Original article

Activity of enzymes and fitness variation

M. Milošević and D. Marinković

Faculty of Science, University of Belgrade, Yugoslavia

(received 31-3-1987, accepted 25-4-1988)

Summary — This study concerns an analysis of variation of a group of enzymes (*i.e. 6-Pgd, G-6pd, α-Gpdh, Adh, Hk, Idh* and *Me*) and fitness characteristics such as fecundity, egg-to-adult development, rate of embryonic development, body mass, and mobility of *Drosophila melanogaster* flies, selected 10 generations for a fast and slow preadult rate of development. As a consequence of this divergent selection, mutual relationships between metabolic and fitness properties have been investigated. The observed results show that significant correlations exist between enzyme activities and studies fitness components, which might be due to selective changes in structural and regulatory genetic variants.

Drosophila – selection for rate of development – enzyme activities – fitness components

Résumé — **Activité des enzymes et variabilité de la valeur adaptative chez *Drosophila melanogaster*.** *Ce travail se rapporte à l'analyse de la variabilité d'un groupe d'enzymes (*G-Gpd, G-6pd, α-Gpdh, Adh, Hk, Idh, et Me*) ainsi qu'à l'analyse de composantes de la valeur adaptative telles que la fécondité, le développement de l'œuf chez l'adulte, la vitesse de développement embryonnaire, le poids corporel et la mobilité de mouches* D. melanogaster *sélectionnées pendant 10 générations pour une vitesse de développement préadulte élevée ou basse. Les relations mutuelles entre propriétés métaboliques et caractères d'adaptation ont été examinées au terme de cette sélection divergente. Les résultats obtenus mettent en évidence des corrélations significatives entre des activités enzymatiques et des composantes de la valeur adaptative, qui pourraient être la conséquence de modifications de variants génétiques de structure ou de régulation dues à la sélection.*

Drosophila – *sélection pour la vitesse de développement – activités enzymatiques – composantes de la valeur adaptative*

Introduction

The question of adaptive significance of enzyme polymorphisms has recently been oriented to the problem of the phenotypes on which selection might act. This has pointed to the possible role of regulatory gene variation in the processes of evolutionary adaptation (*e.g.,* Ayala and McDonald, 1980; Anderson and Gibson, 1985). Many studies have demonstrated that genetic variation of enzyme activities could be used to distinguish the

effects of regulatory genes from those of structural ones (*e.g.*, Gibson, 1970; Ayala and McDonald, 1980; Marinković *et al.*, 1986). The variation is based on the differences in the amounts of given gene products, which could be explained by differences in regulatory genes, rather than by gene duplication. It has been suggested that variation of regulatory genes may provide an even more important source for adaptive evolutionary change than structural gene variation (Britten and Davidson, 1969; MacIntyre and O'Brien, 1976; and others). A large amount of variation of enzyme activities has been documented in *Drosophila* species, even for monomorphic structural genes (Ward and Herbert, 1972; Mc Donald and Ayala, 1978; Laurie-Ahlberg *et al.*, 1980; Van Delden, 1982; Marinković *et al.*, 1984b, 1986; Marinković and Ayala, 1986).

In our previous studies, efforts have been focussed on the correlations between rates of preadult development and activity levels of a number of studied enzymes (*i.e. G-6pd, 6-Pgd, α-Amy, Adh, α-Gpd, Hk, Idh, Me, Sod*) in *Drosophila melanogaster* and *Drosophila subobscura* individuals (Marinković *et al.*, 1984a, b; Marinković, 1985; Milošević, 1987). In progeny of wild individuals a significant difference has been found in activity levels between fast- and slow-developing groups. The fastest-developing group of both species had a majority of highly active enzymes. Specific patterns of intercorrelations between enzyme activities in fast, intermediate, or slow preadult developmental classes suggest that different regulatory gene variants with pleiotropic effects on multiple enzymes might influence the variation in developmental dynamics.

In the present paper, different fitness characteristics are investigated to discern multiple relationships between regulatory, metabolic, developmental, and phenotypic levels in *D. melanogaster*. A continuous 10-generation selection for extremely fast, and slow egg-to-adult developmental rate has been performed, and selected groups of *D. melanogaster* individuals have been analysed for enzyme activity, fertility, mobility, and body weight. To complete the information about studied correlations, we have also examined a sample descended from a natural population for enzyme activity variation, but from the aspect of differential fertility and body mass of their F_1 progeny.

Materials and Methods

The selection experiment was initiated with the progeny of about 300 wild *D. melanogaster* flies caught in June 1984 at Jastrebac Mountain, 150 km South of Belgrade. Starting from more than 2,100 such progeny (G-0 generation), continuous 10-generation selection for extremely fast and slow preadult development was performed under constant laboratory conditions (20°C, relative humidity ca 60%). Five groups of flies were run simultaneously for each line, each in 4 culture bottles with about 200–600 individuals per generation (see Table I). About 10% of the fastest (or slowest) developed individuals were transferred to new cultures and allowed to intercross with one another; 25 such 7-day-old females were randomly chosen per replicate to initiate the following generation. They laid their eggs for 6 h in each of 4 250 cm³ culture bottles with corn-yeast medium, so that development of their progeny occurred in non competitive conditions. To reduce inbreeding and genetic drift, flies were intercrossed among the 5 fast-line groups (as well as among the 5 slow-line groups), in every second generation. In the first intercrossing generation (G-1), 25 males from replicate 1 were placed in a bottle with 25 virgin females from group 2, 25 males from replicate 2 were placed in a bottle with that many virgin females from group 3, and so on. In subsequent intercrossing generations (G-4, G-6, G-8), flies from different replicate cultures were intercrossed such as to provide eventually for interchanges among all replicate cultures.

Table I. Duration of preadult development of *D. melanogaster* (in days, in 5 replicates) during 10-generational selection for fast and slow developmental rate.

G-0			G-1/F			G-1/S			G-3/F			G-3/S			G-4/F		
N	X̄	S.E.	N	X̄	S.E.	N	X̄	S.E.	N	X̄	S.E.	N	X̄	S.E.	N	X̄	S.E.
590	20.05	± 2.34	230	17.64	± 1.11	242	18.33	± 8.68	240	17.15	± 1.20	150	19.73	± 1.31	541	19.10	± 1.41
551	21.80	± 2.19	213	17.04	± 1.21	277	18.25	± 9.99	160	16.93	± 1.44	213	19.62	± 1.42	437	18.78	± 1.32
334	19.18	± 1.74	266	16.77	± 1.30	257	18.09	± 9.40	170	16.87	± 1.48	281	20.29	± 1.41	582	18.99	± 1.39
350	18.79	± 1.72	302	16.86	± 1.42	176	18.28	± 1.06	210	16.98	± 1.41	238	20.47	± 1.44	729	18.90	± 1.31
304	18.71	± 1.87	355	17.87	± 1.24	179	18.58	± 1.22	230	17.38	± 1.23	267	20.20	± 1.41	214	18.42	± 1.58
2129	19.97	± 2.37	1366	17.26	± 1.35	1131	18.29	± 1.02	1010	17.09	± 1.35	1149	20.11	± 1.44	2503	18.90	± 1.39

G-4/S			G-6/F			G-6/S			G-7/F			G-7/S			G-8/F		
N	X̄	S.E.	N	X̄	S.E.	N	X̄	S.E.	N	X̄	S.E.	N	X̄	S.E.	N	X̄	S.E.
405	21.71	± 1.66	516	19.51	± 1.97	376	20.12	± 1.39	493	18.78	± 1.57	371	23.06	± 1.91	338	17.91	± 1.46
454	21.28	± 1.56	573	19.76	± 1.72	448	20.18	± 1.60	398	18.59	± 1.47	407	22.19	± 1.98	330	17.80	± 1.39
398	21.52	± 1.66	605	19.24	± 1.70	385	20.28	± 1.50	460	18.80	± 1.62	399	23.69	± 1.83	403	17.66	± 1.46
354	21.31	± 1.67	525	19.27	± 1.77	534	19.97	± 1.42	458	18.29	± 1.45	351	23.24	± 1.84	362	17.91	± 1.37
382	21.84	± 1.70	559	20.08	± 1.54	374	19.75	± 1.41	398	18.24	± 1.47	377	23.09	± 1.89	341	18.16	± 1.28
1993	21.53	± 1.66	2778	19.58	± 1.77	2117	20.05	± 1.48	2207	18.55	± 1.54	1899	23.05	± 1.96	1774	17.88	± 1.41

G-8/S			G-9/F			G-9/S			G-10/F			G-10/S		
N	X̄	S.E.	N	X̄	S.E.	N	X̄	S.E.	N	X̄	S.E.	N	X̄	S.E.
306	21.16	± 1.36	228	17.28	± 1.32	214	19.98	± 1.20	299	16.52	± 1.13	374	20.16	± 1.29
378	22.01	± 1.31	278	17.77	± 1.26	224	20.95	± 1.35	212	16.63	± 1.18	363	19.76	± 1.17
208	21.71	± 1.61	196	17.04	± 1.35	238	20.01	± 1.24	273	17.22	± 1.36	298	19.99	± 1.94
313	21.56	± 1.32	228	17.60	± 1.16	313	20.80	± 1.46	354	16.45	± 1.22	348	19.72	± 1.14
255	20.69	± 1.28	230	17.16	± 1.22	287	21.00	± 1.39	362	16.57	± 1.28	247	20.14	± 1.07
1460	21.46	± 1.44	1160	17.40	± 1.29	1276	20.59	± 1.41	1500	16.66	± 1.27	1630	19.95	± 1.16

In the G-1, G-5, and G-10 generations, 3 x 100 7-d-old males were taken from one of 2 extreme developmental phenotypes of the first 3 replicates in both selected lines, weighed, homogenized, and analysed for their enzyme activity. The assay procedures have been described by Avise and McDonald (1976), Stam and Laurie-Ahlberg (1982), and Marinković *et al.* (1984b). The homogenization buffer was 0.01 M KH_2PO_4, 1 mM EDTA, pH 7.4. The suspension was centrifuged for 5 min at 12,000 *g* at 4°C. All enzyme assays were performed at 30°C, with a Gilford model 250 spectrophotometer. The absorption spectrum was recorded at 10-s intervals, and reaction rates were calculated as initial changes of optical density units per 2-min interval. Seven enzymes were assayed from the supernatant fraction in each analysed generation. These enzymes are controlled by the following structural loci in *D. melanogaster* :

6-phosphogluconate dehydrogenase (6-Pgdh; 1-0.64);
Glucose-6-phosphate dehydrogenase (G-6-Pdh; 1-63);
Alpha-glycerophosphate dehydrogenase (α-Gpdh; 2-20.5);
Alcohol dehydrogenase (Adh; 2-50.1);
Hexokinase (Hk; 2-73;5);
Isocitrate dehydrogenase (Idh; 3-27.1);
Malic enzyme (Me; 3-53.1).

The obtained enzyme activity values were ajusted by the Lowry test to mg protein per ml solution (Lowry *et al.,* 1951). These adjusted enzyme activity rates are proportional to the relative activities expressed in optical density units, as well as to the values adjusted on the mg of body mass.

At the termination of the selection experiment, several characteristics were measured in both selected groups, most of them simultaneously. The offspring of these selected lines were analysed

for the rates of embryonic development, by counting the number of emerged first instar larvae at 2-h intervals. Larvae were hatched from eggs collected at 6-h intervals, in small Petri dishes with corn-yeast medium. After that, the larvae pupated inside 200 cm² bottles, and the rate of eclosion or total preadult development, was also measured. Each selected line of such experiments consisted of 5 replications. *i.e.* of more than 2,000 individuals. The randomly collected samples of "fast" and "slow" flies were tested for fertility at the age of about 5 d from eclosion, and other samples were tested for individual mobility, as well as for longevity. Body weight was also measured individually.

Another year's sample of *D. melanogaster* flies, F_1 progeny from the same Jastrebac Mountain natural population, collected in June 1985, were investigated (almost synchronously with the previous studies) for the relationships between some of the analysed fitness characteristics and enzyme activity variation. These characteristics are : female fecundity, body weight and rate of embryogenesis. Here the enzyme assays were performed in smaller samples of 10 flies with certain extreme phenotypes, so that reaction rates of 7 enzymes might relatively differ from reaction rates obtained by previously used homogenates with 100 flies each in our selection experiment.

Flies from the 1985 sample were also used for electrophoretic analysis of 7 gene-enzyme systems, *i.e.* of *G-6pdh, 6-Pgdh, α-Gpdh, Adh, Hk, Me,* and *Idh.*

Results

Fig. 1 presents the average developmental time in 2 lines of 10-generational selection for extremely different rates of egg-to-adult development. Table I gives the numerical results
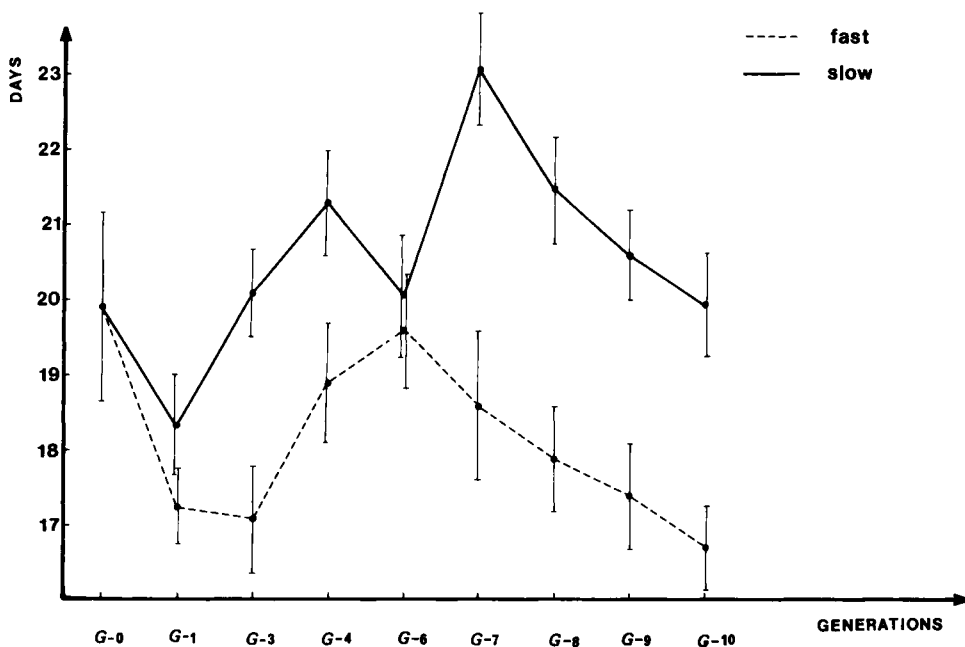

Fig. 1. Average duration of preadult development of *D. melanogaster* individuals (in days) during multigenerational selection for fast and slow development rate.

obtained in 5 replicates, from G-0 to G-10. It is evident that selection progress includes some oscillations of the mean developmental times which might be explained by different environmental effects on the selected phenotypes (Botella & Mensua, 1986). However, after G-7, the divergence became relatively established ($P < 0.001$), and increased up to 60 h between fast and slow lines. A linear regression analysis including all replicates of fast and slow selection lines from G-0 to G-10, led to estimates of heritability H_F^2 00.123 ($c_1 - 0.0078$, $c_2 - 1.2573$), and H_S^2 = 0.185 ($c_1 = 0.0011$, $c_2 - 1.3020$).

Table II presents specific activities of 7 studied enzymes in G-0, G-1, G-5, and G-10 generations of selection for 2 different rates of preadult development of *D. melanogaster*. Despite the fact that some enzymes (such as *α-Gpd, Adh,* and *Me*), had relatively higher activities than other enzymes (such as *Hk*), it can be seen that there is a significant difference in all of the studied enzymes between flies selected to be fast and those to be slow in their development. The combinations of studied enzyme activities are significantly different in 2 developmental groups of flies (measured by χ^2 comparisons); this difference is especially pronounced in G-5 and G-10 generations of selection. Decreased activity occurs among flies with longer development, which is pronounced in 5 enzymes in g-1 and G-10 generations, and in 6 out of 7 enzymes in the G-5 generation of divergent selection. Fast/slow ratio is greater than 1 in these 3 sets of generational comparisons, but significantly so only in the G-5 generation, as well as when all comparisons are accumulated ($t_{20} = 2.43$; $P < 0.05$).

Table III presents the analyses of variances in activities of 7 enzymes (A) between and (B) within G-1, G-5, and G-10 generations of selection for fast and slow prea-

Table II. Average activity levels of 7 enzymes in *D. melanogaster* flies (3 replicates) during multigenerational selection for extremely fast and slow preadult development.

Enzymes	P	Generations of selection						$\chi^2_{F:S}$ (d.f. = 8)
		G-1		G-5		G-10		
		Fast	Slow	Fast	Slow	Fast	Slow	
Adh	656	649	522	562	342	467	691	**
α-Gpd	727	845	809	664	621	816	735	**
G-6pd	255	136	215	164	140	328	269	**
6-Pgd	211	197	190	352	187	133	95	**
Idh	538	483	367	367	448	435	609	**
Me	601	541	570	743	599	676	534	**
Hk	110	33	25	175	130	79	58	**
Fast/Slow ratio		1.08 ± 0.10		1.31 ± 0.14		1.11 ± 0.12		1.17 ± 0.7
d.f.		6		6		6		20
t-test		n.s.		2.21 *		n.s.		2.43 *

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

Relative activities are expressed in O.D. Units/mg protein/ml.

Table III. Analyses of variances of enzyme activities (A) between and (B) within G-1, G-5, and G-10 of selection for extremely fast and slow preadult development of *D. melanogaster.*

Source of variation	d.f.	MS	F
A. Generations of selection (G-1, G-5, G-10)	2	257094	20.94 **
Rates of development	3	12280	12.42 **
Chromosomes	12	989	0.03
Error	36	30394	
B. Rates of development in G-1	1	2404	1.58
Error	40	1522	
Rates of development in G-5	1	21226	2.06 *
Error	40	10209	
Rates of development in G-10	1	67480	3.56 **
Error	40	18983	

* $P < 0.1$; ** $P < 0.05$.

In analysis (B), the mean activities of 7 studied enzymes (with 3 replicates) are adjusted within "fast" and "slow" lines. In analysis (A), only enzymes controlled by structural genes from the same chromosome (I, II, or III) are adjusted within each of 2 lines.

dult development of *D. melanogaster.* In analysis (A), 3 levels of variation were studied : (1) between generations of selection, (2) between developmental lines, and (3) between enzymes controlled by structural loci from the 1st, 2nd, and 3rd chromosomes. There is a significant contribution of the selection process to the observed enzyme activity variation ($F_{2,3}$ = 20.9; $P < 0.02$), as well as of the fast and slow developmental phenotypes ($F_{3,12}$ = 12.4; $P < 0.01$). The enzyme activity variation of corresponding chromosomal groups of genes turns out not to be significant. In analysis (B), the mean values of 7 studied enzymes are adjusted (with their replicates) within fast and slow selected lines, showing a significant difference in G-5 and G-10 generations of selection.

Fig. 2 presents dynamics of embryonic development measured simultaneously in 10 replications for each "fast" and "slow" selected line. This analysis was done in the progeny of the last selected G-10 generation. The average length of embryonic development was 27.9 ± 0.8 h in the "fast", and somewhat longer in the "slow" line, *i.e.* 29.7 ± 0.9 h. There is a marginally significant difference in the dynamics of embryogenesis between these 2 groups of individuals (see also Marinković and Tucić, 1981; Smit *et al.,* 1981).

Fig. 3 presents the longevity studies. The average longevity of the fast line was 29.2 ± 2.7 d, *ve.* 30.5 ± 3.2 d for the slow line. A x^2 test shows a significant difference in variation of the 2 sets of individuals (χ^2_{28} = 18.6; $P < 0.05$).

The measurement of fertility, which is clearly an important component of fitness in *Drosophila,* comprises female fecundity measured as number of eggs produced by a single female per 24 h. Female fecundity was insignificantly greater in the slow line (34.8 ± 4.2) compared to the fast-line flies (29.1 ± 3.2).
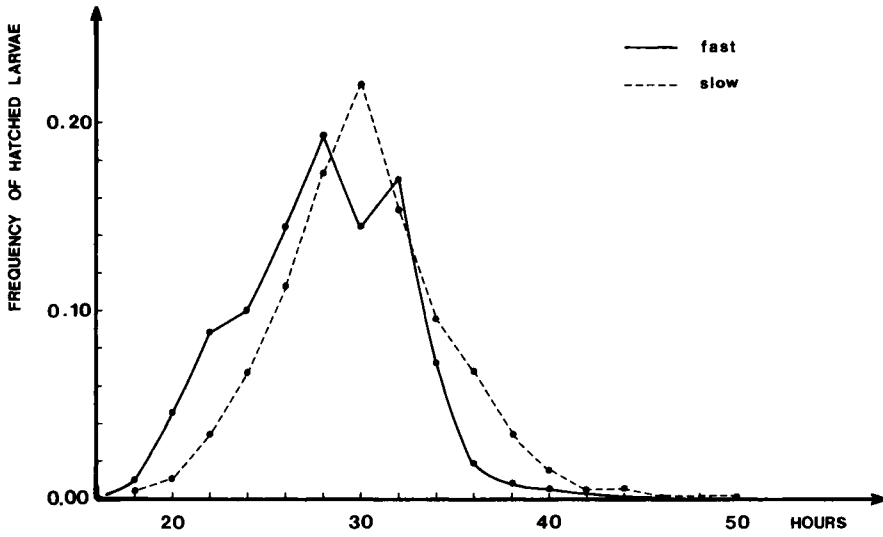
Fig. 2. The dynamics of embryonic development in *D. melanogaster* individuals selected 10 generations for fast and slow preadult development (N_{fast} = 2232, N_{slow} = 2439).
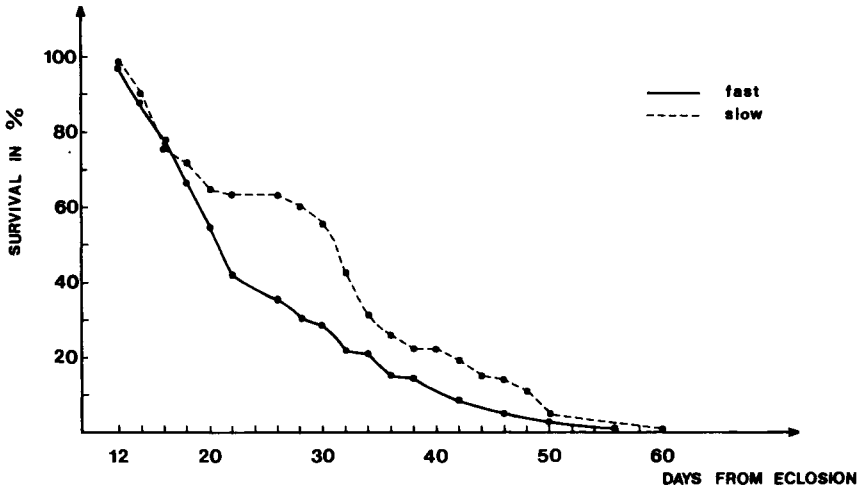

Fig. 3. Life-span of *D. melanogaster* individuals selected over 10 generations for fast and slow preadult development.

Mobility of adult flies was analysed among other fitness components, after the selection proceeded. About 400 individuals were investigated by means of the model of a double maze with 5 chambers (Kerić, 1981). Table IV presents the results of such an experiment, where samples of adult flies were placed simultaneously in the starting chambers and allowed to move through the next chambers at 3-min intervals. It can be seen that flies selected for extremely fast egg-to-adult development moved farther in the maze than the slow group. The observed distribution along the maze, on 3 successive days, was analysed by the appropriate Chi-square method, which gave a significant difference between fast and slow groups. Here it should be mentioned that in an earlier experiment with *D. subobscura,* individuals with the slowest embryonic development were more mobile than those with the fastest development (Marinković and Milošević, 1983).

Table V presents the averages of adult body weights that were measured in the 10th generation of selection for fast and slow preadult development. The observed differences were marginally significant, and it might be concluded that the slowest group of flies had a larger body mass, compared to the fastest long-term selected individuals. The average

Table IV. Mobility of *D. melanogaster* adults from G-11 generation of selection for fast and slow preadult development.

	Chambers					Total
	I	II	III	IV	V	
Fast	42	23	21	9	9	104
	15	29	20	14	24	102
	21	14	26	13	24	98
%	26	22	22	12	18	
Slow	17	6	8	1	1	33
	17	9	4	1	2	33
	22	5	5	1	0	33
%	57	20	17	3	3	

$\chi^2 = 269.0$ (d.f. $= 14$; $P < 0.001$).

Relative distribution of flies in 5 chambers, after 3 min (tested 5th, 6th, and 7th d after eclosion).

Table V. Average body weight of *D. melanogaster* flies from G-11 generation of selection for "fast" and "slow" preadult development ($N = 200$ flies).

	Average weight	
	Males	Females
Fast	0.95 + 0.05 mg	1.35 + 0.06 mg
Slow	1.04 + 0.02 mg	1.53 + 0.07 mg

$t = 1.80$ $(P < 0.1)$; $t = 2.00$ $(P < 0.05)$.

body weight of flies with extreme rates of egg-to-adult development in the G-1 generation, in an earlier study, was found to be equal or higher in the fast group of *Drosophila* individuals (Marinković *et al.*, 1984b).

A separate investigation was conducted with non selected groups of *D. melanogaster* individuals on the relationship between naturally occurring variation of some adaptively significant traits, and the variation of enzyme activities as a metabolic property. These individuals were from the same natural population as the flies from the 10-generational selection experiment, but their parents were collected in the following season, *i.e.* summer 1985.

Fig. 4 shows the average levels of enzyme activities in such groups with fast, medium, and slow embryonic development. The observed differences between developmental phenotypes were found to be significantly correlated to enzyme activity variation for *Adh, α-Gpd, Idh, Me,* and *Hk.* However, specific associations of activity levels for 7 studied enzymes could be observed among flies with a fast, intermediate, or slow embryonic rate of development, pointing to quite complex genetic–physiological relationships.

Fig. 5 presents the average activities of 7 enzymes/mg protein/ml in samples of *D. melanogaster* flies, a progeny of wild females, that differed in average body mass. Three classes acording to body weight were obtained, each with 10 individuals, with minimal ($\overline{x}$ = 0.72 mg), medium ($\overline{x}$ = 0.89 mg), and maximal ($\overline{x}$ = 1.1 mg) weight. As can be seen from the figure, most of the enzymes were found to vary independently of body mass. Yet variations of *6-Pgd* and *Hk* showed a marginally significant increase in the heaviest males. Only α-Gpd variation corresponds to body mass, *i.e.* males with minimal body weight had higher average activity of this enzyme per unit of body mass, than those with maximal body weight (χ^2 = 217.8; df = 6, P < 0.001).

Fig. 6 shows the variation of enzyme activities with respect to differential female fecundity. The experiment was performed on 3 samples, containing 10 females each, again the progeny of wild parents collected in summer 1985, that were tested for egg production individually at 24-h intervals. A group of such females that had produced 18 eggs on average was designated as "minimal" fecundity, a group with 32 eggs as "medium" fecundity, and 61 eggs as "maximal" fecundity group. The minimal fecundity group had significantly higher average enzyme activities of *Adh, α-Gpd, G-6pd, Idh,* and *Me.* The medium fecundity group was very similar to the minimal, except for *G-6pd* and *Idh.* This result might be explained as the possible consequence of the egg production processes at metabolic level. Also, a large amount of variation was observed in assays of *D. melanogaster* females, and it was preferable to use males for enzyme assay procedures (Stam and Laurie-Ahlberg, 1982). In this study we analysed males in all other experiments. Assuming that observed differences in fecundity *versus* enzyme activity have some adaptive meaning, that might be the possible force maintaining polymorphism of structural and regulatory genes in this species.

In this sample of non selected flies (which corresponds to the G-0 generation in our selection experiment), the evidence of allozyme frequencies for studied gene-enzyme systems was also obtained. Only *α-Gpdh* and *Hk-2* loci turned out to be polymorphic (the frequencies of their 2 commonest alleles are about 0.7 and 0.3), while other loci were almost monomorphic (*Adh* : 0.98 *vs.* 0.02; 6-*Pgdh* : 0.97 *vs.* 0.03), or completely monomorphic *(G-6pdh, Me, Idh).* This might confirm the hypothesis that the differences in enzyme activity of *Adh, 6-Pghd, G-6pdh, Me,* and *Idh* found between lines selected for
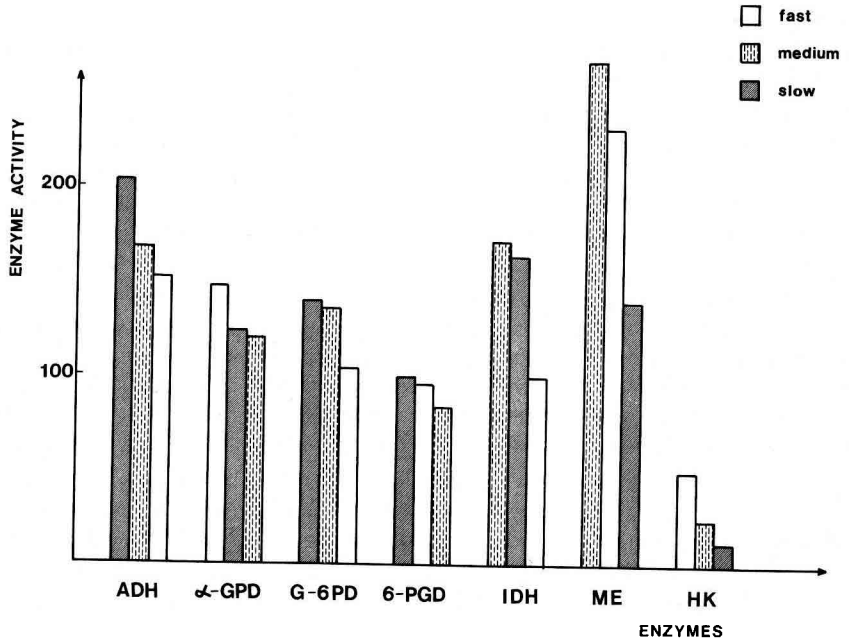
Fig. 4. Activity level (per mg protein/ml) of 7 enzymes in samples of *D. melanogaster* flies with extremely fast, medium, and slow rate of embryonic development ($N = 20$).
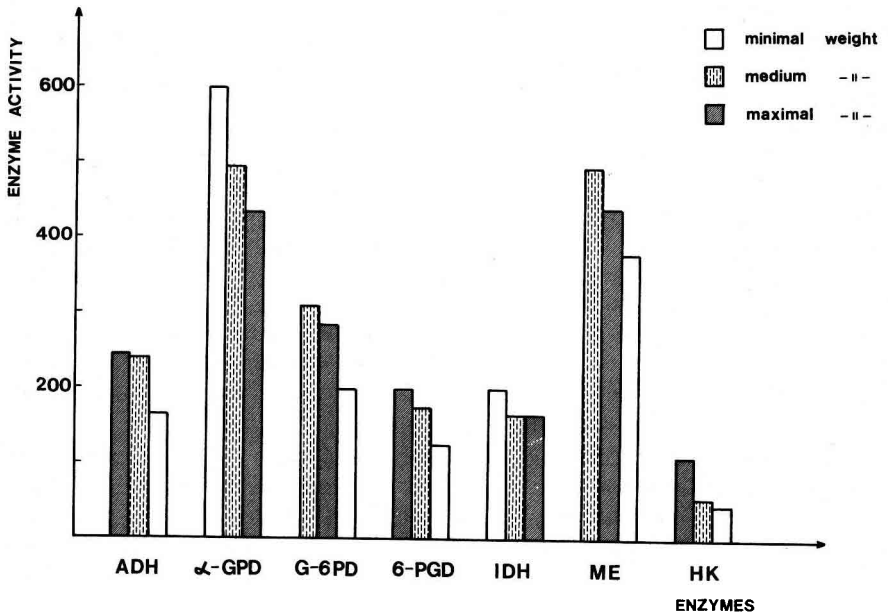

Fig. 5. Activity of 7 enzymes (per mg protein/ml) in *D. melanogaster* males with differential mean body mass ($N = 30$).
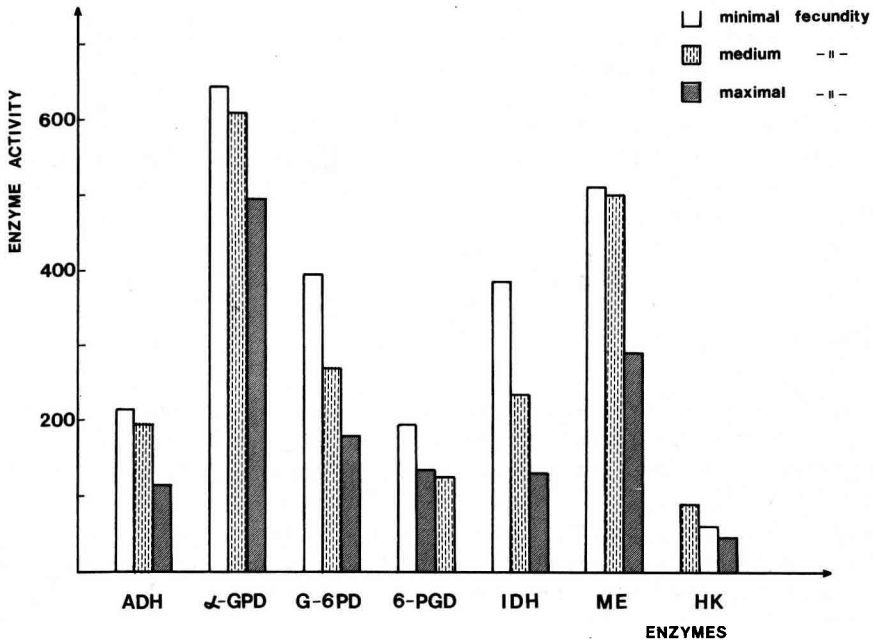
Fig. 6. Activity of 7 enzymes (per mg protein/ml; in *D. melanogaster* females with differential average fecundity (*N* = 30).

Table VI. Product moment correlation coefficients between enzymes, with respect to different fitness properties in *D. melanogaster*.

Adh	Enzyme					
	α-*Gpd*	*G-6pd*	*6-Pgd*	*Me*	*Hk*	*Idh*
Adh	0.47 *	− 0.13	− 0.03	0.59 *	0.07	0.68 **
α-*Gpd*		0.12	0.09	0.67 **	− 0.02	0.79 **
G-6pd			− 0.23	0.33	0.27	0.27
6-Pgd				0.57 *	0.61 *	0.05
Me					0.62 **	0.72 **
Hk						0.32
Idh						

* *P* < 0.05 ; ** *P* < 0.01.

fast and slow rates of development might be due to the differences in regulatory genes. However, in our previous study of F_1 progeny from another *D. melanogaster* population (from Titova Mitrovica, September 1984, Marinković *et al.*, 1986) it was found that the differences in genotypic constitution between "fast" and "slow" individuals are highly significant at the α-*Gpdh*, *6-Pgdh*, *Adh* (as well as at *Sod*, *Aldox* and *Acph*), suggesting that they could also be attributed in this case to structural variation at the corresponding loci (see also Cavener, 1983).

Discussion

Rate of development in *Drosophila* is proposed as an important component of fitness (Dobzhansky *et al.*, 1964), which includes activities of many genes, and has a relatively low heritability (*e.g.*, Brncic and Budnik, 1974; De Oliviera and Cordeiro, 1981; Marinković and Tucić, 1981).

In the G-0, G-1, G-5, and G-10 generations of our selection for fast and slow preadult development, the assay procedures for 7 enzymes were applied in corresponding groups of *D. melanogaster* individuals. As has been already described in *Results,* the combinations of studied enzyme activities were found to be ostensibly different in 2 selected groups of flies, which was especially pronounced in the G-5 and G-10 generations of this selection. Greater activity of a majority of enzymes was found in the fast group and, *vice versa,* lower activity was observed in the slow groups of flies. These results, as well as our previous studies (*e.g.*, Marinković *et al.*, 1984b, 1986) might confirm the hypothesis that greater enzyme activity may speed up the growth rate of some individuals, and that a lower activity, on an average, slows down the growth rate. Specific patterns of intercorrelations between enzyme activities definitely exist in each of the preadult developmental classes. Associations of enzyme activity variation and other fitness components have also been investigated in this analysis.

Many studies have shown the importance of regulatory gene variation, influencing enzyme activities (*e.g.* Britten and Davidson, 1969; Gillespie and Langley, 1974; Stein and Stein, 1976; McDonald and Ayala, 1978; and so on). The difference in the amount of enzymes might be due to a modification of the rate of synthesis of a polypeptide, or to the rate of degradation by specific binding of macromolecules at control sites adjacent to the structural loci. Cluster *et al.* (1987) found a larger rDNA activity in *D. melanogaster* flies with extremely fast preadult development, and with 3 out of 4 studied enzymes showing a greater activity than among individuals with the slowest egg-to-adult development. There is also experimental evidence that interactions between chromosomes, through regulation of the rate of transcription of certain structural genes, or through post-transcriptional or post-translational processes, should be taken into account (McClin, 1965; MacIntyre and O'Brien, 1976; McDonald *et al.*, 1977; Cochrane and Richmond, 1979, etc.).

Significant difference in activity rates of most studied enzymes in our analysis could be observed in samples with different preadult developmental rates (Table II), as well as with different body weight (Fig. 5), female fecundity (Fig. 6), and rate of embryonic development (Fig. 4). This suggests that dynamics of analysed enzymatic processes could be

the basis of differences in fitness components of studied *D. melanogaster* flies. In the last generation of selection several fitness characteristics were compared, and some were specifically associated with the rate of egg-to-adult development, as well as with the enzyme activity variation. Since there is no single conclusion that might be applied for all 7 enzyme activity variants, we will discuss the observed results separately.

ADH enzyme is one of the most investigated models of specific metabolic and adaptive significance. The 2 common alleles, *AdhF* and *Adhs*, influence the difference in enzyme concentration that has been presumed to be responsible for the observed variation in enzyme activity of *Adh FF* and *SS* structural genotypes, other than catalytic properties of protein products (Gibson, 1972). However, a regulatory gene has been mapped closely linked to the structural locus (Thompson *et al.,* 1977). Activity variation in *Adh* was measured during selection for extremely fast and slow egg-to-adult development, and significant differences were found between the developmental phenotypes with respect to analysed generation. However, fast/slow ratio in G-5 generation is greater (1.64) than in G-10 (0.68). A significant positive correlation was found between *Adh* and α-*Gpd* activity in the G-5 generation of selection, as well as in samples of differentially fertile females. Cavener and Clegg (1981) found that relative fitness of α-*Gpd* genotypes is strongly dependent upon the corresponding genotypes at the *Adh* locus. The lack of such correlation in the G-10 generation of selection, for example, might be attributed to interactions of other genes.

Among 7 analysed enzymes in *D. melanogaster* assays, α-*Gpd* is one of the most active. This is a cytoplasmic enzyme with several metabolic functions, very important in flight metabolism (O'Brien, 1972; Zera, 1981). During selection, the enzyme was somewhat more active in the fast line (fast *vs.* slow = 1.07 in G-5 and 1.11 in G-10, respectively). Significantly higher activity has been observed in females of minimal fecundity *versus* those of maximal (ratio 1.30), as well as in flies of minimal body weight (1.38).

A structural gene for the Hk enzyme, as *Adh* and α-*Gpd,* has been mapped on the second *D. melanogaster* chromosome. HK is known as a polymorphic, glucose-metabolizing enzyme (Ayala *et al.,* 1972). Variation of its activity closely corresponds to the variation of *Adh* and α-*Gpd* in different developmental phenotypes during 10-generational selection, yet a group of males with maximal body weight has significantly higher activity.

It has been suggested that enzymes involved in the glucose metabolism cycle in *Drosophila*, such as *Hk, Idh,* and *Me,* tend to have lower variability (Gillespie and Kojima, 1968; Kojima *et al.,* 1970). However, in the present study a high level of enzyme activity variation was found for these enzymes. *Idh* and *Me* structural genes were mapped on the third *D. melanogaster* chromosome, but there is no evidence of a correlation between the activity level of these 2 enzymes. *ME* tends to be more active in the fast selection line, *i.e.* fast/slow ration G-5 was 1.36 and 1.68 in G-10. *Idh* was significantly more active in the "slow" line (F/S ratio 0.82 in G-5, and 0.71 in G-10 generation). Both enzymes are highly active in the minimal fecundity class of females (ratio for *Me* is 1.70, and 2.66 for *Idh*).

G-6-Pdh and *6-Pgdh* are located on the first *D. melanogaster* chromosome, and both are known as polymorphic loci in this species. A strong epistatic interaction has been found between common variants of the loci (Bijlsma, 1978). There are differences between individuals that are hemi- and homozygous for a common, *versus* null *G-6-Pdh* allele, but not upon the allelic state of *6-Pgdh* (Hughes and Lucchesi, 1977). It is suppo-

sed that enzyme activity levels might be influenced by closely linked genes that are acting as regulatory genes (Bijlsma and Van Delden, 1977). In our present study the variation of *G-6pd* and *6-Pgd* has been very often alternative.

Product-moment correlation coefficients were calculated for enzyme activity averages in samples of *D. melanogaster* individuals with differential fitness properties. Table V summaries such relationships between 4 enzymes. The activities of some of the studied enzymes are significantly positively associated (*e.g. Idh,* and *Me, Idh* and *α-Gpd, Me* and *α-Gpd, Adh* and *Idh, etc.*). On the other hand, there is marginally significant negative association between *Adh* and *G-6pd,* as well as *G-6pd* and *6-Pgd.*

Dissimilarity in the activity pattern of studied enzymes in samples with different prea-dult developments, body weights and female fecundities suggests that dynamics of ana-lysed enzymatic processes could be the basis of differences in fitness components of *D . melanogaster* flies. The variability of corresponding regulatory genes and their interac-tions might be a model system for understanding some aspects of adaptive significance of enzyme polymorphism in *Drosophila* as well as in other organisms. Even in enzymes coded by monomorphic loci (and these are our study *G-6pdh, Me, Idh,* and almost so *6 - Pgdh* and *Adh*), a large amount of variation could be maintained by balancing selection that was acting at the regulatory gene level.

The present results demonstrate that complex relationships between fitness characte-ristics and developmental, metabolic, and genetic properties could be evaluated. This knowledge may significantly change our understanding of how individual organisms and populational systems respond to evolutionary forces, and how complex genetico-physio-logical adaptations are built up during the processes of organic evolution.

Acknowledgments

References

Anderson D.G. & Gibson J.B. (1985) Variation in *Adh* activity *in vitro* in flies from natural populations of *D. melanogaster. Genetics* 67, 13-19

Avise C.J. & McDonald J.F. (1976) Enzyme changes during development of holometabolic and hemimetabolic insects. *Comp. Biochem. Physiol.* 53, B, 393-397

Ayala F.J. & McDonald J.F. (1980) Continuous variation : possible role of regulatory genes. *Geneti-ca,* 52/53, 1-15

Ayala F.J., Powell J.R., Tracey M.I., Mourao & Perez-Salas S. (1972) Enzyme variability in *D. willis-toni* group. IV. Genetic variation in natural populations of *D. willistoni. Genetics* 80, 113-138

Bijlsma R. (1978) Polymorphism at *G-6-Pdh* and *6-Pgdh* in *D. melanogaster.* II. Evidence for interac-tion in fitness. *Genet. Res.* 31, 227-237

Bijlsma R. & Van Delden W. (1977) Polymorphism at the *G-6-Pdh* and *6-Pgdh* loci in *D. melanogaster*. I. Evidence for selection in experimental population. *Genet. Res.* 30, 221-226

Botella M. & Ménsua J.L. (1986) Selection for faster and slower mean developmental time in crowded cultures of *D. melanogaster. Drosoph. Inf. Serv.* 63, 35-36

Britten R.J. & Davidson E.H. (1969) Gene regulation for higher cells : a theory. *Science* 165, 349-357

Brncic D. & Budnik M. (1974) Rate of development and viability of hybrids between *D. pavani* and *D. gaucha* under competitive conditions. *Ecology* 55, 662-666

Cavener D.R. (1983) The response of enzyme polymorphism to developmental rate selection in *D . melanogaster. Proc. Natl. Acad. Sci. USA* 57, 645-649

Cavener D.R. & Clegg M.T. (1981) Multigenetic response to ethanol in *D. melanogaster. Evolution* 35, 1-10

Cluster P.D., Marinković D., Allard R.W. & Ayala F.J. (1987) Correlations between development rates, enzyme activities, ribosomal DNA spacer-length phenotypes, and adaptation in *D. melanogaster. Proc. Natl. Acad. Sci. USA* 84, 610-614

Cochrane B.J. & Richmond R.C. (1979) Studies of esterase-6 in *D. melanogaster*. I. The genetics of posttranslational modification. *Biochem. Genet.* 17, 167-183

De Oliviera A.K. & Cordeiro A.R. (1981) *Acph* in *D. melanogaster* selected for extremes of developmental rate and a recessive active allele. *Rev. Bras. Genet.* 4, 135-148

Dobzhansky T.H., Lewontin R.C. & Pavlovsky O. (1964) The capacity for increase in chromosomally polymorphic and monomorphic population of *D. melanogaster. Heredity* 19, 597-614

Gibson J.B. (1970) Enzyme flexibility in *D. melanogaster. Nature* 227, 959-960

Gibson J.B. (1972) Differences in the number of molecules produced by two allelic electrophoretic enzyme variants in *D. melanogaster. Experientia* 28, 975-976

Gillespie J.H. & Kojima K. (1968) The degree of polymorphism in enzymes involved in energy production compared to that in nonspecific enzymes in two *Drosophila ananassae* populations. *Proc. Natl. Acad. Sci. USA* 61, 582

Gillespie J.H. & Langley C.H. (1974) A general model to account for enzyme variation in natural populations. *Genetics* 76, 837-848

Hughes M.B. & Lucchesi J.C. (1977) Genetic rescue of a lethal "null" activity allele of *6-Pgdh* in *D . melanogaster. Science* 196, 1114-1115

Kekic V. (1981) Maze for the study of phototactic behaviour in *Drosophila. Drosoph. Inf. Serv.* 56, 178-179

Kojima K., Gillespie J.H. & Tobari Y.N. (1970) A profile of *Drosophila* species enzymes assayed by electrophoresis. I. Number of alleles, heterozygosities, and linkage disequilibirium in glucose-metabolizing systems and some other enzymes. *Biochem. Genet.* 4, 627

Laurie-Ahlberg C.C., Maroni G., Bevley G.C., Lucchesi J.C. & Weir B.S. (1980) Quantitative genetic variation of enzyme activities in natural populations of *D. melanogaster. Proc. Natl., Acad. Sci. USA* 77, 1073-1077

Lowry D.H., Rosebrough N.J., Farr A.L. & Randal R.J. (1951) Protein measurements with the folinphenol reagent. *J. Biol. Chem.* 193, 265-275

MacIntyre R.J. & O'Brien S.J. (1976) Interacting gene-enzyme systems in *Drosophila. Annu. Rev. Genet.* 10, 281-318

Marinković D. (1985) Genetic variation and dynamics of development processes. *Acta Biol. Yugoslavica (Genetika)* 17, 191-203

Marinković D. & Ayala F.J. (1986) Genetic variation for rate of development in natural populations of *D. melanogaster. Genetica* 71, 123-132

Marinković D. & Milošević M. (1983) Mobility of *D. subobscura* flies with different rates of their embryonic development. *Drosoph. Inf. Serv.* 59, 76-77

Marinković D. & Tucić N. (1981) Age associated changes and developmental programmes in *Dro-*

sophila. In : Advances in Genetics, Development, and Evolution of Drosophila (D. Lakovaara ed.), Plenum Press, New York, pp. 197-209

Marinković D., Milošević M. & Andjelković M. (1984a) Regulatory polymorphism in midgut alpha-amylase activity and developmental rate of D. subobscura. Genetica 64, 115-122

Marinković D., Milošević M. & Milanović M. (1984b) Enzyme activity and duration of preadult development in D. melanogaster and D. subobscura. Arch. Biol. Sci. 36, 13-24

Marinković D., Milošević M. & Milanović M. (1986) Enzyme activity and dynamics of Drosophila development. Genetica 70, 43-52

McClintock B. (1965) The control of gene action in maize. Brookhaven Symp. Biol. 18, 162-181

McDonald J.F. & Ayala F.J. (1978) Genetic and biochemical basis of enzyme-activity variation in natural population. I. Adh in D. melanogaster. Genetics 89, 371-388

McDonald J.F., Chambers G.K., David J. & Ayala F.J. (1977) Adaptive response due to changes in gene regulation : a study with Drosophila. Proc. Natl. Acad. Sci. USA 74, 4562-4566

Milošević M. (1987) Activity of enzymes and quantitative-genetic variability in Drosophila melanogaster. Ph. D. thesis, Faculty of Science, University of Belgrade

O'Brien S.J. (1972) The α-glycerophosphate cycle in D. melanogaster. II. Genetic aspects. Biochem. Genet. 7, 127-138

Smit Z., Rizova M., Mihailović N., Janković V. & Tucić N. (1981) Contribution to the evolutionary theory of ageing. III. Relationship between duration of preimaginal development and longevity in D. melanogaster. Acta Biol. Yugoslavica (Genetica) 13, 158-170

Stam L.F. & Laurie-Ahlberg C.C. (1982) A semi automated procedure for the assay of 23 enzymes from D. melanogaster. Insect Biochem. 12, 537-544

Stein G. & Stein U. (1976) Chromosomal proteins : their role in the regulation of gene expression. BioScience 26, 488-498

Thompson J.N., Ashburner M. & Woodruff R.C. (1977) Presumptive control mutation for Adh in D. melanogaster. Nature 270, 363

Van Delden W. (1982) The Adh polymorphism in D. melanogaster. Selection at an enzyme locus. In : Evolutionary Biology (H.K. Hecht, B. Wallace and T. Prauce, eds.), Plenum Press, New York, vol. 15, pp. 187-222

Ward R.D. & Herbert P.D.N. (1972) Variability of alcohol dehydrogenase activity in a natural population of D. melanogaster. Nat. New Biol. 236, 243-244

Zera A.J. (1981) Extensive variation at the α-glycerophosphate dehydrogenase locus in species of waterstriders. Biochem. Genet. 19, 797-812