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of resistance, despite a considerable decrease in insecticide concentration following treatment (P.M. Harrison, unpublished data).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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2 A.W.A. Brown and R. Pal, Insecticide Resistance in Arthropods. WHO, Geneva 1971.
3 G.P. Georghiou and C.E. Taylor, Proc. XV. int. Cong. Ent., Washington, DC 1976; p. 759.
4 C.E. Taylor and G.P. Georghiou, J. econ. Ent. *72*, 105 (1979).
5 H.N. Comins, in: Genetics in Relation to Insect Management. Ed. M.A. Hoy and J.J. McKelvey, Jr. Rockefeller Foundation, 1979.
6 G.J. Shanahan and N.A. Roxburgh, P.A.N.S. *20*, 190 (1974).
7 J.T.A. Arnold and M.J. Whitten, Bull. ent. Res. *66*, 561 (1976).
8 J.A. McKenzie, J.M. Dearn and M.J. Whitten, Aust. J. biol. Sci. *33*, 85 (1980).
9 G.G. Foster, M.J. Whitten, C. Konovalov. J.T.A. Arnold and G. Maffi, Genet. Res. *37*, 55 (1981).
10 G.J. Shanahan, J. Aust. Inst. agric. Sci. *31*, 11 (1965).
11 M.J. Whitten, J.M. Dearn and J.A. McKenzie, Aust. J. biol. Sci. *33*, 725 (1980).

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

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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*

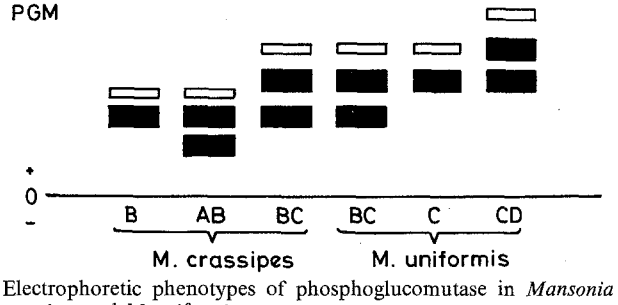
	Homozygotes			Heterozygotes		
	A	B	C	AB	AC	BC
Observed number	0	35	0	6	0	3
Expected number	0.20	35.48	0.005	5.37	0.20	2.69

The mosquitoes used for the present study were reared from larvae collected from the wild in Peninsular Malaysia. Adult mosquitoes were used for horizontal starch-gel (12% Sigma hydrolyzed starch) electrophoresis, employing the 'TEMM' buffer system and the enzyme visualization method of Spencer et al. with slight modification[6].

A total of 4 codominant *Pgm* alleles are found in the present samples of *M. crassipes* and *M. uniformis*. As in most culicine mosquitoes, each allele in *M. crassipes* and *M. uniformis* determines a 2-band electrophoretic pattern (fig.). Of these alleles, two (Pgm^B and Pgm^C) are common to *M. crassipes* and *M. uniformis*, while Pgm^A appears to be present only in *M. crassipes* and Pgm^D only in *M. uniformis*. The allele frequencies in *M. crassipes* are $Pgm^A = 0.068$, $Pgm^B = 0.898$ and $Pgm^C = 0.034$, while those in *M. uniformis* are $Pgm^B = 0.045$, $Pgm^C = 0.932$ and $Pgm^D = 0.023$.

Table 2. Frequencies of PGM phenotypes in wild-caught *Mansonia uniformis*

	Homozygotes			Heterozygotes		
	B	C	D	BC	BD	CD
Observed number	0	38	0	4	0	2
Expected number	0.09	38.22	0.02	3.69	0.09	1.89



Electrophoretic phenotypes of phosphoglucomutase in *Mansonia crassipes* and *M. uniformis*.

Of 6 possible phenotypes, only 3 are found in both species. The distribution of the various phenotypes in *M. crassipes* is summarized in table 1, while that in *M. uniformis* is summarized in table 2. In both species the frequencies are in good accordance with Hardy-Weinberg expectations ($\chi^2 = 0.57$ for *M. crassipes* and $\chi^2 = 0.24$ for *M. uniformis*). It is, however, significant that for the 2 common alleles Pgm^B and Pgm^C, the frequencies are significantly different in these *Mansonia* mosquitoes. Pgm^B allele has the highest frequency in *M. crassipes* while Pgm^C allele has the highest frequency in *M. uniformis*. Each of these alleles controls a phenotype of intermediate mobility in these mosquitoes. This agrees with earlier reports for other mosquitoes that the most frequent allele is generally the one controlling a phenotype with an intermediate electrophoretic mobility[3,4,7]. This phenomenon has been taken as supporting evidence for the idea that protein polymorphism is not primarily influenced by random genetic drift acting on a number of neutral isoalleles[8,9]. The present result, in which the common alleles in 2 related species of *Mansonia* mosquito govern different intermediate phenotypes with the highest frequency, renders further support to this hypothesis.

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2 L. Bullini and M. Coluzzi, Parasitologia *20*, 7 (1978).
3 L. Bullini and M. Coluzzi, Parasitologia *15*, 221 (1973).
4 H.S. Yong, K.L. Chan, S.S. Dhaliwal, W.H. Cheong, G.L. Chiang and J.W. Mak, Theor. appl. Genet. *59*, in press (1981).
5 R.H. Wharton, Bull. No. 11, Inst. Med. Res., Fedn of Malaya, 1962.
6 N. Spencer, D.A. Hopkinson and H. Harris, Science *204*, 742 (1964).
7 H.S. Yong, K.L. Chan, S.S. Dhaliwal, J.J.S. Burton, W.H. Cheong and J.W. Mak, Experientia *36*, 1062 (1980).
8 M.G. Bulmer, Nature *234*, 410 (1971).
9 L. Bullini and M. Coluzzi, Nature *239*, 160 (1972).

Precipitate formation of some sulfonated tetrazolthiomethyl cephalosporins with basic chemicals

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Summary. Cephalosporins containing a thiomethyltetrazole-sulfomethyl or sulfoaminoethyl substituent at the three-position react in aqueous solution with protamine or quinine to form a precipitate. This phenomenon may provide some insight into their pharmacokinetics and modes of action especially as it relates to the high and prolonged serum levels and high degree of serum protein-binding.

Strongly acidic substances, particularly those containing a sulfonic acid and a large electronegative charge form complexes with highly basic materials. This phenomenon gained wide clinical application, as exemplified by the long-acting insulin complexes with either protamine or histone[1], the extremely long acting homidium-suraminate[2] and the depôt-formulation of procaine-penicillin G. The sulfonated and colored azo dyes are known to combine firmly with serum albumin. Evans blue (4 SO_3H groups) is used on the basis of this property for the determination of total blood volume[1]. A similar mechanism is involved in the prompt neutralization of the overdosage symptoms of heparin by the injection of protamine[3,4]. Similar complexing of certain tetrazolsulfonic acid containing cephalosporins with protamine or quinine was observed and is described in this communication.

Materials and methods. 6 sulfonic acid containing cephalosporins were included in this study. 3 non-sulfated cephalosporins (cefuroxime, cefamandole and SK&F 82956) also were included in the experiment as negative controls. 2 sulfonic acid containing drugs, yatren and suramin served as positive controls. The chemical structures are shown in the table.

Compounds were dissolved in deionized water in concentrations of 1000, 500 and 250 µg/ml, whereas protamine sulfate and quinine bisulfate were used in 1% concentra-