

Annual Report

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Title: **A MODEL FOR GENETIC DIVERSITY AQUATIC INSECTS OF THE MOBILE/TENSAW RIVER DELTA.**

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Institution: University of South Alabama

Research Category: SGER

Project Period: 10/01/2001-12/31/2003

Objectives of the Research Project

The objective of this project is to identify a limited number of aquatic insects that can serve as model species for genetic diversity studies of the Delta region and similar aquatic habitats. The results of this genomic inventory, in addition to results generated from companion studies, will be used as a springboard to apply for larger grants (e.g., EPA, NSF) for a complete species inventory and an extensive genomic documentation of Delta insects (companion studies: The paradox of the Delta, JW McCreadie & PH Alder - ACES funded project, May 2000; Total Insect Bio-inventory Project of the Mobile / Tensaw Delta - JW McCreadie & PH Alder - ACES study, starting date, August 2001).

The ideal characteristics for a model species include: high genetic diversity at several loci; good vagility to eliminate the possibility of localized inbreeding through low dispersal levels; significant aquatic stages; significant population sizes to avoid natural/historical causes of inbreeding and to facilitate collecting and; widespread populations throughout the Delta region. Ideally, the species will represent different trophic levels for potential use in biomonitoring studies. The successful identification of model species in this project will lead to subsequent projects, including taxon-specific gene flow studies, and biomonitoring of impacted regions. Specific goals to accomplish our objective are:

1. The collection of samples of 4 populous, widespread and highly vagile aquatic insect species
2. Development or adaptation of DNA extraction techniques for each candidate species
3. Identification of 10 or more polymorphic nuclear DNA loci within each candidate species
4. Preliminary assessment of population structures within the Delta

Progress Summary/Accomplishments

Model Species:

We have collected and identified 3 candidate species for genetic studies: *Ranatra nigra* (water scorpion), *Pelocoris femoratus femoratus* (nocorid water beetle) and *Erythemis simplicicollis* (dragon fly). A fourth species, drawn from collections of numerous

damselfly species, will be chosen once species identification have been verified through voucher specimens.

DNA extraction:

We have assessed the DNA extraction techniques of Sambrook, Frisch and Maniatis (1989), Robertson (1993), Brockhouse (unpublished) and Brockhouse et al., (1993) on *Ranatra nigra* and *Pelocoris femoratus*. The first three methods are for fresh/frozen specimens. Robertson's method would be particularly valuable since it is extremely rapid. However, only the Brockhouse et al., (1993) methods yielded usable DNA. This method involves ethanol fixation of the specimen, followed by prolonged protease/detegent digestsion. We have found that alcohol fixation is necessary for both species to prevent contamination of the DNA with large amounts of extraneous co-purifying material which inhibits subsequent enzymatic steps. Unfortunately, this procedure is time consuming and laborious. We are continuing to assess alternate, more rapid, methods.

Polymorphic DNA loci:

We have cloned 4 loci from *Ranatra nigra* (internal transcribed spacers (*cf.* Brockhouse et al., 1993), actin intron, elongation factor I intron, and beta-tubulin intron (*cf.* Palumbi, 1996). To date, we have assessed the internal transcribed spacers for polymorphisms. This locus show length polymorphisms that are easily scored by agarose gel electrophoresis. Five alleles have been identified to date.

The remaining loci will be assessed for sequence variation by the population heteroduplex assay described in our proposal, augmented by a new technique developed for the identification of human disease-related polymorphisms (Lishanski et al., 1994). Briefly, PCR products from individuals will be mixed, heated and reannealed to form "heteroduplexes". The MutS protein, purified from *E. coli*, will be mixed with the PCR products. This protein binds to mismatches and forces the bound DNA molecule to migrate more slowly in polyacrylamide gel electrophoresis. Individuals with differing DNA sequences will be visible by the different migration pattern. The DNA will then be sequenced, and the difference identified. We have conducted preliminary experiments with this technique and it has so far proven highly satisfactory.

Personnel:

Procedural delays in hiring a qualified senior technician for this project have resulted in the slower than expected progress. However, a person with the appropriate skills has been identified, and is waiting visa reclassification to begin employment in the project. We are confident that the project will be back on its time-line shortly.

To date, we have relied on graduate and undergraduate directed-studies students for assistance. Mark Nelder (MS student working on *Pelocoris femoratus*), Teresa Saultes (BS student) and Samar Nasar (BS student; both working on *Ranatra nigra*) have been trained in DNA extraction techniques, gel electrophoresis and PCR DNA cloning. Samar Nasar will be returning to the project as an MS candidate in the summer of 2003 (pending acceptance into the MS program).

References Cited:

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Future Activities

More genes will be cloned from *R. nigra*, and cloning will soon begin from the other candidate species. Polymorphisms will be discovered using the assay described above, and the alleles will be sequenced at the University of South Alabama DNA sequencing facility. We anticipate that the senior technician will soon be able to join the project.

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