



# Texas Imported Fire Ant Research and Management Project

*Final Progress Report - October 2001*

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## **Endocrine Regulations of Queen Reproduction in the Imported Fire Ant**

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**Funding Amount/2 Years:** \$70,000

### **Major accomplishments to date:**

#### **1. Complete the characterization of vitellogenin (yolk protein precursor).**

**Objective completed** – Manuscript published:

Lewis D. K., Campbell J. Q., Sowa S. M., Chen M.-E., Vinson S. B. and Keeley L. L. 2001. Characterization of vitellogenin in the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Apocrita: Formicidae). *J. Insect Physiol.* **47**, 543-551.

The results to date, suggest that the vitellogenin may consist of two subunits (171 kDa and 182 kDa). The 171 kDa subunit is present in pupae pre-eclosion, and in newly eclosed females. It disappears by day two and by day 4 post-eclosion, the 181 kDa protein is beginning to appear. The 181 kDa protein is present until the onset of egg formation when the 171 kDa protein also appears. It is speculated that the 171 kDa protein may stimulate egg formation.

The results with cloning the VG gene fail to identify two VG genes, hence it would not appear that there are two independent VG protein subunits. Rather, VG may represent a single gene product that is differentially processed relative to growth stages and egg formation.

We have separated the two bands and are in the process of obtaining peptide maps of each and partial sequence data to determine if they are truly two separate proteins, or if they represent one protein that has undergone differential processing.

#### **2. Complete the characterization of the vitellogenin gene from the fat body and the vitellogenin-receptor gene from the ovary.**

Characterization of the VG gene is complete, and a manuscript is in preparation.

The full length fire ant vitellogenin gene has been cloned and sequenced. It was deduced from four separate overlapping clones. The open reading frame and 3' untranslated region consists of approximately 5600 base pairs and shows the strongest homology with vitellogenin gene from the parasitoid wasp *Pimpla nipponica*, the boll weevil *Anthonomus grandis*, and several lepidopteran species. Northern blot analysis indicated that VG is present in the queen, alate reproductive females, workers and pupae but not in males. Consistent with previous SDS-PAGE analysis, VG expression in aged, alate reproductive females was low in newly-eclosed, untanned pupae and increased by approximately 5-fold in tanned pupae. VG expression remained at the pupal level in adults until day 20, when it increased by 30% more. It remained at this level through day 30, the end of the monitoring period. Previously, we reported that alate reproductive females did not show ovarian development until 7 weeks following eclosion; however, in two separate studies, ovarian development was apparent at days 15-20. This research demonstrates that the VG gene is active and the transcript levels are high even during the period of pupation when no eggs are forming. These results demonstrate that the regulation of VG synthesis is not at the level of gene expression, as in most insects, but at the level of protein synthesis or uptake.

Two low-density lipoprotein receptor (LDLR) genes (2.3 and 2.4 kb) are partly sequenced. The VG receptor (VGR) is a member of the LDLR superfamily. The 2.3 kb sequence has been identified as the ovarian VGR gene. The 2.4 kb sequence corresponds with a LDLR-related protein from the chicken and does not appear related to the VGRs. The base sequence analysis of the 2.3 kb gene is completed and the amino acid sequence deduced. Also, a unique sequence in the 2.3 kb gene is being used to develop a probe for in situ studies to identify the tissue expression of the gene.

### **3. Investigate the endocrine regulations of vitellogenin gene expression and synthesis by the fat body and vitellogenin uptake by the ovaries.**

As reported above, the VG gene appears to be expressed continuously from late pupa into the adult reproductive female even though eggs are not being formed. Preliminary studies were performed to determine the sensitivity of VG synthesis to topically applied JH. JH in acetone was applied to alate virgin queens and to decapitated alate virgin queens, and acetone was used as a solvent control. The presence of either JH or acetone stimulated dealation at 24 h post-treatment, some egg formation and the synthesis of both VG subunits after 6 hr. Treatment of decapitated queens resulted in a similar result. In these studies the alate virgin queens were of unknown age.

The study was repeated with alate queens of known age. We tested solvents other than acetone to apply JH since acetone causes a response. Other solvents were ethyl acetate and dimethylsulfoxide (DMSO). Both ethyl acetate and DMSO caused VG expression above the normal baseline activity. However, ethyl acetate caused mortality. DMSO has been used subsequently as a JH solvent.

Although JH (methoprene) causes only marginal increase in VG expression relative to DMSO, VG levels in the hemolymph increase distinctly in response to JH. The data suggest that the VG gene is expressed constitutively in adults of reproductive females but the translation of the VG-mRNA into VG may be JH-dependent. The presence of VG in the hemolymph increases independently of egg formation. This suggests that the regulation of egg formation and Vg synthesis have separate endocrine controls and there may be a hierarchy of hormones acting on VG synthesis and, then, uptake for regulating reproduction in fire ant queens.

Studies using the in vitro system are in progress. VG-antibodies are being used to confirm if the SDS-PAGE 170 and 180 kDa putative VG polypeptides synthesized and released by cultured fat

body are related to the egg vitellin. We are also doing studies with aged, virgin, alate reproductive females to determine at what age the fat body becomes competent for VG synthesis in vitro. Once these studies are completed, we will undertake in vitro treatments of fat body with endocrine extracts and synthetic hormones.

**Goals achieved:**

Objective 1 is completed and a manuscript describing the VG and VN is published. Objective 2 is nearly completed for the VG gene and the cloned sequence for the VGR gene is identified. Objective 3 has been initiated for VG synthesis and in vitro studies are in progress.

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

These results show that treatment with JH results in egg formation in queens, but the presence of a positive response to acetone treatments also makes this conclusion questionable since all previous investigators used acetone as their solvent. This suggests that new hormones may be involved. If confirmed, such a finding would suggest new control strategies. Furthermore, discovery that the VGR is a member of the LDLR family also suggests a novel approach to fire ant control based on recent findings in human medicine. The LDLRs are important in human health since LDLs are the "bad" lipoproteins of vertebrate cholesterol metabolism, and numerous LDLR inhibitors are becoming available as treatments for arteriosclerosis. Inhibition of LDLR functions in insects could suppress egg formation, and other physiological processes that use lipids such as the use of lipid for energy during flight. It might be possible to suppress both the mating flights and egg formation by use of LDLR inhibitors. Finally, if JH does stimulate reproduction, and flight muscle histolysis, it may be possible to formulate a JH analog to cause premature flight muscle histolysis, thus preventing mating flights.

**Publication citations, Paper Presentation Citations and other Citable Products:**

Lewis D. K., Campbell J. Q., Sowa S. M., Chen M.-E., Vinson S. B. and Keeley L. L. 2001. Characterization of vitellogenin in the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Apocrita: Formicidae). *J. Insect Physiol.* **47**, 543-551.

Keeley, L.L. (2001) Physiological Investigations of Events Leading to Egg Yolk Production in Fire Ant Queens. Presentation at the Fire Ant Symposium, Annual Fire Ant Conference, San Antonio.

Lewis, D.K., Campbell, J.Q., Sowa, S.M., Chen, M.-E., Vinson, S.B., and Keeley, L.L., (2001) The Biochemical Characteristics of Vitellogenin in the Red Imported Fire Ant, *Solenopsis invicta* (Hymenoptera: Formicidae). *Southwestern Entomologist*, Proceedings of the Fire Ant Symposium, Annual Fire Ant Conference, San Antonio. (In review).

Keeley, L.L. Reproduction in the Queen Fire Ant – Computer animation for Web or PowerPoint presentations