Selection for insecticide resistance in the Australian sheep blowfly, *Lucilia cuprina*[1]

J.A. McKenzie and M.J. Whitten

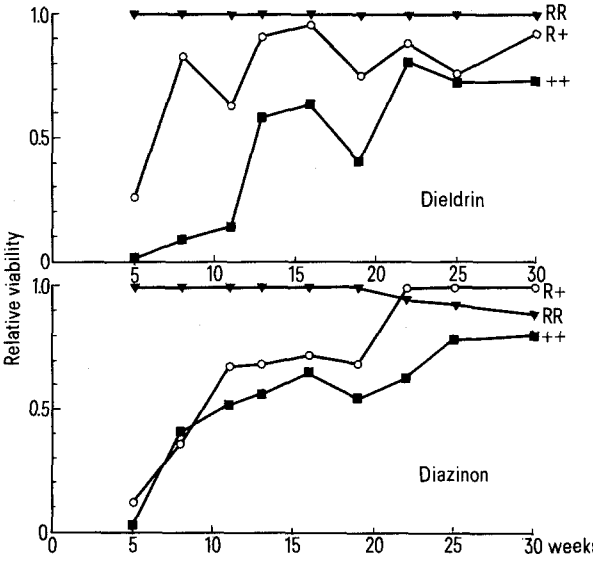
Department of Genetics, University of Melbourne, Parkville 3052 (Victoria, Australia), 15 April 1981

Summary. Egg implant studies on sheep demonstrate that the relative fitness of resistance genotypes of the sheep blowfly, *Lucilia cuprina*, changes over time for the 2 insecticides dieldrin and diazinon. The results suggest that selection may occur following exposure of larvae to sub-lethal concentrations and help to explain the relative rates of the development of resistance to dieldrin and diazinon by *L. cuprina*.

The development of insecticide resistance is of interest to evolutionary biologists concerned with assaying the ability of organisms to adapt by way of genetic response to chemical modification in their environment. The majority of cases of resistance of economic or medical importance can be explained by single allelic substitutions at individual loci[2,3] and models of the evolution of resistance and for strategies of insecticide usage are based on the assumptions of fixed fitness sets, defined by the presence or the absence of the insecticide, for genotypes[3-5].

In the Australian sheep blowfly, *Lucilia cuprina*, resistance has developed by separate single gene responses to the insecticides dieldrin[6] and diazinon[7,8]. The gene for dieldrin resistance *(Rdl)* is unlinked to that for diazinon resistance *(Rop-1)*[9] and there is no cross resistance between them[6]. Before resistance had developed to either of these insecticides (1957 for dieldrin and 1967 for diazinon)[6], the period of protection (that is, total absence of fly strike or myiasis) afforded to treated sheep against fly strike was 12-14 weeks[10]. After insecticide resistance had become widespread, the period of protection was reduced to 2-4 weeks for both insecticides[6,10]. Recent work[11] has suggested that selection for resistance may extend beyond the period when insecticide residues in the fleece and epidermal tissues of sheep have dropped below the level necessary for effective protection against blowfly strike. These results indicate selection will be concentrated at the larval stage of the life cycle and question the appropriateness of constant fitness models, as selection can occur at decreasing sub-lethal concentrations. The present paper reports on estimation of the relative viabilities of the different genotypes (homozygote resistant RR, susceptible + + and heterozygote R+) during development at different times subsequent to treatment of sheep with either dieldrin or diazinon.

Materials and methods. 6-month-old Merino sheep from a flock at the University of Melbourne farm Strathfieldsaye 250 km east of Melbourne were left as untreated controls or treated with either dieldrin (0.02% w/v) or diazinon (0.04% v/v) using hand-held spraying equipment in December 1979. Individuals were identified by numbered metal ear tags and the experimental flock was maintained under normal grazing conditions. At regular intervals between treatment and the subsequent shearing in September 1980, at least 2 sheep were randomly chosen from each experimental class and 'artificial' myiases were established at 3 sites (shoulder, mid-back and rump) along the dorsal mid-line of each sheep. A 1-2 cm long incision was made on the skin using a scalpel and 200-400 F_2 eggs were added from an original parental cross between a strain homozygous for both *Rdl* and *Rop*-1, and a homozygous susceptible strain. A moist cotton dental plug was placed over the eggs and the fleece drawn over the plug and secured with a rubber ring. A further egg sample was established on liver under standard laboratory conditions. After 48 h, larvae near maturity were removed from myiases, placed on liver and allowed to complete development under standard laboratory conditions. Upon emergence in the laboratory, flies were treated with discriminatory doses of insecticide to



Relative larval viabilities over time post treatment of resistant (RR), heterozygotes (R+) or susceptible (+ +) dieldrin or diazinon genotypes on sheep treated with the related insecticide.

The number (and proportion) of each genotype harvested from artificial strikes on sheep treated 11 weeks previously with either dieldrin or diazinon compared to controls with estimates of the relative viabilities of each genotype

Treatment	Dieldrin genotypes			Diazinon genotypes		
	+ +	R+	RR	+ +	R+	RR
Dieldrin	31 (0.07)	181 (0.40)	239 (0.53)	Not scored	Not scored	Not scored
Diazinon	Not scored	Not scored	Not scored	60 (0.16)	155 (0.40)	167 (0.44)
Control	90 (0.30)	117 (0.38)	98 (0.32)	70 (0.23)	136 (0.44)	102 (0.33)
Relative proportion treatment/control	0.23	1.05	1.66	0.70	0.91	1.33
Relative viability	0.14	0.63	1.00	0.52	0.68	1.00

distinguish + + from R+, and R+ from RR genotypes[8,11].

Results and discussion. The data for each period were pooled over strike sites, sheep of a given treatment, and for controls on sheep and liver, as consistent trends were apparent in each case. A typical example of the data generated, relative viability estimates for that period (table) and changes in relative viability over time (fig.) are provided. 2 observations are immediately apparent from the figure. Firstly, the period of selection for resistance genotypes is considerably greater than the period of protection from natural fly strike obtained before the development of resistance, despite a considerable decrease in insecticide concentration following treatment (P.M. Harrison, unpublished data). Secondly there are distinct differences in the responses to the 2 insecticides. In the case of dieldrin, the heterozygote has an intermediate viability over the course of the experiment but for diazinon the resistance is essentially recessive for the first 20 weeks whereupon there is an indication of heterozygote advantage. It should be noted that the relative viabilities of the resistance genotypes depicted in the figure presuppose discriminating dose levels which permit unambiguous identification of the 3 possible dieldrin or diazinon genotypes. This is correct for dieldrin but there may be a small degree of misclassification of RR as R+ for diazinon. Thus, the viability of the diazinon R+ heterozygote could in fact be lower than depicted. Selection may therefore act longer, the R+ genotype could be essentially recessive and the small heterozygous advantage observed after 20 weeks might be questioned. However, given this qualification, the consistency of trend suggests the general reliability of the measures and the relative viability estimates using the larval stage are consistent with the observation that dieldrin resistance developed more quickly (2 years) than diazinon resistance (10 years)[6].

The present system of artificial implants allows an estimation of relative fitness over time and has the potential to assist in the derivation of strategies of insecticide usage which may minimize the chance of resistance developing in the case of future insecticides. In the first instance this will specifically relate to the relative survival rate of R+ and + + genotypes, as an initially rare resistance allele will occur only in heterozygotes. Since resistance to insecticides constitutes one of the major drawbacks to the chemical control of insect pests, it may be useful for those concerned with the development of new insecticides to be conscious of the relationship between pesticide persistence and the development of resistance.

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Multiple phosphoglucomutase alleles in two species of *Mansonia* mosquito[1]

H.S. Yong, W.H. Cheong, G.L. Chiang, S.S. Dhaliwal and J.W. Mak

Department of Genetics and Cellular Biology, University of Malaya, Kuala Lumpur (Malaysia), 28 April 1981

Summary. Multiple phosphoglucomutase (E.C.2.7.5.1) alleles are found in the mosquitoes *Mansonia crassipes* and *M. uniformis.* The present study reveals 4 *Pgm* alleles, of which Pgm^B and Pgm^C are common to both species while Pgm^A is present only in *M. crassipes* and Pgm^D only in *M. uniformis.* The frequencies in both species accord well with Hardy-Weinberg expectations. The most frequent allele is that controlling a phenotype with an intermediate electrophoretic mobility, viz. Pgm^B in *M. crassipes* and Pgm^C in *M. uniformis.*

The applied and theoretical significance of electrophoretic studies on mosquito enzymes has been recently reviewed and discussed by Bullini and Coluzzi[2]. As much as 40% of the loci examined in natural populations have been found to be polymorphic. The 2 most variable gene-enzyme systems are esterase and phosphoglucomutase. As many as 5–7 electrophoretically detectable common alleles have been reported for the phosphoglucomutase locus in *Aedes aegypti*[3], *Aedes albopictus*[4], and *Culiseta longiareolata*[3].

Despite their importance in public health, there appears to be no previous report on the gene-enzyme systems in *Mansonia* mosquitoes. We report here the presence of multiple phosphoglucomutase (E.C. 2.7.5.1) alleles in 2 Malaysian species; *Mansonia crassipes* and *Mansonia uniformis.*

Mosquitoes of the genus *Mansonia* are the main vectors of human filariasis due to *Brugia malayi* in Southeast Asia[5]. They are also vectors of *Brugia pahangi* and *Dirofilaria* of both wild and domestic animals. As they breed typically in open swamps, they cannot be effectively controlled by conventional method such as insecticide spray. This calls for the use of biological control methods including genetic control, for which a knowledge of the genetic make-up of these mosquitoes is a prerequisite.

Table 1. Frequencies of PGM phenotypes in wild-caught *Mansonia crassipes*

	Homozygotes			Heterozygotes		
	A	B	C	AB	AC	BC
Observed number	0	35	0	6	0	3
Expected number	0.20	35.48	0.005	5.37	0.20	2.69