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Genetic variation in the dietary sucrose modulation of enzyme activities in *Drosophila melanogaster**

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SUMMARY

Genetic variation in the modulating effect of dietary sucrose was assessed in *Drosophila melanogaster* by examining 27 chromosome substitution lines coisogenic for the *X* and second chromosomes and possessing different third isogenic chromosomes derived from natural populations. An increase in the concentration of sucrose from 0·1 % to 5 % in modified Sang's medium C significantly altered the activities of 11 of 15 enzyme activities in third instar larvae, indicating that dietary sucrose modulates many, but not all, of the enzymes of *D. melanogaster*. A high sucrose diet promoted high activities of enzymes associated with lipid and glycogen synthesis and low activities of enzymes of the glycolytic and Krebs cycle pathways, reflecting the physiological requirements of the animal. Analyses of variance revealed significant genetic variation in the degrees to which sucrose modulated several enzyme activities. Analysis of correlations revealed some relationships between enzymes in the genetic effects on the modulation process. These observations suggest that adaptive evolutionary change may depend in part on the selection of enzyme activity modifiers that are distributed throughout the genome.

1. INTRODUCTION

The activity levels of several enzymes in the larvae of *Drosophila melanogaster* are modulated by nutrients. The oxidative NADP-enzymes G6PD (see Table 1 for the enzyme abbreviation index), PGD, ME and IDH are influenced by changes in dietary sucrose and lipid (Geer *et al.* 1981); whereas, tissue activities of ADH, GPDH, sn-glycerol-3-phosphate oxidase, mitochondrial malate dehydrogenase, and fatty acid synthetase are sensitive to variations in dietary sucrose and ethanol (Geer, McKechnie & Langevin, 1983; McKechnie & Geer, 1984). Not all enzymes are subject to regulation by dietary sucrose, ethanol or lipid; nor are the modulator effects qualitatively or quantitatively the same for all enzymes (Geer *et al.* 1981, 1983; McKechnie & Geer, 1984). These observations suggest that the actions of the modulating nutrients are specific to some degree.

Allozymes with different properties are known for several structural gene loci

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in *D. melanogaster* (reviewed by O'Brien & MacIntyre, 1978). Studies of artificial populations show that allozymes may contribute differentially to the fitness of the individual in a certain environment (Cavener & Clegg, 1981*a*, *b*; Wilson *et al.* 1982 and references therein) but attempts to relate allozyme frequencies with environmental factors in natural populations have met with mixed success. For example, five enzyme polymorphisms in *D. melanogaster* exhibit latitudinal clines but other than a correlation of the frequency of ADH with rainfall amount, no consistent correlations between allozyme frequencies and environmental factors are evident (Oakeshott *et al.* 1982 and references therein). Thus, the environmental factors responsible for the latitudinal clines remain to be identified. Genetic modifiers of enzyme levels in *D. melanogaster* that are associated with the structural gene locus have been reported (Laurie-Ahlberg *et al.* 1982 and the references therein). By comparing enzyme activities in adults of lines sharing a common isogenic background but differing by chromosomes derived from natural populations, genetic modifiers of some enzyme activity levels have been shown to be distributed throughout the genome (Laurie-Ahlberg *et al.* 1982, Wilton *et al.* 1982 and references therein). Conceivably, adaptive evolutionary change may be more dependent on the variation provided by the polymorphism of enzyme activity modifiers than on structural gene variation.

The natural diet is an important component of the environment, and the selection of variants of genes that control the nutritional modulation of metabolism could be important to the adaptation of a species to the environmental niche. Besides the observation that the magnitude of the induction of G6PD by dietary sucrose is influenced by autosomal modifiers (Geer *et al.* 1981), little is known about the genetics of dietary modulation in *D. melanogaster*. The current investigation of *D. melanogaster* was conducted (1) to investigate the specificity of sucrose modulation on enzyme activities (2) to determine if genetic modifiers alter the degree of sucrose modulation of different enzymes to different degrees and (3) to ascertain if there are sucrose induced coordinate shifts in the activities of metabolically related enzymes.

2. METHODS AND MATERIALS

Twenty-seven isogenic chromosome substitution lines i_1/i_1; i_2/i_2; $+_3/+_3$ were constructed at North Carolina State University (Laurie-Ahlberg *et al.* 1980). Except for the Ho–R standard line, *i* refers to a chromosome from the inbred line, Ho–R, and + refers to a chromosome from one of four natural populations in the United States (Kansas, North Carolina, Rhode Island and Wisconsin). The +, but not the *i* chromosomes, vary within a set of lines and each line is homozygous for all three major chromosomes. Eggs were collected and sterilized by previously described methods (Geer & Newburgh, 1970) and the lines were placed under axenic culture conditions on modified Sang's medium C throughout the experimental work at Knox College. The composition of standard modified Sang's medium C is 3·5 g casein, 1·5 g agar, 1 g sucrose, 0·2 g yeast RNA, 30 mg cholesterol, 0·05 mg biotin, 1·6 mg calcium pantothenate, 8 mg choline chloride, 1 mg folic acid, 1·2 mg nicotinic acid, 0·25 mg pryridoxine-HCl, 1 mg riboflavin, 0·2 mg thiamine-HCl,

25 mg $MgSO_4$ 7 H_2O, 0·1 mg $NaHCO_3$, 0·183 g KH_2PO_4, 0·189 g K_2HPO_4, 40 mg NaOH (pH adjusted to 6·85) and water to 100 ml. The sucrose content of one test diet was 0·1 % (0·1 g/100 ml medium) and 5 % in another test diet. A third diet contained 5 % sucrose and 0·05 % lecithin (50 mg/100 ml medium).

Test cultures were set up by placing 10–15 female-male pairs of four day old adults that had been reared on modified Sang's medium C in 6-dram shell vial cultures containing 5 ml of the standard medium for 24 h. After 4 days of growth, larvae were transferred to different test diets by the method of Geer *et al.* (1976) and cultured for two days before biochemical analysis. The test cultures contained 30–80 larvae per vial and were maintained at 22·8 °C and 50 % relative humidity with a 16 h light – 8 h dark lighting schedule. Cultures contaminated with microorganisms were discarded. Cultures for each test diet for the 27 lines of *D. melanogaster* were prepared in six blocks. The blocks for the three test diets were staggered in time so that larvae of the 27 lines that were cultured on the 0·1 % sucrose diet were harvested on day one; larvae cultured on 5 % sucrose medium where harvested on day two; and larvae cultured on 5 % sucrose-lecithin were harvested on day three. This sequence was performed a total of six times to yield six blocks of each diet using different adult females and males and different batches of freshly prepared media for each block. Homogenates of larvae were prepared and stored at −80 °C until they were shipped from Knox College to North Carolina State University for enzyme and protein analysis. All shipments were packed in dry ice and received the next day in good condition. The samples were stored at North Carolina State at −80 °C until thawed for analysis.

To prepare homogenates, 75 mid-third instar larvae (25 each from three cultures) were placed in a 1·5-ml Eppendorf tube and washed three times with distilled water. The larvae were homogenized in 0·5 ml of 0·01 M-KH_2PO_4-K_2HPO_4 buffer, pH 7·4, with 60 turns of a hand-operated Teflon pestle, then allowed to stand for 20 min at 4 °C before centrifuging at 12000 × g for 20 min. The supernatant was removed and aliquots dispensed into 0·25-ml tubes containing 0·01 M-K_2HPO_4-KH_2PO_4 buffer, pH 7·4, the same buffer with 2 mM ethylenediamine tetraacetic acid (EDTA), the same buffer with 0·2 mM dithiothreitol (DTT), or the same buffer with both 2 mM EDTA and 0·2 mM DTT. The diluent type that the samples of homogenate were stored in before the assay of each enzyme and for protein were as indicated by Stam & Laurie-Ahlberg (1982).

The assay procedures for the enzyme activities in Table 1 have been described by Stam & Laurie-Ahlberg (1982). The assays were performed with a computer-interfaced centrifugal fast analyser, with all of the assays for each enzyme being performed on a single day. The design gives a total of six observations per line for each of three test diets, where each observation is made on a different set of 75 larvae reared at a different time ('block') and homogenized independently of the other sets for that line. One sample from each block-by-diet-by-line combination was used for the determination of total protein concentration. The model for analysis for variance is:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + \epsilon_{ijk},$$

were α_i is the effect of the ith block ($i = 1, \ldots, 6$), β_j is the effect of the jth diet

($j = 1, 2$), γ_k is the effect of the kth line ($k = 1, \ldots, 26$) and effects in parentheses are interactions. The block and line effects are considered to be random and the diet effect is considered to be fixed. This design gives a total of 156 observations per diet when completely balanced. However, some samples were lost during the experiment, creating a small amount of unbalance; the actual total observations per diet varied among enzymes, averaging about 146.

Table 1. *Enzymes assayed in this study*

Enzyme	Abbreviation	E.C. number	Map position[a]
Alcohol dehydrogenase	ADH	1.1.1.1	2–50.1
Aldolase	ALD	4.1.2.13	3–91.5
Arginine kinase	AK	2.7.3.3	3L
Aldehyde oxidase	AOX	1.2.3.1	3–56.6
Fumarase	FUM	4.2.1.2	1–19.9
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49	1–63
sn-Glycerol-3-phosphate dehydrogenase	GPDH	1.1.1.8	2–20.5
Hexokinase	HEX	2.7.1.1	1–29.2, 2–75.3
NADP-Isocitrate dehydrogenase	IDH	1.1.1.42	3–27.1
NADP-Malic enzyme	ME	1.1.1.40	3–53.1
Phosphoglucomutase	PGM	2.7.5.1	3–43.4
6-Phosphogluconate dehydrogenase	PGD	1.1.1.44	1–0.64
Phosphoglucose isomerase	PGI	5.3.1.9	2–58.7
Phosphoglycerokinase	PGK	2.7.2.3	2–7.6
Transaldolase	TA	2.2.1.2	?

[a] Treat-Clemmons & Doane (1982).

The enzyme activities per larva were adjusted for total protein per larva by linear regression, as follows:

$$\hat{Y}_{ijk} = Y_{ijk} - b_j(P_{ijk} - \bar{P}...),$$

where $\hat{Y}$ is the adjusted activity value, Y is the observed activity per larvae, P is the corresponding protein per larva, and b_j is the regression coefficient for the jth diet. The b_j values were obtained by taking the regression of activity on protein over lines within each block and pooling the sums of squares and products over blocks.

3. RESULTS

Each enzyme was assayed in six different blocks for the 27 lines. The analysis of variance of the enzyme activities given in Table 2 indicated that the block of time during which larvae were reared and sampled was a significant source of variation for 12 of the 15 enzymes for the 0·1 % *v.* 5 % sucrose diet comparison and 9 of the 15 enzymes activities for the 5 % sucrose *v.* 5 % sucrose-lecithin comparison (Table 2). These results indicate the importance of the unidentified variation in environmental or experimental conditions as well as the effectiveness of the blocking design. Since WI12 lacks ME and exhibited extreme sucrose-induced responses for some enzymes (Fig. 1), it was excluded from Tables 2–5 to avoid extreme skewing of comparisons.

Table 2. *Significance Levels of F-Tests From Analyses of Variance*

	Diets 0·1 *v.* 5·0 sources of variation						Diets 5·0 *v.* 5·5 sources of variation					
Enzyme	Block	Diet	$B \times D$	Line	$B \times L$	$D \times L$	Block	Diet	$B \times D$	Line	$B \times L$	$D \times L$
G6PD	*	**	****	****	ns	*	ns	ns	****	****	*	ns
FUM	***	****	**	****	ns	****	ns	ns	ns	****	ns	ns
PGM	****	ns	****	****	ns	**	**	*	ns	****	ns	ns
PGK	***	ns	ns	****	ns	ns	****	ns	ns	****	ns	ns
PGI	ns	ns	*	****	ns	**	**	ns	***	****	ns	ns
ADH	ns	****	**	****	ns	***	ns	ns	*	****	ns	ns
ALD	*	****	****	****	ns	****	***	ns	**	****	ns	ns
AOX	****	****	*	****	ns	**	*	ns	ns	****	ns	ns
GPDH	****	****	ns	****	ns	**	**	ns	ns	****	*	ns
ME	****	ns	****	****	ns	****	***	ns	****	****	ns	ns
HEX	****	****	ns	**	**	*	ns	ns	ns	*	ns	ns
IDH	**	****	ns	****	ns	****	*	ns	ns	****	ns	ns
AK	**	***	ns	****	ns	ns	****	ns	ns	****	ns	ns
PGD	**	****	ns	****	ns	ns	ns	ns	ns	****	ns	ns
TA	ns	**	**	**	ns	ns	ns	ns	*	ns	ns	ns
PROT	**	ns	*	****	ns	ns	****	ns	ns	***	ns	ns

Enzyme activities are adjusted by regression on protein.
Diets: 0·1–0·1 % sucrose, 5·0 = 5·0 % sucrose, 5·5 = 5·0 % sucrose + lecithin.
ns $P > 0{\cdot}05$, *$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$, ****$P < 0{\cdot}0001$.

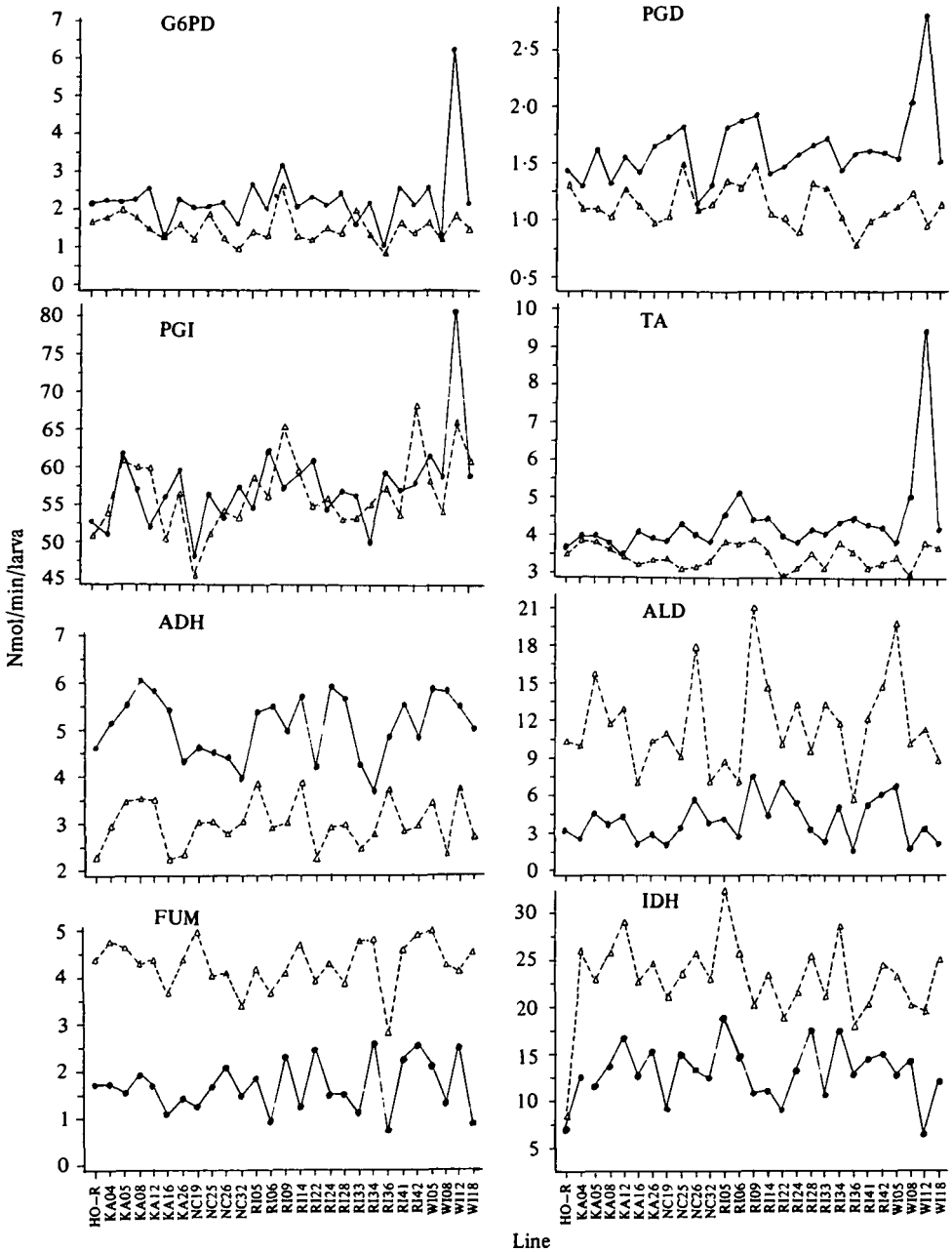

Fig. 1. The plots of enzyme activities of larvae of chromosome substitution lines fed a 0·1% sucrose (△---△) or 5% sucrose (●——●) diet.

The low sucrose and high sucrose diets, as indicated by the diet component, exerted markedly different influences on the test larvae of the test strains; the activities of 11 of the 15 enzymes differed significantly for the two diets (Table 2). In contrast, supplementation of the high sucrose diet with lecithin had little

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effect; only one of the activities of the 15 enzymes was significantly altered. The activities of G6PD, ADH, GPDH, PGD and TA were increased by a high dietary sucrose concentration; whereas, the activities of FUM, ALD, AOX, HEX, IDH, and AK were repressed by the sucrose supplement (Table 3). These observations indicate that dietary sucrose modulates some enzyme activities in a positive way and others in a negative manner. That the activities of PGM, PGK, PGI and ME were not affected by the change in sucrose concentration implies that sucrose acts

Table 3. *Diet means*

	Protein-adjusted enzyme activities (Nmol/min/larva)			% of 0·1 diet		
	Diet			Diet		
	0·1	5·0	5·5	0·1	5·0	5·5
G6PD	1·50	2·12	1·97	100	141	131
FUM	4·26	1·65	1·56	100	39	37
PGM	6·48	7·87	6·85	100	121	106
PGK	22·50	22·23	21·43	100	99	95
PGI	56·16	56·54	57·09	100	101	102
ADH	2·97	5·05	5·10	100	170	172
ALD	11·63	4·03	3·36	100	35	29
AOX	0·464	0·322	0·309	100	69	67
GPDH	3·35	8·10	8·19	100	242	244
ME	7·14	8·11	7·40	100	114	104
HEX	2·45	1·92	1·88	100	78	77
IDH	23·06	13·24	13·06	100	57	57
AK	31·68	26·27	26·45	100	83	83
PGD	1·14	1·59	1·61	100	139	141
TA	3·43	4·16	4·26	100	121	124
PROT	45·84	42·66	43·90	100	93	96

Diets: 0·1 = 0·1 % sucrose, 5·0 = 5·0 % sucrose, 5·5 = 5·0 % sucrose + lecithin.
PROT: micrograms general protein per larva.

with some degree of specificity. That the protein contents of the larvae fed the three test diets did not differ significantly suggests that the larvae of the test groups had attained similar stages of growth regardless of the diet. The variation among lines, the line component, was significant for all of the enzyme activities and for the protein contents in the 0·1 % *v.* 5 % sucrose diet comparison, and for all but one enzyme activity for the 5 % sucrose-lecithin comparison (Table 2). The line or genetic component is diet-independent and is different from the Diet × Line ($D \times L$) component. The $D \times L$ component reflects variation in the extents of dietary induction/repression by sucrose or lecithin supplements for the 27 chromosomal substitution lines. For the 0·1 % *v.* 5 % sucrose comparison there was significant genetic variation in the magnitude of the sucrose induction/repression for 11 of the 15 enzymes but the indicator of larval growth, protein content, had no significant $D \times L$ interaction (Table 2). Since diet-induced genetic variation was not significant for protein content, the genetic variants responsible for dietary modulation of enzyme activities in the chromosomal substitution lines were not

simply a reflection of a general modulation of protein synthesis by dietary sucrose. A comparison of the high sucrose and high sucrose-fat diets showed no significant $D \times L$ variation for any of the enzyme activities or the protein content.

The importance of the $D \times L$ interactions can be evaluated in another way. The correlation over lines between diets (Table 4) provides a relative measure of the

Table 4. *Correlations over line means between diets*

Enzyme	0·1, 5·0	0·1, 5·5	5·0, 5·5
G6PD	0·58**	0·59**	0·82***
FUM	0·36	0·32	0·76***
PGM	0·54**	0·65***	0·70***
PGK	0·49*	0·39*	0·59**
PGI	0·42*	0·68***	0·78***
ADH	0·42*	0·45*	0·75***
ALD	0·72***	0·55**	0·83***
AOX	0·66***	0·54**	0·73***
GPDH	0·31	0·38	0·83***
ME	0·71***	0·61**	0·94***
HEX	0·45*	0·41*	0·67***
IDH	0·78***	0·84***	0·91***
AK	0·55**	0·52**	0·71***
PGD	0·47*	0·52**	0·72***
TA	0·14	0·52**	0·21
PROT	0·51**	0·25	0·64***

Line means are protein-adjusted enzyme activities
Diets: 0·1 = 0·1 % sucrose, 5·0 = 5·0 % sucrose, 5·5 = 5·0 % sucrose, +lecithin.
*$P < 0·05$, **$P < 0·01$, ***$P < 0·001$.

prominence of the $D \times L$ interaction. Although the magnitude of the genetic effects within each diet plays a role to some extent, the smaller the correlation the more important the $D \times L$ interaction. Without exception the correlation estimates for the enzyme activities of the 0·1 % sucrose *v.* 5 % sucrose comparison were smaller than the corresponding correlations of the 5 % sucrose *v.* 5 % sucrose-lecithin comparison. This result provides more evidence that there is variation among the third chromosome substitution lines for genetic elements which influence the magnitudes of sucrose modulation of enzyme activities.

A survey of the enzyme activities of the different lines fed the 0·1 % and 5·0 % sucrose test diets revealed a striking $D \times L$ interaction. The activities of the pentose phosphate cycle (the pentose shunt) enzymes G6PD, PGD and TA for WI12 were induced by the high sucrose diet to levels about two-fold higher than any other line while the levels on the low sucrose diet were not unusual (Fig. 1). Since WI12 lacks ME, these observations suggest that the high degree of inducibility of the pentose shunt enzymes in WI12 reflects a compensation for the ME deficiency. The unusually high sucrose inductions of GPDH and PGI in this line may also reflect a compensatory mode of carbohydrate metabolism.

Other less striking deviates from the norm were evident in a visual examination of the enzyme activity plots lines (Fig. 1). The induction of G6PD by sucrose was observed for all lines except KA16, WI08, RI33 and RI36, but the other pentose

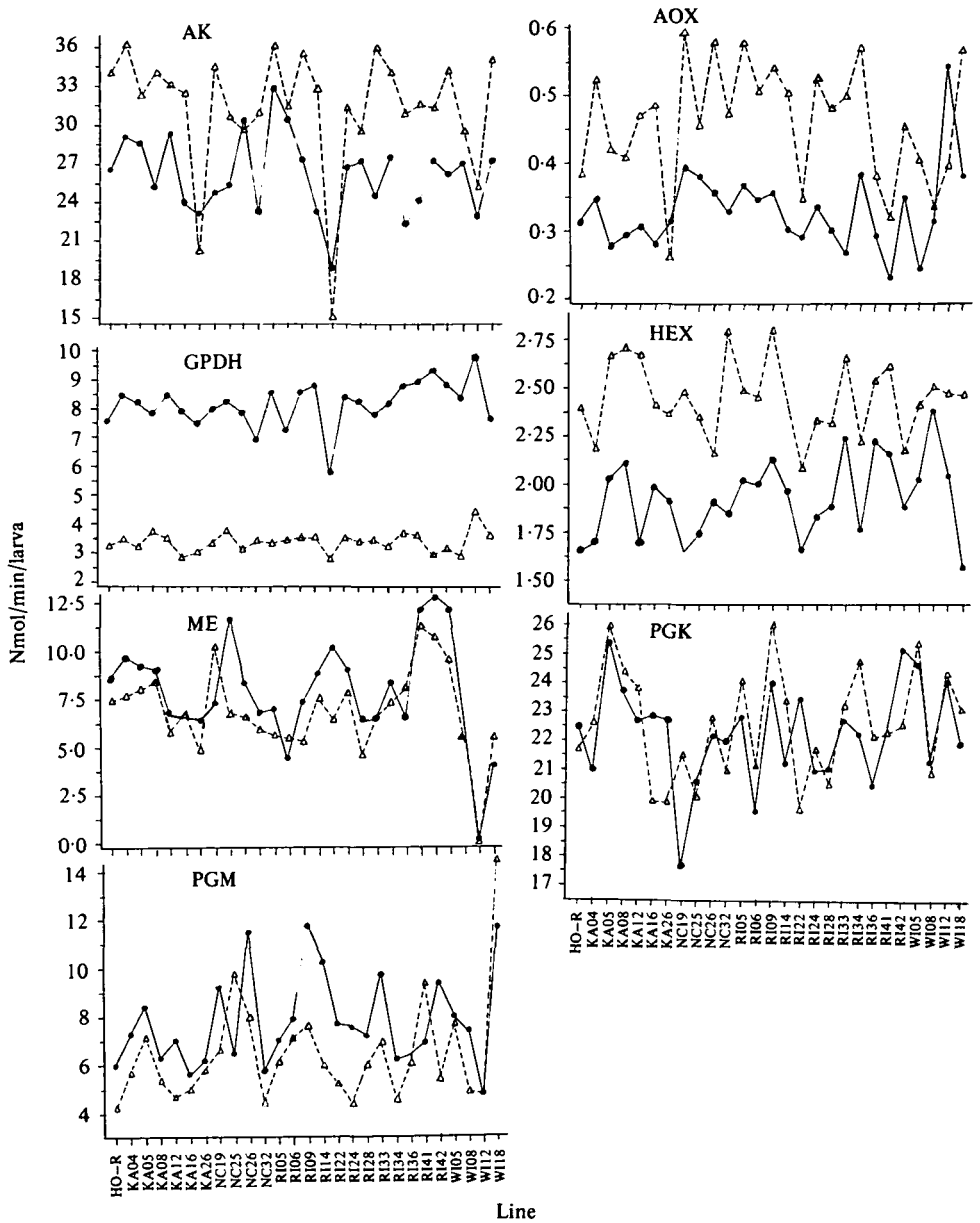

Fig. 2. The plots of enzyme activities of larvae of chromosome substitution lines fed a 0·1% sucrose (△---△) or 5% sucrose (●——●) diet.

shunt enzymes, PGD and TA, were induced to some degree by dietary sucrose in these lines, suggesting that the activity of the shunt may have been altered by sucrose despite the apparent lack of G6PD induction. Sucrose induction of PGD in NC26 was quite low; whereas, sucrose did not induce TA in KA12. NC19 had low PGI activities for the test diets. Although lines NC25 and NC19 did not show particularly abnormal levels of G6PD and PGD induction in the present study,

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Table 5. *Correlations using protein-adjusted line means* ($n = 26$) *above diagonal: over line means within each diet below diagonal: over the ratio of line means*, 5·0/0·1 (see text)

	G6PD	FUM	PGM	PGK	PGI	ADH	ALD	AOX	GPDH	ME	HEX	IDH	AK	PGD	TA	Diet	
		0·41*	0·27	0·51**	0·31	−0·01	0·58**	0·02	0·24	−0·02	0·31	−0·07	0·31	0·49*	0·27	0·1	G6PD
G6PD	—	0·63***	0·30	0·33	−0·10	0·14	0·68***	0·12	0·15	0·33	−0·26	0·16	0·35	0·06	−0·22	5·0	G6PD
		0·74***	0·34	0·55**	−0·02	0·19	0·68***	0·27	0·39*	0·46*	−0·37	0·25	0·25	0·38	0·39*	5·5	G6PD
			0·14	0·43*	0·13	−0·02	0·55**	0·14	−0·12	0·44*	−0·17	0·14	0·30	0·02	0·06	0·1	FUM
FUM	0·42*	—	0·02	0·54**	−0·18	−0·19	0·81***	0·03	0·10	0·68***	−0·13	0·16	0·09	−0·18	−0·19	5·0	FUM
			0·21	0·54**	−0·08	0·12	0·90***	0·17	0·17	0·68***	−0·36	0·11	0·01	0·05	0·05	5·5	FUM
				0·15	0·13	0·04	0·10	0·17	0·37	0·01	0·08	0·10	0·18	0·18	0·12	0·1	PGM
PGM	0·14	0·17	—	0·07	0·08	−0·01	0·28	0·25	0·15	−0·08	0·00	−0·29	0·21	0·08	0·14	5·0	PGM
				0·24	0·13	0·19	0·16	0·05	0·49*	0·08	0·00	0·12	0·09	0·08	0·41*	5·5	PGM
					0·58**	0·56**	0·67***	0·31	0·25	0·21	0·37	0·26	0·60**	0·07	0·61**	0·1	PGK
PGK	−0·06	0·32	−0·05	—	0·38	0·08	0·58**	−0·42*	0·10	0·40*	0·20	0·03	0·03	−0·16	−0·25	5·0	PGK
					0·46*	0·30	0·52**	0·23	0·38	0·37	0·00	0·32	0·18	0·10	0·33	5·5	PGK
						0·41*	0·45*	−0·01	0·05	0·01	0·15	0·29	0·10	−0·00	0·38	0·1	PGI
PGI	−0·17	−0·31	−0·42*	0·34	—	0·24	0·13	−0·48*	−0·15	−0·01	0·42*	−0·06	−0·27	0·24	0·36	5·0	PGI
						0·43*	−0·02	−0·04	0·12	−0·12	0·23	0·22	−0·02	0·04	0·23	5·5	PGI
							0·17	0·24	0·54**	0·20	0·32	0·42*	0·40*	−0·08	0·50**	0·1	ADH
ADH	−0·36	−0·19	0·03	0·44*	0·37	—	−0·02	−0·38	0·45*	0·02	0·32	0·20	0·30	0·18	0·10	5·0	ADH
							−0·00	−0·10	0·57**	0·21	0·42*	0·36	0·08	0·38	0·29	5·5	ADH
								0·12	−0·09	0·25	0·06	0·01	0·18	0·11	0·06	0·1	ALD
ALD	0·60**	0·71***	0·02	0·42*	0·01	−0·23	—	−0·10	0·08	0·60**	−0·07	−0·01	0·02	−0·14	−0·19	5·0	ALD
								0·11	0·05	0·59**	−0·38	0·03	0·01	−0·04	−0·06	5·5	ALD
									0·23	−0·12	−0·07	0·43*	0·58**	0·24	0·41*	1·1	AOX
AOX	−0·06	0·08	−0·15	0·49*	0·22	0·19	0·04	—	−0·06	−0·24	−0·50**	0·15	0·41*	0·11	0·18	5·0	AOX
									0·06	−0·15	−0·31	0·62***	0·41*	0·08	0·39*	5·5	AOX

Table 5 (*cont.*)

	G6PD	FUM	PGM	PGK	PGI	ADH	ALD	AOX	GPDH	ME	HEX	IDH	AK	PGD	TA	Diet	
										0·07	0·46*	0·08	0·49*	0·09	0·35	0·1	GPDH
GPDH	−0·09	0·11	0·30	0·14	−0·30	0·23	−0·23	0·01	—	0·32	0·35	0·37	0·37	0·20	0·16	5·0	GPDH
										0·40*	0·15	0·25	0·22	0·28	0·71***	5·5	GPDH
											−0·07	−0·22	0·22	−0·48*	−0·14	0·1	ME
ME	0·19	0·67***	0·02	0·43*	−0·04	−0·15	0·51**	0·21	0·01	—	−0·08	−0·07	−0·16	−0·23	−0·28	5·0	ME
											−0·38	−0·10	−0·16	−0·12	0·10	5·5	ME
												−0·07	0·41*	0·23	0·23	0·1	HEX
HEX	−0·20	0·10	0·13	0·20	0·15	0·24	−0·10	0·11	0·54**	0·05	—	0·14	−0·02	0·40*	0·50**	5·0	HEX
												0·07	0·03	0·24	0·02	5·5	HEX
													0·16	0·07	0·34	0·1	IDH
IDH	−0·15	0·08	−0·15	0·23	0·13	0·29	0·00	0·40*	0·18	0·06	0·22	—	0·48*	0·19	0·29	5·0	IDH
													0·39*	0·19	0·41*	5·5	IDH
														0·29	0·53**	0·1	AK
AK	0·43*	0·37	0·08	0·53**	0·16	0·17	0·44*	0·53**	−0·02	0·44	0·20	0·01	—	0·16	0·20	5·0	AK
														0·24	0·26	5·5	AK
															0·14	0·1	PGD
PGD	−0·09	−0·28	−0·02	−0·08	0·13	0·03	−0·12	0·34	0·15	−0·39*	0·34	0·18	0·05	—	0·58**	5·0	PGD
															0·28	5·5	PGD
																0·1	TA
TA	−0·15	−0·01	0·01	0·31	0·46*	0·41*	0·04	0·29	0·19	−0·01	0·63***	0·22	0·26	0·33	—	5·0	TA
																5·5	TA

*$P < 0·05$, **$P < 0·01$, ***$P < 0·001$. Diets: 0·1 = 0·1 % sucrose, 5·0 = 5·0 % sucrose, 5·5 = 5·0 % sucrose + lecithin.

Cochrane, Lucchesi & Laurie-Ahlberg (1983) reported that these lines are essentially non-inducible by sucrose. However, several factors differed between the two studies, including sucrose concentrations, culture conditions and the age of the larvae. The conflicting observations suggest that genetic effects on the dietary modulation process may be sensitive to environmental and/or developmental factors.

General and extreme $D \times L$ interaction is detected in the surveys of the activities of ADH, ALD, FUM and IDH in the test lines (Fig. 1). Although the sucrose-stimulated increase in ADH activity averaged 2·1 nanomoles/min/larva, the response varied from 30 % increases in NC32 and RI34 to a 250 % increase in WI12. The sucrose-repression of ALD ranged from a reduction in activity of 3·1 nmol/min/larva in NC32 to 16·5 nmol/min/larva in RI14. The FUM activity in NC19 larvae fed 5 % sucrose was 26·5 % that of larvae fed 0·1 % sucrose; whereas, the activity of WI12 larvae fed 5 % sucrose was 62·5 % that of larvae fed 0·1 % sucrose. Compared to a slight change in Ho–R larvae the activity of WI18 larvae fed 5 % sucrose was only 24 % that of WI18 larvae fed 0·1 % sucrose. Besides these extreme differences in responses to dietary sucrose, $D \times L$ variation was particularly marked for PGM, AOX, ME and HEX (Fig. 2).

Whether the pattern of genetic correlation between enzymes is similar for the different test diets was explored by the analysis reported in Table 5. That the activity levels expressed by the lines were significantly correlated for many enzymes was indicated by the correlations over line means given in the upper half of the table. A significant correlation between a particular pair of enzymes tended to occur in more than one diet, suggesting a similar correlational structure among the three diets. However, the comparison between diets is not statistically powerful because of the rather small number of lines used in the analysis. Thus, the 95 % confidence interval for the difference between any two correlations in the upper half of Table 5 is 0·53. By this criterion, only 6 of the 315 between-diet comparisons are significant, which is about the number expected by chance. Therefore, some substantial differences in correlational structure between diets could have gone undetected.

The correlations between enzymes over the extent of induction/repression of the activities, defined as the ratio of the 5 % sucrose to the 0·1 % sucrose activity level, are provided in the lower half of Table 5. Many of these correlations are significant, indicating that there are some coordinate genetic effects on the dietary modulation process.

DISCUSSION

The current study indicates that the sucrose modulation of enzyme activities in larvae is influenced by modifiers situated on third chromosomes derived from natural populations of *D. melanogaster*. The structural genes of six of the enzymes examined in the current investigation are located on the third chromosome. Variants affecting sucrose modulation of these enzymes could be associated with the structural gene locus, but 6 of 8 enzymes whose structural genes are located on chromosomes other than the third chromosome exhibited significant $D \times L$

variation for the 0·1 % sucrose *v.* 5 % sucrose comparison. This result indicates that variants that are not linked to the structural gene modify the sucrose effects on G6PD, FUM, PGI, HEX, GPDH and ADH. Since factors that modify the sucrose modulation of enzyme activity were prevalent among the chromosome 3 substitution lines in the present study, it is probably safe to predict that there are modifiers of sucrose modulation on all of the major chromosomes of *D. melanogaster*. Although the diet was not varied for the adult flies, Wilton *et al.* (1982) found a high correlation of enzyme activities of five mitochondrial enzymes and several cytosolic enzymes between 48 second- and 48 third chromosome substitution lines of *D. melanogaster*. Our observations suggest that genetic variants that influence sucrose modulation tend to cause coordinate shifts of some enzyme activities, but it would be premature to suggest that coordinate regulation involves the mechanism that dictates the constant ratio of glycolytic and mitochondrial enzymes observed in rat and locust tissues by Pette, Klingenberg & Bucher (1962*a*), Pette, Luh & Bucher (1962*b*).

Lipid synthesis is increased dramatically and the concentration of NADPH is amplified in *D. melanogaster* larvae by the administration of a high concentration of dietary sucrose (Geer, Krochko & Williamson 1979*a*; Geer *et al.* 1983). Furthermore, the flux of carbon from sucrose to lipid via glycolysis is reduced by dietary lipid (Geer *et al.* 1976), suggesting that glycolysis is tightly coupled to lipid synthesis in larvae. In the current study the activities of HEX, ALD and PGK were repressed but the activity of PGM was stimulated by a high sucrose level, which could mean that carbon flow from dietary sucrose was diverted in part to glycogen via PGM at the expense of the glycolytic pathway. A reduction of the activity of FUM, a Krebs cycle enzyme, by a high sucrose diet concurrent with elevations in the activities of GPDH, which supplies glycerol-3-phosphate for lipid synthesis, and the pentose shunt enzymes G6PD and PGD, which supply NADPH for lipid synthesis, suggests that carbon from sucrose is also selectively channelled to lipid synthesis under this dietary regime. The activity of PGI did not respond to a high sucrose diet in most lines in the present study. However, PGI is at a pivotal point in carbohydrate metabolism and must remain responsive to the conflicting demands of glycolysis and the pentose shunt that result from the repression of glycolytic enzyme activities and induction of the pentose shunt enzyme and GPDH activities by dietary sucrose.

An interaction between the pentose shunt enzymes and ME was observed in the present investigation. The activities of G6PD, PGD and TA in WI12, a ME-deficient strain, were induced more than twice as much by a high sucrose diet than in any other line. Since ME normally supplies about 33 % of the total NADPH to larvae (Geer, Lindel & Lindel, 1979*b*), one possible interpretation is that the pentose shunt activity was elevated in ME-deficient larvae when a high sucrose diet was fed in order to compensate for the ME deficiency in terms of meeting the demand for NADPH resulting from sucrose-stimulated lipid synthesis.

Enzyme activities are only general indicators of the flux through biochemical pathways and occasionally are misleading. However, the correlations noted between the effects on enzyme activity levels in the present study and previous observations in our laboratory on the flux through pathways (Geer *et al.* 1976; Geer,

Woodward & Marshall, 1978; Geer *et al.* 1979*b*) suggest that the sucrose modulation of enzyme activities in the larvae of *D. melanogaster* reflects physiological requirements. By mediating the activities of biochemical pathways, dietary modulation of enzyme activities may facilitate the adaptation of the animal to a variety of environmental conditions. Thus, selection for genetic variants that modify the dietary modulation of enzyme activities could extend the adaptation of the species to an even broader ecological niche. The current study indicates that genetic modifiers of the dietary modulation process are commonly polymorphic and suggests that selection could alter the activity of a particular enzyme independent of selection of variants at the structural gene locus. Modulation of the levels of enzymes associated with lipogenesis by dietary sucrose and lipid have been observed in the vertebrate liver (Johnson & Sasson, 1967; Hizi & Yagil, 1974; Kelley *et al.* 1975). Consequently, selection of variants that modify dietary modulation could be prominent in the environmental adaptation of many animal species. Considering the current observations, genetic and biochemical studies designed to explore the ecological significance of enzyme polymorphism in populations should not ignore nutritional influences on enzyme activities.

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