Development of DNA-Based Assays for Identifying Parasitoids (Hymenoptera:Aphelinidae) of Silverleaf Whitefly

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Through a research contract with the California Department of Food and Agriculture, a DNA-based assay is being developed for identifying taxa of Aphelinidae. Five parasitoid taxa with the following accession numbers: *Encarsia formosa* M92030, and *Eretmocerus* spp. M94001, M94002, M94003, M92019 have been received. To study the genetic variability amongst these parasitoids we used the variable regions located within a genetic region called the rRNA transcript. This region includes genes which are conserved and show little variability (changes in nucleotide sequences) through evolutionary time. However, the rRNA transcript also possesses highly variable regions located between the genes, called internal transcribed spacers, ITS1 and ITS2. Also, there is another variable region within one of the genes called the 28S rDNA-D2 expansion region. We have successfully amplified, by polymerase chain reaction (PCR), the ITS2 and 28S rDNA-D2 of each of the above taxa. We have cloned these genetic regions of each taxa with exception of M92019, recently procured. Partial nucleotide sequences of these cloned genetic regions have been completed. Comparisons of these sequences amongst taxa have identified genetic differences that could be used to differentiate the taxa.

Sequences of approximately 600 base-pairs of the 28S rDNA-D2 amongst taxa show that there are more than 65 nucleotide differences between *Encarsia formosa* and the various accessions of *Eretmocerus*. However, there were few to no differences found in this genetic region amongst the *Eretmocerus* taxa. We have completed sequencing two conserved terminal regions of ITS2 of the *Eretmocerus* taxa and found five nucleotide differences amongst them. These differences amongst the *Eretmocerus* within the ITS2 region analyzed suggests that M94001, M94002, and M94003 have diverged to the point of being separate species. Further sequencing of the interior of the ITS2 region would reveal many more nucleotide differences.

In summary, the genetic differences between *Encarsia* and *Eretmocerus* are significant and at a level for easy differentiation using a genetically-based assay. The level of difference amongst *Eretmocerus* taxa is much closer than that between *Encarsia* and *Eretmocerus*. Based on nucleotide sequences examined thus far the *Eretmocerus* taxa appear to be at an equivalence of a species complex. The genetic distances are similar to those determined for another group of pteromalid parasitoids in the *Nasonia* species complex (a separate study). Additional *Eretmocerus* taxa are to be 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. Additionally, in a related study on whitefly genealogy, we and Ray Gill, California Department of Food & Agriculture, have collected numerous species of whiteflies in California. Many of these whiteflies are already parasitized. Since we have saved the DNA of these insects, we will be able to re-examine their DNA in order to determine which species of parasitoids are parasitizing the various California native and introduced species of whiteflies.

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In: Bezark, L.G. (Editor). 1995. *Biological Control Program Annual Summary, 1994.* California Department of Food and Agriculture, Division of Plant Industry, Sacramento, California