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GENERAL GENETICS

Molecular Characteristic of Stable and Unstable *white* Gene Alleles in Highly Mutable Lines from Natural *Drosophila melanogaster* Populations

M. A. Voloshina[a, b], Yu. A. Koromyslov[a], O. V. Vaulin[a], and I. K. Zakharov[a, b, *]

[a] *Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, Novosibirsk, 630090 Russia*

[b] *Novosibirsk National Research State University, Novosibirsk, 630090 Russia*

**e-mail: zakharov@bionet.nsc.ru*

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Abstract—Mutations in the *white* locus emerged in highly mutable isofemale *Drosophila melanogaster* lines from the populations of Novosibirsk 2013 (NS3 line), Nalchik 2014 (N119 line), and Sakhalin Island 2014 (S46 line). A single white-eyed male found in the NS3 line was sterile. Phenotypically mutant derivatives (*white* gene alleles) differing in eye color (pure white, different shades of yellow (honey), orange (apricot), cherry, and red (wild type)) emerged during the N119 and S46 line breeding in the laboratory. Molecular genetic study of the structure of wild type *white* locus in initial lines and *white*-mutant derivatives *de novo* emerging from them, as well as other *white* lines from the fund of the Laboratory of Population Genetics of the Institute of Cytology and Genetics (Siberian Branch, Russian Academy of Sciences), was conducted. The pairs of primers flanking different *white* gene regions were selected. Six such pairs overlapped the coding part of the gene. Molecular genetic analysis demonstrated that most DNA defects were limited to the region which includes the first exon (34 lines). Among them, four mutant events were accompanied by an insertion of DNA fragments of approximately 800 bp; one mutation event was accompanied by a deletion of approximately 200 bp; in 29 cases, no PCR product was obtained (this can indicate that as a minimum one of the primer binding sites is damaged). The inserted DNA fragments have no homology with known *D. melanogaster* sequences presented in the NCBI database. The complete *white* gene deletion with the manifestation of mutant “white eyes” phenotype was registered in four cases (and only in the N119 line derivatives). Normal PCR product was obtained in 22 cases for all six DNA fragments. Among them, there are both alleles phenotypically mutant by the eye color (white, cherry, or orange) and revertants to the wild type (red). The abundance of defects in the beginning of the gene can indicate a multiplicity of mobile genetic element insertion sites in this part of the *white* gene in *D. melanogaster*.

Keywords: white gene, mutation, genetic instability, *Drosophila melanogaster*

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INTRODUCTION

Genetic monitoring of natural *Drosophila melanogaster* populations on the territory of the Soviet Union, which began in the late 1930s by domestic geneticists [1], made it possible to detect the periods of increased concentration of sex-linked mutations in the *yellow body* [2–9], *singed bristles* [4, 10–12], and *white eyes color* [2–4] genes. We should assign the fashion on “abnormal abdomen” anomaly (observed in natural *Drosophila melanogaster* populations in 1968–1975 [13]) to a special category, since the real reason and molecular genetic mechanisms of a sharp increase in the concentration and ubiquitous distribution of the abnormal abdomen anomaly remained unexplained and unclear. The periods of increased concentration of certain mutations (“fashion” on mutations) were usually accompanied by increased mutability of these genes [2–9, 14, 15]. Several different hypotheses were put forward by researchers as explanations of detected unusual population genetic phenomena. According to some authors, this phenomenon was initially associated with insertion mutagenesis and behavior of hypothetical mobile elements in *Drosophila* [16–21]. Mobile (transposable) genetic elements (ME, MGE or TE) are integral (but still remaining facultative) components of the genomes of all studied organisms. The discovery of MGE in *Drosophila* and subsequent development of methodological tools associated with this make it possible to detect experimentally sequences of different MGE types in the genome of animals and plants [22–26]. It is noteworthy that genetic instability of alleles of the *singed* (in the period of fashion on mutations 1968—1975) and *yellow*

(1982–1991) genes isolated from natural *Drosophila melanogaster* populations was caused by the transposition activity of MGE such as *P* and *hobo* [27–30]. However, highly mutable genes are also found in natural *D. melanogaster* populations outside the periods of the fashion on mutations; for example, highly mutable alleles *singed*, *dusky*, *miniature*, and *yellow-1* were isolated in 1986–1990 [15, 31–33].

The occurrence of mutations in the *white* gene was extremely rare and did not exceed the spontaneous level of emergence for half a century of constant monitoring of natural *D. melanogaster* populations in the Laboratory of Population Genetics of the Institute of Cytology and Genetics (Siberian Branch, Russian Academy of Sciences). The unstable state in the *white* locus in the *D. melanogaster* in laboratory line (the *white-crimson* allele) was for the first time found and described by Melvin Green in the 1960s–1970s [16, 18, 19, 34]. The mutable X^Z chromosome, in which the mutation by the *yellow* and *singed* genes occurred with the frequencies about 10^{-4}, was isolated from a natural *D. melanogaster* population of Zaporozhye in 1986. The *white* locus hypermutability and the presence of a hot spot of chromosome rearrangements in the X chromosome terminal segment were a specific feature of the X^Z chromosome (the mutation frequency reached 10^{-3}) [35]. In 2014, we found and isolated mutations by the *white* gene that were extremely unstable (highly mutable) during the study of natural *D. melanogaster* populations of Nalchik and Sakhalin. Altogether, 64 *D. melanogaster* lines with different origin were studied in this work: three initial wild type lines (the S46 and N119, which gave series of unstable *white* mutations, and the NS3 line, which is unstable with respect to other different X chromosome mutations); the alleles mutant by the *white* gene in the S46 and N119 lines (34 lines) and reversions to the wild type obtained from them (14 lines); and 13 mutant *white* lines of different origin from the fund of the Laboratory of Population Genetics of the Institute of Cytology and Genetics (Siberian Branch, Russian Academy of Sciences).

MATERIALS AND METHODS

Drosophila melanogaster lines. Visible mutations, mutability, and structure of the *D. melanogaster white* locus were analyzed in the lines isolated from geographically distant natural populations for the period of 2010–2016—Novosibirsk oblast, Kabardino-Balkaria (Nalchik), and Sakhalin Island (Tomari and Yuzhno-Sakhalinsk)—as well as the lines from the fund of the Laboratory of Population Genetics of the Institute of Cytology and Genetics (Siberian Branch, Russian Academy of Sciences). The flies caught in nature were viewed under a binocular microscope. We will further call the progeny of one female fertilized in nature with subsequent brotherly–sisterly breeding as isofemale line. Mutant flies identifiable in nature and phenotypically emergent and their revertants to the wild type were founding parents of the line—derivatives formed as a result of consecutive crossings with their sisters/brothers during the breeding in the laboratory. This allows one to preserve the natural genotype and cytotype of the lines, which makes it possible not to involve the gene pool and cytotype of laboratory lines in the crossings. A part of sex-linked mutations multiplied in the crossings of mutant males with females of the laboratory line with linked X chromosomes (C(1)DX,*ywf*/Y, where the male X chromosome with the mutation is inherited patroclinically). The mutation symbolic is given according to accepted nomenclature [36]. Isolated mutations and revertants and mutant alleles obtained from them were analyzed by standard genetic and molecular methods. The mutation mapping was conducted according to the standard schemes [37, 38].

In the laboratory, the flies were kept at 18–24°C on a standard feed for *Drosophila*, which includes sugar, raisin, yeast, and agar-agar.

Molecular methods. Molecular genetic study was conducted for the initial *D. melanogaster* S46 and N119 lines, their *white*-mutant derivatives, revertants by the *white* locus, *white* lines from the fund of the Laboratory of Population Genetics, and the NS13 line from a Novosibirsk population characterized by a high mutability. A single mutant *white* male which emerged in the NS13 line was sterile, although its viability was normal.

DNA was isolated from 3–5 flies by standard methods [39]. Six primer pairs whose PCR products completely overlap the *white* gene region corresponding to mRNA and introns were selected. For the primer selection, the *white* gene sequences presented in the DNA database were used: [EMBL: X02974] for *D. melanogaster* and [EMBL: U64875] for *D. simulans*. The total length of the studied fragment was approximately 6 kb (from the position 7483 to the position 13350 relative to the sequence X02974, excluding primer binding sites). The primer composition, their localization on the sequence [EMBL: X02974], and used annealing temperature are given in Table 1.

The reaction mixture for polymerase chain reaction (PCR) had the following composition: 1× PCR buffer, 4 mM $MgCl_2$, 0.4 mM each dNTP, 1 μM each primer, and 1 unit *Taq* polymerase. The temperature regime for PCR: denaturation at 94°C for 1 min; annealing (the annealing temperature is indicated in Table 1) for 1 min; polymerization at 72°C for 1 min; the polymerization stage in the last cycle continued for 5 min at 72°C. PCR products were separated by electrophoresis in 1% agarose gel stained by ethidium bromide and detected by means of the gel photographing in ultraviolet light.

During the study, changes in the length of the first studied DNA fragment were found in several samples;

Table 1. List of studied *white* DNA regions relative to sequence [EMBL: X02974]

Designations of studied gene region and primer sequences			Annealing temperature, °C	Appropriate structural region of *white* gene	Fragment size. Nucleotide positions*
	forward	reverse			
W1	wf1 5'-TCTTT-CGCCA-CCGTT-TGTAG-3'	wr1 5'-ATACA-AGCCG-AGGTG-CTAAG-3'	54	First exon–first intron	7483–8435
W2	wf2 5'-TAAGT-TGGCC-AACAA-CATTG-3'	wr2 5'-GATGG-CCACA-ATATG-GAAAG-3'	51	First intron	8208–9745
W3	wf3 5'-CTGTC-AAAGC-ATCGC-AGCAG-3'	wr3 5'-AGCCT-GGCTA-ATCCC-TGCTC-3'	57	First intron	9364–10663
W4	wf4 5'-AACAT-GGTGG-AGGAA-CCTTG-3'	wr4 5'-ACACC-GATGA-TCGTG-TGCTG-3'	56	First intron –third exon	10391–11574
W5	wf5 5'-CCGCA-GGGCA-TCCAA-GTATC-3'	wr5 5'-CAGGA-AGAGG-AAGAT-GGCTC-3'	56	Third exon–fifth exon	11344–12554
W6	wf6 5'-CGTAA-AAGTG-CGACT-TATTC-3'	wr6 5'-GGCAA-TAAAC-AGTAA-ACACG-3'	51	Fourth exon–DNA region after 6th exon	12254–13350

* Nucleotide positions are given by the sequence [EMBL: X02974] flanked by primer pair (excluding primer sequence).

Table 2. Cases of emergence of *white* mutations in X chromosome from natural *Drosophila melanogaster* populations

Population, year	Lines studied	X chromosomes studied	Number of lines in which *white* emerged *de novo*	Of them, number of lines, in which *white* is unstable
Novosibirsk, 2013	27	~15000	1*	–
Nalchik, 2010–2013	520	~60000	0	0
Nalchik, 2014	220	~25000	1	1
Sakhalin (Tomari), 2014	109	~20000	4**	1
Sakhalin (Yuzhno-Sakhalinsk), 2015	257	~30000	3	0
Sakhalin (Tomari), 2016	267	~30000	1	0

* A male had normal viability; however, it was sterile.
** In addition to the cases of the emergence of *de novo white* mutations, one female from Tomari population 2014 was heterozygous by the *white* mutation (the allele was genetically stable).

therefore, their sequencing was conducted in order to understand the reasons for such change. The primers selected for PCR of this fragment and additional reverse primer IR2 5'-TCCGC-AATTA-ATAGC-TCCTG-3' were used for sequencing. Sequencing was conducted directly from PCR product both from forward and reverse primers using the resources of the Genomics Center for Collective Use (Siberian Branch, Russian Academy of Sciences) (Novosibirsk, http://sequest.niboch.nsc.ru).

RESULTS

Mutability in white Locus in Natural Drosophila melanogaster Populations in 2010–2016

For more than half a century of the constant study of natural *Drosophila melanogaster* populations, the occurrence of the *white* gene mutations was extremely rare and did not exceed the spontaneous level of emergence. The population genetic data on the estimation of the frequency of occurrence of the *white* mutations in the studied natural *D. melanogaster* populations for the period 2010–2016 are summarized in Table 2.

Among more than 15000 studied X chromosomes of the NS3 line (isolated from Novosibirsk populations 2013), a single yellow-eyed male was found in one family in the progeny of the NS3 line males crossed with the C(1)DX,*ywf*/Y females and with females of "hybrid line with *brown* line," as well as its mutant derivatives. We assume that the *white* gene "*honey*" allele, which had normal viability, emerged; however, it was sterile (the male lived for a month, and although fresh females were regularly seated to it, it did not give any progeny.

In the Nalchik population (regularly studied for two decades), no cases of emergence of alleles of this gene were registered until 2014, when the single (and as it turned out highly mutable) $w^{h\text{-}N119}$ allele was found. A completely different picture was observed in the *D. melanogaster* populations of Sakhalin studied the last three years. The *white* mutation emerged in many lines in them and was even found in the Tomari population (the female heterozygous by the *white* mutation). Probably, this indicates the existence of a high level of total mutability in this island population, since both the concentration of visible mutations and the frequency of their occurrence are also increased for other genes (unpublished data).

N119 Line from Nalchik 2014

Among the F_3 descendants of wild type female from the natural Nalchik population 2014, one male mosaic by the eye color was found: the left eye was honey-colored (slightly yellowish shade weakly different from pure white), while a spot with cherry color against the background of honey-colored facets was observed for the right eye. A single mosaic male was found among 1800 progeny males (that is, N119-X chromosomes), which is convincing evidence that the mutation emerged *de novo*.

The fact that we analyzed Nalchik population should be also noted here; several tens of thousands of males were annually viewed; however, no mutations in the *white* locus were found (Table 2).

Mutant $w^{h\text{-}N119}$ flies had white eyes with barely perceptible yellowness (honey). The allele was designated $w^{h\text{-}N119}$. The male that newly emerged was crossed with wild type females from the same line (sisters), as well as with females with linked X chromosomes of the line C(1)DX,*ywf*/Y. As a result, respectively, two lines were obtained: the $w^{h\text{-}N119}$-X chromosome in natural cytotype and the $w^{h\text{-}N119}$-X chromosome in the laboratory C(1)DX,*ywf* line cytotype. The $w^{h\text{-}N119}$ was highly mutable in both lines. The exceptional male descendants that had pure white, orange, cherry, or normal eye color appeared during the line reproduction. We emphasize that manifestation of instability detected during the crossing with the laboratory lines is frequently considered as a consequence of hybrid dysgenesis. However, this explanation in this case is not consistent with the facts, since the unstable state of the

alleles was also manifested and persisted in the initial lines that were never crossed with the laboratory lines. On the basis of this, it can be argued that we deal not with an artifact induced by the crossing with the laboratory lines, but observe true processes that occur in natural populations. The important property of initial *white*$^{h\text{-}N119}$ allele (to be unstable on a natural gene and cytoplasmic background of initial isofemale line) should be underlined here. The frequency of the w^{N119} allele mutation in different derivatives was high and varied in a wide range (from 1 to 30%).

The frequency of the *white*$^{h\text{-}N119}$ allele mutation observed in the crossings with C(1)DX,*ywf*/Y females reached 30% in individual derivatives and was on average 9%. However, it should be noted that the trend toward a decrease in the mutation frequency was observed for all lines with time.

We note one more important peculiarity of the *white*$^{h\text{-}N119}$ allele. It was unstable in both generative and somatic cells: flies with the eyes mosaic in color emerged in the lines. The frequency of their detection varies in different derivatives and is on average about 10^{-2}.

Mutation Properties in white Locus of Mutant Derivatives of N119-X Chromosome

Many mutants with different eye color were obtained from the *white-N119* (Table 3); they can be divided into three groups by the property of instability.

Group 1: mutants that for a long time preserved instability at the level 2%; initial allele on a natural gene background and mutants with the eye color of different shades of orange (apricot) and yellow (honey).

Group 2: initially unstable mutants, but losing this property several generations later. Some initial allele derivatives, as well as a part of revertants to the wild type and mutants with pure white eyes, behaved so.

Group 3: stable alleles. They include most of the revertants to the wide type and mutated to the alleles with cherry and pure white eyes.

Transposition of white Gene in Chromosome 3 in N119 Line

The detected *white* gene transposition in the chromosome 3 also indicates a high mutational (recombination) activity in the line N119. The mutant male with cherry eye color designated as *white-ch2* (emerging in F1 from the initial w^{N119} male) was crossed with females with linked X chromosomes (C(1)DX,*ywf*/Y). A single female with orange eyes (apricot) was found among its white-eyed daughters (also C(1)DX,*ywf*/Y). A part of the daughters in the progeny of this exceptional female crossed with its brother with cherry eye color also had orange eyes. When crossing the female with orange eyes (with linked X chromosomes, C(1)DX,*ywf*/Y) with the male with stable *white* allele from the fund (line $w^{-[1-80]}$), one half of females and males F_1 had white eyes, while the other half had orange eyes; this made it possible to conclude that the "orange eye" gene in this line is in the autosome.

Since the mapping of the w^{ch2} gene transposition was conducted according to a standard scheme of dominant markers [37], we do not give a detailed scheme of crosses in this work. As a result, it was established that the w^{ch2} gene transposition occurred in chromosome 3. The flies homozygous by the transposition (Tn::w^{ch2}) survive and have intense orange eye color.

We note that the *rudimentary* mutation (truncated circumcised wings) (*r*: 1 – 54.5) emerged in the same hybrid line in the X chromosome obtained from the laboratory line $w^{-[1-80]}$) of the fund of the Laboratory of Population Genetics of the Institute of Cytology and Genetics (Siberian Branch, Russian Academy of Sciences) and remained stable before that.

Wide Spectrum of Visible Mutations in N119-X Chromosome

In addition to highly mutable state in the *white* locus, several cases of the emergence of mutations in the N119-X chromosome were also found in other loci for the study period. Six independent cases of the occurrence of the *yellow* mutation were found in the N119-X chromosome. Only one of the emerging alleles was unstable. We note a characteristic feature of three occurring mutational events. Simultaneously with the emergence of *yellow* mutation in the *white*$^{-[h\text{-}N119\text{-}05\text{-}(+)5]}$ line derivatives, the second mutation affecting dorsocentral (dc) bristles (dc3 and dc4 are reduced, dc1 and dc2 are thin and decreased) emerged in two cases, while the absence (or strong degree of reduction) of scutellaria bristles mutation emerged in one case (since no genetic identification was conducted, probably these are the alleles of the *scute* gene (*sc*: 1 – 0.0) located near the *yellow*). The emergence of the second mutation ("decreased muddy wings," *dusky*) was registered in the *white*$^{-[h\text{-}N119\text{-}8\text{-}1]}$ line (light orange eyes) in three independent cases. In both cases, the *dusky* locus was stable, while the *white* locus continued to maintain a high mutability. A single male with singed bristles (*singed-strong* mutation (*sn*: 1–21.0), the allele was designated as sn^{s18}) was found in the *white*$^{-[h\text{-}N119\text{-}1]}$ line. The mutant male was crossed with the C(1)DX,*ywf*/Y females, and 30 descendant males were obtained in F_1; unfortunately, all of them were sterile! Two emerging visible mutations—(1) "truncated wings, thickened veins, longitudinal L2 vein interrupted at the wing edge" and (2) "non-straightened wings"—were unidentified.

Table 3. List of studied lines, genetic properties of alleles, and molecular characteristic of *white* gene PCR products

Group	*white* allele[1]*	Method of line conducting[2]*	Mutational properties (germ cells/somatic cells) of *white* locus[3]*	*white* gene regions[4]*						Note
				W1	W2	W3	W4	W5	W6	
Nalchik 119	w^{+N119}	♀, ♂	Stable	+	+	+	+	+	+	Initial *white*$^{+N119}$ wild type line
	$w^{-h\text{-}N119}$	♀, ♂	Unstable ($10^{-2}/10^{-2}$)	–	+	+	+	+	+	PCR products of three families were studied; similar results were obtained
	$w^{-h\text{-}N119}$	♀C(1)DX,*ywf*/Y	Unstable ($10^{-2}/10^{-2}$)	–	+	+	+	+	+	PCR products of four families were studied; similar results were obtained
	$w^{+[h\text{-}N119\text{-}1(+)]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	+	+	
	$w^{+[h\text{-}N119\text{-}3(+)]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	?	+	
	$w^{+[h\text{-}N119\text{-}14\text{-}9(+)]}$	♀C(1)DX,*ywf*/Y	Stable	–	+	+	+	+	+	
	$w^{+[h\text{-}N119\text{-}2\text{-}6(+)]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	+	+	
	$w^{+[h\text{-}N119\text{-}3\text{-}1(+)]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	+	+	
	$w^{+[h\text{-}N119\text{-}4\text{-}7(+)]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	+	+	
	$w^{+[h\text{-}N119\text{-}8\text{-}1(a)\text{-}1(dy)\text{-}1(+)]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	+	+	
	$w^{+[h\text{-}N119\text{-}8\text{-}1(a)\text{-}9\text{-}2(+)]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	+	+	
	$w^{+[h\text{-}N119\text{-}16\text{-}1a\text{-}8(+)]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	+	+	
	$w^{-[h\text{-}N119\text{-}1(w)]}$	♀C(1)DX,*ywf*/Y	Unstable ($10^{-3}/0$)	+	+	+	+	+	+	
	$w^{-[h\text{-}N119\text{-}3\text{-}5(w)]}$	♀C(1)DX,*ywf*/Y	Stable	–	–	–	–	–	–	
	$w^{-[h\text{-}N119\text{-}2\text{-}4(w)]}$	♀C(1)DX,*ywf*/Y	Stable	–	–	–	–	–	–	
	$w^{-[h\text{-}N119\text{-}1(a)\text{-}9\text{-}1(w)]}$	♀C(1)DX,*ywf*/Y	Stable	–	–	?	+	+	+	
	$w^{-[h\text{-}N119\text{-}5\text{-}3(w)]}$	♀C(1)DX,*ywf*/Y	Stable	–	–	–	–	–	–	
	$w^{-[h\text{-}N119\text{-}2\text{-}2a\text{-}6(w)]}$	♀C(1)DX,*ywf*/Y	Stable	–	–	–	–	–	–	
	$w^{a[h\text{-}N119\text{-}1(w)\text{-}1(a)]}$	♀C(1)DX,*ywf*/Y	Stable	–	+	+	+	+	+	
	$w^{a[h\text{-}N119\text{-}8\text{-}1(a)\text{-}9]}$	♀C(1)DX,*ywf*/Y	Unstable ($10^{-1}/0$)	+	+	+	+	+	+	
	$w^{ch[h\text{-}N119\text{-}2(ch)]}$	♀C(1)DX,*ywf*/Y	Stable	–	+	+	+	+	+	PCR products of two families were studied, similar results were obtained. *white* gene transposition in chromosome 3 occurred in line

Table 3. (Contd.)

Group	*white* allele[1]*	Method of line conducting[2]*	Mutational properties (germ cells/somatic cells) of *white* locus[3]*	*white* gene regions[4]*						Note
				W1	W2	W3	W4	W5	W6	
	$w^{ch[h\text{-}N119\text{-}8\text{-}1(a)\text{-}6(ch)]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	+	+	
	$w^{ch[h\text{-}N119\text{-}2\text{-}5(ch)]}$	♀C(1)DX,*ywf*/Y	Stable	–	+	+	+	+	+	
	$w^{a[h\text{-}N119\text{-}5\text{-}1(a)]}$	♀C(1)DX,*ywf*/Y	Unstable ($10^{-2}/0$)	Insertion	+	+	+	+	+	Several *yellow* alleles occurred in line
	$w^{a[h\text{-}N119\text{-}8\text{-}1(a)]}$	♀C(1)DX,*ywf*/Y	Unstable ($10^{-1}/10^{-2}$)	–	+	+	+	+	+	*dusky* alleles emerged independently three times in line
	$w^{a[h\text{-}N119\text{-}8\text{-}1(a)\text{-}1(dy)]}$	♀C(1)DX,*ywf*/Y	Unstable ($10^{-1}/10^{-2}$)	–	+	+	+	+	+	
	$w^{a[h\text{-}N119\text{-}1(w)\text{-}3(a)]}$	♀C(1)DX,*ywf*/Y	Stable	Deletion 200 bp	+	+	+	+	+	
	$w^{a[h\text{-}N119\text{-}2\text{-}2(a)]}$	♀C(1)DX,*ywf*/Y	Unstable ($10^{-2}/0$)	–	+	+	+	+	+	
	$w^{a[h\text{-}N119\text{-}2\text{-}3(a)]}$	♀C(1)DX,*ywf*/Y	Stable	–	+	+	+	+	+	
	$w^{a[h\text{-}N119\text{-}16\text{-}1(a)]}$	♀C(1)DX,*ywf*/Y	Stable	Insertion	+	+	+	+	+	
	$w^{a[h\text{-}N119\text{-}16\text{-}1(a)\text{-}7(a)]}$	♀C(1)DX,*ywf*/Y	Stable	Insertion	+	+	+	+	+	
Sakhalin 46	w^{+S46}	♀, ♂	Stable	+	+	+	+	+	+	Initial *white*$^{+S46}$ wild type line
	$w^{-[S46\text{-}1(w)]}$	♀, ♂	Unstable ($10^{-3}/10^{-3}$)	–	+	+	+	+	+	
	$w^{-[S46\text{-}2(w)]}$	♀, ♂	Unstable ($10^{-3}/10^{-3}$)	–	+	+	+	+	+	
	$w^{-[S46\text{-}2\text{-}30(w)]}$	♀, ♂	Unstable ($10^{-3}/10^{-3}$)	–	+	+	+	+	+	
	$w^{-[S46\text{-}2\text{-}31(w)]}$	♀, ♂	Unstable ($10^{-2}/10^{-2}$)	–	+	+	+	+	+	PCR products of two families were studied; similar results were obtained
	$w^{-[S46\text{-}1\text{-}15(w)]}$	♀, ♂	Unstable ($10^{-3}/10^{-3}$)	–	+	+	+	+	+	
	$w^{h[S46\text{-}9(h)]}$	♀, ♂	Unstable ($10^{-3}/10^{-3}$)	–	+	+	+	+	+	Incomplete dominance[5]*
	$w^{h[S46\text{-}1\text{-}12(h)]}$	♀, ♂	Unstable ($10^{-3}/0$)	–	+	+	+	+	+	Incomplete dominance
	$w^{h[S46\text{-}1\text{-}13(h)]}$	♀, ♂	Stable	–	+	+	+	+	+	Incomplete dominance
	$w^{+[S46\text{-}1\text{-}7(+)]}$	♀, ♂	Stable	+	+	+	+	+	+	Incomplete dominance
	$w^{+[S46\text{-}1\text{-}14]}$	♀, ♂	Stable	+	+	+	+	+	+	Incomplete dominance
	$w^{+[S46\text{-}2\text{-}27(+)]}$	♀, ♂	Stable	+	+	+	+	+	+	Complete dominance
	$w^{+[S46\text{-}2\text{-}32]}$	♀, ♂	Stable	–	+	+	+	+	+	Incomplete dominance

Table 3. (Contd.)

Group	*white* allele[1]*	Method of line conducting[2]*	Mutational properties (germ cells/somatic cells) of *white* locus[3]*	*white* gene regions[4]*						Note
				W1	W2	W3	W4	W5	W6	
	$w^{+[S46\text{-}1\text{-}17]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	+	+	
	$w^{ch[S46\text{-}2\text{-}24(ch)]}$	♀, ♂	Stable	–	+	+	+	+	+	Incomplete dominance
	$w^{ch[S46\text{-}2\text{-}21(ch)]}$	♀, ♂	Stable	–	+	+	+	+	+	Incomplete dominance
	$w^{ch[S46\text{-}2\text{-}37(ch)]}$	♀, ♂	Stable	–	+	+	+	+	+	Incomplete dominance
	$w^{ch[S46\text{-}2\text{-}25(ch)]}$	♀, ♂	Stable	–	+	+	+	+	+	Incomplete dominance
	$w^{ch[S46\text{-}2\text{-}29(ch)]}$	♀, ♂	Stable	Insertion	+	+	+	+	+	Incomplete dominance
Novosibirsk	$w^{+[NS3]}$	♀, ♂	Stable	+	+	+	+	+	+	Highly mutable NS3 line
Fund	$w^{-[1\text{-}80]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	+	+	
	$w^{-[1\text{-}80a]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	+	+	
	$w^{-[1\text{-}80b]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	–	+	
	$w^{-[1\text{-}81]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	–	+	+	
	$w^{-[1\text{-}83]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	–	–	
	$w^{-[1\text{-}84]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	+	+	
	$w^{a[1\text{-}90]}$	Muller 5	Stable	?	+	–	–	+	+	
	$w^{-[1\text{-}94]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	+	+	
	$w^{co[1\text{-}97]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	+	–	
	$w^{-[1\text{-}99]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	+	+	
	$w^{-[1\text{-}170]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	+	+	
	$w^{-A35\,[1\text{-}171]}$	♀C(1)DX,*ywf*/Y	Stable	+	–	+	+	+	+	
	$w^{-[1\text{-}177]}$	♀C(1)DX,*ywf*/Y	Stable	+	+	+	+	+	–	

[1]* Designations of the *white* allele phenotype (first sign in superscript): w^+, wild type, dark red eye color; w^-, white eyes; w^a, white-apricot, apricot eye color, different shades of orange; w^{ch}, white-cherry, cherry eye color; w^h, white-honey, honey, different shades of light yellow; w^{co}, white-coral, coral eye color.
[2]* ♀, ♂, brother–sister crossings. ♀C(1)DX,*ywf*/Y, crossings of the line males with the laboratory line females with linked X chromosomes (C(1)DX,*ywf*/Y); Muller-5, laboratory *Basc* line (X chromosome markers: *Bar*, *white-apricot*, *scute*).
[3]* The conclusion about instability is made based on the analysis of at least 200 X chromosomes.
[4]* Designations are the same as in Table 1. The presence (+)/absence (–), ambiguity of the result (?), or peculiarities of PCR product (insertion or deletion) in the appropriate *white* gene parts.
[5]* Incomplete dominance: females homozygous by this *white* mutation allele differ in the eye color from females heterozygous by this mutation and *white*⁻ allele (pure white eyes).

S46 Line Isolated from Sakhalin Island 2014 Population

Two males with white eyes were found among F_2 descendants of a wild type female fertilized in nature from the Sakhalin 2014 *D. melanogaster* population. It is possible that they emerged as a result of single mutational event. They were crossed with their sisters, and two lines ($white^{-[S46-1]}$ and $white^{-[S46-2]}$) were obtained. The mutant flies with yellow (*white-honey*) and cherry (*white-cherry*) eyes emerged during the line reproduction. The frequency of the w^{S46} allele mutation was 0.5% in the initial line (without crossing with the laboratory line).

The instability in both generative and somatic cells (somatic mosaics regularly appear in the line) was a peculiarity of the w^{S46} allele.

If the mutant derivatives of the "honey eye color" phenotype (*white-honey*) obtained from the w^{S46} preserve the increased mutability property (the mutation frequency reaches the values of 1%), the instability property is lost in the emerging mutant derivatives of the "cherry eye color" phenotype (*white-cherry*).

Mutations of Other Loci in S46-X Chromosome

The second (autosomal) mutation (curved wings) was in the initial *white-S46* line. The pure line by the mutation of the wings was designated as *white-S46A*.

In addition to the *white* mutations, the emergence of two more sex-linked mutations (decreased muddy wings (*dusky*) and decreased rough eyes, violation of regularity of facets (*lozenge*) (*lz*: 1 – 27.7)) was registered during the study of mutational properties of S46 line.

Molecular Characteristics of white Locus Alleles

The *white* gene was divided into six overlapping regions beginning from the start surroundings and until the place of transcription termination (the total length of the studied gene region was approximately 6 kb; see Table 1). PCR was carried out for each region and each DNA sample. On the basis of the presence/absence of the appropriate PCR product or its length change, the conclusion about the possible character of events leading to such results was made. The conclusion about the insertion of DNA fragment was made with an increase in PCR product size; the conclusion about the presence of deletion was made with a decrease. The absence of PCR product could indicate different events: the deletion affecting as a minimum one of the primer binding sites or the insertion of a large DNA region in the studied gene region. With the standard size of studied fragments (1–1.5 kb), the insertion of a DNA region with a length more than 2 kb could also block the appropriate fragment production. The summarized results of the study in a complete view are given in Table 3. In a number of cases, PCR product was absent for all *white* gene regions, clearly indicating the presence of an extended deletion which completely (or almost completely) excises the gene. The mutant phenotype in some cases corresponded to normal gene structure, as, for example, for the following lines from the fund: $w^{-[1-80]}$, $w^{-[1-80a]}$, $w^{-[1-80b]}$, $w^{-[1-84]}$, $w^{-[1-94]}$, $w^{-[1-99]}$, $w^{-[1-170]}$ (Table 3). Sequencing of DNA fragments was conducted for six cases in order to clarify the characteristics of the insertion or deletion. Sequences differing in the mobility from "normal" ones were studied by this method. The following regions of the *white* DNA were sequenced: for the allele $w^{[1-81]}$, W5 region; for the alleles $w^{a[h-N119-5-1(a)}$, $w^{a[h-N119-5-3(a)}$, $w^{a[h-N119-1(w)-3(a)]}$, $w^{a[h-N119-16-1(a)]}$, $w^{a[h-N119-16-1(a)-7(a)]}$, and $w^{ch[S46-2-29(ch)]}$, W1 region.

It was detected for the sample $w^{[1-81]}$, whose DNA fragment size was noticeably shorter than normal, that the obtained PCR product is a nonspecific PCR product and refers to the left arm of chromosome 3. No sequencing results were obtained for the sample $w^{a[h-N119-16-1(a)]}$. For remaining four samples, defects occur in similar sites in the first exon. The first exon includes the positions 7460–7757 of the *D. melanogaster* X02974.2 nucleotide sequence. The samples $w^{a[h-N119-5-1(a)]}$ and $w^{a[h-N119-16-1(a)-7(a)]}$ had an insertion with the size of approximately 700–1000 bp in near the position 7496. The sample $w^{ch[S46-2-29(ch)]}$ had an insertion with the size of 200 bp near the position 7683. The sample $w^{a[h-N119-1(w)-3(a)]}$ had a deletion of the region 7496–7683. All gene defects in the samples studied by sequencing included the site with a composition GCAAT, which is probably a recognition site for a hypothetical mobile element. The method of the analysis did not allow us to read completely the sequences of insertions, except for the allele $w^{ch[S46-2-29(ch)]}$ (the insertion with the size of 200 bp). The point is that sequencing can be masked close to those primer, from which the reading is conducted (the region with a length up to 150 bp), and the signal intensity becomes insufficient for sequences located at a distance more than 750 bp from the primer binding site. The sequence reading is complicated by extended blocks of the same nucleotides. As a result, insertions in the *white* gene with a length of approximately 700–1000 bp of the samples $w^{a[h-N119-5-1(a)]}$ and $w^{a[h-N119-16-1(a)-7(a)]}$ were read incompletely, but only by approximately 200 bp.

No high degree of homology was detected between the fragments of insertions and sequences from NCBI DNA database relating both to *D. melanogaster* and to another species. The similarity at the level approximately 88% was registered for the fragment of insertion in the $w^{a[h-N119-16-1(a)-7(a)]}$ for the regions of both chromosome 3 arms. Apparently, this is a random similarity with random regions of the extended genome. The sequences became equal to each other for the short read region of insertions. Their similarity between themselves was detected. The homology for most of the nucleotide sequence was traced for the

$w^{a[h\text{-}N119\text{-}16\text{-}1(a)\text{-}7(a)]}$ (in direct orientation) and $w^{ch[S46\text{-}2\text{-}29]}$ (in reverse orientation). For the insertion in the allele $w^{a[h\text{-}N119\text{-}5\text{-}1(a)]}$ (in reverse orientation), the established similarity with two remaining insertions accounts for the second half of the sequence. The largest part of defects (including the absence of PCR product) falls within the first DNA region.

DISCUSSION

The eye coloration in the *D. melanogaster* is determined by the synthesis and accumulation in the pigment cells of red pigments (drosopterins) that are synthesized from guanidine and brown pigments (ommochromes), whose precursor is tryptophan. The transport of these precursors inside the cell is provided by the products of the *white*, *brown*, and *scarlet* genes belonging to a superfamily of ABC transporters (ATP-binding cassette transporters). According to the existing models, the products of the *white* and *brown* genes interact and produce a guanidine-specific transporter, while those of the *white* and *scarlet* genes produce a tryptophan-specific transporter [40].

The substrate specificity of the transporter is provided by the properties of transmembrane domain [41]. It was detected that the *white* alleles emerging as a result of the point mutations induced by mutagens are associated with amino acid substitutions in different White protein domains; this underlies different violations in the functions of guanine and tryptophan transporters expressed in different eye coloration phenotypes [40].

In the case of insertion mutagenesis, the same locus can be a target for different types of ME, and this property is associated with and depends on the peculiarities of the mechanism of their excision, at which the remaining sequence fragments "mark" the insertion site [42–45]. The insertion of *P*, *I*, *F*, *copia*, *FB*, *Zam*, *Stalker*, *jockey*, or *B104* mobile elements results in phenotypically different *white* alleles [46–49]. This is not a complete list of mobile elements, whose activity is a mutational and recombination factor in the *white* locus.

Molecular genetic analysis of the *white* gene alleles studied in this work demonstrated that most DNA defects were limited to the region which includes the first exon (34 lines). Among them, four mutant events were accompanied by insertions of DNA fragments of approximately 800 bp; one mutation event was accompanied by a deletion of approximately 200 bp; in 29 cases, no PCR product was obtained (this can indicate that as a minimum one of the primer binding sites is damaged).

The obtained intriguing result should be emphasized here: the inserted DNA fragments have no homology with known *D. melanogaster* sequences presented in the NCBI database. A complete *white* gene deletion with the extreme manifestation of the mutant white phenotype (white eyes) was registered in four cases and only in the N119 line derivatives ($w^{-[h\text{-}N119\text{-}3\text{-}5(w)]}$, $w^{-[h\text{-}N119\text{-}5\text{-}3(w)]}$, $w^{-[h\text{-}N119\text{-}2\text{-}2(a)\text{-}6(w)]}$, and $w^{-[h\text{-}N119\text{-}2\text{-}2a\text{-}6(w)]}$) (see Table 3). In 22 cases, normal PCR product was obtained for all six DNA fragments. Among them, there are both phenotypically mutant alleles by the eye color (white, cherry, or orange) and revertants to the wide type (red-brown). The abundance of defects at the beginning of the gene can indicate the multiplicity of preferred insertion sites for nucleotide sequences of unknown origin in this region. We assume that the *white* gene in this case is an indicator of unknown transposon activity in the line N119. Its high activity is also indicated by the emergence of mutations in other X chromosome loci and registered transposition of functionally active *white* gene in the autosome (chromosome 3).

It is known that a sharp difference in manifestation of genetic instability properties in different tissues (including in generative and somatic cells) is typical of some genes [50, 51]. Our data on the instability of the *white* alleles isolated from nature support these observations.

Finally, one more important aspect of the results obtained during this study should be noted. Genetic instability in the studied lines occurred against a natural genetic and cytoplasmic background without the involvement of laboratory lines in the crossings. Thereby, we exclude the factor of hybrid dysgenesis (a kind of creation of artificial conditions for the manifestation of instability). In turn, this allows us to state that natural conditions (periods) for a high level of both locus-specific and general mutability exist in natural populations.

The detected diversity of the *white* alleles obtained *de novo* as a result of the study of unstable *D. melanogaster* lines from nature and their genetic/biological properties makes it possible to complement the extensive list of mutations in the *white* gene, which was started more than a century ago [36, 52–57].

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