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The nature of malathion resistance in a population of *Anopheles culicifacies* Giles*

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Malathion resistance in a population of Anopheles culicifacies *from Maharashtra State in India, which also showed resistance to a number of other organophosphorus compounds, was found to be dominant in its expression. Most of the crossing and back-crossing results involving a susceptible population of the same species from Sri Lanka indicated the possible involvement of more than one genetic factor. The existence of such a broad spectrum of resistance, and experiments involving the use of the synergists triphenyl phosphate and piperonyl butoxide both suggest the presence of at least two mechanisms, one involving the specific carboxylesterase and the other the less specific mixed-function oxidase system.*

Resistance of mosquitos to DDT resulting in the failure of vector control measures has been considered a major contributory factor in the resurgence of malaria in many countries. Some of these countries have now changed to alternative insecticides, in most cases to organophosphorus compounds (OPs) and in some to carbamates. The principal OP in use at the present time is malathion, though fenitrothion is used sometimes where resistance to malathion is apparent.

Malathion resistance has been reported in seven species of anopheline mosquito so far (*1*). In some it is the only resistance, e.g., *Anopheles arabiensis* (*2*), while in others, e.g., *A. albimanus* (*3*), multiple resistance involving other OPs and carbamates has appeared. In some cases, the development of this broad-spectrum resistance has been attributed to selection by the relevant insecticides used for public health or agricultural purposes. However, in many instances resistance has developed to compounds other than the selecting agent owing to cross-resistance, and this has been the case with both malathion and fenitrothion as well as other OPs.

This paper reports a preliminary attempt to study the nature of malathion resistance in a population of *A. culicifacies* from Maharashtra State, India, and its contribution towards a broad spectrum of OP resistance. Malathion resistance in this species was first reported by Rajagopal (*4*).

MATERIALS AND METHODS

Two strains of *A. culicifacies* were used:

— CUL/SRL—a malathion-susceptible population derived from eggs received from Attanagalla, Kirindiwela, Sri Lanka, supplied by the Anti-Malaria Campaign in 1976.

— CUL/IND—a malathion-resistant population derived from eggs received from the village of Wadawali, Palghar Unit, Thana District, Maharashtra State, India, and supplied by the National Malaria Eradication Programme in 1976.

The malathion papers used for testing the adult mosquitos of these strains were the standard impregnated papers (5% malathion in olive oil/ionol solution) provided by the World Health Organization for use in their adult mosquito susceptibility test. The synergists triphenyl phosphate (TPP) and piperonyl butoxide (PB) were kindly supplied by Dr F. J. Oppenoorth of the Laboratory for Research on Insecticides, Wageningen, Holland, and Dr P. Chadwick of the Wellcome Research Laboratories, Berkhamsted, England, respectively.

Mosquito rearing and the subsequent experimental procedures were carried out in the Ross Institute insectaries at the London School of Hygiene and Tropical Medicine, in which the temperature (25–28 °C) and relative humidity (70–80%) were controlled and a 12-hour photoperiod was maintained. Larval rearing was carried out in water at temperatures of 28–30 °C adopting standard procedures. Both populations failed to mate significantly in cages and so the artificial mating technique had to be used continuously to provide material for the experimental work. The females

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of both strains proved reluctant to feed on the standard animal host, guinea pig, and had to be fed mostly on human volunteers.

The standard WHO adult mosquito susceptibility test was used to determine insecticide tolerances and 1-day-old males and unfed females were used as test material.

A preliminary mass selection of the Indian resistant strain soon produced a population showing virtually no mortality after exposure to 5% malathion for 1 hour, the discriminating dose producing an almost complete kill of the Sri Lankan susceptible strain. This situation persisted in successive generations after selection.

The two homozygous populations were crossed, again using the forced-mating technique, and the hybrid offspring were characterized as to their malathion tolerance. They were then back-crossed to the susceptible CUL/SRL strain and the progeny were exposed to the discriminating dose of malathion. Survivors were again back-crossed to CUL/SRL and the procedure repeated for a total of three successive back-crosses. Repeated back-crossing with selection is the recognized procedure for distinguishing between monofactorial and polyfactorial inheritance.

To study the effect of synergists, impregnated papers were prepared using the maximum non-toxic dose of the appropriate compound. Using the standard test procedure, a sample of mosquitos was initially exposed to the synergist and subsequently to the insecticide. A comparable test with the insecticide alone was made on another sample of the same population.

RESULTS AND DISCUSSION

The exposure of 81 mosquitos of the Sri Lankan susceptible population to 5% malathion papers for 1 hour followed by a 24-hour recovery period resulted in the survival of only 1 mosquito. The exposure of 215 of the Indian population and 155 of the hybrids between the two populations resulted in only a 2% mortality in each case. Thus the hybrid seemed to be as resistant as the resistant population, and resistance could be considered dominant at that dosage. Exposure to 5% malathion for 1 hour discriminated between susceptible and both heterozygous and homozygous resistant mosquitos almost perfectly. The expected mortality in the offspring of the back-cross between hybrid and susceptible would be 50% if single-factor inheritance were involved and mortalities in the subsequent back-crosses with selection would be expected to remain at this level.

Tables 1 and 2 list the mortalities in single families reared from the offspring of the first and second back-crosses and relate these to the proportions of individuals reaching maturity, on the principle that the higher the family yield, the less chance there is of distortion of results by the differential loss of particular genotypes during the aquatic stages. In the first back-cross (Table 1), 4 out of 12 families showed a significant departure from the expected 1:1 segregation of heterozygotes and susceptible homozygotes and two of these were in families with low yields. In the second back-cross, only 3 families out of 15 showed a significant departure from the expected ratio on the single gene hypothesis, though each of these was in the high-yield range (Table 2). However, in both back-crosses, most of the mortalities were less than 50% and when they are totalled a highly significant departure is evident. In the third back-cross only 2 families were tested. One of these of 41 adults (a yield of less than 70%) showed a mortality of 83% ($\chi^2_1 = 17.78$) while another of 55 adults from a high-yield (>75%) family showed a mortality of 46% ($\chi^2_1 = 0.45$). Thus considering only those families producing more than 70% yields of pupae from eggs, mortalities in the three successive back-crosses were 41%, 42%, and 46%, respectively.

Control of resistance by two genes has been considered by Lines & Curtis (unpublished results) as a

Table 1. Single family results of exposure to the discriminating dosage of malathion of the offspring of the first back-cross of the hybrid (resistant Indian x susceptible Sri Lankan) to the susceptible Sri Lankan population

Batch no.	Yield of pupae from eggs (%)	Total no. tested	Mortality (%)	χ^2 (1:1 expectation)
1	17	45	24	11.74[a]
2	40	55	44	0.90
3	53	142	35	13.64[a]
4	59	20	30	3.20
5	68	22	41	0.72
6	75	60	42	1.66
7	78	35	60	1.40
8	80	36	33	4.00[a]
9	80	84	33	9.34[a]
10	81	43	40	1.88
11	89	33	45	0.28
12	90	71	45	0.70
Totals		646	39	33.90[a]
	> 70	362	41	10.62[a]

[a] $P < 0.05$.

Table 2. Single family results of exposure to the discriminating dosage of malathion of the second back-cross progeny involving resistant and susceptible *A. culicifacies* populations from India and Sri Lanka

Batch no.	Yield of pupae from eggs (%)	Total no. tested	Mortality (%)	χ^2 (1:1 expectation)
1	42	38	61	1.68
2	48	79	44	1.02
3	67	122	45	1.18
4	70	39	36	3.10
5	72	64	55	0.56
6	72	132	43	2.46
7	73	88	53	0.40
8	74	176	30	27.84[a]
9	74	35	57	0.72
10	74	28	46	0.14
11	75	42	38	2.38
12	89	86	50	0
13	96	24	21	8.16[a]
14	98	63	40	2.68
15	?	37	32	4.56[a]
Totals		1053	43	21.08[a]
	> 70	777	42	19.52[a]

[a] $P<0.05$.

possible explanation of less than 50% mortality from back-crosses. If it were assumed that one or both of two unlinked resistance genes on their own gave only partial protection from the malathion treatment, the observed 41% mortality from the first back-cross could be explained, but it was shown that the mortality would then rise to over 50% at the second back-cross. However, if two incompletely-linked resistance genes were assumed, each giving full protection against the malathion treatment on its own, a good fit to the observed mortalities at successive back-crosses would be obtained. This two-locus hypothesis is attractive also because it agrees well with the biochemical evidence given below. However, another possible explanation of the consistent occurrence of mortality below 50% is a single resistance locus (or two or more very closely linked loci) with reduced viability of the larvae of the susceptible homozygotes relative to the heterozygotes. If this were so, one might expect the families with lower overall larval survival to show a greater deviation from 50% mortality on exposure to malathion but this is not apparent from the data (Tables 1 and 2). The five first back-cross families having ⩾80% larval survival came from a total of 319 eggs which gave 267 fourth-instar larvae. On the extreme hypothesis that all the 52 individuals that died as larvae were susceptible homozygotes, these 52 could be added to the observed 103 susceptible adults giving a ratio of 155 susceptibles : 164 resistants from these five families. This does not differ significantly from a 1:1 ratio, so that this hypothesis is not excluded by the presently available data.

Table 3 shows the mortalities in the resistant homozygotes exposed to malathion alone and to malathion after previous exposure to triphenyl phosphate (TPP), the carboxylesterase (CE) inhibitor, and to piperonyl butoxide (PB), the mixed-function oxidase (MFO) inhibitor. At all the exposure times used, the combination of TPP and malathion produced obvious synergism indicating CE involvement in the malathion resistance. Results with the PB/malathion combination varied. Most dosages produced a small increase in mortality, though one showed a decrease. The involvement of MFOs in the activation of malathion to the toxic malaoxon by the conversion of the P = S bond to P = O is well known. If only MFOs are involved in this activation process, pretreatment with PB should produce continuous and complete antagonism with malathion at all doses. There should be no synergism. This was in fact found to be the case with a population of *A. stephensi* from Iran which, unlike the Indian strain of *A. culicifacies* (*5*), had only a low level of fenitrothion resistance. If, on the other hand,

Table 3. Results of exposures of the Indian malathion-resistant *A. culicifacies* to malathion alone and to malathion following pretreatments with triphenyl phosphate (TPP) and piperonyl butoxide (PB)

Insecticide and synergist	Percentage mortality (no. tested) after the following exposure times (min):									
	30		60		120		240		420	
5% malathion	6	(36)	19	(161)	5	(39)	19	(47)	27	(26)
TPP + 5% malathion	51	(67)	58	(88)	44	(66)	65	(93)	–	
PB + 5% malathion	15	(13)	21	(124)	0	(15)	29	(123)	39	(36)

this enzyme system is also involved in the actual breakdown of malathion, the nature of the response to the PB/malathion sequence could be one of synergism, antagonism, or even ineffectiveness depending on the balance of the opposing effects on activation and breakdown. In this Indian population of *A. culicifacies*, therefore, it seems that both the CE and the MFO mechanisms of resistance coexist, and account for the resistance shown by this population to compounds both with carboxylester bonds, e.g., malathion and malaoxon, and without them, e.g., fenitrothion, chlorphoxim, pirimiphos-methyl, and iodophenphos (Herath & Davidson, unpublished data). Synergist evidence would then suggest the involvement of two genetic factors. Further studies are needed to determine the extent to which the MFO-mediated mechanism inhibited by PB contributes to the malathion resistance.

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RÉSUMÉ

LA NATURE DE LA RÉSISTANCE AU MALATHION DANS UNE POPULATION D'*ANOPHELES CULICIFACIES* GILES

On a observé, dans une population d'*Anopheles culicifacies* de l'Etat de Maharashtra, en Inde, que la résistance au malathion, qui s'accompagnait d'une résistance à un certain nombre d'autres composés organophosphorés, était dominante dans son expression. La plupart des résultats de croisements et de croisements en retour avec une population sensible de la même espèce en provenance de Sri Lanka indiquaient que plusieurs facteurs génétiques étaient susceptibles d'intervenir. L'existence d'un spectre de résistance si large et des expériences comportant l'utilisation des composés synergiques, phosphate de triphényle et pipéronyl butoxyde, donnent à penser qu'il existe au moins deux mécanismes, l'un faisant intervenir la carboxylestérase spécifique et l'autre le système des oxydases à fonction mixte, moins spécifique.

REFERENCES

1. WHO Technical Report Series, No. 655, 1981 (*Resistance of vectors of disease to pesticides.* Fifth report of the WHO Expert Committee on Vector Biology and Control).
2. AKOOD, M. A. S. *The use of serology and of tests for drug and insecticide resistance in studying problems of malaria control in the Sudan.* PhD thesis, University of London, 1980.
3. GEORGHIOU, G. P. Studies on resistance to carbamate and organophosphate insecticides in *Anopheles albimanus. American journal of tropical medicine and hygiene*, **21**, 795-806 (1972).
4. RAJAGOPAL, R. Malathion resistance in *Anopheles culicifacies* in Gujarat. *Indian journal of medical research*, **66**: 27-28 (1977).
5. HERATH, P. R. J. *Studies on insecticide resistance in anopheline mosquitos.* PhD thesis, University of London, 1979.