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The frequencies and activity of locus *β-est* alleles in laboratory populations of *Drosophila melanogaster*, originating from wild flies of the Chernobyl Exclusion Zone.

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Summary

We determined the expression of *β*-specific carboxylesterase and the frequencies of related alleles and genotypes in some lines of *Drosophila melanogaster* originating from various regions of the Chernobyl exclusion zone. The enzymes' activities were studied by alkaline polyacrylamide gel electrophoresis and computer densitometry. We also compared activities of *S*- and *F*-allozymes of *β*-esterase from different populations. As the populations were heterogeneous in locus of *β*-esterase, frequencies of alleles and genotypes in studied populations have been determined. The data characterize the Chernobyl population (natural) and *Odessa* population (wild type laboratory strain).

Key words: *β-esterases, allozyme expression, frequencies of the alleles and genotypes, populations of* Drosophila melanogaster.

Introduction

The effect of environmental factors on population genetics brings about changes in various biochemical parameters. These changes therefore can be used to monitor systems of populations in harsh environmental conditions [1]. One of the most effective and widely used techniques to study molecular polymorphism is alkaline polyacrylamide gel electrophoresis (PAGE) [2, 3]. Our object was to determine the frequencies and expressions of S- and F-allozymes of *β*-esterase in *Drosophila* imagoes and to study genetic structure of locus *β-est* in populations of flies derivating from various regions of the Chernobyl exclusion zone.

Materials and Methods

We studied the F_{14} generation of *Drosophila melanogaster* Meigen originally taken from natural populations of the three regions of Chernobyl Exclusion Zone with the level of radiation 50 (two places) and 2100 mkR/hour. All flies of derived laboratory populations (*Sadovaya*, *Polesskaya,* and *Priozernaya*) were raised at the temperature of 25ºC using the standard medium [4]. As a control group we used flies of *Odessa* wild type strain (cultured since 1980).

To study the polymorphism of β-esterase and of its allozymes' expressions, we used electrophoresis followed by histochemical identification of enzymatic fractions. We prepared tissue extracts from three-days-old imagoes, males and females separately. The activities of *β*-specific carboxylesterases in the gel block were then identified by means of α-and β-naftilacetate and diazonium salts. From the obtained electrophoregrams results, we measured the optical densities (for 7 males and 8 females accordingly) to calculate the relative and specific activity of β-esterase allozymes.

To perform the calculations we quantified the average content of water-soluble proteins in a fly ($[\overline{P}]$) after Lawry *et al*., and the average weight of a fly ($\overline{m}$) for males and females accordingly. To obtain an activity index we divided the average optical density ($\Delta\overline{Do}$) by the average protein content or body weight. It was then expressed as optical density per 1 mg of protein or 1 mg of body weight. The data were treated statistically [5]. In addition, the use of electrophoregrams allowed us to find the frequencies of β-esterase expression phenotypes, and the frequencies of the genotypes and alleles which determine its S-and F-allozymes. The expected frequencies of genotypes and alleles were calculated using the Hardy-Weinberg equation (N = 15) [6]. To conduct the research we used the reagents of foreign firms, Russian electrophoretic set "VE-4", and licensed software "Spectrum Analyzer images" (Podzharsky, Rybalko, 2004, Ukraine).

Results

As one can see from Table 1, for both sexes the highest levels of expression of S-allozyme of β-specific esterase per imago were found in the *Odessa* population. At the same time, the the *Sadovaya* differences in total activities of the allozyme among males and females of different Chernobyl populations are less pronounced. As to the the F-allozyme, its activities in females of *Sadovaya*, *Polesskaya*, and *Priozernaya* populations were virtually identical. In males, though, the expression of the allozyme was significantly lower in flies of Polesskaya population than in those of *Sadovaya* and *Priozernaya*.

The S-allozyme activity indexes per 1 mg of body weight are almost identical in Odessa and *Sadovaya* populations for both males and females correspondingly. It was also that way for males of *Polesskaya* and *Priozernaya* populations, though the level of expression of S-allozyme here was approximately twice lower. Interestingly, the activity rate of F-allozyme varied greatly from 2.12 (*Sadovaya*, males) to 0.35 relative units (*Sadovaya*, females).

The specific activity of S-allozyme per 1 mg of water-soluble proteins of male flies from *Odessa* population was slightly higher than that of Chernobyl lines. This was not so in females. Moreover, the highest expression rate of S-allozyme per 1 mg of proteins was detected in the females of *Priozernaya* population. The same pattern was observed in F-allozyme specific activity: it was highly similar in males of *Priozernaya* and *Sadovaya* populations and twice as large in males of *Polesskaya* population.

Table 1. Comparative characteristic of allozymes of β-specific esterase activity in male and female imago of different populations of *Drosophila melanogaster.*

Populations	Parameters	Imago ($\bar{x} \pm s_{\bar{x}}$, n = 7 – 8)			
		Males		Females	
		S-allozyme	*F*-allozyme	*S*-allozyme	*F*-allozyme
Odessa	$\Delta\overline{Do}/ex$	2.159 ± 0.211*	–	1.212 ± 0.147*	–
	$\Delta\overline{Do}/\overline{m}$	2.633 ± 0.258*	–	1.082 ± 0.139	–
	$\Delta\overline{Do}/[\overline{P}]$	5.515 ± 0.541*	–	2.617 ± 0.338*	–
Sadovaya	$\Delta\overline{Do}/ex$	1.579 ± 0.174*	1.333 ± 0.145*	0.849 ± 0.144*	0.613 ± 0.019
	$\Delta\overline{Do}/\overline{m}$	2.507 ± 0.276	2.116 ± 0.230*	0.976 ± 0.191	0.352 ± 0.158*
	$\Delta\overline{Do}/[\overline{P}]$	4.472 ± 0.461*	3.776 ± 0.543*	2.182 ± 0.426*	1.574 ± 0.056
Polesskaja	$\Delta\overline{Do}/ex$	1.053 ± 0.112*	0.568 ± 0.044*	0.783 ± 0.056*	0.585 ± 0.021
	$\Delta\overline{Do}/\overline{m}$	1.224 ± 0.121*	0.661 ± 0.061*	0.719 ± 0.059*	0.537 ± 0.033*
	$\Delta\overline{Do}/[\overline{P}]$	3.463 ± 0.345*	1.603 ± 0.353*	2.377 ± 0.196	1.778 ± 0.109
Priozernaja	$\Delta\overline{Do}/ex$	1.207 ± 0.040*	0.939 ± 0.120*	1.172 ± 0.161	0.777 ± 0.057
	$\Delta\overline{Do}/\overline{m}$	1.567 ± 0.084*	1.220 ± 0.156*	1.105 ± 0.215	0.733 ± 0.058*
	$\Delta\overline{Do}/[\overline{P}]$	4.160 ± 0.224*	3.243 ± 0.415*	3.615 ± 0.703*	2.395 ± 0.189*

Note: $\Delta\overline{Do}/ex$ – total activity, calculated through an optical density (relative units) per specimen, $\Delta\overline{Do}/\overline{m}$ –the relative activity per 1 mg of body weight per specimen, $\Delta\overline{Do}/[\overline{P}]$ – specific activity per 1 mg of water-soluble proteins per speciemen; – – an allozyme is absent; * – differences in interlinear parameters – general, relative and specific activity – are significant with $P < 0.05$.

Thus, our data indicate stable significant differences in esterases activities in *Drosophila* flies of different origin. They probably arise from genotype changes that affect the regulatory patterns controlling β-esterase allozymes' expression.

Unlike the *Odessa* line (obtained in our laboratory through inbreeding and artificial selection), all three post-Chernobyl populations appeared to be heterogeneous by the locus of the marker enzyme.

As one can see from Figure 1 (A and B), genotype frequencies vary in populations originating from different regions of the exclusion zone. Thus, *Sadovaya* consisted of about 29% homozygous dominants (β-Est^S / β-Est^S) and about 71% heterozygotes (β-Est^S / β-Est^F) (*β-Est^S / β-Est^F*, while recessive homozygotes (β-Est^F / β-Est^F) were absent (their expected frequency was ~ 0.13). *Polesskaya* population consisted equally of dominant homozygotes (50% against expected 56%) and heterozygotes (50% against expected 38%), while of recessive homozygotes there were none, as in *Sadovaya* population. A completely different distribution of genotypes was observed in *Priozernaya* population (~ 0.06 dominant homozygotes, 0.40 heterozygotes, and 0.53 recessive homozygotes, which nearly coincides with the expected frequencies). Hence, the frequency of S-allele in the *Priozernaya* population was ~ 0.27, and that of F-allele ~ 0.73, in *Polesskaya* population - 0.75 and 0.25, and in *Sadovaya* population - ~ 0.64 and ~ 0.36, respectively.

Thus, our experiments regarding frequencies of alleles and genotypes in populations heterogeneous by β-esterase locus expressly indicate the varying degree of adaptability of different genotypes. The data also suggest that there are some interpopulation differences in adaptation of flies of a genotypic class to similar conditions of culturing.

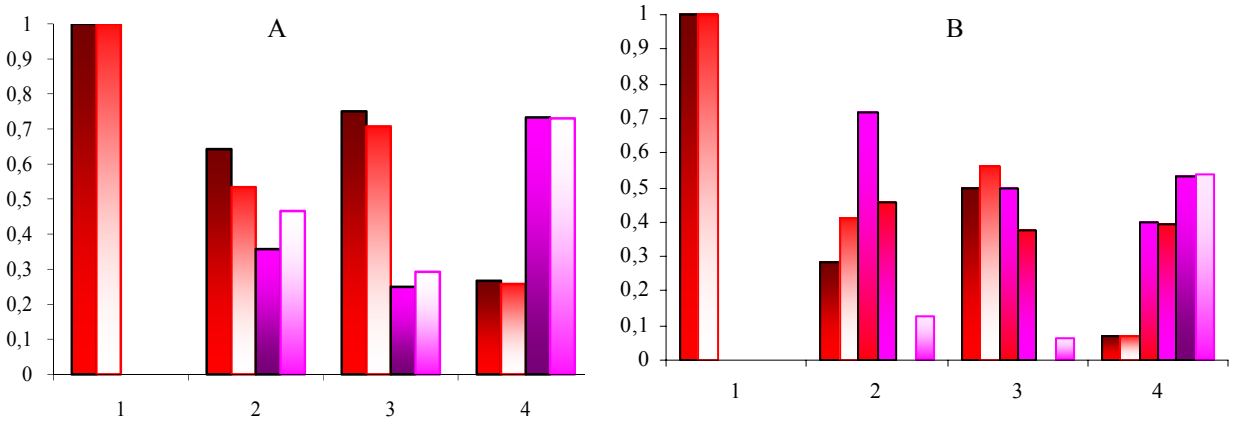

Figure 1. The frequencies of alleles and genotypes in locus of β-specific esterases in different populations of *Drosophila melanogaster*: A: 1 – the observed frequency of S-allele, 1′ – the expected frequency of S-allele, 2 – the observed frequency of F-allele, 2′ – the expected frequency of F-allele; B: 1 – the observed frequency of dominant homozygotes, 1′ – the expected frequency of dominant homozygotes, 2 – the observed frequency of heterozygotes, 2′ – the expected frequency of heterozygotes, 3 – the observed frequency of recessive homozygotes, 3′ – the expected frequency of recessive homozygotes. 1 – *Odesskaja,* 2 – *Sadovaya*, 3 – *Polesskaya*, 4 – *Priozernaya*.

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Rediscovery of *Drosophila wheeleri* in Sonora, Mexico?

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Drosophila wheeleri and *D. aldrichi* are sibling species commonly associated with *Opuntia* cactus in the southwestern United States and Mexico and are members of the large *D. repleta* group. *D. aldrichi* has a much broader distribution than *D. wheeleri*, uses different host cacti, and very likely comprises a complex of cryptic species (Wasserman, 1992; Beckenbach *et al.,* 2008; Oliveira *et al.,* 2008). Samples of *D. aldrichi* have been identified in southern Mexico, as well as El Salvador,