Comparative genomics

Insecticide resistance in mosquito vectors

Resistance to insecticides among mosquitoes that act as vectors for malaria (*Anopheles gambiae*) and West Nile virus (*Culex pipiens*) emerged more than 25 years ago in Africa, America and Europe; this resistance is frequently due to a loss of sensitivity of the insect's acetylcholinesterase enzyme to organophosphates and

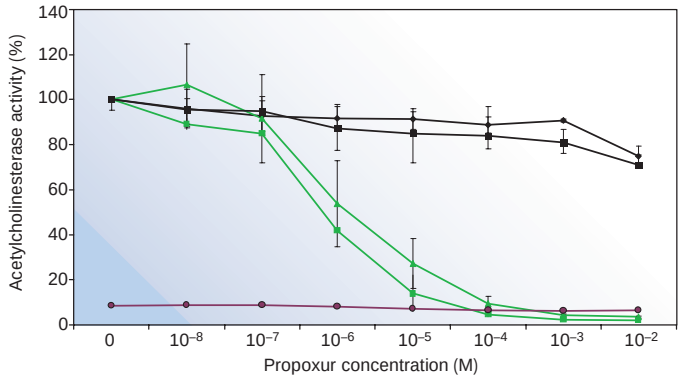

Figure 1 Residual acetylcholinesterase activity of susceptible (green squares) and resistant (black diamonds) mosquitoes assayed in homogenates and lysates from transfected S2 cells in the presence of increasing concentrations of the carbamate insecticide Propoxur. S2 cells were transfected with the recombinant pAc5.1/V5-His vector (Invitrogen) either alone (purple circles) or with expression of either sensitive acetylcholinesterase-1 (green triangles) or insensitive G119S-mutant enzyme (black squares). Residual enzyme activity was assayed after incubation with Propoxur for 15 min (ref. 5). Three independent experiments were carried out using different volumes of cell lysate.

carbamates[1]. Here we show that this insensitivity results from a single amino-acid substitution in the enzyme, which we found in ten highly resistant strains of *C. pipiens* from tropical (Africa and Caribbean) and temperate (Europe) areas, as well as in one resistant African strain of *A. gambiae*. Our identification of this mutation may pave the way for designing new insecticides.

Acetylcholinesterase terminates synaptic transmission by hydrolysing the neurotransmitter acetylcholine; its inactivation by insecticides leads to paralysis and death. Mosquitoes, however, show widespread and strong resistance to this type of insecticide. They have two genes that encode different isoforms of acetylcholinesterase: *ace-1*, which has no homologue in the fruitfly *Drosophila melanogaster* and is closely linked to resistance in *C. pipiens*; and *ace-2*, a homologue of the unique *Drosophila ace* gene[2]. The generally mild insensitivity of acetylcholinesterase-2 in *D. melanogaster* is due to the combined weak effect of several mutations[3].

To identify mutations involved in resistance in mosquitoes, we determined the complete *ace-1* messenger RNA coding sequence of two *Culex pipiens* strains: one susceptible and one resistant (results not shown). *C. pipiens ace-1* encodes a putative 702-amino-acid protein, which is 81% identical to its *A. gambiae* homologue and 39% identical to *D. melanogaster* acetylcholinesterase-2. Complementary DNAs from the susceptible and resistant strains differ at 27 nucleotide positions, only one of which generates an amino-acid substitution: the GGC (glycine) codon at position 119, according to the nomenclature for *Torpedo* acetylcholinesterase[1], is replaced by an AGC (serine) codon in resistant mosquitoes (mutation G119S).

From three-dimensional modelling, we find that this mutated residue lies within the active 'gorge' of the enzyme, close to the catalytic site and abutting the oxyanion hole (results not shown). To evaluate the biochemical effect of the mutation *in vitro*, we assayed the catalytic properties and insecticidal sensitivity of wild-type and mutant recombinant acetylcholinesterase-1 that was expressed in S2 *Drosophila* cells. The G119S mutant showed the same insensitivity to Propoxur insecticide as resistant-strain acetylcholinesterase-1 (Fig. 1). A single mutation in *ace-1* must therefore be responsible for the insensitivity of the enzyme.

To determine whether the G119S mutation is present in other *C. pipiens* strains with insensitive acetylcholinesterase, we sequenced exon 3 of *ace-1* in several resistant and susceptible strains derived either from the temperate or the tropical/subtropical form of the *C. pipiens* species complex (*C. p. pipiens* and *C. p. quinquefasciatus*, respectively). All of the resistant strains carried the G119S substitution, regardless of their origin. Moreover, although 23 nucleotides were polymorphic, a unique haplotype was found to be associated with the resistance within each subspecies (see supplementary information). This indicates that the same G119S mutation has occurred independently at least twice in *C. pipiens*, once in each subspecies.

We also investigated the recent emergence of insensitive acetylcholinesterase in the main African malaria vector *Anopheles gambiae*[4], with the use of the *ace-1* genomic sequences of a resistant (YAO) and a susceptible (KISUMU) strain. The coding sequences differed at 18 nucleotide positions, two of them being non-synonymous. In the YAO strain, one mutation that resulted in the replacement of a valine residue by alanine in the amino-terminal region has no equivalent in *Torpedo* acetylcholinesterase and did not seem to affect the enzyme's catalytic properties (results not shown). The other was the same G119S substitution as in *C. pipiens* (results not shown), indicating that this single point mutation has occurred independently at least three times in the *ace-1* gene: twice in the *C. pipiens* complex and once in *A. gambiae*.

The discovery of the *ace-1* mutation that is responsible for insecticide resistance in mosquitoes opens the way to new strategies for pest management. The development of new insecticides that can specifically inhibit the G119S mutant form of acetylcholinesterase-1 will be crucial in overcoming the spread of resistance.

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COMMUNICATIONS ARISING

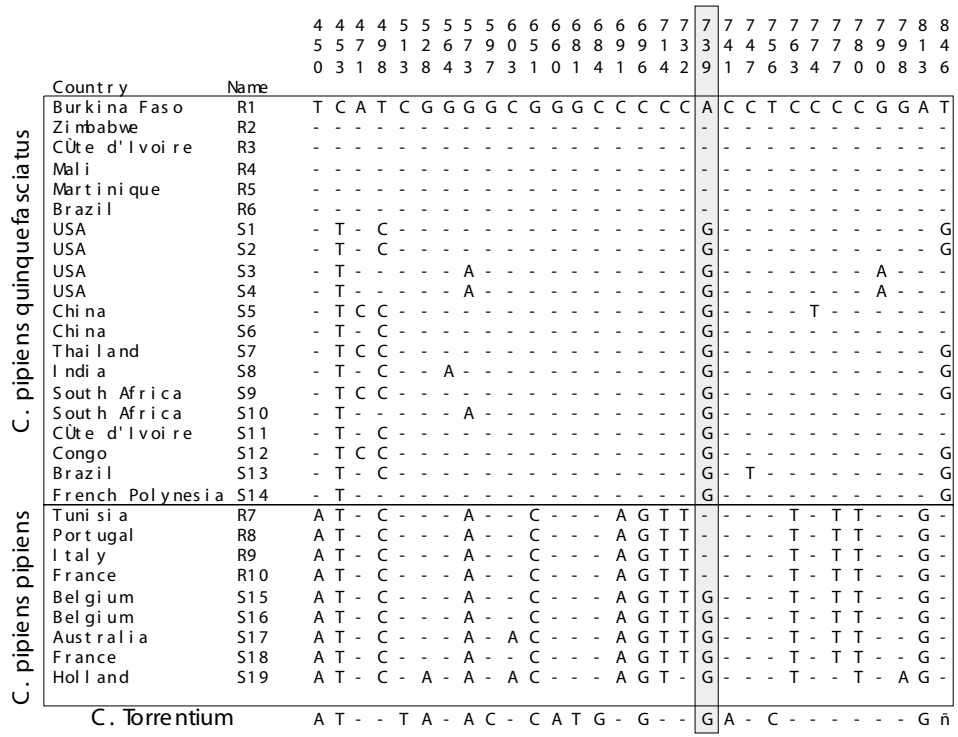

Figure S1. Nucleotide polymorphism in exon 3 of *ace-1* in *Culex pipiens* mosquitoes. Samples are listed according to subspecies (*C. p. pipiens* or *C. p. quinquefasciatus*), presence of insensitive AChE (Ri) or not (Si), and country of origin. Only polymorphic sites are indicated, and a dash indicates similarity with the top sequence. The position of the G119S mutation is boxed. *Culex torrentium*, the closest known species to the *C. pipiens* complex, is presented as an outgroup. See Supplementary Information for references for all samples.

Table S1. Name, country of origin and reference of the strains used in Figure S1.

Taxa	Name in Fig. S1	Real name	Country	Reference
C. p. quinquefasciatus	R1	BO	Burkina-Faso	M. Raymond, unpublished
	R2	HARARE	Zimbabwe	M. Raymond, unpublished
	R3	SUPERCAR	Côte d'Ivoire	1
	R4	DJI	Mali	M. Raymond, unpublished
	R5	MARTINIQUE	Martinique	2
	R6	RECIFE	Brazil	Collected in 1995 by A.-B. Failloux (Pasteur Institute, Paris, France)
	S1	PRO-R	USA	3
	S2	S-LAB	USA	3
	S3	TEM-R	USA	4
	S4	TRANS-P	USA	5
	S6	LING	China	6
	S7	MAO	China	7
	S6	THAI	Thailand	8
	S8	MADURAI	India	9
	S9	BSQ	South Arica	Collected in 1991 by A. J. Cornel (Sth Afr. Inst. Med. Res., South Africa)
	S10	BED	South Arica	Collected in 1991 by A. J. Cornel (Sth Afr. Inst. Med. Res., South Africa)
	S11	BOUAKE	Côte d'Ivoire	10
	S12	BRAZZA	Congo	11
	S13	BRESIL	Brazil	M. Raymond, unpublished
	S14	MOOREA	French Polynesia	12
C. p. pipiens	R7	ESPRO	Tunisia	13
	R8	PRAIAS	Portugal	14
	R9	PADOVA	Italy	15
	R10	BARRIOL	France	16
	S15	BRUGES-A	Belgium	17
	S16	BRUGES-B	Belgium	17
	S17	KILLCARE	Autralia	18
	S18	BLEUET	France	19
	S19	HETEREN	Holland	M. Raymond, unpublished
C. torrentium	-	UPPSALA	Sweden	20

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