Highlights and Impacts of FY '05 Projects Funded by the Texas Imported Fire Ant Research and Management Project

Project Investigator's Name: S. B. Vinson; Co-PI: Patricia Pietrantonio

Project Title: Developing the concept of reproductive control as a method to reduce the imported fire ant population.

Significant Accomplishment	Impact on Imported Fire Ant Management
A). Developed a bioassay that is able to show changes in egg production in queens subjected to various treatments. This has resulted in identifying 3 different controls (1. Something associated with the sperm initiates the initial egg laying as well as wing muscle degeneration. We have focused on the later [see genetic program]. 2. The presence of a dominant queen in a colony suppresses egg production [see B+C below]. 3. When larvae pupate the meconium is fed to the queen and stimulates reproduction [see D below]).	A) This bioassay has been used to identify aspects of the ant's biology that may allow us to isolate factors that stimulate or control reproduction. **GOAL** – to identify factors that suppress reproduction that might be synthesized; the genes responsible can be identified, sequenced and used to suppress IFA reproduction (possibly through bacterial transformation); or design analogs of stimulants that may be able to suppress reproduction.
B). Determined the effects of queen dominance among the multiple queens of polygynous colonies. Determined that unrelated queens aggregate by choice and the aggregation results in increased egg production, possibly due to more efficient feeding by workers. However, the queens exhibit differential worker response and reproductive output (one queen [dominant] attracts the majority of workers suggesting that pheromone levels are involved and possibly regulated by the dominant queen).	B). Even the dominate queen in a polygynous colony rarely become physogastric, as is typical of monogyne queens. Egg production is: monogyne queen > dominate polygyne queen > subordinate polygyne queen; but weight is: monogyne queen > subordinate polygyne queen > dominate polygyne queen; further suggesting that there is some control over egg production. This is under investigation with tools developed by Dr Coates (See C).
C). Currently we are quantifying the Vg's in the hemolymph, tissues and eggs (including trophic eggs) of 1) dominant queens 2) subordinate queens 3) physiograstric queens and 4) virgin queens to determine if the synthesis, storage or up take of any specific Vg is influenced by these conditions so we can focus on some key possible controls.	C). The **goal** here is to determine which Vg is affected by the different types of queens which will allow us to develop a more specific bioassay to focus on isolating one of the factors that control egg production.
D). We developed a bioassay to determine if meconium stimulates egg production or laying. Using this bioassay we have shown that egg production is stimulated by a factor in the liquid component of the meconium that is released just before the	D). The **goal** here is to identify the reproductive stimulant. Once identified we can determine how it acts and we can design analogs that interfere with the stimulant process.

meconial pellet. This is now being fractionated with the help of the Pheromone Project.	

Source and
Amount of Funds Leveraging Current Fire Ant Project

We are now in a position to develop some good proposals and plan one for ARP.

Publications:

Cassill, D., Indira Kuriachan and S. B.Vinson. 2005.Cooperative breeding in fire ants: A test of reproductive skew models. Journal of Bioinformatics (in press). (This is a very significant paper as it is the first to provide data that suggests that there may be alternatives to Kin Selection theory).

Kuriachan, I. and S. B. Vinson. Queen dominance and its effects on reproduction and survival of the polygynous colonies of the red imported fire ant: a physiological perspective. (Manuscript is written and is being submitted to the "Archives of insect Physiology"). (This manuscript shows that the dominate queen suppresses the egg production of other queens in the colony, but the colony as a whole produces more eggs. However the subordinate queens are heaver suggesting that something is released by the dominate queen to suppress egg production).

Cassill, D. and S. B. Vinson. Worker ant response to meconium and the role of meconium products in IFA colony biology. (Being written for submission to insect Socio. in August) (Reveals that meconium products are fed to the queen which stimulates egg laying just as new "nurse workers" are produced).

Highlights and Impacts of FY '05 Projects Funded by the Texas Imported Fire Ant Research and Management Project

Project Investigator's Name: Patricia Pietrantonio Co-PI: Dr. S. Bradleigh Vinson
Personnel: Post-doctoral research associate Mei-Er Chen, student Chris Jagge

Project Title: Developing the concept of reproductive control as a method to reduce the imported fire ant population

Significant Accomplishment (Pietrantonio's lab)	Impact on Imported Fire Ant Management
1. Targeted cloning of G protein-coupled receptors from fire ant mated queen ovaries **a.** We have completed the cloning of the **Short Neuropeptide F (sNPF) Receptor from mated queen ovaries**. In 2004 we reported the cloning of a 200 bp fragment. The receptor is 387 amino acid residues long and has 35.4% identity and 47.5% similarity to the already known orthologue from the fruit fly, *Drosophila melanogaster*.	**1. Targeted cloning of G protein-coupled receptors from mated fire ant queen ovaries** **a.** Until our discovery, only the fruit fly short NPF receptor had been cloned. In locust it is known that the short NPF peptide hormone promotes ovarian development. If this is the case in fire ants **we have found a potential suitable target to inhibit fire ant reproduction by inhibiting ovarian development**, consistent with the objective of this proposal. G protein-coupled receptors are proven drugable targets and at least one commercial pesticide has targeted a GPCR in insects, chlordimeform. Further, in the fruit fly the **Short NPF peptide regulates feeding behavior** (eating or non-eating responses to feeding stimuli) in both larvae and adults, thus we began investigating this aspect in fire ant mated queens, see below.
b. Annotation of the short neuropeptide F receptor from the honey bee genome Based on the sequence of the fire ant cDNA short NPF receptor we identified the genomic sequence within the honey bee genome that contains the ortholog short NPF receptor, which we predicted and that had not been yet annotated. The predicted receptor has a sequence of 393 amino acid residues and has 59.1% identity and 75.4% similarity to the fire ant receptor. The honey bee receptor gene contains one intron.	**b.** Since the honey bee scientific community is much greater than that working on fire ants, our discovery of the **honey bee neuropeptide F receptor** will allow researchers to find out further information on the significance of the short NPF receptor on social insects that can then further help in understanding the fire ant. This is crucial because significant monetary resources are allocated to the understanding of honey bee feeding, foraging and reproduction and this receptor is likely to be the focus of organismal and molecular studies in the honey bee.

2. Starvation reduces the relative amount of short NPF receptor transcript in fire ant queens A starvation assay of fire ant mated queens for 5 and 10 days revealed that the short neuropeptide F receptor transcript decreased significantly, **more than 50% in the mated queen brain** in comparison to the amount of receptor transcript present in fed queens. We used the transcript of the structural protein filamin as a control for gel loading. There was not difference in the amount of receptor transcript in the brain between 5- or 10-day starved queens, confirming experiments by other researchers that claimed that 2-4 days is a reasonable period to consider a fire ant "starved". **Significantly we could not detect a significant transcript decrease in the OVARY of starved queens, indicating that the decrease of transcript in the brain is not unspecifically related to starvation (meaning: no nutrients = no mRNA synthesis) but instead likely a specific feedback signaling mechanism involved in feeding regulation.**	**2. Evidence for implication of the short Neuropeptide F receptor in control of feeding in fire ant queens** Our results indicate that the "starvation signal" feedbacks to the "short Neuropeptide F receptor gene regulation machinery" in a negative fashion, confirming the possibility of feeding regulation by the short neuropeptide F receptor in the fire ant, similar to that found in the fruit fly, *Drosophila*.
3. Tissue localization of the short neuropeptide F receptor The short Neuropeptide F receptor was localized by RT-PCR in brain, midgut, hindgut, Malpighian tubule, fat body and ovaries of fire ant mated queens. Localization of the receptor by whole mount *in situ* hybridizations is under way but definite results are not yet available.	**3.** As a target the short NPF receptor it is both a central nervous system target and a target in peripheral tissues, thus manipulation of this target through insecticide development or biological interference may have profound implications for fire ant survival.
4. We have obtained from Korea (Dr. Kweon Yu, Korea research Institute of Bioscience and Biotechnology Research, Daejeon) **an antibody against the short NPF peptide** to begin to study the hormone signal distribution in the fire ant. There is sequence conservation among various species of insects on the sequence of the peptide thus we expect that the antibody will recognize the fire ant short NPF peptide.	**4.** Availability of the antibody will allow us to study the short NPF peptide (hormone) distribution by immunohistochemistry in the fire ant.

5. Glucose Transporter 8 (GLUT8)

We found that this glucose transporter is most highly expressed in the fat body of mated queens consistent with its role in glucose transport from the hemolymph into the fat body for energy storage when converted into trehalose, triglycerids or glycogen.

We have also focused on the **localization of the Glut8 transporter transcript in reproductive tissues**. In both, alate female (virgin queen) ovaries and in male testes there are two different transcript sizes of 4.9kb and 4.0kb. These transcripts were detected with a highly specific probe for Glut8 only. We also found that the transporter transcript of 4.9 kb is expressed **twice as much in the male testes than in virgin queen ovaries.**

In the mated queen brain there is only one transcript size of 6.1kb for this transporter. This variety in transcript sizes for a glucose transporteris only known from the fire ant since work in vertebrates only shows one size in all tissues studied.

5. GLUT8 may be involved in sperm maturation in the fire ant male

In mammals GLUT8 transcripts are predominantly found in the **testes** and associated predominantly with the acrosomal region of mature sperm spermatozoa. In humans GLUT8 transcripts are undetectable in testicular carcinoma or in men treated with high doses of estrogen; GLUT8 transporters are not found in testes from pre-pubertal rats. Taken together these results indicate that GLUT8 could be regulated by gonadotropins. The GLUT8 **may be an effective target for the inhibition of sperm cell function** and consequently reproduction in the male of the fire ant.

6. Sequences submitted or to be submitted to GenBank (new and corrected)

1. sNPF receptor fire ant (new) (GenBank accession # DQ026281)
2. glucose transporter 8 fire ant (new) (GenBank accession # AY911645)
3. sNPF receptor honey bee (annotated from not yet analyzed genomic sequence AADG04000000, in paper 1, right column).
4. glucose transporter 8 *Anopheles gambiae* malaria mosquito (corrected amino acid sequence EAA11842 in paper 2, see right column).

Publications Submitted

1. Chen, Mei-Er and **Pietrantonio, P. V.** 2005. The short neuropeptide F receptor from the red imported fire ant, *Solenopsis invicta* Buren (for *Archiv.Insect Biochem. Phys.*). Submitted June 2005.

2. Chen, Mei-Er, Steve P. Holmes and **Pietrantonio, P. V.** The glucose transporter 8 from the red imported fire ant *Solenopsis invicta* Buren. 2005. (For *Archiv. Insect Biochem. Phys. Will be submitted July 1st.*).

Published

3. Chen, M.-E., Lewis, D.K., Keeley, L.L., and **Pietrantonio, P.V.** 2004. cDNA cloning and transcriptional regulation of the vitellogenin receptor from the imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae.). *Insect Mol.Biol. 13:195-204.*

	Dissemination of information **National meeting** Mei-Er Chen, D. Lewis, P. Pietrantonio and L. L. Keeley. 2003. cDNA cloning and transcriptional regulation of the vitellogenin receptor from the imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae) Paper 0376. Oral presentation by M.E. Chen at the Section B student competition (Ph.D.), 2003 ESA Annual meeting, Cincinnati, Ohio. **International visitors** 1. Participated in meetings with the Chinese Delegation and presented talks on "The short NPF receptor, GLUT8 transporter and Vg receptor from the red importer fire ant *Solenopsis invicta* Buren." (Mei-Er Chen and P. Pietrantonio). April 2005. 2. Met with visiting professor Dr. Cheng-Jen Shih, National Taiwan University who works on eradication efforts of fire ants in Taiwan. Gave overview of my program. March 2005.
7. **a. Vitellogenin (Vg) receptor regulation: histochemistry and western blots for Vg receptor.** **b. Attempt to use inverse PCR to clone the promoter region of the vitellogenin receptor gene.** a. We developed an antibody against the Vg receptor but this antibody failed to detect the receptor specifically, there were many bands on western blots. Similarly the antibody failed in histochemistry. b. We attempted to clone the regulatory region of the Vg receptor; the latter was previously cloned in our laboratory. After 4 months of effort without positive results we decided to focus in the above projects that were more promising in output. We will continue this effort upon renewal of the proposal by attempting modifications of the methodology.	**7. Understanding the regulation of vitellogenin (yolk protein, Vg) uptake in the eggs of the fire ant** **a.** Our publication showed that the Vg receptor transcript is present even in virgin queen ovary, although in newly emerged queens at low concentration. This presence before mating is unusual in insects. However, we do not know if the receptor protein is simultaneously present. We designed the experiments on the left to study the Vg receptor protein biology through immunolocalization studies. These experiments were needed to elucidate if the Vg receptor protein is present in virgin queens, either on the oocyte plasma membrane or inside the oocyte cytoplasm (and not yet targeted to the plasma membrane), or if the receptor protein is synthesized the novo from the pre-existing detected transcripts upon mating of the virgin queens. **b.** Knowledge of the promoter region is important in order to identify the hormone response elements (DNA sequences) and potential transcription factors that are involved in receptor regulation.
Source and **Amount of Funds Leveraging Current Fire Ant Project**	

No other external funding for this project but the project has benefited from federal grants available to the PI in equipment and supplies. Also see above antibody.