THE GENETICS OF AN ACID PHOSPHATASE IN *DROSOPHILA MELANOGASTER* AND *DROSOPHILA SIMULANS*[1]

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UNTIL very recently, genes whose effects are described at the protein level have been unknown in Drosophila. Gel electrophoresis, however, has remedied this situation. Within the past few years, the following gene-enzyme systems have been elucidated by means of this technique: Amylase (KIKKAWA 1964), Esterase 6 (WRIGHT 1963), Esterase C (BECKMAN and JOHNSON 1964a), Glucose-6-phosphate dehydrogenase (YOUNG, PORTER and CHILDS 1964), Alcohol dehydrogenase (JOHNSON and DENNISTON 1964; GRELL, JACOBSON and MURPHY 1965), Alkaline phosphatase (BECKMAN and JOHNSON 1964b), Leucine aminopeptidases A and D (BECKMAN and JOHNSON 1964c), and 6-phosphogluconic acid dehydrogenase (KAZAZIAN, YOUNG, and CHILDS 1965).

Electrophoretic techniques are being used in solving fundamental problems unique to higher organisms. For example, it is now feasible to study linkage relationships and controlling mechanisms of genes whose enzymes catalyze sequential biochemical reactions in higher organisms. In evolutionary genetics, too, questions concerning the evolution of genes and the existence of biochemical polymorphisms are now open to experimental analysis (see WRIGHT and MACINTYRE 1963; HUBBY and THROCKMORTON 1965).

The present paper reports the discovery of still another gene-enzyme system, an acid phosphatase, in the two sibling species, *D. melanogaster* and *D. simulans*. It also provides evidence that the responsible genes in the two species are homologous, even though at this time no allele is known to be shared by both.

MATERIALS AND METHODS

Electrophoretic procedures: Methods used in preparing gels, homogenizing and inserting flies into the gels, and electrophoresis are described in WRIGHT (1963). In the present investigation, two continuous buffer systems were employed. The Tris-hydrochloric acid system is the same as that of WRIGHT (1963). The Tris-EDTA (ethylenediaminetetraacetic acid)-boric acid system consists of two solutions. The gel buffer is composed of a mixture of 0.9 M Tris, 0.02 M EDTA and 0.5 M boric acid in equal parts. For each 120 ml of buffer, 1.0 g of starch is routinely added to the amount recommended by the manufacturer in order to give the gel more mechanical strength. The final molarity of the gel buffer is adjusted to 0.05 before preparing the starch. The buffer in the electrode chambers contains equal parts of the following solutions: 0.054 M Tris, 0.012 M EDTA and 0.03 M boric acid. The pH of both gel and electrode chamber buffers should

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be between 8.4 and 8.6. The third continuous buffer system consists of Tris and boric acid alone. A solution of two parts of 0.25 M Tris to one part of 0.25 M boric acid is used in the electrode chambers. The pH of this buffer is 8.6. The starch is prepared in this same solution diluted to 0.05 M.

The histochemical stain used to localize the acid phosphatase in the starch gel after electrophoresis has been described by LAWRENCE, MELNICK and WEIMER (1960). The dye used in the present study is Fast Blue BB (Nutritional Biochemical Corp.) at a concentration of 2 mg/ml. At room temperature the stain solution is stable for 6 to 8 hours, and the bands generally appear after 4 hours. The appearance of the bands can be speeded up in two ways. First, if the gels are preincubated in acetate buffer at pH 5.0 for 15 to 20 minutes and then stained, the bands can be seen after 1 to 2 hours. Second, if the gels are incubated in a stain solution at 37°C, the bands become visible within 2 hours.

Sources of the enzyme: Single 2nd instar larvae, 3rd instar larvae, pupae and adults of both *D. melanogaster* and *D. simulans* are good enzyme donors. Individual flies at any of these stages can be easily classified as to their acid phosphatase phenotypes. In general, however, pupae seem to have the highest amount of enzyme activity of all developmental stages. There appears to be no difference between the zymograms of adult males and females. Occasionally, the electrophoretically "slowest" band of *D. melanogaster* appears very diffuse if electrophoresis is not prolonged. This results from an amylase acting on the starch gel and interfering with the appearance of enzymes which remain near the origin (see WRIGHT 1963).

Stocks used in the investigation: Wild-type stocks of *D. melanogaster* from scattered geographic locations were sampled to determine their characteristic acid phosphatase phenotypes. These locations were Louisiana, Oregon, California, New York (two strains recently collected from wild populations by POLIVANOV 1964), South Africa and Israel. Several stocks of *D. simulans* from the following areas were sampled: Guatemala, Alabama, California and Louisiana. A characteristic phenotype was assigned to each strain on the basis of no fewer than 12 individuals separately analyzed.

In order to determine the mode of inheritance of the electrophoretic variants of the enzyme and to map the locus or loci responsible, it is necessary to have wild-type and genetically marked stocks both of which are monomorphic for the different variants. Such monomorphic stocks were obtained from single-pair matings. In each case, after it was apparent that the mating was successful, the parents were analyzed together with suitable "control" flies (i.e. flies of known phenotype.). Furthermore, the acid phosphatase phenotypes of at least six offspring from each pair mating were determined. If the parents and the offspring all showed the same acid phosphatase phenotype, the stock initiated from the single-pair mating was considered monomorphic. Thus, from *D. melanogaster*, a stock monomorphic for one variant was derived from the Commack, New York Stock. Mutant stocks monomorphic for the other variant are *Ins(2L+2R)Cy*, *al² Cy sp²/al dp b pr cn c px sp* (2nd chromosome), *h th st ss, ru h th st cu sr e⁸ ca*, and *ca bv* (all 3rd chromosome recessive mutant alleles). In *D. simulans*, the monomorphic wild-type strain was derived from the Guatemala stock. The alternative monomorphic, genetically marked stocks are *dh b py sd pm* (2nd chromosome recessive mutant alleles) and *jv st pe* (3rd chromosome recessive mutant alleles).

RESULTS

Acid phosphatase phenotypes of D. melanogaster and D. simulans: Electrophoretic variants, as they appear in starch gels prepared in the Tris-hydrochloric acid buffer, can be grouped in both species into three patterns. There are two very intense single band patterns (one "fast," the other "slow") and a third pattern consisting of somewhat paler "fast" and "slow" bands together with a heavier band midway between them. The third pattern has been called the "hybrid" pattern since it is characteristic of the F_1 offspring of a "fast" × "slow" mating. Figure 1 shows the three patterns of both species. It has been demon-

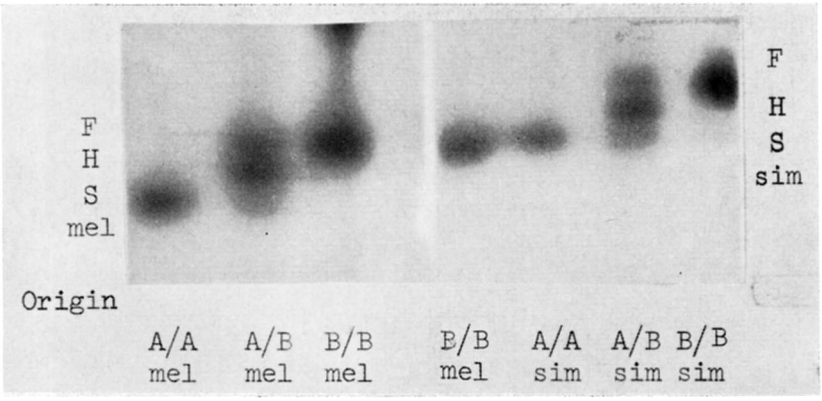

FIGURE 1.—Zymogram of the three phenotypic patterns of the acid phosphatase found in *D. melanogaster* and *D. simulans*. Starch prepared in Tris-hydrochloric acid, 0.05 M, pH 8.6. "F", "H" and "S" designate the positions of the "fast", "hybrid" and "slow" bands respectively. Directly below each of the patterns is the genotype responsible for its formation. "A" stands for the *Acph-1*^{A} allele; "B" for the *Acph-1*^{B} allele.

strated that the enzymes of *D. simulans* migrate farther than those of *D. melanogaster*. This is indicated on the diagram of Figure 5 (compare, for example, Numbers 1 to 3 with 4 to 6) which schematically represents the positions of the various bands. When "slow" individuals of *D. simulans* are electrophoresed next to "fast" *D. melanogaster* flies, the *simulans* enzyme migrates slightly faster than the "fast" *melanogaster* phosphatase.

Figure 2A is a zymogram of "fast" and "slow" individuals of both species in starch prepared in the Tris-EDTA-boric acid buffer. Enzymes of *D. melanogaster*, when exposed to the same voltage drop for the same length of time in starch of this and the Tris-hydrochloric acid buffer migrate equivalent distances from the origin. This is not true of the phosphatases of *D. simulans;* "fast," "hybrid" and "slow" enzymes of this species do not migrate as far in the Tris-EDTA-boric acid buffer system. In the Tris-EDTA-boric acid system, the "slow" band of *D.*

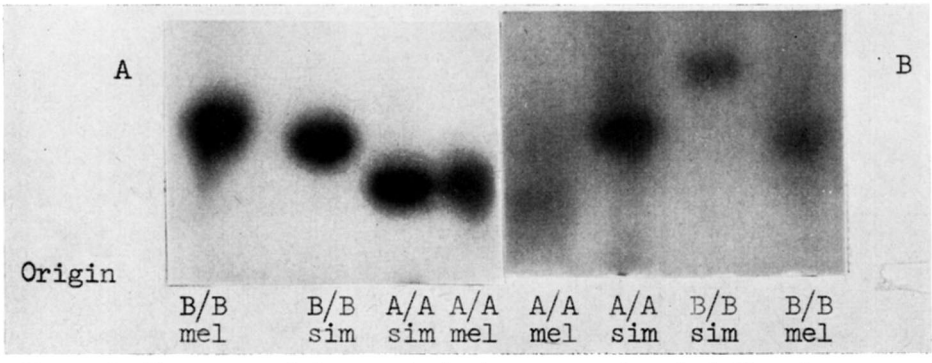

FIGURE 2.—Zymograms of the two *Acph-1* homozygotes from both *D. melanogaster* and *D. simulans* in (A) starch prepared in Tris-EDTA-boric acid buffer, 0.05 M, pH 8.6, and (B) in starch prepared in Tris-boric acid buffer, 0.05 M, pH 8.6.

simulans apparently has the same mobility as the "slow" band of *D. melanogaster*. The "fast" acid phosphatase of *D. simulans* migrates at a rate intermediate to those of the "fast" and "slow" enzymes of *D. melanogaster*. The intermediate rate of migration can also be demonstrated by homogenizing a "fast" *D. simulans* individual together with either a "fast"or "slow" adult of *D. melanogaster*: The resultant zymograms of these mixtures in Tris-EDTA-boric acid gels clearly show two bands and demonstrate, therefore, that the enzymes occupy different positions.

The reduced rates of migration of these acid phosphatases from *D. simulans* in starch prepared in the Tris-EDTA-boric acid buffer appear to be due to the effect of the EDTA alone. Figure 2B shows the positions of the "fast" and "slow" enzymes of each species in starch made in a Tris-borate buffer not containing EDTA. In this buffer system, the relative electrophoretic mobilities of the acid phosphatases of the two species are very probably the same as in Tris-hydrochloric acid starch.

The "slow" variant of the acid phosphatase in *D. melanogaster* was found in a single stock, the polymorphic wild-type strain from Commack, New York. All the other wild-type stocks and 20 mutant stocks analyzed contained only the "fast" form of the enzyme. On the other hand, only "slow" variants of *D. simulans* were found in both Guatemala and Alabama wild-type stocks. Monomorphic "fast" stocks of this species are from Morro Bay, California and Louisiana. The New Orleans, Louisiana strain is polymorphic for all three enzyme patterns. All the stocks of both species were analyzed in both buffer systems. In no instance did enzymes from a *D. melanogaster* stock behave like those of *D. simulans* in gels prepared in Tris-EDTA-boric acid buffer. Conversely, none of the enzymes of *D. simulans* stocks failed to migrate at a slower rate in the Tris-EDTA-boric acid system.

Genetics of the electrophoretic variants: All zymograms used in the genetic analyses were made in Tris-hydrochloric acid starch. Data collected from crosses between lines monomorphic for both "fast" and "slow" variants in each species are summarized in Table 1. Both male and female F_1 offspring from the initial intercrosses invariably contained the three enzymes of the "hybrid" pattern. Backcross and F_2 progeny fell into two or three phenotypic classes in both species in numbers statistically consistent with a monogenic mode of inheritance. Thus, in both species, individuals exhibiting the single band pattern are homozygotes. The "hybrid" pattern, then, is characteristic of heterozygotes. The gene has been named *Acid phosphatase-1*, symbolized *Acph-1*. Alleles responsible for "slow" and "fast" enzymes are designated as $Acph\text{-}1^A$ (*melanogaster* or *simulans*) and $Acph\text{-}1^B$ (*melanogaster* or *simulans*) respectively.

"Acph", of course, stands for "acid phosphatase" and, consequently, conveys some information about the phenotype. The pH optimum of this enzyme definitely would appear to be below 7. This is indicated by its absence in zymograms of alkaline phosphatases of *Drosophila melanogaster* (see BECKMAN and JOHNSON 1964b). In addition, when the pH of the gel is lowered by preincubation in acetate buffer at pH 5.0, the bands appear much more rapidly than if such preincubation

TABLE 1

Crosses made to determine the mode of inheritance of the acid phosphatase variants of D. melanogaster *and* D. simulans

Cross	Female parent	Male parent	Acid phosphatase phenotype of offspring		
			Slow (A/A)	Fast, Slow (A/B)	Fast (B/B)
D. melanogaster					
1.	Commack, N.Y. (*A/A*)	*h th st ss* (*B/B*)	0	9 female	0
			0	9 male	0
2.	F_1 (*A/B*)	*h th st ss* (*B/B*)	0	25	23
3.	F_1 (*A/B*)	Commack (*A/A*)	21	27	0
4.	F_1 (*A/B*)	F_1 (*A/B*)	38	57	33
D. simulans					
1.	Guatemala (*A/A*)	*jv st pe* (*B/B*)	0	9 female	0
			0	9 male	0
2.	F_1 (*A/B*)	*jv st pe* (*B/B*)	0	36	24
3.	F_1 (*A/B*)	Guatemala (*A/A*)	23	33	0
4.	F_1 (*A/B*)	F_1 (*A/B*)	31	64	25

Deduced genotypes are in parentheses. "*A*" and "*B*" stand for the *Acph-1*^{A} and *Acph-1*^{B} alleles of each species.

is omitted. However, the exact pH optima of the various forms of this enzyme remain to be determined. Acid phosphatases are a rather heterogeneous group of enzymes but can generally be characterized by several properties in addition to a pH optimum below 7 (BURSTONE 1962). In all tests made so far, the Drosophila enzyme reported here exhibits these characteristic properties. Thus, it is not inhibited by citrate or EDTA but is completely inhibited by 0.01 M sodium fluoride (E. SENA, personal communication).

"Acph" also distinguishes the present gene from that controlling an alkaline phosphatase which was labeled "Aph" by BECKMAN and JOHNSON (1964b). It is to be expected, in view of the nonspecific methods of enzyme detection generally employed in studies such as this, that more acid phosphatase gene-enzyme systems will be discovered in Drosophila. For this reason, the first acid phosphatase to be described is designated as "Acid phosphatase-1" so that subsequent systems can be easily fitted into an already existent scheme of nomenclature.

The order of discovery is also used as a basis for naming the alleles; thus, *Acph-1*^{A} and *Acph-1*^{B} are the first two alleles to be discovered in both species. BRAEND (1965), in considering problems of nomenclature posed by systems in which electrophoretic mobility is the phenotype, has suggested that alleles should be given letter designations ranging from "F" to "S" with "F" and "S" being the alleles whose protein products have the "fastest" and "slowest" rates of migration respectively. This system is inadequate for a number of reasons. First, this scheme of alleles, in order to be consistent, would have to be revised every time an allele was found whose protein product migrated "faster" than that of the original "fast" allele or "slower" than that of the "slow." Secondly, there is no guarantee that relative migration rates of the protein products of a

set of multiple alleles will be the same in every gel and/or buffer system (e.g. compare the zymograms of Figures 1 and 2). This could force an investigator to discuss his results in descriptive terms not consistent with his own photographic or diagrammatic evidence. Finally, alleles may also be defined by tests supplementing electrophoretic mobility. For example enzymes with the same activities and rates of migration in gel systems may differ in heat stability or sensitivity to various inhibitors (see WRIGHT and MACINTYRE 1965). In the genetic analysis, cumbersome additions would have to be made to the original allele designations to once again preserve the consistency of the scheme of nomenclature. In summary, it seems that the confusion which may result from adherence to a phenotypic scheme in naming alleles can be avoided by designating the alleles according to their order of discovery, as has been done in the present study.

The presence of an intermediate or "hybrid" band together with both "parental" bands in the pattern of the heterozygote suggests that the molecular form of the acid phosphatase-1 enzyme is at least a dimer in both species. Tests have shown that the "hybrid" band is not an artifact. *Acph-1*^{A} and *Acph-1*^{B} homozygotes were homogenized together, and the mixture was allowed to stand for as long as 24 hours at 4°C. The mixed homogenate was then electrophoresed. In each test, only the parental or typically "homozygote" bands appeared. The absence of an intermediate or "hybrid" band suggests that the hybrid enzyme is formed *in vivo*. The general appearance of the heterozygote zymogram (see especially Figure 3A) also suggests, assuming that each allele forms a polypeptide subunit of the enzyme, that the subunits are combined at random. Thus, the subunits appear to exist in a 1:2:1 pattern of parental:hybrid:parental enzymes in the *Acph-1* heterozygote. Work is underway to test this hypothesis by quantitative tests.

In electrophoretically detected gene-protein systems of Drosophila, heterozygous phenotypes fall into two classes. In one class, only the two parental bands are seen in zymograms and these are generally of about equal intensity. Examples of this class can be found in the following systems: Esterase 6 (WRIGHT 1963), Protein-1 (HUBBY 1963), Glucose-6-phosphate dehydrogenase (YOUNG *et al.* 1964), Amylase (KIKKAWA 1964), Esterase C (BECKMAN and JOHNSON 1964a) and Leucine aminopeptidase D (BECKMAN and JOHNSON 1964c). In the other class, a more intense intermediate or "hybrid" band is found in addition to the two parental or "homozygote" bands. The *Acph-1* system is of this sort. Other systems of this kind are: Alcohol dehydrogenase (JOHNSTON and DENNISTON 1964; GRELL *et al.* 1965), Alkaline phosphatase (BECKMAN and JOHNSON 1964b), Xanthine dehydrogenase (GLASSMAN 1965), 6-phosphogluconate dehydrogenase (KAZAZIAN *et al.* 1965) and Isocitrate dehydrogenase (W. J. YOUNG, personal communication) and, probably, Leucine aminopeptidase A (BECKMAN and JOHNSON 1964c).

Assuming that the acid phosphatase exists at least as a dimer, the various electrophoretically distinct enzymes can now be named. Thus, in each species, the "fast" enzyme will be designated Acid phosphatase-1^{BB}, Acph-1^{BB} or simply, BB (since it is thought to be composed of two subunits specified by the *Acph-1*^{B}

allele). Then, the "hybrid" enzymes are Acid phosphatase-1^{AB}, Acph-1^{AB} or AB, and the "slow" forms, Acid phosphatase-1^{AA}, or Acph-1^{AA} or AA. When discussing the enzymes of interspecific hybrids, the subunits derived from the alleles of each species will be designated with the appropriate superscripts. Thus A^m and B^m will refer to the A and B subunits specified by *Acph-1*^{A} and *Acph-1*^{B} alleles from *D. melanogaster*. Also, A^s and B^s will designate subunits derived from the *Acph-1*^{A} and *Acph-1*^{B} alleles of *D. simulans*.

Localization of the Acph-1 *genes:* In both species, crosses were made between homozygous *Acph-1*^{A} wild-type and homozygous *Acph-1*^{B} flies carrying various 2nd and 3rd chromosome mutant genes. The data in Tables 2 and 3 summarize the results from several backcrosses of the resultant *Acph-1* heterozygotes and the mutant stocks. That male heterozygotes are routinely obtained rules out sex-linkage for the *Acph-1* locus. Crosses 1 and 2 of Table 2 clearly show that the *Acph-1* gene segregates with mutant genes on Chromosome 3 in *D. melanogaster*. The analysis of crossover chromosomes from Cross 3 involving the *ru, h, th, st, cu, sr, e*^{s}, and *ca* mutant alleles indicates that the Acid phosphatase-1 locus is near the end of the right arm of the chromosome, probably to the right of *ca* (claret eye color, 100.7). Results from Cross 4 place the *Acph-1* gene between *ca* and *bv* (brevis, 104.3); from the 101 crossover chromosomes analyzed, its locus is at

TABLE 2

Crosses made in locating the Acph-1 *gene in* D. melanogaster

Cross	Female parent	Male parent	Offspring: Genotype—mutant genes	Acph-1 A/B	Acph-1 B/B
1.	*h th st ss* (*B*) / *h th st ss* (*B*)	+ + + + (*A*) / *h th st ss* (*B*)	*h th st ss* / *h th st ss*	0	12
			h th st ss / + + + +	12	0
2.	*Cy (In2LR)* (*B*) / *al dp b pr cn c px sp* (*B*)	+ + + + + + + + + (*A*) / *al dp b pr cn c px sp* (*B*)	*al dp b pr cn c px sp* / *al dp b pr cn c px sp*	7	5
			al dp b pr cn c px sp / + + + + + + + + +	5	7
3.	+ + + + + + + + + (*A*) / *ru h th st cu sr e*^{s} *ca* (*B*)	*ru h th st cu sr e*^{s} *ca* (*B*) / *ru h th st cu sr e*^{s} *ca* (*B*)	*ru* + + + + + + + + / *ru h th st cu sr e*^{s} *ca*	7	1
			ru h + + + + + + + / *ru h th st cu sr e*^{s} *ca*	6	0
			ru h th st cu sr e^s + / *ru h th st cu sr e*^{s} *ca*	4	0
			+ + + + + + + *ca* / *ru h th st cu sr e*^{s} *ca*	0	4
4.	+ + (*A*) / *ca bv* (*B*)	*ca bv* (*B*) / *ca bv* (*B*)	*ca* + / *ca bv*	13	51
			+ *bv* / *ca bv*	30	7

"*A*" and "*B*" stand for *Acph-1*^{A} and *Acph-1*^{B} respectively

TABLE 3

Crosses made in locating the Acph-1 *gene in* D. simulans

Cross	Female parent	Male parent	Offspring: Genotype—mutant genes	Offspring: Acph-1 A/B	Offspring: Acph-1 B/B
1.	$\frac{jv\ st\ pe\ (B)}{jv\ st\ pe\ (B)}$	$\frac{+\ +\ +\ (A)}{jv\ st\ pe\ (B)}$	$\frac{jv\ st\ pe}{jv\ st\ pe}$	0	12
			$\frac{+\ +\ +}{jv\ st\ pe}$	12	0
2.	$\frac{+\ +\ +\ (A)}{jv\ st\ pe\ (B)}$	$\frac{jv\ st\ pe\ (B)}{jv\ st\ pe\ (B)}$	$\frac{jv\ +\ +}{jv\ st\ pe}$	27	13
			$\frac{jv\ st\ +}{jv\ st\ pe}$	30	10
3.	$\frac{+\ +\ +\ (A)}{jv\ st\ pe\ (B)}$ virgins	$\frac{jv\ st\ pe\ (B)}{jv\ st\ pe\ (B)}$	$\frac{+\ +\ +}{jv\ st\ pe}$	125	64
			$\frac{jv\ st\ pe}{jv\ st\ pe}$	46	146

"*A*" and "*B*" stand for the *Acph-1*^{A} and *Acph-1*^{B} alleles respectively. Cross 3 was conducted following the standard procedures given in BRIDGES and BREHME (1944). The samples analyzed were taken from the pooled offspring from the first 10 days of the hatch.

101.4 ± 0.1 (where 0.1 is the standard error. 20% of the crossovers occurred between *ca* and *Acph-1*. 20% of the 3.6 map units between *ca* and *bv* is 0.7. 100.7, the locus of *ca*, plus 0.7 equals 101.4).

Chromosome 3 of *D. simulans* is rather poorly covered by mutant alleles; hence the mapping analysis could not be as thorough as that of *D. melanogaster*. Cross 1 of Table 3, however, clearly shows that, as in *D. melanogaster*, the gene for the acid phosphatase is on Chromosome 3. The analysis of recombinant progeny from testcross number 2 suggests that the *Acph-1* gene lies somewhere to the right of *st* (scarlet eye color, 40.0). To determine the location of *Acph-1* relative to the mutant gene nearest the right end of Chromosome 3, *pe* (peach eye color, 101.0), testcross 3 was set up following the stipulations of BRIDGES and BREHME (1944) for the collection of linkage data for a two-point test. All the phenotypically wild-type and *jv st pe* progeny were collected. In each class, the flies collected on the different days were pooled, and large samples were taken from both groups. In these samples, (see Table 3) the observed frequency of recombination between *pe* and *Acph-1* was 28.8%. The fact that this estimate was 28.8% (110 crossovers between *pe* and *Acph-1* out of 381 chromosomes analyzed) eliminates the possibility that *Acph-1* lies to the left of *pe*, for if this were the case, recombinant chromosomes in the samples would represent double crossovers between *st* and *pe*. These should only occur at a frequency of about 10% at the very most. Therefore, after converting the 28.8% recombination value to map distance (see HALDANE 1919), its locus was placed at 134.0 ± 2.1 (standard error) on Chromosome 3 of *D. simulans*. Thus, in both species, the *Acph-1* locus lies just to the right of the gene for claret eye color.

Zymograms of the species hybrids: Adult hybrids from crosses between *D. simulans* and *D. melanogaster* are invariably sterile. Nevertheless, because of the "allelic interaction" in the acid phosphatase gene-enzyme system, revealed by the hybrid enzyme found in the heterozygotes, an examination of the species hybrids should yield information bearing on the possible homology of the *Acph-1* genes of the two species. With two alleles in each species, four intercrosses (and their reciprocals) can be made using homozygous flies. All eight crosses were attempted, but adult hybrids were obtained only from three; *Acph-1^B/Acph-1^B* (*simulans*) × *Acph-1^B/Acph-1^B* (*melanogaster*), *Acph-1^B/Acph-1^B* (*simulans*) × *Acph-1^A/Acph-1^A* (*melanogaster*), and *Acph-1^A/Acph-1^A* (*simulans*) × *Acph-1^A/Acph-1^A* (*melanogaster*). In each of the three crosses, *D. simulans* was the female parent. An intercross between the two species was considered successful if at least two, and sometimes three, conditions were met. First, from hybridizations in which *D. simulans* is the female parent, most of the adult offspring are males (STURTEVANT 1920). This criterion was met by the three intercrosses listed above because more than 95% of the offspring were males. Second, hybrid offspring must be sterile. Five groups of three of these males each were placed in vials with virgin females from *D. melanogaster*, and five more with *D. simulans* virgin females. No larvae appeared in any of the ten vials after 10 days; consequently, the second criterion, sterility of the species hybrid, was met. Finally, in those intercrosses in which the *D. simulans* female parents were homozygous for morphologically visible recessive mutant genes and the *D. melanogaster* males homozygous wild type, the hybrid offspring must be wild type. This condition was met in all cases.

Zymograms of two of the three classes of hybrids are shown in Figures 3 and 4. In starch gels prepared in Tris-hydrochloric acid buffer, all three types gave a typical heterozygote pattern, i.e. bands in each of the parental positions and a more intense intermediate or hybrid band (see Figure 3). Figure 4 shows that, in

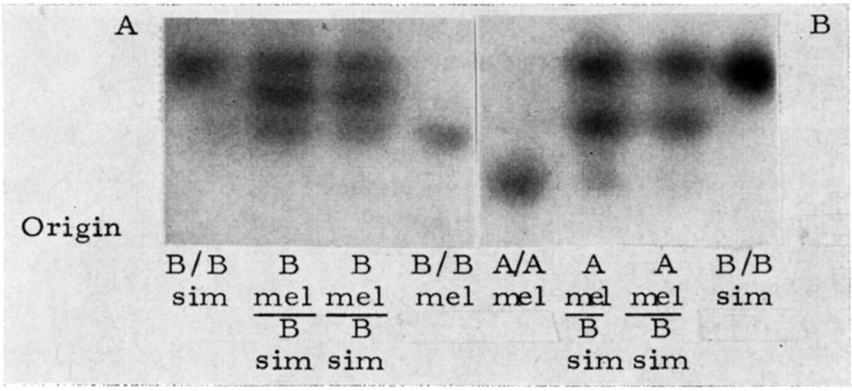

FIGURE 3.—Zymograms of interspecific hybrids from the following crosses: (A) *Acph-1^B/Acph-1^B* (*simulans*) females × *Acph-1^B/Acph-1^B* (*melanogaster*) males and (B) *Acph-1^B/Acph-1^B* (*simulans*) females × *Acph-1^A/Acph-1^A* (*melanogaster*) males. Gels were prepared in Tris-hydrochloric acid buffer, 0.05 M, pH 8.6.

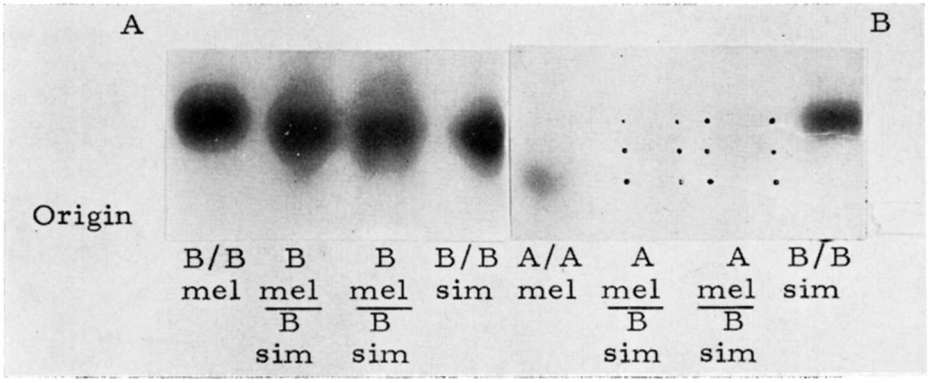

FIGURE 4.—Zymogram of the same interspecific hybrids as described in Figure 3. These gels, however, were prepared in Tris-EDTA-boric acid buffer, 0.05 M, pH 8.6. The dots indicate the positions of the bands obtained from *Acph-1^{B}/Acph-1^{B}* (*simulans*) × *Acph-1^{A}/Acph-1^{A}* (*melanogaster*) hybrids.

these species hybrids, Acph-1 enzymes containing either one or two subunits specified by the *D. simulans* allele have characteristically reduced mobilities in Tris-EDTA-boric acid starch gels. Figure 6 compares the interpreted zymograms of the interspecific hybrids in the two buffer systems. In the Tris-EDTA-boric acid starch, the heterozygote pattern of the "A^{m}/A^{s}" hybrid (Number 1 in Figure 6) is reduced to a single band at the position of the Acph-1^{AA} band of *D. melano-*

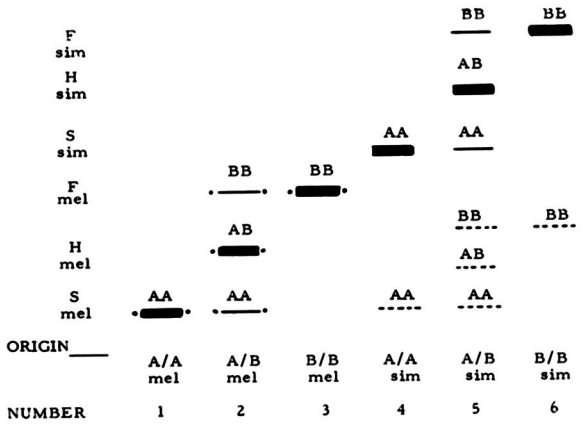

FIGURE 5.—Representations of composite zymograms of Acid phosphatase-1 enzymes of *D. melanogaster* and *D. simulans*. "F", "H", and "S" on the left ordinate refer to the "fast", "hybrid" and "slow" enzymes respectively. The genotypes producing each pattern are indicated directly below the line of origin. "A" and "B" beneath the lower line are designations for the codominant alleles, *Acph-1^{A}* and *Acph-1^{B}*, of each species. "A" and "B" above each band in the diagram refer to the proposed polypeptide subunits specified by the *Acph-1^{A}* and *Acph-1^{B}* alleles of the two species. Solid lines represent the positions of the bands of each genotype in starch prepared in Tris-hydrochloric acid buffer. Dotted lines indicate the positions of the same enzymes in starch made in a Tris-EDTA-boric acid buffer. The pattern indicated by .——. denotes that an enzyme had the same rate of migration in both buffer systems.

gaster. The effect, as in *D. simulans* itself, has been to change the mobility of the "A^s" subunit of *D. simulans*, and thus, the mobilities of the "hybrid" (one "A^s" subunit from *D. simulans*) and "fast" (two "A^s" subunits from *D. simulans*) enzymes to that of the "A^m" subunit of *D. melanogaster*. Thus, all of the enzymes occupy the same position in the zymogram. In the "A^m/B^s" hybrid (Number 2 in Figure 6) in which the *Acph-1*^{B} allele is from *D. simulans*, the Acph-$1^{B^sB^s}$ enzyme (i.e. Acph-1 with two *D. simulans* subunits) migrates at the same rate as the Acph-1^{BB} enzyme from *D. simulans* stocks in Tris-EDTA-boric acid starch. The Acph-$1^{A^mB^s}$ enzyme in the hybrid maintains an intermediate position (see Figure 4B). The somewhat fuzzy bands of the "B^m/B^s" hybrids in Tris-EDTA-boric acid starch (Figure 4A) have been interpreted to represent three bands very close together (see Number 3 in Figure 6). Thus, in this instance, the "slowest" band in Tris-EDTA-boric acid starch is actually the same enzyme, i.e., the Acph-$1^{B^sB^s}$ enzyme with both "B" subunits from the *D. simulans Acph-1*^{B} allele, that has the "fastest" rate of migration in Tris-hydrochloric acid starch (see Figure 6). The Acph-$1^{B^mB^m}$ "parental" enzyme retains its same mobility in

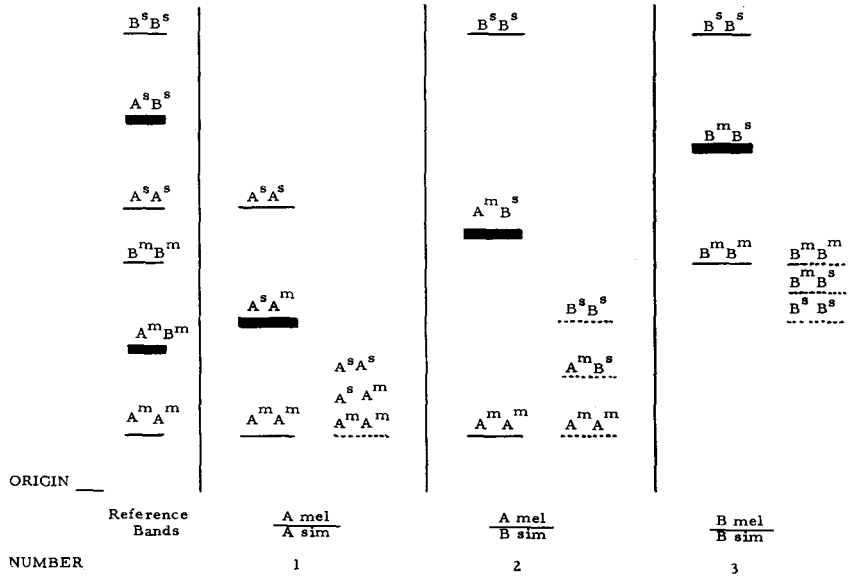

FIGURE 6.—Representations of zymograms of Acid phosphatase-1 enzymes from interspecific hybrids between *D. melanogaster* and *D. simulans*. The six "reference bands" on the left designate the positions of the enzymes characteristic of each species in starch prepared in Tris-hydrochloric acid buffer. The genotypes of the three hybrids are indicated below the line of origin. "A" and "B" beneath the lower line are designations for the codominant alleles, *Acph-1*^{A} and *Acph-1*^{B}, of each species. The proposed polypeptide subunits, A^m, B^m (specified by *Acph-1*^{A} and *Acph-1*^{B} alleles from *D. melanogaster*), A^s and B^s (specified by *Acph-1*^{A} and *Acph-1*^{B} alleles from *D. simulans*) comprising the enzymes of the interspecific hybrids are indicated directly above each of the bands. In each pattern, solid lines on the left represent positions of the enzymes in starch prepared in Tris-hydrochloric acid buffer. Dotted lines on the right indicate the positions of the same enzymes in starch made in a Tris-EDTA-boric acid buffer.

the Tris-EDTA-boric acid starch. The implications of these various patterns in the zymograms of the species hybrids will be discussed in greater detail below.

DISCUSSION

Modern methods for protein purification, exquisite techniques for determining amino acid sequences, and the realization that the latter are direct translations of base sequences in DNA have revived interest in the old problem of gene homology. It is now possible to describe evolutionary changes at the molecular level with a precision unknown in past decades. Fine as our techniques may be, it must be borne in mind that "gene homology" and "gene evolution" are meaningful only with respect to genes related by descent. Homology is not proven by similar enzymatic activities or electrophoretic mobilities. There is no reason from what we now know that convergent evolution does not occur at the molecular level. It should be stressed, then, lacking exact knowledge of the primary structures of the proteins under consideration, that cytological and genetic evidence as well as evidence provided by phenotypic similarity should be used to substantiate any hypothesis of gene homology.

WRIGHT and MACINTYRE (1963) have recently discussed the criteria for establishing gene homologies. The *Acph-1* genes in *D. melanogaster* and *D. simulans* fulfill the criteria. Both genes are located in homologous chromosomal elements (Element E, STURTEVANT and NOVITSKI 1941) and apparently occupy identical positions in a sequence of genes whose homologies have already been established (see STURTEVANT and PLUNKETT 1926). The third criterion, that of phenotypic similarity, is met on the basis of enzyme activity, electropositive migration in pH 8.6 gels and dimerization. Furthermore, true homology of the two genes is supported by the formation of interspecific hybrid dimers. Presumably, the sequences of amino acids in those parts of the polypeptide chains where subunit bonding takes place are very similar, if not identical, in the two species. Thus, the genetic and phenotypic evidence both indicate, at this point, that the *Acph-1* genes in *D. melanogaster* and *D. simulans* are homologous. Further purification and structural analysis of the various enzymes in the two species, together with the results from the genetic tests above, may be expected to provide unimpeachable evidence for this conclusion.

Even though the number of populations analyzed so far is still rather small, there seems to be a basic difference between the two species in the behavior of the Acid phosphatase-1 enzymes in gels containing EDTA. EDTA might be removing a metal ion or ions attached to the Acph-1 enzymes of *D. simulans* but not attached to those of *D. melanogaster*. This phenotypic difference between the enzymes may or may not reflect a corresponding difference between the alleles responsible for their formation. Thus, one can imagine that, even though the amino acid sequences in the enzymes of both species are identical in that region where a metal ion could be attached, only *D. simulans* has a mechanism through which attachment actually occurs. The electrophoretic mobilities of the enzymes of the interspecific hybrids, however, are not in accord with such a model. One

might expect that the hybrids would contain such an "attachment system." If potential "attachment sites" are indeed present on all the molecules, the mobilities of the Acph-1 enzymes specified completely by the allele from *D. melanogaster* in the hybrid pattern in Tris-hydrochloric acid starch should be affected. No such affect on the *D. melanogaster* "parental" band of the hybrid's heterozygote pattern was observed (see Figure 3). Other more involved models could be proposed to account for this difference between the enzymes of the two species in their rates of migration in starch prepared in the two buffer systems. However, the simplest working hypothesis at this time is that all the changes in mobility do indeed result ultimately from differences in the alleles of the structural genes for the *Acph-1* enzyme. Therefore, since the *Acph-1* alleles from *D. simulans* probably differ structurally from both those alleles of *D. melanogaster*, then apparently, no allele has yet been discovered that is common to both species. A larger sample of populations from *D. melanogaster* and *D. simulans* is clearly needed, but so far this contrasts with the results of WRIGHT and MACINTYRE (1963) who found that there are several probably identical Esterase 6 alleles shared by these two species.

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SUMMARY

Electrophoretic variants of an acid phosphatase are described; they are controlled by codominant, interacting alleles of a single autosomal locus in the two sibling species, *D. melanogaster* and *D. simulans*. In both species the genes, designated *Acph-1*, are located on chromosome 3, just to the right of the homologous claret genes. Further evidence for the hypothesis of gene homology is provided by the appearance of typically heterozygous patterns in the species hybrids. However, no allele has yet been found that is common to both species. The reduced rates of electropositive migration of the Acid phosphatase-1 enzymes from *D. simulans* in starch containing EDTA are described and discussed.

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