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Molecular analysis of genetic loci that are involved in the mechanism of development insecticide resistance in the most important olive fruit pest, the olive fly *Bactrocera oleae.*

ABSTRACT

The olive fruit fly, *Bactrocera oleae*, is among the most important agricultural pests, causing severe economic damage in olive cultivation. The control of the fly is based mainly on the use of organophosphate (OP) insecticides. Apart from the damaging effects that insecticides may have in the environment, their intensive and non-prudent use has also resulted in the development and spread of insecticide resistance in natural insect populations. The aim of the present dissertation was, firstly, the study of OP resistance spread of *B. oleae* populations and, secondly, the comprehension of the molecular genetic basis of resistance.

The primary genetic locus that is involved in OP resistance is the acetylcholinesterase (*Ace*) gene. In the first part of the thesis, the organization of the *Ace* locus of *B. oleae* is studied. A series of genomic library screening and PCR reactions determined the intron-exon organization of the *B. oleae Ace* locus, which was proved to be very similar to that of *Drosophila melanogaster*. The *B. oleae* acetylcholinesterase gene is comprised of ten exons, stretched in an area of over 75 kb of DNA. Furthermore, *in silico* analysis of the enzyme demonstrated that it maintains the post-modifications of AChE of insects, indicating the high degree of conservation of this genetic locus.

OP-resistance in the olive fly was previously shown to be associated with two mutations in the catalytic site of the *Ace* gene. These mutations conferred a 16-fold resistance in natural olive fly populations as compared to laboratory reared ones. The frequency of these mutations was monitored in *Bactrocera oleae* individuals of increasing resistance. Despite the difference in resistance among the individuals, there was no significant frequency variation and no correlation between mutation frequencies and resistance level. Consequently, there must be other contributing factors, such as other mutations, to the variation of resistance. The presence of additional mutations in the *Ace* gene was investigated in highly resistant insects. Most of mutations that were isolated yielded nothing but silent nucleotide substitutions. However, a short deletion of three glutamines in the carboxyl-terminal domain of the protein (termed BoaceΔ642-644 or Δ3Q) demonstrated particular curiosity. Three diagnostic tests were developed for monitoring the mutation. The analysis of wild olive fly populations showed a significant correlation between mutation frequency, resistance level and OP use. Moreover, biochemical assays on individual flies showed that the remaining activity of Δ3Q enzyme was higher than the wild type enzyme.

This is the first description of a mutation localized outside the catalytic gorge of AChE with possible involvement in insecticide resistance. It is speculated to affect the GPI-anchoring efficiency or the stability of the protein. In order to investigate the putative role of Δ3Q, the wild type and the mutant enzymes were expressed in COS cells, together with a mutant in which all five consecutive glutamines were experimentally deleted (Δ5Q). The study and biochemical characterization of the three constructs (wt, Δ3Q, Δ5Q), as well as their ability to GPI anchor addition, indicated that the Δ3Q mutation affects the post-translational modifications of AChE (GPI anchoring, stability/degradation). This suggests an entirely new

mechanism of insecticide resistance to OPs, in which a more efficient GPI modification of the enzyme may result in more anchored molecules in the synaptic cleft than the wild-type fly and, therefore, a reduced sensitivity to the insecticide. Finally, the absence of homozygote ($\Delta 3Q^{-/-}$) individuals in genetic crosses of heterozygotes ($\Delta 3Q^{-/+}$) advocates for a high fitness cost of the Δ3Q mutation.