Contracted Research

Title: Develpment of DNA-Based Assays for Identifying Parasitoids of Sweetpotato Whitefly

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The research outlined in the contract with CDFA is to identify regions of genetic variability among aphelinids (Hymenoptera) used in a biocontrol program against *Bemisia tabaci* Biotype B (=silverleaf whitefly) (Homoptera: Aleyrodidae), species complex. The aphelinids are in the genus *Encarsia* and *Eretmocerus*. This past year our focus was on *Eretmocerus*. Our mission was to identify *Eretmocerus* taxa using a genetically based assay. We tested seven parasitoid taxa provided by Charles Pickett, CDFA-Sacramento, with the following accession numbers: *Eretmocerus* spp. M92019, M94001, M94002, M94003 and M94019, M94120 and M95012. Initial cultures were reared by the USDA-APHIS-PPQ in Mission, Texas.

We successfully amplified by polymerase chain reaction (PCR) the ITS2 and 28S D2 of each of the above taxa. We cloned these genetic regions for all taxa. These clones are maintained in a stock library at our laboratory in Albany, CA. Full nucleotide sequences (both top and bottom strands) of these cloned genetic regions have been completed. Comparisons of these sequences among taxa have identified genetic differences that could be used to differentiate the taxa.

Sequences of >600 base-pairs of the 28S rDNA-D2 among taxa show that there are >40 informative site differences between *Encarsia formosa* Gahan and the various accessions of *Eretmocerus*. Among *Eretmocerus*, there was considerable difference (>20 informative sites) between Old World and New World accessions. Differences between New World 94-001, 002, and 003, were negligible.

The 28S-D2 region described above is a highly conserved region of genetic variation. Therefore we also completed sequencing the more variable ITS2s region for the provided aphelinid taxa. The size of this region varied among taxa with a range of about 440 bases for 94001, 94002 and 94003 to 462 bases for 92019 and for *Encarsia*, 569 bases. The size differences reflect sections of nucleotides that are present in some taxa and absent in others. There were vastly higher numbers of differences in overall nucleotides than were seen among taxa in the 28S-D2 region. These differences were exploitable for the development of an assay to identify these taxa, especially *Encarsia* and Old and New World *Eretmocerus*. There were few base differences in ITS2s among New World 94001, 94002 and 94003. However, there were enough to differentiate these taxa using an assay that combined PCR and digestion of the amplified DNA with Hha I. Using this assay this upcoming year, we plan to identify *Eretmocerus* which have become established in field releases in California.

In summation, the level of difference between Old World and New World *Eretmocerus* are vast. Based on ITS2 sequences *Eretmocerus* 94001, 94002, and 94003 appear to be a closely related complex. Old World *Eretmocerus* are genetically distinct enough from the New World *Eretmocerus* examined thus far to perhaps warrant membership in a separate subgenus. These genetic differences are in concordance with a recently discovered physical antennal character that differentiates Old and New World *Eretmocerus*.

Additional *Eretmocerus* taxa are being procured from Charles Pickett and added to the analysis in order to build a genealogical framework for this group of parasitoids. Such information is useful for selecting lineages of parasitoids that may be the most efficient parasitoids of *Bemisia* whiteflies. By building a large database of aphelinid nucleotide sequences, it will be possible to assay whitefly nymphs (native and exotic) by a simple PCR + RFLP analysis to identify the parasitoid. This could be operational for any whiteflies collected in California in future outbreaks or for assessing biocontrol programs. In addition to enlarging the database of aphelinid nucleotide sequences, we will also attempt to develop a radiological assay that might identify parasitoids at the egg, or early nymphal stages.

Also, based on nucleotide sequences of more conserved regions, an inferred phylogeny for the Chalcidoidea (Hymenoptera) has been constructed. Cloning and nucleotide sequencing of a region of the 28S and 18S rRNA genes indicates that lineages of chalcidoids diverged during the Late Cretaceous concurrently with radiation of major whitefly lineages. The ability to parasitize whiteflies evolved a number of different times among chalcidoids. The phylogeny also suggests that Aphelinidae is paraphyletic and, as such, does not comprise a coherent assemblage of taxa. Lastly, genetic distances based on numbers of nucleotide differences between taxa may be a reliable approach to systematic classification of these wasps.

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