and evolution of Regina and the thamnophiine snakes. Mol Phylogenet Evol 2001; 21:408-423. PubMed doi:10.1006/mpev.2001.1024

8. Fitzpatrick BM, Placyk JS, Niemiller ML, Casper GS, Burghardt GM. Distinctiveness in the face of gene flow: hybridization between specialist and generalist gartersnakes. Mol Ecol 2008; 17:4107-4117. PubMed doi:10.1111/j.1365-294X.2008.03885.x

9. Jayne BC, Bennett AF. Scaling of speed and endurance in garter snakes: a comparison of cross-sectional and longitudinal allometries. J Zool (Lond) 1990; 220:257-277. doi:10.1111/j.1469-7998.1990.tb04307.x

10. Arnold SJ, Bennett AF. Behavioural variation in natural populations. III: antipredator displays in the garter snake, Thamnophis radix. Anim Behav 1984; 32:1108-1118. doi:10.1016/S0003-3472(84)80227-4

11. Bennett AF. Individual correlation of morphology, muscle mechanics, and locomotion in a salamander. American Physiological Society 1989;R1200-R1208.

12. Arnold SJ. Behavioral variation in natural populations. I. Phenotypic, genetic and environmental correlations between chemoreceptive responses to prey in the garter snake, Thamnophis elegans. Evolution 1981; 35:489-509. doi:10.2307/2408197

13. Arnold SJ. Behavioural variation in natural populations. VI. Prey responses by two species of garter snakes in three regions of sympathy. Anim Behav 1992; 44:705-719. doi:10.1016/S0003-3472(05)80297-0

14. Manier MK, Seyler CM, Arnold SJ. Adaptive divergence within and between ecotypes of the terrestrial garter snake, Thamnophis elegans, assessed with F-ST-Q(ST) comparisons. J Evol Biol 2007; 20:1705-1719. PubMed doi:10.1111/j.1420-9101.2007.01401.x

15. Gregory PT. Feeding habits and diet overlap of three species of garter snakes (thamnophis) on Vancouver Island. Can J Zool 1978; 56:1967-1974. doi:10.1139/z78-264

16. Kephart DG, Arnold SJ. Garter snake diets in a fluctuating environment: a seven year study. Ecology 1982; 63:1232-1236. doi:10.2307/1938848

17. Lutterschmidt DJ, Mason RT. Geographic Variation in Timekeeping Systems among Three Populations of Garter Snakes (Thamnophis sirtalis) in a Common Garden. Physiol Biochem Zool 2008; 81:810-825. PubMed doi:10.1086/589900

18. Huey RB, Peterson CR, Arnold SJ, Porter WP. Hot rocks and not-so-hot rocks - retreat-site selection by garter snakes and its thermal consequences. Ecology 1989; 70:931-944. doi:10.2307/1941360

19. Brodie ED, III. Correlational selection for color pattern and antipredator behavior in the garter snake Thamnophis ordinoides. Evolution 1992; 46:1284-1298. doi:10.2307/2409937

20. Brodie ED, III. Homogeneity of the genetic variance-covariance matrix for antipredator traits in two populations of the garter snake Thamnophis sordidines. Evolution 1993; 47:844-854. doi:10.2307/2410188

21. Arnold SJ. Quantitative genetics and selection in natural populations: Microevolution of vertebral numbers in the garter snake Thamnophis elegans. In: Weir BS, Eisen EJ, Goodman MM, Namkoong G, editors. Proceedings of the Second International Conference on Quantitative Genetics. Sunderland, Mass: Sinauer; 1988. p 619-636.

22. Jayne BC, Bennett AF. Selection on locomotor performance capacity in a natural population of garter snakes. Evolution 1990; 44:1204-1229. doi:10.2307/2409283

23. Cohn MJ, Tickle C. Developmental basis of limblessness and axial patterning in snakes. Nature 1999; 399:474-479. PubMed doi:10.1038/20944

24. Burke AC, Nelson CE, Morgan BA, Tabin C. Hox genes and the evolution of vertebrate axial morphology. Development 1995; 121:333-346. PubMed

25. Wellik DM, Capecchi MR. Hox10 and Hox11 genes are required to globally pattern the mammalian skeleton. Science 2003; 301:363-367. PubMed doi:10.1126/science.1085672

26. Gomez C, Ozbudak EM, Wunderlich J, Baumann D, Lewis J, Pourquie O. Control of segment number in vertebrate embryos. Nature 2008; 454:335-339. PubMed doi:10.1038/nature07020
27. Thewissen JG, Cohn MJ, Stevens LS, Bajpai S, Heyning J, Horton WE, Jr. Developmental basis for hind-limb loss in dolphins and origin of the cetacean bodyplan. *Proc Natl Acad Sci USA* 2006; **103**:8414-8418. PubMed doi:10.1073/pnas.0602920103

28. Chan YF, Marks ME, Jones FC, Villarreal G, Jr., Shapiro MD, Brady SD, Southwick AM, Absher DM, Grimwood J, Schmutz J, *et al*. Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a Pitx1 enhancer. *Science* 2010; **327**:302-305. PubMed doi:10.1126/science.1182213

29. Shapiro MD, Bell MA, Kingsley DM. Parallel genetic origins of pelvic reduction in vertebrates. *Proc Natl Acad Sci USA* 2006; **103**:13753-13758. PubMed doi:10.1073/pnas.0604706103

30. Crews D, Garstka WR. The ecological physiology of a garter snake. *Sci Am* 1982; **247**:158-168. doi:10.1038/scientificamerican1182-158

31. Crews D, Camazine B, Diamond M, Mason R, Tokarz RR, Garstka WR. Hormonal independence of courtship behavior in the male garter snake. *Horm Behav* 1984; **18**:29-41. PubMed doi:10.1016/0018-506X(84)90048-5

32. Whittier JM, Mason RT, Crews D. Mating in the red-sided garter snake, thamnophis-sirtalis-parietalis - differential-effects on male and female sexual-behavior. *Behav Ecol Sociobiol* 1985; **16**:257-261. doi:10.1007/BF00310989

33. Whittier JM, Mason RT, Crews D. Plasma steroid-hormone levels of female red-sided garter snakes, thamnophis-sirtalis-parietalis - relationship to mating and gestation. *Gen Comp Endocrinol* 1987; **67**:33-43. PubMed doi:10.1016/0016-6480(87)90202-4

34. Whittier JM, Mason RT, Crews D, Licht P. Role of light and temperature in the regulation of reproduction in the red-sided garter snake, thamnophis-sirtalis-parietalis. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 1987; **65**:2090-2096. doi:10.1139/z87-320

35. Nelson RJ, Mason RT, Krohmer RW, Crews D. Pinealobectom blocks vernal courtship behavior in red-sided garter snakes. *Physiol Behav* 1987; **39**:231-233. PubMed doi:10.1016/0031-9384(87)90014-X

36. Mendonça MT, Tousignant AJ, Crews D. Pinealecctomy, melatonin, and courtship behavior in male red-sided garter snakes (*Thamnophis sirtalis parietalis*). *J Exp Zool* 1996; **274**:63-74. PubMed doi:10.1002/(SICI)1097-010X(19960101)274:1<63::AID-JEZ7>3.0.CO;2-D

37. Crews D, Robker R, Mendonca M. Seasonal fluctuations in brain nuclei in the red-sided garter snake and their hormonal-control. *J Neurosci* 1993; **13**:5356-5364. PubMed

38. Mendonça MT, Crews D. Effects of ovariectomy and estrogen replacement on attractiveness and receptivity in the red sided garter snake (Thamnophis sirtalis parietalis). *Journal of Comparative Physiology a-Sensory Neural and Behavioral Physiology* *Journal of comparative physiology A Sensory neural and behavioral physiology* 1996; **178**:373-381.

39. Mendonça MT, Tousignant AJ, Crews D. Seasonal changes and annual variability in daily plasma melatonin in the red-sided garter-snake (Thamnophis sirtalis-parietalis). *Gen Comp Endocrinol* 1995; **100**:226-237. PubMed doi:10.1006/gcen.1995.1152

40. Mendonça MT, Tousignant AJ, Crews D. Courting and noncourting male red-sided garter snakes, Thamnophis sirtalis parietalis: Plasma melatonin levels and the effects of pinealecetomy. *Horm Behav* 1996; **30**:176-185. PubMed doi:10.1006/hbeh.1996.0022

41. Jayne BC, Bennett AF. The effect of tail morphology on locomotor performance of snakes: a comparison of experimental and correlational methods. *J Exp Zool* 1989; **252**:126-133. doi:10.1002/jez.1402520204

42. Walton M, Jayne BC, Bennett AF. The energetic cost of limbless locomotion. *Science* 1990; **249**:524-527. PubMed doi:10.1126/science.249.4968.524

43. Arnold SJ, Phillips PC. Hierarchical comparison of genetic variance-covariance matrices. II. Coastal-inland divergence in the garter snake *Thamnophis elegans*. *Evolution* 1999; **53**:1516-1527. doi:10.2307/2640897

44. Kelley KC, Arnold SJ, Gladstone J. The effects of substrate and vertebral number on locomotion in the garter snake *Thamnophis elegans*. *Funct Ecol* 1997; **11**:189-198. doi:10.1046/j.1365-2435.1997.00077.x

45. Garland T, Jr., Bennett AF. Quantitative genetics of maximal oxygen consumption in a garter snake. *Am J Physiol* 1990; **259**:R986-R992. PubMed

46. Dohm MR, Garland TJ. Quantitative genetics of scale counts in the garter snake *Thamnophis sirtalis*. *Copeia* 1993; **1993**:987-1002. doi:10.2307/1447076
A proposal to sequence the genome of a garter snake

47. Mason RT. Reptilian Pheromones. In: Gans C, Crews D, editors. Hormones, Brain, and Behavior. Biology of the Reptilia. Physiology E, vol 18. Chicago: University of Chicago Press; 1992. p 114-228.

48. Parker MR, Mason RT. Low Temperature Dormancy Affects the Quantity and Quality of the Female Sexual Attractiveness Pheromone in Red-sided Garter Snakes. J Chem Ecol 2009; 35:1234-1241. PubMed doi:10.1007/s10886-009-9699-0

49. Mason RT, Crews D. Female mimicry in garter snakes. Nature 1985; 316:59-60. PubMed doi:10.1038/316059a0

50. Mason RT, Crews D. Pheromone mimicry in garter snakes. In: Duvall D, Muller-Schwarze D, Silversstein RM, editors. Chemical Signals In Vertebrates 4: Plenum Publishing Corporation; 1986.

51. Shine R, Phillips B, Waye H, LeMaster MTMR. Benefits of female mimicry in snakes. Nature 2001; 414:267. PubMed doi:10.1038/35104687

52. Halpern M. Nasal chemical senses in reptiles: structure and function. In: Gans C, Crews D, editors. Hormones, Brain, and Behavior. Biology of the Reptilia. Physiology E, vol 18. Chicago: University of Chicago Press; 1992. p 423-523.

53. Halpern M. Nasal chemical senses in snakes. In: Ewert JP, Capranica RR, Ingle DJ, editors. Advances in Vertebrate Neuroethology. New York: Plenum Press; 1983. p 141-176.

54. Halpern M, Kubie JL. Chemical access to the vomeronasal organs of garter snakes. Physiol Behav 1980; 24:367-371. PubMed doi:10.1016/0031-9384(80)90100-6

55. Brodie ED, III, Brodie ED, Jr. Predator-prey arms races. Bioscience 1999; 49:557-568. doi:10.2307/1313476

56. Brodie ED, Jr., Ridenhour BJ, Brodie ED, III. The evolutionary response of predators to dangerous prey: Hotspots and coldspots in the geographic mosaic of coevolution between newts and snakes. Evolution 2002; 56:2067-2082. PubMed

57. Hanifin CT, Brodie ED. Phenotypic mismatches reveal escape from arms-race coevolution. PLoS Biol 2008; 6:e60. PubMed doi:10.1371/journal.pbio.0060060

58. Feldman CR, Brodie ED, Brodie ED, Jr. Pfalse RE. The evolutionary origins of beneficial alleles during the repeated adaptation of garter snakes to deadly prey. Proc Natl Acad Sci USA 2009; 106:13415-13420. PubMed doi:10.1073/pnas.0901224106

59. Geffeney SL, Fujimoto E, Brodie ED, III, Brodie ED, Jr., Ruben PC. Evolutionary diversification of TTX-resistant sodium channels in a predator-prey interaction. Nature 2005; 434:759-763. PubMed doi:10.1038/nature03444

60. Geffeney S, Ruben PC, Brodie ED, Jr., Brodie ED, III. Mechanisms of adaptation in a predator-prey arms race: TTX resistant sodium channels. Science 2002; 297:1336-1339. PubMed doi:10.1126/science.1074310

61. Herzog HA, Jr., Bowers BB, Burghardt GM. Development of antipredator responses in snakes: V. Species differences in ontogenetic trajectories. Dev Psychobiol 1992; 25:199-211. PubMed doi:10.1002/dev.420250305

62. Herzog JHA, Burghardt GM. Development of antipredator responses in snakes III. Stability of individual and litter differences over the first year of life. Ethology 1988; 77:250-258. doi:10.1111/j.1439-0310.1988.tb00208.x

63. Herzog HA, Jr., Burghardt GM. Development of antipredator responses in snakes I. Defensive and open-field behaviors in newborns and adults of three species of garter snakes (Thamnophis melanolagaster, T. sirtalis, T. butleri). J Comp Psychol 1986; 100:372-379. doi:10.1037/0735-7036.100.4.372

64. Burghardt GM. Behavioral ontogeny in reptiles: whence, whither, and why? The Development of Behavior: Comparative and Evolutionary Aspects. New York: Garland STPM Press; 1978. p 149-174.

65. Burghardt GM. Influence of prenatal maternal experience. Science 1971; 171:921-923. PubMed doi:10.1126/science.171.3974.921

66. Burghardt GM. Intraspecific geographical variation in chemical cue preferences of newborn garter snakes (Thamnophis sirtalis). Behaviour 1970; 36:246-257. doi:10.1163/156853970X00330

67. Burghardt GM. Chemical cue preferences of inexperienced snakes: comparative aspects. Science 1967; 157:718-721. PubMed doi:10.1126/science.157.3789.718

68. Gregory TR, Nicol JA, Tamm H, Kullman B, Kullman K, Leitch IJ, Murray BG, Kapraun DF, Greilhuber J, Bennett MD. Eukaryotic genome size databases. Nucleic Acids Res 2007; 35(Database issue):D332-D338. PubMed doi:10.1093/nar/gkl828

69. Vinogradov AE. Genome size and GC-percent in vertebrates as determined by flow cytometry: the triangular relationship. Cytometry 1998; 31:100-109. PubMed doi:10.1002/(SICI)1097-
70. Matsubara K, Tarui H, Toriba M, Yamada K, Nishida-Umehara C, Agata K, Matsuda Y. Evidence for different origin of sex chromosomes in snakes, birds, and mammals and step-wise differentiation of snake sex chromosomes. Proc Natl Acad Sci USA 2006; 103:18190-18195. PubMed doi:10.1073/pnas.0605274103

71. Organ CL, Moreno RG, Edwards SV. Three tiers of genome evolution in reptiles. Integr Comp Biol 2008; 48:494-504. doi:10.1093/icb/icn046

72. Shedlock AM, Botka CW, Zhao S, Shetty J, Zhang T, Liu JS, Deschavanne PJ, Edwards SV. Phylogenomics of nonavian reptiles and the structure of the ancestral amniote genome. Proc Natl Acad Sci USA 2007; 104:2767-2772. PubMed doi:10.1073/pnas.0606204104

73. Kordis D. Transposable elements in reptilian and avian (sauropsida) genomes. Cytogenet Genome Res 2009; 127:94-111. PubMed doi:10.1159/000294999

74. Novick PA, Basta H, Floumanhaft M, McClure MA, Boissinot S. The evolutionary dynamics of autonomous non-LTR retrotransposons in the lizard Anolis carolinensis shows more similarity to fish than mammals. Mol Biol Evol 2009; 26:1811-1822. PubMed doi:10.1093/molbev/msp090

75. Piskurek O, Nishihara H, Okada N. The evolution of two partner LINE/SINE families and a full-length chromodomian-containing Ty3/Gypsy LTR element in the first reptilian genome of Anolis carolinensis. Gene 2009; 441:111-118. PubMed doi:10.1016/j.gene.2008.11.030

76. Castoe TA, Hall K, Guibotsy Mboulos ML, Gu W, de Koning AP, Poole AW, Vemulapalli V, Daza JM, Feschotte C, Pollock DD. Discovery of highly divergent repeat landscapes in snake genomes using high throughput sequencing. Genome Biol Evol (In press).

77. Fry BG, Vidal N, Norman JA, Vonk FJ, Scheib H, Ramjan SFR, Kuruppu S, Fung K, Hedges SB, Richardson MK, et al. Early evolution of the venom system in lizards and snakes. Nature 2006; 439:584-588. PubMed doi:10.1038/nature04328

78. Ikeda N, Chijiwa T, Matsubara K, Oda-Ueda N, Hattori S, Matsuda Y, Ohno M. Unique structural characteristics and evolution of a cluster of venom phospholipase A(2) isozyme genes of Protobothrops flavoviridis snake. Gene 2010; 461:15-25 PubMed doi:10.1016/j.gene.2010.04.001

79. Secor SM, Diamond J. Adaptive responses to feeding in Burmese pythons: pay before pumping. J Exp Biol 1995; 198:1313-1325. PubMed

80. Secor SM, Diamond J. A vertebrate model of extreme physiological regulation. Nature 1998; 395:659-662. PubMed doi:10.1038/27131

81. Castoe TA, de Koning AP, Kim HM, Gu W, Noonan BP, Naylor G, Jiang ZJ, Parkinson CL, Pollock DD. Evidence for an ancient adaptive episode of convergent molecular evolution. Proc Natl Acad Sci USA 2009; 106:8986-8991. PubMed doi:10.1073/pnas.0900233106

82. Castoe TA, Jiang ZJ, Gu W, Wang ZO, Pollock DD. Adaptive evolution and functional redesign of core metabolic proteins in snakes. PLoS ONE 2008; 3:e2201. PubMed doi:10.1371/journal.pone.0002201

83. Jiang ZJ, Castoe TA, Austin CC, Burbrink FT, Herron MD, McGuire JA, Parkinson CL, Pollock DD. Comparative mitochondrial genomics of snakes: extraordinary substitution rate dynamics and functionality of the duplicate control region. BMC Evol Biol 2007; 7:123. PubMed doi:10.1186/1471-2148-7-123

84. Kordis D, Gubensek F. Bov-L long interspersed repeated DNA (LINE) sequences are present in Viperidae snakes. Eur J Biochem 1997; 246:772-779. PubMed doi:10.1111/j.1432-1033.1997.00772.x

85. Kordis D, Gubensek F. Unusual horizontal transfer of a long interspersed nuclear element between distant vertebrate classes. Proc Natl Acad Sci USA 1998; 95:10704-10709. PubMed doi:10.1073/pnas.95.18.10704

86. Kordiš D, Gubensek F. Horizontal transfer of non-LTR retrotransposons in vertebrates. Genetica 1999; 107:121-128. PubMed doi:10.1023/A:1004082906518

87. Piskurek O, Okada N. Poxviruses as possible vectors for horizontal transfer of retroelements from reptiles to mammals. Proc Natl Acad Sci USA 2007; 104:12046-12051. PubMed doi:10.1073/pnas.0700531104

88. Gilbert C, Schaack S, Pace JK, II, Brindley PJ, Feschotte C. A role for host-parasite interactions in the horizontal transfer of transposons across phyla. Nature 2010; 464:1347-1350. PubMed doi:10.1038/nature08939

89. Pace JK, II, Gilbert C, Clark MS, Feschotte C. Repeated horizontal transfer of a DNA transposon in
A proposal to sequence the genome of a garter snake. *Proc Natl Acad Sci USA* 2008; **105**:17023-17028. [PubMed](https://doi.org/10.1073/pnas.0806548105)

90. National Human Genome Research Institute. Other sources of bacterial artificial chromosome (BAC) libraries. [http://www.genome.gov/11008350](http://www.genome.gov/11008350)

91. Bronikowski laboratory. [http://eco.bcb.iastate.edu](http://eco.bcb.iastate.edu)

92. Schwartz TS, Tae H, Yang Y, Mockaitis K, Van Hemert JL, Proulx SR, Choi JH, Bronikowski AM. A garter snake transcriptome: pyrosequencing, de novo assembly, and sex-specific differences. *BMC Genomics* 2010; **11**:694. [PubMed](https://doi.org/10.1186/1471-2164-11-694)

93. Castoe TA. A Consortium for Snake Genomics. [http://www.snakegenomics.org](http://www.snakegenomics.org).

94. Gnerre S, Maccallum I, Przybylski D, Ribeiro FJ, Burton JN, Walker BJ, Sharpe T, Hall G, Shea TP, Sykes S, *et al*. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. *Proc Natl Acad Sci USA* 2011; **108**:1513-1518. [PubMed](https://doi.org/10.1073/pnas.1017351108)

95. Flicek P, Birney E. Sense from sequence reads: methods for alignment and assembly. *Nat Methods* 2009; **6**(Suppl):S6-S12. [PubMed](https://doi.org/10.1038/nmeth.1376)

96. Flicek P, Amode MR, Barrell D, Beal K, Brent S, Chen Y, Clapham P, Coates G, Fairley S, Fitzgerald S, *et al*. Ensembl 2011. *Nucleic Acids Res* 2011; **39**:D800-D806. [PubMed](https://doi.org/10.1093/nar/gkq1064)

97. Fujita PA, Rhead B, Zweig AS, Hinrichs AS, Karolchik D, Cline MS, Goldman M, Barber GP, Clawson H, Coelho A, *et al*. The UCSC Genome Browser database: update 2011. *Nucleic Acids Res* 2011; **39**(Database issue):D557-D562. [PubMed](https://doi.org/10.1093/nar/gkp972)

98. Finch CE, Pike MC, Witten M. Slow mortality rate accelerations during aging in some animals approximate that of humans. *Science* 1990; **249**:902-905. [PubMed](https://doi.org/10.1126/science.2392680)

99. Flatt T, Schmidt PS. Integrating evolutionary and molecular genetics of aging. *Biochim Biophys Acta* 2009; **1790**:951-962. [PubMed](https://doi.org/10.1016/j.bbabio.2009.08.015)

100. Harshman LG, Zera AJ. The cost of reproduction: the devil in the details. *Trends Ecol Evol* 2007; **22**:80-86. [PubMed](https://doi.org/10.1016/j.tree.2006.10.008)

101. Sparkman AM, Arnold SJ, Bronikowski AM. An empirical test of evolutionary theories for reproductive senescence and reproductive effort in the garter snake *Thamnophis elegans*. Proceedings of the Royal Society B-Biological Sciences 2007;274(1612):943-950.

102. Bronikowski AM. The evolution of aging phenotypes in snakes: a review and synthesis with new data. *Age (Omaha)* 2008; **30**:169-176. [PubMed](https://doi.org/10.1007/s11357-008-9060-5)

Scientists who have provided comments or letters of support for sequencing the garter snake genome

**Scientists who have provided supporting letters**

Eric N. Smith (University of Texas at Arlington), Andre Pires da Silva (University of Texas at Arlington), Chris Parkinson (University of Central Florida), Frank Burbrink (CUNY – Staten Island), Brice Noonan (University of Mississippi), Stephen Secor (University of Alabama), Blair Hedges (Pennsylvania State University), Steve Mackessy (University of Northern Colorado), Lisle Gibbs (Ohio State University), John Weins (SUNY – Stony Brook), Andrew Shedlock (Harvard University), Bob Mason (Oregon State University), Dan Brown (University of Florida), Deb Leutterschmidt (Georgia State University), Gordon Burghardt (University of Tennessee)

**Additional Supporting Scientists**

Jonathan Losos (Harvard University), Patrick Weatherhead (University of Illinois at Urbana-Champaign), Edward Braun (University of Florida), Bryan Fry (University of Melbourne), Jack Sites (Brigham Young University), Ulrich Kuch (Senkenberg Museum, Frankfurt), Cedric Feschotte (University of Texas at Arlington), A.P. Jason de Koning (University of Colorado School of Medicine), Anita Molhotra (University of Wales, Bangor), David B. Wake (University of California, Berkeley), Tod Reeder (San Diego State University), Roger S Thorpe (University of Wales, Bangor), Peter Uetz (J. Craig Venter Institute)