Arch. Biol. Sci., Belgrade, 63 (4), 1273-1286, 2011 DOI:10.2298/ABS1104273S

ADAPTIVE SIGNIFICANCE OF AMYLASE POLYMORPHISM IN *DROSOPHILA* .XV.: EXAMINATION OF GENOTYPE-BY-ENVIRONMENT INTERACTIONS ON THE VIABILITY, DEVELOPMENTAL TIME AND STABILITY OF *DROSOPHILA SUBOBSCURA* HOMOZYGOUS FOR *AMY* DURING EXPOSURE TO NUTRITIONAL CHANGES

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Abstract - Due to the direct interaction between enzyme and substrate, the amylase system can provide valuable information on the relationship between homozygosity and developmental homeostasis under a changing environment in several *Drosophila* species, The adaptive significance of the relationship between genetic variability and environmental change manifests through the well-known polymorphism of the amylase locus (*Amy*). We examined the effect of gradual and abrupt changes in starch concentration in the nutritional substrate, on the developmental time, egg-to-adult viability and phenotypic plasticity in the progeny of *Drosophila subobscura* that was homozygous for "fast" (Amy^F/Amy^F) and "slow" (Amy^S/Amy^S) *Amy* alleles. Our findings show that gradual and abrupt nutritional changes exert a significant effect on developmental time and viability. A high heterogeneity among genotypes in fluctuating asymmetry (FA) and no direct association between FA and fitness components under the two experimental regimes of environmental change were observed.

Key words: Environmental change, *Amy* locus, developmental time, viability, developmental stability, fluctuating asymmetry

UDC 636.2:575

INTRODUCTION

In modern biology, it is generally accepted that organisms in populations cannot be regarded separately from their environments. The degree of success of the responses of different genotypes to environmental changes are significant for the dynamics of the population gene pool. They give rise to a diversity of life-history strategies within populations (Levins, 1968; Roff, 1992; Stearns, 1992; Byers, 2005). Individuals of the same species that develop in different environments can significantly differ phenotypically. Such phenotypic differentiation among groups of individuals in different habitats can be caused either by genetic differences between the groups or exclusively by environmental effects.

Adaptive significance and genetic variability which correlate with environmental change are manifested through the well-known polymorphism of the amylase locus (*Amy*) in several *Drosophila* species (Milanović et al., 1989; Matsuo et al., 1999; Stamenković-Radak et al., 2003). Because α-amylase interacts directly with the nutritive substrate (it hydrolyses the internal α-1,4 glycoside bond of starch to maltose, glucose and α-dextrin to produce energy), *Amy* is a potential target gene of adaptive evolution. In particular, the regulation of the *Amy* locus

has been the focus of a great deal of research. Factors, such as the response to carbohydrates (Hoorn and Scharloo, 1978; Benkel and Hickey, 1987), tissue specificity (Powell et al., 1980; Klarenberg et al., 1986), and stage-specific expression patterns (Yamazaki, 1986; Inomata and Yamazaki 2000) are all involved in the regulation of this gene**.** Matsuo and Yamazaki (1984) showed that amylase regulation (inducibility) is positively correlated with fitness in the productivity and life-span of individuals. Allozyme variants may differ in fitness under some environmental or metabolic conditions.

Intra- and inter-population variability exists in the structural allozyme polymorphism, as well as in the polymorphism that underlies the regulation of gene expression of the Palearctic species *Drosophila subobscura* (for a review see Milanović and Andjelković, 1993). Biochemical and physiological differences caused by the genetic structure of the *Amy* locus are revealed through the variability of fitness components among *D. subobscura* flies reared on different carbohydrate sources.

Fluctuating asymmetry (FA), as a random deviation from perfect bilateral symmetry, has been widely used as a measure of environmental or genetic disturbance during development. There is considerable debate about the utility of FA as a measure of developmental stability (DS) (Whitlock, 1996; Van Dongen, 1998; Houle, 2000); since FA often increases with stress (Clarke, 1993; Badyaev et.al, 2000), including toxins and parasites (Clarke and McKenzie, 1987; Polak, 1993; Bjorksten et al., 2000 a), it is generally thought to be a reliable measure of DS, particularly when more than one trait is measured (Palmer and Strobeck, 2001). It has also been suggested that developmental stability is closely related to fitness through sexual or natural selection in general. A number of studies (Møller,. 1997; Martin and Hosken, 2002, Woods et al., 2002) reported a significant negative association between fluctuating asymmetry (FA) and individual fitness components.

Several studies have reported that the asymmetry of insect bilateral traits increases when organisms develop under environmentally stressful conditions (Parsons, 1992; Clarke, 1998; Markow, 1995; Hoffmann et al., 2005; Imasheva et al., 1998). However, there are many cases where stressful conditions did not cause changes in asymmetry (Leung and Forbes, 1996; Bjorksten et al., 2000 b). Those contrasting results provoke further research, particularly in the relation between FA and fitness.

Another way of responding to environmental variation is by phenotypic plasticity. A concept that places phenotypic plasticity in the context of a genotype-specific response is the norm of reaction. A norm of reaction is an array of phenotypes that will develop from a genotype under an array of different environments (Woltereck, 1909). Phenotypic plasticity demonstrates the two meanings of adaptation: the plastic response is itself an example of a physiological adaptation and it is widely held that the ability to be plastic is adaptive in the sense of increasing fitness. Investigations into the genetic and environmental factors involved in adaptation to a new environmental condition are significant in order to understand the origin and maintenance of species diversity.

The selection process, degree of inbreeding, interaction between genotype and environment, and the complexity of the environment make it difficult to define the mechanisms involved in the response to different environmental conditions as well as defining the consequence of these mechanisms in the life cycle.

The response of homozygous *Amy* genotypes to a particular environmental change through components of fitness, phenotypic plasticity for these components and developmental instability is not sufficiently supported by experimental data. The present study examines how the above-mentioned responses of *D. subobscura* that are homozygous for the two most common *Amy* alleles respond to abrupt or gradual changes of starch concentration in the growth substrate. We also analyzed the possible association of preadult developmental time and egg-to-adult viability with phenotypic plasticity and FA variability under conditions of substrate change.

MATERIALS AND METHODS

A large sample of *Drosophila subobscura* flies collected in the field (Goč Mountain, Serbia) was used to obtain lines homozygous for slow *Amy* allele (Amy^S/Amy^S genotypes) or fast allele (Amy^F/Amy^F genotypes). The genotypes were determined by the method of polyacrylamide gel electrophoresis (Doane, 1967).

The obtained lines were reared on a common standard medium for *Drosophila* (9% sugar, 10% cornmeal, 2% agar and 2% yeast) with nipagin as a mold inhibitor. All cultures were kept and all experiments were performed under constant laboratory conditions, (19°C, relative humidity 60%, light intensity cca. 300 lux, light/dark daily intervals 12h/12h).

Two lines of each homozygous genotype were used in the experiment. In one experimental group, eggs from each line were transferred from the standard substrate to substrates with increasing concentrations of starch concentration (3%, 6%, and 9%) in each successive generation. In the next generation in another experimental group a sample of eggs from the same lines was transferred from the standard substrate directly to the substrate with the highest starch concentration (9%). This served as an experimental model of an abrupt substrate change. The substrates contained 5% yeast, 1.5% agar, and 3%, 6%, or 9% starch, as indicated. Each line in each experimental group had three replicas, containing 60 eggs in 60 mL bottles. After the egg transfer, the time and number of adult flies emerging in each subsequent day was recorded. The average developmental time was calculated according to these data. The egg-to-adult viability was calculated as a percentage of the number of adults with respect to the number of eggs. Prior to statistical analysis *arcsine* transformation was done on the viability data. Two-way ANOVA (Zar, 1999) was performed, with the genotype and starch concentrations serving as the variability of phenotypic plasticity. Phenotypes produced by particular homozygous *Amy* genotypes on the different starch concentrations are shown as a norm of reactions.

An analysis of wing size was carried out on 30 flies of both sex from each experimental group and line. The left and right wing from each fly was cut and prepared on a slide for measurement. The wing length was taken as the distance from the intersection of the third longitudinal vein with the anterior crossvein to the wing tip where the third vein ends. The wing width was taken as the distance between the ends of the second and the fifth longitudinal veins. The measurements were made under a binocular microscope, with a Leica/Cannon Image analysis system. The fluctuating asymmetry (FA) statistics were performed according to Palmer and Strobeck (2001). The asymmetry of each trait was measured as the absolute (unsigned) (L-R) difference (known as Palmer index FA1), and as the signed value of this measure (known as Palmer FA4 index). The non-parametric tests, Kolmogorov-Smirnov and χ^2, were used to test departures from normality. The size dependence of FA for each sex and trait was tested by multiple regression analysis. A one-sample t-test was used to test for the departure of the mean of (R – L) from an expected mean of zero. The measurement error was estimated for all samples by two-way ANOVA on a sample of 30 individuals that were measured twice (as recommended by Palmer and Strobeck, 2001).

RESULTS

Egg-to-adult developmental time

The means of egg-to-adult developmental times are given in Table 1. Results of a two-way ANOVA analysis (Table 2) show that the developmental time significantly differs among the genotypes analyzed in both experimental groups. The developmental times of the genotypes on different substrates and on the same substrate, changed with the starch concentration. The flies which completed development on the standard substrate exhibited significantly longest development (23 days) compared to the flies grown on 3% starch which had the shortest development (19 days), whereas flies grown on the 6% starch substrate (20 days), and on the 9% starch substrate in both experimental groups (21 to 22 days, respectively). In general, the genotypes differ significantly in develop-

Table 1. Developmental time and egg-to-adult viability of *Drosophila subobscura* homozygous for *Amy* locus on different substrates under two experimental regimes

genotype	change in substrate	starch concentration (%)	developmental time±S.E.		viability±S.E.	
$S_{57/8}$	gradual	standard	22,80	±0,046	56,00	±3,849
		3	19,81	±0,162	42,22	±6,261
		6	20,37	±0,079	70,00	±0,000
		9	21,50	±0,085	51,11	±5,879
	abrupt	9	22,51	±0,078	68,33	±3,469
$S_{218/4/1}$	gradual	standard	23,39	±0,121	72,00	±4,073
		3	20,11	±0,118	83,89	±4,340
		6	22,82	±0,115	54,44	±5,300
		9	21,59	±0,074	78,89	±6,407
	abrupt	9	23,32	±0,149	75,00	±5,092
$F_{69/6/8}$	gradual	standard	23,09	±0,062	67,11	±2,475
		3	20,49	±0,110	50,28	±8,198
		6	19,72	±0,045	76,11	±0,556
		9	21,20	±0,055	76,67	±5,092
	abrupt	9	22,68	±0,082	53,33	±24,286
F_{80}	gradual	standard	22,50	±0,056	53,33	±6,158
		3	19,38	±0,069	41,11	±10,643
		6	19,37	±0,045	74,44	±4,006
		9	22,05	±0,102	35,00	±9,179
	abrupt	9	22,46	±0,073	51,67	±6,736

Table 2. Two-way ANOVA for fitness components of *Drosophila subobscura* homozygous for *Amy* locus on different substrates under two experimental regimes

	developmental time				viability		
	df	MS	F		MS	F	
genotype	3	4,80	39,35	***	0,15	12,22	***
substrate	3	23,54	192,84	***	0,04	3,49	*
genotype×substrate	9	1,83	14,96	***	0,07	6,07	***
Error	32	0,12			0,01		

*p<0.05; **p<0.001

mental time on all starch substrates and the interaction between the genotype and the environment is the main source of variability.

Norms of reaction for the average values of developmental time are shown in Fig. 1a. Each line represents the data for a particular homozygous *Amy* genotype under gradual and abrupt starch concentration changes. The effect of gradual starch concentration is significant for developmental time and the genotypes respond with a short developmental time.

Developmental time under abrupt starch concentration change (standard substrate →9% starch

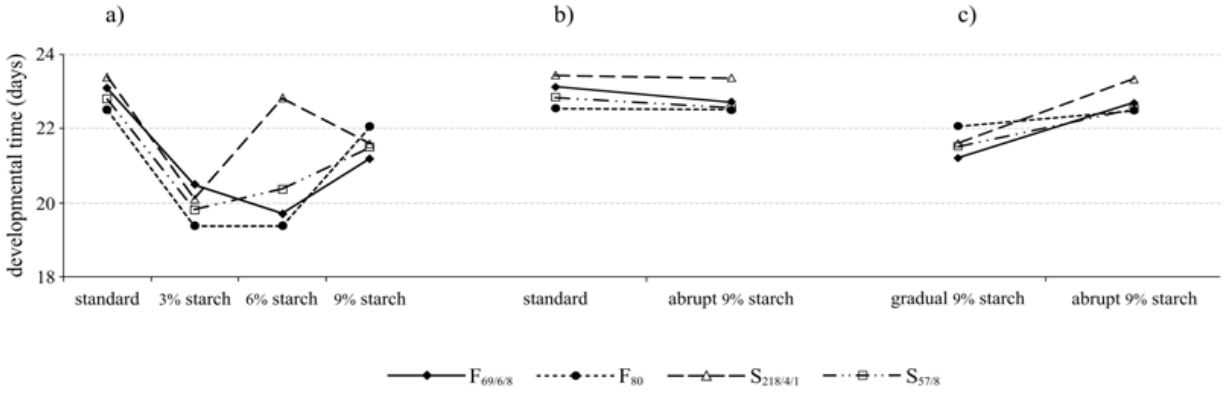

Fig. 1 A norm of reactions for developmental time homozygous *Amy*-genotypes of *Drosophila subobscura* under gradual and abrupt starch concentration changes

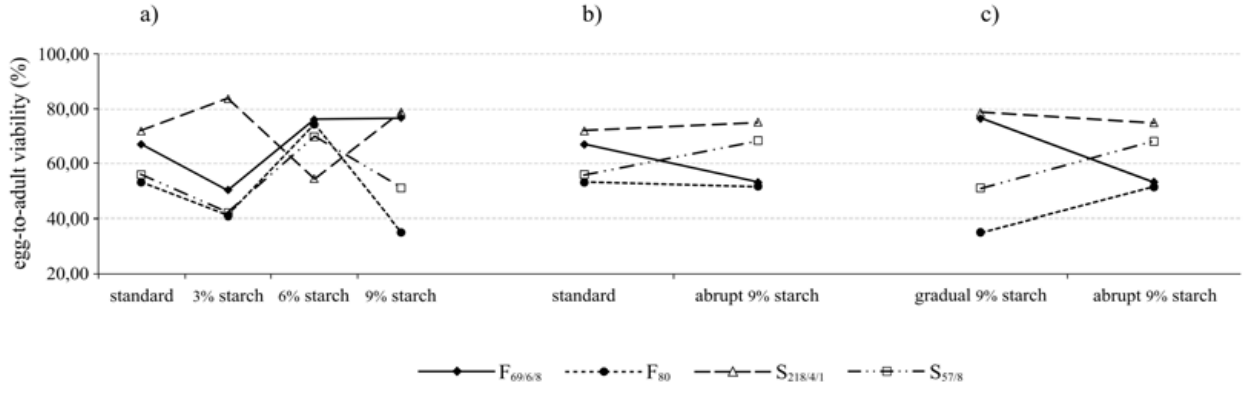

Fig. 2 A norm of reactions for egg-to-adult viability homozygous *Amy*-genotypes of *Drosophila subobscura* under gradual and abrupt starch concentration changes

concentration) remains in the same ranges and there are no significant interactions between the genotype and the environment (Fig. 1b). The homozygous genotypes are plastic in developmental time when they develop on 9% starch concentration in gradual and abrupt environmental changes and their norms of reaction intersect (Fig. 1c) and the genetic variance in phenotypic plasticity exists.

Egg-to-adult viability

The means of egg-to-adult viability are given in Table 1. The viability ranges from 35% to 84%. The viability on the 3% starch substrate is on average 45% lower for all genotypes except for genotype $S_{218/4/1}$ (84%) which has the highest viability on all substrates. The significantly lowest viability (35%) was found in genotype F_{80}. The results of a two-way ANOVA analysis (Table 2) show a more significant effect of the genotypes and their interactions with the substrate, than the substrate itself. The genotypes differ in viability among the substrates. The flies on the 3% starch substrate show a significantly lower viability than the individuals that completed development on the 6% starch substrate under gradual concentration change.

The genetic component (genotype) showed a significant effect on the mean viability on 9% starch both under the gradual and abrupt change, and on 3% and 9% starch under abrupt change. In these conditions, genotype $S_{218/4/1}$ showed the highest viability and genotype F_{80} the lowest.

In Fig. 2a the norms of reaction for average viability are shown. The norms of reaction of the mean values for egg-to-adult viability under gradual environmental change indicate the existence of genetic variability. Homozygous genotype $S_{218/4/1}$ has a different response in terms of viability at gradual starch concentration changes. The effect of genotype and environment interaction is significant, which confirms the phenotypic plasticity of *D. subobscura* individuals and their genetic variability for viability in subsequent generations on different starch concentrations.

Egg-to-adult viability under abrupt starch concentration change (standard substrate →9% starch concentration) indicates a significant interaction between the genotype and the environment for homozygous genotypes (Fig. 2b). Homozygous genotypes are phenotypically plastic in viability when they develop on 9% starch concentration under gradual and abrupt environmental changes and their responses are adaptable (Fig. 2c).

Developmental stability

Table 3 shows the two body size parameters, the wing width and length in the two experimental groups. Sexual dimorphism in the wing size is a characteristic of *D. subobscura*, so the analysis of the wing size was done on each sex separately. Both the length and width of the wings were found to increase with the starch concentrations.

Three-factorial ANOVA (Table 4) shows the significant individual effects of genotype and substrate on the wing size, but also of their interactions, in all cases except for the wing length under the changing starch concentrations. On average, the homozygous genotypes with the "slow" *Amy* allele have significantly longer wings, whereas the wing width appeared to be independent in the homozygous combination.

The FA statistics for the wing length and width of the pooled sexes in both experimental groups are shown in Table 5. The pooled FA data of both sexes are presented, as no significant differences were found when sexes were analyzed separately.

The measurement error shows significant interactions between the wing size and individual FA both for the length ($MS=12.327$, $p < 0.001$) and width ($MS=20.455$, $p < 0.001$). All samples had a normal distribution. After sequential Bonferroni correction, multiple regression analysis showed no significant correlation between $|R-L|$ and $(R+L)/2$, indicating that FA does not vary with trait size in these samples. The left-right (L-D) size differences showed that directional assymetry (DA) is present in some cases. After the sequential Bonferroni correction, only one genotype, F_{80} on 9% starch concentration, was significant ($p < 0.05$) for length. Because of the presence of DA, we chose the FA4 index of FA (Palmer and Strobeck, 2001.), because it is not biased by DA.

An analysis of variance of FA4 for the wing length under abrupt starch concentration change (3% and 9% starch) showed significant individual effects of the environment and genotype, and their interactions as well. In the case of the wing width, a significant source of FA variability was the interaction between the genotype and the environment under gradual starch concentration change, with genotype F_{80} having the highest width FA variability.

An analysis of variance of the mean absolute values $|R-L|$ of the wing length shows a significant environmental (i.e. starch concentration) effect, while the genotype had a significant effect on the developmental stability of the wing width. Under the gradual starch concentration change, genotype F_{80} always had the significantly lowest mean absolute $|R-L|$ values of wing width. Under the abrupt starch concentration change, genotype $S_{218/4/1}$ had the highest mean absolute $|R-L|$ values of wing width. On the 9% starch

Table 3. Wing length and width of *Drosophila subobscura* of females and males homozygous for *Amy* locus on different substrates under two experimental regimes

			female			
			length		width	
genotype	change in substrate	starch concentration (%)	mean (R+L) /2±SE		mean (R+L) /2±SE	
$S_{57/8}$	gradual	standard	600,69	±4.56	386,45	±2,91
		3	633,06	±4.17	407,84	±2.76
		6	643,05	±2.66	411,72	±1.73
		9	635,53	±3.45	411,50	±2.35
	abrupt	9	641,22	±2.20	413,59	±1.82
$S_{218/4/1}$	gradual	standard	592,93	±4.90	402,46	±3,32
		3	652,25	±2.28	441,84	±1.33
		6	669,19	±2.97	452,16	±1.72
		9	622,14	±4.69	421,07	±3.66
	abrupt	9	661,07	±2.34	445,79	±1.58
$F_{69/6/8}$	gradual	standard	589,21	±3.87	393,41	±2.53
		3	651,66	±5,51	441,39	±2.33
		6	655,46	±2,28	436,53	±1.39
		9	645,13	±3,50	429,10	±2.09
	abrupt	9	636,93	±2,67	426,63	±1.53
F_{80}	gradual	standard	580,41	±4.18	376,72	±2,81
		3	656,17	±2.10	423,96	±1.39
		6	650,07	±3.26	418,08	±2.42
		9	643,63	±4.83	410,31	±3.19
	abrupt	9	638,78	±1.91	413,10	±1.51
			males			
$S_{57/8}$	gradual	standard	569,20	±5.54	362,03	±3,93
		3	592,18	±3.80	377,06	±2,33
		6	601,55	±3.42	381,86	±1.98
		9	587,74	±3.00	377,22	±2.28
	abrupt	9	585,55	±5.24	368,71	±3.10
$S_{218/4/1}$	gradual	standard	548,91	±3.53	371,51	±2,35
		3	587,80	±2.45	396,82	±1.36
		6	608,79	±2.54	411,01	±1.82
		9	608,83	±3.16	409,06	±1.79
	abrupt	9	598,19	±2.05	406,11	±1.51
$F_{69/6/8}$	gradual	standard	544,08	±2.87	364,85	±1.82
		3	593,86	±4,07	397,77	±2.46
		6	595,65	±2,42	395,25	±1.10
		9	593,46	±2,78	392,02	±1.96
	abrupt	9	575,88	±2,40	385,47	±1.25
F_{80}	gradual	standard	536,17	±4.98	347,21	±3,59
		3	599,62	±2.73	389,32	±1.56
		6	595,09	±2.44	380,24	±1.50
		9	604,09	±6.55	385,33	±4.27
	abrupt	9	592,00	±3.84	381,28	±2.28

Table 4. Three-way ANOVA for wing length and wing width of *Drosophila subobscura* homozygous for *Amy* locus on different substrates under two experimental regimes

gradual substrate change							
		wing length			wing width		
	df	MS	F		MS	F	
substrate	3	164454,30	384,51	***	65938,70	366,35	***
genotype	3	616,63	1,44		32003,60	177,81	***
sex	1	532369,90	1244,74	***	259381,00	1441,08	***
substrate ×genotype	9	4882,85	11,42	***	2083,20	11,57	***
substrate×sex	3	4544,74	10,63	***	2147,21	11,93	***
genotype×sex	3	1848,10	4,32	***	670,22	3,72	***
substrate×genotype×sex	9	1970,80	4,61	***	836,99	4,65	**
Error	928	427,70			179,99		
9% concentration starch in gradual and abrupt substrate change							
substrate	2	174489,60	381,34	***	68177,81	332,33	***
genotype	3	2386,61	5,22	**	22583,91	110,08	***
sex	1	369311,30	807,12	***	179872,30	876,78	***
substrate ×genotype	6	6202,42	13,56	***	1892,71	9,23	***
substrate×sex	2	5890,77	12,87	***	2742,71	13,37	***
genotype×sex	3	1263,44	2,76	*	734,94	3,58	*
substrate×genotype×sex	6	2313,09	5,06	***	876,85	4,27	***
Error	696	457,57			205,15		
abrupt substrate change							
substrate	2	188031,40	457,65	***	81626,15	492,66	***
genotype	3	2362,16	5,75	***	24166,56	145,86	***
sex	1	466551,30	1135,53	***	225833,50	1363,03	***
substrate ×genotype	6	6609,14	16,09	***	3116,08	18,81	***
substrate×sex	2	4269,18	10,39	***	2253,03	13,60	***
genotype×sex	3	1848,45	4,50	**	470,34	2,84	*
substrate×genotype×sex	6	531,23	1,29		408,01	2,46	*
Error	696	410,87			165,69		

*p<0.05; **p<0.01; ***p<0.001

Table 5. Data on wing width and length and their asymmetry after gradual and abrupt starch concentration changes. The analysis included 30 males and 30 females in each experimental group of *Drosophila subobscura* homozygous for *Amy* locus

			length				
	change in substrate	starch concentration (%)	mean |R-L |±SE		mean (R - L)±SE		var (R-L)=FA_4
$S_{57/8}$		standard	3,71	±0,41	-1,40	±0,60	21,92
	gradual	3	3,86	±0,36	0,46	±0,62	22,72
		6	3,51	±0,38	-0,98	±0,58	20,15
		9	4,09	±0,43	-1,05	±0,67	27,12
	abrupt	9	4,05	±0,49	-0,25	±0,72	30,77
$S_{218/4/1}$		standard	3,76	±0,37	-0,31	±0,60	21,92
	gradual	3	4,96	±0,57	-0,25	±0,86	44,20
		6	3,55	±0,41	-0,36	±0,62	22,97
		9	5,22	±0,39	-1,35	±0,76	34,93
	abrupt	9	4,39	±0,42	-2,02	±0,66	26,15
$F_{69/6/8}$		standard	3,67	±0,51	-1,81	±0,66	25,81
	gradual	3	4,25	±0,54	-0,23	±0,77	35,65
		6	3,61	±0,38	-1,20	±0,59	20,61
		9	3,61	±0,43	-0,84	±0,63	23,61
	abrupt	9	3,84	±0,34	-1,76	±0,56	18,58
F_{80}		standard	2,97	±0,26	-0,52	±0,46	12,65
	gradual	3	2,86	±0,34	-1,60	±0,46	12,76
		6	3,83	±0,46	-1,87	±0,63	23,84
		9	4,49	±0,46	-2,38	±0,68	27,62
	abrupt	9	4,38	±0,47	-3,41	±0,59	21,11
			width				
			mean |R-L |±SE		mean (R - L)±SE		var (R-L)=FA_4
$S_{57/8}$		standard	3,50	±0,33	1,14	±0,54	17,70
	gradual	3	2,94	±0,29	1,03	±0,46	12,81
		6	2,99	±0,29	-0,36	±0,48	13,87
		9	3,25	±0,37	-0,22	±0,56	18,85
	abrupt	9	2,89	±0,32	0,80	±0,48	14,10
$S_{218/4/1}$		standard	3,39	±0,38	0,04	±0,58	20,11
	gradual	3	2,82	±0,32	1,18	±0,46	12,66
		6	3,00	±0,29	-0,46	±0,48	14,08
		9	3,18	±0,34	0,90	±0,52	16,34
	abrupt	9	3,33	±0,32	0,98	±0,52	16,28
$F_{69/6/8}$		standard	2,96	±0,34	0,21	±0,51	15,91
	gradual	3	3,29	±0,35	0,05	±0,55	18,30
		6	3,27	±0,40	1,17	±0,57	19,21
		9	3,63	±0,39	0,45	±0,61	22,20
	abrupt	9	3,20	±0,39	0,54	±0,57	19,16
F_{80}		standard	2,49	±0,28	0,94	±0,41	9,94
	gradual	3	2,36	±0,27	0,96	±0,39	8,97
		6	3,00	±0,30	1,78	±0,44	11,42
		9	2,94	±0,31	0,81	±0,48	13,93
	abrupt	9	3,22	±0,32	0,56	±0,52	16,49

substrate, under both experimental regimes of starch concentration change, the genotypes did not differ in the variability of the mean absolute values |R-L| of the wing width.

DISCUSSION

In *Drosophila*, the expression of amylase is affected by environmental conditions. Amylase is induced when substrate starch is present (Abe, 1958; Yamazaki and Matsuo, 1984; Matsuo and Yamazaki, 1984) and suppressed when the end-product glucose is present (Hickey and Benkel, 1982; Benkel and Hickey, 1987). This gene polymorphism has its clearly defined biochemical phenotype, and the level of amylase activity is regulated by the components and conditions of the nutrient composition. The biochemical and physiological differences caused by the genetic structure of the *Amy* locus appear evident from previous results through the variability of fitness components among *D. subobscura* flies reared on different carbohydrate sources (Andjelković et al., 2003). The viability of homozygotes with the "slow" *Amy* allele (Amy^S/Amy^S) genotype was significantly better than that of the homozygotes with the "fast" *Amy* allele (Amy^F/Amy^F) genotype on substrates with a higher concentration of starch as a stressful factor. The unselected control population showed a significantly better viability than either of the selected lines, which can be explained by the higher heterozygosity over the genome, associated with higher metabolic efficiency.

The results of Milanović and Andjelković (1992) and Hoorn and Scharloo (1978) showed that in terms of enzyme-specific activities, Amy^S/Amy^S homozygotes have, on average, a significantly higher enzyme activity than Amy^F/Amy^F genotypes, and the effect of food components on amylase activity has been observed. As we used homozygous Amy^S/Amy^S and Amy^F/Amy^F lines in the present experiment, it can be assumed that the measured fitness components were also affected by the levels of enzyme activity of these genotypes. It could be an indirect way of adapting to a gradual or abrupt change of starch concentration in the substrate. A previous study with the same genotypes (Savić, 2004) showed that differences in the amylase activity between genotypes, when reared on standard laboratory substrate, disappear with a change of starch concentration. Being significantly higher in the Amy^S/Amy^S genotypes than in the Amy^F/Amy^F on the standard substrate, the activity of both genotypes was shown to decrease under the conditions of either gradual or abrupt starch concentration change. The reason may be the combining with other metabolic pathways in the utilization of the nutritional substrate. We addressed this problem in the present study using *Drosophila subobscura* lines homozygous for each of the two *Amy* alleles and analyzed their response to either a gradual or abrupt change in the nutritional substrate through two fitness components and fluctuating asymmetry of the wings.

The developmental time of *D. subobscura* lines in our experiments was shortened with starch concentration change. Also, the flies had a lower viability on the 3% starch substrate than on 6% starch In some way this could be a consequence of adaptation to the increased starch concentrations. The significant variability in the egg-to-adult viability among the genotypes on 9% starch, either under the gradual or abrupt change, points to a significant genotypic variance. Norms of reaction obtained for the mean values of analyzed characters indicate the significant environmental effect, but also the present genetic variability of *D. subobscura.*

The causes of increased developmental instability and its relationship to fitness are still under dispute as studies give controversial results. Many authors who have looked for the correlations between fluctuating asymmetry and various fitness components have found no such effects (e.g. Breuker and Brakefield, 2002; Goncalves et al., 2002; Martin and Hosken, 2002; Rivera et al., 2002; Siikamaki et al., 2002; Kolliker-Ott et al., 2003; Kruuk et al., 2003). Nonetheless, many other studies report significant associations between levels of fluctuating asymmetry and different fitness attributes (Bergstrom and Reimchen, 2003; Frechette et al., 2003; Hendrickx et al., 2003; Mallard and Barnard, 2003).

Under the experimental conditions of either a gradual or abrupt starch concentration change, the genotypes homozygous for any of the two *Amy* alleles show a significantly higher wing length, while the genotype-by-environment interactions interaction is more significant for wing width variability. It follows that genotypes respond differently in different environments. The results on the wing size and developmental time obtained in our experiments differ from those of other authors, who found a positive relationship between these two variables (Roff, 2002; Woods et al., 2002). Our results show that the developmental time is longest on the standard substrate and 9% starch under both experimental conditions, with flies having narrower and shorter wings. Also, wing length is significantly affected by starch concentration, while wing width variability is under a significant genetic effect.

Under stressfull conditions, such as an abrupt change in starch concentration, the selection pressure is higher. Environmental factors may be stressful because they are in limited supply or because they are in excess supply, and the response to each condition is genotype-specific. In accordance with this, the genotypic specificities under gradual or abrupt substrate changes in the present study, shown through the significant genotype-by-environment interactions interaction as a source of variability of FA of wing size and fitness, suggest that homozygotes differ in their genetic constitution, having different combinations of homozygous alleles at loci which contribute to developmental stability and/or fitness. Fluctuating asymmetry is certainly associated with increased homozygosity, but for specific alleles (Hoffmann and Woods, 2003).

The objective of our present study was to give an answer to whether the *Amy* genotypes of *D. subobscura* differ in fitness components and developmental stability (FA) under gradual or abrupt substrate change and if there is any association between these two kinds of response. The amylase system used in the study of the relationships among homozygosity, developmental homeostasis, phenotypic plasticity and FA under environmental change is convenient because of the direct interaction of enzymes with the substrate. The results show a high heterogeneity among genotypes in FA and no direct association between FA and fitness component variability under the two experimental regimes of environmental change. Møller (1999) claims that asymmetry is generally negatively correlated with fitness components, growth, fecundity, and survival. Although developmental instability measured as FA is expected to be negatively correlated to fitness and positively to stress, empirical evidence shows a much more complex and inconsistent relationship (Bourguet, 2000; Hoffmann and Woods, 2003; Zakharov, 2003).

The results presented here show that the genetic component (genotype) has a significant effect on the mean egg-to-adult viability on 9% starch, under either the gradual or abrupt changes, and on the 3% and 9% starch substrates under abrupt change. Although change in viability was found, the expected increase of FA was not observed. It is possible, albeit not clear, that a selection factor such as starch concentration produces adaptation and reduces asymmetry in viable offspring. Symmetrical flies, thus developmentally stable, may have genotypes that encode to higher metabolic efficency. This may be a link between the fitness and FA results obtained here, and should be studied further. Although some authors strongly recommend FA as a measure of fitness decline in populations, sublethal stress caused by change in the substrate and its relationship with developmental instability has not been proven in all cases and genotype-by-environment interactions are important, and should be considered if FA is used as an indicator of the impact of a gradual or abrupt environmental change. Also, the existence of phenotypic plasticity is very important in distinguishing and better understanding the responses and processes of adaptation to environmental change through generations.

The effect of genetic variability is significant for the phenotype mean value response to gradual or abrupt environmental change. Environmental changes are important and have significant impact

on genotype mean values and therefore to phenotypic plasticity of analyzed characteristics through several generations.

Acknowledgement - This work was supported by the Ministry of Science and Education of the Republic of Serbia, Grant No. 173012.

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