Stress tolerance and metabolic response to stress in *Drosophila melanogaster*

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A potentially important physiological response to stress may be alteration in the gross regulation of energy metabolism. Different genotypes may respond differently to environmental stress, and the variation in these norms of reaction may be of key importance to the maintenance of genetic variation in metabolic traits. In the study reported here, a set of genetically defined lines of *Drosophila melanogaster* were exposed to four stresses (acetic acid, ethanol, starvation and thermal stress) in order to assess the magnitude of environmental effects and genotype × environment interactions. In addition to scoring metabolic traits, distributions of survival times under each stress were also quantified. Although both metabolic traits and survival times exhibited strong differences among genotypes, the correlations between enzyme traits and survival were generally weak. Many of the genetic correlations exhibit significant heterogeneity across environments. The results suggest that transient environmental stress may play an important role in the evolution of this highly intercorrelated set of metabolic traits.

Keywords: *Drosophila melanogaster*, metabolism, norm of reaction, plasticity, stress.

Introduction

Genotype × environment interaction may play a critical role in the maintenance of genetic variation, especially variation that is directly related to tolerance of stress. The study of genotype × environment interaction has an extensive literature (reviewed in Via *et al.*, 1995), generally focusing on the problem of how phenotypic plasticity evolves and to what extent it is adaptive. Another important aspect of genotype × environment interaction is its relevance to the question of maintenance of quantitative genetic variation. Changing the rank order of fitness of genotypes in different environments can certainly maintain polymorphism in classical population genetic models, and genotype × environment (G × E) interaction can likewise be a powerful force, maintaining variation in the face of drift and other forces that may erode that variation. The challenge is to quantify G × E on traits that may relate to a stress, to quantify the fitnesses of the relevant genotypes, and to parameterize models that incorporate environmental fluctuation as a factor that is relevant to the maintenance of variation.

One such model was proposed by Gillespie & Turelli (1989), who considered the importance of G × E on maintenance of variation with a pure additive model in which the genotypes had varying fitness under different environments. In this model, the identity of the fittest genotype differs from one environment to another, resulting in a situation in which genotypes with more heterozygous loci are generally fitter. Such a pattern maintains greater steady-state variation than a population in any one of the environments. Although theory may suggest that G × E can play an important role in maintenance of variation, empirical test of this hypothesis is not easy. Studies like those of Gupta & Lewontin (1982) show that G × E interaction is very common in mapping genotypes to phenotypes, but the relative commonness of significant G × E in this kind of study does not necessarily mean that the patterns of variation across environments promote polymorphism. As Gillespie & Turelli (1989) put it, there is no way to survey experimentally the full range of environments, and the artificial subset of environments that is chosen for experimental tests may not reflect what goes on in the full range of environments in nature.

One way that stressful environments may affect the maintenance of variation is through effects on patterns of additive, dominance and epistatic genetic variance. It is relatively easy to find examples of such changes in the literature, although no consist-

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ent trends in such changes have been observed (reviewed in Hoffmann & Parsons, 1991). Blows & Sokolowski (1995) scored developmental time and absolute viability of six second chromosome isogenic lines of *Drosophila* in a series of environments. Although they found essentially no change in additive variance across environments, dominance and epistasis were significantly elevated at environmental extremes. Such patterns may reflect the past operation of selection on the population, and clearly affect the impact of stress on standing levels of genetic variation.

There are many examples in the literature of genes whose expression is affected by particular environmental conditions; for example, many changes in the growth medium for yeast or *Escherichia coli* result in an increase in the expression of targeted enzymes, a phenomenon referred to as induction. Cases of induction in *Drosophila* include responses to elevated ethanol (McKechnie & Geer, 1984; Geer *et al.*, 1988; Pecsenye *et al.*, 1997) and sucrose (Geer *et al.*, 1981, 1983; Pecsenye *et al.*, 1996). Sometimes induction can be quite simple, resulting in increased expression of the first enzyme involved in metabolism of the substrate. This appears to be the case when increased expression of alcohol dehydrogenase (ADH) is found in *Drosophila* when ethanol is added to the medium (Geer *et al.*, 1988). Metabolic consequences can be more complex, including, in the case of an ethanol diet, a shift towards lipid synthesis on elevated ethanol medium (Geer *et al.*, 1985). Given the complexity of the intermediary metabolism network, it should not be surprising that some diet-induced changes involve more than one kind of switch. Comparison of amylase activities on low and high starch diets shows that amylase expression is higher on the high starch diet, showing both an induction of amylase on the high starch food (Yamazaki & Matsuo, 1984) and a repression of amylase by glucose on the low starch food (Hickey *et al.*, 1994). In addition to dietary-induced changes, metabolism is altered by changes in gene expression under anoxia (Ma & Haddad, 1997) and thermal stress (Oudman *et al.*, 1992).

The study presented here examines differences among lines in their tolerance of stress, partitions the genetic differences to the three chromosomes, and quantifies the magnitude of variation in metabolic traits across stressful environments. It goes on to examine to what extent variation in metabolic traits is correlated to survival, and quantifies the heterogeneity in correlation patterns among traits. The results are interpreted in light of the role of

gene × environment interaction in maintaining quantitative genetic variation.

Materials and methods

Drosophila *culturing*

A set of chromosome-replacement lines of *Drosophila* described in Wu *et al.* (1995) was used for all tests reported in this paper (Table 1). The lines were generated by exchanging chromosomes among four founding lines by use of balancer chromosomes. The four founding lines were the marker stock *rucuca*, a stock from Zimbabwe (Z30), a stock from France (Fr) and a stock from Highgrove, CA (Hg). The 20 lines have different combinations of chromosomes 1, 2 and 3, between the Z30 stock and the other two. In addition, three of the stocks are recombinants with the *rucuca* stock (which bears seven third-chromosome recessive markers) in order to partition the third chromosome. Females were allowed to lay eggs in bottles at standard density and, upon emer-

Table 1 Chromosome replacement lines of *Drosophila melanogaster* used in the tests (Wu *et al.*, 1995). Fr, Hg and Z30 refer to chromosomes from isogenic lines from France, Highgrove (California) and Zimbabwe, respectively (lines 1, 4 and 6). Lines 2, 3, 7 and 20 are recombinants with the *rucuca* third chromosome marker stock (line 5). Table entries for lines 8 to 19 give the line of origin of the first, second and third chromosomes, respectively

Line	Genotype
1	Fr
2	Fr *cu sr e ca*
3	Fr *ru h th st*
4	Hg
5	*ru cu ca*
6	Z30
7	Z30 *cu sr e ca*
8	Fr Fr Z30
9	Fr Z30 Fr
10	Fr Z30 Z30
11	Z30 Fr Fr
12	Z30 Fr Z30
13	Z30 Z30 Fr
14	Hg Hg Z30
15	Hg Z30 Hg
16	Hg Z30 Z30
17	Z30 Hg Hg
18	Z30 Hg Z30
19	Z30 Z30 Hg
20	Z3030 *ru h th st*

gence, male progeny were split into five groups as described below.

Tests of stress tolerance

Flies were placed in the stressful environments for two purposes: (i) to measure changes in metabolism; and (ii) to measure stress tolerance. For the enzyme assays, flies from 19 of the 20 lines were reared at constant density in 0.25 L bottles (line 20 was skipped because it is only useful for mapping the third chromosome arms, and 19 lines × 5 replicates fit perfectly on a microtitre plate). Upon emergence, adult males were allowed to recover from anaesthesia for one-half day on a standard cornmeal–sucrose–agar medium, and were then transferred to the stress medium. The control group was maintained on standard medium at 25°C. One group of flies was placed in vials with Whatman filter paper soaked in 3% acetic acid. Another group was placed in vials with Whatman paper soaked in 3% ethanol. The fourth group was placed on standard medium, but 45 h before homogenization for measurement of enzyme activities they were transferred to empty vials where the cotton ball was kept moist with water. The fifth group was placed on standard medium at 32°C. Henceforth, the environmental treatments will be referred to as Control, Acetic, EtOH, Starve and Temp. Each treatment was represented by at least five rearing bottles and test vials per line, and the effect of the rearing container was quantified as a vial effect in the analysis of variance (see below).

For the survival tests, adult males were collected within 1 day of emergence and were allowed to recover from anaesthesia on standard medium for 2 days. Flies were transferred to stressful media in groups of 20 flies per vial. Each group of 20 flies was from a unique rearing bottle. Approximately every 2 h, the vials were examined and the number of dead flies was recorded. The primary measure of stress tolerance is the mean survival time under each stress. For the acetic acid and ethanol stresses, initial trials were carried out with a range of concentrations in order to determine the concentrations that yielded ≈50% mortality in 48 h. These were 11% acetic acid and 18% ethanol, which were administered on Whatman paper in solutions with 3% sucrose (Chakir *et al.*, 1996). Starvation trials were performed as for the enzyme tests, and the thermal stress was carried out by placing vials in a waterbath at 37°C.

Measurement of metabolic traits and enzyme kinetics

Adult males were maintained on the stressful environments for 6 days, at which time flies from all treatments were weighed and homogenized. Procedures for preparing whole-fly tissue homogenates, dispensing into microtitre plates and scoring enzyme kinetics with a microtitre plate reader are described in detail in Wang & Clark (1995). The metabolic traits that were scored include total protein, triacylglycerol content, glycogen content and the activities of the enzymes ADH, glucose-6-phosphate dehydrogenase (G6PD), glycerol-3-phosphate dehydrogenase (GPDH), hexokinase (HEX), malic enzyme (ME), 6-phosphogluconate dehydrogenase (PGD), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM) and trehalase (TRE). Anaesthetized groups of four flies were weighed to the nearest 0.001 mg and were moved to a cold room (4°C) where each group was individually homogenized, centrifuged and distributed into 15 microtitre plates. Microtitre plates were frozen at −70°C until kinetic assays were run. Plates were thawed for a standardized time and, after adding appropriate reagents with a gauged pipettor, a microtitre plate reader recorded the optical density of each well of the microtitre plate at a series of times. Enzyme activities were calculated from standards as nmoles of substrate converted to product per fly per minute.

Statistical methods

The tests the study aimed to perform fell into three main categories: effects of stress on enzyme activities; effects of stress on survival; and the relationship between the two. For all three, the following null hypotheses were tested: (i) no differences among genotypes; (ii) no effect of environmental treatments; (iii) differences in phenotype were independent of replaced chromosomes; (iv) no genotype × environment interactions. These were all tested with standard methods of analysis of variance using the SAS procedure GLM. For the ANOVA, the model fitted was:

$$y_{ijkl} = \mu + G_i + E_j + (GE)_{ij} + V_{ijk} + e_{ijkl},$$

where G_i is the effect of genotype i ($i = 1$–19), E_j is the effect of environmental treatment j (where $j = 0,1,2,3,4$ for Control, Acetic, EtOH, Starve and Temp, respectively), $(GE)_{ij}$ is the interaction of genotype i with environment j, V_{ijk} is the effect of vial k in genotype i and environment j, and the error term is e_{ijkl}. Environment and genotype are fixed effects and the vial term is a random effect. There

are allometric relationships between enzyme activities and body size, so significance tests were also performed, adjusting out the effects of weight and total protein by analysis of covariance. It should be noted that although the analysis of covariance generally gave smaller error variance, in the majority of cases the tests of significance for ANOVA and ANCOVA in these experiments yielded the same results.

Correlations among metabolic traits were considered in detail in Clark (1997). The present study focused on the correlations of changes across environments, and on the correlations between metabolic traits and survival. This was assessed by Spearman rank correlations of the 20-line means.

Results

Metabolic phenotypes

Four samples from each of the 19 genetic lines under each of five environments, for a total of 380 samples, were scored for weight, protein, lipid, glycogen and activities of nine enzymes in intermediary metabolism (metabolic traits were not measured for line 20). The study examined whether the phenotypes became more variable across lines in the stressful environment than they were in the control environment. This might be the case if the lines differed in their ability to maintain homeostasis. In 25 of the 52 comparisons (13 traits × 4 environments) the among-line variance was greater in the stressful environment than the control environment, indicative of no major trend in variation in homeostasis. The tendency for natural selection to reduce variation in the population is greater, all else being equal, the greater the genetic variance in the phenotypes. When genotypes are exposed to a range of environments, the relevant variance must be taken across some sort of average over the environments. When trait means for each line were averaged over environments, the among-line variance of these mean phenotypes was less than the among-line variance in the control environment in 45 of the 52 comparisons. This occurs because the rank order of genotypes differs from one environment to another, and this is the pattern of G × E that can serve to maintain variation, or at least retard its rate of loss.

The changes in phenotype caused by each stressful environment are plotted in Fig. 1. Each error bar reflects the variation across lines for each metabolic trait. Several features of the data are readily apparent from this plot. In proportion to the control flies, the lines were more stable in live weight than they were in triacylglycerol content. The trait that showed the greatest heterogeneity across lines in its change relative to controls was trehalase activity, and glycogen content was among the most stable. Note that the mean ADH activity appears to have increased under all stressful environments, although not every line exhibited this increase. Statistical significance of these trends will be tested below.

Stress tolerance

Survival times were scored for a grand total of 14 393 flies, for an average of ≈150 flies per line per stressful environment. The survival times differed widely among lines on all stress treatments (Table 2), whereas fewer than 1% of the control flies died during the survival time tests. Even a quick scan of this table shows that the rank order of the lines in their stress tolerance differs among treatments, a pattern that is indicative of a genotype × environment interaction. The pattern of G × E is seen graphically by the crossing lines in Fig. 2.

Tests of significance

Analysis of variance was used to ascertain significance of the effects of each stress treatment (Table 3). The experiment had replicated measurements of flies drawn from more than one vial so that the effects of the microenvironmental variation among vials could also be quantified. The 'vial' term, given in the ANOVA model in the Methods section, does not appear in Table 3 because, as is generally expected in such an experiment, in most cases the vials were heterogeneous compared to variation within vials. Biologically this vial effect is not important to this experiment, but the among-vial error is important to quantify for significance testing. The error mean square reported in Table 3 is the pooled error and vial mean square. The residuals were adequately fitted by a normal distribution, and variances were not significantly heterogeneous. All 13 metabolic characters exhibited significant differences on one environment or another, and all but one of the traits exhibited significant differences among genotypes. Significant genotype × environment interaction was seen for all traits except PRO, ADH, HEX and PGD. A particular pattern of variation is illustrated in Fig. 3, showing two lines that differ in their degree of environmental lability. This example illustrates a contrast between two lines, one of which was evidently highly sensitive to the acetic acid environment (line 5, *ru cu ca*), exhibiting many
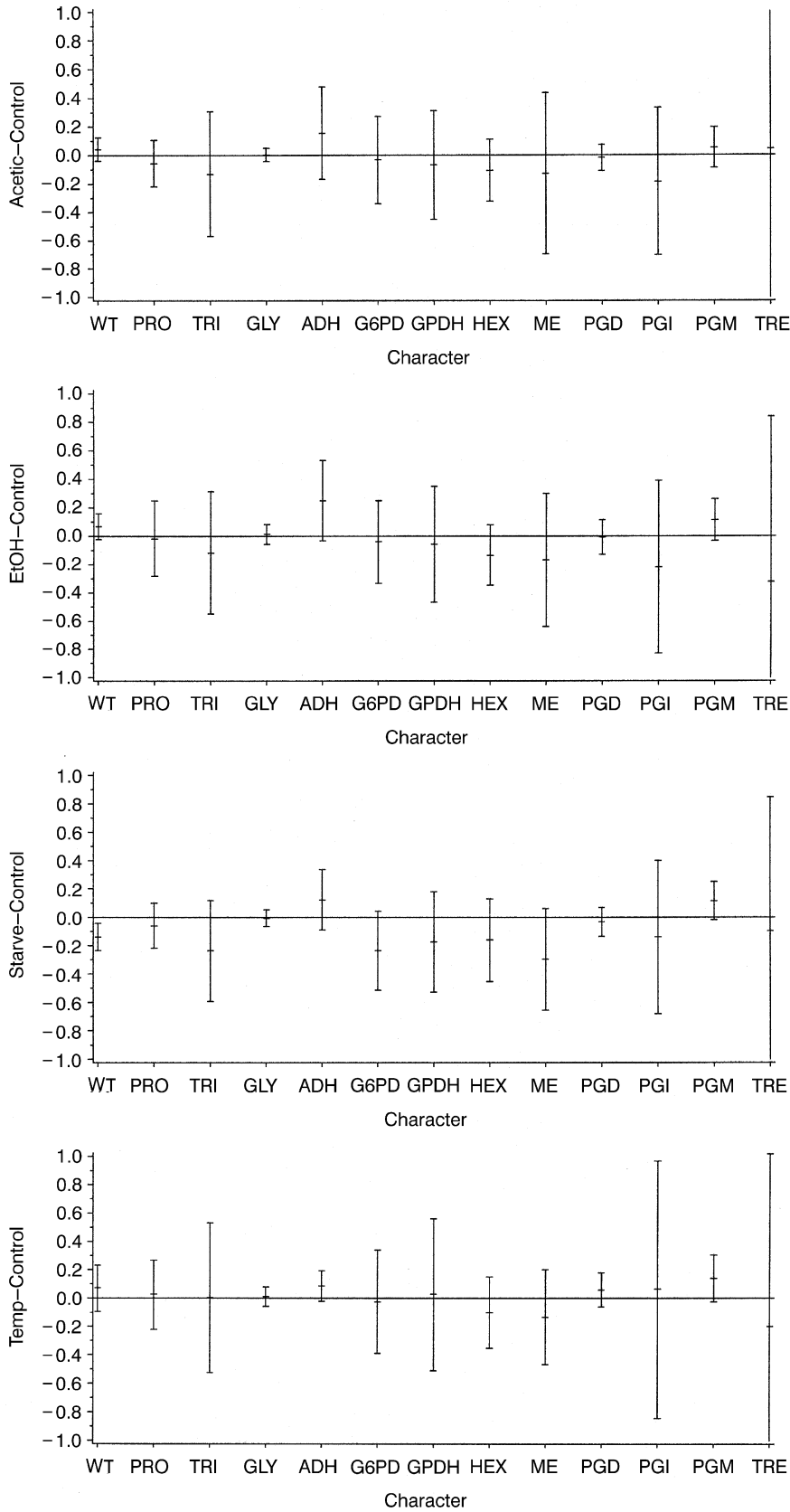
Fig. 1 The effects on metabolic traits of the four environmental stresses on posteclosion male *Drosophila melanogaster*. Error bars represent the standard deviation across the 19 lines for which metabolic traits were measured. The *y*-axis scale is the proportional change of mean phenotypes relative to the control. Abbreviations on the *x*-axis are: WT, live weight; PRO, total protein; TRI, triacylglycerol; GLY, glycogen; ADH, alcohol dehydrogenase; G6PD, glucose-6-phosphate dehydrogenase; GPDH, glycerol-3-phosphate dehydrogenase; HEX, hexokinase; ME, malic enzyme; PGD, 6-phosphogluconate dehydrogenase; PGI, phosphoglucose isomerase; PGM, phosphoglucomutase; and TRE, trehalase.

metabolic traits that differed strongly from the control environment. The other line (line 1, Fr) in this example appears to be well buffered against the stressful environment, in that its metabolic measurements appear to have changed little from the control medium.

Table 2 Line means of survival time (h) for *Drosophila melanogaster* exposed to various stresses. Two-day-old adult males were placed in the stressful environments and followed at ≈ 2 h intervals until all flies succumbed. Sample size was ≈ 150 flies per treatment per line

Line	Acetic acid	EtOH	Starve	Temp
1	53.0 ± 1.44	42.8 ± 1.76	39.1 ± 1.17	33.5 ± 2.05
2	54.3 ± 1.46	51.2 ± 1.74	39.2 ± 1.48	27.5 ± 3.11
3	53.7 ± 1.57	32.5 ± 1.53	38.3 ± 1.56	27.4 ± 3.11
4	60.2 ± 1.40	51.3 ± 1.77	60.4 ± 2.29	38.3 ± 1.87
5	44.1 ± 0.93	41.6 ± 1.65	58.3 ± 2.73	32.6 ± 2.31
6	44.8 ± 1.24	48.0 ± 1.77	60.4 ± 2.43	36.7 ± 1.61
7	41.4 ± 1.31	42.3 ± 1.80	49.5 ± 1.88	31.3 ± 2.35
8	52.5 ± 1.54	46.1 ± 1.85	61.5 ± 3.50	28.4 ± 3.32
9	57.0 ± 1.61	39.5 ± 1.63	54.5 ± 1.88	20.7 ± 3.51
10	48.5 ± 1.46	41.6 ± 1.73	71.1 ± 2.99	28.8 ± 2.69
11	55.9 ± 1.53	25.6 ± 1.34	42.6 ± 1.57	32.1 ± 2.49
12	49.8 ± 1.45	39.4 ± 1.74	63.6 ± 2.11	31.5 ± 3.11
13	52.8 ± 1.29	45.0 ± 1.74	50.7 ± 1.96	37.8 ± 1.52
14	55.6 ± 1.43	53.3 ± 2.04	62.5 ± 3.37	32.2 ± 2.63
15	46.9 ± 1.09	33.7 ± 1.71	56.3 ± 2.32	33.1 ± 2.32
16	45.5 ± 1.23	45.3 ± 1.71	67.2 ± 2.64	39.0 ± 0.49
17	55.4 ± 1.76	47.6 ± 1.66	63.4 ± 2.66	22.7 ± 2.54
18	49.8 ± 1.21	43.7 ± 1.89	61.0 ± 4.58	25.9 ± 2.10
19	59.7 ± 1.67	39.3 ± 1.76	58.6 ± 2.28	35.0 ± 2.33
20	59.2 ± 1.50	44.4 ± 1.75	70.8 ± 3.41	29.1 ± 3.02

The phenotypic measurements used for the calculations in Table 3 are taken on a per-fly basis. This approach implicitly assumes that the relationship between live weight and each trait is the same across rearing media (e.g. a doubling in weight doubles enzyme activity). The one treatment that significantly affected weight was starvation. Table 3 looks very similar if the analysis is repeated calculating each activity on a per mg live weight basis. The effects of live weight and total protein were also removed from the other traits by analysis of covariance, treating live weight and total protein as covariates. This is better than calculations on a per-mg basis, because analysis of covariance allows for allometric relationships between metabolic traits and body weight where the slope differs from one. The final result is that the analysis of variance and covariance yielded tables that were remarkably similar in the pattern of significant tests.

Individual chromosome effects and epistasis

The set of lines listed in Table 1 allows partitioning of effects into the three chromosomes. For a given pair of parental lines, the eight genotypes having the various combinations of chromosomes 1, 2 and 3 allow the orthogonal contrasts to ascribe effects to each chromosome. Figure 4 shows how individual chromosome effects can be deduced. In this particular example, any line having a third chromosome from the French line has a higher activity than does the line with a Zimbabwe third chromosome. Table 4 reports the variance partitioning, showing the percentage of the variance ascribed to each

Fig. 2 Line means of survival time of *Drosophila melanogaster* in each stressful environment. The crossing of the lines on the plot when traversing from one environment to another is indicative of genotype × environment interaction (but note that significant G × E can occur even when lines do not cross).
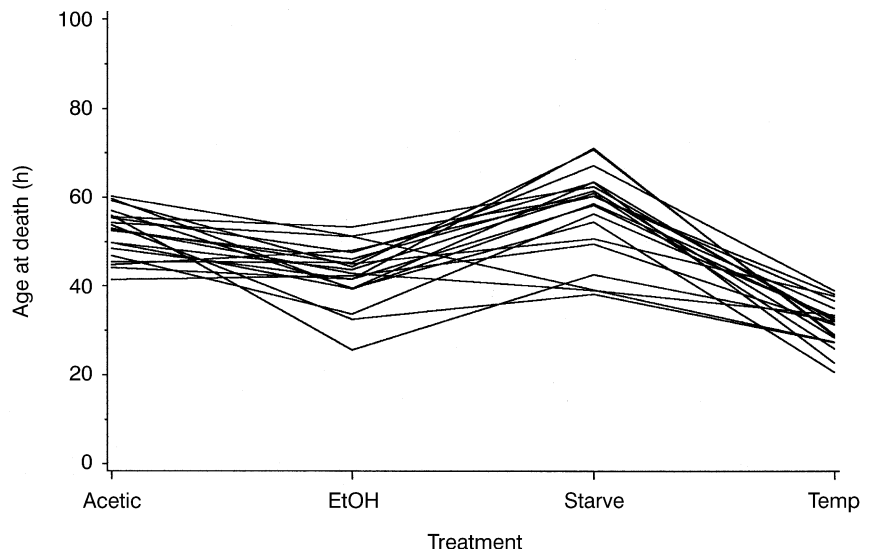
Table 3 Effects of posteclosion stress on metabolic traits and survival in *Drosophila melanogaster*

Trait	Genotype	Environment	G × E	Error
Acetic acid vs. control				
WT	0.857***	0.117	0.127	0.091
PRO	70.560	387.600**	45.830	56.010
TRI	298.600***	965.200***	217.400***	79.480
GLY	0.328**	0.117	0.200	0.146
ADH	4.608*	50.330***	3.300	2.532
G6PD	6.623***	0.336	1.011*	0.482
GPDH	916.500**	245.400	322.000	572.400
HEX	0.919***	3.587***	0.239	0.230
ME	68.580***	108.600***	7.691*	4.222
PGD	0.281	0.381	0.145	0.225
PGI	46.040*	325.300***	44.900*	24.590
PGM	37.120**	394.200***	32.950*	17.040
TRE	853.500***	38.460	828.700***	208.200
SURV	507.700***	1195.000***	378.700***	579.900
Ethanol vs. control				
WT	1.046***	0.063	0.591*	0.316
PRO	71.690	75.460	115.200	94.420
TRI	364.200***	915.900*	143.800	134.300
GLY	0.215*	0.909*	0.428***	0.136
ADH	3.833	135.700***	2.209	3.788
G6PD	7.277***	0.300	1.308***	0.362
GPDH	1531.000***	197.800	429.400	523.800
HEX	1.104***	8.991***	0.117	0.236
ME	55.540***	100.000**	13.830	9.684
PGD	0.380**	0.092	0.168	0.179
PGI	61.770**	513.000***	48.500*	28.820
PGM	47.280***	1819.000***	37.330**	15.640
TRE	512.100***	3681.000***	974.400***	118.500
SURV	183.800***	1022.000***	43.150***	14.370
Starvation vs. control				
WT	0.894***	7.557***	0.045	0.516
PRO	110.100	406.200*	41.210	93.300
TRI	687.400***	2339.000***	117.900	121.700
GLY	0.988	0.111	0.341***	0.113
ADH	2.441*	19.340***	1.334	1.572
G6PD	5.826***	15.830***	1.548***	0.375
GPDH	1151.000***	1427.000*	406.800	392.000
HEX	1.693***	6.573***	0.483**	0.209
ME	51.430***	230.800***	14.080***	3.967
PGD	0.292*	0.224	0.201	0.179
PGI	79.020***	114.600*	37.500	28.830
PGM	64.820***	1191.000***	29.630**	11.670
TRE	1176.000***	84.040	689.400*	350.000
SURV	438.600***	2907.000***	355.100***	366.400
Thermal stress vs. control				
WT	1.381***	2.017***	0.168	0.122
PRO	123.800	40.750	84.690	109.300
TRI	628.500***	0.546	213.400**	97.880
GLY	0.192	0.312	0.442***	0.145
ADH	2.085***	14.030***	0.287	0.571
G6PD	4.062***	2.103*	0.987**	0.439
GPDH	1643.000***	116.600	609.600*	313.500
HEX	1.303***	5.324***	0.301	0.286
ME	57.140***	109.900***	11.270***	3.945
PGD	0.422*	2.827***	0.227	0.241
PGI	62.850*	3.257	98.260***	31.890
PGM	57.120***	2226.000***	43.140***	13.980
TRE	693.800***	238.300	1873.000***	118.900
SURV	319.200***	7299.000***	129.400***	350.300

Degrees of freedom: Environment (4), Genotype (18), G × E (72), and Error (285).

Fig. 3 Changes in the 13 phenotypic characters of *Drosophila melanogaster* caused by posteclosion exposure to 3% acetic acid for 5 days compared to the control medium. The *y*-axis value is the scaled difference in the line means on acetic acid vs. control medium [(acetic acid mean − control mean)/control mean]. Only two different genetic lines are illustrated, showing one line that appears to be very stable across the two environments, and another genetic line that is very labile.
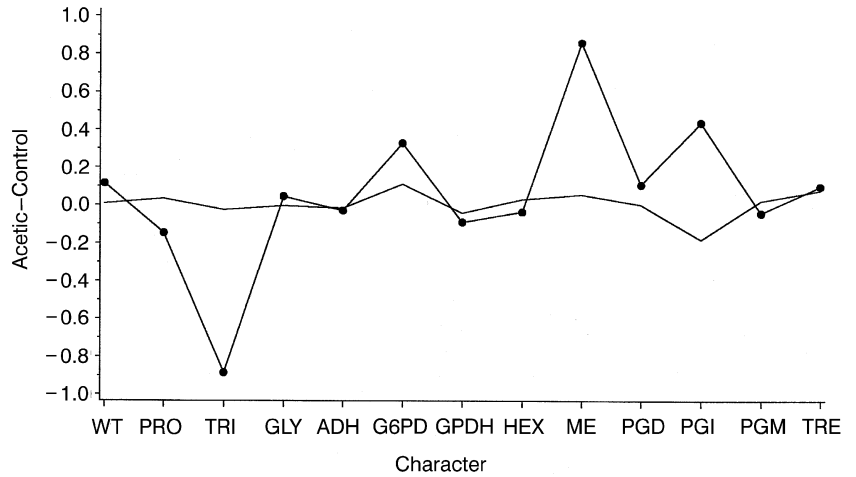
chromosome, to interactions among chromosomes, and to interactions between environmental treatments and each chromosome. Significance values were not adjusted for multiple contrasts, in part because there are several ways to consider what is the scope of the experiment-wide tests, but even the most conservative correction for multiple comparisons leaves about half the tests shown in Table 4 as significant. From Table 3 it was clear that there should be both genetic effects and genotype × environment interactions, and many of these could be partitioned to individual chromosomes. A significant chromosome × environment interaction term implies the existence of a gene or genes on the given chromosome that mediate the degree of phenotypic change when comparing one environment with another. Such genes mediate the environmental lability of the trait. The striking observation from Table 4 is the large size of the epistatic effects, and how frequently they are significant. The generally abundant epistatic effects for these metabolic traits

were also seen for the effects of pairs of random *P*-element insertions (Clark & Wang, 1997), and suggest that the network of coordinated regulation of these genes is highly interconnected.

Genetic correlations

The stability of genetic correlations across the stress environments received a quantitative analysis in Clark (1997), leaving two further aspects to consider. First, what are the correlations among pairs of traits in the difference between control and stressed conditions? If acetic acid stress causes an increase in G6PD, for example, does it also increase PGD activity? Fig. 5 shows the significant correlations among changes in activities across the test environments. Not surprisingly, the pentose phosphate shunt enzymes, G6PD and 6PGD did, in fact, show coordinated changes. One of the most striking results of this analysis was for the pair of enzymes PGM and PGI, both of which share glucose-6-phos-

Fig. 4 Mean hexokinase activity of the eight recombinant lines of *Drosophila melanogaster* between the F and Z30 chromosomes. Note that the presence of the F third chromosome results in a significantly elevated HEX activity, despite the fact that the structural gene *HEX-C* is on the second chromosome at 2–74.5.
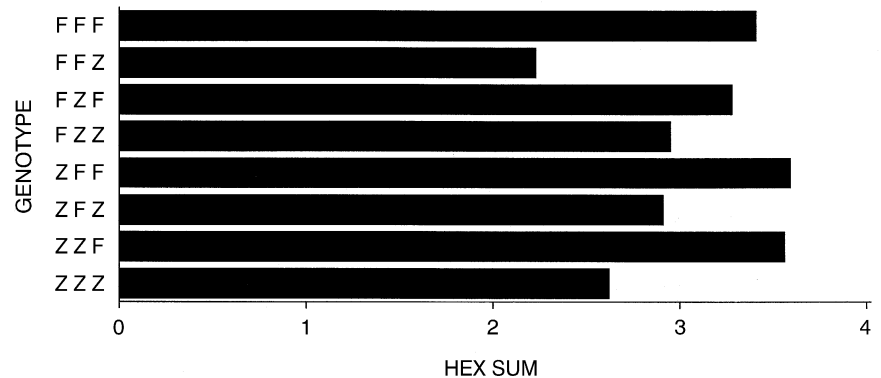
Table 4 Variance components from ANOVA partitioned by chromosome of *Drosophila melanogaster*. Total variance was partitioned by restricted maximum likelihood and percentage contribution of each factor is reported along with the significance. The Epistasis effect includes interactions among chromosomal effects (1×2, 1×3, 2×3 and $1 \times 2 \times 3$). The terms $T \times 1$, $T \times 2$ and $T \times 3$ are interactions between treatments (stress environments) and each of the three chromosomes

	Chromosome				$G \times E$			
Trait	1	2	3	Epistasis	$T \times 1$	$T \times 2$	$T \times 3$	Error
Acetic acid vs. Control								
WT	9.9**	0.0	33.6***	13.1***	2.3	1.2*	0.0	39.8
PRO	0.0	0.0	0.0	3.6*	0.0	0.0	0.0	96.4
TRI	0.0	0.0	0.0	18.3**	3.1	3.0*	0.5	75.1
GLY	5.5	0.0	0.0	10.2	0.0	1.4	0.0	82.9
ADH	0.0	0.0	0.0	9.2*	0.0	0.0	0.0	90.8
G6PD	5.1***	0.0	1.5**	48.1***	1.0	0.0	0.0	44.3
GPDH	1.4	0.0	0.0	9.7*	0.0	0.0	0.0	88.9
HEX	6.2***	0.0	4.6*	26.1***	0.0	0.0	0.3	62.8
ME	0.0	5.2*	0.0	53.4***	2.4	0.0	0.9	38.2
PGD	0.0	0.0	0.0	1.5	0.0	0.0	0.0	98.5
PGI	0.0	1.6*	0.0	5.1	0.0	4.1	0.0	89.2
PGM	0.0	0.0	0.0	10.9*	10.9**	0.0	0.0	78.1
TRE	0.0	0.0	0.0	21.3***	4.0**	15.3***	0.5	58.9
SURV	0.5	4.0***	4.4***	6.8***	1.5*	3.9***	3.4	75.5
Ethanol vs. Control								
WT	1.2	0.0	12.1***	8.7*	0.0	0.0	0.0	78.0
PRO	0.0	0.0	0.0	0.5	0.0	2.7	0.0	96.8
TRI	3.1*	2.1	0.0	8.6	0.0	0.0	0.0	86.2
GLY	0.0	0.0	0.0	3.5*	4.1*	0.0	0.0	92.4
ADH	0.6	0.0	0.0	2.7*	1.5	0.0	0.0	95.2
G6PD	2.9*	0.0	0.3	60.0***	3.0*	0.0	0.0	33.9
GPDH	0.6	0.0	3.1*	16.8**	0.0	0.0	0.0	79.5
HEX	4.4***	0.0	4.3*	36.5***	0.0	0.0	0.0	54.7
ME	0.0	0.9**	0.0	35.7***	0.0	0.0	1.6	61.8
PGD	3.5*	2.0	0.0	4.3*	0.0	0.0	0.0	90.2
PGI	2.0*	0.0	3.2**	6.7	2.8	7.7**	0.0	77.6
PGM	0.0	0.0	2.4*	18.9***	12.5***	6.0*	0.0	60.3
TRE	0.0	0.0	0.0	14.0**	12.6***	15.4**	1.4	56.6
SURV	4.4	10.4***	3.2*	13.5**	1.1	2.4*	1.1	63.7
Starvation vs. Control								
WT	17.8***	2.3***	49.6***	7.5***	0.0	0.0	0.0	22.7
PRO	3.4*	0.0	0.0	4.9*	0.0	0.0	0.0	91.6
TRI	0.0	1.1*	0.0	21.5***	0.0	0.0	0.0	77.4
GLY	0.0	0.0	0.0	0.0	15.0***	0.0	0.0	85.0
ADH	0.0	0.0	0.0	6.4*	0.9	2.1	0.0	90.6
G6PD	3.0***	2.8***	0.0	47.2***	0.2	0.0	0.0	46.9
GPDH	0.0	0.0	1.4**	14.7***	1.4	0.0	0.0	82.5
HEX	11.5***	0.0	1.7*	27.8**	0.0	1.1*	3.6*	54.3
ME	4.2***	3.5***	0.0	42.2***	0.0	0.0	0.0	50.1
PGD	2.6	0.1	0.0	1.8	0.0	0.0	0.0	95.5
PGI	0.0	7.0**	0.0	9.5*	1.3	0.0	0.0	82.2
PGM	0.0	2.1*	0.0	18.3***	4.1	0.0	0.0	75.5
TRE	0.0	5.5**	0.0	16.6***	12.6***	0.0	0.0	65.3
SURV	3.2***	0.9	13.4**	7.6**	2.8*	8.3***	2.2*	61.7

Table 4 *Continued*

| Trait | Chromosome | | | | G × E | | | |
	1	2	3	Epistasis	T × 1	T × 2	T × 3	Error
Thermal stress vs. Control								
WT	0.0	2.4**	33.7***	22.1***	2.6	0.0	0.0	39.2
PRO	2.9*	0.0	0.0	2.8	0.8	0.0	0.0	93.5
TRI	8.5***	7.3***	0.0	11.8**	2.6	2.7	0.0	67.1
GLY	0.0	1.0	1.3	3.0	8.6**	0.0	0.2	85.8
ADH	0.0	0.0	0.0	27.2***	0.0	0.0	0.0	72.8
G6PD	2.3***	0.0	0.0	41.7***	0.5	2.6*	1.5	51.4
GPDH	3.2***	0.0	0.0	33.4***	0.0	4.9**	0.6	58.0
HEX	7.5***	0.0	3.2**	29.1***	0.0	0.0	0.0	60.3
ME	4.1***	4.0***	0.0	45.8***	0.0	0.0	3.7	42.4
PGD	6.4**	0.0	0.0	1.7*	0.2	0.0	0.0	91.6
PGI	0.0	0.0	2.2*	7.1*	5.5**	2.5	0.3	82.3
PGM	0.0	0.0	3.3*	18.8***	11.6***	0.0	7.7	58.6
TRE	2.0**	0.0	0.0	4.2	6.9**	13.4***	8.5	65.0
SURV	1.3	4.1***	4.0***	11.2***	1.4*	0.7	2.7*	74.7

phate as a substrate. This is a branchpoint, so if the changes were effectively a switch favouring one or the other branch of the pathway, one might expect that increases in one activity would be associated with a reduction in the activity of the other. Figure 6 shows very clearly that this is the kind of switching that occurred.

Correspondence between effects of stresses on enzyme activities and survival

Survival times on the stressful media were not significantly correlated with any of the metabolic traits (details not shown). However, when we compared the changes in metabolic traits in response to stresses to the survival times, several correlations were
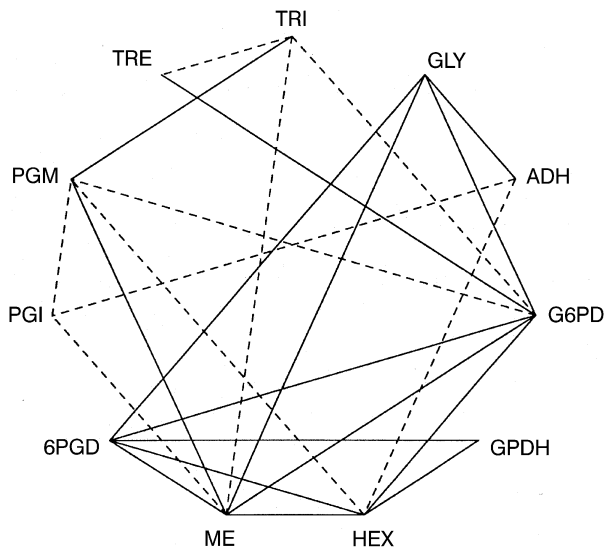

Fig. 5 Correlations in changes in line means of metabolic traits that occur across the four stress treatments applied to *Drosophila melanogaster*. Positive correlations between pairs of traits are indicated by solid lines, and occur when the pair of traits covaries across treatments. Negative correlations are indicated by dashed lines. Significance is determined after adjusting for multiple comparisons.
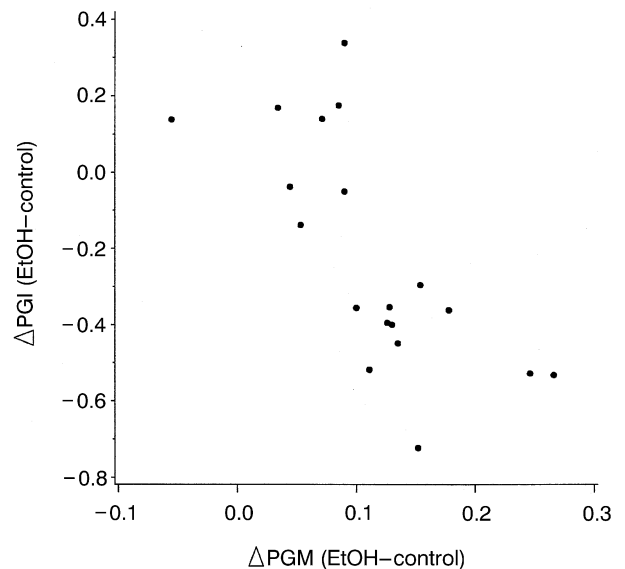

Fig. 6 A particular example of the coordinated changes identified in Fig. 5. Note that ethanol results in an increase in PGM activity in most lines, and a decrease in PGI. Those lines with the greatest increase in PGM appear to have the greatest decrease in PGI, consistent with a branchpoint switch.

Table 5 Correlations between survival time and stress-induced changes in metabolic traits in *Drosophila melanogaster*. Figures are Spearman rank correlations of line means of survival and the difference in line mean of metabolic traits in the stressed medium vs. the control medium

	Acetic	EtOH	Starve	Temp
WT	0.080	0.086	0.026	0.094
PRO	0.314**	0.069	0.143	0.318**
TRI	−0.134	0.091	0.323**	−0.042
GLY	0.046	0.087	−0.149	0.216*
ADH	0.096	0.047	−0.099	0.012
G6PD	0.082	0.210*	0.054	0.176
GPDH	−0.167	−0.018	0.250*	−0.042
HEX	−0.042	0.044	0.227*	0.169
ME	−0.017	0.130	0.236*	0.060
PGD	0.147	0.092	−0.003	0.287*
PGI	0.076	−0.325**	0.142	−0.124
PGM	−0.174	0.318**	0.047	0.220*
TRE	0.168	0.048	−0.350**	−0.045
Acetic	—	0.478***	0.466***	0.014
EtOH	0.478***	—	0.229*	0.308**
Starve	0.466***	0.229*	—	0.129
Temp	0.014	0.308**	0.129	—

$*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

significant (Table 5). These correlations are tested with only 19 lines, so the power to detect significant effects is quite low. Nevertheless, some of the correlations made good physiological sense. Survival under starvation was higher in the lines that had the biggest change in triacylglycerol, implying that the lines most able to utilize fats were the longest survivors. There was no association between survival under ethanol stress and the relative induction of ADH activity, but the PGI–PGM switch was very strong in the ethanol treatment, and lines that shifted towards PGM had higher survival. The survival times under the various stresses were generally positively intercorrelated.

Discussion

It remains an open question how much evolution is driven by rare bouts of exposure to stressful environments, but it is clear that such exposure can have a major impact on underlying genetic variation. This study showed that simple metabolic stresses resulted in measurable changes in every metabolic trait examined. The 20 genotypes that were studied differed markedly in their responses to the stressful environments, with some lines seeming to have very poor homeostatic ability, varying wildly in many

components of metabolism, whereas other lines remained relatively stable across environments. One might suppose that such homeostasis is advantageous, but several correlations between survival under stress and magnitude of short-term metabolic changes suggest that it may not be so simple.

The immediate physiological changes that occur in response to a stressful environment may be related to the genetic responses that occur during long-term artificial selection for tolerance of the same stress. Lines of flies that have been selected for resistance to knockdown by ethanol fumes, for example, generally have a higher frequency of high-activity ADH alleles (Oakeshott *et al.*, 1985; Hoffmann & Cohan, 1987). The Chateau Tahbilk winery population of *D. melanogaster* was shown to have greater resistance to ethanol vapour than flies caught outside the winery, again suggesting that the natural selection on the stressful substrate reinforced the short-term physiological response (Hoffmann & McKechnie, 1991). Desiccation acclimation also is probably mediated by the same genetic variation responsible for long-term desiccation resistance (Hoffmann, 1990, 1993). Lines of *Drosophila* reared at 28°C are more tolerant of heat shock than are lines reared at lower temperatures (Cavicchi *et al.*, 1995), and lines that have been selected by cold pretreatment are generally more tolerant of cold-shock (Chen & Walker, 1993).

In some cases, the short-term physiological response to stress clearly involves the same mechanism as long-term selection response. This might particularly be expected in the case of unusual metabolites in the food, where elevated levels of an enzyme might be needed for either detoxification or for utilization of the food. The ability to regulate expression of enzymes in a way that adapts to changes in the available food would appear to be advantageous. There have been extensive studies of the effects of dietary carbohydrate and ethanol on enzyme activities in *Drosophila*, and many aspects of the regulation are beginning to be understood. Many of the changes appear to be associated with $NADH : NAD^+$ ratio, including GPDH (Geer *et al.*, 1983), ADH and FAS (Geer *et al.*, 1985). In some cases, the evidence is very clear that dietary changes affect transcript levels; for example, ethanol in the medium clearly induces elevated transcription of *Adh* in *Drosophila* larvae (Geer *et al.*, 1988). Interpretation of increased enzyme activities after a challenge with synthetic medium as induction should be made with caution. *In vitro* selection with bacterial systems has produced phenomenal response, and sometimes the metabolic basis can be understood in

terms of simple mechanisms. Richard Lenski's long-term selection on maltose medium has resulted in a 100-fold increase in fitness relative to control populations (Leroi *et al*., 1994; Travisano *et al*., 1995). The observed response is almost certainly caused by changes in glucose uptake, because the metabolic steps subsequent to maltose hydrolysis are identical (Bennett & Lenski, 1996).

Simple and direct tests of genotype × environment interaction, as detailed in Table 3, demonstrate the generality of the statement that different genotypes respond to changes in the environment in different ways. The fact that the among-line variance in phenotypes averaged across environments is nearly always less than the among-line variance within any one environment is caused by a changing rank-order of genotypes in different environments. If the genetic basis for the phenotypes were simple, it is clear that directional selection on the phenotype would eliminate variation faster in one environment than it would in a fluctuating environment. But the data presented here also demonstrate the genetic complexity of the genotype × environment interactions. Specifically, the identification of interactions to individual chromosomes shows that responses to the environment map to different loci for different traits and for different lines. Even more striking was the prevalence and magnitude of epistatic interactions, which also appeared to change across environments.

Pleiotropic effects of allelic variation give rise to genetic correlations, which may or may not fit into preconceived ideas about the regulation of metabolism. There is no difficulty in telling stories *post hoc* for particularly strong coordinated changes in metabolic traits (such as G6PD and 6PGD). The stability of genetic correlations across environments becomes an issue when one tries to imagine the trajectories of multivariate selection. Classically, these models assume that the genetic covariance matrix is stable. Wilkinson *et al*. (1990) performed directional selection on thorax size in *D. melanogaster* for 23 generations and obtained significant direct effects as well as significant changes in the genetic covariance matrices among morphological traits. In another test of metabolic traits, Geer & Laurie-Ahlberg (1984) compared correlations across media that differed in composition, and they found the genetic correlations to be very stable. Geer *et al*. (1991) saw strong positive correlation between ADH activity, flux from ethanol to fatty acid, and survival on 4.5% ethanol.

In order to begin to determine whether the pattern of genotype × environment interactions is important to the maintenance of variation, it is first necessary to establish an association between metabolic changes and fitness. Historically this has not been easy. For one thing, important sources of variation are often regulatory, and those regulatory factors are often pleiotropic. Chakir *et al*. (1996) found strong correlation between tolerance of ethanol and tolerance of acetic acid in the medium, an effect that was most strongly mediated by a gene on the third chromosome (the *Adh* structural gene is on chromosome 2). Another source of difficulty in identifying fitness effects of changes in metabolism is that it is not possible to change just a single factor in metabolism. Geer *et al*. (1991) showed that lines of *Drosophila* with the highest ADH activity also deposit the most fat and have the highest survival rate on ethanol. Although one might suspect that the higher ADH activity better enables these lines to detoxify the substrate, Geer *et al*. (1991) presented evidence that it is the elevated lipid that provided the survival advantage. There was a positive correlation in the present study between survival under starvation and changes in triacylglycerol storage, but no association between changes in ADH activity and ethanol tolerance. When *D. melanogaster* were selected for increasing levels of starvation resistance, highly starvation-resistant lines were readily obtained (Chippendale *et al.*, 1996). Not surprisingly, the selected lines also had increased lipid storage, and the timing of development during the larval phase was altered. The manifold changes that are observed when organisms are faced with new metabolic stresses underscore the fact that there are many physiological solutions to the same problem. Adding genetics to this, each physiological response may be mediated by different genes in different individuals. The results reported here support the notion that the complexity of pleiotropic effects and epistatic interactions make it all the more likely that genotype × environment interactions play an important role in the maintenance of genetic variation.

Acknowledgements

References

BENNETT, A. F. AND LENSKI, R. E. 1996. Evolutionary adaptation to temperature. V. Adaptive mechanisms and

correlated responses in experimental lines of *Escherichia coli*. *Evolution*, **50**, 493–503.

BLOWS, M. W. AND SOKOLOWSKI, M. B. 1995. The expression of additive and nonadditive genetic variation under stress. *Genetics*, **140**, 1149–1159.

CAVICCHI, S., GUERRA, D., LA TORRE, V. AND HUEY, R. B. 1995. Chromosomal analysis of heat-shock tolerance in *Drosophila melanogaster* evolving at different temperatures in the laboratory. *Evolution*, **49**, 676–684.

CHAKIR, M., CAPY, P., GENERMONT, J., PLA, E. AND DAVID, J. R. 1996. Adaptation to fermenting resource in *Drosophila melanogaster*: ethanol and acetic acid tolerances share a common genetic basis. *Evolution*, **50**, 767–776.

CHEN, C-P. AND WALKER, V. K. 1993. Increase in cold-shock tolerance by selection of cold resistant lines in *Drosophila melanogaster*. *Ecol. Entomol.*, **18**, 184–190.

CHIPPENDALE, A. K., CHU, T. J. F. AND ROSE, M. R. 1996. Complex trade-offs and the evolution of starvation resistance in *Drosophila melanogaster*. *Evolution*, **50**, 753–766.

CLARK, A. G. 1997. Stress and metabolic regulation in *Drosophila*. In: Bijlsma, R. and Loeschcke, V. (eds) *Environmental Stress, Adaptation and Evolution*, pp. 117–132. Birkhäuser, Basel.

CLARK, A. G. AND WANG, L. 1997. Epistasis in measured genotypes: *Drosophila* P-element insertions. *Genetics*, **147**, 157–163.

GEER, B. W. AND LAURIE-AHLBERG, C. C. 1984. Genetic variation in the dietary sucrose modulation of enzyme activities in *Drosophila melanogaster*. *Genet. Res.*, **43**, 307–321.

GEER, B. W., WILLIAMSON, J. H., CAVENER, D. R. AND COCHRANE, B. J. 1981. Dietary modulation of glucose-6-phosphate dehydrogenase (EC 1.1.1.4.9) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) in *Drosophila melanogaster*. In: Bhaskaran, G., Friedman, S. and Rodriguez, J. G. (eds) *Current Topics in Insect Endocrinology and Nutrition*, pp. 253–282. Plenum Press, New York.

GEER, B. W., McKECHNIE, S. W. AND LANGEVIN, M. L. 1983. Regulation of sn-glycerol-3-phosphate dehydrogenase in *Drosophila melanogaster* larvae by dietary ethanol and sucrose. *J. Nutr.*, **113**, 1632–1642.

GEER, B. W., LANGEVIN, M. L. AND McKECHNIE, S. W. 1985. Dietary ethanol and lipid synthesis in *Drosophila melanogaster*. *Biochem. Genet.*, **23**, 607–622.

GEER, B. W., McKECHNIE, S. W., BENTLEY, M. M., OAKESHOTT, J. G., QUINN, E. M. AND LANGEVIN, M. L. 1988. Induction of alcohol dehydrogenase by ethanol in *Drosophila melanogaster*. *J. Nutr.*, **118**, 398–407.

GEER, B. W., McKECHNIE, S. W., HEINSTRA, P. W. H. AND PYKA, M. J. 1991. Heritable variation in ethanol tolerance and its association with biochemical traits in *Drosophila melanogaster*. *Evolution*, **45**, 1107–1119.

GILLESPIE, J. H. AND TURELLI, M. 1989. Genotype–environment interactions and the maintenance of polygenic variation. *Genetics*, **121**, 129–138.

GUPTA, A. P. AND LEWONTIN, R. C. 1982. A study of reaction norms in natural populations of *Drosophila pseudoobscura*. *Evolution*, **36**, 934–948.

HICKEY, D. A., BENKEL, K. I., FONG, Y. AND BENKEL, B. F. 1994. A *Drosophila* gene promoter is subject to glucose repression in yeast cells. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 11,109–11,112.

HOFFMANN, A. A. 1990. Acclimation for desiccation resistance in *Drosophila melanogaster* and the association between acclimation responses and genetic variation. *J. Insect. Physiol.*, **36**, 885–891.

HOFFMANN, A. A. 1993. Selection for adult desiccation resistance in *Drosophila melanogaster* fitness components, larval resistance and stress correlations. *Biol. J. Linn. Soc.*, **48**, 43–55.

HOFFMANN, A. A. AND COHAN, F. M. 1987. Genetic divergence under uniform selection. III. Selection for knockdown resistance to ethanol in *Drosophila melanogaster* populations and their replicate lines. *Heredity*, **58**, 425–433.

HOFFMANN, A. A. AND McKECHNIE, S. W. 1991. Heritable variation in resource utilization and response in a winery population of *Drosophila melanogaster*. *Evolution*, **45**, 1000–1015.

HOFFMANN, A. A. AND PARSONS, P. A. 1991. *Evolutionary Genetics and Environmental Stress*. Oxford University Press, Oxford.

LEROI, A. M., LENSKI, R. E. AND BENNETT, A. F. 1994. Evolutionary adaptation to temperature. III. Adaptation of *Escherichia coli* to a temporally varying environment. *Evolution*, **48**, 1222–1229.

MA, E. AND HADDAD, G. G. 1997. Anoxia regulates gene expression in the central nervous system of *Drosophila melanogaster*. *Mol. Brain Res.*, **46**, 325–328.

McKECHNIE, S. W. AND GEER, B. W. 1984. Regulation of alcohol dehydrogenase in *Drosophila melanogaster* by dietary alcohol and carbohydrate. *Insect Biochem.*, **14**, 231–242.

OAKESHOTT, J. G., COHAN, F. M. AND GIBSON, J. B. 1985. Ethanol tolerances of *Drosophila melanogaster* populations selected on different concentrations of ethanol supplemented medium. *Theor. Appl. Genet.*, **69**, 603–608.

OUDMAN, L., VAN DELDEN, W., KAMPING, A. AND BIJLSMA, R. 1992. Interaction between the *Adh* and *αGpdh* loci in *Drosophila melanogaster*: adult survival at high temperature. *Heredity*, **68**, 289–297.

PECSENYE, K., LEFKOVITCH, L. P., GILES, B. E. AND SAURA, A. 1996. Differences in environmental temperature, ethanol and sucrose associated with enzyme activity and weight changes in *Drosophila melanogaster*. *Insect Biochem. Mol. Biol.*, **26**, 135–145.

PECSENYE, K., BOKOR, K., LEFKOVITCH, L. P., GILES, B. E. AND SAURA, A. 1997. Enzymatic responses of *Drosophila melanogaster* to long- and short-term exposures to ethanol. *Mol. Gen. Genet.*, **255**, 258–268.

TRAVISANO, M., VASI, F. AND LENSKI, R. E. 1995. Long-term experimental evolution in *Escherichia coli*. III. Varia-

tion among replicate populations in correlated responses to novel environments. *Evolution*, **49**, 189–200.

VIA, S., GOMULKIEWICZ, R., DEJONG, G., SCHEINER, S. M., SCHLICHTING, C. D. AND VAN TIENDEREN, P. H. 1995. Adaptive phenotypic plasticity consensus and controversy. *Trends Ecol. Evol.*, **10**, 212–218.

WANG, L. AND CLARK, A. G. 1995. Physiological genetics of the response to a high-sucrose diet by *Drosophila melanogaster*. *Biochem. Genet.*, **33**, 149–164.

WILKINSON, G. S., FOWLER, K. AND PARTRIDGE, L. 1990. Resistance of genetic correlation structure to directional selection in *Drosophila melanogaster*. *Evolution*, **44**, 1190–2003.

WU, C.-I, HOLLOCHER, H., BEGUN, D. J., AQUADRO, C. F., XU, Y. AND WU, M.-L. 1995. Sexual isolation in *Drosophila melanogaster*: A possible case of incipient speciation. *Proc. Natl. Acad. Sci. U.S.A.*, **92**, 2519–2523.

YAMAZAKI, T. AND MATSUO, Y. 1984. Genetic analysis of natural populations of *Drosophila melanogaster* in Japan. III. Genetic variability of inducing factors of amylase and fitness. *Genetics*, **108**, 223–235.