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SPECTRUM OF SPONTANEOUS MUTATIONS IN NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER* FROM UKRAINE

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We analyzed mutation counts in five generations of laboratory culture of isofemale lines established in 2009 from 7 natural populations of Drosophila melanogaster from Ukraine. A mutant male white (w: 1–1.5) was revealed among the third generation progeny of the Chornobyl Nuclear Power Plant cooling pond population. The sepia (se: 3–26.0) mutation was observed in 27 individuals in the third and fourth generations progeny of the Chornobyl city population. Mutants spread (sprd: 3–65,0) were revealed in a total of 54 individuals in the third and fourth generations of inbreeding progeny from Kyiv population. The molecular nature of these mutations remains to be studied.

Keywords: monitoring, mutations, white, sepia, spread

Introduction. Studies of spontaneous mutation are important in that they contribute to the understanding of population genetics aspects and evolutionary processes in populations. Historically, *Drosophila melanogaster* has been a widely used model in spontaneous mutation research. This species has been reported to have rather high spontaneous mutation rates [1], however estimates differ depending on methods employed [2, 3]. Estimates based on DNA sequence alignment are also ambiguous [4, 5].

Studies of *D. melanogaster* populations from the former USSR territory have shown oscillating rates of some spontaneous mutations, with the basic rates getting elevated from time to time [6]. The molecular nature of most of these mutations had not been studied, though they were believed to be caused by TE activity [7, 8].

Understanding of the molecular nature of mutations is important in population and evolutionary studies. After all, it is the nature of a mutation that determines its evolutionary and practical significance in each particular case. Still, research in this area is limited [9].

During 2005–2010, studies of *D. melanogaster* natural populations from Ukraine have not revealed incidents of "mutation outbursts" [10, 11], although heritable deviations were found in each of the studied populations. In laboratory culture of flies from 7 natural populations collected in 2009, we found eye

coloration and wing orientation mutations. Here we present the results of hybridological and molecular analysis of the revealed mutations.

Materials and methods

We studied flies from natural populations collected in Kyiv, Odesa, Uman, Varva, Magarach (Yalta), and the Chornobyl zone of alientation. Flies were collected during August-September, 2009. In the Chornobyl zone, we sampled two populations from locations with different background radiation (30 uR/h in an apple garden and 500 uR/h near the nuclear power plant cooling pond). The locations also differed by the sampling biotopes [11].

All collected flies were first examined under a stereoscopic microscope to screen for visible phenotype deviations. 30 females from each population were used to establish isofemale lines, each being screened for visible phenotype deviations for 5 generations of laboratory culture. Diviant individuals were removed from the isofemale lines and further studied to test for heritability of the phenotypic alternations.

For hybridological analysis, we used flies from the mutant *D. melanogaste*r strain collection of the General and Molecular Genetics Department of Taras Shevchenko National University of Kyiv. Details on these strains are presented in Table 1.

To investigate the molecular nature of revealed mutations, we used PCR amplification with further sequencing of regions in question. PCR products were sequenced on 3130 Genetic Analyzer (Applied Biosystems, USA). Sequence alignment was performed using BLAST [12].

Flies were reared on the standard medium at room temperature [13].

Statistics were calculated with reference to [14].

Results and Discussion

Mutation spectra and rates

We analyzed a total of 36433 flies being progeny of wild females sampled from 7 populations from Ukraine and cultured in laboratory for 5 generations (Table 2). We found mutations in three different loci which had not been reported for Ukrainian populations before [6]. The mutation "white eyes" was found only in progeny of the

Table 1. Fly strains used in hybridologic analysis

Strain	Mutation			Phenotype
	Symbol	Name	Localization	
C – S	*C-S*	*Canton S*		Wild type
w	*w*	*white*	1–1.5	Eye colour: white
se	*se*	*sepia*	3–26.0	Eye colour: brown
y w ; If/CyO; Sb/Tb	*y* *w* *If* *CyO* *Sb* *Tb*	*yellow* *white* *Irregular facets* *CurlyO* *Stubble* *Tubby*	1–0.0 1–1.5 2–107. 2–66.1 3–58.22 3–90.6	Body colour: yellow Eye colour: white Irregular facets Wings curled upward Small and thicker bristle Larvae, pupae and adult are short and thickset
st ss e	*st* *ss* *e*	*scarlet* *spineless* *ebony*	3–44.0 3–58.5 3–70.7	Eye colour: bright red Bristles only a little larger than hairs Body colour: dark

Chornobyl NPP cooling pond population in the fourth generation; its frequency in this generation equalled 0.07% (one male in 1,428). The mutation "brown eyes" was only found in flies originating from the Chornobyl city (apple garden) population in the third and fourth generations with the frequency of 1.6% (25 mutant flies in 1,566) and 0.1% (2 flies in 1,530), respectively. The mutation "spread wings" was only found in flies from Kyiv in the third, fourth, and fifth generations. The mutant frequencies were for F3 – 2.27% (31 flies in 1,363), F4 – 2.2% (23 flies in 1,026), and F5 – 1.0% (6 flies in 595) (see Table 2). Complementation tests revealed that the mutation "white eyes" was allelic to the gene white (*w*: 1–1.5) and thus was sex-linked, the "brown eyes" was allelic to the gene sepia (*se*: 3–26.0) and thus was located in the chromosome 3. To identify the chromosomic position of the mutation "spread wings", we used a strain with marked chromosomes (see Table 1). This mutation was also found to localize in the chromosome 3. According to recombination tests, the mutation "spread wings" mapped on the genetic map between the genes *spineless* (*ss*: 3–58.5) and *ebony* (*e*: 3–70.0) with the recombination frequencies 7.5% and 5.2%, respectively, which allowed us to identify this allele as one of the *spread* (*sprd*: 3–65.0) gene.

The revealed visible mutation spectrum somewhat differed from that observed in 2005–2008 [10, 11]. For instance, the mutations *w* and *sprd* are reported for the first time. Meanwhile, the mutation *abnormal abdomen*, which had previously been typical for all populations, was not found in 2009, as were a number of other mutations. The mutation *se*, on the other hand, had persisted through both periods of study.

Table 2. Phenotypic deviation spectra and frequencies in the studied *D. melanogaster* populations

Population		F_1		F_2		F_3		F4		F5	
		total	mutants	total	mutants	total	mutants	total	mutants	total	Mutants
Chernobyl City	♀	996	0	1173	17	796	17*	797	2*	844	0
	♂	936	0	1112	14	770	9*	733	0	761	0
Kyiv	♀	593	0	535	0	701	16**	534	10**	388	3**
	♂	560	0	495	0	662	15**	492	13**	251	3**
Uman'	♀			684	0	495	0	529	0	523	0
	♂			621	0	458	0	518	0	500	0
Cooling pond	♀	830	0	716	0	739	0	878	0	845	0
	♂	790	0	710	0	689	1***	804	0	853	0
Yalta (Magarach)	♀	685	0	693	0	575	0	715	0	580	0
	♂	625	0	680	0	545	0	706	0	567	0
Odesa	♀			284	0	155	0	221	0	209	0
	♂			270	0	161	0	221	0	214	0
Varva	♀			523	0	460	0	370	0	462	0
	♂			487	0	416	0	362	0	436	0

– sepia; **– spread; *– white*

Therefore, certain changes in the visible mutation spectra can be traced in the studied populations of *D. melanogaster*.

Molecular nature of the mutations

We analyzed the molecular nature of two out of the three revealed mutations. For the *sepia* mutation, we designed three primer pairs with overlapping PCR amplicons: two pairs for the coding part of the gene (3L:8,513,652..8,514,596) and one for the regulatory region –350+46. BLASTing of the amplicon sequences didn't reveal any changes, suggesting that the mutation had happed in some other (distal) regulatory parts of the gene.

The gene *white* in *D. melanogaster* is encoded by a 5 kb (X:2,684,632..2,690,499) region. We approached the analysis of this sequence from a functional standpoint. Of the 1,779 known alleles, w^1 has spontaneous origins and has been obtained independently at least 635 times [15]. So first we designed primers for the distal part of the coding sequence of the gene, as this allele deviation is known to be caused by MGE insertions in the region X:2,690,518..2,690,530. No changes in the resulting amplicon sequence were found. Further we turned our attention to yet another allele with known 14,448 spontaneous occurrences – the allele w^{67c23}. This allele results from the large deletion $Df(1)w^{67c23}$ with breakpoints in 3C2;3C5 on the cytologic map. Using the Coordinates Converter [15], the deleted sequence was approximately identified, and the primer pair was designed so that no PCR product formed in the presence of this deletion. Nonetheless, we did obtain the PCR product. Sequence analysis of this product revealed three nucleotide substitutions (G-A, C-A, and T-A at different sites). All the three substitutions were silent (did not alter the amino acids) and could not have been the cause of the deviation. Thus, we didn't find any mutations in the analyzed parts of the gene, and this topic requires further investigation.

We were unable to study the molecular nature of the changes in the *sprd* gene, as this gene has yet not been mapped on the physical map [15] and its sequence is unknown. Further research is necessary to clarify this question as well.

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ХАРАКТЕРИСТИКА СПОНТАННОГО МУТАЦИОННОГО ПРОЦЕССА В ПРИРОДНЫХ ПОПУЛЯЦИЯХ *DROSOPHILA MELANOGASTER* УКРАИНЫ

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Проанализировано выход мутаций в пяти поколениях лабораторного разведения самок (изосамковых линий) из 7 природных популяций *Drosophila melanogaster* Украины 2009 года сбора. Мутантный самец *white* (*w*: 1–1,5) был найден в третьем поколении потомков популяции Водойом охладитель ЧАЭС. Мутация *sepia* (*se*: 3–26.0) наблюдалась у 27 особей в третьем и четвертом поколениях потомков самок природной популяции г. Чернобыль. Мутацией *spread (sprd:* 3–65,0) характеризовались 54 особи в третьем и четвертом поколениях имбридинга самок популяции г. Киева. Проведенный молекулярно биологический анализ не позволил установить природу полученных мутаций.

Ключевые слова: мониторинг, мутации, ***white, sepia, spread.***

ХАРАКТЕРИСТИКА СПОНТАННОГО МУТАЦІЙНОГО ПРОЦЕСУ В ПРИРОДНИХ ПОПУЛЯЦІЯХ *DROSOPHILA MELANOGASTER* УКРАЇНИ

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Проаналізовано вихід мутацій у п'яти поколіннях лабораторного розведення самок (ізосамкових ліній) із 7 природних популяцій *Drosophila melanogaster* України 2009 року збору. Мутантного самця *white* (*w*: 1–1,5) було знайдено у третьому поколінні нащадків популяції Водойми охолоджувача ЧАЕС. Мутацію *sepia* (*se*: 3–26.0) спостерігали у 27 особин у третьому та четвертому поколінні нащадків самок природної популяції м. Чорнобиль. Мутацію *spread (sprd:* 3–65,0) знайдено у 54 особини третього та четвертого покоління інбридингу самок популяції м. Києва. Проведений молекулярно-біологічний аналіз не дозволив встановити природу отриманих мутацій.

Ключові слова: моніторинг, мутації, ***white, sepia, spread.***