



Texas Imported Fire Ant Research and Management Project

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Sensory Reception in the Fire Ant Antenna

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Summary of Work to be Done:

1) Determine the composition of the antennal gland secretions, which may contain pheromones involved in worker-queen interactions, and possibly also in interactions between workers and between male and female alates. 2) Study the structure and function of the fire ant basiconic and coeloconic sensilla, which show significant differences on female and on male antennae.

Major accomplishments:

1) Analysis of gland secretions.

Antennal segments of workers and queens were analyzed by SDS polyacrylamide gel electrophoresis. The protein profiles showed prominent low-molecular weight bands in the

region where odorant- and pheromone-binding proteins have been found in other insect antennae. Isoelectric focusing of queen antenna extracts show that these proteins have acidic isoelectric points, consistent with odorant- and pheromone-binding proteins. Separate analysis of antennal segments showed the following. The distal antennal club segment (segment 10) had proteins at 18.2, 16.2 and 13.8 kDa, which we refer to as Siap1, Siap2 and Siap3, respectively. The club segment containing the antennal glands (segment 9) had the same three proteins, as well as an additional 23.4 kDa protein, Siap0. The protein patterns in alate queens were similar, except the amount of Siap0 was about twice that of workers. Siap1 and Siap2 from segment 9 and segment 10 were separately subjected to in-gel trypsin digestion, and the resulting fragments were analyzed by MALDI-TOF mass spectrometry. Seven tryptic fragments were observed in each protein. Siap1 in segment 9 appears to be identical to Siap1 in segment 10, and Siap2 in segment 9 appears to be identical to Siap2 in segment 10. However, Siap1 and Siap2 clearly have different structures. Electrospray mass spectrometry was used to sequence one peptide in Siap2 of segment 9. The sequence showed a high degree of similarity to a putative odorant-binding protein previously found in honey bee antennae. New methods were developed to analyze cysteine-containing peptides isolated from the antenna, which will permit complete sequence analysis of Siap0-3.

In collaboration with R. Deslippe, Texas Tech Univ., we have analyzed the queen secretion containing a pheromone that induces workers to kill sexual larvae. We found the secretion contains low-molecular weight proteins.

Antennae were modified with amine-specific fluorescent reagents to test for the presence of protein in the gland secretion using fluorescence microscopy. No labeling was observed with fluorescamine. NBD-Cl did not label the gland secretion, but it strongly labeled the base of sensillae on the club segments. FITC showed some weak labeling of gland secretions.

2) Structure of basiconic and coeloconic sensilla.

High resolution scanning and transmission electron micrographs of basiconic sensilla showed pores near the tip. The finding of tip pores, along with the previously mapped location of the basiconic sensilla at the part of the antenna the ants touch to surfaces, implicates the basiconic sensilla as the primary antennal contact chemoreceptors. A 3-dimensional map of the positions of the basiconic sensilla on a worker antenna is posted at <http://lonestar.utsa.edu/rrenthal/Basiconic.htm>.

We looked for evidence of a contractile apparatus in the basiconic sensilla that might be involved in uptake of substances from surfaces. Antennal clubs were stained with fluorescent-tagged phalloidin, which binds to filamentous actin. On examination by fluorescence confocal microscopy, we found no evidence of an actin cytoskeleton in the basiconic sensilla. However, extensive actin staining was observed in the epithelial cells underlying the sensilla, consistent with their known role in secretory vesicle traffic supporting the extracellular antennal lymph in the sensilla.

Analysis of the patterns of coeloconic sensillae on worker antennal clubs shows evidence of right-left asymmetry. (Fluctuating asymmetry has been linked to developmental stress.) This finding is in contrast to the basiconic sensilla, which we previously showed display right-left symmetry.

Goals achieved/Milestones/Highlights:

- Identified an odorant-binding protein for the first time in ant antennae.
- Discovered a protein which appears to be a component of the antennal gland secretion.

- Located pores in basiconic sensilla, implicating them as major antennal contact chemoreceptors.

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

The results of these studies will provide information about new pheromones that could be useful as baits, and will suggest new methods of disrupting communications between workers and between alates.

Publication citations, Paper Presentation Citations and other Citable Products:

Publication:

Isidoro, N., Romani, R., Velasquez, D., Renthal, R., Bin, F. & Vinson, S.B. "Antennal glands in queen and worker of the fire ant, *Solenopsis invicta* Buren: first report in female social Aculeata (Hymenoptera, Formicidae)" *Insectes Sociaux* 47:236-240 (2000)

Submitted:

Renthal, R., Velasquez, D., Hoog, S., Carroll, C. & Weintraub, S.T. "Analysis of antennal proteins of the red imported fire ant." submitted to *Southwestern Entomologist* (2001)

Manuscript in preparation:

Renthal, R., Velasquez, D., Olmos, D., Hampton, J., & Wergin, W.P. "Sensilla Structures and Patterns on the Antenna of the Red Imported Fire Ant "

Presentations:

Renthal, R., Velasquez, D., Olmos, D., Farnsworth, P & Vinson, S.B., "Antenna morphology of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae)," *Entomological Society of America 1999 Annual Meeting*, (1999)

Renthal, R., Velasquez, D. & Hoog, S. "Protein composition differences in antennal club, flagellum and scape of the red imported fire ant." *Imported Fire Ant Conference* p. 20 (2000)

Vinson, S.B., Renthal, R., Velasquez, D., Isidoro, N., Romani, R. & Bin, F. "Glands in the antennae of fire ant females." *Imported Fire Ant Conference* p. 18 (2000)

Renthal, R., Velasquez, D., Hoog, S. & Weintraub, S.T., "Analysis of antennal proteins of the red imported fire ant." *Entomological Society of America 2000 Annual Meeting* (2000)

Renthal, R., Velasquez, D., Hoog, S., Carroll, C. & Weintraub, S.T. "Analysis of antennal proteins of the red imported fire ant." *Imported Fire Ant Conference* (2001)