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Genetic Basis of Resistance to Diazinon in Victorian Populations of the Australian Sheep Blowfly, *Lucilia cuprina*

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Abstract

Populations of *L. cuprina* from the Bairnsdale, Glenrowan, Hamilton and Torrita areas of Victoria were found to have similar high diazinon resistance status, following near fixation of a single allele at the R_1 locus. Pure-breeding resistant strains were derived from each area and used as base populations for a selection program on adults. An approximate doubling of the level of resistance to diazinon was achieved in each strain after eight generations of selection. Relaxation of selection over seven generations showed the response had generated a stable plateau in all but the Torrita selected strain, which regressed towards the resistance level of the original base population.

The genetic mechanism associated with the response to selection in each strain was autosomal but unique for each selected strain. Genetic effects directly influenced the resistance phenotype or acted through modification of the major locus.

The possible reasons for the uniform-resistance status of the field populations are discussed in terms of the observed selection responses and the likely selection regimes operating in the laboratory and field.

Other keyword: insecticide.

Introduction

The development of insecticide resistance has been recorded in a large number of species (Georghiou and Taylor 1977). Studies have used dosage-mortality curves to define the resistance status of populations and the effectiveness of laboratory-selection programs. The results demonstrate that resistance of economic significance in the field can essentially be regarded as a single-locus phenomenon (Milani 1963; Brown and Pal 1971; Kerr 1977), although a polygenic component may be associated with an initial response to selection (Hoskins and Gordon 1956) and, perhaps, with increased resistance subsequent to fixation at the major locus (de Zulueta *et al.* 1968). In almost all studies, however, little is known of the formal genetics of the organism. Consequently, it has not been possible to relate changes in resistance to gene frequency changes, or the relative importance of different environmental factors or insecticide management practices in determining these changes. The Australian sheep blowfly, *Lucilia cuprina*, affords an opportunity to examine these questions due largely to recent background work on the genetics and ecology of this species (Foster *et al.* 1975; Whitten *et al.* 1975).

Organophosphorus insecticides, in particular diazinon, have been used against *L. cuprina* since 1958 and resistance was first detected in 1965 (Shanahan and Roxburgh 1974). Genetic analysis carried out on populations from New South Wales and

Queensland demonstrated the existence of several resistance alleles (R_{1A} and R_{1B}), in addition to the original allele at a locus (R_1) on chromosome 4 (Arnold and Whitten 1976).

It was decided to examine the genetic basis of resistance to organophosphorus insecticide in Victorian populations of *L. cuprina* and the relationship between insecticide usage and the frequency of resistance alleles in different regions of the State. Four areas were chosen for study which prior examination indicated differed in terms of climate and insecticide practice. Preliminary examination of data from field populations in the four regions using dosage-mortality curves (the DMC is the calculated best-fitting regression line of mortality in probits on dosage in logarithms) showed that, contrary to our expectations, individuals from all four areas were uniformly resistant. In an attempt to account for this observation, the genetic basis of the resistance was examined using two approaches. Firstly, a detailed analysis of the frequency of resistance genes in field populations was carried out using a chromosome-substitution technique. Secondly, strains derived from field populations were subjected to selection for further resistance in the laboratory. The genetic basis of response to this selection was investigated to see if any genetic variation for resistance existed in the field.

Materials and Methods

Testing Procedure

Topical application of 0·5 μl of solutions of diazinon to the dorsal surface of the thorax of the fly, using a Drummond micropipette, followed the procedure of Arnold and Whitten (1976) except that deodorized kerosene was used as the solvent. All flies were reared under optimal conditions.

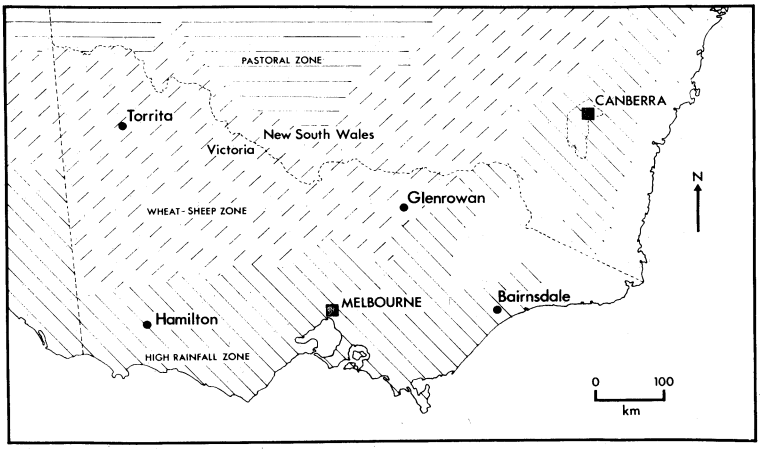

Fig. 1. Map of south-eastern Australia showing the sampling sites—Bairnsdale, Glenrowan, Hamilton and Torrita—in relation to the Australian sheep industry climatic zones (after Bureau of Agricultural Economics 1972).

Insecticide concentration levels were selected to yield DMCs for the resistant field strains, laboratory-susceptible strains and hybrids between these. In each case the DMCs provided an acceptable linear fit indicating that each strain was genetically homogeneous for any major resistance factor. The data enabled selection of discriminating dose levels [+ +–0·01 %(v/v)–*R*+–0·025 %(v/v)–*RR*] which are similar to those defined by Arnold and Whitten (1976) for the R_{1A} allele. Progeny

samples from individual field inseminated females were tested with discriminating doses of diazinon and the phenotypic mating combination of a female and her mate ascertained from the levels of mortality. These data allowed gene frequencies to be estimated for each area.

Strains

Field samples were made from populations of *L. cuprina* in the south-east (Bairnsdale), north-east (Glenrowan), south-west (Hamilton), and north-west (Torrita) areas of Victoria (Fig. 1) during the 1977/1978 spring to autumn period using liver-baited traps (Vogt and Havenstein 1974).

Pure-breeding resistant strains were derived for each area by pooling progeny of matings of homozygous resistant genotypes [i.e. survived 0·03%(v/v) diazinon]. These strains formed the base populations for the selection program.

A multiple marker strain (M5) with a single visible marker on each autosome was used in the genetic-localization studies. The recessive morphological markers—chromosome 2, *bp* (black pupa); 3, *ru* (rusty body); 4, *gl* (golden facial pubescence); 5, m_1 (m_1 vein incomplete); 6, *y* (yellow eye)—are described elsewhere (Whitten *et al.* 1975). The marker strain had a comparable DMC (Fig. 2) to that of the standard reference susceptible strain, SWT (Arnold and Whitten 1975).

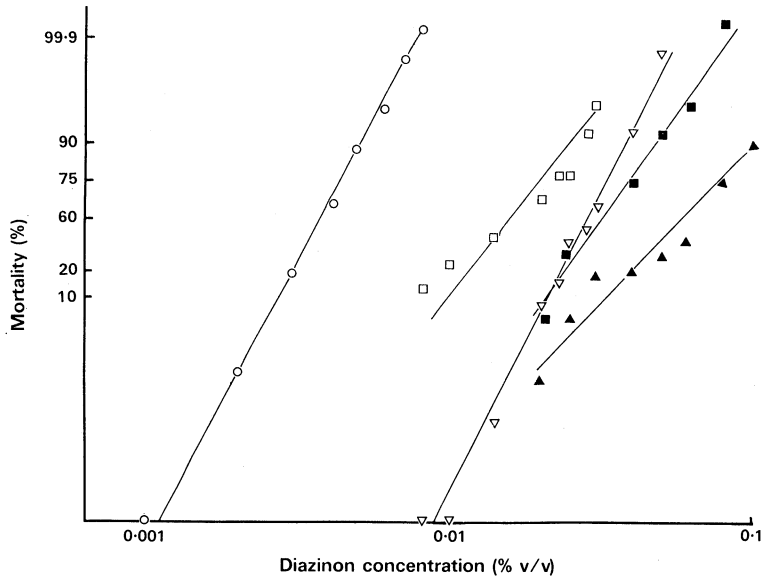

Fig. 2. Adult female dosage mortality regression lines for selected (▲) and unselected (■) Hamilton strains, for the M5 strain (○) and for F_1's between M5 and the selected (▽) and unselected (□) Hamilton strains. Each point is based on the mortality of 40 females and the LC_{50} values (and 95% confidence levels) are: Hamilton selected strain, 0·061 (0·053–0·072); Hamilton unselected strain, 0·030 (0·028–0·032); M5 strain, 0·0037 (0·0035–0·0040); F_1 between M5 and selected Hamilton strain, 0·028 (0·027–0·030); F_1 between M5 and unselected Hamilton strain, 0·016 (0·013–0·018).

Genetic Characterization of the Base Populations

Females of the pure-breeding strains of each area were crossed to males of a pure-breeding R_{1A} strain (Arnold and Whitten 1975). DMCs were obtained for the progeny of each cross and on the backcross progeny of F_1 (Bairnsdale $\times R_{1A}) \times R_{1A}$.

Selection Procedure

In all, 100 virgin males and 100 virgin females were collected from each base population. Males and females were tested with 0·03 and 0·04% diazinon respectively and survivors used to produce

the next generation. The procedure was continued for four generations and then for four more generations with the selective doses increased to 0·04 and 0·06% respectively.

Unselected controls were maintained for each population by breeding from similar numbers to the number of survivors of the related selected population at each generation. At the end of the eight generations of the selection program, DMCs were obtained for selected and unselected strains from each area and then selection pressure was relaxed for seven generations.

Table 1. Estimates of the resistant allele frequency (*R*) in four areas of Victoria (1977–78)

Area	No. of chromosomes tested	*R* frequency	Area	No. of chromosomes tested	*R* frequency
Bairnsdale	1134	0·98	Hamilton	766	0·98
Glenrowan	102	0·98	Torrita	120	1·00

Genetic Localization

Selected and unselected strains from each area were used in the crossing protocol outlined below. Since crossing-over does not occur in male *L. cuprina* (Whitten *et al.* 1975) the use of F_1 males ensures that marker assortment is equivalent to the assortment of entire chromosomes. From F_1 and M5 DMCs (Fig. 2) concentrations of 0·004 and 0·023% were chosen to kill approximately 50% of the selected strain backcross generation in *gl* (0·004%) or gl^+ (0·023%) segregant classes respectively. These phenotypic classes indicate the absence or presence of the R_{1A} allele as the R_1 and *gl* loci are both on chromosome 4 (Arnold and Whitten 1976). Testing was carried out on the 16 phenotypic classes, of progeny derived from both selected and unselected strains, for each segregant class.

Table 2. LC_{50} values (with 95% confidence limits) for diazinon for progenies of crosses between R_{1A} males and females of the designated lines

LC_{50} values are derived from testing 40 females at each of 10 concentrations of diazinon in the range 0·01–0·08% (v/v)

Source of females	LC_{50} (% v/v)	95% confidence limits (% v/v)
Bairnsdale strain	0·047	0·042–0·053
Glenrowan strain	0·048	0·043–0·055
Hamilton strain	0·042	0·039–0·049
Torrita strain	0·046	0·042–0·053
R_{1A} strain	0·038	0·034–0·043
F_1 (Bairnsdale strain × R_{1A})	0·039	0·037–0·043

Progeny Testing

If analysis of the genetic-localization data implicated a particular chromosome in the selection response, appropriate crosses were set up to substantiate this observation. For instance, if chromosome 6 was deemed to contribute to the response, in both *gl* and gl^+ classes, a cross (M5 × *bp ru gl* m_1 y^+) was established to generate *y* and y^+ progeny classes, i.e. segregating for chromosome 6, but with an otherwise uniform genetic background. Comparative DMCs were then obtained for each class. Similarly, crosses were devised to test any chromosomal or interactive effect.

Results

The populations of *L. cuprina* from each area surveyed consisted largely of pure-breeding resistant individuals (Table 1). Crosses, and a backcross, involving a

homozygous R_{1A} strain and pure-breeding strains of each area producedon ly resistant progeny. The resistance phenotypes were consistent with each of the pure-breeding strains being homozygous for the R_{1A} allele (Table 2). The strains of each area formed the base populations for the selection program.

Response of All Populations to Selection

A rapid response to selection was observed for all populations (Fig. 3). Only female data are presented but similar responses were observed for males of each strain. After eight generations of selection no strain appeared to have reached a selection limit but, given the magnitude of the responses, genetic analysis was considered appropriate at this stage. A similar methodology was used for all strains. Accordingly, detailed data and their analysis are presented for the Hamilton strains only. A general summary of the other strains is then provided.

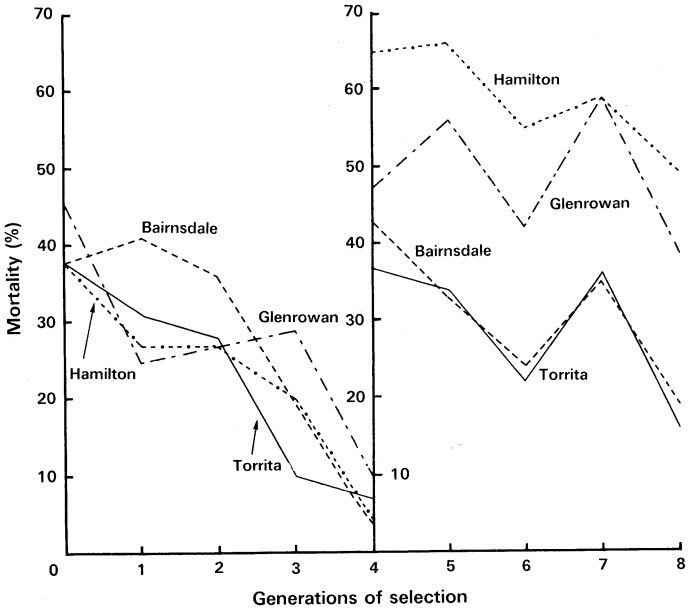

Fig. 3. Response to selection in females of strains homozygous for R_{1A} derived from Bairnsdale, Glenrowan, Hamilton and Torrita areas following selection with 0·04% (v/v) (generations 0–4) and 0·06% (v/v) (generations 4–8) diazinon.

Response of the Hamilton Strains

Analysis of the response to selection

DMCs of the selected and unselected Hamilton strains, the marker (M5) strain and of F_1's between M5 and the selected and unselected Hamilton strains are provided (Fig. 2). The regression lines represent the best least-squares fit following probit transformation of the mortality data (Bliss 1935). The results substantiate the genetic nature of the response. The level of resistance in the Hamilton population did not change significantly during the experiment [LC_{50} values for generations 0 and 8 were 0·034 and 0·030%(v/v) diazinon respectively].

Table 3. Percentage mortalities of the different phenotypic classes, of backcrosses between M5 and Hamilton strains, following testing with diazinon

Diazinon concentration for *gl* classes 0·004% (v/v), for gl^+ classes 0·023% (v/v). Two trials were conducted, each using 20 females

Phenotype	*gl* classes				gl^+ classes			
	Unselected		Selected		Unselected		Selected	
bp ru m_1 *y*	95	85	100	90	100	95	95	95
bp ru + *y*	75	80	65	55	55	73·3[A]	95	95
bp + m_1 *y*	75	60	75	80	100	80	95	95
bp + + *y*	45	50	80	30	80	70	80	75
bp ru m_1 +	75	95	65	85	70	65	90	90
bp ru + +	85	70	55	25	93·3[A]	85	65	55
bp + m_1 +	90	90	20	50	80	100	80	70
bp + + +	65	70	20	10	65	50	35	45
+ *ru* m_1 *y*	50	95	80	100	85	85	100	90
+ *ru* + *y*	70	75	80	70	85	20	95	85
+ + m_1 *y*	70	50	95	90	75	75	70	40
+ + + *y*	45	65	60	75	65	55	40	50
+ *ru* m_1 +	45	60	60	70	65	55	80	70
+ *ru* + +	80	40	5	10	80	75	80	85
+ + m_1 +	55	70	20	30	90	60	65	75
+ + + +	50	55	10	45	60	65	40	40

[A] 15 females used.

Table 4. Analyses of variance, after angular transformation, of the mortality data of Table 3 to test the influence of autosomes (other than chromosome 4) in the Hamilton selection strain response

*** $P < 0·001$; ** $P < 0·01$; * $P < 0·05$

Source of variation	d.f.	*gl* class		gl^+ class	
		Unselected *F*	Selected *F*	Unselected *F*	Selected *F*
Chromosome	15	1·94	7·66**	1·95	13·01***
bp (2)	1	—	0·11	—	10·03**
ru (3)	1	—	9·83**	—	68·75***
m_1 (5)	1	—	22·00***	—	28·59***
y (6)	1	—	63·17***	—	35·12***
2×3	1	—	4·09	—	11·56**
2×5	1	—	0·08	—	3·67
2×6	1	—	1·82	—	9·91**
3×5	1	—	6·35*	—	5·13*
3×6	1	—	1·41	—	2·62
5×6	1	—	0·45	—	2·37
2×3×5	1	—	0·01	—	0·09
2×3×6	1	—	1·09	—	6·77*
2×5×6	1	—	0·66	—	3·27
3×5×6	1	—	2·41	—	0·44
2×3×5×6	1	—	1·48	—	6·75*
Error	16				
Error mean square	83·55		108·46	127·00	30·85

The genetic basis of the response of the selected strain was determined using the following genetic localization protocol:

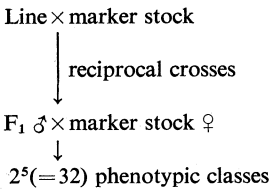

Table 5. Analyses of variance, after angular transformation, to test the influence of chromosome 4 (additional to R_{1A} locus) in the Hamilton selection strain response

*** $P < 0.001$

Source of variation	d.f.	Class *gl* *F*	Class *gl*$^+$ *F*
Between strains	1	1·89	0·001
Between genotypes within strains	30	5·18***	4·10***
Between trials within genotypes	32		
Error mean square		95·98	78·93

The mortalities of each of the 16 phenotypic classes were recorded for both *gl* (++ with respect to resistance locus) and *gl*$^+$ (+R with respect to resistance locus) classes (Table 3). Factorial analysis of variance demonstrated differences between the selected and unselected Hamilton strains (Table 4). For the selected strain, chromosomes 3, 5 and 6 significantly influenced the resistance phenotype in both backgrounds, while chromosome 2 effects were apparently only in the *gl*$^+$ class, that is in the presence of the R_{1A} allele. These effects can be implicated as causal in the phenotypic response as they are not apparent in the Hamilton strain.

The effect of chromosome 4, other than for the R_{1A} allele, was analysed by comparing the selected and unselected Hamilton strains in both *gl* and *gl*$^+$ classes. In the *gl* class both chromosome 4's of each strain derive from M5. Therefore, the comparison of the strains in this class may be seen as a control. No difference was observed between the strains (Table 5). The influence of chromosome 4, additional to the R_1 locus, was assessed by comparison in the *gl*$^+$ class. The analysis showed chromosome 4 effects to be unimportant to the response in the selected line (Table 5). The significant genotype effect, in both *gl* and *gl*$^+$ classes, reflected the influence of other chromosomes on the response (Table 4).

Progeny testing

DMCs were obtained for the progeny of crosses designed to test the influence of chromosomes 3, 5 and 6, and of chromosome 2 in the presence of the R_{1A} allele.

These results substantiated those of the statistical analyses (Table 6).

Table 6. LC_{50} values of diazinon for progenies of test crosses designed to substantiate the influence of chromosomes 2, 3, 5 and 6 in the selection response of the Hamilton strain

Values are derived from testing 20 females at each of six concentrations in the ranges 0·002–0·008% (v/v) and 0·01–0·03% (v/v) diazinon for the *gl* and gl^+ classes respectively. ** $P < 0.01$; * $P < 0.05$ for differences between LC_{50}'s of selected and unselected chromosomes

Chromosome No.	Class	LC_{50} (% v/v) Marker chromosome progeny	LC_{50} (% v/v) Selected chromosome progeny
2	*gl*	0·0033	0·0034
	gl^+	0·0182	0·0252**
3	*gl*	0·0036	0·0051*
5	*gl*	0·0035	0·0044*
6	*gl*	0·0036	0·0046**

Response of Strains from All Areas

The genetic architectures of the selection responses of strains from the different areas were unique to each strain (Table 7). Since it is not possible to analyse the influence of chromosome 4 in the absence of the R_{1A} allele with the present experimental procedure, the allocated effect of chromosome 4 for the Bairnsdale and Glenrowan selected strains is provisional. Tests to determine the nature of the chromosome 4 effect are in progress. Analyses of the unselected strains showed only the R_{1A} allele influenced the resistance phenotype in each case, no other chromosome contributing to the resistance level.

Table 7. Summary of the genetic basis for the response to selection in the strains derived from the different areas

+ indicates significant effect; − indicates no detectable effect; * indicates effect only in presence of R_{1A} allele

Strain	Chromosome 2	3	4	5	6
Bairnsdale	+	+	+	−	*
Glenrowan	−	−	+	−	−
Hamilton	*	+	−	+	+
Torrita	−	+	−	+	+

A consideration of the genetic basis of the selection responses has concentrated on the influence of the autosomes. The genic content of the X chromosome in *L. cuprina* represents a minute fraction of the genome (Whitten *et al.* 1975). Furthermore, no X chromosome influence was apparent when the mortalities of F_1 males, from reciprocal crosses between the M5 and selected strains, were compared.

Relaxed selection

Selected strains of the Bairnsdale, Hamilton and Glenrowan populations maintained the same level of resistance after seven generations of relaxed selection. However,

the strain from the Torrita population had returned to the resistance level of the base population (Table 8). The unselected strains maintained the same resistance levels during the seven generation period, as they had done for the course of the experiment.

Table 8. LC_{50} values of the selected strains from each area following relaxation of selection
Values are derived from testing 40 females at each of nine concentrations of diazinon in the range 0·01–0·10% (v/v). * Significantly different ($P < 0·05$) from unselected line

Strain	Generation LC_{50} (% v/v)		Strain	Generation LC_{50} (% v/v)	
	Generation 0	Generation 7		Generation 0	Generation 7
Bairnsdale	0·060*	0·054*	Hamilton	0·061*	0·064*
Glenrowan	0·061*	0·065*	Torrita	0·091*	0·049

Discussion

A uniform distribution of resistant phenotypes is observed for populations of *L. cuprina* from different areas of Victoria, despite differences in insecticide usage and sheep management practices between the areas. The resistance status of a population is usually considered a function of the level of insecticide usage with resistant phenotypes being at a disadvantage in the absence of insecticide (Brown and Pal 1971; Curtis *et al.* 1978). The latter condition may not necessarily apply (Bøggild and Keiding 1958; Keiding 1967) and there is evidence in *L. cuprina* that the fitness of phenotypes associated with the R_1 locus may be modified by the genetic background (McKenzie, unpublished data). Modifications of this kind could help explain the high frequency of the resistance allele in all of the Victorian populations surveyed. Genetic mapping studies, allelism tests with R_{1A} and toxicological data all indicate that the resistance allele in Victoria is similar to, if not identical with, the R_{1A}.

Laboratory selection for increased resistance has been successful for a number of organisms including *L. cuprina* (Harrison 1967; Shanahan and Roxburgh 1974). However, the present study represents one of the few in which a detailed genetic interpretation can be associated with the response. Eight generations of selection have produced an approximate doubling of the LC_{50} values in each of the selection lines relative to the base populations, but these similar levels of phenotypic response have resulted from different underlying genetic mechanisms (Table 7). These mechanisms may be considered in terms of genes directly influencing the tolerance phenotype or those which modify the major locus to produce an effect. The latter may prove to be of particular relevance in the definition of the biochemical basis of diazinon resistance in *L. cuprina*. At this stage genetic effects have only been localized to the chromosome level but more precise localization is practical, whether the effects be due to single gene or polygenic mechanisms (Thoday 1961; MacBean *et al.* 1971). Further genetic analysis of the Glenrowan selected line may be particularly rewarding since the genetic effects are concentrated on the same chromosome as the major resistance locus. The response may, therefore, involve a change at the resistance locus or may be due to gene(s) elsewhere on the chromosome. In the latter case, the linkage relationships of these gene(s) to the R_1 locus could be of considerable evolutionary interest.

The frequency of the chromosomes involved in the responses to selection needs to be ascertained for each of the base populations used in the selection prodecure as only the effect of the R_1 locus was apparent in the initial analysis of the base

populations. The goodness of fit of the DMCs for each field population supports this contention. However, given the rates of response, the selection of these chromosomes can be rapid (Fig. 3) and can, at least in some circumstances, lead to a new stable resistance plateau (Table 8). The phenomena are not manifest in the field as each population has a similar resistance profile. It may be inappropriate to talk in terms of initial frequencies of the chromosomes involved in the response to selection because these selected chromosomes may contain polygenic combinations which have been synthesized during the course of the experiment. It will be difficult to distinguish experimentally between these two alternatives but an attempt to do so is in progress because of their relevance to our understanding of the selection response.

It is probable that selection in the field will be most intense at the larval stage of the life cycle (Roxburgh and Shanahan 1973; Arnold and Whitten 1975). Therefore the laboratory-selection regime, and the response observed, may not be applicable to the field as there is general evidence of a genotype yielding a different resistance phenotype at different stages of the life cycle (Arnold and Whitten 1975; Margham and Wood 1976). In the present study, however, the resistance status of the larval stages of the selection strains has increased (Roache, unpublished data), suggesting a broad correlation of response across the life-cycle stages for the selection procedure used. Whether similar genetic mechanisms are involved at each life-cycle stage is not known but it is feasible, though logistically demanding, to determine this. The relevance of such a procedure will depend on the response, at both larval and adult stages of the life cycle when selection for increased resistance is applied at the larval stage. This work is currently in progress in our laboratory.

The laboratory procedures used in these experiments enhance the prospect of polygenically determined phenotypic response, while in the field the selection process is more likely to favour changes at a single genetic locus (Whitten *et al.*, unpublished data). It should be noted, however, that before the occurrence of an appropriate mutation at a single locus, and perhaps after the fixation of the favoured allele, polygenic mechanisms may explain the phenotypic response observed in the field (Hoskins and Gordon 1956; de Zulueta *et al.* 1968; Brown and Pal 1971; Georghiou 1972).

The potential importance of the genetic background to the resistance phenotype raises one final point for consideration. Arnold and Whitten (1975) have defined different alleles at the R_1 locus on the basis of DMC comparisons of adult and larval stages of the life cycle. On the basis of the present results it would seem desirable for allelic comparisons to be carried out in a standardized genetic background. Only then can the effect of intra-allelic interactions on the phenotype be defined more precisely.

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