A HOMOLOGOUS GENE-ENZYME SYSTEM, ESTERASE 6, IN DROSOPHILA MELANOGASTER AND D. SIMULANS[1]

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THE existence in natural populations of a protein polymorphism involving two forms of a nonspecific esterase has been established in *Drosophila melanogaster* (WRIGHT 1961, 1963). The two forms of the esterase, Esterase 6, are distinguishable by their different electrophoretic mobilities in starch gel, Esterase 6^F migrating more rapidly toward the anode than Esterase 6^S. The inheritance of these two forms is controlled by a pair of codominant alleles, Esterase 6^F (*Est* 6^F, 6^F, *F*) and Esterase 6^S(*Est* 6^S, 6^S, *S*), located at 36.8± on the third chromosome. Individuals homozygous for the *Est* 6^F allele produce a strong, single, Esterase-6 band which migrates faster than a similar, strong, single, Esterase-6 band produced by individuals homozygous for the other allele, *Est* 6^S. Zymograms of heterozygotes, *Est* 6^F/*Est* 6^S, exhibit both the Esterase-6^F band and the Esterase-6^S band. Both alleles have been found together in numerous laboratory stocks and in at least two different wild populations.

This paper is a report of a search for a similar gene-enzyme system in *Drosophila simulans* that was undertaken in an effort to extend existing evidence of gene homologies between *D. melanogaster* and *D. simulans* to include a case which would provide some information about the nature of the protein products of the homologous genes. In addition, the search was initiated in an attempt to establish the existence of homologous, polymorphic gene-enzyme systems in wild populations of these two closely related species. The discovery of such homologous polymorphic systems would raise interesting questions concerning the stability of such polymorphisms over long periods of evolutionary history and perhaps provide a valuable tool for the investigation of the selective forces involved in their maintenance.

MATERIALS AND METHODS

The wild type and visible mutant stocks of *Drosophila simulans* used in this investigation are listed in Table 1, along with the form or forms of Esterase 6 found in each particular strain.

Except for a few slight modifications, the techniques used were the same as those described by WRIGHT (1963). For single fly zymograms, single flies were crushed with a glass pestle on 5 × 7 mm pieces of Whatman 3 MM filter paper

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TABLE 1

Forms of Esterase 6 found in various strains of Drosophila simulans

Strains	Source	Esterase 6
Wild-type strains		
New Orleans	Baltimore*	Slow
Lima, Peru	Baltimore	Slow
South Africa	Baltimore	Slow
Morro Bay	Pasadena†	Fast
Guatemala-3	Pasadena	Fast
Kushla-F, Alabama	Pasadena	Fast
La-3	Baltimore	Slow and Fast
La-4	Baltimore	Slow and Fast
Mutant stocks		
dh b py sd pm	Pasadena	Fast
jv st pe	Pasadena	Fast
v	Baltimore	Intermediate

* Stock Collection—Department of Biology, The Johns Hopkins University, Baltimore, Maryland.
† Stock Collection—Division of Biology, California Institute of Technology, Pasadena, California.

which were presoaked in 0.05 M Tris(hydroxymethyl)aminomethane buffer, pH 8.7. These pieces of filter paper along with the crushed flies were then inserted directly into the starch gel blocks. These blocks were exposed either for 5½ hours at room temperature to a voltage gradient of 5 to 6 v/cm or for 16 hours at 4°C to a voltage gradient of approximately 3 v/cm.

RESULTS

Esterase 6 in Drosophila simulans: Zymograms of multiple-fly homogenates clearly show that various *D. simulans* strains contain nonspecific esterases with electrophoretic mobilities in starch gel which are identical with Esterase 6^F and Esterase 6^S of *D. melanogaster* (Figures 1 and 2). Since these *D. simulans* proteins have the same enzymatic activity and the same electrophoretic mobility as the *D. melanogaster* Esterase 6 proteins, they can be considered to be homologous and, therefore, are also designated as Esterase 6 enzymes.

A survey of eight laboratory wild-type stocks and three visible mutant stocks of *D. simulans* showed that three strains contained only the slow migrating form of Esterase 6, five contained only the fast migrating form, and two exhibited both the slow and fast forms (Table 1). Furthermore, it is clear that at least some natural populations are polymorphic for these two forms of Esterase 6, for collections of wild *D. simulans* in the vicinity of Baltimore reveal the presence of individuals which contain both Esterase 6^F and Esterase 6^S.

A third form of Esterase 6 with an electrophoretic mobility intermediate to Esterase 6^F and Esterase 6^S (Figure 2) was discovered in a vermilion stock (Table 1) and also in wild collections made in the vicinity of Baltimore. This form of Esterase 6 has been designated as Esterase 6^I, and to date has not been found in *D. melanogaster*.

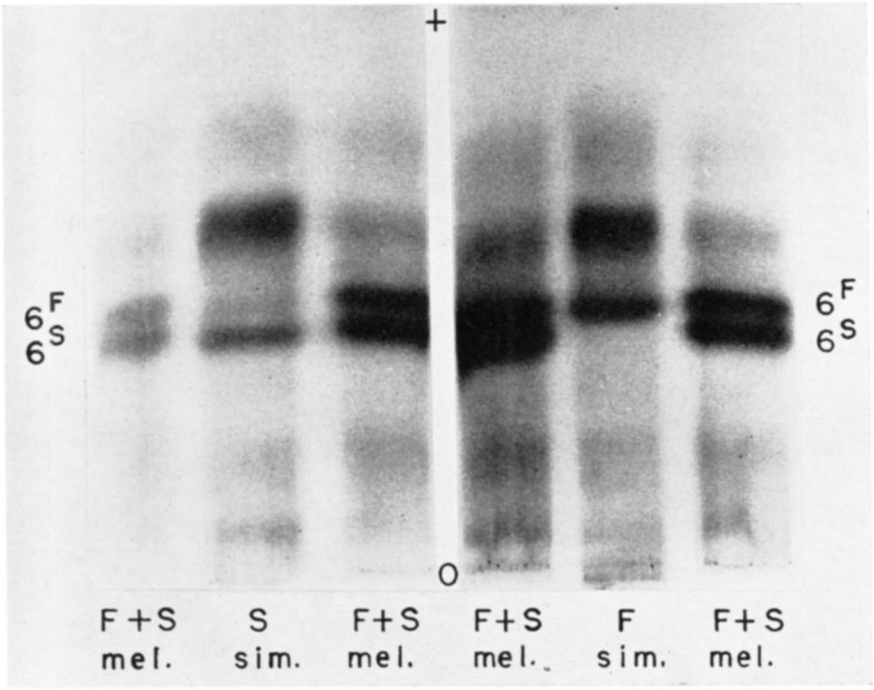

FIGURE 1.—Left: A zymogram of a multiple-fly homogenate of adults from the New Orleans strain of *D. simulans* between two zymograms of a multiple fly homogenate of an equal mixture of *Est* 6^F homozygotes and *Est* 6^S homozygotes of *D. melanogaster*. Right: A zymogram of a multiple-fly homogenate of adults from the Morro Bay strain of *D. simulans* between two zymograms of the same mixture of *D. melanogaster*.

Mendelian inheritance of Esterase 6 in D. simulans: The results of the crosses listed in Table 2 show that the manner in which Esterase 6^F and Esterase 6^S are inherited in *D. simulans* is identical to the mode of inheritance of the homologous enzymes in *D. melanogaster* (WRIGHT 1963), i.e., the structure of Esterase 6 is

TABLE 2

Crosses made to determine the mode of inheritance of Esterase 6^F *and Esterase* 6^S *in* D. simulans
(Deduced genotypes in parentheses)

Cross no.	Female parent		Male parent		Esterase 6 phenotype of offspring		
	Source	Esterase 6 phenotype	Source	Esterase 6 phenotype	Fast	Fast and slow	Slow
1	New Orleans ♀ ♀	Slow (S/S)	Morro Bay ♂ ♂	Fast (F/F)	0	20 ♀ ♀ 20 ♂ ♂	0
2	Morro Bay ♀ ♀	Fast (F/F)	New Orleans ♂ ♂	Slow (S/S)	0	20 ♀ ♀ 20 ♂ ♂	0
3	F_1 ♀ ♀	(F/S)	New Orleans ♂ ♂	(S/S)	0	54	42
4	F_1 ♀ ♀	(F/S)	Morro Bay ♂ ♂	(F/F)	50	44	0
5	F_1 ♀ ♀	(F/S)	F_1 ♂ ♂	(F/S)	21	48	26

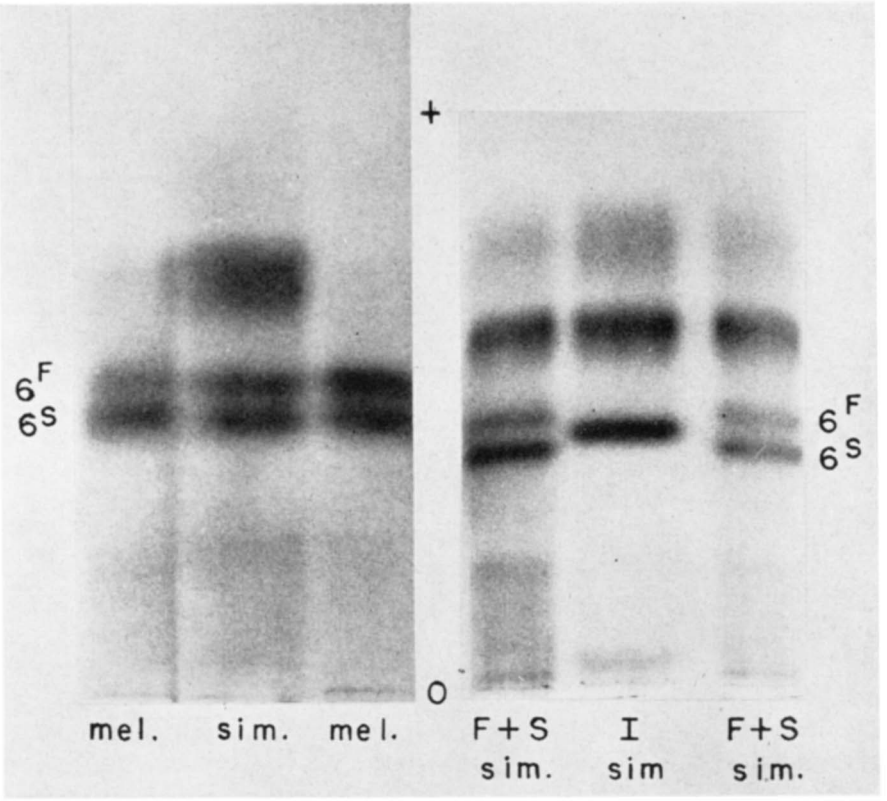

FIGURE 2.—Left: A zymogram of a multiple-fly homogenate of an equal mixture of adults from the New Orleans and Morro Bay strains of *D. simulans* between two zymograms of the same mixture of *D. melanogaster* described for Figure 1. Right: A zymogram of a multiple-fly homogenate of adults from the vermilion stock of *D. simulans* between two zymograms of a mixture of *D. simulans* similar to that described for the left starch block of Figure 2.

controlled by codominant alleles at an autosomal locus. Individuals homozygous for one of the alleles, designated as Esterase 6^F (abbreviated *Est* 6^F, or 6^f, or F), produce only the fast migrating form of Esterase 6, and similarly individuals homozygous for a different allele, designated as Esterase 6^S (*Est* 6^S, 6^s, S), produce only the slow migrating form. That these two alleles are codominant is shown by the fact that heterozygotes (*Est* 6^F/*Est* 6^S) produce both Esterase 6^F and Esterase 6^S.

The production of Esterase 6^I in *D. simulans* may be controlled by a third codominant allele, to be designated as Esterase 6^I (*Est* 6^I, 6^i, I), at the *Est 6* locus, but this has not been established conclusively, for it has not yet been possible to effect consistently a complete separation of Esterase 6^I from Esterase 6^F and from Esterase 6^S in single-fly zymograms. That Esterase 6^I is a third allele is suggested by the fact that individuals do exist which produce neither Esterase 6^F nor Esterase 6^S but only Esterase 6^I. In addition, the complete separation of Esterase 6^I from Esterase 6^S has been successfully accomplished in a number of single-fly

zymograms of F_1 progeny from matings of *Est 6^S/Est 6^S* homozygotes with individuals from the vermilion stock (presumably *Est 6^I/Est 6^I* homozygotes). In these single-fly zymograms the two Esterase-6 bands, Esterase 6^I and Esterase 6^S, are approximately half as intense as the Esterase-6 bands exhibited by homozygotes (including presumed *Est 6^I/Est 6^I* homozygotes) in single-fly zymograms. This reduction in staining intensity of bands is typical of heterozygotes and indicates that a single dose rather than a double dose of each allele is present (see the section on Gene Dosage in WRIGHT 1963). These results, then, are consistent with the hypothesis that the production of Esterase 6^I is controlled by a third allele at the *Est 6* locus. However, until the expected segregation ratios are obtained in F_2 and backcross progeny, no definite statement can be made concerning the allelism of *Est 6^I* with *Est 6^F* and *Est 6^S*.

Locus of Esterase 6 alleles in D. simulans: The F_1 offspring from Crosses 3 and 4 of Table 2 show that the *Est 6* alleles are not sex-linked, eliminating the X chromosome (Element A) as the linkage group for *Est 6*. Homozygous *Est 6^S* flies were crossed both to *dh b py sd pm* homozygotes (recessive visible mutants on the second chromosome—Elements B and C) and to *jv st pe* homozygotes (recessive visible mutants on the third chromosome—Elements D and E). That homozygotes from both of these visible mutant stocks were homozygous for *Est 6^F* was verified by running single-fly zymograms of all the parents of these crosses after larvae had appeared in the cultures. F_1 male progeny from each cross, heterozygous for the visible mutants and for *Est 6*, were then backcrossed to the respective, visible mutant, virgin females as outlined in Table 3, Crosses 1 and 2. The results of these crosses show conclusively that the locus of *Est 6* is somewhere on the third chromosome, and an analysis of recombinant progeny from Cross 3, Table 3, indicated that the precise location is between *jv* and *st*. Of the two crossover products in this region, the phenotypically more reliable class, because of the variable expressivity of javelin, is *jv* + + where the selected flies definitely show the gnarled and blunt bristles. Therefore this crossover class was used to determine the location of *Est 6* between *jv* and *st*. On the basis of 197 $\frac{jv\ (Est\ 6?)\ +\ +}{jv\quad Est\ 6^F\quad st\ pe}$ flies run as single-fly zymograms, 62.9 percent of the crossovers had occurred between *jv* and *Est 6* and 37.1 percent between *Est 6* and *st*. According to the loci assigned to *jv* (0.0) (STURTEVANT and NOVITSKI 1941) and to *st* (40.0) (STURTEVANT and PLUNKETT 1926), the *Est 6* alleles of *D. simulans* are located at 25.2 on the third chromosome (on Element D).

DISCUSSION

Criteria for establishing gene homologies: The problems involved in establishing gene homologies between two or more species have been considered by STURTEVANT (1921a,b), SPENCER (1949); PATTERSON and STONE (1952, pp. 261–264), and DOBZHANSKY (1959). The criterion for determining the homology of recessive genes between two species that produce viable hybrids is definitive: recessive genes which are noncomplementary in hybrids are homologous, and

TABLE 3

Crosses made to establish the locus of Esterase 6 in D. simulans

Cross no.	Female parent Genotype	Male parent Genotype	Offspring: Genotype of visible mutant genes	Offspring: Number of $Est6^F/Est6^F$	Offspring: Number of $Est6^S/Est6^F$
1	$\frac{dh\ b\ py\ sd\ pm\ (6^F)}{dh\ b\ py\ sd\ pm\ (6^F)}$	$\frac{dh\ b\ py\ sd\ pm\ (6^F)}{+\ +\ +\ +\ +\ (6^S)}$	$\frac{dh\ b\ py\ sd\ pm}{dh\ b\ py\ sd\ pm}$	8	10
			$\frac{dh\ b\ py\ sd\ pm}{+\ +\ +\ +\ +}$	8	10
2	$\frac{jv\ st\ pe\ (6^F)}{jv\ st\ pe\ (6^F)}$	$\frac{jv\ st\ pe\ (6^F)}{+\ +\ +\ (6^S)}$	$\frac{jv\ st\ pe}{jv\ st\ pe}$	18	0
			$\frac{jv\ st\ pe}{+\ +\ +}$	0	18
3	$\frac{jv\ st\ pe\ (6^F)}{+\ +\ +\ (6^S)}$	$\frac{jv\ st\ pe\ (6^F)}{jv\ st\ pe\ (6^F)}$	$\frac{jv\ st\ +}{jv\ st\ pe}$	24	0
			$\frac{+\ +\ pe}{jv\ st\ pe}$	2*	22
			$\frac{+\ st\ pe}{jv\ st\ pe}$	20	16
			$\frac{jv\ +\ +}{jv\ st\ pe}$	73	124

* Probable misclassification and should be $\frac{jv\ +\ pe}{jv\ st\ pe}$.

those that do complement one another are nonhomologous. This criterion, however, is, of course, not applicable to those species which do not produce hybrids, nor is it applicable when dominant genes are considered in those species that do hybridize (except dominant genes that are also recessive lethals—see STURTEVANT and PLUNKETT [1926]). In order to determine homologies in these cases, investigators have primarily relied on three other criteria: the location of the gene in homologous, chromosomal elements; the position of the gene in a sequence of homologous genes; and the correspondence of the phenotypes of the mutant genes. This last criterion has been particularly useful when mutant genes with pleiotropic phenotypes have been examined. Even though these criteria each have their pitfalls (SPENCER 1949; PATTERSON and STONE 1952), convincing cases of homology have been made for many genes when all three criteria have been applied together.

It should, however, be quite apparent that the case for any putative gene homology would be considerably strengthened, possibly definitely established, if the corresponding protein products of the two genes could be compared. Certainly,

if the amino acid sequence of the protein products of genes from two species were shown to be identical, there would be little doubt about their homology, and, furthermore, if the genetic code is indeed universal, one could infer identical base sequences for the two genes within the limits of the degenerate code. Although the purification and subsequent determination of the primary structure of proteins (which is still a major undertaking involving many specialized techniques) will provide the most complete information on putative homologies of proteins, other properties, especially other properties of enzymes, such as size (molecular weight and number of amino acid residues), state of polymerization, heat stability, electrophoretic mobility, antigenic cross-reactiveness, substrate specificities, inhibitor sensitivities, cofactor requirements, and peptide fingerprints can and should be used to make valid comparisons. It is quite obvious that a comparison of the properties of protein products of putative homologous genes is merely a refinement of the third criterion listed above (the examination of the correspondence of the phenotypes of mutant genes) and should be regarded in a similar fashion, i.e., that any gene homology established solely on the basis of corresponding protein properties without corroborative genetic evidence is highly suspect.

The homology of the Est 6 *locus in* D. melanogaster *and* D. simulans: Crosses between *D. melanogaster* and *D. simulans* produce viable, but sterile hybrids (STURTEVANT 1920). Using the criterion of noncomplementarity of homologous genes in these hybrids, STURTEVANT showed that many phenotypically similar recessive mutations in *D. melanogaster* and *D. simulans* are homologous (STURTEVANT 1921a,b,c, 1929; STURTEVANT and NOVITSKI 1941). Furthermore, he demonstrated that not only are these homologous genes located on the same chromosomal elements in the two species, but that in most cases the genes lie in the same relative order along these elements (e.g. see Figure 3).

Since the *Est 6* alleles in both species are codominant, the criterion of noncomplementarity in hybrids could not be used to establish their homology. However, it has been determined that the *Est 6* gene is located in the left arm of the third chromosome (Element D) of both species. In addition, in both *D. melanogaster* and *D. simulans* not only is the *Est 6* gene on the same chromosomal element, but it also occupies the same relative position in the linear sequence of homologous genes previously established by STURTEVANT. Figure 3 illustrates this important relationship. The discrepancies in the relative distances between the various homologous genes in the two species probably has little bearing on the problem of establishing gene homologies, for many factors, including genetic factors, influence the frequency of crossing over (BODMER and PARSONS 1963; LAWRENCE 1963).

The above genetic comparisons along with the fact that in both species, alleles exist which produce fast and slow migrating forms of a nonspecific esterase with identical electrophoretic mobilities provides a strong case for the homology of the *Est 6* genes in *D. melanogaster* and *D. simulans*. Further investigations of the comparative properties of these enzymes should make this homology even more convincing.

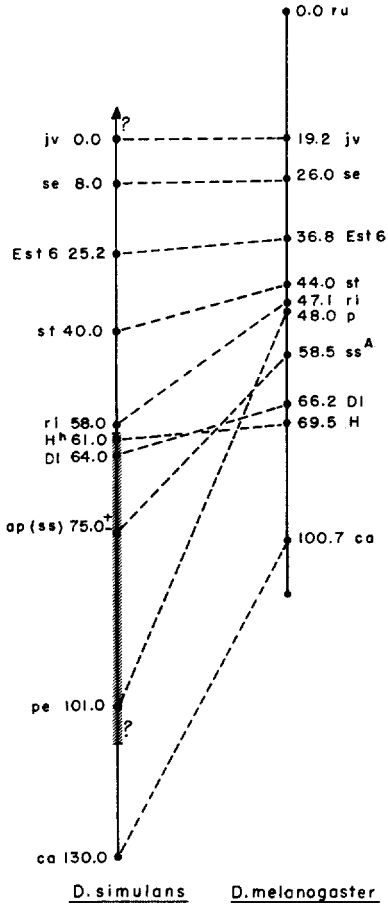

FIGURE 3.—A comparison of the loci of homologous genes on the third chromosome of *D. simulans* and *D. melanogaster*. The connecting line represents established homologies. The crosshatched portion of the *D. simulans* chromosome represents a section that is inverted relative to the *D. melanogaster* chromosome. The genetic location of the right end of this inversion is unknown, and no genes have yet been found distal to *jv* on the left end of the *D. simulans* chromosome.

The origin of the homologous protein polymorphisms: The discovery of homologous Esterase 6 polymorphisms in wild populations of the sibling species, *D. melanogaster* and *D. simulans*, immediately leads to speculations concerning their origins. The first possibility is that these are long term, stable polymorphisms which existed in an ancestral species and were maintained as speciation proceeded (see DOBZHANSKY and PAVLOVSKY 1959 and MULLER 1959 for discussions on stable polymorphisms). On the other hand, the other hypothesis states that these homologous polymorphisms arose independently and much more recently in the two species (a case of convergent evolution), i.e., the simultaneous existence of *Est* 6^F and *Est* 6^S alleles in populations of both species is merely a reflection of the

common mutational properties of the homologous genes. It is doubtful that conclusive evidence will ever be forthcoming which will make it possible to decide which of the two hypotheses is the correct one. However, complete information on the structure and function of all the proteins involved may supply evidence which favors one or the other possibility. If, for instance, a comparison of the amino acid sequence of Esterase 6^F and Esterase 6^S in both species reveals that a single mutational event which substitutes one amino acid for the other is all that is necessary to derive Esterase 6^F from Esterase 6^S (or vice versa), then the likelihood that these polymorphisms represent cases of convergent evolution will be increased. However, if such a comparison of amino acid sequences reveals that a similar series of mutational events, or better yet, a similar series of mutational events in a precise sequence, must have occurred in both species to produce one allele from the other, then one would find it more difficult to reject the hypothesis that these polymorphisms arose in an ancestral species. Perhaps an investigation of the spontaneous rate of mutation of one allele to the other would yield results which would not only serve to support the idea that these are cases of convergent evolution, but would also put the thought that a series of mutational events is involved beyond the realm of possibility.

That natural populations of both *D. simulans* and *D. melanogaster* monomorphic for one or the other allele may exist could be inferred from the fact that a significant number of laboratory stocks of wild strains have been found to contain only one of the alleles. This then could be construed as evidence for the hypothesis that not only have these polymorphisms arisen separately in the two species but that they have arisen more than once within each species. Unfortunately, the authors have virtually no information on how these various laboratory stocks of wild type strains were established. If a single female founder was used to start any of these strains, there is a good chance that they would be monomorphic even though the natural population was polymorphic. Furthermore, these strains are usually maintained by small mass transfers of parents which subjects them to a certain amount of inbreeding, and also selective forces in the culture bottles may be such that a monomorphic rather than polymorphic population is more advantageous. For these reasons, the existence of monomorphic laboratory wild-type strains should not be considered as reliable evidence for the existence of monomorphic, natural populations. Further collections from wild populations will have to be made before this point can be clarified.

In closing, one should state that there is as yet no evidence on whether or not the same selective forces are operative in the two species in the maintenance of these homologous polymorphisms, but it is to be hoped that this question will be answered eventually. It might be suggested that since both *D. melanogaster* and *D. simulans* are cosmopolitan species, man-made selective pressures (e.g. insecticides) may be responsible for the origin and maintenance of these polymorphisms. However, pertinent to the last suggestion is the fact that preliminary results from the investigation of *Drosophila willistoni*, a close, noncosmopolitan relative of *D. melanogaster* and *D. simulans*, indicate that a similar polymorphism for the Esterase 6 enzymes may also exist in this species.

SUMMARY

The existence of a pair of codominant alleles which control the production of two forms of a nonspecific esterase has been established in *Drosophila simulans*. These alleles are located at 25.2 on the third chromosome. Evidence is presented in support of the conclusion that these alleles in *D. simulans* are homologous to a similar pair of alleles in *D. melanogaster*. In addition, it has been shown that natural populations of both *D. simulans* and *D. melanogaster* are polymorphic for these homologous alleles.

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