VARIATION IN ACTIVITIES OF AMYLASE ALLOZYMES ASSOCIATED WITH CHROMOSOME INVERSIONS IN *DROSOPHILA PSEUDOOBSCURA, D. PERSIMILIS* AND *D. MIRANDA*

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ABSTRACT

Different electrophoretic alleles of amylase show associations with particular chromosome *3* inversions in *D. pseudoobscura* and *D. persimilis*. Relative adult amylase activities were compared in 37, 37 and 10 strains of *D. pseudoobscura, D. persimilis* and *D. miranda*, respectively. Strains carrying the same electrophoretic allele were compared by crossing these lines individually to a reference strain carrying a different electrophoretic mobility allele. This procedure allows comparisons among species, inversions, electromorphs and strains for genetic variation in amylase activity. F_2 analysis established that the activity variation co-segregates with the structural amylase locus. This type of variation could be due to either structural gene differences or differences in closely linked, *cis*-acting regulatory regions. Variation has been detected among and within electrophoretic mobility classes. Moreover, this variation is clearly nonrandom and reveals more of the genetic structure associated with the chromosomal inversion phylogeny of *D. pseudoobscura* and *D. persimilis*.

——Some of the findings are: (1) Similar electromorphs in *D. pseudoobscura* and *D. persimilis* usually show different activities. These species show nearly complete differentiation of amylase alleles, based on activities. (2) *D. persimilis* has the broadest range of variation in amylase activity, about four-fold between the highest and lowest alleles. *D. pseudoobscura* and *D. miranda* are also polymorphic for activity, but have more constrained ranges of variation. *D. miranda* alleles show on the average about four times the activity of *D. pseudoobscura* alleles. (3) Some association of electrophoretic mobility and activity has been found. Alleles *1.09* of *D. persimilis*, as well as *1.43* and *1.55* of *D. miranda*, have relatively high activity. It may be that these high activity alleles are part of an adaptation to cooler habitats. (4) Within electrophoretic classes, associations of activities with inversions have been found. These are especially strong in *D. persimilis*. The *1.00* alleles in the ST, KL, MD and WT inversions, the *0.92* allele in the ST and MD inversions and the *1.09* allele in the WT and KL inversions have levels of activities that depend upon the arrangement in which they are located. These results demonstrate that suppression of recombination in inversion heterokaryotypes can result in extensive genic divergence between inversions.

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CHROMOSOMAL inversion polymorphisms are a common form of genetic variation in natural populations of Drosophila. For polymorphic loci near or within naturally occurring inversions, associations of different alleles with particular gene arrangements are frequent (PRAKASH and LEWONTIN 1968, 1971; KOJIMA, GILLSPIE and TOBARI 1970; MUKAI, METTLER and CHIGUSA 1971; PRAKASH and MERRITT 1972; PRAKASH and LEVITAN 1973, 1974; POWELL 1973; LANGLEY, TOBARI and KOJIMA 1974; ZOUROS *et al.* 1974; PRAKASH 1974, 1976). The amylase locus (*Amy*) in the sibling species group, *D. pseudoobscura*, *D. persimilis* and *D. miranda*, is such a locus on chromosome 3. Since the inversions of this chromosome are overlapping, the phylogeny has been constructed for *D. persimilis* and *D. pseudoobscura*; the *D. miranda X* chromosome corresponds to the third in *D. pseudoobscura*, but it differs considerably from the inversions of *D. pseudoobscura* and *D. persimilis* and its derivation is unclear. Figure 1 gives

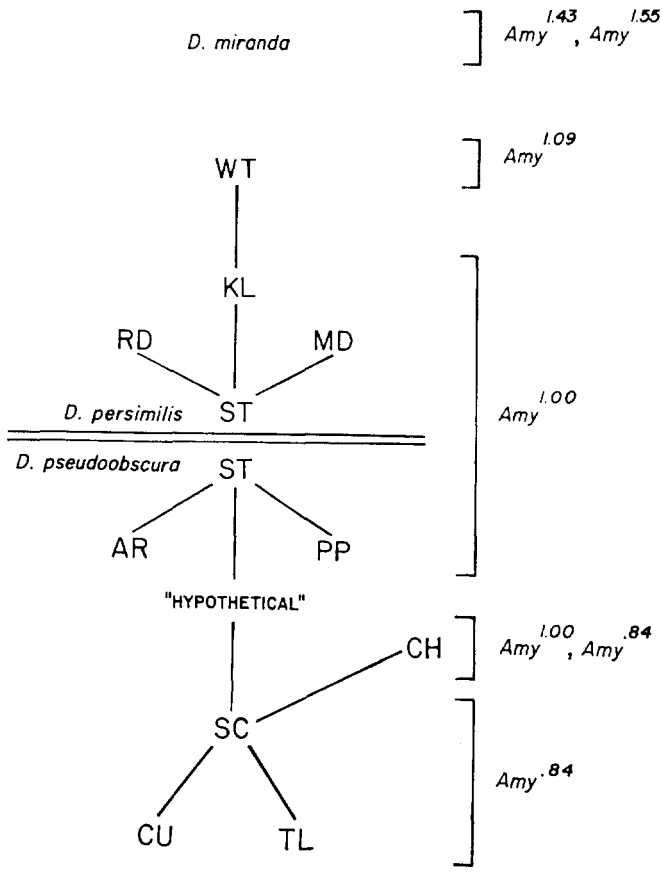

FIGURE 1.—Inversion phylogeny and commonly associated amylase electrophoretic alleles in *Drosophila pseudoobscura*, *D. persimilis* and *D. miranda*. The phylogeny is modified from DOBZHANSKY and EPLING (1944) and SPIESS (1965). Amylase alleles are from PRAKASH and LEWONTIN (1968) and PRAKASH (1977a).

the phylogenetic relationships along with the commonly associated amylase alleles of the inversions used in this study.

The distinction between *D. persimilis* and *D. pseudoobscura* at the amylase locus can be attributed largely to the associations of *Amy* alleles with inversions that are characteristic of each species. The associations have been described in PRAKASH and LEWONTIN (1968). The Santa Cruz phylad inversions (CH, TL, SC and CU), found only in *D. pseudoobscura*, have a high frequency of the *0.84* electrophoretic allele, while the Standard phylad inversions of both *D. pseudoobscura* (AR, PP and ST) and *D. persmilis* (KL, MD and RD) predominantly have the *1.00* electrophoretic allele. The WT arrangement of *D. persimilis*, however, has a high frequency of the *1.09* allelic class not found commonly in other gene arrangements. Other electrophoretic alleles are somewhat rarer, occurring in *D. pseudoobscura* and *D. persimilis* in different frequencies (PRAKASH 1977b). *Amy* alleles *0.92* and *1.05* are found at frequencies of less than 5% in *D. persimilis*. In *D. pseudoobscura*, these alleles are very infrequent. The slow mobility *0.74* electrophoretic allele is found in less than 5% of *D. pseudoobscura* genomes; it occurs to a even lesser extent in *D. persimilis*. *D. miranda* has a set of unique amylase allozymes much faster in mobility than the allozymes of *D. persimilis* and *D. pseudoobscura* (PRAKASH 1977b).

Because recombination is drastically reduced over the entire third chromosome in inversion heterokaryotypes, the inversion types segregate as independent genetic units. Both laboratory experiments and field investigations have convincingly shown inversion frequencies to be under the influence of natural selection (DOBZHANSKY 1970). In natural populations, inversions show extensive altitudinal and geographical variation across the ranges of both *D. pseudoobscura* and *D. persimilis* (DOBZHANSKY and EPLING 1944; ANDERSON *et al.* 1975; SPIESS 1950). From fitness studies coupled with the reduction in gene exchange between arrangements, DOBZHANSKY (1951) hypothesized that the genes within an inversion type are co-adapted. At present, we know very little about the actual genetics of this proposed process of coadaptation. We know only that, at the organism level, chromosome 3 inversions show fitness differences; whereas, at the genic level, alleles of structural genes on chromosome 3 show particular associations with certain gene arrangements.

Recently, varying conditions of gel electrophoresis have revealed more extensive genetic variation and different genetic structures for the polymorphic loci, esterase-5 and xanthine dehydrogenase of *D. pseudoobscura* and *D. persimilis* (COBBS and PRAKASH 1977; MCDOWELL and PRAKASH 1976; COYNE 1976; COYNE, FELTON and LEWONTIN 1978; SINGH, LEWONTIN and FELTON 1976). We have uncovered amylase allozyme activity variation that profoundly alters the observed pattern of variation at this locus. In this study, we examine the association of amylase activities among allozymes, inversions and species. We show that this type of variation co-segregates with the structural amylase allele. Large differences in amylase activities can be found both between and within electrophoretic mobility classes. Our results demonstrate that the profile of electro-

phoretic mobility variation gives a very incomplete picture of the genetic divergence that has occurred among inversions and species.

MATERIALS AND METHODS

Strains: A total of 37 lines of *D. pseudoobscura* from a population in Mather, CA., were assayed. These strains were made isogenic for the entire third chromosome by using balanced lethal stocks and represent the 5 common inversions found in this locality. The CU, $Amy^{1.05}$ strain is from Amecameca, Mexico, and was isolated from a heterozygous $Amy^{0.84}/Amy^{1.05}$ isofemale strain obtained from W. ANDERSON. Four different amylase electromorphs were represented in the sample.

A total of 38 lines of *D. persimilis*, made homokaryotypic by sib-mating, were used from 3 different populations. No interpopulational variation was detected. In order to avoid genetic heterogeneity within strains, most lines were additionally inbred for 8 or more generations by single brother-sister matings, including the reference strains WT 10 and KL 8. Some chromosome *3* isogenic lines were provided by E. SPIESS. In all, 5 different inversions and 5 different electromorphs were included in the sample.

Ten lines of *D. miranda* were selected from 2 different populations. Each line was inbred for 4 to 5 generations by sib-mating. Two electromorphs were included in the sample.

Measurements of relative activities: For comparisons of relative activities, strains with a particular electrophoretic allele were crossed independently to a reference strain with a different mobility allele. The F_1 offspring of these crosses are each heterozygous for a different strain-specific allele and a given reference allele. Electrophoresis was performed in 0.075 M Tris-borate buffer (pH 8.9) on crude extracts of these F_1 adults for 4 to 6 hr, and 5% acrylamide gels were made in 0.075 M Tris-borate with 0.02 M $CaCl_2$. For activity determinations, the gels were incubated against a 1% starch–5% acrylamide plate (pH 7.4) at 16° to 25° for 12 to 16 hr. Each gel pocket contained the extract from independent samples of 2 to 5 adults, depending on the total amylase activity of the F_1 strains. After incubation, the starch gel plate was stained with I-KI solution and fixed in 7% acetic acid. The amylase activity expression of the 2 different electrophoretic alleles was represented by the intensity of the clear starch digested area in the plate. A number of independent samples were scored for each strain.

Each amylase assay plate was scanned on a Beckman R-112 densitometer, using a clear portion of the glass plate as a baseline. The linearity of response was calibrated by serial dilutions of crude extracts for selected crosses that displayed a range of activity differences. Assays in the nonlinear range could be recognized on the scan printouts and were disregarded. The scans were quantified, using a Dupont Curve Resolver. The procedure involves the comparison of the 2 allozyme curves in heterozygotes. We derive a quantitative activity parameter termed *percent relative activity*, obtained as follows: The total area under the curves of both allozymes is considered 100%. The activity of the assayed strain's allozyme is thus recorded as its percentage contribution to the total activity. Independent lines with the same amylase allozyme are compared when measured against the same reference allozyme. The values obtained conform well to the assumptions of parametric analysis of variance. A nested analysis of variance (Anova) for unequal sample sizes (SOKAL and ROHLF 1969) and the Statistical Analysis System (SAS Institute) were used in analyzing the data.

Several F_1 heterozygous lines were subjected to a variety of different assay conditions before extensive data were gathered. Assay temperatures of 5° to 30° did not alter the relative activity expression of amylase allozymes. Electrophoretic runs of longer times and at pH 7.4 did not affect the observed activity differences. Even though males and females clearly differ in total activity per fly, no consistent effect of sex could be demonstrated for relative amylase activities in heterozygotes. Finally, no significant effects of adult age were detected in relative activities of amylase allozymes on flies ranging in age from 2 to 30 days, even though activity decreases in older adults. The adults used in this study were between 2 and 14 days old and were maintained at 18°. F_2 activity data were assayed in the same manner except, individual F_2 *Amy* heterozygotes had to be used that were the offspring of the assayed F_1 adults.

RESULTS

Drosophila pseudoobscura

Amy$^{1.00}$ strains: The activities of *D. pseudoobscura* strains with an amylase allozyme of 1.00 electrophoretic mobility relative to the reference 0.84 allozyme of CH 2 are given in Table 1. In all, five different gene arrangements were examined. The gene arrangements PP, ST and AR belong to the Standard phylad, which shows a preponderance of the *1.00* electrophoretic allele. The TL 1.00 strain represents a "wrong" association since the *1.00* allele is uncommon in this

TABLE 1

Relative activities of D. pseudoobscura Amy$^{1.00}$ *alleles in different strains and gene arrangements as assayed in F_1 heterozygotes obtained by crossing these strains individually to a reference strain (CH2) with an* Amy$^{0.84}$ *allele*

Gene arrangement	Strain	Allele	Number of samples assayed	Percent relative activity ± 1 S.D.*
PP	1	1.00	8	50.5 ± 2.9
PP	3	1.00	6	51.2 ± 3.0
PP	11	1.00	4	53.2 ± 2.8
PP	13	1.00	5	54.6 ± 3.6
PP	18	1.00	6	50.2 ± 2.4
PP	19	1.00	6	51.0 ± 2.0
ST	2	1.00	7	57.3 ± 4.6
ST	4	1.00	14	53.6 ± 4.3
ST	7	1.00	11	54.5 ± 3.5
ST	8	1.00	7	53.1 ± 3.1
ST	9	1.00	6	57.2 ± 2.9
ST	10	1.00	9	56.2 ± 3.0
AR	1	1.00	7	54.3 ± 3.2
AR	3	1.00	8	58.1 ± 4.8
AR	6	1.00	6	55.5 ± 2.3
AR	7	1.00	5	56.2 ± 1.8
AR	8	1.00	9	56.2 ± 2.9
AR	9	1.00	7	60.1 ± 3.9
AR	10	1.00	10	56.5 ± 4.3
CH	4	1.00	15	58.8 ± 4.5
CH	5	1.00	7	55.3 ± 2.6
CH	7	1.00	15	58.3 ± 2.7
TL	3	1.00	7	57.3 ± 2.3

Nested analysis of variance

Source	D.F.	S.S.	M.S.	F
Gene arrangements	4	890.1	222.53	9.693, ($p < 0.001$)
Strains	18	413.3	22.96	1.899, ($p = 0.038$)
Samples	161	1946.6	12.09	
Total	183	3250		

* The total activity is set at 100% and includes the activity of both reference 0.84 allozyme and the 1.00 allozyme. Percent relative activity is then the contribution of the strain specific 1.00 allozyme to the total activity. S.D. is the standard deviation of the samples.

arrangement. The 1.00 allozyme appears more active in some strains and, in others, equivalent to the 0.84 allozyme of the reference CH 2 strain. The nested analysis of variance in Table 1 reveals significant heterogeneity among both inversions and strains within inversions, with more of the variance contributed by the former. It is most likely that the PP gene arrangement accounts for the significant differences between gene arrangements. The mean relative activity of PP is 51.8%, while the means of ST, AR and CH are 55.3%, 56.7% and 57.5%, respectively. In order to determine if the activity differences between the 1.00 and 0.84 allozymes are due to differences at or near the amylase locus, the F_1 offspring from the crosses of the three CH strains and the ST 7 strain to the CH 2 reference strain were allowed to produce F_2 progeny. The relative activities of the heterozygous amylase segregants were examined in these F_2 individuals. Free recombination should occur in F_1 females of crosses between the three CH strains and the CH 2 0.84 reference strain. (The results are given in Table 11). The 1.00 amylases, behaving as they do in F_1 heterozygotes, are consistently higher in activity than the 0.84 enzyme in F_2 heterozygotes. Thus, this difference in activity co-segregates with the structural alleles. In 41 F_2 segregants of CH homokaryotypes, which were heterozygous for the *0.84* and *1.00* allele, no individual was found that could be considered a recombinant for the activity differences between the electrophoretic alleles.

In view of the level of resolution afforded in the quantitative measure involving just one reference strain, it seems unwise to speculate any further about the variation between the strains in Table 1. Some of this variation could be due either to background genetic effects or to experimental error. Further genetics would be required to test differences between certain strains, and this does not seem worthwhile for the small differences here.

Amy$^{0.84}$ *strains*: The other commonly occurring electromorph in *D. pseudoobscura* is *Amy*$^{0.84}$, found mainly in the Santa Cruz phylad. By using the PP 1 and AR 1 strains as references, both of which carry *Amy*$^{1.00}$ alleles, a number of different strains with the 0.84 allozyme have been assayed. Table 2 gives the mean relative activities for these strains of the TL, CH, AR and ST arrangements. The *0.84* allele is uncommon in the Standard phylad. Analyses of variance were performed separately on the two data sets. Both analyses reveal significant variation among inversions ($P = 0.003$, d.f. = 3,9; $P = 0.032$, d.f. = 3,7), using PP 1 and AR 1 as references, respectively. Variance among strains within inversions was much less significant ($P = 0.04$, d.f. = 9,105; $P = 0.16$, d.f. = 7,69), using PP 1 and AR 1, respectively. A nested analysis of covariance on the means in Table 2 further supports the differences among inversions. The strain means as assayed against both references have a total mean-square correlation of 0.79. The mean-square correlation due to inversion differences is very strong (0.98). Among strains within inversions, however, the mean-square correlation is negative and not strong (−0.43). From inspection of the data, it appears that AR and CH, as compared to TL, are primarily responsible for the differences detected among inversions. The mean activities of the TL strains are 54.8% and 53.7% against PP 1 and AR 1, respectively, while the CH mean was 49.8% and 49.4%.

TABLE 2

Relative activities of D. pseudoobscura $Amy^{0.84}$ *alleles in different strains and gene arrangements as assayed in* F_1 *heterozygotes obtained by crossing these strains individually to reference strains (PP 1 and AR 1) with* $Amy^{1.00}$ *alleles*

Gene arrangement	Strain	Allele	Reference PP 1		Reference AR 1	
			Number of samples assayed	Percent relative activity ± 1 S.D.	Number of samples assayed	Percent relative activity ± 1 S.D.
TL	1	*0.84*	4	53.5 ± 3.1	8	53.7 ± 2.7
TL	2	*0.84*	10	54.4 ± 2.3	8	52.5 ± 1.8
TL	4	*0.84*	14	58.1 ± 2.6		—
TL	5	*0.84*	7	54.2 ± 2.1		—
TL	6	*0.84*	8	53.5 ± 2.9	6	56.5 ± 2.9
TL	7	*0.84*	10	54.9 ± 4.2	4	54.3 ± 2.6
TL	8	*0.84*	5	54.2 ± 3.6	7	54.8 ± 4.2
TL	9	*0.84*	7	56.3 ± 4.4	7	53.0 ± 2.9
TL	10	*0.84*	8	53.9 ± 1.8	7	51.0 ± 3.5
CH	1	*0.84*	5	49.8 ± 2.8	8	50.7 ± 4.0
CH	2	*0.84*	15	49.7 ± 2.5	6	48.0 ± 3.1
AR	4	*0.84*	14	47.8 ± 3.3	8	47.4 ± 4.4
ST	3	*0.84*	10	51.8 ± 3.9	11	53.3 ± 2.2

AR 4 has mean activities of 47.8% and 47.4%, being slightly less active than the reference allozymes. ST 3 appears to be more like the TL strains.

Rare alleles: Strains carrying other structural alleles were also examined (Table 3). The 0.92 allozyme of ST 5 is less active than the reference 1.00 allozyme of either AR 1 or PP 1. ST 5 has the lowest activity of any strain examined. The 1.05 activity of CU 58 is very similar to the common activity class of the 1.00 allozyme in strains of *D. pseudoobscura*.

Drosophila persimilis

$Amy^{1.00}$ *strains*: Amylase activities of strains with the *1.00* electrophoretic allele from four different gene arrangements, assayed against WT 10 with a *1.09* electrophoretic allele, are given in Table 4. The *1.00* electromorph is com-

TABLE 3

Relative activities of D. pseudoobscura *rare alleles*, $Amy^{0.92}$ *and* $Amy^{1.05}$, *as assayed in* F_1 *heterozygotes obtained by crossing these strains individually to various reference strains**

Gene arrangement	Strain	Allele	Reference strain	Number of samples assayed	Percent relative activity ± 1 S.D.
ST	5	*0.92*	PP 1	6	43.2 ± 2.5
			AR 1	10	41.3 ± 3.0
			KL 8	7	29.4 ± 3.6
CU	A-58	*1.05*	CH 2	5	57.4 ± 2.8

* Reference strains PP 1, AR 1 and KL 8 carry a *1.00* electrophoretic *allele*. CH 2 carries an *0.84* allele.

TABLE 4

Relative activities of D. persimilis $Amy^{1.00}$ *alleles in different strains and gene arrangements as assayed in* F_1 *heterozygotes obtained by crossing these strains individually to a reference strain (WT 10) with an* $Amy^{1.09}$ *allele*

Gene arrangement	Strain	Allele	Number of samples assayed	Percent relative activity ± 1 S.D.
KL	H22	*1.00*	9	30.2 ± 4.6
KL	H1	*1.00*	10	29.6 ± 7.8
KL	H63	*1.00*	8	31.3 ± 2.7
KL	H125	*1.00*	10	28.8 ± 3.5
KL	H 77	*1.00*	14	30.6 ± 4.5
KL	H95	*1.00*	14	29.8 ± 5.8
KL	H74	*1.00*	7	30.9 ± 2.9
KL	H128	*1.00*	8	30.1 ± 3.9
KL	33	*1.00*	11	27.2 ± 4.2
KL	4	*1.00*	13	27.8 ± 4.4
KL	155	*1.00*	12	31.5 ± 3.5
KL	H43	*1.00*	8	29.9 ± 5.4
KL	H132	*1.00*	10	30.5 ± 5.5
KL	H128	*1.00*	8	30.1 ± 3.9
KL	8	*1.00*	9	30.9 ± 5.7
MD	33	*1.00*	12	51.5 ± 2.2
MD	H118	*1.00*	8	51.9 ± 1.6
MD	H86	*1.00*	11	46.0 ± 2.5
MD	H29	*1.00*	11	41.1 ± 3.5
MD	H48	*1.00*	7	50.0 ± 2.3
RD	H103	*1.00*	10	29.2 ± 5.1
RD	H44	*1.00*	11	29.9 ± 7.5
WT	1	*1.00*	9	36.1 ± 3.8

mon within KL, MD and RD, but much less frequent in WT. Clearly, strains of the KL, RD and WT gene arrangements with the 1.00 allozyme show much less activity than the 1.09 allozyme of WT 10 (Table 4; Figure 2, pockets D-F). This 1.09 reference allozyme is about two and one-third times as active as the 1.00 allozyme from strains of KL and RD. Many of the MD 1.00 strains, however, are about equal to the WT 10 1.09 reference strain in amylase activity (Table 4; Figure 2, pockets B, C). The analysis of variance shows significant variation among inversions ($P < 0.001$, d.f. = 3,18) and among strains within inversions ($P < 0.001$, d.f. = 18,208). The analysis of variance excluding MD strains also reveals heterogeneity among inversions ($P = 0.003$, d.f. = 2,15), but not among strains of RD and KL ($P = 0.82$, d.f. = 15,155). The 1.00 allozyme of WT 1 has greater activity than the 1.00 allozyme in the RD and KL gene arrangements. Within the MD 1.00 strains, there is significant variation ($P < 0.001$, d.f. = 4,45). The MD H29 strain displays considerably lower activity than the other 1.00 strains. MD H86 may also be different.

$Amy^{1.09}$ *strains*: It is clear from Table 4 that WT 10 carries an amylase *1.09* allele that expresses far more activity than the KL, RD and WT strains with the *1.00* electrophoretic allele. Ten strains of 1.09 electromorph found commonly

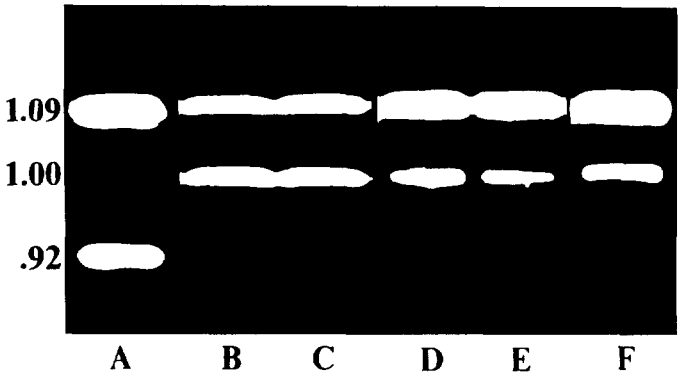

FIGURE 2.—Composite photograph of gels showing amylase activities in F_1 heterozygotes of *D. persimilis*. Each pocket contained independent samples from the following crosses: A; MD 155, $Amy^{0.92}$ × WT 10, $Amy^{1.09}$. B,C; MD 33, $Amy^{1.00}$ × WT 10, $Amy^{1.09}$. D,E; KL 4, $Amy^{1.00}$ × WT 10, $Amy^{1.09}$. F; RD H44, $Amy^{1.00}$ × WT 10, $Amy^{1.09}$.

only in the WT arrangement and four strains of the KL arrangement with the 1.09 electromorph were studied. The data for both WT and KL 1.09 strains are presented in Table 5. The 1.09 allozyme is, in all of these strains, much more active than the 1.00 allozyme of the KL 8 reference strain. Comparison of Tables 4 and 5 shows that the 1.09 strains, in general, are more active than the 1.00 allozyme in strains of the KL, RD and WT arrangements. The nested analysis of variance reveals differences between the KL and WT arrangements. Significant variation is found between inversions ($P < 0.001$, d.f. = 1,12), whereas differences among strains within these two arrangements are not significant

TABLE 5

Relative activities of D. persimilis $Amy^{1.09}$ *alleles in different strains and gene arrangements as assayed in* F_1 *heterozygotes obtained by crossing these strains individually to a reference strain (KL 8) with an* $Amy^{1.00}$ *allele*

Gene arrangement	Strain	Allele	Number of samples assayed	Percent relative activity ± 1 S.D.
WT	M2	*1.09*	7	72.4 ± 5.8
WT	M15	*1.09*	7	69.1 ± 6.1
WT	M8	*1.09*	14	69.6 ± 3.5
WT	106	*1.09*	11	71.6 ± 4.9
WT	18	*1.09*	9	70.7 ± 5.4
WT	M16	*1.09*	7	69.3 ± 4.6
WT	11	*1.09*	8	71.0 ± 4.3
WT	M6	*1.09*	11	67.4 ± 5.4
WT	M10	*1.09*	14	68.9 ± 5.6
WT	H84	*1.09*	8	68.6 ± 4.1
KL	H31	*1.09*	10	66.5 ± 4.7
KL	R2	*1.09*	13	64.6 ± 4.8
KL	R4	*1.09*	11	62.3 ± 4.2
KL	R17	*1.09*	13	62.2 ± 2.7

($P = 0.50$, d.f. = 12,129). Even though some of the differences between KL and WT strains are slight, the 1.09 strains from KL have the lowest activities of any of the 1.09 lines in Table 5.

Other Amy *alleles*: The amylase activities of various alleles from available strains of the ST arrangement are presented in Table 6. The allozymes in the ST arrangement are generally lower in activity than allozymes in other arrangements. The 1.00 allozyme of ST M4 is not equivalent in activity to the 1.00 activities of other arrangements in *D. persimilis* (cf. Table 4). It is also evident from Table 6 that the 0.92 allozyme activity is considerably different in the ST and MD arrangements. The 1.05 allozyme in KL, when compared indirectly through the MD 106 reference, has an activity level very much like that of the KL 1.09 allozyme. Strains MD 155 and ST H2 have been assayed against both KL 8 and WT 10 reference strains; while these two 0.92 strains have different activities, their expressions relative to KL 1.00 and WT 1.09 are consistent with the results obtained in Table 5 showing WT 1.09 to be two and one-third times as active as KL 1.00.

Drosophila miranda

Table 7 gives the amylase activity data collected on nine inbred strains of *D. miranda* that carry the 1.43 electrophoretic allele, the common electromorph in this species. They were assayed against a reference strain S 204 with an $Amy^{1.55}$ allele. Some variation was detected among just these line strains. Most 1.43 strains showed slightly greater activity than the reference 1.55 allozyme (Figure 3, pockets B and C), but two strains, M 566 and M 174, have consistently less activity than the reference allozyme (Figure 3, pocket D). The one-way analysis of variance reflects significant differences between strains ($P < 0.001$, d.f. = 8,65). We have thus detected two distinct activity classes among just nine 1.43 strains of *D. miranda*.

Species hybrids

The three species in this study are closely related sibling species. Hybridization between these species is possible and, in the case of *D. pseudoobscura* and

TABLE 6

Relative activities of D. persimilis Amy *alleles in the ST gene arrangement and a KL,* $Amy^{1.05}$ *allele as assayed in* F_1 *heterozygotes obtained by crossing these strains individually to various reference strains**

Gene arrangement	Strain	Allele	Reference strains KL8	WT10	MD106
			Percent relative activity ± 1 S.D.		
MD	155	*0.92*	(6) 64.1 ± 3.8	(14) 43.9 ± 2.6	
MD	H106	*0.92*		(14) 39.9 ± 3.0	
ST	H2	*0.92*	(10) 39.6 ± 3.3	(11) 21.7 ± 3.7	
ST	M4	*1.00*		(4) 19.9 ± 5.8	(10) 33.4 ± 3.1
KL	H82	*1.05*			(9) 56.6 ± 6.1

* Reference strains KL 8, WT 10 and MD 106 carry electrophoretic alleles *1.00*, *1.09* and *0.92*, respectively. Numbers of samples assayed are given in parentheses.

TABLE 7

Relative activities of D. miranda Amy$^{1.43}$ *alleles in different strains as assayed in* F_1 *heterozygotes obtained by crossing these strains individually to a reference strain (S-204) with an* Amy$^{1.55}$ *allele*

Strain	Allele	Number of samples assayed	Percent relative activity ± 1 S.D.
S M3	*1.43*	9	56.1 ± 3.0
S A3	*1.43*	6	54.3 ± 2.6
S 206	*1.43*	8	54.2 ± 3.1
S 143	*1.43*	10	55.3 ± 2.8
M 566	*1.43*	11	43.9 ± 2.6
M 214	*1.43*	7	56.4 ± 3.1
M 427	*1.43*	8	55.0 ± 1.6
M 174	*1.43*	8	43.1 ± 1.1
M 55	*1.43*	7	53.7 ± 2.1

D. persimilis hybrids, backcrosses are successful, thus permitting genetic analysis of amylase expression. By using species hybrids, we have investigated the pattern of *cis*-specific amylase expression between related species in the same manner as was done within species.

D. persimilis—D. miranda: Two strains of *D. miranda* were successfully hybridized to the WT 10 reference strain. Data on amylase comparison are presented in Table 8. Both of the 1.43 strains of *D. miranda* exhibit activities slightly greater than the activity of the WT 1.09 allozyme. This result suggests that the reference *Amy* alleles *1.55* of S 204 and *1.09* of WT 10 are about equivalent in amylase expression (Figure 3, pocket A).

D. persimilis—D. pseudoobscura: Using the highly active *D. persimilis* WT 10 1.09 allozyme, a comparison of a number of *D. pseudoobscura* strains hy-

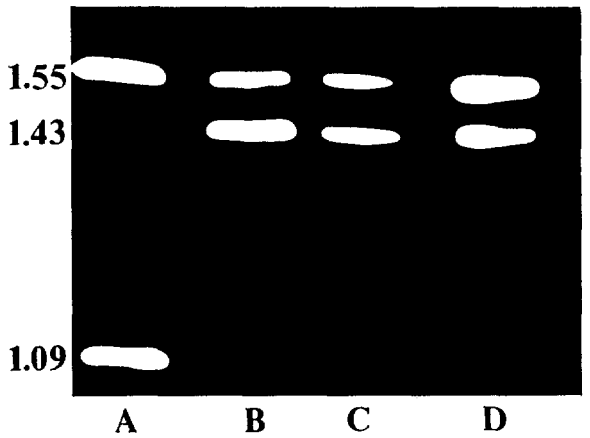

FIGURE 3.—Composite photograph of gels showing amylase activities in F^1 heterozygotes of *D. miranda* and *D. persimilis* (WT 10). Each pocket contained independent samples from the following crosses: A; S 204, *Amy*$^{1.55}$ (*D. miranda*) × WT 10, *Amy*$^{1.09}$ (*D. persimilis*). B,C; S M3, *Amy*$^{1.43}$ × S 204, *Amy*$^{1.55}$. D; M 566, *Amy*$^{1.43}$ × S 204, *Amy*$^{1.55}$.

TABLE 8

Relative activities of D. miranda $Amy^{1.43}$ *alleles in two different strains as assayed in heterozygotes of* F_1 *interspecific hybrids obtained by crossing these strains individually to a reference* D. persimilis *strain (WT 10) with an* $Amy^{1.09}$ *allele*

Strain	Allele	Number of samples assayed	Percent relative activity ± 1 S.D.
S A3	*1.43*	4	54.7 ± 1.7
M 55	*1.43*	6	54.8 ± 3.1

bridized to this strain is given in Table 9. Clearly, the 1.09 allozyme has about four times the activity of *D. pseudoobscura* strains, all of which appear very similar (Table 9; Figure 4). The activity difference between the tested *D. pseudoobscura* strains and the *D. persimilis* reference allozyme is so great that the error variance is too large to detect any variation among the *D. pseudoobscura* strains.

We next turn our attention to the 0.84 strains within *D. pseudoobscura.* Data in Tables 1 and 2 show that most of these lines are very similar to the 1.00 strains of *D. pseudoobscura.* By comparing these 0.84 strains to the 1.00 strains of *D. persimilis,* we can complete the profile for amylase activity variation between the three species. Table 10 gives relative activities for the 0.84 allozyme of *D. pseudoobscura* strains, as assayed against the reference 1.00 allozyme in the KL 8 strain of *D. persimilis.* The results in Table 10 confirm what could be expected indirectly from the previous data: the 1.00 KL allozyme consistently expresses about 1.5 times the activity of the 0.84 *D. pseudoobscura* amylases (Figure 5). The nested Anova reveals the same general pattern of variance, among inversions ($P = 0.06$, d.f. = 3,8) and strains ($P = 0.20$, d.f. = 8,93),

TABLE 9

Relative activities of D. pseudoobscura $Amy^{1.00}$ *and* $Amy^{0.84}$ *alleles in various strains and gene arrangements as assayed in* F_1 *interspecific hybrids of heterozygotes obtained by crossing these strains individually to a* D. persimilis *reference strain (WT 10) with an* $Amy^{1.09}$ *allele*

Gene arrangement	Strain	Allele	Number of samples assayed	Percent relative activity ± 1 S.D.
PP	3	*1.00*	5	17.2 ± 5.9
PP	5	*1.00*	10	22.7 ± 6.9
PP	11	*1.00*	5	16.4 ± 8.0
PP	13	*1.00*	6	23.7 ± 8.5
ST	7	*1.00*	5	18.6 ± 4.5
ST	9	*1.00*	7	20.1 ± 5.3
AR	1	*1.00*	4	19.3 ± 4.8
AR	5	*1.00*	6	18.7 ± 6.4
AR	7	*1.00*	5	21.2 ± 6.0
AR	10	*1.00*	6	18.8 ± 4.9
CH	5	*1.00*	6	19.7 ± 6.0
TL	6	*0.84*	6	21.2 ± 9.8

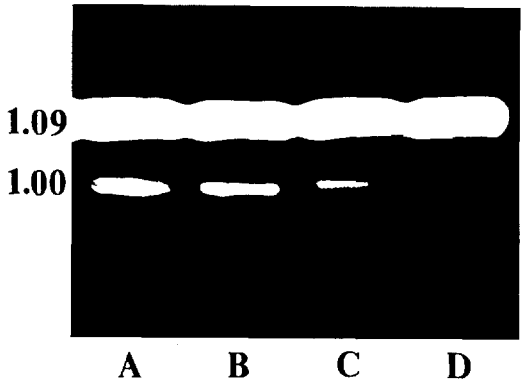

FIGURE 4.—Photograph of gel showing amylase activities in F_1 heterozygotes of *D. pseudoobscura–D. persimilis* hybrids. Each pocket contained independent samples from the following crosses: A,B; AR 7, $Amy^{1.00}$ (*D. pseudoobscura*) × WT 10, $Amy^{1.09}$ (*D. persimilis*). C,D; PP 3, $Amy^{1.00}$ (*D. pseudoobscura*) × WT 10, $Amy^{1.09}$ (*D. persimilis*).

found for these lines when assayed with *D. pseudoobscura* reference allozymes (*cf.*, Table 2). Note also that the activity of the ST 5 0.92 allozyme, when assayed against the three reference strains AR 1, PP 1 and KL 8 (Table 3), reflects the activity variation between KL 1.00 and AR, PP 1.00. Thus, the pattern of variation is largely consistent between and within species, with no significant contribution from any interaction between alleles or inversions involved in the crosses. The main activity differences are associated in a *cis*-specific way with the particular electrophoretic alleles and chromosome inversions. This can be further illustrated by comparing various tables. The major class of TL 0.84 activities is equivalent to those of the 1.00 ST and AR strains

TABLE 10

Relative activities of D. pseudoobscura $Amy^{0.84}$ *alleles in different strains and gene arrangements as assayed in* F_1 *interspecific hybrids of heterozygotes obtained by crossing these strains individually to a* D. persimilis *reference strain (KL 8) with an* $Amy^{1.00}$ *allele*

Gene arrangement	Strain	Allele	Number of samples assayed	Percent relative activity ± 1 S.D.
TL	1	*0.84*	5	42.2 ± 5.5
TL	2	*0.84*	5	39.0 ± 4.5
TL	4	*0.84*	15	39.2 ± 4.0
TL	5	*0.84*	7	37.7 ± 4.8
TL	6	*0.84*	7	37.6 ± 3.7
TL	7	*0.84*	11	38.1 ± 3.0
TL	8	*0.84*	7	40.0 ± 2.6
TL	9	*0.84*	7	37.0 ± 3.2
CH	1	*0.84*	10	37.5 ± 4.9
CH	2	*0.84*	12	35.5 ± 2.6
AR	4	*0.84*	15	32.7 ± 3.8
ST	3	*0.84*	4	41.0 ± 6.2

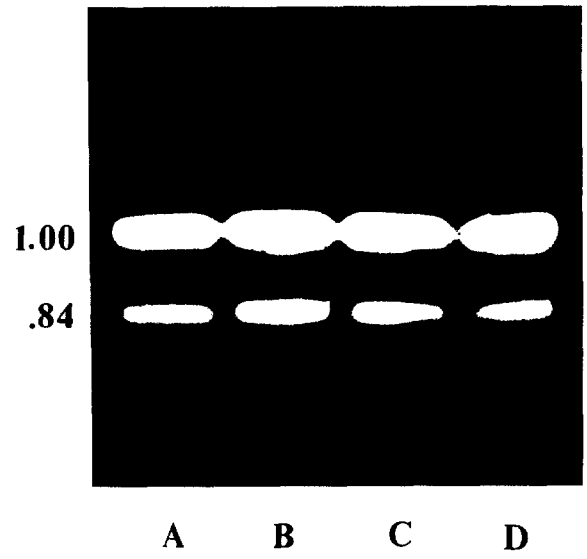

FIGURE 5.—Photograph of gel showing amylase activities in F_1 heterozygotes of *D. pseudo obscura–D. persimilis* hybrids. Each pocket contained independent samples from the following crosses: A,B; TL 4, $Amy^{0.84}$ (*D. pseudoobscura*) × KL 8, $Amy^{1.00}$ (*D. persimilis*). C,D; ST 3, $Amy^{0.84}$ (*D. pseudoobscura*) × KL 8, $Amy^{1.00}$ (*D. persimilis*).

(Tables 1 and 2). The 1.09 strains of WT are equivalent among themselves (Table 5) and are four times as active as the 1.00 strains of *D. pseudoobscura* (Table 9). The WT 1.09 strains are two and one-third times as active as the KL 1.00 strains, all of which are equivalent in amylase activity. Thus, the 1.00 allozymes of KL should be about 1.6 times as active as the 0.84 TL strains when these alleles are present together in heterozygotes. As expected, the KL 8 1.00 allozyme is about 1.5 times as active, on the average, as strains of *D. pseudo-obscura* TL 0.84 (Table 10).

Quantitative comparisons of *D. pseudoobscura* alleles with those of *D. miranda* were not undertaken since the activity differential of amylases between these species was too great for accurate comparisons.

Segregational analysis

F_2 segregational analyses were done on selected strains representative of the activity differences detected in F_1 heterozygotes (Table 11). These data indicate that the activity co-segregates with the amylase locus. In only two of sixteen *t* test comparisons of F_1 *versus* F_2 heterozygotes was there a significant difference between means, and these cases are not dramatically different. F_2 analyses with homokaryotypes of WT, KL and CH failed to reveal any F_2 heterozygote that may be considered recombinant for amylase expression and the structural *Amy* allele. These data strongly suggest that the activity differences are at the amylase locus or a closely linked region.

TABLE 11

Comparisons of relative activities of Amy *alleles in F_1 and F_2 heterozygotes*

Parental cross	Allele	Generation	Number of samples assayed	Percent relative activity ± 1 S.D.	*t* value
CH 2 × ST 8	*1.00*	F_1	7	53.1 ± 3.0	2.35 (p=0.04)
		F_2	7	57.1 ± 3.3	
CH 2 × CH 5	*1.00*	F_1	6	55.3 ± 2.6	2.01 N.S.
		F_2	6	58.5 ± 2.9	
CH 2 × CH 7	*1.00*	F_1	15	58.3 ± 2.8	0.26 N.S.
		F_2	10	58.6 ± 3.8	
CH 2 × CH 4	*1.00*	F_1	15	58.8 ± 4.5	0.42 N.S.
		F_2	25	59.5 ± 4.1	
KL 8 × TL 7*	*0.84*	F_1	11	36.1 ± 3.0	0.22 N.S.
		F_2	15	36.5 ± 5.1	
KL 8 × ST H2	*0.92*	F_1	10	39.6 ± 3.3	0.11 N.S.
		F_2	8	39.4 ± 5.5	
KL 8 × KL R2	*1.09*	F_1	13	64.6 ± 4.7	0.06 N.S.
		F_2	14	64.7 ± 4.6	
WT 10 × MD H106	*0.92*	F_1	14	39.9 ± 3.0	0.58 N.S.
		F_2	9	40.9 ± 5.5	
WT 10 × WT 1	*1.00*	F_1	9	36.1 ± 3.4	2.06 (p=0.05)
		F_2	15	39.3 ± 3.9	
WT 10 × MD 33	*1.00*	F_1	12	51.5 ± 2.2	1.95 N.S.
		F_2	8	49.3 ± 2.9	
WT 10 × KL H95	*1.00*	F_1	14	29.8 ± 5.9	1.92 N.S.
		F_2	11	33.8 ± 4.3	
WT 10 × KL 155	*1.00*	F_1	12	31.5 ± 3.5	1.31 N.S.
		F_2	11	29.4 ± 4.3	
WT 10 × KL 4	*1.00*	F_1	13	27.8 ± 4.4	0.50 N.S.
		F_2	9	28.9 ± 6.2	
WT 10 × KL 33	*1.00*	F_1	11	27.2 ± 4.1	0.62 N.S.
		F_2	10	28.2 ± 3.2	
WT 10 × AR 7*	*1.00*	F_1	5	21.2 ± 6.0	0.36 N.S.
		F_2	6	20.0 ± 5.2	
WT 10 × PP 12*	*1.00*	F_1	6	23.7 ± 8.5	0.93 N.S.
		F_2	9	20.1 ± 6.4	

CH 2, KL 8 and WT 10 in the first column represent reference strains of electrophoretic mobilities 0.84, 1.00 and 1.09, respectively. The strain carrying the assayed allele is on the right. F_1 data are from the previous tables. F_2 data are assayed on single F_2 heterozygous segregants from $F_1 \times F_1$ mass matings.

* Backcrosses of *D. persimilis* males with the F_1 interspecific female hybrids between *D. pseudoobscura* and *D. persimilis*.

DISCUSSION

The associations of electrophoretic alleles with inversions were first described by PRAKASH and LEWONTIN (1968). Subsequently, inversion-associated allozymes have been reported in other Drosophila species. It now appears that geographical differentiation of inversions plays a major role in geographical

variation for associated allozymes. Based upon both the association of alleles and the detectable frequencies of "wrong" alleles in particular arrangements, PRAKASH and LEWONTIN (1968) postulated that associations of alleles within chromosome 3 inversions were evidence of co-adaptation at the genic level. NEI and LI (1975), however, using theoretical arguments, have claimed that the data on associations do not significantly depart from those expected under a mutation-drift hypothesis. ISHII and CHARLESWORTH (1977) have reviewed reported associations between allozymes and chromosome arrangements in Drosophila. They infer that the rates of double crossovers or conversions between inversion heterozygotes are probably too low to refute a hitch-hiking explanation for most associations. It is unclear whether any gene-frequency studies can resolve the question of whether these loci are part of the genic co-adaptation, hypothesized for chromosome 3 inversions. Our data pertain to this problem since we show that inversions are more genetically isolated than electrophoretic data have suggested.

Activity variation of amylases in species of the *D. pseudoobscura* group displays a striking nonrandomness similar to allozyme associations. If we examine the variation separately within each electrophoretic mobility class, then this procedure gives us essentially another tool for exploring the genetic structure of the locus, the structural region and the linked regulatory region. For *Amy*, there is essentially a hierarchy in the way genetic variation could be structured. At the upper level of sibling species, gene exchange does not occur, so that similarities and differences are due to mutation, selection and drift. Chromosome inversions are similar in this respect to different species, in that genes are isolated into separate pools. In *D. melanogaster*, the majority of recombination in simple inversion heterozygotes seems to be due to gene-conversion events (CHOVNICK 1973). The amylase locus in *D. pseudoobscura* is located within the inverted sequence of most gene arrangements used in this study (YARDLEY 1974). Recombination between different inversions must be small for amylase and closely linked loci—perhaps as low as the rate of gene conversions (10^{-5}) observed for the rosy heteroalleles in *D. melanogaster*. Electrophoretic allele associations with inversions may develop because of this lack of gene exchange among chromosomal types. For this reason, the electrophoretic structure of the locus can be considered in parallel to the chromosomal structure. At the lowest level, variation could potentially exist between strains within an inversion type and electrophoretic mobility class.

Distinction between similar electromorphs of D. pseudoobscura and *D. persimilis*

It is important to examine the pattern of variation between *D. persimilis* and *D. pseudoobscura*, since they share a set of amylase alleles. This aspect of our study is similar to the studies of Cobbs and Prakash (1977) and COYNE (1976), who showed more extensive divergence between these species for the esterase-5 and xanthine dehydrogenase loci, respectively. Our results are even more dramatic simply because these species are more similar for *Amy* electrophoretic alleles than they originally were for esterase-5 and xanthine dehydrogenase

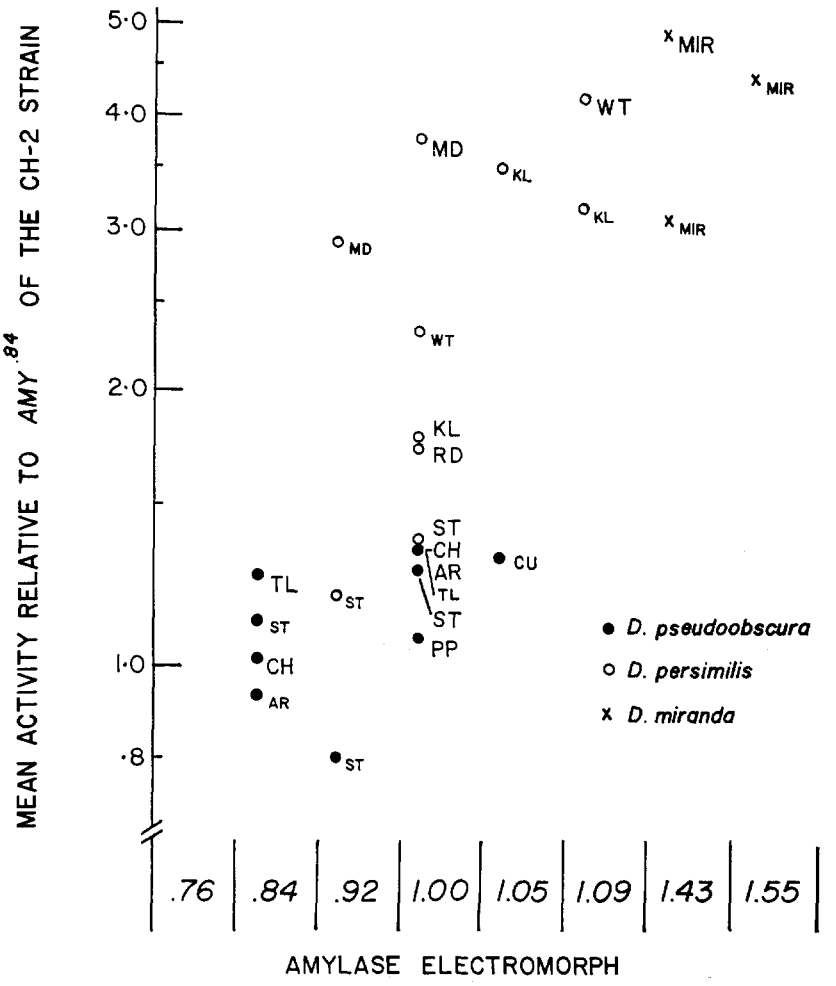

FIGURE 6.—Mean activity ratios of strains (in *D. pseudoobscura*, *D. persimilis* and *D. miranda*) within an inversion type and electrophoretic mobility class relative to CH 2, $Amy^{0.84}$. Chromosome arrangements in large letters have the corresponding electromorph in high frequency; smaller letters represent either uncommon electromorphs or "wrong" associations. Points representing *D. miranda* are based on discernible activity classes, the large letters being the main class. Means of inversions that were assayed against CH 2 were calculated directly. Other inversion means were estimated indirectly from comparisons with other reference strains (*e.g.*, KL 8 × CH 2, WT 10 × KL 8, PP 1 × CH 2). This graph was constructed based on the observation that most variation was detected among inversions and serves to give an overview of the pattern of variation between electromorphs and inversions. (Consult the tables for statistical analyses of the data.)

alleles. We have demonstrated near-complete genetic divergence for amylase activities between these two species (Figure 6). The *1.00* amylase alleles in KL, RD, MD and WT inversions of *D. persimilis* are all expressed differently from any of the *1.00* alleles in *D. pseudoobscura*. Significantly, the only simi-

larity remaining between these species is that of the *1.00* electrophoretic allele of ST, the only inversion shared between the species.

Not only did the major *1.00* class break down between species, but our lines with rarer alleles were also heterogeneous in a nonrandom way. We were able to examine three *D. persimilis* $Amy^{0.92}$ and one *D. pseudoobscura* $Amy^{0.92}$ strains (Figure 6). Here again, the activity of the 0.92 electromorph in *D. persimilis* differs from the activity of the 0.92 electromorph in the ST arrangement of *D. pseudoobscura*. Unlike the activities of the 1.00 electromorph in this arrangement, the ST strains of the two species are different in activity for the 0.92 allozyme. We have also examined two 1.05 electromorphs in the CU arrangement of *D. pseudoobscura* and a KL arrangement of *D. persimilis*. Again the species differ in the activity expression of this electromorph.

Activity variation associated with different chromosome versions

A large part of our results are summarized in Table 12, which gives the percent of components of variance for inversion and strain differences within the common Amy allelic classes of *D. pseudoobscura* and *D. persimilis*. Substantial portions of the activity variance are almost always due to inversion differences, but the picture is somewhat different within each species (Figure 6). In *D. pseudoobscura*, the main 1.00 class largely resists further subdivision into activity classes. Many of the PP lines, however, are slightly lower in activity than the 1.00 strains of other arrangements. The majority of the 1.00 strains of the chromosomes in the Standard phylad are not detectably different from strains with the "wrong" 1.00 allozyme in the Santa Cruz plylad. The genetic picture is similar for the 0.84 electromorph. The activity variation is not large but the major detectable variation is found among inversions. There is no relationship between inversion relatedness and activity differences. For example, the 0.84 allozyme seems to differ between AR and ST, as does the 0.84 allozyme between CH and TL. These pairs of arrangements differ by one inversion event. On the other hand, ST and TL differ by four events, yet are very similar in the 0.84 amylase expression.

TABLE 12

Components of variance for gene arrangements and strains with common Amy *electrophoretic alleles in* D. pseudoobscura *and* D. persimilis

Species	Gene arrangements	Total d.f.	Total sum of squares	Variance component		Reference strain
				Gene arrangements	Strains	
D. pseudoobscura	TL,CH,ST,AR	104	2,059	24.7%	6.2%	KL 8
		117	2,169	54.3%*	6.4%†	PP 1
		79	1,221	37.6%†	7.5%	AR 1
	ST,PP,AR,TL,CH	183	3,250	29.6%**	7.3%†	CH 2
D. persimilis	MD,KL,RD,WT	229	17,737	80.7%**	3.0%**	WT 10
	KL,RD,WT	180	4,502	19.2%*	0.0%	
	WT,KL	142	4,334	43.5%**	1.4%	KL 8

†($P<0.05$). *($P<0.01$). **($P<0.001$).

Within *D. persimilis*, we find a greater range of activity variation and clearer distinctions between inversions. This result is most dramatic among 1.00 strains (MD, KL, RD, WT) where over 80% of the variance is attributable to inversion differences. Moreover, this 1.00 data set does not include the M4 strain of the ST arrangement, which is also greatly different from the other arrangements of *D. persimilis* (Table 6). In the case of the *0.92* electromorph, a major difference in amylase expression also exists between the ST strain and the two strains of MD. The 1.09 strains have high activity in general, but those of the KL arrangement are all less active than any of the WT 1.09 strains examined. We have already noted that the WT-1 line with a *1.00* allele is more active than any KL 1.00 strain. Thus, WT chromosomes generally show more amylase activity than KL chromosomes having the same electrophoretic allele. Thus, in *D. persimilis*, the activity of every amylase electromorph is strongly dependent upon the arrangement in which it is located.

We have been unable to detect much variation among strains with the same electrophoretic allele within an inversion type. Among the KL 1.00, RD 1.00 and WT 1.09 strains, no significant variation was detected within each of these inversion types. Only within the MD arrangement, among the 1.00 strains, does significant variation appear.

The original study of electrophoretic variation of amylase in these species showed both the association of electrophoretic alleles with inversions and the presence of "wrong" alleles in low frequencies in different arrangements (PRAKASH and LEWONTIN 1968). When activity variation is examined among different gene arrangements, we find that the extent of differentiation between inversions is far greater; that is, inversions carrying the same electromorph can be quite different genetically in the expression of the amylase enzyme. Furthermore, the electromorphs involved in the so called "wrong" associations, which have suggested that a common allele in one gene arrangement occurs in low frequency in another, are actually genetically distinct between inversions in many cases. *Inversions, like species, are more genetically different than is apparent from electrophoretic data.* Our results support the general notion that inversions, through the repression of recombination, can be effective in the isolation of sub-gene pools within a species.

Genetic nature of activity variants

We have shown significant genetic variation in the expression of amylase among sibling Drosophila species. The activity variants described here may be due to differences in the enzymes themselves, such as some kinetic parameter or stability property which affects the amounts of enzymes present. Amount differences could also be due to regulatory variants that affect translation and/or transcription. We have no information to distinguish between these alternatives. The 1.09 allozyme has high activity regardless of whether it is in the KL or WT gene arrangement. This allozyme is structurally different from the 1.00 allozyme, so that some physical properties of the enzymes might account for this difference. Likewise, the 1.43 and 1.55 allozymes are unique to *D. miranda*.

It may be that high activity of these allozymes has resulted from changes in the structural gene. On the other hand, the *1.00* allelic class is highly polymorphic for activity and this class has resisted further subdivision under extensive electrophoretic conditions of varying gel concentration and pH. Both the 0.92 and 1.00 allozymes have elevated activities in the MD arrangement as compared to their activities in other arrangements. This may indicate regulatory differences in different gene arrangements. The alternative explanation would be that *1.00* alleles in different gene arrangements are different structural gene mutations with altered enzyme properties within the same mobility class.

The activity variation described here can not be attributed to the midgut activity polymorphism recently reported for amylase in *D. pseudoobscura* (POWELL and LICHTENFELS 1979; POWELL 1979). The characteristics of our *cis*-acting activity variants are not consistent with those reported for the midgut polymorphism. In contrast to midgut amylase activity polymorphism, our variants are not temporally varying in adults, are associated with chromosome *3* inversion polymorphism and segregate exclusively with the structural amylase locus.

Implications of activity variation in the three species

The widest range of activity variation by far is found in *D. persimilis*, ranging four-fold between ST and WT, and MD strains (Figure 6). While there is variation present in *D. miranda* and *D. pseudoobscura*, it is more constrained in its range than that in *D. persimilis*. *D. miranda* alleles are distributed on the high activity side of *D. persimilis*, while *D. pseudoobscura* alleles are mainly lower than the lowest activity *D. persimilis* alleles. This pattern reflects the ecological intermediacy of *D. persimilis*, whose range is included within that of *D. pseudoobscura* and itself includes the more limited range of *D. miranda*. *D. persimilis* prefers cooler and moister habitats than *D. pseudoobscura*, yet *D. persimilis* is not confined to as cool or as moist areas as *D. miranda* (DOBZHANSKY and EPLING 1944). If the amylases of *D. miranda* are compared with *D. pseudoobscura*, the level of activity expression in *D. miranda* is four to five times that of *D. pseudoobscura*. The *1.09* electrophoretic allele that characterizes only the WT arrangement has the highest activity level of any alleles in *D. persimilis* being about equal to the *1.43* and *1.55* alleles of *D. miranda* (Figure 6). Both natural population collections (DOBZHANSKY and EPLING 1944; DOBZHANSKY 1948) and laboratory studies (MOHN and SPIESS 1963) clearly show that WT is the predominant arrangement of the colder habitats within the range of *D. persimilis* and seems like *D. miranda in* its temperature preference. Thus, there is some correlation between amylase activity and habitat due to the associations of particular amylase alleles with species and inversions.

The pattern of activity variation within the three species is rather surprising. That is, why is the range of variation in *D. persimilis* so much greater than that of *D. miranda* and *D. pseudoobscura*? In *D. pseudoobscura*, for both the 0.84 and 1.00 allozymes, genetic variants in amylase activity can be detected. Likewise, such variants can also be found in *D. miranda*. Yet, within each of these two

species, no variation was found outside a rather limited range, relative to the range of amylase expression in *D. persimilis*. One possible explanation for the large range of variation in *D. persimilis* could be that the amylase region is located close to inversion breakpoints. Differences in amylase activity among inversions could then be due to position effects. We do not consider this as a likely explanation, since the breakpoints of many inversions (DOBZHANSKY and EPLING 1944) are far from the suggested position of the amylase locus (YARDLEY 1974). Another possibility, suggested by this pattern, is that genetic variants causing activity modifications are selected against if they fall outside of some normal range. This range might be dictated by some of the differences in environmental variables faced by these species. This reasoning implies that high activity variants are not present in detectable frequencies in *D. pseudoobscura* because high activity is selected against, while in *D. miranda* the reverse could be true: low activity amylase variants are disadvantagous. Again, this speculation is critically based on the fact that such variants have become established in *D. persimilis*. Of course, there may be other important distinctions between alleles not revealed in our activity assays.

The historical uncertainty of the origins of inversions within the phylogeny allows different interpretations for the patterns of activity associations. It may be that *D. persimilis* inversions are "old" and, therefore, have had more time for genetic divergence of amylase alleles; whereas, *D. pseudoobscura* inversions are of more recent origin. However, at several chromosome 3 loci, genetic differentiation does exist among inversions of *D. pseudoobscura*. For *Pt-10* and *Amy*, the Santa Cruz and Standard phylads show the major differentation in electrophoretic alleles (PRAKASH and LEWONTIN 1968). *AP-3* is similarly differentiated between phylads except for the PP arrangement, which has an allele found predominantly in the Santa Cruz phylad (PRAKASH 1976). *D. persimilis* shows no differentiation of electrophoretic alleles at the *AP-3* locus. At the *Pt-12* locus, all arrangements of *D. persimilis* have similar allele frequencies, except ST and WT; whereas, in *D. pseudoobscura*, the ST arrangement is differentiated from the AR and PP arrangements (PRAKASH and LEWONTIN 1971). Thus, the amylase activity divergence between the ST, KL and MD inversions of *D. persimilis* is not mirrored by electrophoretic differentiation at the amylase or other chromosome 3 loci.

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