CROCODYLIAN GENETICS AND GENOMICS: THE LAST FIVE YEARS

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The last five years have witnessed a tremendous increase in the numbers and kinds of studies dealing with crocodylian systematics, population genetics, immunology and at long last genomics. I will be talking about perspectives and approaches that are now being employed, not emphasizing specific studies (which we will hear a great deal about over the next couple of days), but rather the kinds of molecular markers and analyses that being used to better understand crocodylian biology. I will also discuss some of the problems that are impacting molecular biologists studying this group of organisms and try to predict what we may expect to see in crocodylian molecular research over the next decade.

REPTILIAN GENOMICS - WHEREFORE ART THOU?

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Reptiles are the sister group of mammals and thus represent a phylogenetic group uniquely able to inform inferences of the mammalian genome. The genome of the first non-avian reptile has recently been sequenced by whole genome shotgun sequencing, assembled and released (the Green Anole, Anolis carolinensis). Reptiles have unique challenges relative to many mammalian groups, especially for genome mapping. In this presentation, we will: 1) give a bit of history about the reptilian genome projects, 2) cover some of the unique aspects of reptiles relative to mammals for genome mapping and characterization, and 3) provide some information to guide future efforts to obtain a crocodilian genome sequence.

DEVELOPMENT OF MICROSATELLITE DNA LOCI FOR CROCODILLIANS (OR ANY OTHER EUKARYOTE)

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We will present techniques to construct genomic DNA libraries highly enriched for microsatellite DNA loci. The techniques used here derive from the protocol of Glenn & Schable (2005, Methods in Enzymology 395:202-222), which have been optimized and tested in our research labs as well as classroom settings. Reducing the number of manipulations involved has been a key to success, decreasing both the failure rate and the time necessary to isolate loci of interest. During the past 5 years, we have used these protocols to isolate microsatellite DNA loci from more than 125 species representing three kingdoms. Using these protocols, the total time to identify candidate loci for primer development from most eukaryotic species can be accomplished in as little as one week and is routinely done in 2-3 weeks in our lab. The techniques are also scalable, and have now been used to isolate hundreds of microsatellite loci from Crocodylus porosus. If any researchers require microsatellite DNA from any crocodilian species for which primers are not available (e.g., Gavialis and Tomistoma), they need only let us know – we will be happy to isolate any loci needed.
THE APPLICATION OF QUANTITATIVE GENETICS IN COMMERCIAL CROCODILE FARMING.

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Quantitative genetic parameters for economically-important traits have been estimated for known breeding crocodiles at Darwin Crocodile Farm in the Northern Territory, Australia. The repeatability estimate for the number of viable hatchlings produced (r = 0.67 SE 0.06) was significantly different to the previously reported estimate (Isberg et al. 2004; r = 0.34 SE 0.02), whilst heritability estimates for inventory head length (indicator of growth rate; h² = 0.55 SE 0.16) and scale rows (h² = 0.43 SE 0.04) were quite robust in comparison. The heritability estimate for juvenile survival (h² = 0.70 SE 0.10) was significantly higher than previous reported, although a clutch effect was not able to be included due to confounding of the current dataset. Phenotypic data from this pedigree will be combined with genotypic data (generated by Miles et al. 2007; this proceedings) for quantitative trait loci (QTL) analysis. It is hoped that QTL will be identified to assist saltwater crocodile producers more accurately select future breeding animals.

CROCODILIAN IMMUNOLOGY RESEARCH: CURRENT STATE AND FUTURE DIRECTIONS

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Crocodilians often exhibit territorial disputes which can result in serious injuries. However, despite the fact that these animals inhabit aqueous environments rich in potentially pathogenic microbes, these injuries generally heal without serious infection. For this reason, our research is focused on the crocodilian immune system. We are involved in the investigation of a broad spectrum of projects covering the investigation of the innate and adaptive immune systems of the American alligator. We are engaging a proteomics approach to assess the systemic and leukocyte-specific differential protein expression induced during the inflammatory response. Other projects are aimed at assessing the ability of the alligator to produce antibodies to a specific immunological challenge. Still other projects focus on the isolation and characterization of antimicrobial peptides from alligator leukocytes. Data will be presented from these projects, with comments on how the advancement of crocodilian genomics research can be used expand the knowledge of crocodilian immunity.

A PALEONTOLOGICAL, TEMPORAL FRAMEWORK FOR COMPARATIVE GENOMIC STUDIES OF CROCODYLIANS.

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Crocodilians have an extensive fossil record, making them ideal for the study of time-related issues in evolutionary biology. A prerequisite is a robust phylogeny estimate for fossil species, which currently outnumber their living relatives by at least eight to one. Morphological and molecular phylogenetic
analyses for Crocodylia result in broadly congruent results (with a few notable exceptions), and we are able to identify basal members of most extant lineages in the fossil record. Crown-group Crocodylia first appears in the Campanian stage, ~80 million years ago. Alligatoroidea and Crocodyloidea are both known from unambiguous fossils in the Late Cretaceous; fossils of similar age are regarded on morphological grounds as gavialoids, though divergence time estimates from molecular data indicate caution in these interpretations. The Alligator-Caiman split had occurred by the early Paleocene, and the earliest taxa closer to either Tomistoma or Crocodylus + Osteolaemus are from the early Eocene. Divergences among speciose genera (Crocodylus, Caiman) are more recent phenomena, with both first appearing in the Neogene, though the greatest range extensions within Crocodylia are expected for caimans, whose fossil record is comparatively sparse. These data points allow us to test various calibration-based methods for studying evolutionary rates and divergence times.

ARE CROCODILES REALLY MONOPHYLETIC? DNA EVIDENCE FOR SUBGROUPS IN CROCODYLIA

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The phylogenetic placement of the African slender snouted crocodile, Crocodylus cataphractus, was addressed using molecular and morphological techniques. Although it has often been recognized as being a "basal" form, morphological studies have traditionally placed C. cataphractus within the genus Crocodylus, while molecular studies have suggested that C. cataphractus is very distinct from other Crocodylus. In the present study we addressed the relationship of this species to its congeners using partial sequences of two nuclear genes (C-mos 302bp and ODC 294bp), and two mitochondrial genes (ND6-tRNAGlu-cytB 347 bp and control region 457 bp). Analyses of these molecular data sets, both as individual gene sequences and as concatenated sequences, support the hypothesis that C. cataphractus is not a member of Crocodylus or Osteolaemus. Examination of 165 morphological characters supports and strengthens our resurrection of an historic genus, Mecistops (Gray 1844) for cataphractus.

SEX-SPECIFIC DIFFERENCES IN RECOMBINATION IN Crocodylus porosus: A SPECIES WITH TEMPERATURE-DEPENDENT SEX DETERMINATION.

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Although higher female recombination has been reported in many species of mammals, sex-specific differences in recombination rate have not been investigated in any species with environmentally determined sex, such as crocodiles. We have recently reported the first genetic linkages in the saltwater crocodile, Crocodylus porosus, and indeed, any reptile. Furthermore, all non-zero map distances found in this preliminary study were higher in females than in males, mostly by a substantial margin. The conventional Haldane-Huxley explanation that recombination is higher in the homogametic sex clearly does not work for a species without sex chromosomes. Indeed, the result challenges the relevance of sex chromosomes to sex-specific recombination differences in general. Comprehensive linkage maps
covering the whole genome of *C. porosus* are now being developed and will enable further investigation and confirmation of sex specific recombination.

**PHYLOGENY AND SYSTEMATICS OF *Crocodylus acutus***

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Despite the diversity with the genus *Crocodylus* in the Americas, and the broad geographic range of the American crocodile, little is known about the expansion and diversification of the group. *Crocodylus acutus* is an abundant crocodile that is widespread throughout the neotropics, and is found in freshwater, brackish water, and saltwater habitats. There are a few noticeable morphological, physiological and ethological differences along its distribution (Brazaitis, 1973; Ross and Mayer, 1983; Seijas, 2003). It is not clear whether the nominal *C. acutus* truly refers to only one biological species. These differences and the variety of habitats where this putative species occurs, may suggest that subspecies could be recognized, or that perhaps there is more than one species present. In order to study the historical biogeography of widespread *Crocodylus* species in the New World, and clarify the systematic relationships of the group, we amplify 1820 bp from two mitochondrial genes, 1200 bp of Cyt b and 620bp of COI from populations spanning Mesoamerica and several countries in South America, including Colombia, Ecuador and Peru. Our molecular systematic analyses place *Crocodylus intermedius* inside the *C. acutus* lineage and shows small genetic distance between them. As a result of these findings, *C. intermedius* Graves 1819 probably does not merit full specific rank, nor do any of internal clades in those traditionally referred to *C. acutus* Cuvier 1807. From our phylogeographic reconstruction and the estimated time of divergence, we propose a cryptic Late-Pliocene diversification to explain the acutus- intermedius lineage into four evolutionary units, (i) Intermedius clade, (ii) South American Pacific clade, (iii) El Darién clade, (iv) Northern clade.

**Hibridación del cocodrilo cubano *Crocodylus rhombifer* en la Reserva de la Biosfera Ciénaga de Zapata, Cuba.**

Roberto Ramos Targarona, Manuel Alonso Tabet, Roberto Rodríguez Soberón, y John Thorbjarnarson.

El propósito de este trabajo fue presentar la existencia de cocodrilos con caracteres fenotípicos atípicos en la zona de la parte central de la porción sur-occidental de la Reserva de la Biosfera Ciénaga de Zapata, donde habitan simbáticamente las dos especies de cocodrilos que ocurren en Cuba: cocodrilo cubano, *Crocodylus rhombifer* y el cocodrilo americano, *Crocodylus acutus*. La sugeneración de las dos especies de cocodrilos se han hibridado en la naturaleza data de informaciones de la mitad del siglo XX, por cazadores comerciales de cocodrilos del lugar que distinguieron con el nombre de “mixturados” o “cruzados” a determinados ejemplares que consideraban el producto del apareamiento entre el cocodrilo cubano y el cocodrilo americano. Los estudios realizados desde 1979 a 1992 sobre la presencia del cocodrilo cubano en la Ciénaga de Zapata mostraron que en algunas localidades existían las dos especies y fue evidente de la proporción de cocodrilo cubano y cocodrilo americano, que había un área central (núcleo) donde el cocodrilo cubano fue más abundante, un área transitoria y una extensa región costera de la península donde el cocodrilo americano se encuentra exclusivamente. Posteriormente en los estudios de estimados poblacionales realizados en los años 1993, 1996 y 2002, una de las más
importantes consideraciones fue la presencia de las poblaciones simpátricas del cocodrilo cubano y del cocodrilo americano y la posible presencia de estos "mixturados" o actualmente denominados "ambiguos" por no estar comprobada esa hibridación y analizando la posible ocurrencia de una variación extrema de las especies. Los cocodrilos capturados fueron generalmente asignables a alguna de las dos especies en estudio mediante una tabla construida con las más importantes características externas visibles que diferencian estas dos especies. A los individuos que demostraron una mezcla de caracteres más allá del rango de variación fueron denominados "Ambiguos" sin hacer ningún comentario acerca del posible origen genético de esos individuos. El los estudios de 1993 y 1996 se identificaron un 3,4 % (230 animales capturados) y un 7,4 % (243 animales capturados) de animales ambiguos respectivamente. En el 2002 se perfecciono dicha tabla aplicándola a los 32 cocodrilos capturados, de los cuales ninguno se considero cocodrilo cubano puro: 2 ejemplares tenían un 80% de los caracteres fenotípicos del cocodrilo cubano, otros 2 un 70%, 5 un 50% y los demás por debajo de un 50%. Se describe los híbridos obtenidos en cautiverio mediante el cruce de estas especies. El desconocimiento de la frecuencia y aspectos biológicos (genéticos, reproductivos y ecológicos) de esos posibles híbridos silvestres hacen difícil conocer sobre las causas de su ocurrencia de que si es por un proceso evolutivo o es debido al impacto humano. Si realmente la hibridación esta ocurriendo, la introgresión genética traería como consecuencia la pérdida de la integridad genética de la especie y su posible desaparición como especie.

INVESTIGACIÓN Y MANEJO DEL COCODRILLO AMERICANO (CROCODYLUS ACUTUS) EN CUBA.

Roberto Rodríguez Soberón¹, Manuel Alonso Tabet¹ y Roberto Ramos Targarona²

Cuba ocupa el quinto lugar a nivel mundial en área de manglares y se considera al cocodrilo como una especie bandera para la conservación de estos importantes sistemas costeros. Actualmente Cuba tiene las poblaciones más saludables de cocodrilos americanos, dentro de los 17 países en que la especie está presente. Desde los años '70 en Cuba se vienen realizando estudios sobre la ecología y estatus poblacional del cocodrilo americano. En 1984 estos estudios recibieron un importante impulso con la formación del Programa Nacional de Cocodrilos, auspiciado por la Empresa Nacional para la Conservación de la Flora y la Fauna (Ministerio de la Agricultura). En 1987 se dio inicio a un programa de investigaciones sobre esta especie en los refugios de fauna Delta del Cauto y Monte Cabaniguán, situados de manera contigua, al sur de las provincias orientales de Las Tunas y Granma. Entre los tópicos abordados por este proyecto de investigaciones se encuentran la ecología de la reproducción, la dinámica poblacional, el estudio de la dieta, la conducta y la influencia de las temperaturas de incubación en la determinación del sexo. Este ha funcionado como proyecto piloto para la puesta a punto de nuevos métodos de investigación y adiestramiento de los especialistas cubanos dedicados al estudio y conservación de los cocodrilos. Otras áreas donde se han realizado investigaciones son Isla de la Juventud, Sur de la provincia de Pinar del Río, Ciénaga de Zapata, en la provincia de Matanzas y Desembocadura del Río Máximo, en la provincia de Camagüey.

La puesta en marcha de seis zoocriaderos de C. acutus, a establecido las bases para la materialización de un programa de uso sostenible basado en el rancheo, lo que unido a los resultados de los programas de investigación en vida silvestre y su implementación en los planes de manejo de áreas protegidas, hizo posible la aprobación de la correspondiente propuesta a CITES para el traspaso al Apéndice II de la población cubana de C. acutus. Actualmente se abre una nueva línea de trabajo, en colaboración con entidades académicas cubanas (Universidad de La Habana) e internacionales (Wildlife Conservation Society, Instituto Smithsonian de Investigaciones Tropicales de Panamá) para el estudio genético de las

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poblaciones cubanas de C. acutus y su posible intercambio genético con la especie endémica Crocodylus rhombifer, tema de crucial importancia para la conservación de ambas especies en nuestro contexto nacional. Las perspectivas futuras del trabajo con el Cocodrilo americano en Cuba son: ampliar las investigaciones en la ciénaga de Birama, extender los temas y métodos de investigación a otras importantes poblaciones de la especie en Cuba, desarrollar el programa de uso sustentable a través del rancheo y crear un centro regional de Adiestramiento en métodos de investigación y manejo de cocodrilos en la ciénaga de Birama.

PRELIMINARY RESULTS ON THE RELATIONSHIPS AMONG Crocodylus acutus AND C. rhombifer FROM CUBA

Collaborative work:
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Panamá
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Based on morphological characters, two putative species of crocodiles have been reported for Cuba: Crocodylus rhombifer Cuvier 1807 and Crocodylus acutus Cuvier 1807. Crocodylus rhombifer is an endemic species, with a very restricted distribution range: Cienega de Zapata and Cienega de Lanier in Cuba. Crocodylus acutus has a wider distribution, from Florida, México, Central America to Venezuela and north of Perú, including Cuba, Jamaica and La Española. In addition to the two morphological species, evidence also indicates the presence of individuals with intermediate phenotype called “ambiguous”, that are presumably of hybrid origins.

The present project propose to answer the following questions:
- What is the relationship between the Cuban C. acutus and the continental C. acutus?
- What is the relationship of the Cuban C. rhombifer with the other Crocodylus species?
- What is the population structure on morphologically characterized C. acutus and C. rhombifer
- What is the origin of the “ambiguous” populations (potential hybrids)?

For this study, three populations were selected based on morphological characterization:
- Delta del Cauto, Birama - C. acutus
- Zanja del Brigadista - C. rhombifer
- Maniadero - ambiguous (potential hybrids)

We used sequence data from a 800 bp fragment of the mitochondrial Cytochrome b gene, and microsatellite data from loci Cj18, Cj20, Cj119 y Cj131 developed by Nancy Fitzsimmons et al. (2000).

Our preliminary data shows that the mitochondrial haplotypes found in Cuban “acutus” populations are more closely related to “rhombifer” populations, than to other C. acutus from
the Continent. Moreover, very preliminary results using microsatellite data indicate that populations of these two species are mixed in a much higher proportion than previously thought. We will continue to explore these questions using more nuclear markers and including additional individuals from Cuba and the Continent.

GENETIC VARIABILITY OF THE AMERICAN CROCODILE IN THE COAST OF JALISCO, MÉXICO.

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During November and December of 1994, 38 Crocodylus acutus were captured from four localities along the coast of Jalisco: Estero de la Manzanilla (MA), Reserva Cuixmala (CX), Estero Majahuas (MJ) and Laguna Boca Negra (BT). These four sites cover most of the Jalisco coastline which is 342 km in length. Blood samples from each individual were taken for analysis of genetic variation using allozyme and DNA fingerprint methods. Sixteen proteins (21 loci) were detected by electrophoresis in polyacrylamide or starch gels, and all were monomorphic. In contrast, genetic variability was detected in each population using multilocus DNA fingerprint methods and heterozygosity levels were estimated. The same allozyme allele per locus was found in each population and minisatellite variation was shared by all populations. However, the causes for the lower observed level of variability, whether low sample size, a recent bottleneck or inbreeding, cannot be determined without further study. On the other hand, a metapopulation is considered in the area due to high migration rate between populations by the ocean.

GENETIC STRUCTURE OF THE AMERICAN CROCODILE (CROCODYLUS ACUTUS) IN FLORIDA AND EVIDENCE OF HYBRIDIZATION INFERRED FROM MITOCHONDRIAL AND NUCLEAR MARKERS

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Phylogenetic relationships among members of the genus Crocodylus have been difficult to assess partly due to relatively recent divergence times. We infer discrete assignment of haplotypes using partial sequences from mtDNA genes (ND4, tRNA-Hist and tRNA-Ser) and the control region. Resulting data contribute to identifying specific taxonomic units and revealing hybridization. Evidence of hybridization between the Cuban crocodile and the American crocodile was detected in wild and captive individuals from Florida and the Caribbean. Microsatellite markers were used to test for population subdivision among American crocodiles in Florida and to compare diversity values with previous studies of crocodilian populations. Distance-based and likelihood measures reveal unexpected population differentiation for American crocodiles in Florida (ST = 0.211, P = 0.008; FST = 0.144, P < 0.001; AMOVA). Our results suggest that the Florida population segment has increased in size, but is still threatened by genetic fragmentation and hybridization.

POPULATION GENETIC STRUCTURE OF THE AMERICAN CROCODILE, CROCODYLUS
ACUTUS, ACROSS ITS DISTRIBUTIONAL RANGE

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The American crocodile, *Crocodylus acutus*, is endangered throughout its range due to habitat loss, fragmentation and commercial harvesting. The IUCN Crocodile Specialist Group (CSG) stated that determining the status of *C. acutus* in Costa Rica is a high priority due to lack of data. The genetic structure of these populations was also considered a high priority for conservation. My proposed research will determine the population genetic structure of *C. acutus* throughout its distributional range. I will determine the structure at a local level in Tamarindo Estuary, Guanacaste, Costa Rica, and relate this to the population’s life history traits. I will determine the genetic structure, extent of gene flow and relatedness at six additional estuaries along the Guanacaste coast and compare that to the genetic structure along the Caribbean coast. I will also determine the genetic structure across its distributional range from southern Florida to Costa Rica to determine the extent of gene flow and relatedness of populations. Genetics will be determined for all populations using 12 microsatellite loci developed for *C. acutus*. Studying the genetic structure of *C. acutus* will provide important data on gene flow between populations, which will enhance the conservation and management of across its range.

COMPARATIVE PHYLOGEOGRAPHY AND CONSERVATION GENETIC ANALYSIS OF AMAZONIAN CAIMAN AND MELANOSUCHUS

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The genetic structure of Caiman crocodilus and *Melanosuchus niger* was investigated using a nearly complete cytochrome b gene and 3’ flanking region, and 8 microsatellite markers. Inferences were based on a sample of 125 and 132 individuals from 9 and 11 collecting localities, respectively, in Peru, Brazil and French Guiana. In both species, significant differences exist between populations from Atlantic drainages not draining into the Amazon River, and those of the Amazon River basin proper. The differentiation between these two regions was most likely the result of continuous range expansion or long distance colonization. A pattern of significant isolation-by-distance is observed in the Amazon basin for *M. niger*, but not for *C. crocodilus* whose populations appear undifferentiated from each other. *Melanosuchus niger* also shows significant differentiation between blackwater and whitewater populations observed within both macrogeographic regions; this pattern of ecological divergence is weakly supported in *C. crocodilus*. Sampled localities of *C. crocodilus* appear predominantly to be at a mutation-drift genetic equilibrium, while *M. niger* is at a significant mutation-drift genetic disequilibrium most likely attributable to a history of over-exploitation. However, demographic and population genetic data suggest the beginnings of a possible demographic recovery after long period of over-exploitation, although signals of recovery are highly regionalized and dependent on management and conservation techniques.

PRELIMINARY PILOT RESEARCH TO ESTABLISH THE PHYLOGENETIC AND SYSTEMATIC OF MESOAMERICAN CAIMANS.

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Mesoamerica (including the Caribbean) has a very complicated geological history. This region has undergone complicated geological changes due to tectonic activity and sea level changes (Coates and Ovando, 1996). Geological and climatic changes have remodeled crocodilian habitats and have influenced the geographical distribution of several species. It is important to recognize that the geographical differences in species richness in the tropics depend on factors such as long-term ecological changes, the physiology and the ethology of each species (Hubbel, 2001; Wright, 2002; Dick, 2003; McKane et al., 2004). Given its abundance and wide geographical distribution Caiman crocodilus Linnaeus, 1758, provides an excellent model to study biogeography and the origin of the biodiversity in the Neotropics. The main goal of this research is to use species-level phylogenetic and phylogeographic analyses to infer both the rate and pattern of cladogenesis of Caiman crocodilus in Mesoamerica. A preliminary pilot study to assess the phylogeographic and biogeographic patterns of C. crocodilus in Mesoamerica, has started with the sequencing of some 1200 bp of the mitochondrial gene Cytb, from 78 samples collected from all over Central America and several countries in South America, including Colombia, Ecuador and Peru. A phenetic analysis performed using the neighbor-joining algorithm in PAUP, using distance method with GTR+G model of nucleotide evolution suggests that Caiman lineage is monophyletic with a clear South American origin. The Mesoamerican Caiman clade has three subclades. One is formed by samples described as Caiman crocodilus chapasius, the other two clades are composed of samples identified as Caiman crocodilus fuscus. The genetic distance between each Caiman crocodilus fuscus sub-clade is the same as the genetic distance between Caiman crocodilus fuscus and Caiman crocodilus chapasius. These preliminary results also suggest that the genus Caiman is more diverse than we believed in the past.

THE AFRICAN DWARF AND OTHER CROCODILES: CONSERVATION, MANAGEMENT & GENETICS

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The African dwarf crocodile (Osteolaemus tetraspis ssp.) is among the most heavily exploited of the Crocodylia, principally as a subsistence resource for rural people of central and west Africa. Despite its importance for food security and as a mainstay of local economies, the dwarf crocodile is also among the least known of the world’s crocodiles. As part of a larger study on dwarf crocodile ecology and population dynamics, we are using genetic techniques to quantify ecological parameters relevant for species management. We are approaching these questions at three different scales, using novel microsatellite primers developed for Osteolaemus and direct sequencing of mt and nuDNA regions. At the level of Evolutionary Significant Units (ESUs), we will assess the possibility that the two currently recognized subspecies (O. t. tetraspis & O. t. osborni) warrant full species status and subsequent independent management consideration. At the regional level, we are concerned with historical population structure and the designation of appropriate conservation units. At the population level, we will measure contemporary population connectivity and dispersal rates to evaluate the utility of spatial harvest management, potential source-sink dynamics and demographic rescue effects.
We will also report on other crocodilian genetic research underway at the American Museum of Natural History’s Institute for Comparative Genomics and the Wildlife Conservation Society.

POPULATION GENETICS OF THE AMERICAN (Crocodylus acutus) AND MORELET'S (Crocodylus moreletii) CROCODILES IN THE YUCATAN PENINSULA, MÉXICO.

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Maintenance of genetic diversity is a primary objective in management of wild and captive populations of threatened species. Consequently the IUCN recognizes the need to conserve genetic diversity as one conservation priority. We analyzed genetic structure and gene flow in wild populations of American crocodile (C. acutus) and Morelet's crocodile (C. moreletii) in the Yucatan peninsula, México using 477 base pairs of the mitochondrial (mt) control region DNA marker. Both species are sympatric in eastern Yucatán peninsula and are considered endangered by the IUCN and listed in the Appendix I of CITES. Skin clips from 115 individuals (51 C. acutus; 63 C. moreletii) were taken for genetic identification. After removing 16 hybrid crocodiles, identified during haplotypes assignment, population statistics were calculated for pure populations with software Arlequin v2.0. AMOVA tests returned minimal partitioning of populations for both species. Wright’s fixation index (FST) of pairwise comparisons between strains grouped by region, resulted in small, not significant FST values, suggesting that geographic subpopulations belong to one genetic population for C. acutus, and likewise for C. moreletii. Careful population management is required to maintain genetic integrity, and reduce the rate of hybridization, in order to conserve pure strains of these endangered crocodiles.

GENETIC STUDIES ON Caiman latirostris IN SANTA FE, ARGENTINA: A REVIEW THROUGH TIME

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Genetic studies on Caiman latirostris (Broad-Snouted Caiman) in Santa Fe, Argentina, started with a cytogenetic analysis in contrast with Caiman yacare. The obtained karyotype in both species was of 42 chromosomes, a pale C-banding pattern and only one NOR bearing chromosome pair. Due to the lack of relevant differences between both karyotypes, we decided to use molecular markers in the analysis of this species. We studied four isozymes: Esterase, Ioccitrate Dehydrogenase, Malate Dehydrogenase, and Superoxide Dismutase in animals from 4 populations of Santa Fe province. Samples used were pieces of kidney, heart and liver. In all cases heterozygosity values of 0 were found, in agreement with other authors. We used a set of 7 RAPD primers. PCR products were tested by electrophoresis on 4 % polyacrylamide gels. Only 13.73 % of 233 RAPD markers analyzed was polymorphic. Results suggest low to intermediate levels of polymorphism, heterozygosity and mean number of alleles per locus for each population. AMOVA indicated that nearly all variation exists within rather than among populations, implying that several alleles are shared among them. Due to RAPD markers may not be as effective at detecting variation as microsatellite amplification in populations of crocodylians, we have
already started an analysis with the last molecular technique mentioned.

EVIDENCE FOR PLACING THE FALSE GHARIAL (*Tomistoma schlegelii*) INTO THE FAMILY GAVIALIDAE; INFERENCE FROM NUCLEAR GENE SEQUENCES

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The extant crocodylians comprise 23 species divided among three families, Alligatoridae, Crocodylidae, and Gavialidae. Currently, based on morphological data sets, *Tomistoma schlegelii* (False Gharial) is placed within the family Crocodylidae. Molecular data sets consistently support a sister-taxon relationship of *T. schlegelii* with *Gavialis gangeticus* (Indian Gharial), which is the sole species in Gavialidae. To elucidate the placement of *T. schlegelii* within the extant crocodylians, we have sequenced 352 bp of the dentin matrix protein 1 (DMP1) nuclear gene in thirty individuals and 424 bp of the nuclear gene C-mos in seventy-four individuals. Molecular analysis of the DMP1 data set indicates that it is highly conserved within the Crocodylia. Of special note is a seven base pair indel (GTGCTTT) shared by *T. schlegelii* and *G. gangeticus*, that is absent in the genera *Crocodylus*, *Osteolaemus*, and *Mecistops*. To date, C-mos is the largest molecular data set analyzed for any crocodylian study including multiple samples from all representatives of the eight extant genera. Analysis of these molecular data sets, both as individual gene sequences and concatenated sequences, support the hypothesis that Tomistoma schlegelii should be placed within the family Gavialidae.

EXPERIENCIAS SOBRE EL MANEJO EN CAUTIVERIO DEL CAIMÁN LLANERO, *CROCODILUS INTERMEDIUS*

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La Estación de Biología Tropical Roberto Franco (EBTRF), se inicia en 1934 con los estudios de fiebre amarilla y enfermedades, debe su nombre al Dr. Roberto Franco, médico bogotano impulsor de los estudios de malaria y enfermedades tropicales en Colombia tropicales llega a la Universidad Nacional en 1947.

Está adscrita a la Facultad de Ciencias de la Universidad Nacional con sede en la ciudad de Villavicencio en 1966 y se dedica al estudio de la fauna herpetológica; tiene una larga tradición, reconocida por la comunidad científica internacional. Es un Centro de Investigación en Biología y Conservación de Testudines (tortugas) y Cocodrilos. Su primer Director el Dr. Federico Medem, zoólogo alemán, se constituyó en pionero de los estudios de reptiles en el Neotrópico con un altísimo reconocimiento científico en la conservación de la fauna silvestre. Presta servicios anexos asociados a la investigación y la docencia.

La EBTRF tiene el núcleo parental más importante del caimán llanero (*Crocodilus intermedius*) que logró su primer reproducción en cautividad en el año de 1991 y es una de las dos especies de cocodrilos en el mundo de mayor interés conservacionista, según la Convención Internacional para el mercado de especies de flora y fauna en peligro de extinción (CITES), por lo que se crea el Programa de Conservación del Caimán Llanero – Procaimán –. Igualmente la EBTRF conserva una colección de 23 especies de tortugas vivas representativas de todo el país algunas de ellas se están reproduciendo. La EBTRF ha impulsado desde 2002 hasta 2007 la socialización del Programa, el manejo de la fauna,

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This study describes seasonal variation in the microscopic anatomy and histochemistry of the oviduct of the American alligator, *Alligator mississippiensis*. The secondary purpose of this study is to look at sperm storage areas and determine if specialized glands for sperm storage exist, or if sperm occur in glands that serve other purposes (albumen or shell formation). Sperm storage is widespread in female reptiles but has only recently been observed in alligators (D. Gist, unpublished data). The sites where sperm storage occurs and the time frame during which sperm are stored are of interest because of the variation known in other vertebrate groups and possible phylogenetic implications. Female alligators are being collected in southern Louisiana at four main points throughout the year: before mating, directly after mating but prior to ovulation (i.e., the sperm storage period), during nesting, and after the nesting period. Microscopic anatomy is described using light microscopy, transmission electron microscopy, and scanning electron microscopy.

EFFECT OF CLIMATE ON SEX RATIO IN *Caiman crocodilus*

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Evidences of climate effect on ecosystems and species have been increasing in last years. However, the potential effects of climate variability on crocodilians sex ratio have not been evaluated in natural conditions. This study’s objective was to determine the effect of climate variability on sex ratio in natural population of caiman, *Caiman crocodilus*, in Caño Negro National Wildlife Refuge located in the Northern region of Costa Rica. We measured temperature in caiman nest to evaluate if there is a relationship between incubation temperature and weather variables. We observed a relationship of nest temperature with minimum temperature and precipitation. Furthermore, we captured caimans to evaluate sex ratio and to predict caiman’s age using Von Bertalanffy model. We analyzed the relationship between precipitation pattern and sex ratio using the oldest caiman age. We observed a negative correlation between number of borned males by year and precipitation during 90’s decade. Viability of caiman populations can be affected in the future if this situation continues.

CROCODILE CONSERVATION IN WEST AFRICA: PLANNING FOR THE FUTURE

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West African crocodiles are among the least known and most exploited crocodilian species. The goal of
this project was to establish a foundation for the conservation of *Mecistops cataphractus* and *Crocodylus niloticus* in West Africa by assessing their conservation status in Ghana and Cote d’Ivoire, building capacity in local practitioners, and examining the specific status of *C. niloticus*. Conservation status for both species was determined through systematic surveys of population, distribution, bushmeat threat, and cultural implications. Capacity building was achieved via 3 short courses and hosting 8 students for extended periods. The systematic analysis will utilize a total evidence, phylogenetic approach employing 8 molecular markers and 12 morphological characters (in progress). *C. niloticus* was present in extremely low abundance (avg < .5 ind./km) while only 12 *M. cataphractus* were encountered in 4 different locales. Strong cultural affinities preclude commercial pressure for bushmeat, despite low-level exploitation for traditional practices and home consumption. Preliminary evidence from previous phylogenetic analysis suggests that West African *C. niloticus* may represent a unique species. This study established a baseline for further research on the status, distribution, and threats facing these crocodiles. It also empowered locals to carry out future conservation initiatives and raised interest and awareness in a region where crocodiles have been largely disregarded.

**HEAVY METAL BURDENS IN LOUISIANA ALLIGATORS**

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Alligator are long-lived predators that are can accumulate environmental contaminants over many years, and as such can be useful in determining the level of pollution in areas they inhabit. Louisiana has a carefully regulated state wide hunt during which up to 40,000 adult alligators are harvested for skin and meat during the month of September.  Alligator tissues from widely separated geographical regions are thus available for study.  Liver, kidney and muscle tissues were collected from 25 separate sites in Louisiana representing “clean” and potentially “dirty” sites.  The tissues were shipped to San Diego for analysis.  A total of 161 alligators were sampled (483 separate tissue samples).  Approximately 0.2 g of each tissue was digested in concentrated nitric acid, diluted 1:50 in distilled water then stored at room temp until analyzed on an Agilent Technologies ICP-MS (inductively-coupled plasma mass spectroscope).  The following elements were analyzed: Magnesium, Aluminum, Vanadium, Chromium, Manganese, Cobalt, Nickel, Copper, Zinc, Arsenic, Selenium, Strontium, Molybdenum, Silver, Cadmium, Tin, Barium, Tungsten, Mercury, Thallium, and Lead.  The results were encouraging in that in every area sampled tissue levels of heavy metals were below what would be considered toxic.  A few individuals however, had very high levels of some metals.  We attributed these outliers to the known habit of alligators of ingesting lead fishing weights, spark plugs and anything else that may get in the way while feeding.  Statistical analysis of the data revealed significant regional differences.  As predicted, areas that have a high concentration of petrochemical plants, i.e. the Mississippi River bank from Baton Rouge.

**USING MICROSATELLITE MARKERS TO REVEAL BREEDING STRATEGIES IN MORELET'S CROCODILE (CROCODYLUS MORELETII) IN BELIZE, CA**

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Microsatellite data was generated from hatchlings from ten nests of Morelet’s Crocodile from New River Lagoon and Gold Button Lagoon in Belize in order to test for evidence of multiple paternity.  Eleven microsatellite loci were amplified for 192 individuals from 10 nests, and for
five adult males and females from Gold Button Lagoon; mitochondrial control region sequence was generated for the adults and for one individual from each nest to test for presence of *Crocodylus acutus*-like haplotypes within the population. Examining five of the eleven microsatellite loci amplified revealed both qualitative and quantitative evidence for multiple paternity in six of ten nests. These data represent the first published evidence of multiple paternity as a mating strategy in the true crocodiles.

**A Comprehensive Genetic-Linkage Map for the Saltwater Crocodile**  
(*Crocodylus porosus*)

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We report the development of more than 250 informative microsatellite markers, and the first phase construction of a comprehensive microsatellite based genetic linkage map for the saltwater crocodile (*Crocodylus porosus*). This map represents the first genetic linkage map in the Order Crocodylia and indeed the first in the Class Reptilia. These markers will be used to map Quantitative Trait Loci (QTL) for economically important selection objectives in farmed saltwater crocodiles. Any QTL identified may then be useful in implementing a Marker-Assisted Selection (MAS) genetic selection program. High-density genetic maps for QTL identification require many polymorphic markers spaced evenly across the genome. Our map, which we anticipate completing by mid-2007, will contain approximately 300 informative genetic markers selected from more than 600 microsatellites that were originally developed and evaluated. All 600 loci will be evaluated for utility in other living crocodilian species.

**GENETIC CHARACTERIZATION OF THE CUBAN CROCODILE** (*Crocodylus rhombifer*) **USING ZOOLOGICAL SAMPLES**

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There is a void in genetic data for the Cuban crocodile (*Crocodylus rhombifer*), a critically endangered species. We used zoological samples to genetically characterize this species in comparison to other New World crocodilians. Partial mitochondrial sequence data was
generated from cyt-\(b\) (406 bp) and tRNAPhe-control region (442 bp). Phylogenetic analyses were preformed by generating Parsimony, Maximum Likelihood and Bayesian-based topologies. We also genotyped 10 polymorphic microsatellite loci in an effort to identify species specific alleles. Distance and model based assignments were used to cluster samples. This data will help in the genetic characterization of wild populations of \textit{C. rhombifer}, in which hybridization could be threatening an already endangered species.