

**MOLECULAR ECOLOGY OF RIBOSOMAL ITS1 POLYMORPHISMS IN
HELICOVERPA ARMIGERA POPULATIONS IN THAILAND**

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We are investigating the molecular ecology of *Helicoverpa armigera* populations in Thailand. The bollworm *Helicoverpa armigera* is a serious agricultural pest in several different areas in Thailand. In cultivated areas, it infests a variety of different host plants including cotton, tomatoes and potatoes. We are using molecular markers to try and determine if there are genetic differences among populations defined geographically or on the basis of host plant preferences. These markers may also be useful for studies of pest movements through migration and/or invasions of new habitats.

In the search for appropriate molecular markers, we have determined the DNA sequence of the internal transcribed spacer region 1 (ITS1) of the ribosomal genes in a number of individuals collected on different host plants and from various geographic areas in Thailand. Using the variability we have uncovered in the DNA sequence data from these individuals, we have devised a method to identify specific marker types based on restriction fragment length polymorphisms. We are currently making additional collections and screening for additional markers to aid in this analysis.

MOLECULAR ECOLOGY OF THE *BACTROCERA TAU* SPECIES COMPLEX

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Evolutionary relationships of *Bactrocera tau* species and populations have been examined using nucleotide sequences of a heat shock cognate 70 like gene (hsc70-like gene). *B. tau* belongs to a group of tephritid fruit flies which are destructive pests in Southeast Asia. The name *B. tau* initially referred to a widely distributed complex of species. By identification of some biological differences, distinguishable groups of *B. tau* have been discerned and designated as eight forms labelled A, B, C, D, E, F, G, and I. However, the evolutionary and genetic relationships among these forms has not been clarified. Establishment of a molecular phylogeny may promote better understanding of the diversity and evolution of fruit flies within this complex.

For this purpose, part of an hsc70-like gene was amplified by PCR and cloned from genomic DNA of *B. tau* specimens. DNA sequences and genetic distances measures were obtained for the hsc70-like sequences. These were used to postulate evolutionary histories based on maximum likelihood, neighbor-joining, and maximum parsimony approaches to reconstruct phylogenetic relationships.

The best resulting phylogenetic tree topologies derived from all of the approaches were congruent. These results suggested that the *B. tau* forms could be divided into three subgroups. The first subgroup consisted of only *B. tau* form A. The second closely related subgroup consisted of forms B, E, F, and G, and the third consisted of forms C, D, and I. However, this same approach did not resolve phylogenetic relationships of *B. tau* (form A) geographical populations. In conclusion, the hsc70-like sequence appears to be suitable for establishing molecular phylogenies at the level of species but not populations in the *B. tau* species complex.

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POLYMORPHISM OF *DROSOPHILA* PHEROMONES: MOLECULAR STUDIES

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The cuticle of *Drosophila melanogaster* is covered with a layer of lipids including long-chain hydrocarbons. Two of them are mainly produced by mature males (7-tricosene, 7T and 7-pentacosene, 7P) and can act on conspecifics either as attractants for females or repellents for other males. The main mature female hydrocarbons are longer and act as contact pheromones after males tap female cuticle (7,11heptacosadiene, 7,11 HD and 7,11nonacosadiene, 7,11 ND). Contrary to those of the rest of the world, females from sub-saharan African strains show low levels of 7,11HD and high levels of its position isomer, 5,9 HD. Other species, as the sympatric sibling species, *D. simulans* are sexually monomorphic, with a large production of 7T and 7P together with 2methyl-branched HCs as in *D. melanogaster* flies of either sex; in 5 such species of the *melanogaster* subgroup, 7T acts as a sex pheromone. The biosynthesis of these compounds shares a number of steps (synthesis of unsaturated fatty acids, desaturation, elongation and decarboxylation) while some steps are sex-specific, especially for female diene production (female-specific desaturation of monoenic fatty acids and their elongation and decarboxylation.) The common steps involve among others D9 desaturase and one or more elongase(s). We have characterized molecularly and functionally a D9 desaturase in *D. melanogaster* and in *D. simulans*, *desat1*, which transforms palmitic acid into palmitoleic acid and seem to lead to w7-HCs. Indeed in position 7 *desat1* mutants show low levels of such unsaturated hydrocarbons. Another desaturase gene has been characterized in *D. melanogaster*, which is translated only in females of the 5,9 HD rich morph. *desat2*, located near *desat1*, encodes another D9 desaturase which uses myristic acid as substrate leading to the expected w5-fatty acids necessary for the diene biosynthesis. We are searching for the enzymes involved in the female-specific steps and a number of preliminary data will be presented and discussed.

POSTER

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PHYTOCHEMICAL VERSUS MOLECULAR EVALUATION OF *PHYLLANTHUS NIRURI* L.

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The significance of *Phyllanthus niruri* in the treatment of various diseases especially liver complication has emphasized the necessity for adequate standardization. The ultimate goal of our research was to establish a phytochemical and a molecular standardization protocol to ensure the quality, safety and efficacy of this herb, as well as to avoid taxonomic confusion and adulteration in commerce. Eight species of *Phyllanthus* were screened in this survey. The thin layer and gas chromatographic profile of the hexane extract was recognized as the best method for phytochemical evaluation. Lignan constituent, i.e. lintetralin, niranthin, hypophyllanthin, phyllanthin and nirtetralin were only available in *P. niruri* and were reliable chemotaxonomic markers for this particular taxon. Meanwhile, Random Amplified Polymorphic DNA (RAPD) technique was used to distinguish the molecular variation. Polymorphisms in all the *Phyllanthus* species were detected by twenty random decamer primers in the polymerase chain reaction (PCR). The comparisons between the molecular and phytochemical evaluation were made. The great resolution contributed by the results obtained in the identification and standardization of *P. niruri* was briefly discussed.