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Quantitative genetic variation of enzyme activities in natural populations of *Drosophila melanogaster*

(population genetics/modifier loci/regulatory elements/protein polymorphism)

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ABSTRACT The genetic component of variation of enzyme activity in natural populations of *Drosophila melanogaster* was investigated by using two sets of chromosome substitution lines. The constitution of a line of each type is: $i_1/i_1;+_2/+_2;i_3/i_3$ and $i_1/i_1;i_2/i_2;+_3/+_3$, where *i* refers to a chromosome from a highly inbred line and + refers to a chromosome from a natural population. The + but not the *i* chromosomes vary within a set of lines. By use of a randomized block design to test and estimate components of variance, 50 of the second- and 50 of the third-chromosome substitution lines have been screened for variation in the activity levels of seven enzymes. Six of the seven enzymes show a significant genetic component in at least one set of lines, and five of the seven enzymes show activity variations attributable to factors that are not linked to the structural gene. These unlinked activity modifiers identify possible regulatory elements. Analyses of covariance show that most of the genetic variation of enzyme activities cannot be accounted for by genetic variation of live weight or protein content. These results and the lack of strong correlations between the genetic effects on the activities of different enzymes indicate that the effects are mainly specific for individual enzymes.

Naturally occurring genetic variants that affect activity levels have been reported for a number of enzymes in *Drosophila melanogaster*. Characterization of these variants has basically involved attempts at classification into three types of genetic units: a structural gene and two types of modifier genes, one that maps very close to the structural gene and one that maps some distance away. Here we define modifier genes as loci that affect enzyme activity levels without affecting the primary structure of the polypeptide(s) at the time of translation. The mechanisms of modifiers may, of course, involve differential rates of transcription, mRNA processing, or translation, which are generally regarded as "regulatory," or other processes such as posttranslational modification, intracellular compartmentalization, or differential rates of degradation.

Although most activity variants in *D. melanogaster* have not been well characterized, probable examples of variants of each of the three types of genetic unit have been identified (1–8). These studies and recent models of the regulation of gene expression in eukaryotes (9, 10) suggest that in natural populations there may be several polymorphic loci distributed throughout the genome that affect the expression of a given structural gene and therefore contribute to variation in the activity of an enzyme. However, at present there is very little quantitative information about the extent of genetic variation of enzyme activities, the relative importance of structural, regulatory, or other types of genes in causing this variation, and the number, organization, and types of activity modifiers that are polymorphic in natural populations. Here we report some preliminary results of a study designed to investigate these questions. This study may ultimately prove useful in the design of experiments to test the adaptive significance of enzyme variability and will also have a bearing on the suggestion that regulatory variation of enzyme activity levels is a more important source of adaptive variation than structural variability (9, 11).

Our basic approach to quantifying the amount of genetic variation of enzyme activity in natural populations of *Drosophila* is to view activity as a quantitative trait and to use standard biometrical methods to partition its variance into genetic and environmental components. In order to localize activity variants, two sets of homozygous lines were constructed in which either second or third chromosomes from natural populations were substituted onto an isogenic background. Within the set of second-chromosome substitution lines, for example, all X and third-chromosome loci are constant but second-chromosome loci vary. This design permits detection of activity variants that are not linked to the structural locus of the enzyme and can therefore easily identify modifier loci.

The enzymes assayed in this study are glucose-6-phosphate dehydrogenase (G6PD; D-glucose-6-phosphate:$NADP^+$ oxidoreductase, EC 1.1.1.49), 6-phosphogluconate dehydrogenase (6PGD; 6-phospho-D-gluconate:$NADP^+$ oxidoreductase, EC 1.1.1.44), fumarate hydratase (FUM; L-malate hydro-lyase, EC 4.2.1.2), α-glycerophosphate dehydrogenase (α-GPDH; *sn*-glycerol-3-phosphate:NAD^+ 2-oxidoreductase, EC 1.1.1.8), alcohol dehydrogenase (ADH; alcohol:NAD^+ oxidoreductase, EC 1.1.1.1), catalase (CAT; H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6), and aldehyde oxidase (AOX; aldehyde:O_2 oxidoreductase, EC 1.2.3.1). Electrophoretic variants are known for each of these enzymes except catalase and have been used to map the locations of the structural genes: G6PD, 1-63.0; 6-PGD, 1-0.64; FUM, 1-19.9; α-GPDH, 2-20.5; ADH, 2-50.1; and AOX, 3-56.6 (3). The structural gene for catalase is probably located in region 75D–76A on the third chromosome; this is the only dosage-sensitive region in the genome (12).

MATERIALS AND METHODS

The procedure for substitution of second chromosomes derived from natural populations onto an isogenic background is shown in Fig. 1. Third-chromosome isogenic substitution lines ($i_1/i_1;i_2/i_2;+_3/+_3$) were constructed in an analogous manner with use of the balancer $i_1/i_1;i_2/i_2;TM6$, *Ubx/Sb*. During construction of these lines, the isogenic background chromosomes were never heterozygous in females and were only heterozygous with balancer chromosomes in males. Fifty second- and

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Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase; FUM, fumarate hydratase; α-GPDH, α-glycerophosphate dehydrogenase; ADH, alcohol dehydrogenase; CAT, catalase; AOX, aldehyde oxidase.

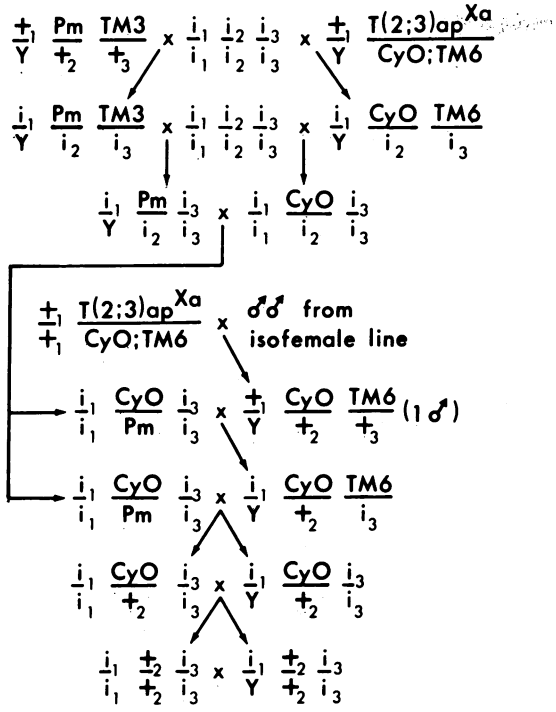

FIG. 1. Procedure for construction of a second-chromosome isogenic substitution line [*Pm* = *In(2LR)bw*^{V1}, *Sp--TM3* = *In(3LR)TM3, Sb Ser--TM6* = *In(3LR)TM6, Ubx--i* = chromosome from *Ho-R* inbred line].

fifty third-chromosome substitution lines were constructed in 1978 and used in this study. The isofemale lines were collected in late summer 1977 in Lawrence, KS, by P. W. Hedrick; in Providence, RI, by M. G. Kidwell; in Cochrane, WI, by D. C. Dapkus; and in Raleigh, NC. The isogenic background stock (*Ho-R*) was originally collected in Hochi, Japan, in 1968, made isogenic with balancer chromosomes, and then maintained by single-pair matings for 130 generations before use in this study. The *Ho-R* stock was kindly provided by W. W. Doane. Other stocks are described by Lindsley and Grell (13).

After construction of the 100 isogenic lines, each one was tested for homozygosity of allozymes at 17 enzyme loci (see ref. 14 for the loci and electrophoretic procedures). The *Ho-R* stock was homozygous at all 17 loci. All of the third-chromosome lines were identically homozygous for the X and second-chromosome allozymes found in *Ho-R* and were homozygous for various different allozymes on the third chromosome, as expected. Similar results were obtained for the second-chromosome lines except that acid phosphatase (*Acph-1*, 3-101.0, ref. 3) was polymorphic in the balancer stock i_1/i_1;*CyO/bw*^{V1}, *Sp*;i_3/i_3 and consequently in some of the second-chromosome lines. Presumably this variation resulted from a male recombination event that occurred in a balancer heterozygote. All of the enzymes for which activities were measured were homozygous for an appropriate allozyme in all lines.

The enzyme activities were measured in two independent experiments, one for second-chromosome lines and one for third-chromosome lines. In each case, the 50 lines were sampled at each of two times ("blocks"). Within each block, and for each line, four bottles with 50 pairs per bottle from that line were set up in random order over a 3-day period. When progeny began emerging, virgin females and males were collected and aged separately for 6 days, at which time they were weighed in sets of 10 and frozen at −70°C. CAT and α-GPDH were assayed from one set of 10 males; ADH, AOX, G6PD, 6PGD, FUM, and general protein were assayed from another set of 10 males and from a set of 10 females. The enzyme assays and activity units are described elsewhere (G6PD and 6PGD, ref 15; FUM, ref. 16; α-GPDH, ref. 17; ADH, ref. 7; CAT, ref. 12; and AOX, ref. 18), and general protein concentration was estimated by the colorimetric method of Bio-Rad (Bio-Rad Laboratories Bulletin 1056).

RESULTS

The ranges of line means from the randomized block experiments for both second- and third-chromosome substitution lines are presented in Table 1. These means are based on the raw data, activity units per set of 10 flies, with sample sizes of one observation per line per block for enzyme activities and protein, three observations per line per block for female live weight, and seven observations per line per block for male live weight. Table 1 shows that the ratios of highest to lowest line mean in males fall between the limits 1.5–3.8 for all enzymes except ADH in second chromosome lines, for which the ratio exceeds 13. The line means for enzyme activities as well as for general protein and live weight have essentially continuous distributions with one exception—a discontinuity in the distribution of ADH activities in second-chromosome lines, which was due to lines carrying either Slow or Fast ADH allozymes, as discussed below.

In the simplest form of the analysis of variance (ANOVA) of these data, treating sexes separately, there are just three sources of variation: blocks, lines (genotypes), and error. Table 2 summarizes the results of these ANOVAs performed on the raw data, activity units per 10 flies. The significance levels of the *F* tests for blocks and lines and the ratio of variance component estimates, $\hat{\sigma}_l^2/(\hat{\sigma}_l^2 + \hat{\sigma}_e^2)$, are given in Table 2 (where $\hat{\sigma}_l^2$ is the line variance component estimate and $\hat{\sigma}_e^2$ is the error mean square). The ratio $\hat{\sigma}_l^2/(\hat{\sigma}_l^2 + \hat{\sigma}_e^2)$ is the proportion of the variation among the observations corrected for block effects that is due to line (genetic) differences.

The ANOVAs on activity units per 10 males show that lines are a significant source of variation for α-GPDH, ADH, AOX, G6PD, and FUM in second-chromosome lines and α-GPDH, ADH, CAT, AOX, and G6PD in third-chromosome lines. The variance component ratio ranges from 0.25 for G6PD to 0.83 for ADH (both in second-chromosome lines) for these cases. Not only do all but one, 6PGD, of the seven enzymes show a sig-

Table 1. Ranges of line means from the screen of second- and third-chromosome substitution lines

Variable (per fly)		Chromosome 2	Chromosome 3
α-GPDH, units × 10	♂♂	95.1–204.8	111.4–178.4
ADH, units × 10^3	♂♂	3.8–50.4	24.7–48.7
	♀♀	2.1–60.9	24.0–47.0
CAT, units	♂♂	2.69–3.92	2.01–4.18
AOX, units × 10^{-2}	♂♂	1.25–3.02	1.27–4.25
	♀♀	1.65–3.62	1.61–5.41
G6PD, units × 10^3	♂♂	10.1–25.4	10.9–41.5
	♀♀	10.9–31.2	15.7–49.5
6PGD, units × 10^3	♂♂	10.1–22.1	11.7–25.4
	♀♀	15.7–37.0	15.7–49.5
FUM, units	♂♂	121.0–220.0	135.0–209.0
	♀♀	151.0–272.0	188.0–303.0
Protein, μg	♂♂	44.0–80.5	50.0–85.0
	♀♀	63.5–140.0	65.0–136.0
Weight, mg	♂♂	0.715–0.938	0.788–1.134
	♀♀	0.980–1.328	1.032–1.642

Table 2. Summary of analysis of variance of activity per 10 flies (ANOVA) and the analysis of covariance of activity per 10 flies with live weight as the covariate (ANOCOV)

Variable		Chromosome 2						Chromosome 3					
		Males			Females			Males			Females		
		F test		$\hat{\sigma}_l^2$	*F* test		$\hat{\sigma}_l^2$	*F* test		$\hat{\sigma}_l^2$	*F* test		$\hat{\sigma}_l^2$
		Blocks	Lines	$(\hat{\sigma}_l^2 + \hat{\sigma}_e^2)$	Blocks	Lines	$(\hat{\sigma}_l^2 + \hat{\sigma}_e^2)$	Blocks	Lines	$(\hat{\sigma}_l^2 + \hat{\sigma}_e^2)$	Blocks	Lines	$(\hat{\sigma}_l^2 + \hat{\sigma}_e^2)$
α-GPDH (2)	ANOVA	***	***	0.61	—	—	—	***	***	0.52	—	—	—
	ANOCOV	***	***	0.66	—	—	—	***	**	0.35	—	—	—
ADH (2)	ANOVA	***	***	0.83	***	***	0.74	NS	*	0.29	***	**	0.35
	ANOCOV	**	***	0.83	*	***	0.80	*	*	0.29	***	***	0.56
CAT (3)	ANOVA	**	NS	0.10	—	—	—	*	**	0.40	—	—	—
	ANOCOV	NS	NS	0.05	—	—	—	*	*	0.32	—	—	—
AOX (3)	ANOVA	*	***	0.52	**	NS	−0.11	***	***	0.81	***	***	0.89
	ANOCOV	NS	***	0.46	NS	NS	−0.01	***	***	0.79	***	***	0.91
G6PD (X)	ANOVA	***	*	0.25	NS	NS	0.19	*	**	0.33	NS	*	0.27
	ANOCOV	***	NS	0.21	***	*	0.27	**	*	0.28	*	*	0.32
6PGD (X)	ANOVA	NS	NS	0.13	***	NS	−0.04	*	NS	0.17	NS	NS	0.13
	ANOCOV	NS	NS	0.08	*	NS	0.16	**	NS	0.15	NS	NS	0.14
FUM (X)	ANOVA	**	**	0.37	***	NS	0.10	NS	NS	0.20	NS	*	0.26
	ANOCOV	NS	**	0.35	NS	NS	0.09	NS	NS	0.06	NS	*	0.28
Protein	ANOVA	***	NS	0.19	***	NS	−0.06	*	NS	0.13	***	NS	0.18
Weight	ANOVA	***	***	0.57	***	NS	0.25	NS	***	0.73	NS	***	0.57

Significance levels of the *F* tests and the ratio of variance component estimates, $\hat{\sigma}_l^2/(\hat{\sigma}_l^2 + \hat{\sigma}_e^2)$, are given. The linkage groups of the structural loci are given in parentheses.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant ($P > 0.05$).

nificant genetic component of activity variation, but also five of the seven enzymes, α-GPDH, ADH, AOX, G6PD, and FUM, show evidence for genetic activity variants that are not linked to the structural locus.

There is also a significant line component for live weight of males in both sets of lines and for females in third-chromosome lines, which suggests that genetic variation of enzyme activities on a per fly basis could be caused simply by variation in live weight. Analysis of the ratio of activity units to live weight does not necessarily address this issue because the ratio may be correlated with weight (which was true for about one-fourth of the ratio variables in this study). A more appropriate analysis is provided by the analysis of covariance (ANOCOV) with weight as the covariate, which essentially adjusts activities by regression to what they would be if weights were the same for each fly. The results of the ANOCOVs are also summarized in Table 2. In most cases the variance component ratio $\hat{\sigma}_l^2/(\hat{\sigma}_l^2 + \hat{\sigma}_e^2)$ for males is less in the ANOCOV than in the ANOVA of activity per 10 flies, which indicates that weight variation does contribute somewhat to the line component of variation of activities on a per fly basis. However, the differences are all small and in only case, G6PD in second-chromosome lines, were lines significant in the ANOVA but not in the ANOCOV. For females, the variance component ratio is usually greater in the ANOCOV than in the ANOVA of activity per 10 flies, which indicates that weight variation of females tends to obscure the line component of variation in activities on a per fly basis. ANOVAs of the ratio, activity per live weight, give results very similar to the ANOCOVs. We conclude that genetic variation in activity per fly is not accounted for by variation in live weight per fly.

In addition to the above analyses of covariance, correlations of activities of different enzymes provide information about the specificity of the genetic effects on activity. Two types of correlations were computed: *r*, the product-moment correlation obtained by pairing enzyme variables between blocks, and r^*, a standardized covariance component estimate. Let $\hat{\sigma}_{xl,yl}$ be the estimated covariance of line effects on enzymes *x* and *y* and $\hat{\sigma}_{xl}^2$, $\hat{\sigma}_{yl}^2$ and $\hat{\sigma}_{xe}^2$, $\hat{\sigma}_{ye}^2$ be the line and error variance component estimates, respectively; then $r = \hat{\sigma}_{xl,yl}/\sqrt{(\hat{\sigma}_{xl}^2 + \hat{\sigma}_{xe}^2)(\hat{\sigma}_{yl}^2 + \hat{\sigma}_{ye}^2)}$ and $r^* = \hat{\sigma}_{xl,yl}/(\hat{\sigma}_{xl}\hat{\sigma}_{yl})$. Because the quantity r^* is not necessarily bounded by −1 and +1 and may not even be defined for negative variance component estimates, no test of the hypothesis that the true value of r^* equals zero is available although a procedure for computing the standard error of r^* is given by Mode and Robinson (19). Because the between-block correlation, *r*, is a true product-moment correlation, testing procedures are standard. For each enzyme pair X and Y, two between-block correlations, $r(X_1, Y_2)$ and $r(X_2, Y_1)$, where the subscripts denote blocks, were computed and averaged by Fisher's *z*-transformation method. Because these two correlations are not independent estimates, the standard error of $\bar{z}$ is not known precisely but lies between $1/\sqrt{47}$ and $1/\sqrt{2(47)}$. The values of *r*, r^*, and the standard error of r^* are given in Table 3 for each pair of enzymes for which lines are a significant component in the ANOCOV. These quantities were computed with activities that had been adjusted by regression on weight, as in the analyses of covariance, in order to avoid spurious correlations due to weight differences among lines.

Most of the r^* estimates in Table 3 are less than SEs in absolute value; only two are greater than twice the value of SE. Also, there is no clear trend in direction of association among enzyme pairs; ten of the *r* values are negative and five are positive. None of the *r* values is significantly different from zero under a "conservative" test (i.e., assuming that the SE of the corresponding *z* transform is $1/\sqrt{47}$) and only one *r* (AOX, FUM, second chromosome) is significant with $P < 0.05$ under a "liberal" test [assume SE of *z* is $1/\sqrt{2(47)}$]. Similar conservative and liberal tests show that the *r* values for third-chromosome enzyme pairs are not significantly heterogeneous and the average, $\bar{r} = -0.024$, is not significantly different from zero. The same results are obtained for the six second-chromosome *r* values, for which $\bar{r} = +0.034$. These results suggest that the genetic effects on activity are specific for the individual enzymes investigated here, although some weak correlations clearly cannot be ruled out.

The line or genetic variance component can itself be partitioned into variance components for state of origin of the

Table 3. Correlation estimates $r^* \pm$ SE and in parentheses, r

	Chromosome 2			
	ADH	α-GPDH	AOX	FUM
ADH	—	−0.041 ± 0.165 (−0.038)	0.254 ± 0.174 (0.158)	−0.097 ± 0.211 (−0.055)
α-GPDH	0.320 ± 0.272 (0.114)	—	0.038 ± 0.201 (0.022)	−0.191 ± 0.220 (−0.096)
AOX	−0.031 ± 0.224 (−0.021)	−0.197 ± 0.205 (−0.100)	—	0.485 ± 0.201 (0.206)
CAT	−0.176 ± 0.370 (−0.058)	0.204 ± 0.273 (0.071)	−0.158 ± 0.212 (−0.084)	—
G6PD	0.574 ± 0.268 (0.173)	−0.404 ± 0.333 (−0.135)	−0.235 ± 0.227 (−0.109)	−0.275 ± 0.364 (−0.086)
	ADH	α-GPDH	AOX	CAT
	Chromosome 3			

Enzyme pairs for second-chromosome lines are above the diagonal and those for third-chromosome lines are below.

wild-type substituted chromosome (Rhode Island, North Carolina, Wisconsin, or Kansas) and for lines within states. States did not provide a significant component of variation for any of the variables in either males or females.

The two enzymes for which the structural element is located on the second chromosome, ADH and α-GPDH, showed allozymic variation among second-chromosome substitution lines, and AOX, located on the third chromosome, showed allozymic variation among third-chromosome lines. CAT, located on the third chromosome by segmental aneuploidy, is electrophoretically monomorphic in all lines. For ADH there is almost no overlap between Fast and Slow lines. The mean ± SD for ADH^F lines is 24.3 ± 9.9 and for ADH^S lines is 8.3 ± 3.8. When the line variance component is partitioned into components for allozymes and for lines within allozymes, both are highly significantly ($P < 0.001$) nonzero. The lines within allozymes component constitutes only about 17% of the total line variance. The large difference in activity between ADH allozymes has previously been reported (20). Only four of the second-chromosome lines carried $\alpha\text{-GPDH}^S$ and, although these four are all in the upper half of the distribution, allozymes are not a significant source of variation. Five allozymic forms of AOX were detected and are designated AOX^1–AOX^5 in order of increasing mobility. The two common forms, AOX^1 and AOX^3, are clustered at opposite ends of the distribution with means ± SD of 3.32 ± 0.84 for AOX^3 and 1.78 ± 0.45 for AOX^1. Both allozymes and lines within allozymes are highly significant sources of variation, with the lines within allozymes component constituting about 18% of the total line variance, which is very similar to the situation for ADH.

Sex differences in mean activities were tested for each block separately because of large block effects for some variables in females. ANOVAs on a per fly basis showed sex to be a significant source of variation for all enzymes in both sets of substitution lines except for ADH in block one, second chromosome. Mean activities for females always exceeded those for males, except for ADH when the direction of the difference was not consistent over blocks. The sex differences of AOX, G6PD, and 6PGD activities appear to be caused by the sex difference in live weight since sex is not a significant source of variation in the ANOCOV with weight as a covariate. For FUM, however, sex is still significant in the ANOCOV.

Coefficients of variation are also greater for females than for males for all variables in both sets of lines. The error variances in the ANOVAs are greater for females than for males, which prevented pooling of sexes for the analysis of line components. Most of the line variance component ratios in Table 2 are greater for males than females and in a few cases the difference is extreme—AOX, FUM, and protein in second chromosome lines. For several enzymes, lines are a significant source of variation in males but not in females while in only one case, FUM for third chromosomes, lines are significant in females but not males. These results illustrate that environmental variation contributes more to the activity variation in females than males, perhaps via egg content.

Finally, Table 2 shows that blocks are a significant source of variation for most variables in each sex and each set of lines. The block differences for second-chromosome lines are in the same direction for all variables, except for G6PD in males, which suggests that they may be attributable to rearing conditions. For third-chromosome lines, however, the block differences do not have a consistent direction among variables, suggesting perhaps that assay conditions are primarily responsible.

DISCUSSION

The ultimate goals of this study are to determine the extent of genetic variation of enzyme activities in natural populations, the relative importance of structural, regulatory, or other types of genes in causing this variation, and the number, organization, and types of modifier loci that are polymorphic in natural populations. Here we have reported substantial genetic variation of the activities of six out of seven enzymes investigated, but at present very little is known about the biochemical and genetic mechanisms that cause the variation.

Five of the seven enzymes investigated show evidence of activity variants that are not linked to the structural gene—G6PD, FUM, α-GPDH, ADH, and AOX. These variants identify modifier loci under the assumption that there is just one structural gene for each of the enzymes and the chromosome containing it has been correctly identified. The evidence for these assumptions is briefly summarized here (see refs. 3, 12, 21, and 22 for documentation): There is either just one isozymic form of the enzyme in homozygotes (G6PD, FUM, and AOX) or the mobilities of all isozymes vary coordinately (α-GPDH and ADH). For ADH, α-GPDH, AOX,and G6PD, both electrophoretic and null mutants map to the same locus (or at least have been localized to the same chromosome) and sensitivity to dosage of the putative structural element has been observed. Also, ADH, α-GPDH, AOX, and G6PD appear to each consist of identical subunits; $NaDodSO_4$ electrophoresis of purified enzyme reveals just one band.

More experiments are obviously required to clarify the biochemical nature of activity variants that are on the same

chromosome as the structural gene and those that are unlinked. Nevertheless, the present evidence suggests that variants of modifier loci may contribute substantially to the total variation of enzyme activity in natural populations. For example, the range of line means of α-GPDH for third chromosomes (111.4–178.4) is nearly as great as for second chromosomes (95.1–204.8), and the line variance component ratios are similar (0.52 for third chromosomes compared to 0.61 for second chromosomes). The results for ADH and AOX, however, suggest that allozymic variation is responsible for most of the activity variation observed. The relative importance of modifier and structural variants may, of course, vary among enzymes.

The degree of specificity of the genetic effects on enzyme activity is a critical point for understanding their significance. The analyses of covariance with live weight as covariate suggest that weight variation does not account for most of the variation in activity per fly. The correlations between the genetic effects on the activities of different enzymes suggest that these effects are mainly specific for the individual enzymes investigated, but the results in Table 3 clearly do not rule out some weak correlations. If the effects were highly correlated, a common genetic cause would be indicated, but even if they are truly uncorrelated, the activity variation of a number of enzymes may nevertheless be caused by polymorphism at the same locus or loci. For example, variation among the isogenic lines at loci that affect the pattern of aging in adults may cause uncorrelated variations in the activities of a number of enzymes because different enzymes have different types of developmental curves. Only detailed developmental, biochemical, and genetic studies of particular pairs of high- and low-activity lines will resolve these problems about the nature of the genetic variation reported here.

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