THE GENETICS OF AN ESTERASE IN DROSOPHILA MELANOGASTER[1]

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IN recent years numerous cases of naturally occurring protein polymorphisms have been reported. These polymorphic proteins very often have been detected by their differential electrophoretic mobilities, and, in most cases, it has been shown that these changes in protein structure are controlled by a pair or series of codominant alleles. For example, such naturally occurring, gene-controlled, polymorphic protein systems have been found in β-globulins of the serum and plasma of man, dairy cattle, Zebu cattle, sheep, horses, goats, swine, mice, and pigeons (see MUELLER, SMITHIES and IRWIN 1962 for bibliography) and among esterases of Tetrahymena (ALLEN 1960, 1961), maize (SCHWARTZ 1960), mice (POPP 1961), swine (AUGUSTINSSON and OLSSON 1959), and humans (HARRIS, WHITTAKER, LEHMANN, and SILK 1960). In consideration of the diversity of organisms which appear in this list, one would expect eventually to find such a polymorphism in Drosophila. It should, therefore, not be surprising that this paper is a report about a pair of alleles found in laboratory stocks as well as natural populations which modify the electrophoretic mobility of an esterase in *Drosophila melanogaster* (see also WRIGHT 1961).

The significance of this discovery in Drosophila is manifold. For example, it contributes substantially to the biochemical genetics of Drosophila, not only because it adds the sixth, specifically identified, gene-enzyme system in Drosophila, but because it provides a method by which other useful gene-enzyme systems may be found. (The other five gene-enzyme systems are: (1) xanthine dehydrogenase-*ry* and *ma-l*—FORREST, GLASSMAN and MITCHELL 1956; GLASSMAN and MITCHELL 1959; (2) tryptophan pyrrolase-*v*—BAGLIONI 1960; (3) dopa oxidase-α and -β—LEWIS and LEWIS 1961a and b, 1962; (4) amylase-*Amy* —KIKKAWA and ABE 1960; KIKKAWA 1960; and (5) ali esterase-*ali*$^+$ and *ali*$^-$—OGITA 1961.) Secondly, the fact that the separate protein products of the two alleles can be readily distinguished, along with the fact that, in contrast to most allelic systems, the substitution of one allele for the other does not in any obvious way interfere with the normal processes of growth, differentiation, and morphogenesis, presents one with a unique experimental system for the investigation of numerous interesting problems of gene action in Drosophila. In addition, the use of experimental populations of Drosophila should prove to be highly instructive concerning the adaptive significance of these gene-controlled protein polymorphisms, and an examination of the polymorphic proteins of sibling species

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should not only provide interesting information about gene homologies but could yield valuable clues concerning the stability of these polymorphisms over long periods of evolutionary history. It should, therefore, be quite apparent that the discovery of a gene-controlled protein polymorphism in a well-worked and very useful experimental organism cannot help but eventually produce some interesting results.

MATERIALS AND METHODS

The following two wild-type stocks of *Drosophila melanogaster* were used in this investigation: (1) Oregon-R-CH: This stock, designated as CH in this paper, was made homozygous for the 1st, 2nd, and 3rd chromosomes in 1957 by making the appropriate crosses with the balancer stock $\frac{\text{M5}}{\text{M5}}$;$\frac{SM1}{Pm}$;$\frac{Ubx}{Sb}$. The CH stock has since been maintained by small mass-transfers and is not now considered to be isogenic. (2) Oregon-R, Amherst Inbred: Stock #1 from Amherst, Massachusetts, designated as Am in this paper. At the time of this investigation this stock had been carried through at least 357 generations of single-pair matings.

Preparations for electrophoresis were made by homogenizing 100 whole adult flies in 0.5 ml 0.05 M Tris(hydroxymethyl)aminomethane (Tris) buffer, pH 8.7, and then the homogenates were centrifuged for five minutes at 12,000 rpm. (International Micro-Capillary Centrifuge, Model MB, head No. 905) to remove the cell debris. Approximately 20 μl of the centrifuged homogenate was absorbed to 5 × 17 mm pieces of Whatman 3 mm filter paper which were then frozen, thawed, and finally inserted into lucite trays containing starch gel 6 mm deep. Single, whole flies were homogenized in tiny homemade glass homogenizers in 10 μl of the same buffer. The entire, uncentrifuged homogenate from one fly was transferred to 5 × 7 mm pieces of filter paper which then received similar treatment.

Connaught (Toronto) partially hydrolyzed starch was used for the starch gel which was prepared with 0.05 M Tris buffer, pH 8.7, in the concentration recommended by the supplier. Tris buffer at the same pH, but 0.25 M, was used for the electric bridge. The samples were exposed for five hours at room temperature to a voltage gradient of 5 to 6 v/cm. The 6 mm thick blocks of starch gel were then sliced horizontally, and slices approximately 1.5 mm thick were used for staining the multiple-fly homogenate runs, whereas slices approximately twice as thick were stained for the single-fly homogenate runs. Routinely, sliced starch strips were incubated for one hour in a solution of 300 mg Fast Blue BB and 30 mg α-naphthyl acetate in 100 ml of 0.1 M phosphate buffer, pH 6.5. For the characterization of the esterases the following substrates were substituted for α-naphthyl acetate: naphthyl AS acetate, β-naphthyl propionate, α-naphthyl butyrate, β-naphthyl laurate, β-naphthyl myristate, β-naphthyl stearate, β-carbonaphthoxy choline iodide, n-benzoyl-DL-phenylalanine-β-naphthol ester, and n-acetyl-DL-phenylalanine-β-naphthol ester. Inhibitors were used by pre-incubating the starch strips for one hour in a solution of the inhibitor in 0.1 M phosphate buffer,

pH 6.5. The strips were then placed in the α-naphthyl acetate-Fast Blue BB staining solution to which the inhibitor also had been added. Control strips from the same starch block were similarly pre-incubated for one hour in the buffer and then stained with the standard staining solution.

RESULTS AND DISCUSSION

Complement and nature of some adult Drosophila esterases: An array of at least ten bands is found when a homogenate of *Drosophila melanogaster* imagoes between two and six days old is subjected to starch gel electrophoresis for five

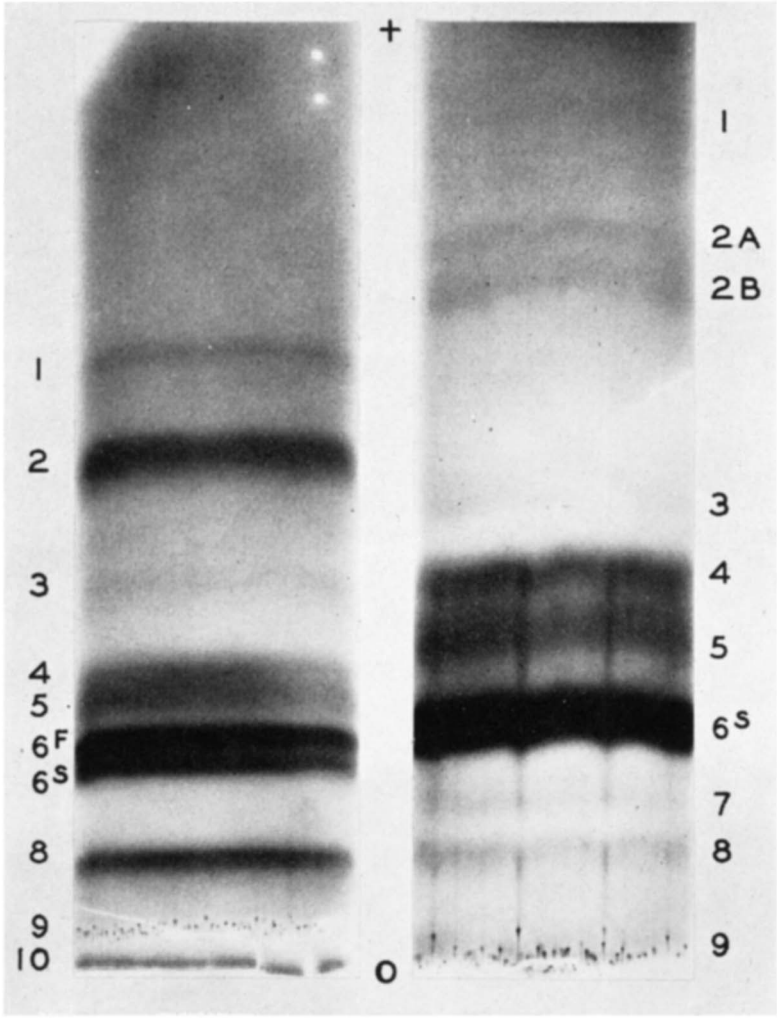


FIGURE 1.—Two zymograms of adult Drosophila esterases. Left: Multiple-fly homogenate of *Est* 6^F/*Est* 6^S heterozygotes. Right: A more concentrated multiple-fly homogenate of *Est* 6^S/*Est* 6^S homozygotes electrophoresed for a longer period of time.

hours at pH 8.7 with a voltage drop of 5 to 6 v/cm and subsequently stained for esterases with α-naphthyl acetate (Figure 1). Under these conditions all ten bands migrate toward the anode. No negatively migrating bands have been observed at pH 8.7. When a similar homogenate is run at pH 4.6, only one wide, heavily staining band, migrating slowly toward the cathode, is found. No attempt has been made to determine why only one band is found at this acid pH.

Except for single-fly runs one can usually depend on demonstrating the presence of Bands 2, 4, 5, 6, 8, 9, and 10 with little or no difficulty in all zymograms at pH 8.7. However, Bands 1, 3, and 7 do not appear consistently in all preparations (note the absence of Band 7 in the left zymogram of Figure 1). These three bands must, however, be included in the array of adult Drosophila esterases, for their presence can be regularly demonstrated if precautions are taken to prevent the elevation of the temperature of the starch blocks during electrophoresis, and if the amount of material inserted is increased beyond the optimum suitable for the resolution of Bands 4, 5, and 6. Furthermore, if the amount of material inserted is increased and the electrophoresis prolonged for an extra hour, i.e., six hours rather than five, it is possible to separate Band 2 into two distinct bands, designated as 2A and 2B (compare left and right zymograms in Figure 1). It is likely that under different conditions, e.g. different pH's, other bands will be separated into more than one component, e.g., preliminary comparisons of the zymogram patterns of third-instar larvae with adults indicate that in the adult, Band 5 is composed of two components. It is hoped that the system of nomenclature introduced here for Drosophila is sufficiently flexible to handle future refinements in resolution.

Of the bands that have been resolved, Band 9 is of interest because of its peculiar particulate appearance. Microscopic examination shows that the diazonium dye is deposited at the anodal ends of tiny tunnels in the starch. Proximal to Band 9 the starch block is quite friable, often breaking when it is sliced (compare the zymograms in Figure 1, where the slices have broken between the Origin and Band 9, with those of Figure 2, which have not broken). Although no attempts have been made to verify the following explanation, it is thought that the channels are formed by the action of an amylase which is located on some kind of particle, and that the formation of the channels permits one or more esterases, which would not normally migrate, to move to the distal ends of the channels where the diazonium dye is deposited. Strain differences in the distance that Band 9 migrates have been observed (compare CH with Am in Figure 2). While no genetic analysis of this difference has been made, it has been observed that the segregation of this difference does not appear to be linked with that of the Esterase-6 alleles (see below). However, an explanation of this apparent genetic difference may be that the Am strain is carrying the strong amylase allele (*Amy*) located on Chromosome 2 (KIKKAWA and ABE 1960; KIKKAWA 1960) whereas the CH stock may contain the weak amylase allele (*amy*). If this is the case, then the difference in the distance of Band 9 from the origin in the two strains may depend more on differential amylase activity than on a difference in electrophoretic mobility.

The esterase activity of Band 9, along with that of Bands 5 and 6, is not sensitive to high concentrations of eserine sulfate, i.e. 10^{-4} M, and, therefore, these three enzymes definitely are not cholinesterases (AUGUSTINSSON 1960). On the other hand, Bands 4 and 8 probably are cholinesterases, for they are completely inhibited by 10^{-6} M eserine sulfate. The nature of the rest of the Bands (1, 2, 3, 7, and 10) either has not been determined or remains doubtful.

Strain differences in the Esterase-6 band: Although both the CH and the Am wild-type stocks were originally derived from the Oregon-R strain, an obvious difference in their esterase patterns is apparent. The CH zymogram shows an intense 6^F band with a lighter 6^S band, while on the other hand, the Am zymogram has a very intense 6^S band with no 6^F band at all. The left zymograms in Figure 2 illustrate typical esterase patterns obtained from multiple-fly homogenates of these two strains.

When multiple-fly homogenates of the F_1 from a series of single-pair matings between the two strains were run, one of the following three patterns always appeared: A. Pattern with no 6^F band but with a very intense 6^S band, identical with the Am strain pattern; B. Pattern with a light 6^F band and an intense 6^S

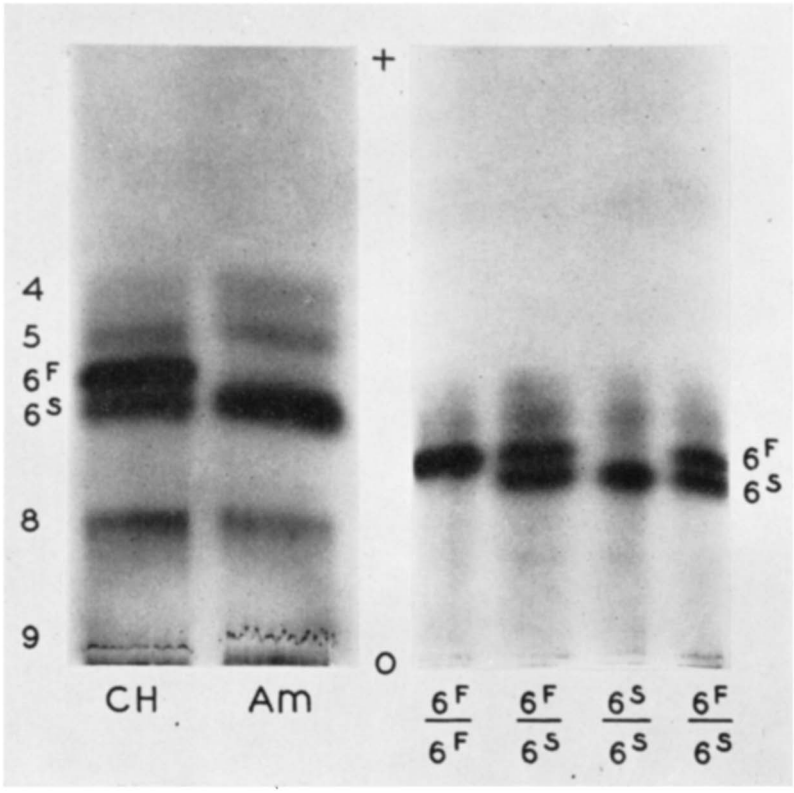


FIGURE 2.—Left: Zymograms of multiple-fly homogenates of Oregon-R-CH and Oregon-R-Amherst Inbred adults showing the different Esterase-6 patterns obtained. Right: Four single-fly zymograms showing the three possible Esterase-6 phenotypes.

band, unlike either of the parental strain patterns; C. Pattern with the two bands, 6^F and 6^S, appearing with an equal medium intensity, also unlike either parental strain pattern.

Multiple-fly homogenates of offspring from a series of single-pair matings of Am × Am invariably produced only one pattern which was identical with the Am parental strain pattern. However, a series of single-pair matings of CH × CH produced the five different patterns listed below: A. No 6^F band with a very intense 6^S band, identical to the Am strain pattern; B. Light 6^F band with an intense 6^S band, identical to F_1 pattern B above; C. Medium 6^F band equal in intensity to a medium 6^S band, identical to F_1 pattern C above; D. Intense 6^F band with a light 6^S band, similar to the CH strain pattern; E. Very intense 6^F band with no 6^S band, a completely new pattern.

If the appearance of these various zymogram patterns for the Esterase 6 Band is controlled by two alleles at a single locus, then the following deductions can be made from the above results: 1. Since Am breeds true, it must carry only one allele at the locus, with all individuals being genotypically and phenotypically identical. 2. Since CH does not breed true, it must carry two alleles at the locus. 3. If the CH strain carries two alleles in its population, then it is made up of three different genotypic classes of individuals and of at least two and possibly three phenotypic classes. 4. The appearance of the Am strain pattern in some of the Am × CH and CH × CH crosses indicates that: a. One of the two alleles in the CH strain is identical with the one allele in the Am strain; b. One of the three genotypic classes of the CH strain is genotypically and phenotypically identical with Am individuals. 5. The appearance of three different F_1 patterns among the Am × CH crosses is a result of three different crosses: the single Am genotype crossed with each of the three different CH genotypes. 6. With three genotypic classes of individuals in the CH population, there are six possible intra-strain crosses. Apparently these six crosses produce five different patterns among their offspring, indicating that two of the six crosses produce the same pattern. 7. If the CH strain is made up of three different genotypic classes, then the CH strain pattern must be a composite derived from a mixture of at least two, and very likely three, phenotypic classes.

Single-fly zymograms: Although the above deductions probably could have been confirmed by an analysis of zymograms from multiple-fly homogenates of offspring from an extensive series of single-pair matings, it was at once apparent that phenotypic classification of single flies would greatly simplify the problem. Fortunately, it was possible to develop a technique that clearly separated the 6^S and 6^F Band in zymograms of single-fly homogenates. (See MATERIALS and METHODS.) (The separation and identification of most of the other esterase bands is not very satisfactory in such single-fly runs.)

To date, regardless of their origin, hundreds of single-fly homogenates have always produced one of three different Esterase-6 zymogram patterns. These are: A. No 6^F band with a very intense 6^S band, comparable to the Am strain pattern; B. Medium 6^F band equal in intensity to a medium 6^S band, comparable with F_1

Pattern C above; C. A very intense 6^F band with no 6^S band, comparable to CH × CH Pattern E above.

Only one pattern, no 6^F band with a very intense 6^S band, was found among more than 60 single-fly homogenates from the Am strain, which is further evidence that all Am individuals are phenotypically and genotypically identical. On the other hand, all three single-fly patterns were found among 133 zymograms produced by CH flies. Of these, 11 (8.3 percent) showed only the strong 6^S band identical to the Am single-fly pattern (see deduction 4 above), 56 (42.1 percent) had only the strong 6^F band, and 66 (49.6 percent) produced both 6^S and 6^F bands in equal intensities. This supports the hypothesis that the CH strain carries two alleles which produce three different phenotypic and genotypic classes in the population.

The data reported in Table 1 are the results of single-fly runs on small samples of offspring from six single-pair matings between Am and CH, five backcrosses, and one F_1 cross in which the phenotype of each parent was determined after larvae had hatched in the cultures. A comparison of the results of the six single-pair matings between Am and CH (Crosses 102 and 103 *vs.* Cross 105 *vs.* Crosses 106, 117, and 181) clearly show that the three phenotypic classes of CH individuals are produced by three different genotypes.

Mendelian inheritance of Esterase 6: Although the data obtained from any one cross in Table 1 are meager, the results from all 12 crosses considered together permit the following genetic interpretation.

Two alleles exist at an autosomal locus that controls the structure of Esterase 6 in *Drosophila melanogaster*. One allele, Esterase 6^F (abbreviated *Est* 6^F, or 6^F, or F) is responsible for the production of a form of Esterase 6 that migrates faster than that produced by the other allele, Esterase 6^S(*Est* 6^S, 6^S, S). Single-fly zymograms of individuals homozygous for *Est* 6^F (F/F) show a single, intense, fast migrating Esterase-6 band (the 6^F Band). *Est* 6^S homozygotes (S/S) have a similar single, intense Esterase-6 band which, however, migrates more slowly (the 6^S Band) (Figure 2). Heterozygotes, *Est* 6^F/*Est* 6^S (F/S), produce single-fly zymograms with both a fast (6^F) and a slow (6^S) Esterase-6 band of equal intensity. Unlike the maize esterases (SCHWARTZ 1960), the heterozygote does not produce a hybrid form of Esterase 6 with an electrophoretic mobility intermediate between the slow and fast forms. In the heterozygote the two alleles show no dominance, i.e., they are codominant alleles, each controlling the production of a similar, but not identical, protein. As yet there is no evidence that the substitution of one Esterase-6 allele by the other in any way affects growth, differentiation, and morphogenesis; for example, no apparent morphological difference was found after a careful comparison of the two types of homozygous imagoes.

Gene dosage, filial allelic ratios, and multiple-fly homogenates: There appears to be a quantitative correlation between the intensity of an Esterase-6 band and the gene dosage responsible for its production, i.e., each gene dose produces approximately equal amounts of enzyme activity. That one dose of 6^F is equal to one dose of 6^S is shown by the equal intensity of the two bands in a $6^F/6^S$ heterozygous single-fly zymogram. Also, two doses of 6^F are equal to two doses of 6^S,

TABLE 1

Crosses made to determine the mode of inheritance of the inter-strain difference in the electrophoretic mobility of Esterase 6. (The genotypes listed in parentheses are deductions that fit the data)

	Female parent		Male parent		Offspring			
Cross no.	Source	Phenotype (genotype)	Source	Phenotype (genotype)	Band 6^F (F/F)	Bands 6^F and 6^S (F/S)	Band 6^S (S/S)	Total
102	Am	Band 6^S (S/S)	CH	Bands 6^F and 6^S (F/S)	0	4	4	8
103	Am	Band 6^S (S/S)	CH	Bands 6^F and 6^S (F/S)	0	5	11	16
105	CH	Band 6^S (S/S)	Am	Band 6^S (S/S)	0	0	12	12
106	CH	Band 6^F (F/F)	Am	Band 6^S (S/S)	0	25	0	25
$106F_1 \times F_1$	$106F_1$ ♀ ♀	Bands 6^F and 6^S (F/S) ♂ ♂	$106F_1$	Bands 6^F and 6^S (F/S)	14	25	7	46
106 Backcross	Am	Band 6^S (S/S)	$106F_1$ ♂	Bands 6^F and 6^S (F/S)	0	9	9	18
117	Am	Band 6^S (S/S)	CH	Band 6^F (F/F)	0	18	0	18
117 Backcross	$117F_1$ ♀	Bands 6^F and 6^S (F/S)	Am	Band 6^S (S/S)	0	11	9	20
117 Backcross	$117F_1$ ♀	Bands 6^F and 6^S (F/S)	CH	Band 6^F (F/F)	11	9	0	20
181	CH	Band 6^F (F/F)	Am	Band 6^S (S/S)	0	16	0	16
181 Backcross	Am	Band 6^S (S/S)	$181F_1$ ♂	Bands 6^F and 6^S (F/S)	0	10	10	20
181 Backcross	CH	Band 6^F (F/F)	$181F_1$ ♂	Bands 6^F and 6^S (F/S)	9	12	0	21

for again the two bands are of equal intensity in a double-fly zymogram made by homogenizing one $6^F/6^F$ homozygote together with one $6^S/6^S$ homozygote. Furthermore, the 6^F band produced by one dose in the single-fly zymogram of a heterozygote (F/S) is about half as dark as that produced by two doses of F in a homozygous (F/F) single-fly zymogram (the same is found for one 6^S *vs.* two 6^S). This is confirmed by comparing a double-fly zymogram made by homogenizing two heterozygotes (F/S) with another double-fly zymogram made by homogenizing two homozygotes: one a F/F homozygote and the other a S/S homozygote. In both of these double-fly zymograms two doses produce the 6^F band and two doses are responsible for the 6^S band with the result that all the Esterase-6 bands are equally dark. This correlation of gene dosage and band intensity can be applied to the analysis of multiple-fly homogenates and has provided a basis for the explanation of all the multiple-fly patterns of Esterase 6 found so far. Below are only a few examples of the application of this correlation to multiple-fly homogenates.

A 20 μl sample of each of the following three homogenates, each made with two hundred flies in one ml of buffer, will give identical patterns with the 6^F and 6^S bands equally intense: (a) 100 F/F homozygous flies with 200 doses of F alleles mixed with 100 S/S homozygous flies with 200 doses of S alleles giving an overall allelic ratio in the homogenate of 2 F:2S alleles; (b) 200 F/S heterozygotes with 200 doses of F alleles and 200 doses of S alleles again with an allelic ratio of 2 F:2 S; (c) 50 F/F homozygotes (100 doses of F) mixed with 100 F/S heterozygotes (100 doses of F and 100 doses of S), and with 50 S/S homozygotes (100 doses of S) gives a combined total of 200 doses of F and 200 doses of S, again a 2 F:2 S allelic ratio.

However, a different pattern with a light 6^F band and an intense 6^S band is obtained when 100 F/S heterozygotes (100 doses of F and 100 doses of S) are mixed with 100 S/S homozygotes (200 doses of S) giving a total of 100 doses of F and 300 doses of S or a 1 F:3 S allelic ratio. A comparison of this 1 F:3 S zymogram with the 2 F:2 S zymogram shows that the 3 S band is darker than the 2 S band, and that the 1 F band is about half as intense as the 2 F bands (Note that the allelic ratio 2 F:2 S has not been reduced to 1 F:1 S so that gene doses and band intensities can be compared between different zymograms.)

If standard conditions are used (i.e., number or weight of flies homogenized per ml of buffer, 20 μl samples of homogenate run in starch for a certain time, standard staining time for approximately equal thickness of starch slices), it is possible to predict Esterase-6 patterns for any mixture of flies with a known allelic ratio, or, on the other hand, it is possible to estimate the allelic ratio of an unknown mixture of flies by the zymogram pattern it produces. Table 2 lists all the Esterase-6 patterns that can be produced by multiple-fly homogenates of offspring from single-pair crosses involving the two known alleles at the Esterase-6 locus. This table makes it possible to interpret all the multiple-fly zymograms obtained from the initial single-pair crosses (see Table 3). Actually of all the multiple-fly zymograms run only the CH strain pattern is open to misinterpretation, for its intense 6^F and light 6^S band pattern is virtually indistinguishable from Pattern

TABLE 2

Columns 1 and 2 designate and describe the Esterase-6 zymogram patterns obtained from multiple-fly (= 100 flies) homogenates of the offspring produced by the crosses listed in Column 3, and that have the genotypic and allelic ratios listed in Columns 4 and 5

(1) Pattern number	(2) Pattern 6^F	(2) Pattern 6^S	(3) Crosses	(4) Filial genotypic ratios	(5) Filial allelic ratio 6^F : 6^S
I	None	Very intense	$S/S \times S/S$	S/S	0 : 4
II	Light	Intense	$F/S \times S/S$	$1\ F/S : 1\ S/S$	1 : 3
III	Medium	Medium	$F/F \times S/S$ $F/S \times F/S$	F/S $1\ F/F : 2\ F/S : 1\ S/S$	2 : 2
IV	Intense	Light	$F/F \times F/S$	$1\ F/F : 1\ F/S$	3 : 1
V	Very intense	None	$F/F \times F/F$	F/F	4 : 0

TABLE 3

An explanation of the various Esterase-6 patterns (Column 2) obtained from multiple-fly homogenates of single-pair inter- and intra-strain crosses. The allelic ratios that produce these patterns are in Column 4. In Columns 5 and 6 are the filial genotypic ratios and parental genotypes that yield these allelic ratios

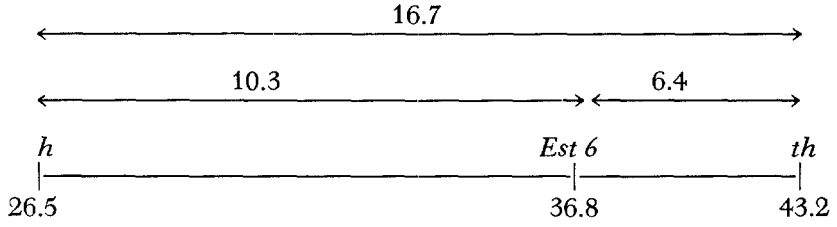
(1) Crosses	(2) Pattern obtained 6^F	(2) Pattern obtained 6^S	(3) Pattern number	(4) Allelic ratio 6^F	(4) Allelic ratio 6^S	(5) Filial genotypic ratios	(6) Parental genotypes
Am × CH	None	Very intense	I	0	4	S/S	$S/S \times S/S$
Am × CH	Light	Intense	II	1	3	$1\ F/S : 1\ S/S$	$S/S \times F/S$
Am × CH	Medium	Medium	III	2	2	F/S $1\ F/F : 2\ F/S : 1\ S/S$	$S/S \times F/S$ $F/S \times F/S$*
Am × Am	None	Very intense	I	0	4	S/S	$S/S \times S/S$
CH × CH	None	Very intense	I	0	4	S/S	$S/S \times S/S$
CH × CH	Light	Intense	II	1	3	$1\ F/S : 1\ S/S$	$S/S \times F/S$
CH × CH	Medium	Medium	III	2	2	F/S $1\ F/F : 2\ F/S : 1\ S/S$	$S/S \times F/F$ $F/S \times F/S$
CH × CH	Intense	Light	IV	3	1	$1\ F/F : 1\ F/S$	$F/F \times F/S$
CH × CH	Very intense	None	V	4	0	F/F	$F/F \times F/F$

* No evidence for the existence of Band 6^F in the Am strain eliminates this alternative. (See next line in the table.)

IV, Table 2. However, the flies that produced this pattern are not offspring from a single-pair mating but arise from a small mass transfer of parents. The CH strain genotypic ratio is .42 *F/F*:.50 *F/S*:.08 *S/S* giving an allelic ratio of 2.7 *F*: 1.3 *S*, which should produce a pattern very similar to Pattern IV.

Locus of the Esterase-6 alleles: The following two stocks from the Johns Hopkins University stock collection were used to determine the linkage group and locus of the Esterase-6 alleles: *pr cn; by; ci ey^R* and *ru h th st cu sr e^s ca*. Zymograms showed that both stocks were homozygous for the *Est 6^S* allele. Both of the above mutant strains were crossed to *Est 6^F* homozygotes to produce *Est 6^F/Est 6^S* heterozygous F_1s which were also heterozygous for the visible markers. Single-fly

zymograms of offspring from the backcross, $\frac{6^S}{6^S}$; $\frac{pr\ cn}{pr\ cn}$; $\frac{by}{by}$; $\frac{ci\ ey^R}{ci\ ey^R}$ females $\times$ $\frac{6^S}{6^F}$; $\frac{pr\ cn}{+\ +}$; $\frac{by}{+}$; $\frac{ci\ ey^R}{+\ +}$ males, demonstrated that the *Est 6* alleles are linked to the *by* (blistery, 3–48.7) locus and are, therefore, located on the third chromosome. A preliminary analysis of the recombinant offspring from the backcross, $\frac{(6^S)\ ru\ h\ th\ st\ cu\ sr\ e^s\ ca}{(6^F)\ +\ +\ +\ +\ +\ +\ +\ +}$ females $\times$ $\frac{(6^S)\ ru\ h\ th\ st\ cu\ sr\ e^s\ ca}{(6^S)\ ru\ h\ th\ st\ cu\ sr\ e^s\ ca}$ males, indicated that the *Est 6* locus is between *h* (hairy, 3–26.5) and *th* (thread, 3–43.2). This cross was then repeated and single-fly zymograms were run only on those offspring which showed that crossing over had occurred between *h* and *th*. The results (Table 4) not only confirm that the *Est 6* locus lies between *h* and *th*, but also shows that 61.7 percent of the crossovers occurred between *h* and *Est 6* and that 38.3 percent occurred between *Est 6* and *th*. Using these percentages and the loci reported in BRIDGES and BREHME (1944) for these two visible markers (*h*-26.5 and *th*-43.2) one can calculate a locus for *Est 6* at 36.8 ±.



Occurrence of Esterase-6 alleles: It is evident that the occurrence of both alleles in laboratory stocks is not uncommon, for of the 18 laboratory strains screened (Table 5), four carried both alleles in their populations. Thirteen of the 18 strains were homozygous for the *Est* 6^S allele, and only one strain, a mutant stock, was homozygous for the *Est* 6^F allele.

TABLE 4

Recombinant chromosomes recovered from the backcross,

$$\frac{\text{h}\ 6^S\ \text{th}}{+\ 6^F\ +}\ \text{females} \times \frac{\text{h}\ 6^S\ \text{th}}{\text{h}\ 6^S\ \text{th}}\ \text{males}$$

Number of recombinant chromosomes tested:	149	
Recombinants between *h* and *Est 6:*		
h 6^F +	47	
+ 6^S *th*	45	
Total	92	= 61.7%
Recombinants between *Est 6* and *th:*		
h 6^S +	26	
+ 6^F *th*	31	
Total	57	= 38.3%
Double recombinants:	0	

TABLE 5

Occurrence of Esterase-6 alleles in laboratory strains

Esterase 6^S allele only			
Wild-type strain	Source	Wild-type strain	Source
Canton-S (Inbred)	New Haven*	Oregon-R (Inbred)	Amherst§
Canton-S	Berkeley†	Oregon-R_4 (Inbred)	New Haven
Canton-S	Baltimore‡	Sevelan (Inbred)	New Haven
Cockaponsett	New Haven	Swedish-B_1 (Inbred)	New Haven
Lausanne-S	Baltimore		
Mutant stock	Source	Mutant stock	Source
ru h th st cu sr e^s ca	Baltimore	*pr cn; by; ci ey^R*	Baltimore
pr cn; by	Baltimore	*sc^{S1} B In-S w^a sc^s; SM1 Cy/Pm, Ubx/Sb*	Baltimore
Esterase 6^F allele only			
Mutant stock	Source		
y; bw; e; ci ey^R	Baltimore		
Both Esterase 6^S and 6^F alleles			
Wild-type strain	Source	Mutant stock	Source
Oregon-R-CH	New Haven	T(2:3)*Mé/ru h th st cu sr e^s Pr ca* (6^F on *Mé* chromosome; 6^S on *rucuca Pr* chromosome)	Baltimore
Samarkand	Berkeley		
Varese	Baltimore		

* Stock Collection—Dept. of Zoology, Yale University, New Haven, Conn.
† Stock Collection—Dept. of Zoology, University of California, Berkeley, Calif.
‡ Stock Collection—Dept. of Biology, The Johns Hopkins University, Baltimore, Md.
§ Stock Collection—Dept. of Biology, Amherst College, Amherst, Mass.

It is also quite clear that both alleles are present in wild populations, for both alleles were found in a small sample taken from a wild population in South Carolina. In addition, both alleles were found to be present in every one of 12 separate, small samples collected at 12 different locations in the eastern part of Baltimore County, Maryland. These collections show that two widely separated populations are polymorphic for two forms of Esterase 6 and because rather small samples were taken, that both alleles are present at relatively high frequencies.

Some properties of Esterase 6^F and Esterase 6^S: No differences in the abilities of the fast and slow forms of Esterase 6 to hydrolyze various substrates have been found. Both hydrolyze α-naphthyl acetate, β-naphthyl propionate, and α-naphthyl butyrate and show no evidence of splitting naphthyl AS acetate, β-naphthyl laurate, β-naphthyl myristate, β-naphthyl stearate, β-carbonaphthyoxy choline iodide, n-benzoyl-DL-phenylalanine-β-naphthol ester, nor n-acetyl-DL-phenylalanine-β-naphthol ester. The Esterase 6 Bands produced when β-naphthyl propionate is used as a substrate appear to be equal in intensity to those produced with α-naphthyl acetate. On the other hand, α-naphthyl butyrate is a much less

suitable substrate, for the Esterase-6 Bands with α-naphthyl butyrate appear to be less than half as dark as those obtained with α-naphthyl acetate or β-naphthyl propionate.

The fast and slow forms of Esterase 6 also show identical reactions to a series of inhibitors with only one exception. Neither is sensitive to 10^{-5} M eserine sulfate, and both are slightly inhibited by 10^{-4} M eserine sulfate. The activity of neither Esterase 6^F nor Esterase 6^S is affected by any of the following inhibitors or activators: 10^{-2} M $AgNO_3$, 10^{-3} M $Pb(NO_3)_2$, 10^{-3} M $CuSO_4$, 10^{-4} M parachloromercuribenzoate, 10^{-3} M cysteine, and 5 percent Na taurocholate. Both are completely inactivated by 10^{-4} M diethyl-p-nitrophenyl phosphate (E600, paraoxon), but when treated with 10^{-5} M, both still show a small amount of activity.

On the other hand, a differential response between the fast and slow forms to the organophosphate inhibitor, Mipafox (N N′-diisopropylphosphorodiamidic fluoride), is quite apparent. At 10^{-4} M Mipafox, the activities of both the fast and slow Esterase 6 are only slightly reduced. However at 10^{-3} M, the slow form shows no activity at all, whereas the fast is still active, although its activity is reduced to about half that of the control. Furthermore, at 10^{-2} M Mipafox, the fast form still exhibits a small amount of activity. This difference in sensitivity to Mipafox has been confirmed by obtaining similar results when both Esterase 6^F and Esterase 6^S were treated simultaneously in the same slice of starch after homogenates of heterozygotes (*Est* 6^S/*Est* 6^F) or equal mixtures of homozygotes had been subjected to electrophoresis.

Yet another difference between Esterase 6^F and Esterase 6^S was discovered when it became apparent that the fast form is more heat labile than the slow. If Esterase 6^F is heated to 50°C for five minutes either before or after separation by electrophoresis, all activity is lost. The identical treatment of Esterase 6^S results in very little loss in activity. These results also have been confirmed by treating homogenates from heterozygotes and equal mixtures of homozyote homogenates.

Thus the two genetically controlled forms of Esterase 6 not only differ in their electrophoretic mobility, but also are differentially sensitive to an organophosphate inhibitor and to heat.

Unfortunately the physiological substrate of Esterase 6 is unknown. Esterase 6 is by no means unique in this regard, for the physiological substrates of many vertebrate esterases are also unknown. This makes the classification of esterases rather arbitrary, for one is usually forced to classify them by their artificial substrate specificities and their sensitivities to various inhibitors (MYERS 1960). The properties of Esterase 6 reported above permit one to classify it as a nonspecific B-esterase (= ali esterase = organophosphate sensitive esterase). However, all the critical tests have not yet been performed to completely exclude it from A-esterases (= Arom esterases = Aryl esterases = organophosphate resistant esterase = E600 esterase = DFPase) or the C-esterases (a group of organophosphate resistant esterases which are not E600 esterases nor DFPases) (MYERS 1960; PEARSE 1960, Chapter 16).

SUMMARY

The existence of a naturally occurring polymorphism involving two forms of a nonspecific esterase has been established in *Drosophila melanogaster*. The inheritance of this protein polymorphism is controlled by a pair of codominant alleles at a locus of 36.8 ± units on the third chromosome. The two forms of the esterase appear to have similar substrate specificities but have been found to differ in electrophoretic mobility, in sensitivity to an organophosphate inhibitor, and in stability to treatment with heat.

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