P-Element-Induced Variation in Metabolic Regulation in Drosophila

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ABSTRACT

Movement of transposable elements has been demonstrated to be a cause of genetic variation that is relevant to quantitative characters in Drosophila. Here a particular class of *P*-element-induced variation known to be mediated through changes in expression of targeted enzyme-encoding genes is examined. Balancer chromosomes and the "jumpstarter" modified *P*-element were used to construct 124 second-chromosome and 139 third-chromosome lines of *Drosophila melanogaster* bearing unique stable *P*-element insertions in a common genetic background. Lines that were homozygous for second-chromosome *P*-element insertions were significantly more heterogeneous than control lines in 10 of 16 characters, whereas third-chromosome insertion lines were heterogeneous in 11 of the 16 traits. The average mutational variance per insertion relative to environmental variance (V_{mI}/V_e) was 5.7×10^{-2}, and estimates varied widely across characters. The distributions of mutational effects tended to be skewed, with a longer tail toward high enzyme activities. Mutational effects deviated from a normal distribution in 15 of the 16 traits and significant outlier lines were found in both a positive and negative direction in several characters. Pleiotropic effects of single *P*-element insertions were quantified by correlation, and, after correcting for simultaneous tests, of the 91 correlations, 37 were significant at the 5% level. The pattern of pleiotropic effects deviated both from the equilibrium genetic correlations quantified in a previous study and from the correlations of mutational effects in a mutation-accumulation experiment, suggesting that multiple forces are at play that shape extant variation.

MUTATIONS provide the source of variation for all adaptive changes, and despite the wealth of knowledge we have concerning molecular aspects of mutation, there remains much to be learned about the effects of mutations on quantitative characters. Properties of new mutations such as the number of loci, the mutation rate per locus, the distribution of effects, dominance, pleiotropy, and epistasis all play key roles in shaping the amount of quantitative variation maintained in a population in mutation-selection balance (BARTON and TURELLI 1989; BARTON 1990). The classical infinitesimal model of quantitative genetic variation assumes that traits are determined by a very large number of genes having small additive effects, resulting in a Gaussian distribution of genotypic values. A contrasting view is that fewer loci with larger effect are involved (ROBERTSON 1968; TURELLI 1984). Empirical results are beginning to give clear support for the latter view (MACKAY *et al.* 1994, and references therein).

The ubiquity of transposable elements and their importance as a source of molecular variation in *Drosophila melanogaster* has been revealed by a number of surveys of restriction site variation. These surveys have been performed in extracted lines by analysis of genomic Southern blots of DNA from wild-caught Drosophila lines probed with various cloned genes, including *Adh* (AQUADRO *et al.* 1986), *Amy* (LANGLEY *et al.* 1988b), *hsp70* (LEIGH BROWN 1983), *rosy* (AQUADRO *et al.* 1988), *white* (MIYASHITA and LANGLEY 1988), *zeste-tko* (AGUADÉ *et al.* 1989a), *Notch* (SCHAEFFER *et al.* 1988), *y-ac-sc* (AGUADÉ *et al.* 1989b; EANES *et al.* 1989b), and *Zw* (the gene encoding G6PD) (EANES *et al.* 1989a). The average density of transposable elements in the genome of *D. melanogaster* was estimated by CHARLESWORTH and LANGLEY (1990) to be 0.005 per kb, and the probability of drawing a pair of chromosomes having the same transposable element at a site was 0.022. Surveys of restriction site variation reveal extensive variation in presence/absence of transposable elements because there are many polymorphic insertion sites, but, although there are many polymorphic sites, each particular insertion is rare. The rareness of particular insertions is consistent with the idea that most insertions are slightly deleterious (LANGLEY *et al.* 1988a). Although *D. melanogaster* appears to have the highest proportion of variation caused by transposable elements (CHARLESWORTH and LANGLEY 1990), other species of Drosophila also exhibit extensive presence/absence polymorphism of transposable elements.

Models of KEIGHTLEY and HILL (1988, 1990) formalized the manner in which selection acting on one trait can result in "apparent selection" on pleiotropically related traits. This in turn affects the equilibrium variance for a population in mutation-selection balance. These observations motivate an effort to quantify the

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pleiotropic effects of mutations induced by *P*-element transposition. It is important to know whether the effects of *P*-element-induced mutations are different from the distribution of spontaneous mutational effects. We especially want to know whether *P*-element insertions generate pleiotropic effects on pairs of enzyme activities known to be coregulated. Extensive knowledge of *P*-element molecular biology and our ability to control transposition make it an ideal tool for this purpose.

As informative as mutation-accumulation experiments have been, they suffer from the need to assume particular models before relevant parameters can be estimated. Comparison of lines of Drosophila tagged by transposable elements has several advantages over other methods for estimating the distribution of mutational effects. Four criteria outlined by MACKAY *et al.* (1992) are as follows: (1) mutations can be examined against an otherwise isogenic background, (2) the mutation rate for *P*-element insertion can be made much higher than the background spontaneous mutation rate, (3) one can assemble a series of lines with known *P*-element insertions independent of their effects on the phenotype and (4) one can recover mutations even if their deleterious effects would make their recovery unlikely by other methods. Several studies have documented the influence of *P*-element transposition on variation in quantitative traits, and the general conclusion is that *P*-elements transpose frequently enough in natural populations and produce sufficiently large effects that transposition provides a significant source of variation (MACKAY 1984, 1985; LAI and MACKAY 1990; MACKAY and LANGLEY 1990; MACKAY *et al.* 1992).

In this study a series of metabolic characters were quantified in lines of Drosophila bearing single *P*-element insertions in an otherwise co-isogenic background. The data indicate that the distribution of mutational effects is leptokurtic, so that, relative to a Gaussian distribution, there was an excess of mutations of large effect. Thirteen of the 16 characters that were measured exhibited a significant heterogeneity among lines, indicating that the mutational target size for enzyme activities is large and that most of the mutational effects must be regulatory. Pleiotropic effects, quantified by correlations of effects of *P*-element insertions, appeared to differ in pattern both from spontaneous mutational effects (CLARK *et al.* 1995) and from correlations in a natural population (WILTON *et al.* 1982; CLARK 1989).

MATERIALS AND METHODS

Drosophila stocks: All stocks were obtained from the Bloomington Stock Center at Indiana University. Symbols for the following stocks are described in LINDSLEY and ZIMM (1992):

1. *y w P[w⁺]*. The second and third chromosomes of this stock were made isogenic by use of balancer chromosomes *SM5* and *TM6*. The stock has *X*-linked recessive mutations *yellow* and *white* and an insertion of a nonautonomous *P*-element with a "CaSpeR" element, containing the *mini-white* gene inserted into the transposase coding region (PIRROTTA 1988).
2. *CyO/Sp; ry Sb P[ry⁺ Δ2-3](99B)/Tm6;Ubx*. This stock has the second chromosome balancer *Curly-of-Oster* and a *P*-element that serves as the source of transposase. The *ry Sb P[ry⁺ Δ2-3](99B)* chromosome (referred to as Δ2-3) has the third intron of the transposase gene removed, which eliminates the tissue specificity of transposase expression and results in a high constitutive rate of transposition (LASKI *et al.* 1986). This element has defective terminal repeats and is itself stable at chromosomal position 99B on the third chromosome (ROBERTSON *et al.* 1988).
3. *w; SM5 TM3/Xa*. This strain was brother-sister mated for 10 generations to make it virtually homozygous for the *X*-chromosome. It has the second chromosome balancer *SM5*, which is marked by the dominant *Curly* wing mutation, and *TM3*, which bears the *Serrate* mutation. *Xa* indicates a 2;3 translocation bearing the dominant wing marker *apterous*^{Xasta}.

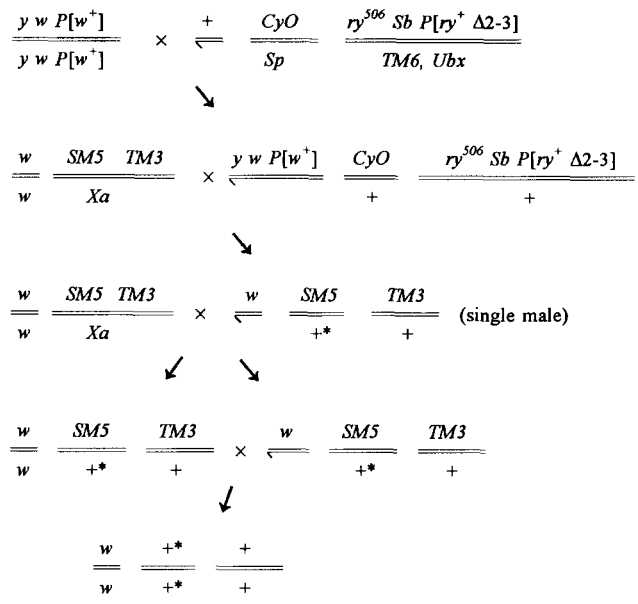


FIGURE 1.—Crossing scheme used to generate random *P*-element insertions on the second and third chromosomes. In all crosses, genotypes of the virgin females are indicated on the left. Flies bearing an insertion of *P[w⁺]* on the second or third chromosome (+*) are identified by presence of eye pigmentation. The balancer chromosomes *SM5* and *TM6* were used to recover the insertions in an isogenic background. Females in the first cross were made isogenic for the second and third chromosomes by use of balancer crosses, and males in the first cross were isogenic for the *X* chromosome.

P-element transposition: By a modification of the "jump-starter" protocol (COOLEY *et al.* 1988; ROBERTSON *et al.* 1988), lines that had random *P*-element insertions were constructed having an otherwise co-isogenic background. The F1 males in the crossing scheme depicted in Figure 1 have Δ2-3 elements whose transposase mobilizes transposition of the *P[w⁺]* elements. Because Δ2-3 is expressed in somatic as well as germline tissues, these flies exhibit somatic variation in *P*-element position, and this is seen as variegation in eye pigmentation. These males also produce gametes bearing different *P*-element insertions. The rest of the crossing scheme is designed

to produce flies that are isogenic for a particular insertion and isogenic for the rest of the genome. The scheme differs from that of ROBERTSON *et al.* (1988) in that only a single *P*-element is mobilized (as opposed to the 17 elements in the *Birm-2* stock). This resulted in a lower rate of transposition, and most cases of transposition resulted in single insertions as opposed to frequent multiple insertions caused by *Birm-2* (MACKAY *et al.* 1992). The lines that are produced in the end bear the X-linked *white* eye mutation, but their eyes generally have some pigmentation because of expression of the *white* minigene on $P[w^+]$. There was considerable range in the degree of eye pigmentation due to the dependence of *white* expression on its location in the genome (HAZELRIGG *et al.* 1984; HAZELRIGG and PETERSEN 1992). Although variation in *white* expression may have an effect on fitness, it seems unlikely that *white* expression *per se* influences activity of metabolic enzymes or fat or glycogen storage. As noted in previous studies, eye color was also frequently sexually dimorphic because of altered dosage compensation.

P-element quantification: Quantitative PCR proved to be a rapid and efficient way to identify lines with single *P*-element insertions. The design makes use of three oligonucleotide primers. A forward primer in the first exon of the *white* gene is used for both PCR products from the endogenous *white* gene and from the *mini-white* gene on the *P*-element vector. The endogenous gene gives a fragment of size 381 bp due to amplification between the exon 1 primer (5′-CAAGCGGTTTACGCCATC-3′) and a reverse primer near the 5′ end of the first intron (5′-TCTGGTAGCTGTGCTCGCTA-3′). The first and third primer would yield a product of 2967 bp from the *white* gene, but this product is not formed because the PCR reaction is outcompeted by the first and second primers. The CaSpeR *P*-element contains a *mini-white* gene that lacks the first intron, so the *mini-white* template gives a PCR product of length 250 bp between the primer in the first exon and a reverse primer in the second exon (5′-TATCATTGCAGGGTGACAGC-3′). Amplification of genomic DNA samples with these three primers produces two fragments, and the density of the two corresponding bands on ethidium bromide-stained gels can be used to quantify the relative amounts of the initial targets. Gels were photographed with Polaroid P/N film, and resulting negatives were scanned with a laser densitometer. The quantitative relation between relative band density and target concentration was tested by mixing a series of concentrations of two genomic DNAs, one with no *P*-elements and another with one *P*-element (Figure 2). By replicating the assays, it was possible to unambiguously identify lines that had single *P*-element insertions.

Control lines: Some of the lines at the end of the crossing protocol of Figure 1 had a *white* eye phenotype. Quantitative PCR showed that some of these had no $P[w^+]$ insertions, whereas others had a $P[w^+]$ insertion that was evidently inserted in a genomic region that prevented virtually all expression. Eight of the lines that had no $P[w^+]$ insertions were used as control lines. They went through the same history of crossing as the insertion lines and hence had the same opportunity for rare double crossover events in balancers and are expected to be as isogenic as the *P*-element insertion lines.

Preparation of homogenates: Five pairs of flies were transferred from each line to a vial with fresh medium and allowed to lay eggs for 3 days. Upon emergence of the progeny, two replicate sets of five males were collected from each vial and transferred to fresh yeasted medium for 6 days of aging. Flies from each line were collected from ≥4 vials on each of 4 days, so that each line was represented by flies from 16 vials. Each sample of males was then weighed to the nearest 1 μg, placed in a coded microcentrifuge tube, and homogenized 15 sec with a motorized Kontes Teflon mortar in 1 ml of

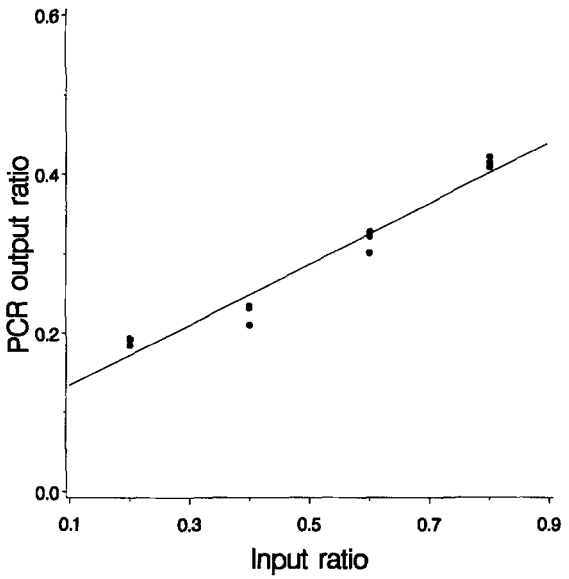


FIGURE 2.—Test of quantitative PCR for scoring *P*-element copy number. In this test, DNA from lines with known differences in *P*-element copy number were mixed in four different relative concentrations. PCR was performed with primers that yielded a band corresponding to the endogenous *X*-linked *white* gene and a different-sized band corresponding to the *miniwhite* gene. Relative densities of these bands corresponded linearly to the initial DNA concentrations.

homogenization buffer (0.01 M KH_2PO_4, 1 mM EDTA, pH 7.4). Homogenates were centrifuged at 2000 rpm for 2 min to pellet cellular and cuticular debris, and 25-μl aliquots of this crude homogenate were pipetted into the same position of 15 96-well microtiter plates. The entire process of homogenizing, centrifugation, and pipetting was performed in a 4° cold room. Homogenates were stored in microtiter plates at −70° until the day of assay. Altogether the project involved distributing 76,860 homogenate samples into 900-microtiter plates (60 sets of 15 plates). Further details of homogenate preparation are described in CLARK and KEITH (1989).

Assays of enzyme activities: For each sample of flies, 16 characters were quantified, including live weight and 15 biochemical quantities that were assayed with a microtiter plate reader. Details of the procedures appeared in CLARK and KEITH (1989) and CLARK and WANG (1994). Each microtiter plate had homogenate samples as well as standards and controls. The 15 identical plates were used to assay triacylglycerol (TRI), glycogen (GLY), total protein (PRO), and the activities of fatty acid synthase (FAS), glucose-6-phosphate dehydrogenase (G6PD, map location *1*-63.0), α-glycerol-3-phosphate dehydrogenase (map location *3*-55.4), glycogen phosphorylase (GP), glycogen synthase (GS), hexokinase (HEX, Hex-A at *1*-29.3 and Hex-C at *2*-75.0), malic enzyme (ME, at *3*-53.1), 6-phosphogluconate dehydrogenase (at *1*-0.64), phosphoglucose isomerase (PGI, at *2*-58.0), phosphoglucomutase (PGM, at *3*-43.4), trehalase (TRE, at *2*-92.0), and alcohol dehydrogenase (at *2*-50.1). The units of TRI and GLY are micrograms per fly, and the units of the enzyme activities are nanomoles NADP (or NAD) reduced per fly per minute. The microtiter plate reader (VMAX, Molecular Devices Inc.) was programmed to record 6–12 optical densities of each well at a prescribed time intervals depending on the assay. Each such run was recorded in a separate data file, resulting in a total of 900 files with 491,904 optical density readings.

Statistical analysis: The data were fitted to linear models that had the classifications Day crossed with Line, and Vial nested within Day and Line, and an error term representing differences between samples taken from the same vial. Null hypotheses of interest included the equivalence of the control lines, equivalence of single *P*-element insertion lines, and deviation of the mean of the insertion lines from the controls. These null hypotheses were tested separately for all 16 characters assayed. In the case of the two traits live weight and total protein, the following model for analysis of variance was used:

$$Y_{ijkl} = \mu + D_i + L_j + (D*L)_{ij} + V_{ijk} + \epsilon_{ijkl}.$$

The remaining 14 traits are expected to be highly correlated with size, so the covariates weight and protein were removed in the following model of analysis of covariance:

$$Y_{ijkl} = \mu + D_i + L_j + (D*L)_{ij} + V_{ijk} + \beta_w(w_{ijkl} - \bar{w}) + \beta_p(p_{ijkl} - \bar{p}) + \epsilon_{ijkl},$$

where μ is the grand mean, D_i is the effect of the ith day, L_j is the effect of the jth line, $(D*L)_{ij}$ is the day by line interaction, V_{ijk} is the effect of the kth vial nested in the jth line and ith day, and ϵ_{ijkl} is the residual error. The covariates in the analysis of covariance were weight (w_{ijkl}) and protein content (p_{ijkl}), whose means were $\bar{w}$ and $\bar{p}$ and whose regression coefficients were β_w and β_p, respectively. In applying this model there is an implicit assumption that variation in weight and protein is the cause of variation in activities. Models with and without weight and protein as covariates were fitted, because these linear models cannot reveal which character is causal.

It cannot be assumed *a priori* that mutational effects will be normally distributed, and some lines may be outliers if tested by standard Gaussian statistical theory. For this reason, no effort was made to remove outliers from the study. Normality of line means, which tests whether mutational effects have a normal distribution, was done with the Shapiro-Wilk statistic (the UNIVARIATE procedure of SAS). The linear models were fitted with the routines VARCOMP and GLM in the statistical package SAS (SAS Institute, 1990). VARCOMP produced restricted maximum likelihood (REML) estimates of variance components and their standard errors. Tests of significance of heterogeneity among lines were made from confidence intervals of variance components and with GLM, which generated functions of mean squares required for hypothesis testing. In addition to testing whether lines were homogeneous, the nested analysis of variance indicated other levels in the experiment at which significant heterogeneity was introduced. Further details concerning the application of linear models appear in RESULTS.

Genetic correlation of *P*-element effects: The genetic correlations were estimated in the manner of CLARK (1989), taking the among-line component of covariance for a pair of traits divided by the product of the genetic standard deviations. The formal sampling theory for these estimators of genetic correlation has not been done, so the following bootstrapping procedure was used to obtain approximate confidence intervals on each element in the correlation matrix. One thousand samples of the data were drawn with replacement, in a manner that retained the structure of the data complete with its departure from being totally balanced. Estimates of genetic correlations were made from each bootstrap sample, and significant of departures from zero were identified from the tail area of the distribution across bootstrap samples of correlation estimates. To determine tablewise estimates of significance, the sequential Bonferroni method was applied (RICE 1989).

RESULTS

Distributions of mutational effects: Because each of the *P*-element insertion lines bears a single *P*-element insertion in an otherwise isogenic background, the distribution of line-means reflects the distribution of mutational effects of the *P*-element insertions. Table 1 presents the descriptive statistics for the distributions of line-means of each trait. Inspection of these figures suggests several hypotheses to test more formally. First, by comparing the controls to the single-insertion lines, it appears that the means did not change in any consistent fashion, and in general the changes were much less than one standard deviation.

The figures in Table 1 are raw values, and subsequent analyses used these raw values for the two characters live weight and total protein. The other characters are expected to scale allometrically with body size, so given that the lines vary in weight, traits that are causally related to weight are also expected to exhibit variation among lines. The statistical tests for heterogeneity among lines in the metabolic traits remove the effects of body size by analysis of covariance. This makes tests of line effects more conservative, because a mutation may affect a metabolic character directly and, as a consequence, may be the indirect cause for a change in live weight. In practice, tests with and without adjusting out the effects of weight and total protein yielded very similar results (CLARK *et al.* 1995).

The distributions of line-means are plotted as normal probability plots in Figure 3. If the data are normally distributed, the points of such a plot fall on a straight line. This plot allows one to quickly identify departures from normality, and the data reflect some consistent departures. Most of the characters exhibit a few lines whose mean trait values are more extreme than would be expected for a normal distribution. These extreme lines produce a downward bend at the lower end (left) of each curve and an upward bend at the high end (right) of each distribution. Some characters show a marked asymmetry in mutational effects, including FAS and ME, both of which seem to have an excess of insertions whose mutational effect is to increase activity. The Wilks-Shapiro test revealed that all traits except GLY departed significantly from the normal distribution. When the 14 metabolic traits of Figure 3 are replotted, using the predicted values obtained from the analysis of covariance and after removing effects of weight and protein, inspection reveals the same tendency toward leptokurtic distributions, and significant departures from normality remain (details not shown). Normal probability plots and the Wilks-Shapiro test of the raw data from control lines revealed no significant departures from normality, indicating that the other sources of error in the experiment did not account for the leptokurtosis.

Mutational variance: Generalized linear models were

TABLE 1

Means and among-line SD for metabolic traits

Trait	Controls	Chromosome *2* insertions	Chromosome *3* insertions
WT	794.37 ± 34.87	791.59 ± 47.93	788.82 ± 46.20
PRO	43.64 ± 2.34	42.30 ± 3.36	42.77 ± 3.62
TRI	62.62 ± 2.57	58.78 ± 6.16	57.40 ± 6.76
GLY	12.75 ± 0.84	12.52 ± 1.53	12.25 ± 1.32
FAS	3.71 ± 0.62	4.08 ± 1.19	4.42 ± 1.24
G6PD	3.70 ± 0.41	3.31 ± 0.76	3.19 ± 0.85
GP	2.14 ± 0.30	2.13 ± 0.30	2.12 ± 0.34
GPDH	53.90 ± 4.50	56.54 ± 4.43	56.85 ± 5.71
GS	2.84 ± 0.14	2.73 ± 0.19	2.70 ± 0.21
HEX	2.80 ± 0.16	2.79 ± 0.29	2.79 ± 0.27
ME	7.69 ± 0.80	7.71 ± 1.07	7.72 ± 1.34
PGD	1.67 ± 0.17	1.67 ± 0.40	1.69 ± 0.46
PGI	11.05 ± 2.00	11.01 ± 1.59	10.83 ± 1.50
PGM	57.06 ± 7.03	59.41 ± 8.24	59.63 ± 9.11
TRE	16.82 ± 1.48	17.07 ± 2.46	17.02 ± 2.03
ADH	6.82 ± 0.84	6.12 ± 1.19	5.99 ± 1.24

Units are micrograms per fly for weight (WT), protein (PRO), and triacylglycerol content (TRI), and nanomoles per milligram per minute for the enzyme activities. The SD reported is the standard deviation among the line means in each class. There were eight control lines with no *P*-element insertions, 124 lines with a single second-chromosome insertion and 139 lines with a single third-chromosome *P*-element insertion.

used both to partition the variance into components of mutational (among-line) effects and various levels of error and to perform hypothesis testing. Analysis of variance and covariance were used to quantify the magnitude of genetic effects among the lines that differ in *P*-element insertion sites. Components of variance attributed to Line, Day, Line × Day interaction, Vial and Error were estimated separately for the control lines and for the homozygous viable single *P*-insertion lines. Table 2 gives restricted maximum likelihood (REML) estimates of the proportion of variance among control lines attributed to each source in the linear model described in MATERIALS AND METHODS. As in previous studies, the difference between vials is generally greater than the difference between flies within a vial. This microenvironmental heterogeneity results in significant Vial effects. Similarly, larger scale environmental effects, such as systematic fluctuations in temperature, humidity, or some aspect of the medium, result in differences from one day of assay to another. These differences are manifested as significant Day effects. The control lines are expected to be virtually identical genetically, so the lack of heterogeneity among lines in all characters but weight is expected.

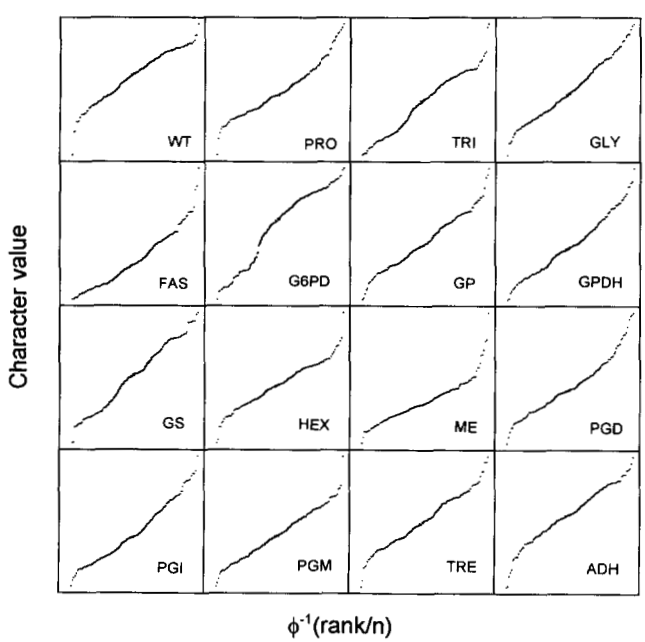


FIGURE 3.—Normal probability plots showing that the distributions of many of the characters among the single *P*-element insertion lines had excessively long tails (outlier lines). The *X*-axis is the inverse normal cumulative distribution function for the rank and the *Y*-axis is the trait value. Points from a normal distribution would fall on a straight line. Note that in most of the cases, there were about as many outliers in the positive as well as negative directions.

Lines that are homozygous for second chromosome *P*-element insertions exhibited significant heterogeneity in 10 of the 16 metabolic traits (Table 3). The proportion of variance attributed to the line effect varied considerably across traits and ranged from 0 to 0.35 (in the case of G6PD). Vial and Day effects were again generally significant, and there were several instances of significant Line × Day interactions, including one with TRE (which had no Line effect). The pattern of variance components of third chromosome insertions (Table 4) was similar to that of second chromosome insertions, but GP, PGI, and PGM exhibit significant

TABLE 2

Variance components from analysis of covariance of metabolic traits

	Control lines				
Trait	Line	Day	Line × Day	Vial	Residual
WT	0.170 ± 0.137	0.336 ± 0.275	0.053 ± 0.060	0.080 ± 0.052	0.360 ± 0.055
PRO	0.0	0.390 ± 0.288	0.0	0.100 ± 0.063	0.511 ± 0.076
TRI	0.0	0.110 ± 0.104	0.033 ± 0.064	0.039 ± 0.074	0.819 ± 0.124
GLY	0.016 ± 0.076	0.432 ± 0.343	0.080 ± 0.079	0.125 ± 0.064	0.346 ± 0.053
FAS	0.0	0.063 ± 0.073	0.0	0.0	0.937 ± 0.123
G6PD	0.0	0.288 ± 0.255	0.062 ± 0.062	0.127 ± 0.073	0.523 ± 0.080
GP	0.0	0.395 ± 0.327	0.033 ± 0.037	0.251 ± 0.088*	0.322 ± 0.050
GPDH	0.0	0.719 ± 0.523	0.009 ± 0.016	0.108 ± 0.039*	0.165 ± 0.025
GS	0.0	0.282 ± 0.233	0.017 ± 0.038	0.305 ± 0.109*	0.396 ± 0.061
HEX	0.0	0.186 ± 0.155	0.047 ± 0.059	0.191 ± 0.092*	0.576 ± 0.088
ME	0.005 ± 0.106	0.241 ± 0.302	0.146 ± 0.130	0.197 ± 0.088*	0.411 ± 0.062
PGD	0.0	0.198 ± 0.183	0.069 ± 0.070	0.002 ± 0.057	0.731 ± 0.113
PGI	0.042 ± 0.151	0.026 ± 0.103	0.191 ± 0.184	0.117 ± 0.082	0.624 ± 0.094
PGM	0.050 ± 0.092	0.135 ± 0.153	0.011 ± 0.054	0.163 ± 0.097	0.640 ± 0.097
TRE	0.0	0.613 ± 0.444	0.0	0.070 ± 0.041	0.317 ± 0.047
ADH	0.040 ± 0.138	0.297 ± 0.285	0.150 ± 0.130	0.084 ± 0.064	0.430 ± 0.069*

Values are means or means ± SE. Variance components were estimated by restricted maximum likelihood (REML) using the SAS procedure VARCOMP. Table entries are the proportion of the total variance attributed to each source.

* Variance components whose estimate exceeds two SEs.

heterogeneity only among third-chromosome lines. Tests of significance of variance components in Tables 2–4 were somewhat more conservative than tests that used F ratios estimated from the GLM procedure. In none of the linear models of Tables 2–4 did the distributions of residual error depart significantly from normality.

Mutational variance ($V_{m|1}$) induced by insertion of a single *P*-element can be estimated from the variance components following the reasoning of MACKAY *et al.* (1992). The difference between the among-line variance components of the single *P*-insertion lines and the control lines provides an estimate for $V_{m|1}$. For purposes of comparison across disparate traits and experi-

TABLE 3

Variance components from analysis of covariance of metabolic traits

	Second chromosome single *P*-element insertion lines				
Trait	Line	Day	Line × Day	Vial	Residual
WT	0.204 ± 0.047*	0.179 ± 0.093	0.178 ± 0.028*	0.112 ± 0.015*	0.327 ± 0.013
PRO	0.0	0.233 ± 0.112*	0.034 ± 0.013*	0.066 ± 0.017*	0.667 ± 0.026
TRI	0.066 ± 0.024*	0.240 ± 0.118*	0.071 ± 0.019*	0.089 ± 0.018*	0.533 ± 0.021
GLY	0.129 ± 0.036*	0.233 ± 0.115*	0.113 ± 0.021*	0.162 ± 0.019*	0.362 ± 0.014
FAS	0.0	0.091 ± 0.045*	0.014 ± 0.013	0.016 ± 0.019	0.878 ± 0.035
G6PD	0.346 ± 0.058*	0.181 ± 0.092	0.069 ± 0.015*	0.085 ± 0.013*	0.319 ± 0.012
GP	0.006 ± 0.021	0.165 ± 0.083	0.060 ± 0.016*	0.253 ± 0.029*	0.516 ± 0.020
GPDH	0.084 ± 0.028*	0.220 ± 0.108*	0.073 ± 0.018*	0.156 ± 0.021*	0.467 ± 0.018
GS	0.0	0.361 ± 0.174*	0.019 ± 0.009*	0.158 ± 0.019*	0.462 ± 0.018
HEX	0.046 ± 0.021*	0.136 ± 0.068*	0.034 ± 0.016*	0.134 ± 0.024*	0.650 ± 0.025
ME	0.161 ± 0.042*	0.026 ± 0.017	0.104 ± 0.023*	0.170 ± 0.024*	0.539 ± 0.021
PGD	0.048 ± 0.023*	0.027 ± 0.017	0.048 ± 0.019*	0.130 ± 0.025*	0.748 ± 0.029
PGI	0.012 ± 0.020	0.189 ± 0.094*	0.081 ± 0.020*	0.147 ± 0.022*	0.571 ± 0.022
PGM	0.008 ± 0.020	0.134 ± 0.067*	0.085 ± 0.020*	0.189 ± 0.026*	0.584 ± 0.023
TRE	0.037 ± 0.017*	0.442 ± 0.211*	0.049 ± 0.012*	0.121 ± 0.016*	0.351 ± 0.014
ADH	0.184 ± 0.040*	0.221 ± 0.111*	0.093 ± 0.018*	0.152 ± 0.018*	0.350 ± 0.014

Values are means and means ± SE. Table entries are the proportion of the total variance attributed to each source (see Table 2 for details).

* Variance components whose estimate exceeds two standard errors.

TABLE 4

Variance components from analysis of covariance of metabolic traits

Trait	Third chromosome single *P*-element insertion lines				
	Line	Day	Line × Day	Vial	Residual
WT	0.122 ± 0.036*	0.197 ± 0.102	0.202 ± 0.029*	0.099 ± 0.014*	0.380 ± 0.013
PRO	0.017 ± 0.013	0.257 ± 0.123*	0.025 ± 0.014	0.038 ± 0.015*	0.662 ± 0.024
TRI	0.048 ± 0.019*	0.293 ± 0.142*	0.081 ± 0.017*	0.066 ± 0.014*	0.512 ± 0.018
GLY	0.023 ± 0.022	0.311 ± 0.153*	0.148 ± 0.023*	0.133 ± 0.016*	0.385 ± 0.014
FAS	0.0	0.088 ± 0.045	0.016 ± 0.012	0.009 ± 0.017	0.887 ± 0.032
G6PD	0.317 ± 0.051*	0.217 ± 0.108	0.088 ± 0.015*	0.079 ± 0.011*	0.299 ± 0.011
GP	0.063 ± 0.026*	0.132 ± 0.068	0.082 ± 0.017*	0.250 ± 0.026*	0.473 ± 0.017
GPDH	0.180 ± 0.032*	0.246 ± 0.129*	0.037 ± 0.010*	0.139 ± 0.017*	0.381 ± 0.014
GS	0.0	0.347 ± 0.167*	0.023 ± 0.009*	0.152 ± 0.017*	0.478 ± 0.017
HEX	0.053 ± 0.020*	0.123 ± 0.063	0.043 ± 0.015*	0.123 ± 0.021*	0.658 ± 0.023
ME	0.213 ± 0.044*	0.064 ± 0.038	0.137 ± 0.023*	0.146 ± 0.018*	0.439 ± 0.016
PGD	0.115 ± 0.029*	0.008 ± 0.008	0.085 ± 0.021*	0.056 ± 0.018*	0.736 ± 0.026
PGI	0.064 ± 0.020*	0.169 ± 0.084*	0.042 ± 0.015*	0.084 ± 0.018*	0.641 ± 0.023
PGM	0.064 ± 0.027*	0.104 ± 0.053	0.120 ± 0.023*	0.162 ± 0.021*	0.550 ± 0.019
TRE	0.024 ± 0.014	0.446 ± 0.213*	0.052 ± 0.011*	0.128 ± 0.015*	0.351 ± 0.012
ADH	0.256 ± 0.046*	0.139 ± 0.074	0.117 ± 0.020*	0.115 ± 0.015*	0.374 ± 0.013

Values are means and means ± SE. Table entries are the proportion of the total variance attributed to each source (see Table 2 for details).

* Variance components whose estimate exceeds two standard errors.

mental designs (LYNCH 1988), V_m is often scaled by the environmental variance (V_e). In this study, V_e was estimated as the sum of the Day, Vial, and residual effects in the control lines. The variance components were calculated on each of 1000 bootstrap samples that were constructed in the same manner as those for estimates of correlation. Each bootstrap sample yielded an estimate of $V_{m|1}/V_e$, and the mean and standard error of this bootstrap distribution are reported in Table 5. Estimates of $V_{m|1}/V_e$ do not differ dramatically between chromosomes *2* and *3*, and, in particular, estimates are not elevated consistently for the chromosome bearing the structural gene. In a survey of many studies of mutational variance for quantitative characters, LYNCH (1988) obtained a rough mean estimate of V_m/V_e of 0.001, and a more recent review arrived at similar estimates (KEIGHTLEY *et al.* 1993). Estimates of $V_{m|1}/V_e$ for effects of *P*-element insertions on metabolic traits vary over a wide range, but, in general, they are higher than estimates obtained from mutation-accumulation experiments (HOULE *et al.* 1992). MACKAY *et al.* (1992) also obtained very high values for V_m/V_e for effects of *P*-element insertions on bristle traits, with an average over sexes of $V_m/V_e \geqslant 0.03$, and the average across traits in our study was 0.057. It is important to note that these *P*-element studies do not yield estimates of mutation rate but instead yield estimates of the average phenotypic effect per *P*-element insertion. This is because the design specifically selects lines that have experienced a transpositional insertion.

Pleiotropic effects of *P*-element insertions: As a first test of multiplicity of effects of single *P*-element insertions, *t*-tests were done to test the null hypothesis that each line was representative of a sample of the control lines. Tests were done independently for each line and each trait, and 110 of the 263 lines were found to show no significant deviation in any trait. However, many lines showed multiple effects, and two lines exhibited

TABLE 5

Estimates of $\hat{V}_{m/1}/\hat{V}_e$ from variance components for second- and third-chromosome *P*-element insertions

Trait	Chromosome *2*	Chromosome *3*
WT	0.055 ± 0.005	0.064 ± 0.005
PRO	0.008 ± 0.002	0.008 ± 0.002
TRI	0.040 ± 0.004	0.066 ± 0.004
GLY	0.042 ± 0.003	0.041 ± 0.003
FAS	0.013 ± 0.002	0.013 ± 0.002
G6PD	0.121 ± 0.007	0.135 ± 0.008
GP	0.019 ± 0.002	0.031 ± 0.003
GPDH	0.026 ± 0.003	0.054 ± 0.004
GS	0.315 ± 0.042	0.282 ± 0.035
HEX	0.020 ± 0.003	0.022 ± 0.002
ME	0.040 ± 0.004	0.065 ± 0.006
PGD	0.019 ± 0.003	0.027 ± 0.003
PGI	0.017 ± 0.002	0.022 ± 0.002
PGM	0.022 ± 0.002	0.039 ± 0.003
TRE	0.024 ± 0.002	0.024 ± 0.002
ADH	0.063 ± 0.005	0.097 ± 0.007

Values are means ± SE. Estimates of mutational variance for single *P*-insertions ($\hat{V}_{m/1}$) and the environmental variance ($\hat{V}_e$) were obtained from the REML variance components. $V_{m/1}$ is the component of variance among lines attributed to single *P*-element insertions.

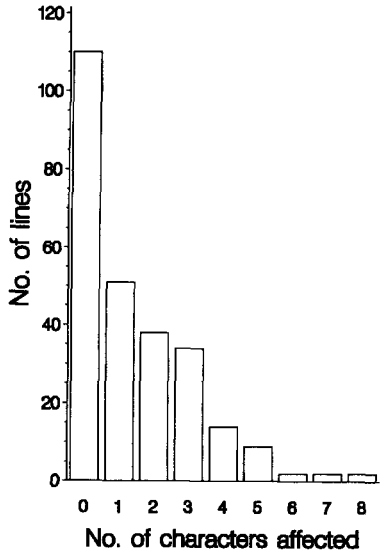


FIGURE 4.—Distribution of the number of significantly deviant characters detected in each *P*-insertion line. Extensive pleiotropy is revealed by the observation of single insertions with multiple effects.

significant deviation in eight distinct traits (Figure 4). It appears that *P*-element effects on multiple metabolic characters are common.

Scattergrams of line means for pairs of traits illustrate the magnitude of pleiotropic effects. In the panels of Figure 5, the points representing the control lines have been enclosed in a polygon. Lines with single *P*-element insertions are plotted as a scatter of open circles, and it is readily apparent that the insertion lines are more variable than the control lines. In general, there appears to be a positive correlation in mutational effects, such that, for example, lines with unusually low triacylglycerol storage are also likely to store little glycogen. One exception appears in the top left panel of Figure 5, where a significant negative correlation between HEX and GS activities is seen.

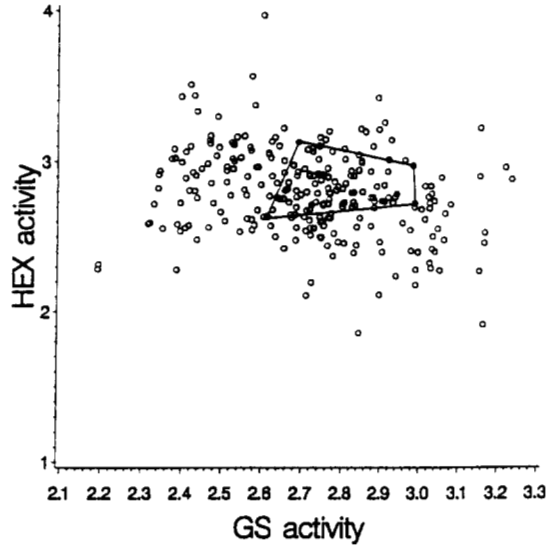
Genetic correlations for mutational effects (r_m) were estimated as the genetic covariance (among-line component of covariance) divided by the product of the among-line standard deviations for the two traits (WILTON *et al.* 1982; CLARK 1989). Covariance components were estimated with the same model of analysis of covariance as was used in Tables 2 and 3, which removes the effects of covariation with live weight and protein content. Although asymptotic sampling theory can yield estimates of the variance of genetic correlations (MODE and ROBINSON 1959), the sampling properties of the variance are not well described and are known to be highly distribution dependent. Empirical variances of genetic correlations were estimated by bootstrapping over the entire data set. The significance of the correlated effects was tested by determining the fraction of bootstrap samples that gave an r_m of opposite sign from the mean estimate. Experiment-wide significance was obtained by Bonferroni correction of these tail probabilities. Results are presented graphically in Figure 6. Correlations were unexpectedly strong, with 37 of the 91 estimates significant at the 5% level. Casual inspection suggests that *P*-element-induced mutations exhibit a pattern of correlations different from both mutation-accumulation lines and from natural populations (CLARK 1989; CLARK *et al.* 1995). Formal statistical comparison of estimates of mutation effects from these two studies will be made in a later report. Despite the appearance of different patterns of significance of correlations, estimates of correlations from the two studies are in fact correlated (Figure 7).

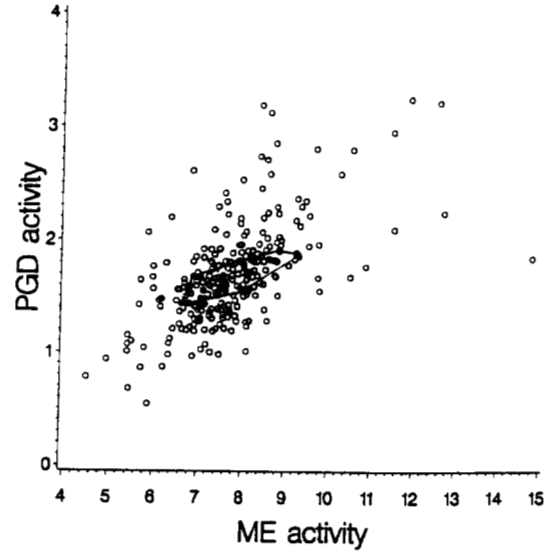
DISCUSSION

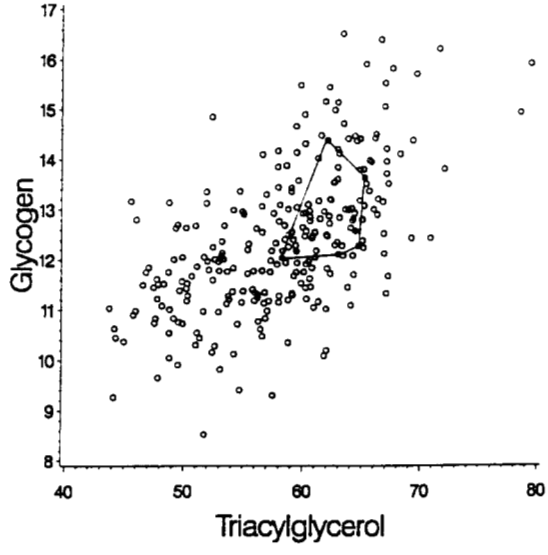
The four main results of this study are as follows: (1) *P*-element insertions have an unexpectedly high chance of influencing metabolic traits, including enzyme activities; (2) the distribution of effects is leptokurtic; (3) the distribution of mutational effects is nearly symmetric, with an equal chance of increasing and of decreasing enzyme activities and (4) the effects are highly pleiotropic and generally affect several characters. To get a better understanding of the maintenance of quantitative genetic variation, it is necessary to quantify aspects of the influx of variance through mutation. Many attributes of mutations need to be considered, and the parameters of models that describe the process of mutation need to be estimated empirically. The number of loci that affect a character, the mutation rate per locus, the distribution of the mutational effects, the degree of dominance, pleiotropy, and epistasis are all features of mutations that influence the equilibrium variance expected in a population in mutation-selection balance (BARTON and TURELLI 1989). Progress in the area of empirical estimation is being made (LYNCH 1988; MACKAY *et al.* 1992, 1994), and a clearer picture is beginning to emerge.

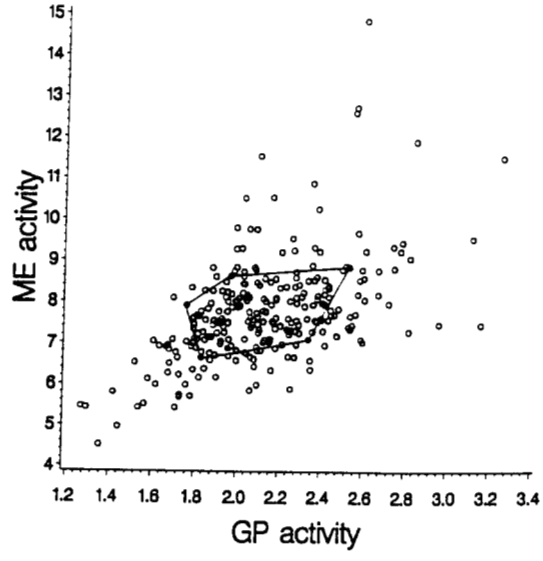
Here we examined the distribution of mutational effects on 16 metabolic characters in a series of lines that differ by single random *P*-element insertions on the second and third chromosome of *D. melanogaster*. By comparing distributions of single-insertion lines to control lines, the distribution of mutational effects could be inferred. The means of mutational distributions were not significantly different from zero, and most of the traits exhibited a significant inflation in variance. The distributions deviated from a normal distribution

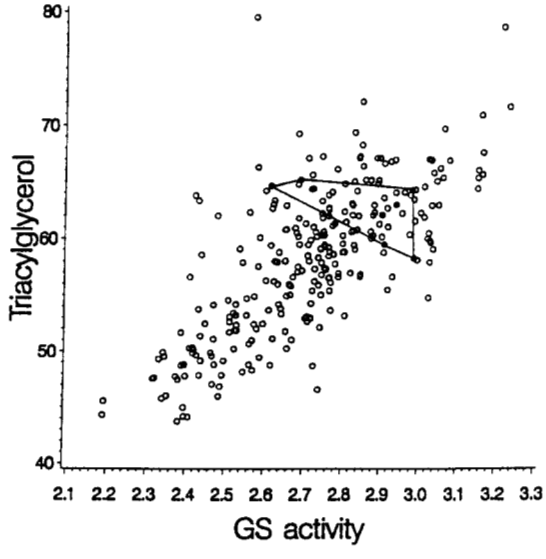
FIGURE 5.—Scattergrams illustrating the pleiotropic effects of single *P*-element insertions on a few pairs of characters. Solid dots are line means of control lines that have no *P*-element insertions but otherwise the same genetic background, and open circles represent lines with single *P*-element insertions. In most cases each dot represents a mean of 16 scores.

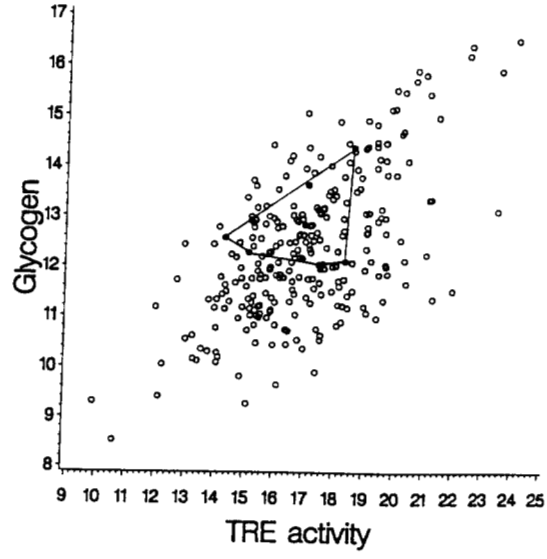

HEX activity
GS activity


PGD activity
ME activity


Glycogen
Triacylglycerol


ME activity
GP activity


Triacylglycerol
GS activity


Glycogen
TRE activity

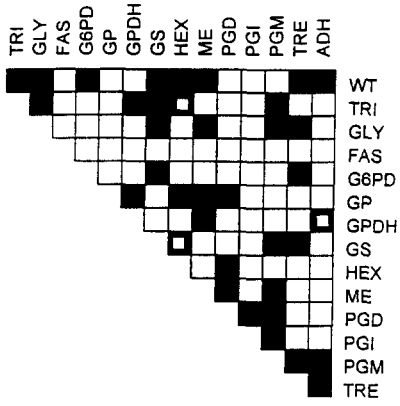


FIGURE 6.—Pattern of genetic correlations of all pairs of traits. Correlations significant at $P < 0.01$ are indicated by a solid square. Correlations with live weight (WT) are based on raw values, and all other correlations are tested after partialling out effects of live weight and total protein. The three cases of significant negative correlation are indicated by the small white squares within the black squares.

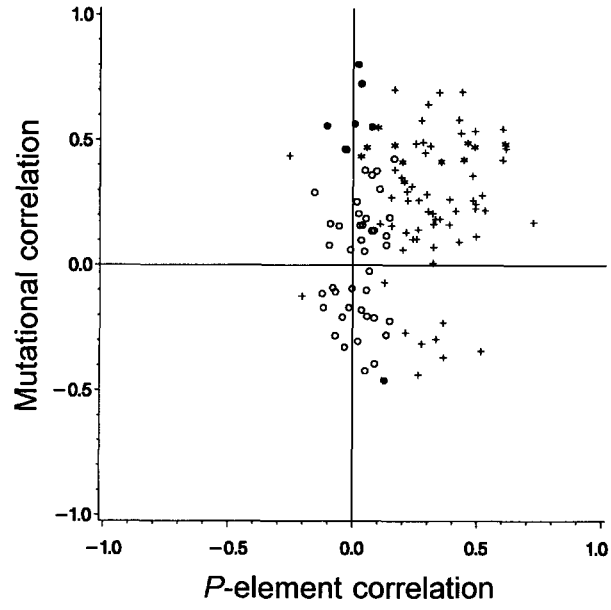


FIGURE 7.—Scattergram of genetic correlations obtained in this *P*-element tagging study and in a mutation-accumulation (MA) experiment (CLARK *et al.* 1995). *, correlations significant at $P < 0.001$ in both experiments; +, correlations significant at $P < 0.001$ in the *P*-element study but not in the MA study; ●, significance in the MA study only; ○, correlations not significant in either study. The Spearman rank correlation for the two sets of correlation estimates is $\rho = 0.256$ ($P = 0.005$).

in most cases, and departures were caused by a few excessively large mutational effects. The high frequency with which random *P*-element insertions affect metabolic characters indicates that the mutational target size is very large. In addition, strong pleiotropic effects appear to be common, with a tendency toward positive correlations, suggesting that positive regulators of one enzyme are likely to influence other enzyme loci positively as well. Despite the tendency for positive correlation, the *P*-element insertional effects exhibited more cases of significant negative correlation than either a mutation-accumulation experiment (CLARK *et al.* 1995) or a sample from a natural population (CLARK 1989).

The chance that a *P* element inserts in a particular gene of size x is, assuming homogeneous probability of insertion, x divided by the genome size. For a gene of 2 kb in length, this probability is only $\sim 10^{-5}$. If it were the case that only insertions within the structural gene could affect enzyme activity, then it would be very unlikely that we would have seen an inflation in variance in any of the metabolic traits. The observed large increase in variance demonstrates that *P*-element insertion at many sites affects each enzyme locus. Enzyme activities, as they were scored here, are influenced by many factors that may regulate any of a number of steps in gene expression, including transcription, transcript processing, message stability, translation, posttranslational processing, and the catalytic activity of the final enzyme. This fact makes it more plausible that *P*-element insertions can result in such a high mutational variance for these traits. The data show significant increases in among-line variances with very little change in the mean activities, suggesting mutability of both positive and negative regulators. The magnitude of variation in mutational variance suggests that the genes encoding the enzymes differ dramatically in the structure of their regulatory variation. The high mutational variances imply that mutations at hundreds or thousands of genes can influence these characters.

The data also suggest that most of the *P*-element-induced mutations have pleiotropic effects on many of the characters. In the absence of selection, and assuming a population sufficiently large that effects of genetic drift can be ignored, the equilibrium covariance should be proportional to that seen among new mutations. As was argued by CLARK *et al.* (1995), a significant difference between the pattern of correlations of newly arisen mutations and an equilibrium population can be interpreted as evidence for natural selection.

The magnitude of pleiotropic effects of *P*-element-induced mutations has important consequences for metabolic characters. Quantitative genetic models of metabolic control can provide a means of generating stabilizing selection on enzyme activity when the target of selection is a property of the pathway (BEAUMONT 1988). The same mechanism that results in dominance of a single metabolic trait (KACSER and BURNS 1981) is also expected to produce consistent degrees of dominance for all pleiotropically related metabolic traits (KEIGHTLEY and KACSER 1987). Despite the highly interactive nature of metabolic regulation, the equilibrium epistatic variance for enzyme activities is expected to be very small (KEIGHTLEY 1989). Metabolic control theory provides a quantitative model for mapping many

discrete characters (enzyme activities) into a global property (such as flux) that may be associated with fitness (CLARK 1991). Whenever genes share a step in a regulatory heirarchy, such as a transcription factor, it is plausible that mutations in that regulatory chain will have pleiotropic effects on expression of genes downstream from the mutation. The extensive pleiotropy that was observed is therefore not entirely unexpected, although details of particular pleiotropic effects await future studies.

These experiments were motivated by the need to quantify distributions of mutational effects and the patterns of pleiotropy. It is clear that single *P*-element insertions can have a large effect on multiple characters, and the magnitude of these effects demands consideration of pleiotropy in models of mutation-selection balance for quantitative characters. But further experiments will be necessary before we can have strict confidence that the phenotype of any particular line is strictly attributable to its *P*-element insertion. Balancer chromosomes are not perfect in their suppression of double crossing over, and any such events would introduce variation from the balancer chromosomes into the homozygous lines. It is possible that dysgenic crosses may allow *P*-elements to insert and excise within a generation, so there may be some mutations not tagged by a *P*-element. These potential technical problems weaken our confidence that any particular phenotype is caused by any particular *P*-element, but when a collection of insertions is examined, the distribution of effects provides a good representation of the distribution of *P*-element insertions, simply because these artifacts are known to be rare. For these reasons, the biggest shortcoming with our design was the small number of control lines (eight). Despite the small number of control lines, however, the primary conclusions—that *P*-elements have surprisingly large, frequent, and pleiotropic effects on metabolic characters—remain intact.

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