

## AVIAN GENOMICS

# Three crocodylian genomes reveal ancestral patterns of evolution among archosaurs

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**INTRODUCTION:** Crocodylians and birds are the two extant clades of archosaurs, a group that includes the extinct dinosaurs and pterosaurs. Fossils suggest that living crocodylians (alligators, crocodiles, and gharials) have a most recent common ancestor 80 to 100 million years ago. Extant crocodylians are notable for their distinct morphology, limited intraspecific variation, and slow karyotype evolution. Despite their unique biology and phylogenetic position, little is known about genome evolution within crocodylians.

**RATIONALE:** Genome sequences for the American alligator, saltwater crocodile, and Indian gharial—representatives of all three extant crocodylian families—were obtained to facilitate better understanding of the unique biology of this group and provide a context for studying avian genome evolution. Sequence data from these three crocodylians and birds also allow reconstruction of the ancestral archosaurian genome.

**RESULTS:** We sequenced shotgun genomic libraries from each species and used a variety of assembly strategies to obtain draft genomes for these three crocodylians. The assembled scaffold N50 was highest for the alligator (508 kilobases). Using a panel of

reptile genome sequences, we generated phylogenies that confirm the sister relationship between crocodiles and gharials, the relationship with birds as members of extant Archosauria, and the outgroup status of turtles relative to birds and crocodylians.

We also estimated evolutionary rates along branches of the tetrapod phylogeny using two approaches: ultraconserved element-anchored sequences and fourfold degenerate sites within stringently filtered orthologous gene alignments. Both analyses indicate that the rates of base substitution along the crocodylian and turtle lineages are extremely low. Supporting observations were made for transposable element content and for gene family evolution. Analysis of whole-genome alignments across a panel of reptiles and mammals showed that the rate of accumulation of micro-insertions and micro-deletions is proportionally lower in crocodylians, consistent with a single underlying cause of a reduced rate of evolution-

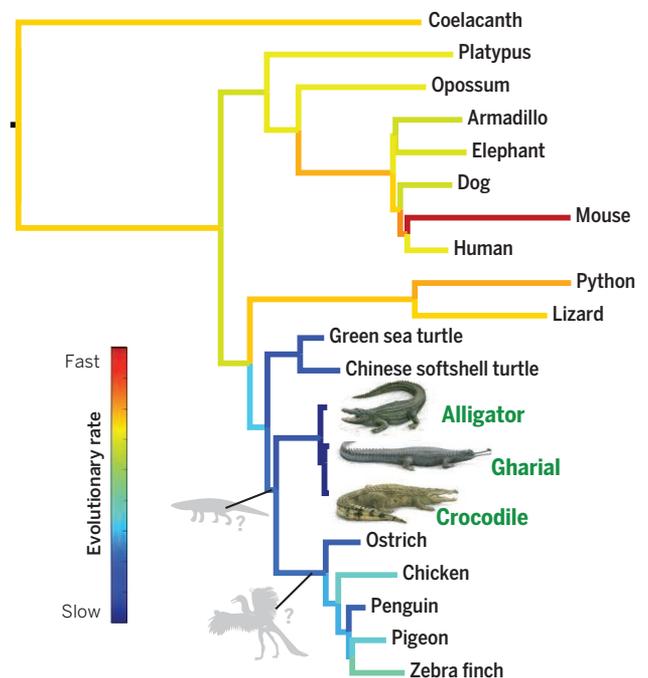
ary change rather than intrinsic differences in base-repair machinery. We hypothesize that this single cause may be a consistently longer generation time over the evolutionary history of Crocodylia.

Low heterozygosity was observed in each genome, consistent with previous analyses, including the Chinese alligator. Pairwise sequential Markov chain analysis of regional heterozygosity indicates that during glacial cycles of the Pleistocene, each species suffered reductions in effective population size. The reduction was especially strong for the American alligator, whose current range extends farthest into regions of temperate climates.

**CONCLUSION:** We used crocodylian, avian, and outgroup genomes to reconstruct 584 megabases of the archosaurian common ancestor genome and the genomes of key ancestral nodes. The estimated accuracy of the archosaurian genome reconstruction is 91% and is higher for conserved regions such as genes. The reconstructed genome can be improved by adding more crocodylian and avian genome assemblies and may provide a unique window to the genomes of extinct organisms such as dinosaurs and pterosaurs. ■

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**Evolutionary rates of tetrapods inferred from DNA sequences anchored by ultraconserved elements.** Evolutionary rates among reptiles vary, with especially low rates among extant crocodylians but high rates among squamates. We have reconstructed the genomes of the common ancestor of birds and of all archosaurs (shown in gray silhouette, although the morphology of these species is uncertain).

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Cite this article as R. E. Green et al., *Science* 346, 1254449 (2014). DOI: 10.1126/science.1254449