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Linkage and Dominance Characteristics of Genes for Resistance to Organophosphorus Acaricides and Allelic Inheritance of Decreased Brain Cholinesterase Activity in Three Strains of the Cattle Tick, *Boophilus microplus*

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Abstract

Resistance to the organophosphorus acaricides diazinon, dimethoate and formothion in the Biarra (B), Mackay (M) and Ridgelands (R) strains respectively of the cattle tick *B. microplus* has been shown previously to be controlled in each strain by a single incompletely dominant autosomal genetic factor. A very similar mode of inheritance of fenthion resistance in strain B has now been demonstrated with no departure in degree of dominance of resistance from the mean value of +0·57 common to these strains exposed to these chemicals.

No F_1 larval progeny from the following crossings were appreciably more resistant than their parents to these chemicals: R × B—bromophos ethyl and fenthion; B × M—carbaryl, chlorfenvinphos, chlorpyrifos, diazinon, dimethoate, ethion, fenthion and formothion; M × R—chlorfenvinphos, diazinon, dimethoate, ethion, formothion. The field importance of this absence of overdominance is discussed.

There were no susceptible double recessive F_2 larval progeny of B × M crossings or F_2 or F_3 larval progeny of R × M crossings when tested against dimethoate to which the three parental types were similarly resistant; 1/16 of the larval progeny would be expected to be completely susceptible if the resistance genes were unlinked.

F_1 adult progeny of B × M and R × M crossings exhibited the incompletely recessive mutant-type decreased brain acetylcholinesterase (AChE) activity common to strains B, M and R, thus satisfying the test for allelism. No ticks with normal levels of brain AChE were detected in F_2 adult progeny of B × M or R × M crossings.

This evidence was strongly suggestive of a series of closely linked genes or alleles controlling dimethoate resistance and a series of alleles controlling decreased brain AChE activity in strains B, M and R.

Introduction

Resistance to organophosphorus compounds in the Ridgelands (R) strain of the cattle tick *Boophilus microplus*, first reported by Shaw and Malcolm (1964), is due to a decrease in acetylcholinesterase (AChE) sensitivity to inhibition (Lee and Batham 1966; Schuntner *et al.* 1968), and in the Biarra (B) strain (Roulston and Wharton 1967) it is due to a marked decrease in AChE sensitivity (Roulston *et al.* 1968). Coumaphos resistance in the Mackay (M) strain (Roulston *et al.* 1969) was reported as being due to increased detoxication only (Roulston *et al.* 1969) but there is evidence now of decreased AChE sensitivity to coroxon in strain M and to dimethoxon in strains B, M and R (Schnitzerling *et al.* 1974; Stone *et al.* 1976).

Resistance to diazinon, dimethoate and formothion in strains B, M and R respectively is controlled in each strain by an incompletely dominant autosomal gene (Stone 1968*a*; Wilson *et al.* 1971; Stone *et al.* 1973). Dimethoate resistance in strain B is also monofactorial (B. F. Stone, unpublished data) and in strain R it is almost

certainly due to gene *R* (Stone 1968*a*) which controls resistance to the dimethoate analogue, formothion. It seems justifiable to conclude that dimethoate resistance in strains B, M and R is due to a similar mechanism, 'decreased AChE sensitivity', which is under the control of B, M and R mutations. 'Decreased brain AChE activity' in these strains is controlled by an incompletely recessive autosomal gene which is either very closely linked to the resistance gene in each strain or is the resistance gene (Stone 1968*b*; Stone *et al.* 1976).

In *B. microplus*, sex determination is due to an XX–XO mechanism; there is cytological evidence of crossing over in both sexes (B. F. Stone, unpublished data) and it is considered that free recombination of resistance genes most probably occurs (Stone 1972).

This present study attempts to elucidate the interrelationships of these various inherited factors.

Materials and Methods

Ticks

The field history and subsequent laboratory purification of strain R was outlined by Stone (1968*a*), of strain B by Roulston and Wharton (1967) and Wilson *et al.* (1971), of strain M by Roulston *et al.* (1969) and of substrain MM_i (referred to here simply as strain M) by Stone *et al.* (1973). Each of these three strains was essentially homogeneous with respect to all resistances tested. The acaricide-susceptible Yeerongpilly reference strain (strain S) of high brain AChE activity has been cultured without contact with acaricides for 17 years. Strain R resulted from dioxathion pressure in the field whilst strains B and M resulted from ethion–coumaphos pressure; some resistance factors for these strains are shown in Fig. 1.

Acaricides

The following chemicals were used: bromophos ethyl* [4-bromo-2,5-dichlorophenyl diethyl phosphorothionate], carbaryl* [1-naphthyl methyl carbamate], chlorfenvinphos* [2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate], chlorpyrifos† [diethyl 3,5,6-trichloropyrid-2-yl phosphorothionate], coumaphos* [3-chloro-4-methyl-7-coumarinyl diethyl phosphorothionate], cyanophos† [4-cyanophenyl dimethyl phosphorothionate], diazinon* [diethyl 2-isopropyl-6-methyl-4-pyrimidinyl phosphorothionate], dimethoate* [dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolothionate], dioxathion* [*S*,*S*′-1,4-dioxan-2,3-ylidene bis(*O*,*O*-diethyl phosphorothiolothionate)], ethion* [tetraethyl *S*,*S*′-methylenebis(phosphorothiolothionate)], fenthion* [dimethyl 3-methyl-4-methylthiophenyl phosphorothionate] and formothion* [*S*-(*N*-formyl-*N*-methylcarbamoylmethyl) dimethyl phosphorothiolothionate]. Coroxon and dimethoxon are the oxygen analogues of coumaphos and dimethoate respectively. All chemicals were at least 98% pure.

Genetic Procedures

The usual notation (Stone 1962*a* *et seq.*) is used to identify the progeny of crosses by their parentage, the female parent being given first: F_1–F_3: F_1–F_3RM, MR, BM, MB, RB, BR; test-crosses: BM/M, MB/M, M/BM, and M/MB.

The procedures used in culturing of tick strains, single pair crossings, dosage–mortality testing and analysis of data were very similar to those described in earlier publications (Stone 1962*a*, 1962*b*, 1968*a*, 1968*c*; Stone and Haydock 1962; Stone *et al.* 1973). The fiducial limits and significance of degree of dominance (*D*) were calculated according to the procedure of Misra (1968). Histochemical methods used and ratings for 'brain' (synganglion) AChE activity were similar to those described elsewhere (Stone 1968*b*; Stone *et al.* 1976).

* Common names recommended by the International Organization for Standardization (ISO/R 1750-1970).

† Common names recommended by the British Standards Institution (BS 1831: 1969 and supplements).

The following formula (M. J. Whitten, unpublished data) was used to estimate an upper limit to the likely recombination values in F_2 and F_3 populations:

$$P = (1-r^2/4)^n,$$

where r is the recombination value, n is the number of progeny tested (derived from random-mating populations obtained by combining about 10 F_1 broods of larvae from single pair crossings after testing for parental homozygosity), and P is the probability. If P is set at some arbitrary value (say 0·01) it is possible to set an upper limit to the value of r.

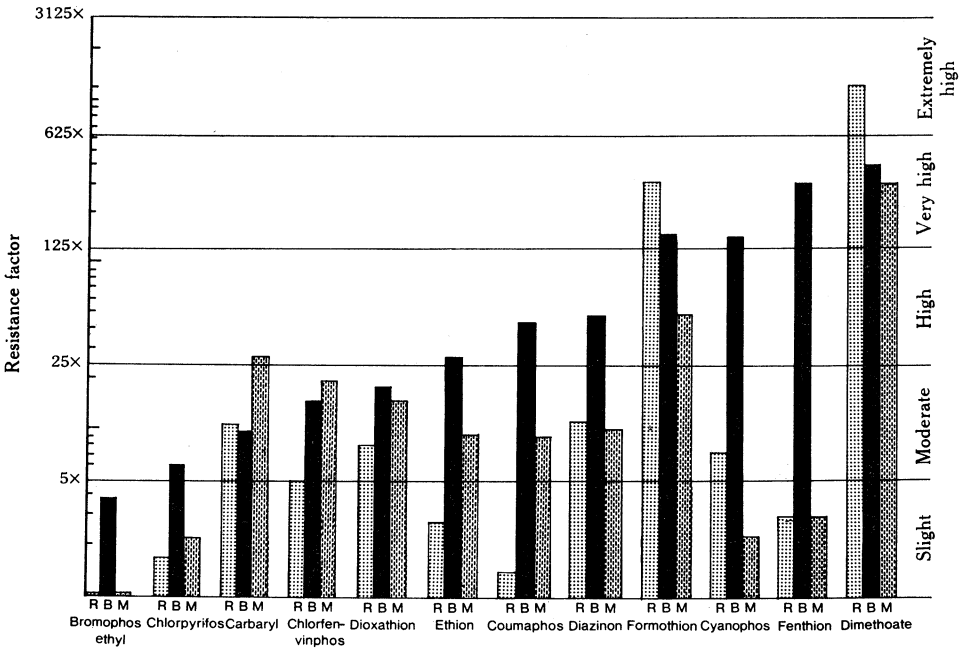

Fig. 1. Resistance factors for larvae of R, B and M strains of *B. microplus* enclosed in chemically impregnated paper packets.

Genetic Expectations

Because a common resistance mechanism, decreased AChE sensitivity, was shared by the three strains it was postulated that the three genes may behave as alleles when genotypes were tested against acaricides to which both parental strains were similarly resistant (e.g. dimethoate, Fig. 1) despite the distinct resistance spectra due to genes at B, M and R loci and the presence of the additional mechanism of detoxication in strain M.

If none of these genes were allelic the genotypes corresponding to the various phenotypes may be represented as in the following tabulation, the genes being referred to simply as *B*, *M* and *R*:

Phenotype	Genotype	Simplified genotype
B	*rrBBmm*	*rrBB* or *BBmm*
M	*rrbbMM*	*rrMM* or *bbMM*
R	*RRbbmm*	*RRbb* or *RRmm*
S	*rrbbmm*	*rrbb* or *rrmm* or *bbmm*
F_1BM or F_1MB	*rrBbMm*	*BbMm*
F_1RB or F_1BR	*RrBbmm*	*RrBb*
F_1RM or F_1MR	*RrbbMm*	*RrMm*

Each F_2(RB, RM or BM) population would then be expected to consist of nine genotypes ranging from *rrbb* . . . to *RRBB*, *rrmm* . . . to *RRMM* or *bbmm* . . . to *BBMM* respectively. The

six double recessive or double dominant genotypes named above would be expected to occur in frequencies up to 1/16 according to the extent of linkage of the loci. The data were examined for departure from expectation based on the most extreme case of completely unlinked loci, i.e. a frequency of 1/16.

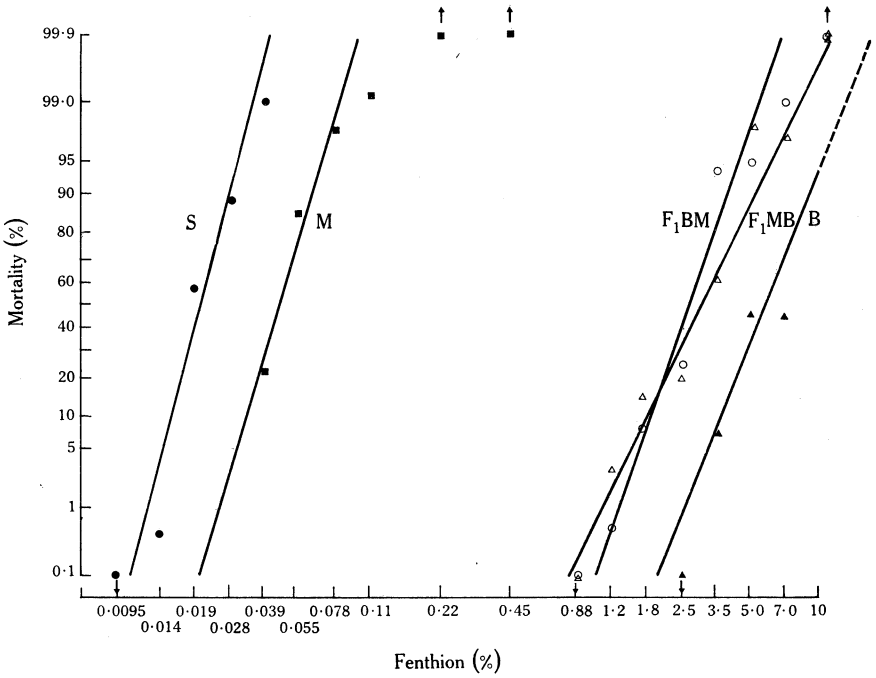

Fig. 2. Mortality of B strain (▲), F_1BM (△), F_1MB (○), M strain (■) and susceptible (S) *B. microplus* larvae (●) enclosed in fenthion packets. Each percentage mortality is based on 100–200 larvae.

Results

(*a*) *Dosage–Mortality Tests*

(i) *F_1 progeny of reciprocal crosses*

The mortalities of F_1BM and F_1MB larvae are compared with those of B, M and S larvae after enclosure in fenthion packets (Fig. 2). LC_{50} values, slopes of *ld–p* lines, resistance factors and degrees of dominance are shown for these genotypes and for F_1BS and F_1SB larvae in Table 1. The slopes of the five *ld–p* lines (common slope = 6·80) and the LC_{50} values for F_1BM and F_1MB were not significantly different but the LC_{50} value for B was significantly different from those for F_1BM, F_1MB and S. The degree of dominance of fenthion resistance in strain B over that in M was not significantly different from that of resistance in B over susceptibility in S except that D_{MB} was just significantly ($P < 0{\cdot}05$) greater than D_{BS} (t = 2·31). The degree of dominance for combined F_1BM and F_1MB was approximately +0·70 compared with approximately +0·61 for combined F_1BS and F_1SB.

An examination of LC_{50} values for B, F_1BM, F_1MB, M, R, F_1RM and S larvae after enclosure in carbaryl, chlorfenvinphos, diazinon, chlorpyrifos, dimethoate,

ethion and formothion packets (Table 2) shows that hybrids were not appreciably more resistant than their more resistant parent although in a few instances they were equally resistant or slightly less resistant than either parent. A comparison of LC_{50} values for B, F_1BR, F_1RB and R larvae after exposure in fenthion and bromophos ethyl packets (Table 3) also showed that hybrids were not more resistant than their more resistant parent. Thus there was no overdominance expressed in hybrids obtained by crossing the B, M and R strains.

Table 1. LC_{50} values, slopes of *ld–p* lines, and resistance factors for B, F_1BM, F_1MB, M, F_1BS, F_1SB and S larvae exposed in fenthion-impregnated packets

Type	LC_{50}[A] (%)	Slope of *ld–p* lines	Resistance factor[A]	Degree of dominance[A]
B	5·61 (4·39–7·75)	6·75	277 (212–364)	
F_1BM	2·63 (2·27–3·02)	7·75	130 (106–159)	+0·69[B] (0·59–0·78)
F_1MB	2·89 (2·42–3·50)	5·58	143 (114–179)	+0·73[B] (0·62–0·83)
M	0·0428 (0·0157–0·0530)	9·16	2·12 (1·61–2·74)	
F_1BS	1·68 (1·40–2·03)	9·74	83·2 (66·4–104)	+0·57[C] (0·48–0·66)
F_1SB	2·16 (1·82–2·57)	4·97	107 (86·2–133)	+0·66[C] (0·57–0·75)
S	0·0202 (0·0170–0·0202)	10·5	1	

[A] 95% confidence limits shown in parentheses. [B] Not significantly different. [C] Not significantly different.

(ii) *Testcrosses*

Single broods of larvae derived from three BM × M, two MB × M, one M × BM and one M × MB crossings were tested in 0·312% fenthion packets. Mortalities ranged from 42 to 55% and were not significantly different from 50%. Samples of larvae from these broods were combined and tested over a range of concentrations (Fig. 3). There was good agreement between observed and expected mortalities over the range of concentrations for which 50% was the expected mortality if fenthion resistance was due to a single gene.

(iii) *F_2 and F_3 progeny*

Fenthion. There was clear evidence of inflexion in the F_2BM and F_2MB *ld–p* lines in the region of 25% mortality and there was reasonably good agreement between observed and expected mortalities (Fig. 4). Thus it was confirmed that fenthion resistance in strain B was due to an incompletely dominant gene; strain M in this instance was behaving essentially as a fenthion-susceptible strain.

Dimethoate. B, M and R ticks were almost equiresistant and the high resistance to dimethoate in particular facilitated the detection of any susceptible ticks. However, among approximately 2500* F_2BM and F_2MB (Fig. 5) larvae tested over the range 0·0024–0·078% there were no dimethoate-susceptible ticks of *bbmm* genotype; 6·25% (1/16) susceptibles would have been expected if genes *B* and *M* were non-allelic and completely unlinked. Similarly there was no evidence of the presence of susceptible larvae of *rrmm* genotype among approximately 700* F_2MR (Fig. 6) or 669* F_3RM larvae tested over the range 0·0097–0·078% dimethoate. There

* It may be calculated from $n = 2500$ that the B and M loci are no more than 9 map units apart ($P = 0·01$). Similarly, from $n = 700$ or 699, M and R loci are no more than 16 map units apart.

Table 2. LC_{50} values,[A] and resistance factors (rf) for B, F_1BM, F_1MB, M, R, F_1RM and S larvae exposed in packets impregnated with carbaryl, chlorfenvinphos, diazinon, chlorpyrifos, dimethoate, ethion and formothion

The hybrids in all tests except those with carbaryl were obtained by mass matings. LC_{50} values are expressed as percentages

Type	Carbaryl		Chlorfenvinphos		Diazinon		Chlorpyrifos		Dimethoate		Ethion		Formothion	
	LC_{50}	rf	LC_{50}	rf	LC_{50}	rf	LC_{50}	rf	LC_{50}	rf	LC_{50}	rf	LC_{50}	rf
B	0·030	8·6	0·31	8·4	0·35	32	0·12	10	0·37	500	4·2	17	0·47	150
F_1BM	0·026	7·4	0·52	14	0·35	32	0·069	5·8	0·13	180	3·0	12	0·13	42
F_1MB	0·026	7·4	0·52	14	0·35	32	0·10	8·3	0·16	220	4·7	19	0·20	65
M	0·040	11	0·45	12	0·12	11	0·039	3·2	0·24	320	2·1	8·4	0·20	65
S	0·0035	1	0·037	1	0·011	1	0·012	1	0·00074	1	0·25	1	0·0031	1
R			0·22	6·0	0·17	15			0·92	1200	0·90	3·6	0·58	190
F_1RM			0·47	13	0·16	15			0·37	500	1·9	7·6	0·26	84

[A] Approximate values only, read from eye-fitted *ld–p* lines.

appeared to be no evidence of the presence of more highly resistant larvae in F_2BM or F_2MB (Fig. 5), F_2MR (Fig. 6), F_2RM or F_3RM.

Therefore the data departed from expectation in this regard and the evidence presented [sections (i), (ii) and (iii)] suggests that genes *B* and *M*, *R* and *M* are closely linked or allelic.

Table 3. LC_{50} values[A] and resistance factors (rf) for B, F_1BR, F_1RB, R and S larvae exposed in packets impregnated with fenthion and bromophos ethyl

Type	Fenthion		Bromophos ethyl	
	LC_{50} (%)	rf	LC_{50} (%)	rf
B	4·7	290	0·80	3·8
F_1BR	3·4	210	0·64	3·0
F_1RB	4·8	300	0·64	3·0
R	0·094	5·9	0·15	0·72
S	0·016	1	0·21	1

[A] Approximate values only, read from eye-fitted *ld–p* lines.

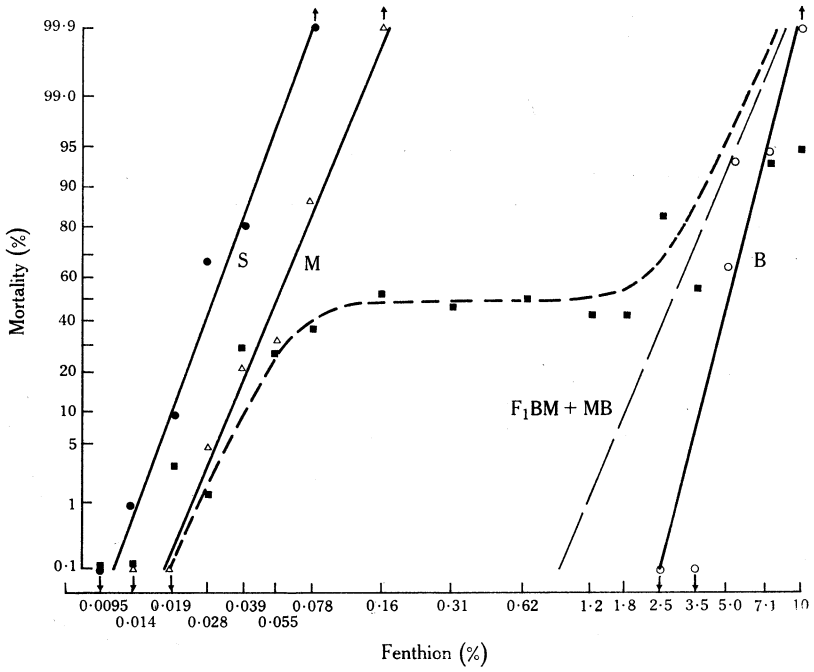

Fig. 3. Mortality of B strain (○), combined (BM + MB) × M testcross (■), M strain (△) and susceptible (S) *B. microplus* larvae (●) enclosed in fenthion packets. Each percentage mortality is based on 100–200 larvae. F_1(BM + MB) *ld–p* line calculated from Fig. 1 data superimposed for comparison. – – – Combined testcross (expected).

(*b*) *Decreased Brain AChE Activity*

(i) *F_1 progeny of reciprocal crosses*

Brains of B, M, F_1BM and F_1MB adult ticks (usually female) tested histochemically for AChE activity by the indoxyl acetate method were graded 0–1

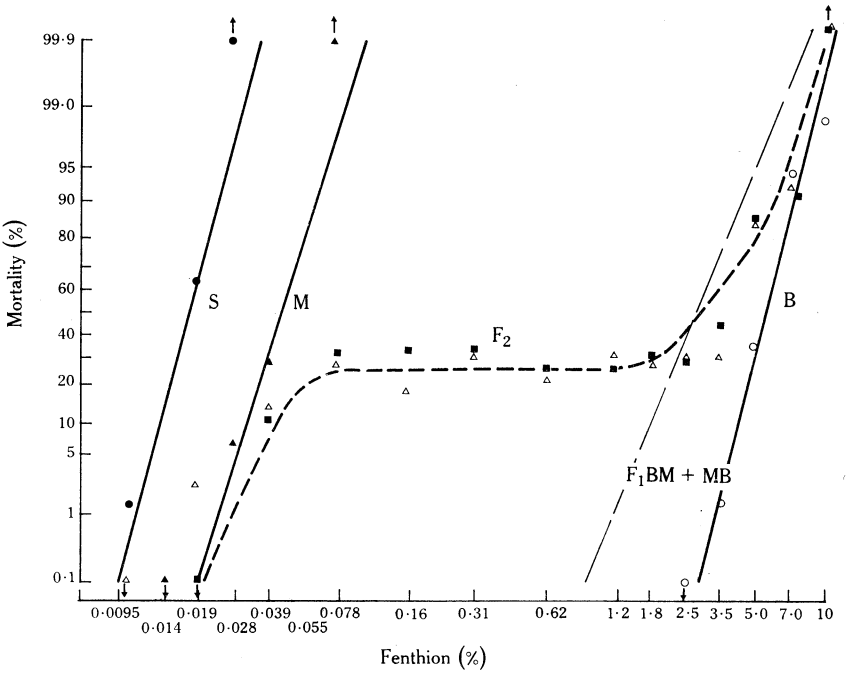

Fig. 4. Mortality of B strain (○), F_2MB (■), F_2BM (△), M strain (▲) and susceptible (S) *B. microplus* larvae (●) enclosed in fenthion packets. Each percentage mortality is based on 100–200 larvae. $F_1(BM+MB)$ *ld-p* line calculated from Fig. 1 data superimposed for comparison. – – – F_2 (expected).

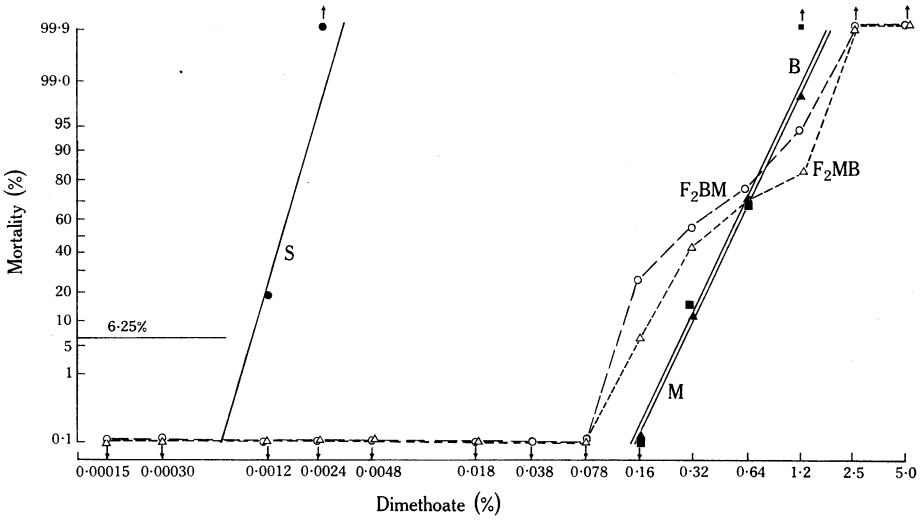

Fig. 5. Mortality of B strain (■), F_2MB (△), F_2BM (○), M strain (▲) and susceptible (S) *B. microplus* larvae (●) enclosed in dimethoate packets. Each percentage mortality is based on 200–300 larvae.

compared with 5 for S brains and by the thiocholine method B brains were 2–3 and S was 5. M, F_1RM and F_1MR rated 0–1 whilst F_1BS, F_1MS and F_1RS rated 3–4 by both methods.

It is clear that AChE in brains of hybrids from B × M and R × M crosses was not nearly as active as in the wild-type S genotype. Thus the recessive genes for 'decreased AChE activity' in strains B, M and R, i.e. dca^B, dca^M and dca^R respectively*, appeared to be allelic as this constitutes an allelism test.

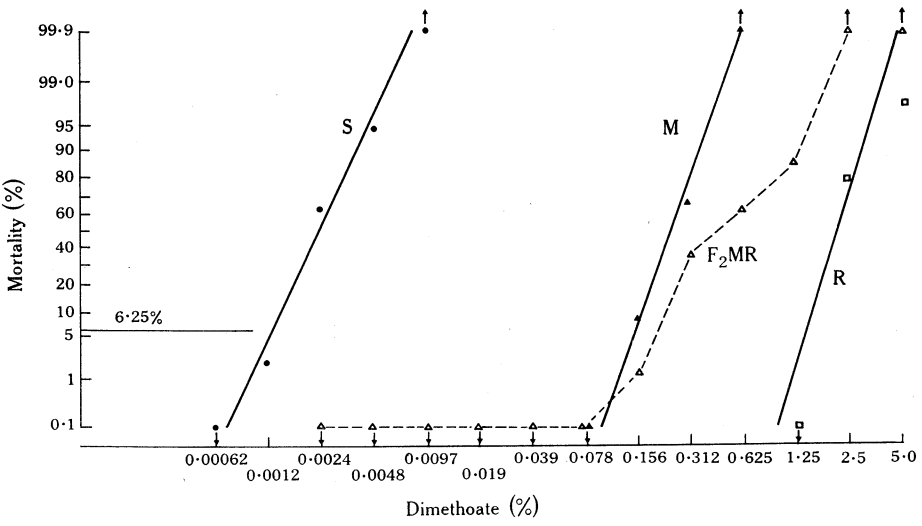

Fig. 6. Mortality of R strain (□), F_2MR (△), M strain (▲) and susceptible (S) *B. microplus* larvae (●) enclosed in dimethoate packets. Each percentage mortality is based on 200–300 larvae.

(ii) *F_2 progeny*

No brains with wild-type high AChE activity were detected among 59 F_2BM and 59 F_2MB ticks examined histochemically by the indoxyl acetate method. All brains were graded as 0–1 whereas it would be expected that 1/16 (7 brains) would have been equal to a 4–5 grading if genes dca^B and dca^M were non-allelic and unlinked. Similarly among another 44 F_2(BM+MB) brains examined by the thiocholine method there were no brains with a rating greater than 2–3; 5 out of 23 F_2MB and 3 out of 21 F_2BM brains were graded 0–1, the balance being 2–3; these values were not significantly different from the expected proportion of M-type ticks if genes dca^B and dca^M were segregating as a pair of alleles. Similarly, it was shown that among 441 F_2MR females there was none with a wild-type high level of brain AChE activity towards indoxyl acetate.

This helped to confirm that dca^B, dca^M and dca^R were allelic.

(*c*) *Degree of Dominance of Resistance in Strains R, B and M*

The mean *D* value was +0·57 derived from data on formothion resistance in larvae of strain R [+0·53 and +0·61 (Stone 1968*a*)], on diazinon resistance and

* *ch* of Stone (1968*b*) in strain R is now renamed dca^R and the other two genes are named thus by Stone *et al.* (1976).

fenthion resistance in strain B [+0·51, +0·49 and +0·62; +0·57 and +0·66 (Wilson *et al.* 1971); Table 1 of this paper respectively], and on dimethoate resistance in strain M [+0·56 and +0·58 (Stone *et al.* 1973)]. Only one of these D values was significantly different ($P < 0·05$) from the mean value of +0·57 when the data were analysed (Stone 1968*c*) and this was for F_1SB with fenthion ($D = +0·66$; $t = 2·11$). However D_{SB} (+0·66) was not significantly different from D_{BS} (+0·57) when allowance was made for both variances (Table 1) so +0·57 is regarded as a very good estimate of the degree of dominance of *B*, *M* and *R* genes for resistance to all the chemicals used.

Discussion

Evidence has been provided that dimethoate-resistance genes in the B and M as well as M and R strains are very closely linked or are allelic; this conclusion is based largely on the absence of any susceptible F_2BM or RM ticks (see p. 253). B and M loci have been shown to be no more than 9 map units apart while M and R loci are no more than 16 map units apart. Therefore B and R loci could not be more than 7, 9, 16 or 25 map units apart depending on sequence.

These genes confer a remarkable uniformity in degree of dominance of resistance over susceptibility (mean value +0·57) despite the distinct characteristics of each strain and the different chemicals used to measure resistance. No firm conclusions can be drawn from this fact in relation to linkage or allelism of the genes but it is quite clear that quantitative comparisons (Results, section *c*) are only possible by the use of a statistically tested formula for degree of dominance (Misra 1968; Stone 1968*c*).

The absence of F_1 and F_2 progeny appreciably more resistant than their parents, from B × M × R crossings (Tables 1–3, Figs 2–6), suggests a lack of augmentative interaction between resistance genes in hybrids and in any recombinants present in the F_2. This result is also of considerable field interest because of the previously expressed concern (Stone 1972) that the natural intermingling of such strains in the field may give rise to even more uncontrollable strains. There appeared to be some justification for this concern following the detection of the Mt Alford strain with increased resistance to chlorpyrifos, diazinon, bromophos ethyl and dioxathion compared with the Biarra strain (Schnitzerling *et al.* 1974). The former strain may have arisen from the latter by the acquistion of detoxication, a resistance mechanism additional to decreased AChE sensitivity. The inheritance of resistance to chlorpyrifos in the Mt Alford strain forms the basis for a separate communication (B. F. Stone, unpublished data).

There is clear evidence that decreased AChE sensitivity in strain B is due to a single incompletely dominant gene (*Dcs*^{B}—Stone *et al.* 1976) which is almost certainly gene *B* which controls resistance to many organophosphates and carbamates. Genes *M* and *R* also are almost certainly concerned with decreased AChE sensitivity, a mechanism known to be common to the three strains (Roulston *et al.* 1968; Schuntner *et al.* 1968; Schnitzerling *et al.* 1974). However, existing techniques used to detect AChE of decreased sensitivity in individual ticks do not permit similar studies on the inheritance of decreased AChE sensitivity in strains M and R which have lower levels of brain AChE than strain B (Stone *et al.* 1976). Hence the presence of a gene corres-

ponding to Dcs^B in each of strains M and R can only be inferred indirectly at the present time.

Decreased AChE activity found in brains of all hybrids from B × M and R × M crosses is conclusive evidence that the genes for decreased AChE activity in the three strains are allelic. Decreased AChE activity is not considered to have a causal or functional relationship to resistance, but its very close, if not inseparable, association with resistance previously demonstrated (Stone 1968*b*; Wharton and Roulston 1970) suggests either a close linkage between the *dca* and resistance loci or that decreased AChE activity and resistance are pleiotropic expressions of the same set of alleles. The case for pleiotropism is considerably strengthened now that decreased sensitivity of the same enzyme has been shown to be a resistance mechanism common to the three strains.

If there were separate loci for activity and sensitivity of AChE, it would be difficult to suggest why a mutant allele for decreased activity, having no conceivable advantage under acaricidal pressure, had been selected together with a mutant gene for decreased sensitivity in all three strains, when the latter alone would have sufficed. Furthermore, there is no reason to suspect that more than one locus would be required to determine the molecular structure of this enzyme and thus its properties including activity in its normal function and sensitivity to inhibitors. Such a situation would have required mutation at only one locus in each of the strains to alter the AChE to a less sensitive, less active form. Hence the notion of a single locus, with a set of alleles determining the molecular structure and thus the activity and the sensitivity of AChE, appears to provide the simplest and most probable explanation of the experimentally demonstrated characteristics of the resistant B, M and R strains of ticks that have been investigated genetically. An additional locus or loci may be involved in the resistance of the M strain which has the added capability to detoxify coumaphos (Stone *et al.* 1976).

Brain AChE in strains B, M, R and S has been shown (Stone *et al.* 1976) to consist of 'critical enzyme component II' only which is considered by Nolan *et al.* (1972) and Nolan and Schnitzerling (1975) to be the critical factor in the resistance mechanism to organophosphorus compounds. Furthermore, these authors regarded component II in strains B and R as modified forms of component II in strain S brought about by minor changes in physical or chemical structure of the enzyme and resulting in both decreased AChE sensitivity and activity. The most likely explanation is that the wild-type gene Dcs^+ mutated to give resistance genes such as Dcs^B in the various strains.

Decreased AChE sensitivity is a versatile resistance mechanism potentially capable of conferring some degree of resistance to all acaricides that act directly, or via a metabolite, by inhibiting the normal AChE of ticks. This explains qualitatively the broad spectrum of cross resistances shown by each of the strains to organophosphorus and carbamate acaricides that were not the chemicals that selected the strains in the field. Across the spectrum there is considerable variation in the relative resistance of the strains (Fig. 1); while this wide variation itself suggests that each strain may have a different form of AChE, evidence on this point must be taken from the following cases that have been investigated biochemically.

In a study of the biochemical genetics of resistance to organophosphorus compounds it was shown that brain AChE in strains B, M and R had a decreased

sensitivity to coroxon amounting to about 1/200, 1/6 and 1/3 respectively of that of strain S (Stone *et al.* 1976). Clearly the sensitivity of M-type AChE is similar to that of R-type AChE to this inhibitor whilst that of B-type AChE is distinctly different. A similar relationship was found using both coroxon and diazoxon to inhibit AChE in larval homogenates of strains B and R (Roulston *et al.* 1969) and R and M (Schnitzerling *et al.* 1974). This relationship is reflected to some extent in the larval resistance profiles for coumaphos and diazinon (Fig. 1) where B is clearly the most resistant strain. The increased resistance of M to coumaphos compared with R is undoubtedly due to increased detoxication (Roulston *et al.* 1969) but it is not known if the similarity in degree of resistance of M and R to diazinon is due to the similarity in AChE sensitivity as no information is available on possible increased detoxication of diazinon by strain M. In general, it is considered unwise to attempt to correlate *in vitro* and *in vivo* findings where so many different factors such as uptake, penetration, toxication, detoxication, transport of toxicant to target site, etc., are involved as well as sensitivity of target enzyme which itself may vary greatly according to the physical and chemical properties of the toxicant.

Although firm conclusions on close linkage or allelism have been drawn for dimethoate resistance only, it seems very likely that these same genes or alleles control cross resistance due to decreased AChE sensitivity to other acaricides. This mechanism is thought to be exclusively responsible for resistance to carbaryl, dioxathion, diazinon and dimethoate in strain R and for resistance to chlorpyrifos, carbaryl, coumaphos, diazinon and dimethoate in strain B as well as at least partly responsible for resistance to coumaphos, diazinon and dimethoate in substrain MM_i (Lee and Batham 1966; Schuntner *et al.* 1968; Schnitzerling *et al.* 1974; Stone *et al.* 1976). It has already been concluded elsewhere in this paper that resistance to formothion is almost certainly under the control of a gene at the R locus. Certainly, the cross resistance profile for formothion is very similar to that for its analogue dimethoate (Fig. 1).

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