

Evolutionary Ecology of Freshwater Turtles

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Introduction

This long-term study explores the evolution of life history traits (e.g. reproductive traits such as mating system, migratory behavior, sex determination, demography) and the footprint that those traits leave on the genetic makeup of populations (e.g. genetic differentiation, gene flow) to understand the ecological and evolutionary significance of trait variation, and to provide information important for the conservation of reptiles, particularly turtles. The project consists of several phases that investigate complementary ecological and evolutionary modules related to this main goal. Those modules are: population genetics, paternity analyses, gene expression during sex differentiation, and molecular evolution of genes and chromosomes.

The species of turtles inhabiting the Horticulture Research Station provide a population sample for comparison with other species/populations for this broader study.

Modules and Specific Aims

1. *Population Genetics:*
 - a. Determine levels of genetic variability and heterozygosity within populations.
 - b. Determine genetic differentiation and gene flow among populations.
 - c. Identify evolutionary units that require protection for endangered species.
2. *Paternity Analysis:*
 - a. Determine the number of alleles present among clutch mates at several nuclear loci (microsatellite DNA).
 - b. Calculate the minimum number of males that might have fathered each clutch based on the fact that each parent can be heterozygous for different alleles at each locus examined.
3. *Gene expression:*
 - a. Identify novel candidate genes that take part in the sex differentiation cascade during development for turtles whose sex is determined by the incubation temperature (TSD), or at conception by their genotype (GSD).
 - b. Compare the gene expression profiles of known and novel genes during embryonic stages prior, during and after the thermosensitive period (when temperature can affect sex determination in some turtle species).
 - c. Identify genes whose expression is differential between males and females prior to or at the onset of the thermosensitive period for TSD turtles which are candidate master switch elements (responsible for the thermal sensitivity in TSD taxa or major sex determining gene).
4. *Molecular evolution of genes and chromosomes:*
 - a. Analyze the sequence of known and novel genes (coding and promoter regions) and their corresponding messages (mRNA) to study the regulation and hierarchy of these elements.
 - b. Compare sequence data from a. above between TSD and GSD turtles to study the evolution of these genes and the evolution of sex determining mechanisms.
- c. Calculate the relatedness of clutch mates taking into account the allele frequencies for the population to adjust the calculation of the number of fathers from b. above.

- c. Determine the location of these genes in the chromosomes and identify those genes that are linked in the same chromosome.
- d. Compare the location of these genes in different turtles to study the evolution of the chromosomes and of the sex determining mechanisms.

Activities

We have trapped and sampled individuals of two turtle species at the Horticulture station: painted turtles (*Chrysemys picta*) and snapping turtles (*Chelydra serpentina*). Many but not all of these individuals trapped were marked by

numerical notching of the carapace scutes for future monitoring of life history characteristics and to avoid duplicate sampling. Blood samples were obtained non-lethally from multiple individuals to carry out genetic analysis, and to obtain chromosomal spreads for the modules 1 and 4 described above. Blood culture produced successful metaphase spreads (Figure 1), which can now be used to paint gene-specific probes for gene mapping. Other analyses are ongoing.

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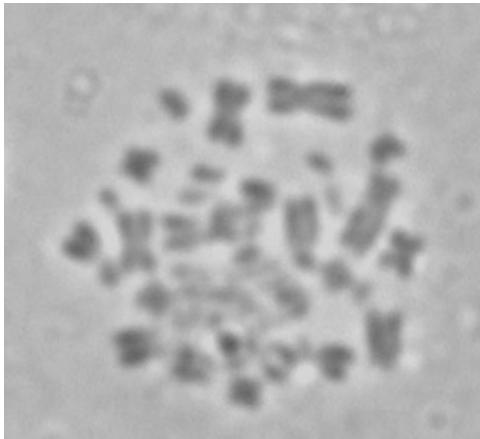


Figure 1. Exemplar metaphase chromosomal spread from sampled turtles.