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Correspondence

Insecticide resistance: a silent base prediction

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Response to a challenging environment proceeds through adaptation, the result of stochastic processes (chance) and of the influence of history (constraint) [1]. Adaptations, such as pesticide resistance, provide an opportunity to study historical constraints. Insecticides, widely used since the mid 1950s, have elicited numerous cases of resistance. Specific amino acid changes at unique or few critical positions of the target protein explain resistance to the major classes of insecticides, such as cyclodienes, organochlorines, pyrethroids, carbamates and organophosphates (OPs) [2–6] and sometimes lead to extremely high resistance levels (>1000 fold).

In mosquitoes, a single glycine (Gly) to serine (Ser) substitution at position 119 (*Torpedo* nomenclature) in the acetylcholinesterase (AChE1) gene confers high levels of resistance to carbamates and OPs [3]. This G119S substitution was selected at least twice independently in *Culex pipiens*, once in *Anopheles albimanus* and once in *Anopheles gambiae*, suggesting that there are only few possibilities to generate high AChE1 insensitivity [7]. Although heavily controlled with carbamates and OPs, the mosquito vector of dengue and yellow fever, *Aedes aegypti*, never developed high levels of resistance.

We first checked whether the G119S mutation in AChE1 is ineffective in this species. We cloned the complete *AChE1* cDNA, encoding a protein 96.4% similar to *C. pipiens* AChE1, (supplemental data) and produced wild-type and G119S mutant recombinant proteins. *Ae. aegypti* AChE1 behaved exactly like *C. pipiens* AChE1: wild-type proteins were inhibited at identical doses of the carbamate insecticide propoxur (IC50 = 5 x 10^{-7} M), while G119S proteins remained insensitive up to 10^{-2} M propoxur (Figure 1). It is, therefore, unlikely that the low resistance in *Ae. aegypti* resulted from particular biochemical properties of its AChE1.

Alternatively, *Ae. aegypti* AChE1 may not be able to evolve to the G119S substitution. Notably, in *Ae. aegypti* glycine 119 of AChE1 is encoded by a GGA codon, whereas GGC was found in all the other species analysed so far [3,7]. This silent third base change represents an extraordinarily heavy constraint. It decreases the probability of a spontaneous G119S substitution by several orders of magnitude . As Ser can be encoded by AGY or TCN, substitution to Ser requires only one mutation when Gly is encoded by GGY, whereas two are required when Gly is encoded by GGR. We thus hypothesized that high levels of resistance cannot emerge if the G119S substitution requires more than a single step mutation, a situation that could be described as a 'codon constraint'. Accordingly, knowing the sequence of codon 119 should allow the prediction of the ability of a given mosquito species to develop high OP-resistance.

We first checked all known acetylcholinesterase amino acid sequences (79 animal species), and found that a glycine is present at position 119 in all species, except in ascidians and *Schistosoma*, which show a serine. This suggests that presence of a glycine is critical, and that no other amino acids are allowed in this position, except serine.

We next analysed the sequence of codon 119 in 26 natural populations of *Ae. aegypti* collected in 12 countries. In all samples, the glycine was encoded by GGA (serine-immutable), which fits with the

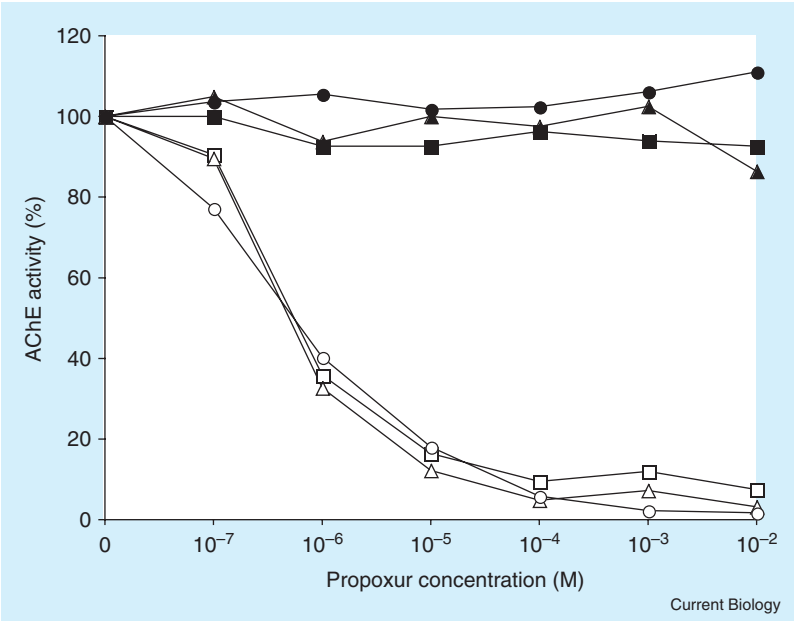

Figure 1. Activity of recombinant AChE1 in the presence of the carbamate propoxur. S2 cells were transfected with a recombinant pAc5.1/V5-His vector (Invitrogene). Three independent experiments were carried out using different volumes of cell lysate. Error bars (< 5%) were omitted for clarity. Susceptible G119 form (open symbols) or resistant form G119S (solid symbols) of *Ae. aegypti* (squares) and *C. pipiens* (triangles) AChE1 or human acetylcholinesterase (circles).

Table 1. Codon for G119 in various mosquito species.

	iAChE1	G119 codon
Mutable (GGY)		
Anopheles albimanus	Y	GGC
Anopheles arabiensis		GGC
Anopheles gambiae	Y	GGC
Anopheles hyrcanus		GGC
Anopheles subpictus		GGC
Anopheles sundaicus		GGC
Culex deserticola		GGC
Culex hortensis		GGC
Culex pipiens pipiens	Y	GGC
Culex pipiens quinquefasciatus	Y	GGC
Culex pipiens molestus	Y	GGC
Culex tigripes		GGT
Immutable (GGR)		
Aedes aegypti	N	GGA
Aedes albopictus	N	GGA/GGG
Aedes cinereus		GGA
Aedes eatoni		GGA
Aedes polynesiensis		GGA
Aedes taeniorynchus	N	GGA
Aedes vexans		GGA
Anopheles claviger		GGG
Anopheles darlingi		GGG
Anopheles funestus		GGA
Anopheles maculatus		GGA
Anopheles minimus		GGG
Anopheles moucheti		GGG
Anopheles nili		GGG
Anopheles pseudopunctipennis		GGA-
Anopheles sacharovi	N	GGG
Anopheles stephensi	N*	GGA
Culiseta annulata		GGA
Culiseta longiareolata		GGA
Culex cinereus	N	GGG
Culex pereexiguus	N	GGA
Culex tarsalis	N	GGA
Culex thelieri	N	GGA
Culex torrentium		GGA
Culex tritaeniorhynchus	N*	GGA
Ochlerotatus cataphylla		GGA
Ochlerotatus caspius	N	GGA
Ochlerotatus detritus	N	GGA
Ochlerotatus japonicus		GGA
Ochlerotatus mariae		GGA
Uranotaenia unguiculata		GGG

Codon 119 was sequenced and classified as serine-mutable (GGY) or immutable (GGR). Species in contact with OP or carbamate are indicated whether (Y) or not (N) a high propoxur AChE1 insensitivity (iAChE) has been reported. See Supplemental Data for sample locations and references.
*Slight propoxur insensitivity reported.

worldwide lack of AChE resistance in this species (Supplemental Data).

To test the predictive power of the Gly 119 codon, we surveyed 44 mosquito species from different genera of two subfamilies (Table 1). The Gly 119 codon was found serine-immutable (GGR) in 31 species, among them several human malaria and virus vectors, all of which are being heavily controlled with insecticides. Thus far, high AChE1 insensitivity has not been reported in any of these species, despite intense international work. The only exception may be *Culex tritaeniorynchus*, in which inseciticide insensitivity has been linked recently to a mutation at another position of AChE1 [8]. Of the species that display a serine-mutable codon, about 50% have already developed high AChE insensitivity through the G119S mutation.

Gly 119 is encoded by GGR in all species of the *Aedes*/*Ochlerotatus* group. This is significantly different ($p < 0.04$) from the frequencies found in *Anopheles* and *Culex* (62.5% and 60%, respectively) and suggests that molecular constraints affect the three main mosquito genera differentially. The nature of the constraints remains unknown but does not reflect global codon usage, as GGR is used in 64.3% of Gly codons in *An. gambiae* and in 53.7% in *Ae. Aegypti* (data not shown). In general, codon constraint could be predictive in cases in which a particular phenotype results from a single amino acid substitution.

In conclusion, we have established that the silent base of a single codon represents a major constraint for insecticide treated species to develop high G119S-linked resistance in natural populations. The G119 codon provides a test for the emergence of high resistance to some insecticides, allowing the implementation of refined management strategies.

Supplemental data

Supplemental data containing experimental procedures are available at http://www.current-biology.com/cgi/content/full/14/14/R552/DC1/

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