INHERITANCE OF DIMETHOATE RESISTANCE IN THE MACKAY STRAIN OF THE CATTLE TICK (*BOOPHILUS MICROPLUS*) IN AUSTRALIA

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Abstract

Reciprocal crossing with a susceptible strain and phenotype analysis of F_1, test cross, and F_2 progeny for resistance showed that dimethoate resistance in the Mackay strain of the cattle tick *Boophilus microplus* was due to an incompletely dominant autosomal gene. The degree of dominance of resistance at LC_{50} was $+0 \cdot 567$; resistance factors for homozygotes and heterozygotes were respectively 221 and 69.

I. INTRODUCTION

Between 1963 and 1968, three distinct types of resistance to organophosphorus compounds (OPC resistance—Brown and Pal 1972) were detected in the cattle tick. These were shown by the Ridgelands strain in 1963 (Shaw and Malcolm 1964), the Biarra strain in 1966 (Roulston and Wharton 1967), and the Mackay strain in 1968 (Roulston *et al.* 1969). There is a wide spectrum of cross resistance to organophosphorus compounds and carbamates in all three strains (Roulston *et al.* 1969) but the Mackay strain is distinguishable from the Ridgelands strain by its slightly greater resistance to coumaphos, dioxathion, and ethion, and from the Biarra strain by its much lower resistance to Cyanox (*O-p*-cyanophenyl *O*, *O*-dimethyl phosphorothioate). Resistance in the Mackay strain (to coumaphos) is due to detoxication† (Roulston *et al.* 1969) whereas in both Ridgelands and Biarra strains resistance is due to acetylcholinesterase (AChE) insensitivity towards inhibitors (Lee and Batham 1966; Schuntner *et al.* 1968; Roulston *et al.* 1968). All three strains are deficient in AChE compared with a susceptible strain (Wharton and Roulston 1970) but resistant homozygotes in the Ridgelands or Mackay strains are distinguishable from those in the Biarra strain by brain AChE histochemistry (Stone 1972).

Resistance to dioxathion, carbophenothion, and formothion in the Ridgelands strain and resistance to diazinon in the Biarra strain were each shown to be due to an incompletely dominant gene (Stone 1968*a*; Wilson *et al.* 1971). This paper reports on similar studies on the inheritance of resistance to dimethoate in the Mackay strain.

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† *Note added in proof.*—Recent evidence shows that AChE insensitivity of the Ridgelands type as well as the mechanism for detoxication is present in this strain (H. J. Schnitzerling, unpublished data).

II. Materials and Methods

The history of the Mackay (M) strain which was established in culture in 1967 was described by Roulston *et al.* (1969). In the laboratory the strain was maintained initially under continuous ethion selection pressure for about 1 year; after two generations without pressure, a single selection for low "brain"* AChE, using acetyl thiocholine substrate, as for the RR substrain of the Ridgelands (R) strain (Stone 1968*b*, 1972), was followed by normal rearing. The aim was to eliminate susceptible ticks and partly resistant hybrid ticks possessing high levels of brain AChE; it was assumed on the basis of dosage–mortality tests that the only OPC-resistant ticks in this strain were of one type, designated the M type. The resulting strain was called MM and was used for all experimental work with the exception of the second test cross. Subsequently sib-mating was carried out for two generations and a homogeneous substrain (MM_i) established from the most homogeneously dimethoate-resistant inbred line. This substrain was used for the second test cross. The acaricide-susceptible Yeerongpilly reference strain (strain S) of high brain AChE activity had been cultured without contact with acaricides for 17 years.

The crossing procedure used was as described by Stone (1962*a*, 1968*a*), and consisted of the removal from steers of engorged nymphs which were then allowed to moult in isolation. They were then mated in single pairs in organza-covered Perspex rings arrached by contact cement to the backs of steers. After engorgement the fertilized females were maintained at 27°C and 80–90% relative humidity for oviposition. Eggs and larvae were maintained under the same conditions.

The following notation was used to identify the progeny of crosses by their parentage, the female parent being given first: $F_1 \ldots . F_4 : F_1 \ldots . F_4MS$ and $F_1 \ldots . F_4SM$; test crosses: MS/S, SM/S, S/MS, and S/SM.

The mortality of larvae 7–14 days old was recorded after exposure for 24 hr in filter paper packets impregnated with oil solutions of dimethoate as described by Stone and Haydock (1962) and Stone (1968*a*). The dosage–mortality data were analysed by the probit method of Finney (1971). Dosage is expressed as percentage in oil on paper (Stone and Haydock 1962) rather than as micrograms per square centimetre Stone (1968*a*, 1968*b*; Wilson *et al.* 1971). This is to enable easier comparison with dosage–mortality data of Roulston *et al.* (1969 *et seq.*). A 1% dose is equivalent to 35 $\mu g/cm^2$.

Degree of dominance of resistance (*D*) was calculated by the formula used by Stone (1968*a*, 1968*c*). Chi-square tests as described by Stone (1962*b*) were used to test significance of departure from expectation.

The results were examined for conformity with a single-gene hypothesis.

III. Results

(*a*) F_1 *Progeny of Reciprocal Crosses*

The mortalities of F_1MS and F_1SM larvae from the first cross and those of MM and S larvae are compared (Fig. 1). LC_{50}, slopes of *ld-p* lines, and resistance factors are given in the following tabulation:

	LC_{50} (%)	Slope of *ld-p* lines	Resistance factor at LC_{50}
MM	0·408	6·42	221
F_1MS	0·131	3·42	71·3
F_1SM	0·123	3·94	66·6
S	0·00184	4·64	1

The slopes for the four *ld-p* lines were significantly different ($P<0·01$) so fiducial limits are not shown. The LC_{50} for F_1MS and F_1SM were not significantly

*This is more correctly a "synganglion" or fusion of numerous ganglia consituting the bulk of the nervous tissue of the tick (Binnington and Tatchell 1973).

different but the LC_{50} for MM was signficantly higher than those for F_1MS or F_1SM. Therefore resistance was controlled by an autosomal factor (*s*) and was incompletely dominant (*D* at LC_{50} = +0·579, +0·556 respectively).

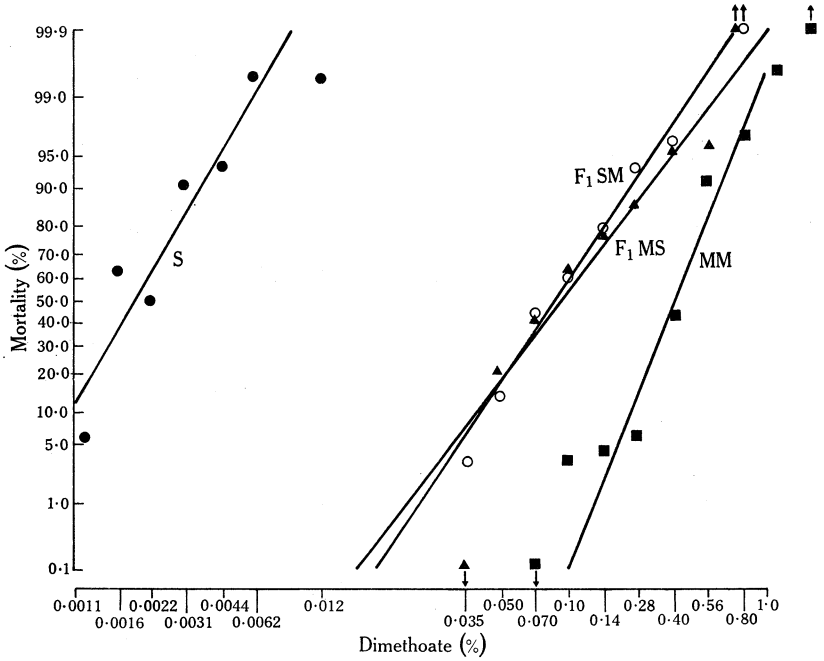

Fig. 1.—Mortality of Mackay strain, hybrid, and susceptible *B. microplus* larvae enclosed in dimethoate packets. Each mortality is based on 100–200 larvae.

(*b*) *First Test Cross*

In general, points obtained by plotting the mortalities of MS/S, S/MS, and SM/S larvae lay reasonably close to the expected test cross line (Fig. 2) constructed by using the F_1(MS+SM) and S data of Figure 1 obtained simultaneously. However, the mortalities for MS/S at the four doses above 0·05% were consistently lower than expected and this showed that the hybrid component in this test cross was more resistant than the F_1(MS+SM) *ld-p* line suggested.

(*c*) *Second Test Cross*

The mortalities obtained for seven individual broods of MS/S, SM/S, and S/SM larvae treated with dimethoate at 0·01% ranged from 47% to 54% and were not significantly different from the expected value of 50%.

(*d*) *F_2 Progeny*

Similar *ld-p* lines (Fig. 3) were obtained for F_2MS and F_2SM larvae and there was clear evidence of inflexion at about 25% mortality as well as reasonably good agreement between the observed mortalities and those expected from a 1 : 2 : 1

segregation of genotypes in an F_2 population. However, F_2MS mortalities in particular were consistently lower than expected at dosages above 0·40%, suggesting the presence of individuals more resistant than were found in the sample of MM larvae tested.

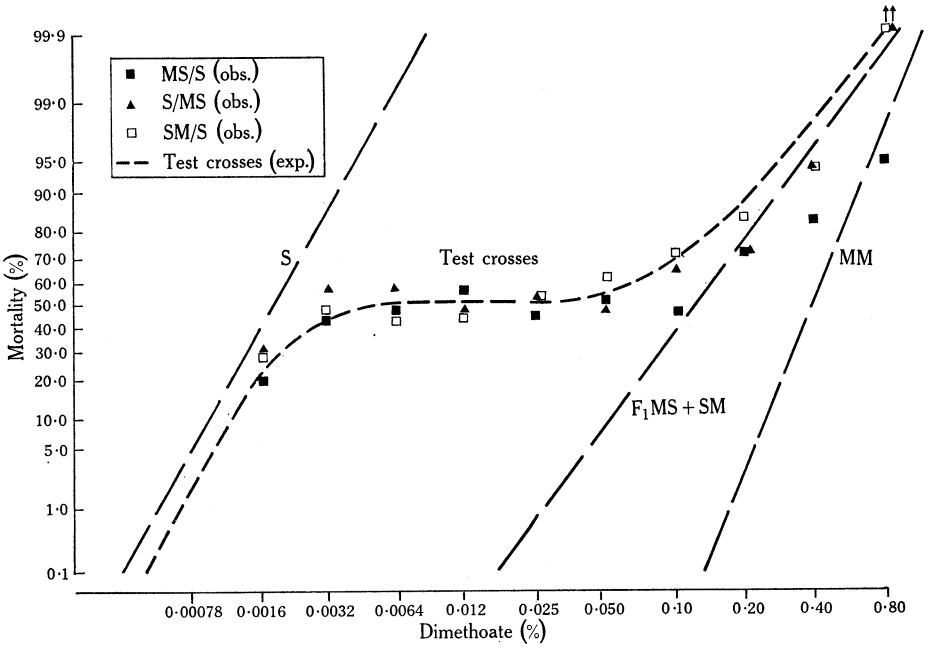

Fig. 2.—Mortality of Mackay strain, hybrid, test cross, and susceptible *B. microplus* larvae enclosed in dimethoate packets. Each percentage mortality is based on 100–200 larvae.

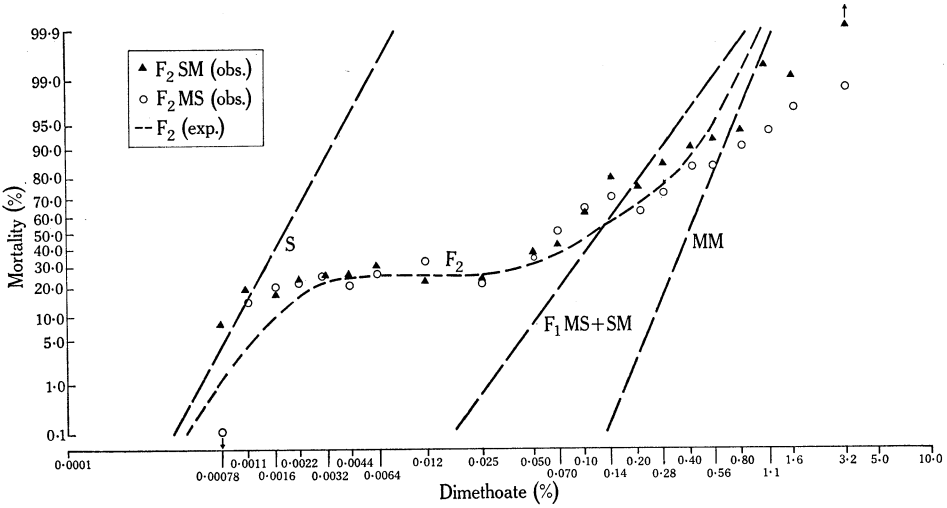

Fig. 3.—Mortality of Mackay strain, hybrid, F_2, and susceptible *B. microplus* larvae enclosed in dimethoate packets. Each percentage mortality is based on 100–200 larvae.

(*e*) F_3 *and* F_4 *Progeny*

The *ld-p* lines for F_3MS, F_3SM, and F_4MS, F_4SM larvae (Fig. 4) were very similar in shape to the F_2 lines, showing the same distinct inflexion at 25% mortality. This indicated that little change had taken place in the phenotypic composition since the F_2 and that F_3MS and F_4SM populations at least, also had a proportion of individuals that were more resistant than the most resistant individuals of the MM reference strain.

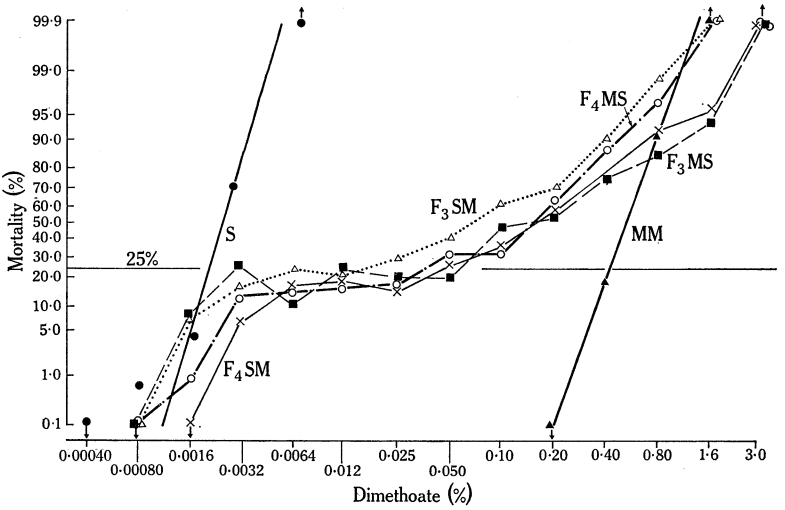

Fig. 4.—Mortality of Mackay strain, F_3, F_4, and susceptible *B. microplus* larvae enclosed in dimethoate packets. Each percentage mortality is based on 100–200 larvae.

It was concluded from the results in Sections III(*b*)–III(*e*) that dimethoate resistance in the Mackay strains is due to a single genetic factor.

IV. Discussion

There is strong evidence for monofactorial inheritance of dimethoate resistance in strain MM, derived from the Mackay strain. There was very close agreement between observed mortalities and those expected in test cross, F_2, F_3, and F_4 progeny. The symbol *M* will be used for the resistance allele in this strain.

Dimethoate did not contribute to selection for resistance in the Mackay strain but it is assumed that similar conclusions would have been reached with respect to inheritance of resistance to the selecting chemicals, ethion and coumaphos, had it been possible to obtain a clear separation of phenotypic responses in dosage–mortality tests with these chemicals. However, the resistance factors for ethion and coumaphos were too low to allow this, whereas the very high cross-resistance to dimethoate provided an excellent separation. In other studies of inheritance of OPC resistance in ticks, this type of procedure has been followed; in strains R and B, formothion and diazinon rather than the field chemicals dioxathion and ethion–coumaphos, respectively, enabled the clearest elucidation of resistance genetics.

In the present study it was most desirable to know that the strain was of high purity because both R type and B type ticks were also very resistant to dimethoate. B type but not R type ticks were excluded by the selection for low brain AChE activity. Although R type ticks were three to four times as resistant to dimethoate as M type while M type were three to four times as resistant to dioxathion, coumaphos, and ethion as R type, this difference did not permit complete exclusion of R type ticks except by inbreeding separate lines. This was not undertaken during the early stage of laboratory culturing of this strain although it was subsequently done to produce strain MM_i. However, there was every indication that strain MM at the time of the first cross had a very high percentage of specific M type ticks possessing their characteristic cross-resistance pattern (J. T. Wilson, personal communication).

It is not known why a proportion of MS/S, F_2MS, F_3MS, and F_4SM larvae were more resistant than expected (Figs. 2, 3, and 4). Although this result could, perhaps, be attributed to increased non-specific tolerance of *MM* genotypes due to recombination of modifying factors following crossing back to susceptible stock, it may also suggest the previously unsuspected presence of a slightly more dimethoate-resistant genotype in the original M population. In view of the earlier discussion on strain purity, *RR*, *RM*, or *MR* were the most likely candidate genotypes because strain R has subsequently been detected in the Mackay area (Wharton and Roulston 1970) and detected in strain MM 2 years later (B.F. Stone, unpublished date). It is possible that one or two of the 30 matings may have been R × S instead of M × S but as the vast majority would have been of the correct type, this possibility in no way invalidates the general conclusions. If confirmation was needed that monofactorial inheritance of resistance was truly characteristic of M type ticks it was obtained by testing the progeny of the second test cross derived from the MM_i strain from which any possible R ticks had been eliminated.

There are now three different types of OPC resistance in which monofactorial inheritance has been demonstrated; these are Ridgelands, Biarra, and Mackay types. The interrelationship of the resistance genes will form the basis of a separate communication.

V. Acknowledgments

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