INSECTICIDE CROSS-RESISTANCE SPECTRA AND UNDERLYING RESISTANCE MECHANISMS OF SRI LANKAN ANOPHELINE VECTORS OF MALARIA

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Abstract. Present status of insecticide resistance was investigated in two major vectors of malaria; *An. culicifacies* and *An. subpictus*, collected from a high malaria transmission area in Sri Lanka during 1996/1998. Adult and larval bioassays were carried out to obtain log-probit mortality lines for malathion, propoxur, permethrin and chlorpyrifos. Respective LD_{50} values were 4.45%, 0.002%, 0.16% and 0.001% for *An. culicifacies* and 0.66%, 0.004%, 0.004% and 0.04% for *An. subpictus*. Adults were also tested for WHO standard discriminating dosages of malathion, propoxur, permethrin, DDT, cypermethrin, deltamethlin and lambda cyhalothrin. Both populations were highly resistant to DDT. *An. culicifacies* was more resistant to malathion and *An. subpictus* was more resistant to chlorpyrifos. About 25% of both populations were resistant to permethrin. *An. culicifacies* was susceptible to propoxur, deltamethrin and lambda cyhalothrin and *An. subpictus* to cypermethrin and lambda cyhalothrin. Adult mosquitos were individually tested for their insecticide detoxifying enzyme activities and altered target-site, acetylcholinesterase. High general esterase activity indicated the presence of amplified esterase genes in both populations. Native gel electrophoresis resolved one elevated esterase isoenzyme, with high affinity to organophosphates, from each species. Malathion carboxylesterase mechanism was present in both populations. Higher glutathione-S-transferase activity was marked in *An. subpictus*. Synergistic studies showed the possible involvement of monooxygenases in resistance in both species. Acetylcholinesterase activity of~80% of both populations was not inhibited by a standard dosage of propoxur. Low resistance to carbamates shows that the impact of agricultural pesticides is not significant in the development of resistance especially in *An. culicifaies*. Pyrethroids, other than permethrin, can be successfully used in vector control programs. Carbamates will be an alternative.

INTRODUCTION

Malaria is one of the vector-borne diseases in Sri Lanka. Its control has been a major concern and focus of attention locally for decades. Although many strategies of control have been implemented the disease resurfaces posing problems such as drug resistance of parasites, and differential resistance of malarial vectors to insecticides. The latter is increasingly becoming a problem in the control programs in Sri Lanka, due to the extensive use of pesticides for both agricultural and vector control purposes. This has resulted in a broad spectrum insecticide resistance in the vector mosquitos.

Commonly used synthetic insecticides can be divided into four major groups; organochlorines, organophosphates, carbamates and pyrethroids. Of these, the use of organochlorines has been discontinued in many countries, including Sri Lanka, because of resistance and concerns for the environment. Insect nervous system is the target site of these insecticides. Cyclodines, a subgroup of organochlorines, binds to the γ-aminobutyric acid (GABA) receptors in the Cl^- channels of the neurones. The rest of the organochlorines (DDT+ its analogues) and pyrethroids bind to Na^+ channel proteins of the nerve membrane. For organophosphates and carbamates the target site is acetylcholinesterase (AChE). Major mechanisms of insecticide resistance involve either an alteration in the rate of insecticide metabolism *ie* detoxication, or an alteration within the target site of the insecticide. Increased metabolism is due to qualitative and/or quantitative changes of enzymes which metabolise insecticides. Esterases, glutathione-S-transferases and monooxygenases are the major groups involved. Target site alteration is often due to highly specific point mutations so that the altered target site does not respond to insecticides but perforrn its normal physiological functions (Karunaratne, 1998).

In Sri lanka, malathion (an organophosphate), which replaced DDT (an organochlorine) in 1975/1977, has been the major insecticide used in adult mosquito control programs. The main larvicidal insecticide has been the organophosphate temephose.

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Pyrethroid spraying in mosquito control programs occurred only recently (1994). Prior to that, the use of mosquito coils, made with volatile pyrethroids, and permethrin impregnated bed nets have been the only exposure of mosquito populations to synthetic pyrethroids. In agriculture, carbamates have been heavily used in addition to organophosphates and pyrethroids.

From vector incrimination studies carried out to date, *Anopheles culicifacies* Giles (sibling species B) and *An. subpictus* Grassi are considered to be major malaria vectors in Sri Lanka (Carter and Jacocks, 1929; Carter, 1930; Amerasinghe *et al*, 1992). Previous studies have shown the development of metabolic resistance in Sri Lankan anopheline populations (Herath *et al*, 1987; Hemingway *et al*, 1991). The overall aim of the present study was to obtain baseline information on pesticide resistance levels and mechanisms of malaria vector populations, where they have been exposed to insecticides for a long period of time.

MATERIALS AND METHODS

Mosquito collections

Mosquitos were collected from two villages; Weththiyaya and Meewalapathaha, located in the Galewela area, Matale District, Sri Lanka, from August 1995 to February 1998. The landscape of the villages is typical of other villages in the Intermediate zone of Sri Lanka with large rice fields and chena areas. Two streams: Welamitiya Oya and Mala Oya, border Weththiyaya and Meewalapathaha respectively. Malaria outbreaks in this area has been well reported and the control of vector mosquitos has been primarily with the use of insecticides (Amerasinghe, 1998).

Adult *An. culicifacies* and *An. subpictus* mosquitos were collected using bovid baited trap huts. Insecticide bioassay experiments were carried out in the field. Fed females were brought live to the laboratory and allowed to lay eggs to obtain larvae. Unfed mosquitos collected by bovid/ human baited night catches, were snap frozen at -20°C and brought to the laboratory for biochemical studies.

Insecticides

Insecticides (97-99.8% pure) used for experiments were a gift from Prof Janet Hemingway, University of Wales Cardiff, UK. Malathion, propoxur, permethrin, DDT, cypermethrin, deltamethrin, lambda cyhalothrin were used to prepare insecticide papers for adult bioassays. Chlorpyrifos was used for larval bioassays. Paraoxon, propoxur and permethrin were used for gel inhibition studies.

Preparation of insecticide papers

Standard World Health Organization (WHO) insecticide impregnated paper preparation method was used (WHO, 1963). Insecticide solutions of different dosages were prepared by mixing technical grade insecticide with a carrier oil. Malathion, propoxur and DDT papers were made in olive oil. Permethrin, cypermethrin, deltamethin and lambda cyhalothrin papers were made in Dow-Corning 556 silicone fluid. Whatman No. 1 filter papers (12 x 15 cm^2) were impregnated with the insecticide/oil solution (0.7 ml) at a fixed spreading rate. An equal volume of acetone (0.7 ml) was used to ensure an even distribution of the oil solution on the paper. Papers were left at room temperature until the acetone had evaporated. They were then foil wrapped and stored at -20°C until used.

Adult bioassays

Adult bioassays were via tarsal contact by exposure to insecticide impregnated papers (WHO, 1963). Batches of 5-25 mosquitos (depending on the availability) were exposed to different dosages of insecticide impregnated papers for one hour in WHO bioassay kits. After a recovery period of 24 hours, mortalities were recorded. For each bioassay at least four concentrations, giving mortality between 0% and 100%, were tested and four replicates were set for each concentration. Controls were exposed to papers impregnated with oil alone. Data were considered if the mortalities observed in controls were less than 20% only. If there were mortalities in controls, data were adjusted accordingly using Abbott's formula (Matsumara, 1985).

Mortality data were plotted against dosages to obtain log-probit mortality lines. To obtain LD_{50} and LD_{90} values, data were subjected to regression analysis using a computer program written by C Schofield (WHO, Geneva). Adult log-probit mortality lines were obtained for three insecticides which represent currently used major insecticide groups *ie* malathion (an organophosphate), propoxur (a carbamate) and permethrin (a pyrethroid). Adult mosquitos were tested for the WHO discriminating dosage of all the insecticides. Synergist studies with the oxidase inhibitor piperonyl butoxide were undertaken with a 1 hour pre-exposure of batches of mosquitos to filter papers impregnated with 0.1 µg/ cm^2 (4%) piperonyl butoxide. The insects were then immediately exposed to insecticide impregnated pa-

pers and mortalities were recorded after the recovery period. Control insects were exposed to 4% piperonyl butoxide for 1 hour and then to oil impregnated control papers.

Larval bioassays

Larval bioassays were performed by exposing batches of 15 fourth instar larvae to known insecticide concentrations in 125 ml of distilled water (WHO, 1981). Insecticide solutions were made in ethanol and 0.5 ml was added to the water. For each bioassay at least five concentrations giving mortality between 0 -100% were tested and four replicates were set for each concentration. Control experiments were done using alcohol alone. After 24 hours exposure larvae were transferred to distilled water and the mortalities were counted after a recovery period of 24 hours. Mortality data were analysed as described for adult bioassays.

Enzyme assays

Adult mosquitos were individually subjected to esterase, glutathione *S*-transferase (GST), acetylcholinesterase (AChE) and protein assays. At least 200 adults of each population were tested. Each insect was homogenized in 150 µl of distilled water and centrifuged at 10,000*g* for 2 minutes. The supernatant was used for various enzyme assays. To obtain specific activities of enzymes, protein concentrations of the homogenates were deterrnined by BIO-RAD protein determination kit, using bovine serum albumin as the standard protein. In a microtiter plate well, 10 µl of the homogenate was mixed with 300 µl of working solution (prepared according to the instructions of the manufacturer) and the absorbance was read at 570 nm after a five minute incubation at 22°C.

Esterase assay

10 µl of homogenate was mixed with 200 µl of 1 mM *p*-nitrophenyl acetate (pNPA) in 50 mM sodium phosphate buffer (pH 7.4). The increase in absorbance was monitored for 1-2 rminutes in a kinetic microtiter plate reader (Bio-Tek, USA) at 405 nm at 22°C. An extinction co-efficient of 6.53 mM^{-1} (corrected for a path length of 0.6 cm) was used to convert the absorbance to moles.

Glutathione S-transferase (GST) assay

10 µl of homogenate was mixed with 200 µl of the substrate solution (95 parts of 10.5 mM reduced glutathione in 100 mM phosphate buffer + 5 parts of 63 mM l-chloro 2,4-dinitrobenzene in methanol). The rate of reaction was measured at 340 nm for 5 minutes. An extinction co-efficient of 5.76 (corrected for path length of 0.6 cm) was used to convert absorbance to moles.

Acetylcholinesterase (AChE) assay

Homogenate (2 x 20 µl aliquots) was added to consecutive microtiter plate wells, each containing 145 µl of 1% Triton X-100 in 0.1 M sodium phosphate buffer (pH 7.8) and 10 µl of dithiobis-2, nitrobenzoic acid in phosphate buffer (pH 7.0). To one set of homogenates, 25 µl of acetylthiocholine iodide (ASChI) and propoxur solution (10 ml of 0.01M ASChI + 20 µl of 0.1M propoxur in acetone) was added. To the other replicate, 25 µl of 0.01M ASChI alone was added. The plate was read at 405 nm for 5 minutes. Results were expressed as the percentage remaining activity in the inhibited fraction compared with the control activity. Remaining activity of more than 80% indicates the presence of altered AChEs in *Anopheles* and *Culex* mosquitos (Penilla *et al*, 1996).

Malathion metabolism

25-50 adult mosquitos were homogenized in 1 ml of 25 mM Tris buffer (pH 7.5), centrifuged at 13,000*g* for 5 minutes and the supernatant was incubated with 300 µM malathion for 2 hours at room temperature. Dithiothreitol (10 mM) was used to protect the enzyme activity. The sample was then extracted with chloroform and loaded onto a TLC plate, run with n-hexane: diethyl ether (1:3), sprayed with 0.5% (w/v) 2,6 -dibromoquinone 4-chloromide in cyclohexane and left at 100°C for 2 hours to detect malathion and its metabolic products. Distilled water incubated with the same concentration of malathion, was run as a control.

Gel electrophoresis

Native polyacrylamide gel electrophoresis (PAGE) was used to identify the elevated esterase isozymes present in both populations. Mass homogenates of 10 - 25 adult mosquitos were made in 250 µl of 50 mM sodium phosphate buffer pH 7.4. Electrophoresis of 10,000g supernatants from crude homogenates was performed in 7.5% acrylamide gels in tris/borate buffer pH 8.0 containing 0.2 mM EDTA. Gels were stained for esterase activity with 0.04% (w/v) α- and β-naphthyl acetate and 0.1% (w/v) fast blue B in 100 mM phosphate buffer pH 7.4. For inhibition studies, gels were incubated, after electrophoresis, in 0.1 mM paraoxon, propoxur or permethrin in 50 mM phosphate buffer pH 7.4 for 15 minutes, and then stained for esterase activity as above in the presence of the insecticide.

RESULTS

Figs 1-4 show log probit mortality curves for malathion, propoxur, permethrin and chlorpyrifos for both vectors; *An. culicifacies* and *An. subpictus*. Calculated LD_{50} and LD_{90} values are shown in Table 1. Chi-square (χ^2) values indicate whether the population is homogeneous ($p>0.05$) or heterogeneous ($p< 0.05$) for resistance to the insecticide concerned. The degree of heterogeneity (*ie* $p<0.001$, 0.01 or 0.05) is also shown. Both vector populations were heterogeneous for their resistance to all the insecticides tested, except for *An. culicifacies* population to the carbamate propoxur. WHO discriminating dosage of propoxur, 0.1% (4 µg/cm^2) gave 100% mortality for *An. culicifacies* indicating that this population is susceptible to propoxur and therefore homogeneous. Percentage survivals, when exposed to WHO discriminating dosage of various insecticides tested, are shown in Table 2. Both mosquito populations were highly resistant to DDT. *An. culicifacies* population was more resistant to malathion than *An. subpictus* population. However, *An. subpictus* larvae were more resistant to the organophosphate chlorpyrifos. About 25% of the populations of both species showed resistance to the pyrethroid permethrin. *An. culicifacies* population was susceptible to propoxur, deltamethrin and lambda cyhalothrin and *An. subpictus* to cypermethrin and lambda cyhalothrin.

Individual esterase activities of both populations are shown in Fig 5. Mean specific activities were 0.39±0.34 µmol min^{-1} mg^{-1} for *An. culicifacies* and 0.23±0.19 µmol min^{-1} mg^{-1} for *An. subpictus*. For the same substrate, a susceptible strain (without elevated esterase activity) and a resistant strain (with elevated esterase activity) of *Culex quinquefasciatus* showed specific activities of 0.02±0.007 µmol min^{-1} mg^{-1} and 0.92±0.08 µmol min^{-1} mg^{-1} respectively (Karunaratne *et al*, 1996). Therefore, it is apparent that both anopheline species studied have high level of general esterase activity. Specific activities of some individuals of *An. culicifacies* were very high, indicating the presence of amplified esterase genes in this population (Hemingway and Karunaratne, 1998).

Elevation of esterase activity was evident from gel electrophoresis studies as well. Native PAGE could resolve one highly elevated esterase isoenzyme from each of the two species; *An. culicifacies* (R_f = 0.83) and *An. subpictus* (R_f = 1.14) (Fig 6). Paraoxon completely inhibited the activity of both bands. Inhibition by propoxur was very little.

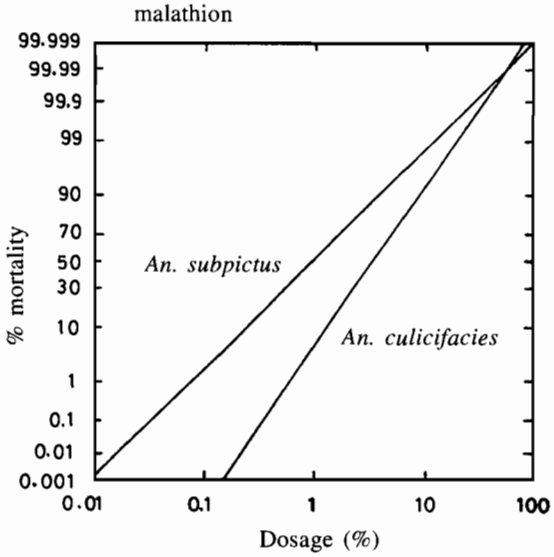

Fig 1–Log-dosage probit mortality lines for the adults of two vector populations tested with malathion.

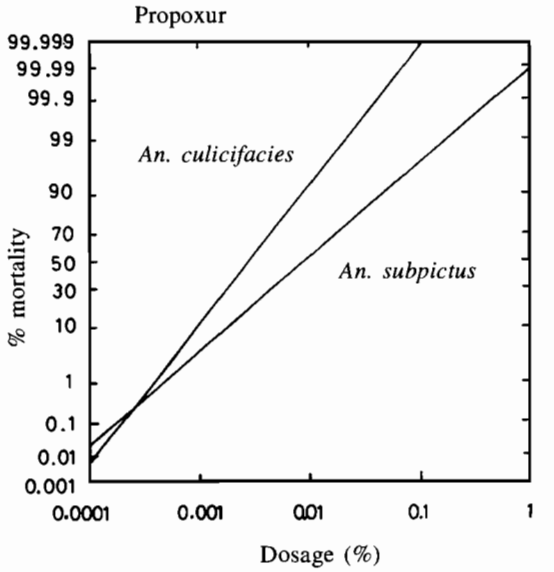

Fig 2–Log-dosage probit mortality lines for the adults of two vector populations tested with propoxur.

Permethrin did not interfere with the activity of both bands. Since these elevated isoenzymes are highly reactive with organophosphorous insecticides, they can cause high resistance to this insecticide group.

Presence of malathion carboxylesterases, altered forms which can metabolize malathion at a much faster rate, was seen in both populations. Thin layer chromatography showed that the crude

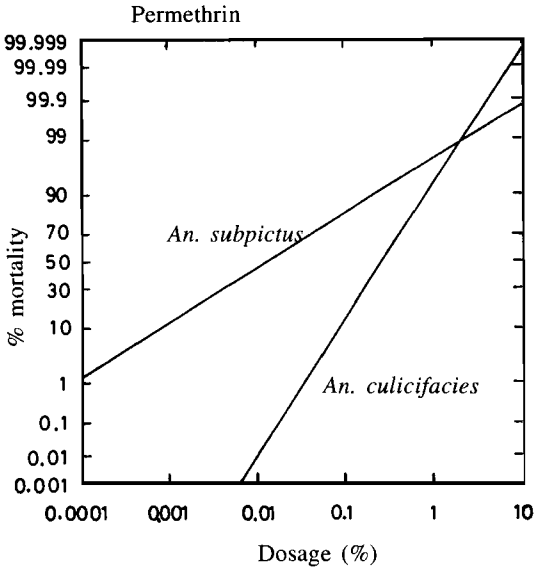

Fig 3–Log-dosage probit mortality lines for the adults of two vector populations tested with permethrin.

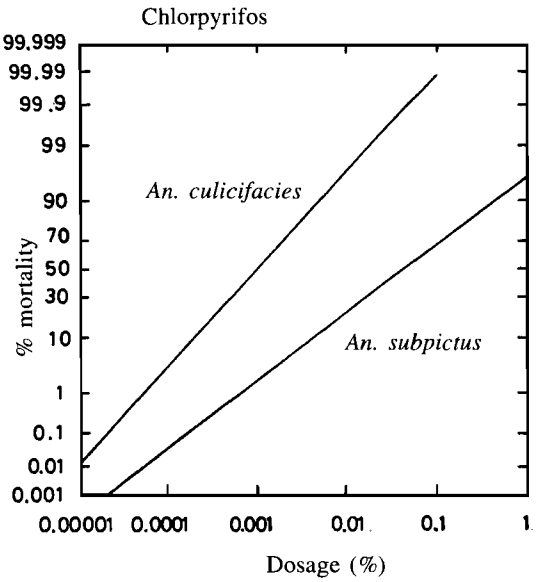

Fig 4–Log-dosage probit mortality lines for the larvae of two vector populations tested with chlorpyrifos.

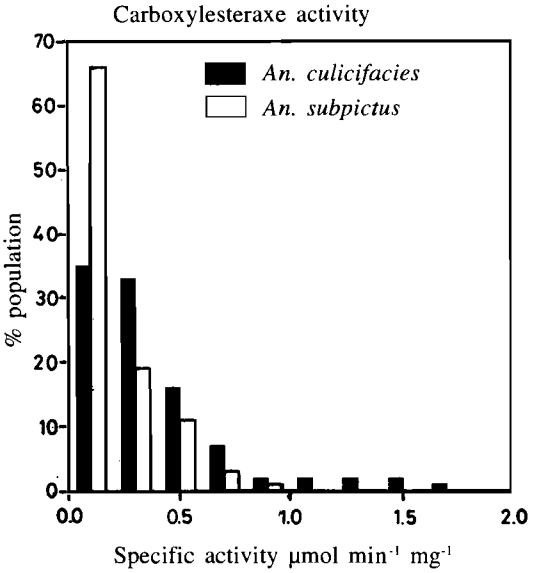

Fig 5–Distribution pattern of carboxylesterase specific activity in the two vector populations for the substrate p-nitrophenyl acetate.

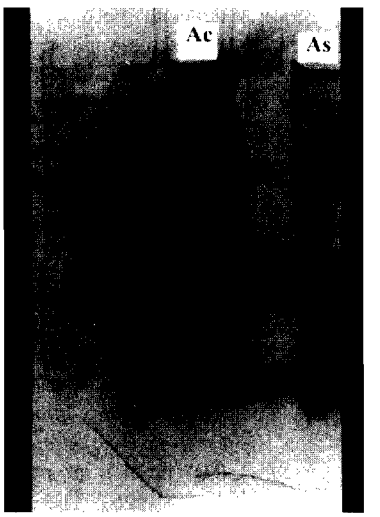

Fig 6–Native Polyacrylamide gel electrophoresis (PAGE) of adult crude homogenates of *An. culicifacies* (Ac) and *An. subpictus* (As), stained for esterase activity with the substrates α- and β- naphthyl acetate.

homogenates of both species had metabolized malathion into both mono- and di- acid products during the incubation.

Individual GST activities of both species are shown in Fig 7. The mean specific activities were 0.24±0.14 µmol min^{-1} mg^{-1} for *An. culicifacies* and 0.30±0.27 µmol min^{-1} mg^{-1} for *An. subpictus*. Susceptible strains of *An. gambiae* and *Cx. quinquefasciatus*, which do not have this mechanism, have shown GST specific activities of 0.42±0.06 µmol min^{-1} mg^{-1} and 0.34±0.07 µmol min^{-1} mg^{-1} respectively (Prapanthadara, 1993; Karunaratne and Hemingway, 1996). Although the mechanism is not well developed in these populations, individuals with much higher activity can be seen especially in *An.*

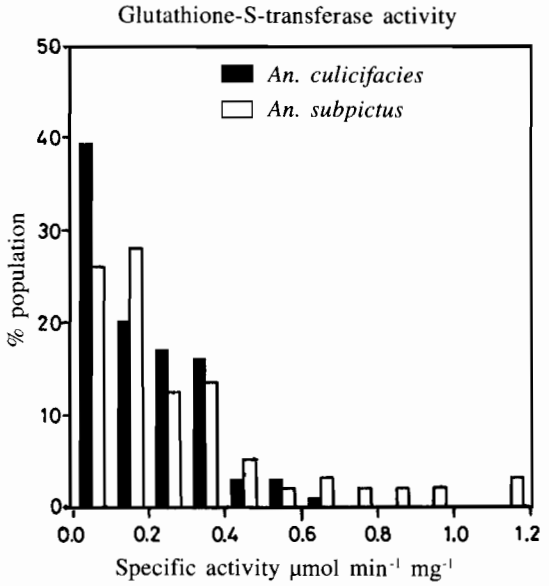

Fig 7–Distribution pattern of glutathione-S-transferase specific activity in the two vector populations for the substrate 1-chloro 2,4-dinitrobenzene.

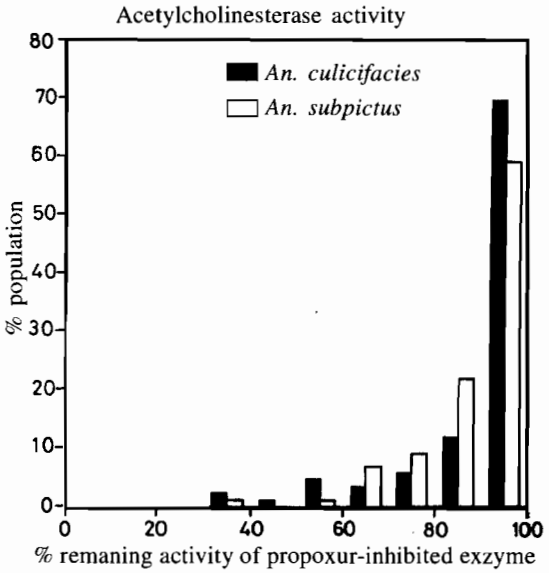

Fig 8–Activity of acetylcholinesterase in the presence of the inhibitor-propoxur.

subpictus population (Fig 7).

Involvement of monooxygenases of two anopheline vectors in insecticide resistance was indirectly tested using the oxidase inhibitor piperonyl butoxide in adult bioassays. Pre-exposure to 4% (standard dosage) piperonyl butoxide papers for one hour considerably reduced the pyrethroid resistance in both species. Mortality of *An. culicifacies* at 0.1% dosage was increased from 29.28 % to 61.11% (n=40). Mortality of *An. subpictus* at 0.01% dosage was increased from 60.9% to 100% (n=32). No mortalities were recorded in controls. Due to the very low field mosquito abundance, the sample size used for this experirnent had to be limited and therefore the results are not very conclusive.

Assays, carried out to test altered AChE mechanism revealed that the standard dosage of propoxur could not inhibit AChE activity of most of the individuals tested indicating that this mechanism is present in both populations (Fig 8). More than 80% remaining activity was shown by 82 % of *An. culicifacies* and 81.4% of *An. subpictus* population.

DISCUSSION

The resistance status of *An. culicifacies* and *An. subpictus* populations studied are shown in Table 3, according to a recent classification of WHO measurements/criteria (Herath, 1997). Presence of different types of resistance mechanisms in both vector populations and the insecticide groups likely to be affected are shown in Table 4.

Results show that resistance to DDT is still very high in both species although DDT spraying was curtailed in 1975/1977 in Sri Lanka. It was reported in late '80s that the resistance to DDT declined slowly after the cessation of its usage, but increased again after 1983 in *An. subpictus*. This was attributed to the selection of GST mechanism, which can metabolize activated products (oxon analogues) of organophosphates, with the spraying of the organophosphate malathion (Hemingway *et al*, 1991; Herath and Jayawardena, 1988). Present study shows that individuals with high GST activity are still present in both populations but only at a low frequency. This together with monooxigenase mechanism must be responsible for the high level of DDT resistance shown. At present resistance to malathion is very high in *An. culicifacies* population in the area studied. When malathion was first introduced in Sri Lanka, 20 minutes exposure to 5% malathion gave 100% mortality in *An. culicifacies* (Herath *et al*, 1987). First survivors for this dosage was detected after two years of malathion spraying in 1979. Resistance to the standard WHO dosage (5% for one hour) was first observed in 1982. Malathion carboxylesterase was found to be the major underlying mechanism for malathion resistance in *An.*

Table 1
LD_{50} and LD_{90} values of the populations of two major vectors for different insecticides (1% = 40 μg/cm²)

	An. culicifacies		*An. subpictus*	
	LD_{50}	LD_{90}	LD_{50}	LD_{90}
Malathion[a]	4.45%	29.33%	0.66%	9.45%
	N=184 (χ^2=11.79, n=3)		N=228 (χ^2=25.32, n=3)	
Propoxur[a]	0.002%	0.013%	0.004%	0.3%
	N=142 (χ^2=2.67, n=2)		N=246 (χ^2=28.21, n=3)	
Permethrin[a]	0.16%	1.66%	0.004%	5.49%
	N=140 (χ^2=7.17, n=2)		N=338 (χ^2=35.73, n=4)	
Chlorpyrifos[b]	0.001%	0.025%	0.043%	0.64%
	N=180 (χ^2=14.95, n=3)		N=180 (χ^2=17.72, n=3)	

[a]adult bioassays, [b]larval bioassays, N= total no. tested, n= degree of freedom

Table 2
Resistance of the vector populations to various insecticides (tested for WHO discriminating dosage*, n=50).

	An. culicifacies	*An. subpictus*
Malathion (5%)*	70.13%	14.95%
Chlorpyrifos (0.01%)*	25.72%	88.28%
Propoxur (0.1%)*	0%	16.71%
Permethrin (0.25%)*	26.19%	27.43%
DDT (4%)*	100%	89.1%
Deltamethrin (0.25%)*	0%	12.25%
Cypermethrin (0.1%)*	18.34%	0%
Lambda- cyhalothrin (0.1%)*	0%	0%

culicifacies (Herath *et al*, 1987) while oxidases played the major role in *An. subpictus* (Hemingway *et al*, 1991). Same workers had shown that oxidase mechanism was absent in Sri Lankan *An. culicifacies* (Herath *et al*, 1987; Herath and Jayawardena, 1988). Present study reveals that both mechanisms; malathion carboxylesterases and oxidases, have been developed in the populations of both species after two decades of malathion spraying. An indication for the presence of elevated activity of general esterases was seen in late 1980s in *An. culicifacies* at Puttalam area, Sri Lanka. However, no elevated isoenzymes were found in electrophoretic studies. Present study shows that the activity levels of general esterases are very high and elevated isoenzymes, highly reactive with organophosphates, are present in both species. Therefore, this mechanism must undoubtedly be playing an important role in organophosphate resistance in both these vectors. Early studies have shown that Sri Lankan *An. culicifacies* develop cross-resistance to other organophosphates as a result of continuous exposure to malathion (Herath *et al*, 1981). Cross-resistance, developed by the long-term exposure to malathion and to the larvicide organophosphate temephose, must be responsible for the high level of resistance observed for chlorpyrifos.

Although broad spectrum organophosphate and carbamate resistance had been reported in *An. nigerrimus*, only organophosphate but not carbamate resistance in *An. culicifacies* and organophosphate resistance with very little carbamate resistance in *An. subpictus* was detected earlier (Herath and Davidson, 1981; Herath and Joshi, 1986; 1989; WHO, 1992). This was mainly due to their breeding habitats (Herath and Joshi, 1989). The susceptibility to carbamates is still conserved especially in *An. culicifacies*, which breeds mainly in river-bed pools and are not directly exposed to agricultural insecticides. Target-site of organophosphates and carbamates was shown to be not altered in Sri Lankan *An. culicifacies* even after about ten years of malathion usage (Herath *et al*, 1987). Present study shows that both *An. culicifacies* and *An. subpictus* populations have high proportions of individuals with altered AChE mechanism. However, the susceptibility of both populations to the carbamate propoxur, as shown by bioassay experiments, can not exist if the target site of carbamates, AChE, is altered. This may indicate that the standard dosage, which has been established mainly by using other anopheline species, is not strong enough to inhibit unaltered AChEs of *An. culicifacies* and *An. subpictus*. Low level of resistance to carbarnates also shows that the impact of pesticides used in agriculture, where carbamates have been used heavily, has not been very signifi-

Table 3
Resistance status of *An. culicifacies* and *An. subpictus* populations for various insecticides tested.

WHO measurement/ criteria to assess resistance status (Herath, 1997)

Method (A)	<10% R	= susceptible
	10-50% R	= intermediate
	>50% R	= Resistant
Method (B)	0-1% R	= Susceptible
	2-20% R	= verification required
	>20% R	= resistant individuals present

Resistance status of two vector populations.

According to the method (A):

	Susceptible	Intermediate	Resistant
An. culicifacies	PR	CH	MAL
	DM, L-C	PM, CM	DDT
An. subpictus	CM, L-C	MAL	CH
		PR	DDT
		PM, DM	

According to the method (B):

	Susceptible	Verification required	Resistant individuals present
An. culicifacies	PR	CM	MAL, CH
	DM, L-C		PM
			DDT
An. subpictus	CM, L-C	MAL	CH
		PR	PM
		DM	DDT

%R= % resistance, CH= chlorpyrifos, CM= cypermethrin, DM= deltamethrin, L-C=lambda cyhalothrin, MAL= malathion, PM= permethrin, PR= propoxur

Table 4
Presence of major resistance mechanisms.

Resistant mechanism	Insecticides/group likely to be affected	*An.culicifacies*	*An.subpictus*
Metabolic resistance			
1. Carboxylesterases			
a) altered	malathion	+ +	+ +
b) elevated	OP, Car, pyr?	+ +	+
2. GSTs	DDT, OP?	+	++
3. Oxidases	DDT, OP, Car, Pyr	+	+
Altered target sites			
1. AChE	OP, Car	++	++
2. "kdr"	DDT, Pyr	-	-
3. "GABA"	Cyclodiene (org chl)	-	-

Car = carbamate, OP = organophosphates, Org chl = organochlorines, Pyr =pyrethroids
+ + (high), + (low) = degree of presence, - = not investigated, ? = not confirmed.

cant in the development of resistance in these two vectors.

Resistance to pyrethroids in *An. culicifacies* but not in *An. subpictus* has been reported earlier from Sri Lanka (WHO, 1992). Present study shows that both populations have developed a significant resistance to permethrin. This may be due to the high usage of permethrin in agriculture and insecticide impregnated bed nets. Mosquito coils, which are made up of volatile pyrethroids and heavily used in Sri Lanka, may also have contributed to the pyrethroid resistance in these mosquitos. However, the resistance to other pyrethroids tested, is still negligible. Since DDT was heavily used in Sri Lanka in the past, genes coding for altered Na^+ channel regulatory proteins, which were selected during that period, may still exist in mosquito populations at a very low frequency. If so, they can give cross-resistance to pyrethroids and be easily selected to a very high frequency with in few years, by pyrethroids used in current malaria control programs. Studies on Na^+ channel receptors, are very important if Sri Lanka wants to continue the use of pyrethroids.

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REFERENCES

Amerasinghe PH. A study on malaria transmission by two anopheline vectors in the Galewela area. 54th Annual Sessions, Sri Lanka Association for the Advancement of Science, [Abstract] December 1998: A-23.

Amerasinghe PH, Amerasinghe FP, Wirtz RA, *et al.* Malaria transmission by indoor resting *Anopheles subpictus* Grassi in a new irrigation project in Sri Lanka. *J Med Entomol* 1992; 29: 577-81.

Carter HF. Further observations on the transmission of malaria by anopheline mosquitos in Ceylon. *Ceylon J Sci* 1930; 2: 159-76.

Carter HF, Jacocks WP. Observations on the transmission of malaria by anopheline mosquitoes in Ceylon. *Ceylon J Sci* 1929; 2: 67-86.

Hemingway J, Karunaratne SHPP. Mosquito carboxylesterases: A review of the molecular biology and biochemistry of a major insecticide resistance mechanism. *Med Vet Entomol* 1998; 12: 1-12.

Hemingway J, Miyamoto J, Herath PRJ. A possible novel link between organophosphorous and DDT insecticide resistance genes in Anophelus: supporting evidence from fenitrothion metabolism studies. *Pesticide Biochem Physiol* 1991; 39: 49-56.

Herath PRJ. Insecticide resistance status in disease vectors and its practical implications. International workshop on insecticide resistance of mosquito vectors, Salatiga, Indonesia, 5-8 August, 1997.

Herath PRJ, Davidson G. Multiple resistance in *Anopheles culicifacies* Giles. *Mosq News* 1981; 41: 325-7.

Herath PRJ, Jayawardena KGI. DDT resistance in *Anopheles culicifacies* Giles and *A. subpictus* Grassi (Diptera: Culicidae) from Sri Lanka: a field study on the mechanisms and changes in gene frequency after cessation of DDT spraying. *Bull Entomol Res* 1988; 717-23.

Herath PRJ, Joshi GP. Factors affecting selection for multiple resistance in *Anoheles nigerrimus* in Sri Lanka. *Trans R Soc Trop Med Hyg* 1986; 80: 649-52.

Herath PRJ, Joshi GP. Pesticide selection pressure on *Anopheles subpictus* in Sri Lanka: comparison with two other Sri Lankan anophelines. *Trans R Soc Trop Med Hyg* 1989; 83: 565-7.

Herath PRJ, Miles SJ, Davidson G. Fenitrothion (OMS 43) resistance in the taxon *Anopheles culicifacies* Giles. *J Trop Med Hyg* 1981; 84: 87-8.

Herath PRJ, Hemingway J, Weerasinghe IS, Jayawardena KGI. The detection and characterisation of malathion resistance in field populations of *Anopheles culicifacies B* in Sri Lanka. *Pesticide Biochem Physiol* 1987; 29: 157-62.

Karunaratne SHPP. Insecticide resistance in insects: A review. *Ceylon Sci (Bio Sci)* 1998; 25: 72-99.

Karunaratne SHPP, Hemingway J. Different insecticides select multiple carboxylesterase isoenzymes and different resistance levels from a single population of *Culex quinquefasciatus*. *Pesticide Biochem Physiol* 1996; 54: 4-11.

Karunaratne SHPP, Hemingway J, Jayawardena KGI, Dassanayaka V, Vaughan A. Kinetic and molecular differences in the amplified and non-amplified esterases from insecticide resistant and susceptible *Culex quinquefasciatus* mosquitoes. *J Bio Chem* 1998; 270: 31124-83

Matsumara F. Toxicology of insecticides. New York: Plenum Press, 1985: 598pp.

Penilia RP, Rodriguez AD, Hemingway J, Estrada JLT, Jimenez JIA, Rodriguez MH. Rotational and mosaic strategies for delaying the development of insecti-

cide resistance in mosquitoes - baseline data for a large scale field trial in Southern Mexico. In: Widley KR, Robinson WH, eds. Proceedings of the 2nd International Conference on Insect Pests in the Urban Environment. BPCC 1996: 401-11.

Prapanthadara L. Purification and characterisation of glutathione-S-transferases involved in DDT resistance from the mosquito *Anopheles gambiae*. University of London 1993: 212pp. PhD Thesis.

World Health Organisation. Criteria and meaning of tests for determining susceptibility or resistance of insects to insecticides. *WHO Tech Rep Ser* 1963; 265: 135-8.

World Health Organisation. Instructions for determining the susceptibility of resistance of mosquito larvae to insecticides. *WHO/VBC 81: 807*. 1981.

World Health Organisation. Vector resistance to pesticides. *WHO Tech Rep Ser* 1992; 818: 62p.