

ACETYLCHOLINESTERASE GENES AND INSECTICIDE RESISTANCE IN APHIDS



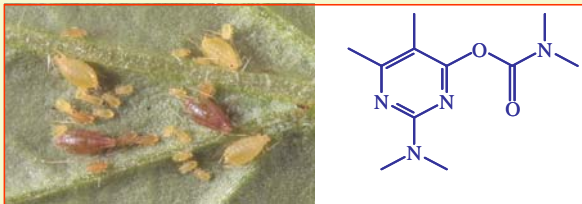
S.L. Dong, M.C. Andrews, F. Li, G.D. Moores, Z.J. Han & M.S. Williamson
Rothamsted Research, Harpenden, UK & Nanjing Agricultural University, Nanjing, PR China

Introduction

The peach-potato aphid (*Myzus persicae*) and cotton aphid (*Aphis gossypii*) are important agricultural pests in many parts of the world, causing feeding damage and transmission of virus diseases on a range of crops. They are controlled with a variety of insecticides, including the dimethylcarbamates (pirimicarb & triazamate), since these are 'soft' insecticides with good aphicidal activity but low toxicity to non-target insects (eg. aphid predators).

The widespread and intensive use of these two carbamates has however led to the selection of resistant populations. Biochemical studies of resistant strains from both species have shown that this often results from alterations in the main synaptic AChE that render it insensitive to these compounds.

In order to understand the molecular basis of this resistance, we have cloned and sequenced the AChE genes from both species and identified single amino acid substitutions that correlate with the resistant phenotypes.

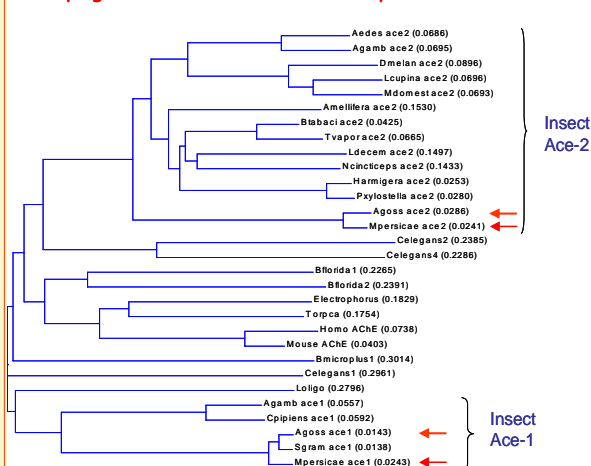


Myzus persicae adults & nymphs, and pirimicarb structure

Our efforts to identify the underlying mutations that confer resistance were hampered by the discovery that aphids contain 2 different AChE genes. We initially cloned the *Drosophila* AChE homologue (now called ace-2), but failed to find any differences between susceptible and resistant strains¹.

We then cloned and sequenced a second AChE gene from both aphid species (termed ace-1). It now seems that most insects contain both ace genes and that ace-1 normally encodes the major synaptic AChE enzyme (except for certain Dipteran insects such as *Drosophila*). The ace-1 and ace-2 proteins share less than 50% amino acid identity, suggesting that the duplication that gave rise to these genes occurred early during the evolution of insects (see the phylogenetic tree below).

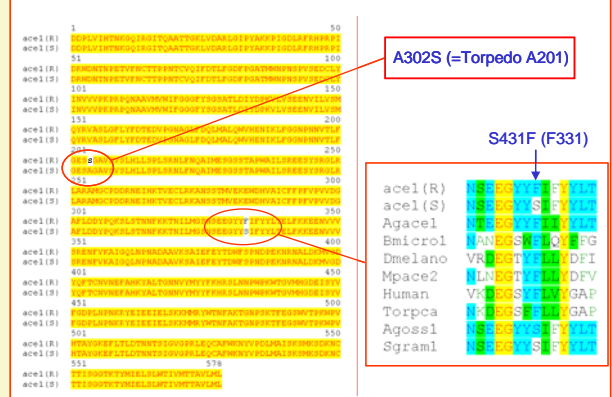
Phylogenetic tree - database Acetylcholinesterases



Comparative sequencing studies of the ace-1 genes from susceptible and resistant strains of *Myzus persicae* and *Aphis gossypii* have identified two point mutations that are associated with resistant phenotypes²⁻⁴.

- 1) A serine to phenylalanine replacement (S431F = Torpedo F331) in strains that are selectively resistant to pirimicarb and other dimethylcarbamates.
- 2) An alanine to serine replacement (A302S = Torpedo A201) that is found in strains that show a broader resistance to OPs and carbamates.

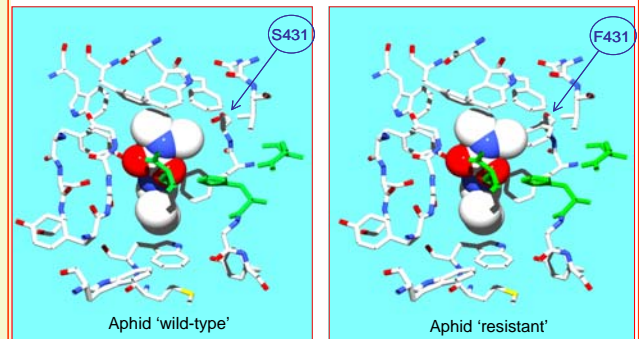
Point mutations associated with resistant phenotypes



We are particularly interested in the S431F mutation since only wild-type aphids appear to contain a serine at this position. Preliminary modelling studies reinforce the view that replacement of this serine by the bulkier tyrosine side-chain restricts pirimicarb binding at the active site (see below).

This in turn may explain why pirimicarb and other dimethylcarbamates are selectively toxic to aphids, since other insects (and vertebrates) already have the 'resistance-conferring' phenylalanine at this position.

Modelling the affect of the S431F mutation on pirimicarb binding



Current Work and Aims

We are expressing the ace-1 and ace-2 genes using the baculovirus system:

- 1) To compare the biochemical and kinetic properties of these enzymes.
- 2) To confirm the resistance functions of the S431F and A302S mutations.
- 3) To explore other unique features of the aphid ace-1 active site and to purify recombinant ace-1 protein for structural studies.

1. Javed *et al* (2003) *Insect Mol Biol* 12, 613
2. Nabeshima *et al* (2003) *Biochem Biophys Res Com* 307, 15
3. Andrews *et al* (2004) *Insect Mol Biol* 13, 555
4. Toda *et al* (2004) *Insect Mol Biol* 13, 549