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THE RESPONSE OF ENZYME POLYMORPHISMS TO DEVELOPMENTAL RATE SELECTION IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Two strains of *Drosophila melanogaster* derived from different geographical localities were subjected to selection for fast and slow developmental rate. Four enzyme polymorphisms were monitored for their response to such selection. Significant changes in allele frequencies were effected for the *αGpdh* and *6Pgd* polymorphisms under fast developmental rate selection. The strain from Rhode Island exhibited a correlated change in body weight during developmental rate selection, whereas the Georgia strain did not.

THE developmental rate of Drosophila is a function of numerous metabolic and developmental processes (CHURCH and ROBERTSON 1966). Indeed, it is well known that a large fraction of the various mutants employed in the genetic analysis of Drosophila have an effect on the time of development. By subjecting a wild-type population to fast and slow developmental rate selection, ROBERTSON (1963, 1964) has shown that genetic variation exists in natural populations of *D. melanogaster* for developmental rate. Genetic variation for enzyme structural genes is a possible candidate for at least a portion of the total genetic variation influencing development time. Allozyme variation that affects metabolic rates may ultimately affect developmental rates. Direct evidence has been given that naturally occurring *G6pd* (glucose-6-phosphate dehydrogenase) and *6Pgd* (6-phosphogluconate dehydrogenase) allozyme variants in *D. melanogaster* affect metabolic rates (CAVENER and CLEGG, 1981a).

In vitro kinetic differences and population genetic studies suggest that metabolic flux differences may exist between *Adh* (alcohol dehydrogenase) and *αGpdh* (α-glycerophosphate dehydrogenase) allozyme variants (GIBSON 1970; DAY, HILLER, and CLARKE 1974; MILLER, PEARCY, and BERGER 1975; VAN DELDEN, KAMPING, and VAN DIJK 1975; OAKESHOTT 1976; CAVENER and CLEGG 1978, 1981b; MCDONALD, ANDERSON, and SANTOS 1980; MCKECHNIE, KOHANE, and PHILLIPS 1982). I report herein the response of the *G6pd*, *6Pgd*, *Adh* and *αGpdh* polymorphisms to fast and slow developmental rate selection in two populations derived from different geographical localities.

MATERIALS AND METHODS

Two wild-type strains were subjected to developmental rate selection. Strain R was collected from the wild in Providence, Rhode Island in 1974 by M. G. KIDWELL. Strain G was collected in Athens, Georgia in 1978 by the author. Five replicates for each of the two strains were initiated in half-pint bottles at generation 0 (G0) with 25 males and 25 females. A random sample of 25 male and 25 female G1 progeny from the first 3 days of the eclosion period were drawn from each replicate to initiate the fast development selected lines. A random sample of 25 male and 25 female G1 progeny eclosing on days 4–10 were used to initiate the slow development selected lines. The fast development selected lines are denoted as RF1, RF2, RF3, RF4, RF5, GF1, GF2, GF3, GF4 and GF5. The slow development selected lines are denoted RS1, RS2, RS3, RS4, RS5, GS1, GS2, GS3, GS4 and GS5. (R = R strain, G = G strain, F = fast selected, S = slow selected and 1–5 are the replicate numbers.) In generations 2 through 18, 25 males and 25 females were randomly chosen from the first 3 days of the eclosion period in the fast selected replicates and used as the parents for the next generation. In generation 2 through 15 the adults emerging during the first 3 days of the eclosion period were discarded in the slow development selected replicates. Twenty-five males and 25 females randomly chosen from among adults emerging during days 4–10 from the slow selected lines were used as the parents for the proceeding generation. For both the fast and slow selected replicates, the parents were left in the bottles for only 1 day and then removed to minimize the variation in the time at which eggs were oviposited. All replicates were maintained on a standard cornmeal-molasses-yeast-agar diet at 23–24°.

Standard starch gel electrophoretic procedures (*e.g.*, AYALA, POWELL and TRACEY 1972) were used to estimate *G6pd*, *6Pgd*, *αGpdh* and *Adh* gene frequencies for the R and G strains prior to the beginning of the selection experiment. During the selection experiment, gene frequencies were determined at generations 5 and 15 for the slow selected replicates and at generations 7 and 18 for the fast selected replicates.

At generations 15 and 18 for the slow and fast selected lines, respectively, the mean developmental rates were determined for each of the replicates. Adults emerging during a 10-day period were collected daily and counted for each of the 20 replicates. At the termination of the selection experiment, a random sample of 25 males and 25 females from each of the replicates were weighed.

RESULTS

Pairwise comparisons of each fast and slow selected lines (Table 1) indicates that in nine of ten cases the fast selected lines had a significantly faster developmental rate than their corresponding slow selected replicate. The average difference in the mean development time between the R slow and fast selected strains was 0.898 days. The difference for the G strain was 1.226 days. In general, the G strain had a longer mean development time than the R strain. It is important to note that even though the total number of adults eclosing per bottle ranged between 282 and 669, there is no correlation between the mean development rate and total number of flies eclosed (product moment correlation = −0.00749).

Selection on developmental rate leads to a correlated change in body weights of both males and females in the R strain (Table 2). Fast selected flies from the R strain were significantly smaller than the slow selected flies. In contrast, no correlated changes in body size were observed for the G strain. Body weights and developmental rates were not estimated prior to the start of these experiments. Therefore, it is not known whether the observed differences reflect changes in both the fast and slow selected lines or changes in only one direction of selection. Germane to this point is the fact that some of the RF

TABLE 1

Developmental time (days) at the termination of developmental rate selection

Replicate	RF		RS			GF		GS		
	Mean	S.E.M.	Mean	S.E.M.	t	Mean	S.E.M.	Mean	S.E.M.	t
1	13.29	0.085	14.45	0.093	9.21**	15.79	0.141	16.17	0.115	2.12*
2	14.54	0.090	15.36	0.096	6.28**	15.58	0.091	16.67	0.105	7.89**
3	13.02	0.149	13.91	0.141	4.34**	16.58	0.102	17.07	0.120	3.15**
4	15.99	0.151	16.19	0.081	1.19 N.S.	15.20	0.094	16.17	0.123	6.28**
5	16.27	0.130	17.67	0.104	8.46**	12.93	0.075	16.11	0.096	25.64**
Replicate mean	14.62		15.52			15.22		16.44		

*·** Significant differences (t test) between fast and slow replicates at the 5 and 1% levels, respectively. N.S., not significant.

TABLE 2

Body weight (mg) of 25 adult flies per replicate after developmental rate selection

Replicate	Female				Male			
	RF	RS	GF	GS	RF	RS	GF	GS
1	28.3	34.0	28.8	28.2	19.6	21.2	19.2	19.8
2	27.5	29.1	26.7	24.9	19.1	21.6	19.2	16.9
3	24.0	29.0	30.0	25.0	16.9	19.4	20.3	17.9
4	24.3	33.1	30.8	29.3	17.6	21.3	20.5	18.8
5	28.8	33.5	28.6	30.7	19.9	20.7	18.3	21.2
Mean	26.58	31.74	28.98	27.62	18.62	20.84	19.50	18.92
S.E.M.	1.02	1.11	0.70	1.16	0.59	0.39	0.40	0.75
t	3.43**		1.00 N.S.		3.16*		0.68 N.S.	

*,** Significant differences between the mean body weights of the slow and fast strains at the 2.5 and 1% levels, respectively. N.S., not significant.

lines exhibited extraordinarily small flies (Table 2), suggesting that body weight selection had occurred in the R strain as a result of fast developmental rate selection.

In total, the allozyme data (Table 3) exhibit a diversity of gene frequency dynamics including stability, random fixation, and replicated directional changes. Two criteria were used to test for a significant directional response of the enzyme polymorphisms to developmental rate selection. The first criterion was a replicated and significant change in gene frequency either between sampling periods or between fast and slow developmental rate selected lines. The second, and more stringent criterion, was a significant gene frequency response in both the R and G strains occurring in the same direction. A paired comparisons *t* test was performed on the change in gene frequencies (Table 4). This test is appropriate for comparing fast and slow lines (as well as comparing temporal samples within lines) inasmuch as one fast replicate and one slow replicate were originally derived from each of five lines for the R and G strains (see MATERIALS AND METHODS). Because of an uncertainty regarding possible violations of the distributional assumptions of the *t* test, the nonparametric Wilcoxon's signed-ranks test was also performed on gene frequency differences (Table 5). This latter test was performed upon pooled G and R strain data; it is, therefore, a test of the robustness of any gene frequency change across strains.

$\alpha Gpdh^S$ and $6Pgd^S$ exhibited a significant decrease in frequency between generation 7 and 18 under fast developmental rate selection (Tables 3 and 5). In the case of *αGpdh*, this resulted in a significant difference between slow and fast replicates at generations 15 and 18, respectively. One complication of the *αGpdh* data is that, during the first seven generations, the frequency of the *S* allele increased dramatically in the GF replicates, then decreased during the last 11 generations of the experiment.

TABLE 3

$G6pd^S$, $6Pgd^S$, Adh^S, and $\alpha Gpdh^S$ gene frequencies of fast and slow developmental selected lines

Generation	R	RF		RS		G	GF		GS	
	0	7	18	5	15	0	7	18	5	15
$G6pd^S$	0.523 (0.040)	0.604 (0.071)[a]	0.688 (0.067)	0.460 (0.070)	0.500 (0.072)	0.389 (0.043)	0.456 (0.073)	0.708 (0.066)	0.440 (0.070)	0.370 (0.071)
		0.891 (0.046)	0.979 (0.020)	0.580 (0.070)	0.458 (0.072)		0.580 (0.070)	0.000 (0.000)	0.146 (0.051)	0.521 (0.072)
		0.738 (0.068)	0.563 (0.072)	0.455 (0.075)	0.333 (0.068)		0.438 (0.072)	0.313 (0.067)	0.200 (0.056)	0.000 (0.000)
		0.720 (0.063)	0.479 (0.072)	0.630 (0.071)	0.500 (0.072)		0.143 (0.054)	0.000 (0.000)	0.460 (0.070)	0.646 (0.069)
		0.708 (0.065)	0.396 (0.071)	0.500 (0.071)	0.375 (0.070)		0.480 (0.071)	0.750 (0.063)	0.238 (0.066)	0.000 (0.000)
	Mean	0.732	0.621	0.525	0.433		0.419	0.354	0.297	0.307
$6Pgd^S$	0.483 (0.042)	0.417 (0.071)	0.417 (0.071)	0.780 (0.059)	0.625 (0.070)	0.569 (0.043)	0.587 (0.073)	0.417 (0.071)	0.520 (0.071)	0.957 (0.030)
		0.478 (0.074)	0.063 (0.035)	0.780 (0.059)	0.500 (0.072)		0.680 (0.066)	0.697 (0.068)	0.438 (0.072)	0.146 (0.051)
		0.667 (0.072)	0.604 (0.071)	0.750 (0.065)	0.688 (0.067)		0.354 (0.069)	0.229 (0.061)	0.740 (0.062)	0.283 (0.066)
		0.540 (0.070)	0.188 (0.056)	0.261 (0.065)	0.500 (0.075)		0.857 (0.054)	0.854 (0.051)	0.380 (0.069)	0.625 (0.070)
		0.292 (0.066)	0.167 (0.054)	0.500 (0.071)	0.550 (0.079)		0.640 (0.068)	0.083 (0.040)	0.642 (0.074)	0.705 (0.069)
	Mean	0.479	0.288	0.614	0.573		0.624	0.456	0.544	0.543
Adh^S	0.398 (0.035)	0.396 (0.071)	0.458 (0.072)	0.400 (0.069)	0.625 (0.070)	0.766 (0.029)	0.370 (0.071)	0.125 (0.048)	0.880 (0.046)	0.667 (0.068)
		0.304 (0.068)	0.313 (0.067)	0.480 (0.071)	0.542 (0.072)		0.740 (0.062)	0.625 (0.070)	0.854 (0.051)	0.792 (0.059)
		0.595 (0.076)	0.667 (0.068)	0.250 (0.065)	0.646 (0.069)		0.667 (0.068)	0.646 (0.069)	0.740 (0.062)	0.313 (0.067)
		0.520 (0.071)	0.708 (0.066)	0.478 (0.074)	0.188 (0.056)		0.762 (0.066)	0.979 (0.021)	0.920 (0.038)	1.000 (0.000)
		0.604 (0.071)	0.875 (0.048)	0.860 (0.049)	1.000 (0.000)		0.540 (0.070)	0.479 (0.072)	0.881 (0.050)	0.625 (0.070)
	Mean	0.484	0.604	0.494	0.600		0.616	0.571	0.855	0.679
$\alpha Gpdh^S$	0.608 (0.045)	0.688 (0.067)	0.542 (0.072)	0.580 (0.070)	0.917 (0.040)	0.350 (0.044)	0.587 (0.073)	0.313 (0.067)	0.160 (0.052)	0.458 (0.072)
		0.348 (0.070)	0.313 (0.067)	0.560 (0.070)	0.458 (0.072)		0.440 (0.070)	0.271 (0.064)	0.250 (0.063)	0.458 (0.072)
		0.619 (0.075)	0.438 (0.072)	0.841 (0.055)	0.854 (0.051)		0.750 (0.063)	0.708 (0.066)	0.540 (0.070)	0.750 (0.063)
		0.540 (0.070)	0.167 (0.054)	0.630 (0.071)	0.646 (0.069)		0.429 (0.076)	0.217 (0.061)	0.040 (0.028)	0.000 (0.000)
		0.813 (0.056)	0.771 (0.061)	0.780 (0.059)	0.729 (0.064)		0.660 (0.067)	0.333 (0.068)	0.375 (0.070)	0.750 (0.063)
	Mean	0.602	0.446	0.678	0.721		0.573	0.368	0.273	0.483

[a] Standard errors are given in parentheses.

TABLE 4

Paired comparisons t *test of the change (difference) in* G6pd^S, 6Pgd^S, Adh^S and αGpdh^S *gene frequencies*

Generation	RF 7 *vs.* 18	RS 5 *vs.* 15	RF/RS 18 *vs.* 15	GF 7 *vs.* 18	GS 5 *vs.* 15	GF/GS 18 *vs.* 15
$\bar{D}$[a] *G6pd^S*	0.111	0.092	0.188	0.065	0.011	0.047
S.E.	0.0834	0.0330	0.0960	0.1561	0.1175	0.269
t	1.33	2.78	1.96	0.42	0.09	0.17
	N.S.[b]	*	N.S.	N.S.	N.S.	N.S.
$\bar{D}$ *6Pgd^S*	0.191	0.042	0.285	0.168	0.001	0.087
S.E.	0.0816	0.0887	0.063	0.1036	0.1657	0.224
t	2.34	0.47	4.51	1.62	0.01	0.39
	P < 0.10	N.S.	*	N.S.	N.S.	N.S.
$\bar{D}$ *Adh^S*	0.120	0.107	0.004	0.045	0.176	0.109
S.E.	0.0477	0.1136	0.135	0.0756	0.0864	0.1406
t	2.53	0.94	0.03	0.60	2.03	0.77
	P < 0.10	N.S.	N.S.	N.S.	N.S.	N.S.
$\bar{D}$ *αGpdh^S*	0.155	0.043	0.275	0.205	0.210	0.115
S.E.	0.0614	0.0768	0.0972	0.0488	0.0698	0.1032
t	2.53	0.55	2.83	4.20	3.01	1.11
	P < 0.10	N.S.	*	*	*	N.S.

[a] $\bar{D}$ equals the mean difference between paired comparisons.
[b] N.S., not significant; d.f = 4 for all comparisons.
* P < 0.05.

TABLE 5

Wilcoxon's signed-ranks test for paired comparisons of allozyme gene frequencies from fast and slow selected lines[a]

Generation	Fast 7 *vs.* 18	Slow 5 *vs.* 15	Fast/Slow 18 *vs.* 15
G6pd	N.S.	N.S.	N.S.
6Pgd	P = 0.012[b]	N.S.	N.S.
Adh	N.S.	N.S.	N.S.
αGpdh	P < 0.005	N.S.	P < 0.05

[a] R and G strain replicates were pooled. N.S., not significant.
[b] Two tailed test probabilities. Sample sizes are equal to ten except in cases where ties exist in rank.

DISCUSSION

ROBERTSON (1963, 1964) and CHURCH and ROBERTSON (1966) extensively investigated developmental rates and body size in Drosophila as a function of diet. They showed that flies selected for short development time on either a low protein diet or a low RNA diet exhibited a correlated decrease in body size. Flies selected for long development time exhibited a correlated increase in body size under the low RNA diet but not under the low protein diet.

Selection for small and large body size also led to correlated changes in developmental rate, but again the presence of a correlated change was dependent upon diet. The results of the developmental selection experiments reported herein demonstrate that correlated changes in size and development time is also dependent upon the genetic constitution of *D. melanogaster* populations.

The fact that, in general, the R strain (from Rhode Island) has a faster developmental rate than the G strain (from Georgia) is in accordance with the geographic cline in developmental rates observed in Drosophila. Flies from colder climates generally develop faster than flies from warmer climates when reared in the laboratory at the same temperature (McFARQUHAR and ROBERTSON 1963). ANDERSON (1966) has also shown that cold temperature selection on *D. pseudoobscura* can lead to a correlated decrease in the time of development relative to populations subjected to warm temperature selection. In these experiments, cold temperature selection also led to a correlated increase in body size. Therefore, it may be concluded that the absence or presence of a significant correlation between body size and developmental rate and the sign of the correlation is dependent upon at least the following factors: the genetic constitution of the population, diet and temperature.

It is evident from the gene frequency data (Table 3) that both random genetic drift and selective processes have led to gene frequency changes. These two processes can be differentiated by testing for replicated directional changes (*i.e.*, selection). The results of a nonparametric test (Table 5) indicate that the large changes in *G6pd*^{S} frequencies (Table 3) are most likely due to random drift. In contrast, the changes in *6Pgd*^{S} and *αGpdh*^{S} frequencies under fast developmental rate selection appear to be due to directional selection (Table 5).

The divergence in *αGpdh* gene frequency between the slow and fast selected lines in both the R and G strains is impressive in view of the differences mentioned above between these two strains. The cause of the reversal in direction of selection for *αGpdh* in the G strain is unknown. One attractive hypothesis is that the *αGpdh* frequencies in the early generations may have been affected by "hitchhiking" with the inversion *In 2L(t)*. This inversion includes the *αGpdh* locus and almost exclusively carries the *αGpdh*^{F} allele, whereas the standard chromosome is polymorphic in populations derived from the Southeastern United States (VOELKER *et al.* 1978). Before the beginning of this project, I estimated the frequency of this inversion to be greater than 5% in the G strain and completely absent in the R strain (D. R. CAVENER, unpublished results). The frequency of *In 2L(t)* was not estimated during the selection experiments; therefore, this hypothesis was not tested.

The fact that *αGpdh* responds to developmental rate selection offers an additional hypothesis for the response of *αGpdh* to ethanol selection in the R strain (CAVENER and CLEGG 1981b). We observed that the 10% ethanol used in our ethanol selection experiments retards the time of development by 1 to 2 days. Because we imposed a discrete 18-day generation schedule for both the ethanol and control replicates in our design, it is quite likely that this resulted in truncation selection for fast developmental rate. This hypothesis

can only account for the initial response of *αGpdh* to ethanol selection inasmuch as this polymorphism showed a biphasic gene frequency trajectory. The second phase of the *αGpdh* response to ethanol selection (*i.e.*, an increase in the $\alpha Gpdh^S$ allele) may be explained by our original hypothesis stating that *αGpdh* responded to selection due to its role in recycling NADH, which increases during the oxidation of ethanol in *D. melanogaster* (S. W. McKECHNIE and B. W. GEER, personal communication). I propose that *αGpdh* responded to developmental rate selection during the first phase when the Adh^S low activity allele was more frequent, yielding a relatively low NADH/NAD ratio. As the NADH/NAD ratio increased in the second phase (due to a dramatic increase in the high activity Adh^F allele), the selection on *αGpdh* may have switched to "NADH selection." A third alternative hypothesis suggested by B. W. GEER (personal communication) is that the response of *αGpdh* to ethanol and developmental rate selection may be related to its role in generating glycerophosphate for fatty acid synthesis. B. W. GEER and S. W. McKECHNIE (personal communication) have shown that triacylglycerol levels are increased significantly by the addition of ethanol to the diet of Drosophila larvae.

The response of *αGpdh* to developmental rate selection may also suggest a possible mechanism for maintaining the *αGpdh* gene frequency cline found in North American populations of this species (VOELKER *et al.* 1978; OAKESHOTT *et al.* 1982). The $\alpha Gpdh^S$ allele is positively correlated with latitude and increases during fast developmental rate selection. These data are consistent with the hypothesis that the *αGpdh* polymorphism is partly responsible for the developmental rate clines observed in Drosophila.

Difference in the *in vivo* flux through the pentose shunt as a function of *6Pgd* genotype has been previously demonstrated (CAVENER and CLEGG 1981a). In addition, the *6Pgd* polymorphism exhibits a strong response to ethanol selection (CAVENER and CLEGG 1981b; D. R. CAVENER, unpublished results). The $6Pgd^{FF}$ genotype, which has a relatively low *in vivo* flux rate, is selected for under ethanol selection and fast developmental rate selection.

The hypothesis that the observed *αGpdh* and *6Pgd* gene frequency changes reported herein are due to a hitchhiking effect with another locus under selection can not be rejected. However, the requirements for hitchhiking selection (*e.g.*, CAVENER and CLEGG 1981b) are considerably more stringent than often implied. The question of whether selection is acting upon an enzyme polymorphism can only be conclusively answered by assessing the *in vivo* biochemical and physiological allozyme phenotypes under the environmental conditions imposed by the selective regimen.

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