BASELINE ESTERASE LEVELS FOR ANOPHELINE AND CULICINE MOSQUITOES

J. HEMINGWAY[1] AND G. P. GEORGHIOU
Department of Entomology, Division of Toxicology and Physiology, University of California, Riverside, CA 92521

ABSTRACT. Baseline general esterase levels were measured spectrophotometrically with the substrates α- and β-NA for several *Anopheles* and *Culex* species. All the insecticide susceptible colonies showed similar levels of general esterase activity, which were much lower than, and clearly distinguishable from, those of organosphosphate-resistant *Culex tarsalis* and *Cx. quinquefasciatus*.

INTRODUCTION

Enzyme mediated insecticide resistance may be associated with changes in the mixed function oxidase, glutathione-s-transferase, esterase or acetylcholinesterase systems of the insect. Elevated levels of one or more esterases have been strongly associated with organophosphorus (OP) resistance in several insect species including *Tribolium castaneum* Herbst (Dyte and Rowlands 1968), *Culex pipiens* L. complex (Stone and Brown 1969, Pasteur and Sinegre 1975, Georghiou and Pasteur 1978), *Culex tarsalis* Coquillett (Matsumura and Brown 1961, Georghiou and Pasteur 1978), *Culex tritaeniorhynchus* Giles (Yasutomi 1971), *Aphis fabae* Scopoli (Beranek 1974), *Myzus persicae* Sulz (Needham and Sawicki 1971), *Nephotettix cincticeps* Uhler (Miyata and Saito 1976), *Heliothis virescens* Fallen (Bull and Whitten 1972) and *Phorodon humuli* (Buchi and Beck 1981). The increase in esterase levels in the resistant (R) compared with the susceptible (S) strain can be easily detected with non-specific substrates such as naphth-1-yl acetate (α-NA) and naphth-2-yl acetate (β-NA) (Van Asperen 1962).

The present study was undertaken to determine the "baseline" general esterase activity (GEA) for a number of susceptible anopheline and culicine mosquitoes for the future comparison with resistant strains.

MATERIALS AND METHODS

The following colonized stocks were used:

Anopheles stephensi Liston; insecticide susceptible strain originating in Pakistan, obtained from the Walter Reed Army Institute of Research, Washington, D.C.

Anopheles albimanus Wied; insecticide susceptible strain obtained from the Gorgas Memorial Laboratory, Panama.

Anopheles aconitus Dönitz; collected near Jakarta, Indonesia, in February 1982.

Anopheles culcifacies Giles; an insecticide susceptible population from India collected in 1978.

Culex quinquefasciatus Say; an insecticide susceptible laboratory strain, and an OP multi-resistant strain (TEM-R) from California, which has been selected by temephos pressure for several years (Ranasinghe and Georghiou 1979).

Culex tarsalis; insecticide susceptible and an OP multi-resistant strain from California. The latter has been selected by methyl parathion pressure for several years (Apperson and Georghiou 1975).

Individual 1-day-old adults were weighed and then homogenized in 1 ml of ice-cold phosphate buffer (0.02M pH7) containing 1×10^{-7} M eserine. Half of the homogenate was added to 2.5 ml of 0.3 mM α-NA in phosphate buffer and the other half to 0.3mM β-NA. These were then shaken and kept at 25°C for 30 min. The reaction was stopped by adding 0.5 ml stain (150 mg Fast Blue B salts in 15 ml distilled water + 35 ml of 5% sodium lauryl sulphate). Color formation was then measured spectrophotometrically at 605 and 555 nm for α- and β-NA respectively (Van Asperen 1962). Activity readings for 100 individuals of each species and strain were plotted, apart from *An. aconitus* for which only 30 individuals were assayed.

RESULTS

Table 1 gives the mean O.D. values for GEA activity in all populations tested assayed with both α- and β-NA. All the populations showed a normal distribution of absorbance values for both substrates. The distribution of GEA in all the insecticide susceptible colonies covered a similar range and most of the means within each category were not significantly different (see Table 1). However, the S strain of *Cx. tarsalis* gave consistently higher values than the other species tested. This is due, in part, to the large size of individuals of this species, as correcting the O.D. values for the weight of the insects tested brings the activity in *Cx. tarsalis* into line with those of the anopheline species.

[1] Current address: Department of Entomology, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, Great Britain.

Table 1. Mean O.D. values and standard deviations for general esterase activity in a number of insecticide susceptible and resistant laboratory colonies of *Anopheles* and *Culex*.

Species	Substrate (O.D. × 10^{-3}) α−NA	β−NA
Susceptibles		
An. aconitus	4.7 ± 0.1	1.8 ± 0.3
An. culicifacies	2.4 ± 0.3	1.0 ± 0.2
An. stephensi	3.5 ± 1.6	1.5 ± 0.5
An. albimanus	3.6 ± 2.3	2.2 ± 0.8
Cx. quinquefasciatus	5.8 ± 1.8	2.3 ± 0.6
Cx. tarsalis	7.9 ± 2.4	2.5 ± 0.4
Resistants		
Cx. quinquefasciatus	31.8 ± 5.8	36.2 ± 4.0
Cx. tarsalis	59.2 ± 20	29.4 ± 3.1

GEA activity in the organophosphate-resistant colonies of *Cx. quinquefasciatus* and *Cx. tarsalis* was significantly higher than that of all the susceptible colonies when measured with either α- or β-NA. There was no significant difference between the β-NA values for the two resistant colonies. However, *Cx. tarsalis* had significantly higher activity with α-NA than did the R *Cx. quinquefasciatus* colony.

DISCUSSION

All the insecticide susceptible colonies of *Culex* and *Anopheles* tested showed lower levels of GEA than the OP-resistant populations of *Cx. quinquefasciatus* and *Cx. tarsalis* tested. In the field, changes in the baseline levels of GEA of these susceptible populations may indicate selection for OP-resistance. However, the lack of an increase in GEA does not necessarily mean that resistance is not developing. In contrast to the situation in *Culex* species, there are cases of malathion resistance in *Anopheles* in which specific esterase based mechanisms have not resulted in a general increase in esterase activity against α- and β-NA. *Anopheles arabiensis* Patton from Sudan and *An. stephensi* from Pakistan both show 24-fold resistance to malathion, which is completely synergized by the carboxylesterase inhibitor triphenyl phosphate, but the resistant strains had slightly, but not significantly, lower levels of GEA with α- and β-NA (Hemingway 1982, Hemingway 1983). Malathion-specific resistance associated with a decrease in α-NA activity has also been noted in the blowfly, *Chrysomya putoria* (Townsend and Busvine 1969) and the Indian-meal moth *Plodia interpunctella* (Beeman and Schmidt 1982).

It has still to be determined whether selection with organophosphorus compounds other than malathion will produce resistance associated with elevated levels of GEA in *Anopheles*. If they do not, then the question of why such mechanisms seem to readily appear in *Culex*, but not in *Anopheles*, and what effect this may have on future developments of resistance and the strategy for control need to be asked.

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