VARIATIONS IN LARVAL ALKALINE PHOSPHATASE CONTROLLED BY *Aph* ALLELES IN DROSOPHILA MELANOGASTER[1]

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DURING the last few years a rather large number of protein polymorphisms have been described in different animal groups. This development is largely due to improvements in the techniques for protein separation, such as the method of starch gel electrophoresis (SMITHIES 1955, 1959). The combination of starch gel electrophoresis with different enzyme staining methods has also made it possible to study with greater efficiency electrophoretic variations in various enzymes (cf. HUNTER and MARKERT 1957). The concept of an isozyme has been defined as one of the different molecular forms of an enzyme (MARKERT and MØLLER 1959). Isozyme variations have been described in a large variety of organisms ranging from protozoans to man (cf. the papers presented at the symposium "Multiple Molecular Forms of Enzymes," 1961). In *Drosophila melanogaster* WRIGHT (1963) has described genetic variations in a nonspecific esterase. Two different electrophoretic esterase variants were found, and the inheritance of this naturally occurring protein polymorphism is controlled by a pair of codominant alleles located on the third chromosome.

This paper reports another new enzyme difference in *Drosophila melanogaster*, electrophoretic variations in the larval alkaline phosphatase (cf. also BECKMAN and JOHNSON 1964).

MATERIALS AND METHODS

Three different mutant stocks were used. They were obtained from The National Science Foundation Stock Center at The Institute for Cancer Research, Philadelphia and carry the stock list notations b 213, b 232 and b 313.

Single Drosophila individuals of different developmental stages were homogenized in a drop of distilled water and the homogenate was absorbed in a small piece of filter paper (about 4 × 6 mm). The animals were placed on ordinary microscope slides, homogenized with a lancet, and thereafter the drop was covered with a small piece of single layer Kleenex (Celluwipes, Kimberly-Clark Corp.). The moisture of the homogenate was then absorbed into the filterpaper through the thin Kleenex paper, thereby filtering out tissue fragments. We have found this to be a simple and efficient way of avoiding streaking of the enzyme bands in the following electrophoresis without centrifuging the homogenate. The filterpapers with the absorbed samples were inserted in a buffered starch gel (thickness 6 mm) and the electrophoresis was performed at room temperature for about 3 hr at a voltage gradient of 6 to 8 v/cm. The discontinuous buffer system described by POULIK (1957) was used throughout this study. Some results were controlled using another type of discontinuous buffer system (cf. BECKMAN and HOLMGREN

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1961). After the electrophoresis the gels were sliced horizontally and stained for alkaline phosphatase using the following stain solution:

100 ml Tris buffer, pH 8.65 (same buffer as the one used in the gel; cf. POULIK 1957)
100 mg Sodium alpha-naphthyl phosphate
100 mg Blue RR salt
10 drops of 10 percent solution of magnesium chloride
10 drops of 10 percent solution of manganese chloride
0.5 g Polyvinylpyrrolidone
2 g Sodium chloride

The larvae of *Drosophila melanogaster* are strong enzyme donators and incubation for 20 to 30 min is usually sufficient to bring out clearly the darkly staining alkaline phosphatase bands. In linkage studies involving the Esterase-6 marker (WRIGHT 1963), one slice of the gel was stained for alkaline phosphatase and the other for esterase.

RESULTS AND DISCUSSION

Electrophoretic variations of alkaline phosphatase zones: The electrophoretic phosphatase patterns vary among the different developmental stages (see Figure 1). In young larvae, up to the 2nd instar, there was practically no alkaline phosphatase activity except for a fast moving faintly staining single band. From about the 3rd instar stage there is a very strong phosphatase band which disappears at the onset of the pupation. In pupae, a phosphatase component is found that has a considerably faster mobility than that of the 3rd instar larvae. In homogenates from adult flies, we have not been able to detect any phosphatase activity when performing the electrophoresis and staining in the Poulik buffer. When staining at a more acid pH the phosphatase bands described above in larvae and pupae do not appear, hence they may be classified as alkaline phosphatases.

The electrophoretic mobility of the larval alkaline phosphatase band showed variations between different laboratory stocks. Three different electrophoretic variants were observed (see Figure 1), one fast moving zone, here called F, one slow moving zone (S), and finally a zone of intermediate mobility (H). Zone H was wider than the F and S zones, and was often accompanied by a faint slow band (see Figure 1, sample 3).

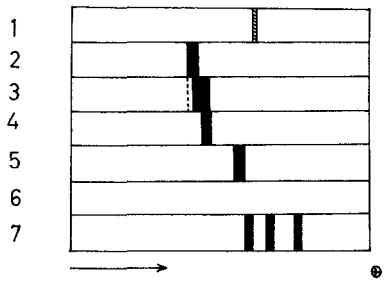

FIGURE 1.—Schematic picture showing the variations in alkaline phosphatase pattern between developmental stages. 1 = young larva, 2 to 4 = 3rd instar larvae, 5 = pupa, and 6 = adult. 7 shows the three main protein components seen in homogenates of individuals from all stages after staining of the gels with amidoblack. Samples 2, 3 and 4 show the larval alkaline phosphatase variants S, H and F respectively.

Inheritance of the larval alkaline phosphatases: In the laboratory stocks b 232 and b 213, only the fast larval alkaline phosphatase component was found, while in stock b 313 all three types were present. From b 313 a strain of S individuals was isolated by testing the larval offspring from single pair matings. These S individuals from stock b 313 were used in crosses with the "fast" strains b 232 and b 213 in order to determine the inheritance of the larval alkaline phosphatase variants. The results from the crosses are given in Table 1, and in Table 2 the F_1, F_2 and backcross data have been summarized. These assembled results show that the variations in the larval alkaline phosphatase are controlled by two codominant autosomal alleles. One allele (Aph^F) controls the synthesis of the fast moving alkaline phosphatase variant, and the other allele (Aph^S) is responsible for the production of the slow variant. Individuals homozygous for the Aph^F allele (F/F) show the fast variant and individuals homozygous for Aph^S (S/S) have the slow component only. Heterozygotes (F/S) produce a pattern that has a component of intermediate mobility (the H phenotype).

TABLE 1

Crosses made to demonstrate the mode of inheritance of the larval alkaline phosphatase variants. Genotypes within brackets are deductions fitting the results

	Female parent		Male parent		Offspring: Phosphatase pattern F (*F/F*)	H (*F/S*)	S (*S/S*)	Total
Cross	Source	Genotype	Source	Genotype				
A	b 313	*(S/S)*	b 232	*(F/F)*	0	14	0	14
A	b 232	*(F/F)*	b 313	*(S/S)*	0	20	0	20
D	b 313	*(S/S)*	b 213	*(F/F)*	0	14	0	14
D	b 213	*(F/F)*	b 313	*(S/S)*	0	7	0	7
	AF_1	*(F/S)*	AF_1	*(F/S)*	7	20	9	36
	DF_1	*(F/S)*	DF_1	*(F/S)*	20	37	21	78
	AF_1	*(F/S)*	b 232	*(F/F)*	29	18	0	47
	b 232	*(F/F)*	AF_1	*(F/S)*	10	9	0	19
	AF_1	*(F/S)*	b 313	*(S/S)*	0	10	7	17
	b 313	*(S/S)*	AF_1	*(F/S)*	0	10	11	21
	DF_1	*(F/S)*	b 213	*(F/F)*	16	28	0	44
	DF_1	*(F/S)*	b 313	*(S/S)*	0	8	15	23

TABLE 2

Summary of the F_1, F_2 and backcross results listed in Table 1

Parental combinations	Offspring: *F/F*	*F/S*	*S/S*	Total
$F/F \times S/S$	0	55	0	55
$F/S \times F/S$	27	57	30	114
$F/S \times F/F$	55	55	0	110
$F/S \times S/S$	0	28	33	61

The electrophoretic pattern of the heterozygote: When using the buffer system by POULIK (1957) the differences in electrophoretic mobility are rather small. The hybrid pattern usually appears as a wide zone of intermediate mobility (see Figure 2). Sometimes a slow faint band can be seen in addition to the wide band. When using another discontinuous Tris buffer system (cf. BECKMAN and HOLMGREN 1961) the separation of the different phosphatase bands in the heterozygous pattern was improved, and the wide phosphatase zone of the heterozygotes could be separated into two zones (see Figure 3). Thus in the heterozygotes there are three different forms of alkaline phosphatase. One of them has the same mobility as the fast (*F*) variant, another one coincides with the slow (*S*) variant and finally there is a variant with an electrophoretic mobility intermediate between the fast and slow variants. The component with intermediate mobility is apparently a hybrid form of alkaline phosphatase. The concentrations of the parental enzymes in relation to the hybrid enzyme show some variations between different crosses. In some individual samples showing a faint stain, only the intermediate component may be clearly visible. Hybrid forms of enzymes have previously been described by SCHWARTZ (1960) in maize esterases, where the heterozygotes have an electrophoretic pattern showing both the parental enzymes and the hybrid substance. Among the inherited electrophoretic protein variations described in various organisms, hybrid substances do not occur frequently. The formation of

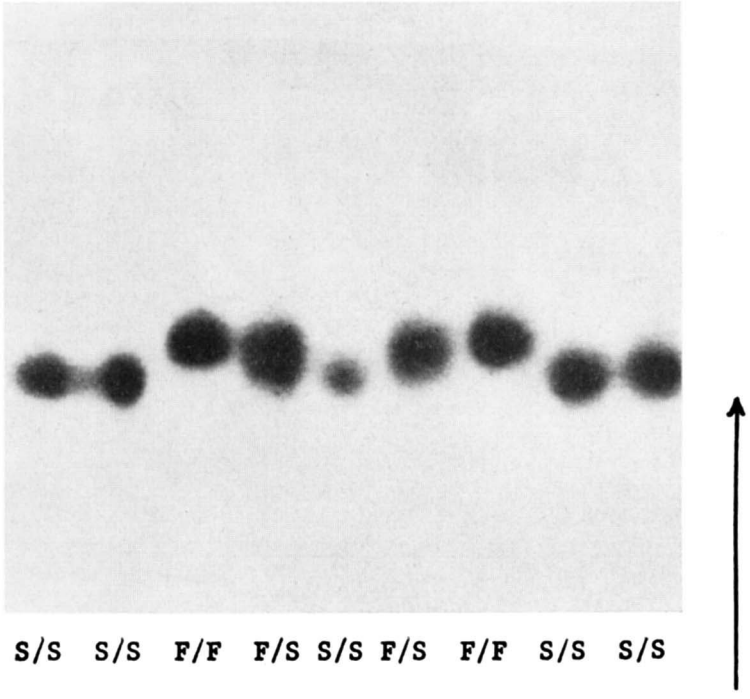

FIGURE 2.—Photograph of starch gel showing the larval alkaline phosphatase patterns *S/S*, *F/F* and *F/S* (the heterozygote). The arrow shows the direction of migration. Enlargement 2/1.

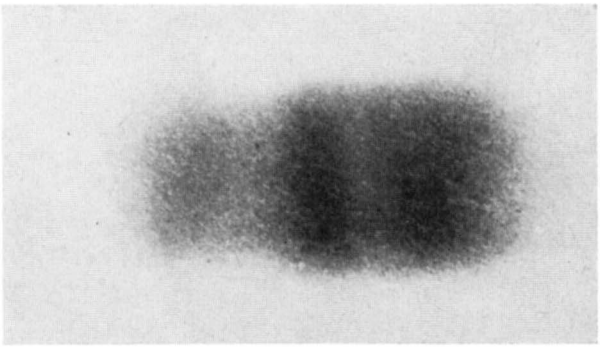

FIGURE 3.—Photograph showing the three different enzyme bands found in the hybrid zymogram pattern. F = fast enzyme variant, S = slow enzyme variant and H = hybrid enzyme. In this particular heterozygote, the fast component has a rather high concentration. The high degree of diffusibility makes the enzyme bands blurred and it is very difficult to obtain good photographs.

hybrid molecules in maize esterases (SCHWARTZ 1960) and human serum haptoglobins (SMITHIES 1955) seem to be associated with protein polymerization. The most likely explanation of our results concerning the larval alkaline phosphatase variations seem to be that the phosphatase enzymes exist as dimers and that the hybrid pattern is a result of the combination of two different types of enzyme monomers.

Linkage group of the Aph *alleles:* The Esterase 6 enzymes (WRIGHT 1963) can be seen also in the larval stage and we have used this marker to test the recombination between the *Aph* and *Est 6* (3–36.8) loci. The results given in Table 3 show that the *Aph* and *Est 6* genes are linked and thus that the *Aph* alleles are located on the third chromosome.

Finally it can be said, although no extensive studies of the occurrence of the *Aph* alleles have been performed, that both alleles seem to be common among laboratory stocks, for in different stocks obtained from the National Science Foundation Stock Center, Philadelphia, we have found those with only the Aph^F allele, others with only the Aph^S allele, and finally some with both alleles.

SUMMARY

An electrophoretic variation in the larval alkaline phosphatase of *Drosophila melanogaster* has been found. The inheritance of this enzyme variation is controlled by a pair of codominant alleles. The locus (*Aph* = alkaline phosphatase) was found to be on the third chromosome. Heterozygotes show in addition to the parental enzymes a hybrid enzyme of intermediate electrophoretic mobility.

TABLE 3

Recombinations between Aph *and* Est 6

Backcross		Offspring			
Female parent	Male parent	*Aph*	*Est 6*	Number	Recombinants
$\frac{Aph^F\ Est\ 6^S}{Aph^S\ Est\ 6^F}$	$\times \frac{Aph^F\ Est\ 6^S}{Aph^F\ Est\ 6^S}$	$\frac{F}{F}$	$\frac{S}{S}$	17	14.9%
		$\frac{S}{F}$	$\frac{F}{S}$	23	
		$\frac{F}{F}$	$\frac{F}{S}$	4	
		$\frac{S}{F}$	$\frac{S}{S}$	3	
$\frac{Aph^F\ Est\ 6^S}{Aph^F\ Est\ 6^S}$	$\times \frac{Aph^F\ Est\ 6^S}{Aph^S\ Est\ 6^F}$	$\frac{F}{F}$	$\frac{S}{S}$	11	0.0%
		$\frac{F}{S}$	$\frac{S}{F}$	8	
		$\frac{F}{F}$	$\frac{S}{F}$	0	
		$\frac{F}{S}$	$\frac{S}{S}$	0	
$\frac{Aph^F\ Est\ 6^S}{Aph^S\ Est\ 6^F}$	$\times \frac{Aph^S\ Est\ 6^F}{Aph^S\ Est\ 6^F}$	$\frac{F}{S}$	$\frac{S}{F}$	7	29.4%
		$\frac{S}{S}$	$\frac{F}{F}$	5	
		$\frac{F}{S}$	$\frac{F}{F}$	3	
		$\frac{S}{S}$	$\frac{S}{F}$	2	
$\frac{Aph^S\ Est\ 6^F}{Aph^S\ Est\ 6^F}$	$\times \frac{Aph^F\ Est\ 6^S}{Aph^S\ Est\ 6^F}$	$\frac{S}{F}$	$\frac{F}{S}$	10	0.0%
		$\frac{S}{S}$	$\frac{F}{F}$	11	
		$\frac{S}{F}$	$\frac{F}{F}$	0	
		$\frac{S}{S}$	$\frac{F}{S}$	0	

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