



Texas Imported Fire Ant Research and Management Project

Progress Report - June 2002

Application of molecular genetic technologies to the management of the Imported Fire Ant

Principal Investigator(s): C. Coates, J. S. Johnston, J. Piedrahita, and S. B. Vinson

Funding Amount/2 Years: \$368,878

Summary of Work to be Done:

1. Identification of Imported Fire Ant populations.
 - The goal was to determine if there are sufficient differences between Imported Fire ants from different areas to allow us to identify different populations. Differences were found and now we are completing the characterization of the population across the Southeast.
2. Identification of the genes involved in Imported Fire Ant sterility.
 - Goal is to isolate the genes that are important in the diploid sterility of male Imported Fire ants. Once identified, knowledge of these genes these may be of value in inducing sterility in fire ants, a prospect that has been enhanced by our ability to produce large numbers of males.
3. Transformation of biological control agents.
 - The first goal of this objective is to completely characterize those organisms associated with the imported fire ant that show potential as a component of an integrated control strategy. The second goal of this objective is to use genetic transformation technologies to enhance the ability of these organisms to be utilized as biological control agents. In the first year of funding of this project, the culture conditions for in vitro rearing of *Thelohania solenopsae*, a microsporidium pathogen of the imported fire ant, are to be established and optimized. Yeast samples isolated from fire ants are to be cultured and genetically transformed. The identification and isolation of additional microorganisms associated with the imported fire ant is to be performed.
4. Transformation of Imported Fire Ants.
 - The goal of this objective is to produce a transgenic imported fire ant strain. In the first year of funding of this objective, modified recombinant baculoviruses and pantropic retroviruses will be produced to express reporter genes for the identification of transgenic fire ants. These modified viruses will be introduced into fire ant queens.
5. Characterization of gene expression in dealate queens.
 - Although not initially funded, this project was initiated late in the financial year using returned funds from the Texas Agriculture Quarantine Program. The goal of this project

is to characterize genes that are specifically, or more highly expressed, in dealate fire ant queens following a mating flight. The initial objective is to produce a subtractive library enriched for genes expressed in dealate queens and characterize the differentially expressed clones.

Major Accomplishments to Date:

1. Identification of Imported Fire Ant Populations.

- Completed a large molecular genetics survey that used 16 highly variable microsatellite loci to show the migration paths of the RIFA and introgression of *S. richteri* (to the east), and *S. xyloni* and *S. sp?* (in the West) genes into the RIFA.
- Completed a survey of genome size variation. Genome size supports the introgression indicated by the molecular data.
- Mapped the 16 microsatellite loci we have been using to chromosomes.
- Developed a rapid cheap identification method for recognition of RIFA.
- Characterized the hybrid *S. geminata* x *S. xyloni* population. The results suggest that *S. xyloni* within the hybrid zone has evolved social hybridogenesis, in which the female offspring of hybridizing queens become workers and contribute to the somatic growth of the colony but are excluded from the female reproductive caste.

2. Identification of the Genes Involved in Imported Fire Ant Sterility.

- Found additional evidence that fertile diploid males are found at frequencies averaging 20%. The diploid males are highly variable ranging from 0 to 80% of all males.

3. Transformation of Biological Control Agents.

- We have been able to get *Thelohania* into an insect cell culture and produced a few spores.
- An isolated yeast species from the imported fire ant, *C. guiller mondi*, was cultured under laboratory conditions. A high frequency transformation procedure was adopted for this species, using a recombinant plasmid expressing a resistance gene, to the antibiotic drug, Zeocin. Transformation of the yeast isolate with a linearized form of this plasmid resulted in the successful genetic transformation of the yeast species.
- A symbiotic gram negative bacterial species was isolated from the midguts of third instar fire ant larvae. This bacterial symbiont can now be cultured under laboratory conditions. Preliminary characterization of this species has included the development of an antibiotic resistance profile and a bacterial lipid profile for species identification. The bacterial isolate has a unique lipid profile that is most similar to *Cedecea davisae*.

4. Transformation of Imported Fire Ants.

- The construction of appropriate recombinant baculoviruses and pantropic retroviruses for use in the imported fire ant was successfully completed. Preliminary introductions of these viruses into the ovaries of fire ant queens was successful and marker gene expression was observed in the ovarian tissue.
- Developed a method to produce large numbers of only fertile males that can be used in transformation experiments.

5. Characterization of Gene Expression in Dealate Queens.

- A substantial subtracted library was constructed and preliminary differential screening completed. Initial sequencing of candidate clones revealed several with significant identity to entries in the genetic database. These include:
- Cytochrome C Oxidase- a potential initiator of cell destruction by apoptosis in the flight muscles and new protein synthesis in the fat body.
- Vitellogenin- the major yolk protein component of the developing eggs.
- Hymenapterocin- an immune peptide molecule that may share the same signalling pathway that initiates apoptosis.
- Royal jelly proteins- members of the royal jelly protein family that may be involved in the changes in the nutritional state of the queen and developing eggs and larvae.

Goals Achieved:

1. Identification of Imported Fire Ant Populations.

- We have been able to identify differences in different Imported fire ant populations that may be useful in identifying possible sources of new infestations.

2. Identification of the Genes Involved in Imported Fire Ant Sterility.

3. Transformation of Biological Control Agents.

- We have been able to culture *Thelohania* in an insect cell culture.
- The successful transformation of the isolated yeast species was achieved. This will allow the further transformation of a fluorescent marker gene prior to reintroduction into laboratory fire ant colonies for further studies, including the characterization of infection sites and mode of transmission.
- Bacterial species in close association with the imported fire ant were isolated, cultured and partially characterized. This will allow the subsequent development of genetic transformation technologies for these species.

4. Transformation of Imported Fire Ants.

- Developed a method to obtain large numbers of fertile males.
- Recombinant Baculoviruses and Pantropic Retroviruses were produced for use in transforming fire ant queens. Direct injection techniques for the ovarian tissue were developed successfully.

5. Characterization of Gene Expression in Dealate Queens.

- A subtractive library was successfully constructed. Initial characterization indicates that it contains a relatively high proportion of differentially expressed genes.
- DNA sequence analysis and database comparisons revealed some interesting candidate genes that appear to be differentially expressed in the dealate queens. Further northern blot and RT-PCR experiments will be used to confirm these findings.

Relevance to the Texas Imported Fire Ant Research Ant Management Project:

We have developed a program focused on the genetic control and modification of the fire ant that may provide new approaches to management. These data will also be useful in determining sources of new infestations and in tracking progress in the fire ants management.

Products; publications submitted/published; presentations/posters presented at state and national technical conferences:

A. Refereed publications:

B. Manuscript (in Press):

Mir, Bashir; S. B. Vinson and J. A. Piedrahita. Artificial maturation of female alates and production of only male progeny in *Solenopsis invicta* (Hymenoptera: Formicidae). *Southwestern Entomologist*.