UDC: 575.117.2:575.118.9:595.773.4

Variability analysis in *Drosophila melanogaster* locus *white* compound heterozygotes at different genetic backgrounds

N.Ye.Volkova, A.A.Aleynikova, L.I.Vorobyova

V.N.Karazin Kharkiv National University (Kharkiv, Ukraine)
genetics@univer.kharkov.ua

In the paper the characteristics of variability in *Drosophila melanogaster* locus *white* compounds at different degrees of genetic background heterozygosity that were experimentally studied and analyzed are represented. It has been found that the variability of *white* locus compounds at different degree of genetic background heterozygosity is revealed in deviation of the sex ratio in first and second generation offspring, in reduced viability of the second generation, and in the emergence of the irregular offspring of different nature. The frequency of X-aneuploidy obtained here is twice higher that is known for spontaneous levels of aneuploidy for this species, somatic mutagenesis frequency is 0,03% and is near spontaneous level for drosophila; the level of heterozygosity has no effect on the conversion rate in *D. melanogaster white* locus in the region between alleles [1] and [a], but there are more chances for conversion at this site if allele [a] comes from mother.

Key words: *Drosophila, white locus, heterozygosity, variability.*

Аналіз мінливості компаундів за локусом *white Drosophila melanogaster* за умови різного рівня гетерозиготності генетичного фону

Н.Є.Волкова, А.А.Алейникова, Л.І.Воробйова

У роботі експериментально досліджено та проаналізовано особливості мінливості компаундів за локусом *white Drosophila melanogaster* при різному ступені гетерозиготності генетичного фону. Встановлено, що мінливість компаундів по локусу *white* в умовах різного рівня гетерозиготності генетичного фону проявляється у відхиленні співвідношення статей серед нащадків першого та другого поколінь, зниженні показника життєздатності та появі нерегулярного потомства різної природи. Отримана частота анеуплоїдії за Х-хромосомою удвічі вища за відому для спонтанного рівня для даного виду; частота соматичного мутагенезу складає 0,03%, що знаходиться у межах спонтанного рівня для дрозофіли; рівень гетерозиготності не впливає на частоту конверсії гена *white D. melanogaster* на ділянці між алелями [1] та [a], але конверсія більш вірогідна у випадку, якщо алель [a] має материнське походження.

Ключові слова: *Drosophila, локус white, гетерозиготність, мінливість.*

Анализ изменчивости компаундов по локусу *white Drosophila melanogaster* при разном уровне гетерозиготности генетического фона

Н.Е.Волкова, А.А.Алейникова, Л.И.Воробьёва

В ходе работы экспериментально изучены и проанализированы особенности изменчивости компаундов по локусу *white Drosophila melanogaster* при разной степени гетерозиготности генетического фона. Установлено, что изменчивость компаундов по локусу *white* в условиях разной степени гетерозиготности генетического фона проявляется в виде отклонения соотношения полов в потомстве первого и второго поколений, снижения показателя жизнеспособности во втором поколении, появления нерегулярного потомства различной природы. Полученная частота анеуплоидии по Х-хромосоме в два раза чаще известной для спонтанного уровня для данного вида; частота соматического мутагенеза составляет 0,03%, что находится в пределах спонтанного уровня для дрозофилы; уровень гетерозиготности не влияет на частоту конверсии гена *white D. melanogaster* на участке между аллелями [1] и [a], но конверсия более вероятна в случае, если аллель [a] имеет материнское происхождение.

Ключевые слова: *Drosophila, локус white, гетерозиготность, изменчивость.*

Introduction

Heterozygosity factor both for individual loci and for the general genetic background has been actively studied for more than 60 years in the context of developmental stability/instability (the ability of individuals to realize optimal phenotype in the existing genetic microenvironment). The influence of heterozygosity degree on quantitative traits expression is particularly evident when comparing the values of traits in inbred and

hybrid organisms, including in connection with heterosis (overdominance) effect. A large number of papers of Genetics and Cytology Department of V.N.Karazin Kharkiv National University were devoted to the study of the viability and stress resistance of inbred and heterozygous organisms. These studies were conducted on different model and agricultural organisms (plants, Drosophila, silkworms). The higher resistance of heterozygous organisms, especially heterosis hybrids, to the action of high temperature and other factors was showed (Shakhbazov, 2001; Shakhbazov et al., 1971).

For example, in the work of Ye.A.Boyko, L.M.Chepel, S.V.Sukhanov, in the study of some quantitative traits of silkworm Bukhara breed stocks it has been shown that an increase of inbreeding degree up to 10 reduces the weight of the cocoon, the weight of silk shell and pupa weight in this stock compared to those with 5 and 7 degrees of inbreeding. Heterosis effect of cocoon weight, silk shell weight and pupa weight has been also shown in interstock hybrids obtained in offspring of stocks with larger difference in the inbreeding degree (Boyko et al., 2008). Previously, with the participation of the same authors, another research had been conducted. The aim was to study the response of breeds and interbreed hybrids of silkworm to low-intensity electromagnetic irradiation. It has been shown that interbreed silkworm hybrids are characterized by higher resistance to the factor studied comparatively to the parental forms. This was expressed as the absence of significant changes in traits expressivity after irradiation of hybrids eggs, while the parental forms under the same conditions demonstrated significant changes of parameters studied (Boyko et al., 2004).

As to the influence of the genetic background heterozygosity degree on expressivity of mutations in *Drosophila*, in the works of L.I.Vorobyova it has been shown that *eyeless* (*ey*) mutation introduction into the genetic background of wild-type inbred stock Canton-S decreases ey trait expressivity in nearly 10 times (Vorobyova, 1988). But first the dependence of mutations manifestations on the environment was shown with the example of genes *balloon in Drosophila ampelophila*, *curved*, *dachs* in *Drosophila melanogaster* (Marshall, Muller, 1917). In continuation of these studies mutations *radius incompletus*, *notch*, *scute*, and *vestigial* were involved in research (Baklanova-Yelkina et al., 1987; Vasilyeva, Ratner, 2000; Zolotyh et al., 2004, Kirpichenko et al., 2002). In a study of I.Zolotykh and A.Nekrasova the influence of the genetic background on Bar expressivity was established. If exactly, it has been shown that the substitution of the genetic background of an unselected stock carrying the mutation may result in both reduction of the trait expressivity, and in its increase (Zolotyh, Nekrasova, 2004).

In other words, it is believed that heterozygosity has some positive effects on quantitative and morphological characteristics of the organism and contributes to their adaptability, but its loss at individual loci and SNPs may lead to genome destabilization and to the emergence of malignancies (Takeuchi et al., 2003; O'Malley et al., 2007; Clarke et al., 2006; Frank et al., 2007). On the other hand, despite the fact that hybridization between individuals, for example, from different populations can lead to heterosis (overdominance) in the first generation, later the so-called outbreeding depression is often observed due to the destruction of coadapted complexes of genes (Dobzhansky, 1950). Nevertheless, if heterozygosity and/or genomic coadaptation do influence the parameters of developmental stability is still not clear (Parsons, 1990). Available data point out the trend of fluctuating asymmetry increase with inbreeding (Palmer, Strobeck, 1986), interspecific or inter-population hybridization, although some studies have reported about exceptions from this model (Clarke et al., 1992).

The gene *white* of *Drosophila melanogaster* (Yurchenko, Golubovsky, 1988; Gene Dmel\w…) is, to date, one of the best-studied of the species. It encodes a subunit of transmembrane ABC-transporter (ATP-binding cassette), which transports 3-hydroxykynurenine – the precursor of ommochromes eye pigments – and/or some pteridines precursors into the pigment cells of the eye and certain other organs (Malpighian vessels and covers the testes in males) and/or into pigment granules (Tearle, 1991; Sullivan, Sullivan, 1975; Mount, 1987; Dreesen et al., 1988; Tearle et al., 1989) When the gene *w* is not active or is deleted all these structures stay colorless. Homozygotes for different alleles of this gene differ in the degree of pigmentation of the eye – from the complete absence of pigment, to dark red. The set of phenotypically different alleles of gene *w* form a single complementation group. However, the locus is clearly divided according to the functional properties of alleles in two regions (domains). The alleles of the left domain that occupy 2/3 of the genetic map (from w^{bwx} to w^{a}) are characterized by dosage compensation and do not suppress mutation expression. Based on the genetic data it has been suggested that the left part of the locus *white* is its structural part. Alleles of the right domain according to its properties are supposed to be regulating ones, they do not possess dosage compensation effect, are able to inhibit mutation expression and retain ones regulatory properties (Yurchenko, Golubovsky, 1988). Compounds of different alleles of this gene may be a good model for the investigation of various factors effects. Moreover, the orthologs of the gene *white* of

Drosophila are known for a wide range of organisms (Gene Dmel\w…): 12 species of *Drosophila*, 3 species of Diptera that are not related to this genus (*Aedes aegypti*, *Anopheles gambiae*, *Culex quinquefasciatus*), 12 species of insects (not dipterous) – *Apis mellifera*, *Nasonia vitripennis*, *Acromyrmex echinatior*, *Atta cephalotes*, *Camponotus floridanus*, *Harpegnathos saltator*, *Linepithema humile*, *Pogonomyrmex barbatus*, *Acyrthosiphon pisum*, *Bombyx mori*, *Pediculus humanus*, *Tribolium castaneum*, 1 species of arthropods (not insects) – *Daphnia pulex*, 3 species that are not arthropods and are the classic model organisms for genetics and developmental biology – *Caenorhabditis elegans*, *Strongylocentrotus purpuratus* (sea urchin) and *Danio rerio*, and according to some researchers the gene homologous to the *Drosophila* gene *white* is in the human genome also (Savary et al., 1996). Proteins synthesized on these genes perform similar (transport) function. For many of them (as for whole proteins and for individual domains) tertiary structure is known (Gene Dmel\w).

In addition, *Drosophila* ommochromes biosynthesis is one of the ways of tryptophan metabolism and its block at intermediate stages leads to accumulation of intermediate metabolites in the body or to other changes in the tryptophan metabolism pathways. In particular, an excess of kynurenine, and 3-hydroxykynurenine can lead to the accumulation of kynurenic, xanthurenic and anthranilic acid – toxic products of nitrogen metabolism. Similar changes are known to be indicators of a number of pathologies, including age-dependent human conditions: chronic hepatitis, diabetes mellitus, acute leukemia, chronic myeloid and lymphocytic leukemia, Hodgkin's disease, rheumatic fever, sclerodermia and schizophrenia (Oxenkrug, 2011; Erhardt et al., 2007).

All of the above identified the aim of this research: to study the variability of *Drosophila melanogaster* locus *white* compounds at different degrees of genetic background heterozygosity. Tasks to achieve this goal were as follows: to carry out reciprocal crosses between the stocks differing in *white* allele and general genetic background; to analyze the variability of F1 and F2 offspring.

Material and methods

***D. melanogaster* laboratory stocks.** We used 4 outbred stocks from the Collection of drosophila stocks of Genetics and Cytology Department of V.N.Karazin Kharkiv National University that is among objects that constitute Ukraine National Heritage: $w^{1}{}_{C\text{-}S}$, $w^{a}{}_{C\text{-}S}$, $w^{1}{}_{Or}$, $w^{a}{}_{Or}$ containing correspondent *white* alleles in the genetic backgrounds of wild type stocks Canton-S and Oregon-R, respectively. These stocks were obtained by consecutive saturating crossings before the experiment. The phenotypic characteristics of mutant alleles correspond to standard ones (Allele Dmel \ w1; Allele Dmel \ wa)

Research methods. Material for analysis was obtained by hybridization (reciprocal crosses):

♀ $w^{1}{}_{Or}$ × ♂ $w^{a}{}_{C\text{-}S}$ ♀ $w^{a}{}_{Or}$ × ♂ w_{Or} ♀ $w^{1}{}_{C\text{-}S}$ × ♂ $w^{a}{}_{Or}$ ♀ $w^{a}{}_{C\text{-}S}$ × ♂ $w^{1}{}_{C\text{-}S}$

♀ $w^{a}{}_{C\text{-}S}$ × ♂ $w^{1}{}_{Or}$ ♀ $w^{1}{}_{Or}$ × ♂ $w^{a}{}_{Or}$ ♀ $w^{a}{}_{Or}$ × ♂ $w^{1}{}_{C\text{-}S}$ ♀ $w^{1}{}_{C\text{-}S}$ × ♂ $w^{a}{}_{C\text{-}S}$

From each of the reciprocal crosses the offspring of the first and second generations was studied. First generation supposes obtaining females who are compounds in the gene *white* (carry different alleles) with more or less heterozygous background and males who are hemizygous for this gene with the same genetic background.

Viability of individuals was determined by the number of adult descendants of one parental pair (Kaidanov, 1979). For this virgin females and males of each experimental group were placed in test tubes with food in amount of 1 ♀ vs 1 ♂. Oviposition period took 7 days. Then parental animals were removed from the tube. The total number of adult descendants was fixed. For each experimental group 30–40 vials were supplied in parallel to analyze F1 and 100 tubes for F2. For each generation data were averaged inside the experimental group. Parental individuals were selected randomly from the collection stock. It should be noted that some authors use different terms to refer to this trait, such as "emergence of adults" (Zhuravlyova et al., 2004), "fecundity" (Levchuk, Tots'kyy, 1998), etc. In our work we were guided by the definition of viability as the chance for individuals of specific genotype to reach reproductive age. In parallel sex ratio (the total number of males and females in the progeny of each experimental group) was analyzed. Also in the offspring of each cross we fixed exceptional individuals and from the peculiarities of eyes pigmentation manifestations we revealed aneuploidy (white-eyed females in F1 crosses like ♀ w^{1} × ♂ w^{a}), gene conversion (red-eyed individuals in F2), or somatic mutations (mosaic effects in the eye pigmentation).

All results were subjected to statistical analysis. For the index of the viability we calculated the arithmetic mean, its error and standard deviation. The fact of the influence of the genetic heterozygosity background on the viability index was adjusted by analysis of variance for quantitative traits (ANOVA). The proportion of exceptional individuals was determined as a percentage of all offspring of the experimental

group. The frequencies of occurrence of exceptional individuals were compared by Fisher exact test. Experimental sex ratio was compared with the theoretically expected (1 : 1) by Chi square test (McDonald, 2014). Microsoft Excel® and Statistica 6.0® software were used for all calculations.

Results and discussion

Sex ratio analysis in the progeny of different reciprocal crosses (table 1 and 2) has shown that in the half cases (all where maternal individuals came from either w^a_{Or} or $w^1_{C\text{-}S}$ stocks) it is significantly different from the theoretically expected 1 : 1 (**in bold**), and the deviation is in favor of females that, probably, indicates the presence of recessive X-linked lethal mutations in the genomes of stocks mentioned.

Table 1.

The variability of the F1 from crosses of stocks with different *white* alleles and depending on the genetic background *(n – number of families analyzed)*

Cross	Females $x_{av}±m_x$, phenotype	Males $x_{av}±m_x$, phenotype	Sex ratio (♀♀:♂♂), individuals	X^2
♀ w^1_{Or} × ♂ $w^a_{C\text{-}S}$ n=38	21,47±0,20 orange eyes	19,77±0,19 white eyes	816 : 751	2,69
♀ $w^a_{C\text{-}S}$ × ♂ w^1_{Or} n=21	22,37±0,30 orange eyes	24,05±0,36 orange eyes	425 : 481	3,46
♀ w^a_{Or} × ♂ w^1_{Or} n=32	23,47±0,25 orange eyes	23,47±0,27 orange eyes	788 : 704	**4,72**
♀ w^1_{Or} × ♂ w^a_{Or} n=22	25,02±0,22 orange eyes	22,05±0,21 white eyes	501 : 441	3,82
♀ $w^1_{C\text{-}S}$ × ♂ w^a_{Or} n=40	32,94±0,34 orange eyes +5 white eyes 0,22% (aneuploidy)	26,92±0,33 white eyes	1285 : 1050	**23,6**
♀ w^a_{Or} × ♂ $w^1_{C\text{-}S}$ n=40	26,24±0,27 orange eyes	23,24±0,23 orange eyes	997 : 883	**6,9**
♀ $w^a_{C\text{-}S}$ × ♂ $w^1_{C\text{-}S}$ n=30	17,53±0,39 orange eyes	17,36±0,31 orange eyes	491 : 486	0,024
♀ $w^1_{C\text{-}S}$ × ♂ $w^a_{C\text{-}S}$ n=30	20,93±0,23 orange eyes	18,43±0,17 white eyes	628 : 553	**4,82**

The average viability of individuals of different sex from individual families of F1 and F2 varies from 17,36±0,31 to 32,94±0,34 and from 6,24±0,02 to 27,92±0,057, respectively. Despite the great diversity of families in the number of descendants left, the sex of an offspring (according to ANOVA) is not a risk factor for its survival.

It should be noted that in the first generation the largest number of offspring was obtained from the cross ♀ $w^1_{C\text{-}S}$ × ♂ w^a_{Or}, and the least – in the cross ♀ $w^a_{C\text{-}S}$ × ♂ $w^1_{C\text{-}S}$ (fig. 1). So, we can say that higher level of genetic background heterozygosity has positive effect on individuals' viability. ANOVA showed that there is a combined effect of mother's and father's genotype ($F=5,73$, $p<0,01$) on the viability index (the number of offspring surviving to reproductive age).

The progeny of the second generation of individual families in average is less numerous than was in the first one (table 2). According to ANOVA it can be said that the viability of compound heterozygotes depends on allele of paternal origin ($F=77,302$; $p<0,01$), on the level of genetic background heterozygosity ($F=10,47$; $p<0,01$) and on the interaction of these two factors ($F=335,804$; $p<0,01$) (fig. 2). Contrary to the F1 results, the most viable offspring was obtained in crosses where w^1 allele was of paternal origin and was supported by less heterozygous background. Such result supports the idea of coadaptive gene complexes destruction in further than F2 generations.

Maternally derived allele interacts with the genetic background in the same manner in viability control (fig. 3).

Table 2.

The variability of the F2 from crosses of stocks with different *white* alleles and depending on the genetic background (n – number of families analyzed**)**

Cross	Females $x_{av}±m_x$, phenotype		Males $x_{av}±m_x$, phenotype		Sex ratio (♀♀:♂♂), individuals	X^2
♀ w^1_{Or} × ♂ $w^a_{C\text{-}S}$ n=100	15,34±0,057 orange eyes	12,58±0,056 white eyes	13,84±0,056 orange eyes	12,7±0,056 white eyes	2708 : 2575	3,4
♀ $w^a_{C\text{-}S}$ × ♂ w^1_{Or} n=99	19,81±0,085 orange eyes	–	9,727±0,055 orange eyes	7,316±0,038 white eyes	1962 : 1680	**21,8**
♀ w^a_{Or} × ♂ w^1_{Or} n=100	24,88±0,057 orange eyes	–	13,71±0,038 orange eyes	10,12±0,031 white eyes	2463 : 2360	2,2
			+1 red eyes (0,02%) (gene conversion)			
♀ w^1_{Or} × ♂ w^a_{Or} n=100	10,96±0,038 orange eyes	7,861± 0,02 white eyes	9,41±0,03 orange eyes	7,48±0,02 white eyes	1770 : 1588	**9,8**
♀ $w^1_{C\text{-}S}$ × ♂ w^a_{Or} n=95	9,33±0,038 orange eyes	10,85±0,042 white eyes	8,06±0,038 orange eyes	8,67±0,038 white eyes	1906 : 1565	**32,4**
	+1 mosaic (1 eye is white, 1 eye is white with horizontal orange bar, fig. 4)					
♀ w^a_{Or} × ♂ $w^1_{C\text{-}S}$ n=95	7,98±0,05 orange eyes	–	3,01±0,01 orange eyes	3,23±0,02 white eyes	750: 475	**61,6**
	+1 red eyes (0,08%) (gene conversion)					
♀ $w^a_{C\text{-}S}$ × ♂ $w^1_{C\text{-}S}$ n=99	17,2±0,06 orange eyes	–	9,02±0,03 orange eyes	7,79±0,03 white eyes	1686:1640	0,62
♀ $w^1_{C\text{-}S}$ × ♂ $w^a_{C\text{-}S}$ n=99	6,1±0,02 orange eyes	4,34±0,03 white eyes	5,92±0,02 orange eyes	5,29±0,03 white eyes	1021:1054	0,52

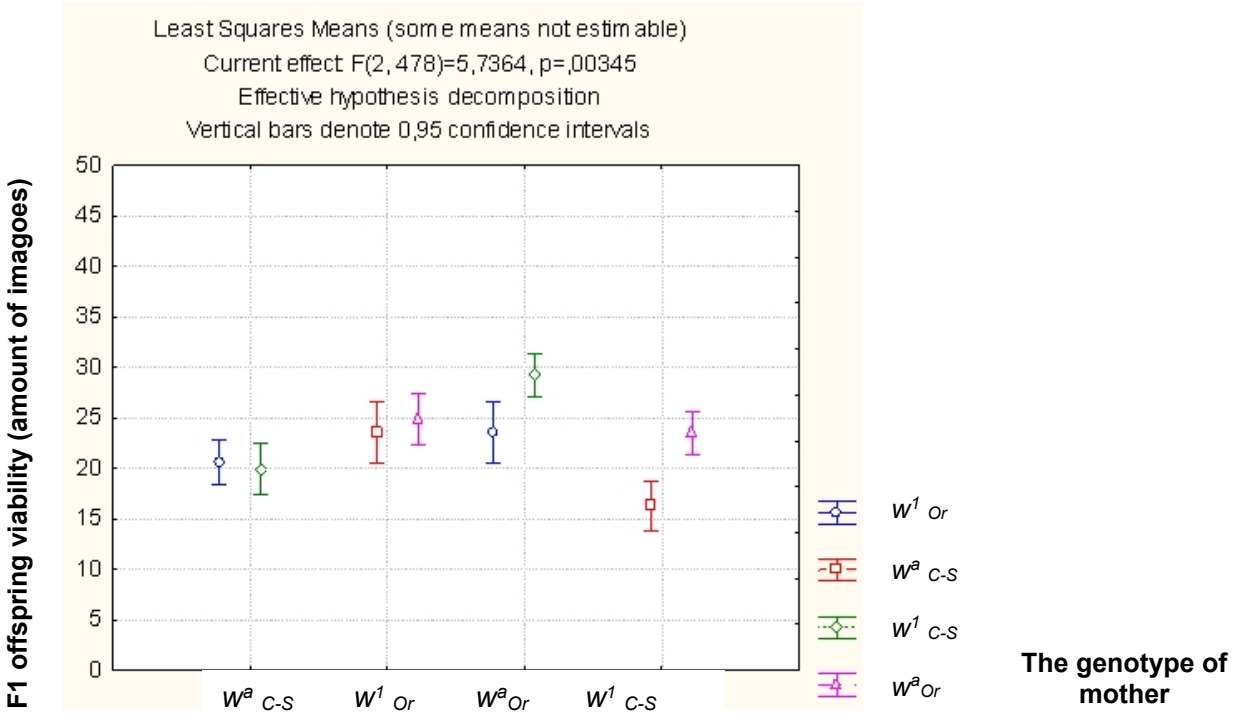


Fig. 1. The average viability of F1 offspring

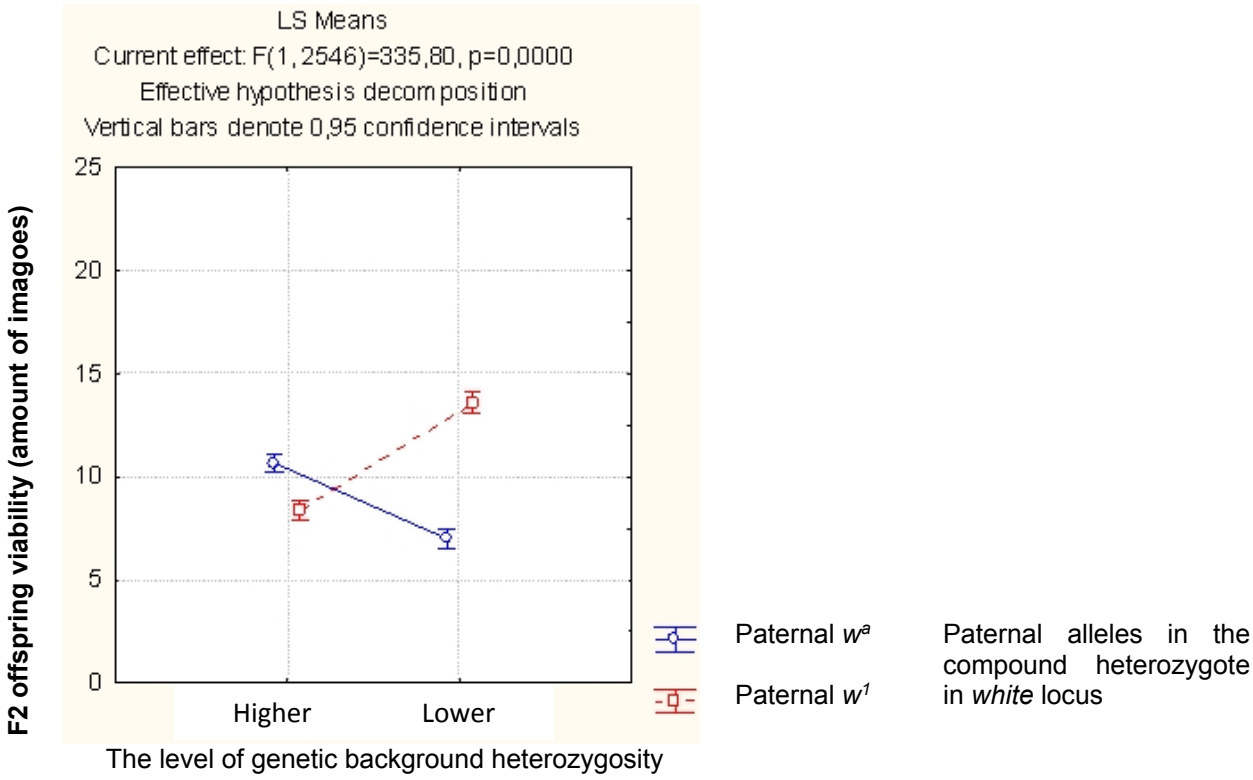


Fig. 2. The influence of paternal origin of *white* allele and the genetic background heterozygosity degree on the viability index in second generation

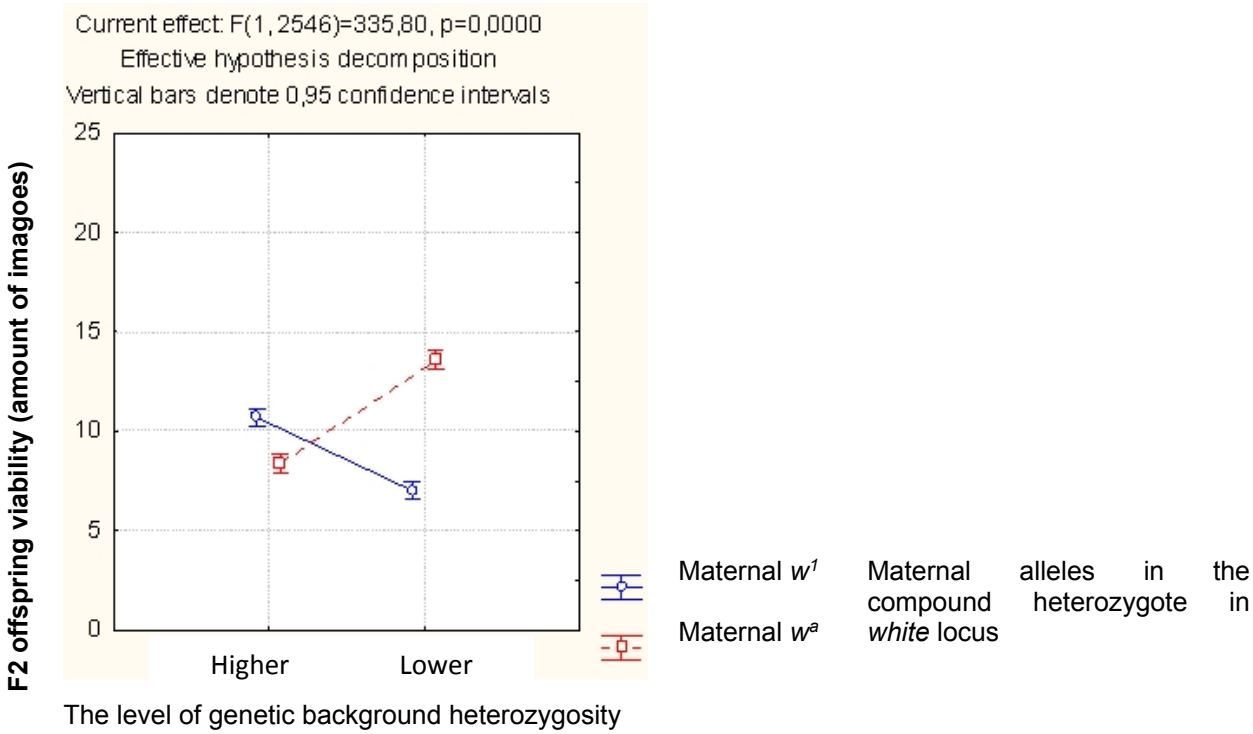
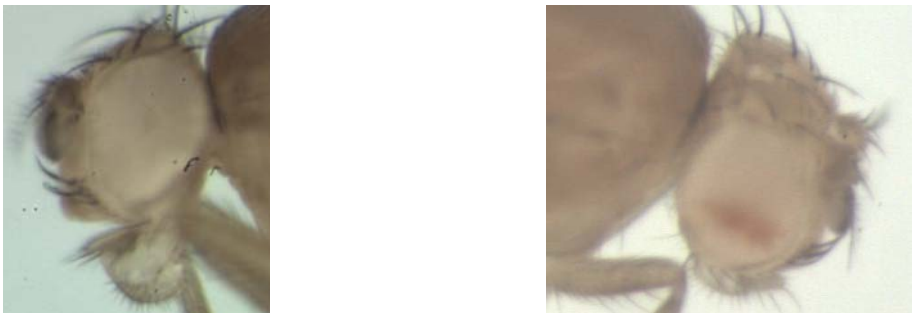


Fig. 3. The influence of maternal origin of *white* allele and the genetic background heterozygosity degree on the viability index in second generation

Analysis of F1 and F2 progeny phenotype showed both regular and irregular individuals for the respective crossings (table 1 and 2). For example, among the F1 offspring from the cross ♀ $w^{1}{}_{c\text{-}s}$ × ♂ $w^{a}{}_{or}$ 5 females (that is 0.22%) with white eyes were detected (they are considered to be aneuploid, and it was confirmed experimentally by their progeny segregation). The frequency of X-aneuploidy obtained here is twice higher that is known for spontaneous levels of aneuploidy for this species (Zeng et al, 2010; Gregg, Day, 1965; Koehler et al., 1996). The phenomenon of non-disjunction of chromosomes, resulting in two X chromosomes appear either in the egg (forming XX gamete), or in the polar body (gamete 0) was discovered by K.Bridges (1916). When eggs XX or 0 are fertilized by X or Y- containing sperms, XXX or XXY females or X0 males will develop, i.e., aneuploid individuals. All of them have normal diploid set of autosomes. The offspring of these individuals most likely will be also aneuploid due to secondary violation of chromosomes segregation in meiosis (Epstein, 2007).

In F2 of the same cross we discovered a mosaics female, whose left eye was white, and the right one was white with a horizontal orange stripe (fig. 4). That is 0.03% and is near spontaneous level of somatic mutagenesis in drosophila (Garcia et al., 2007, 2010). Such a phenotype may result from somatic mutations (including those associated with aneuploidy or with the position variegation effect) in separate precursor cells antennal-eye imaginal disc. Mutations of this type can occur in any cell of a multicellular organism. The expression of the mutant phenotype of somatic mutation will dependent on the stage when it occurs. The earlier such mutation occurs, the more cells will carry it. If somatic mutation arose early in the individual development, then this mutant cell can give rise to a large section of the tissue and all its cells will be mutated. This phenomenon is called a mosaicism. Somatic mutations do not play very important role in heredity (i.e. a new sign appeared as a result of somatic mutation won't be transmitted to offspring cause this mutation is absent in gametes) if the organism reproduces by only sexually and germ-line cells isolate from the somatic ones at early stages of development. But they can cause malignant tumors in humans and animals and therefore reduce viability (Blair, 2003; Frank, 2010).



A. Left eye B. Right eye

Fig. 4. The mosaic phenotype (× 24)

Also, in F2 of crosses ♀ $w^{a}{}_{or}$ × ♂ $w^{1}{}_{or}$ and ♀ $w^{a}{}_{or}$ × ♂ $w^{1}{}_{c\text{-}s}$ we found red-eyed male and female (respectively) that is 0,02% and 0,08% of the offspring, and, apparently, is the result of *white* gene conversion in meiosis of compound F1 females. Note that the two exceptional individuals were detected among the offspring of females that came from $w^{a}{}_{or}$ stock. It should be said, that the frequency of gene conversion obtained is much higher comparatively to the same index known for other loci (Kahn, Sick, 1982).

The gene conversion is a biological process that plays an important role in the evolution of living organisms and their individual development. Apart from the fact that the gene conversion is involved in general (homologous) recombination, it is also one of the manifestations of another fundamental genetic process – DNA reparation. Although the conversion is based on a common mechanism – unpaired (non-complementary) bases correction in DNA recombination heteroduplex, this mechanism may be involved in diverse biological processes, often playing a key role (Rihito et al., 2010; Chen et al., 2007). The process of gene conversion is usually initiated by the formation of hybrid DNA between the two partially complementary strands. As a rule, they usually belong to two double-stranded DNA molecules. Sometimes, as a result of meiosis three copies of maternal allele and only one copy of paternal one arise that indicates the change of one copy of the paternal allele into the maternal one, this phenomenon is called gene conversion. During meiosis joint heteroduplex is formed at crossing over sites between homologous maternal and paternal chromosomes. If these areas are somewhat different violation of their pairing may occur in sites of joining.

These violations will be corrected by DNA reparation system and result in gene conversion (Rihito et al., 2010; Chen et al., 2007). Initially, since it had been discovered by K.Lindegren in 1949, the term "gene conversion" was applicable only to a specific phenomenon – a violation of the standard Mendel segregation in ascosporic tetrads in Ascomycetes. However, after the universal mechanism of unpaired bases correction had been clarified, the term was extended to all the processes when there is a conversion of one allele into another one by recombination heteroduplex correction. Currently, there are two well-known models of recombination leading to conversion: R.Holliday model and J.Zhostak et al. model (Rihito et al., 2010; Chen et al., 2007). According to the hypothesis of A.Carpenter et al. conversion and crossing-over during meiosis are separate processes, differing in time and playing different roles. The activities of conversion and crossover machineries are linked with special complexes in contacts sites of DNA and synaptonemal complex called recombination nodules. They are of two types: early and late. The first ones are related to the conversion, and the second (less frequent) ones – to a crossing-over. The time of their occurrence in meiosis correspond to mentioned processes: conversion takes place in zygotene (the role of the conversion is to verify homology between chromosomes after their temporary synapsis and it is carried out by direct comparison of DNA sequences through the attempt to form a heteroduplex; in the presence of homology the situation is stabilized with the assistance of synaptonemal complex; in the absence of homology chromosomes unsynaps for new attempts of homology search); crossing over events occur in pachytene and lead to the formation of chiasma that are necessary for the correct segregation of homologues in anaphase I (Rihito et al., 2010; Chen et al., 2007).

The frequency of gene conversion obtained does not contradict the data on the structure of the *white* locus (Yurchenko, Golubovsky, 1988; Gene Dmel\w). Cloning and transcription analysis of *white* gene has shown that the fragment of 12 kbp includes all of the known mutations of this locus, and according to recombination analysis the overall gene length corresponds to 0,03 map units. In general, here there is approximate coincidence of the length of the genetic and physical maps, even though in some areas much stronger differences are possible. Locus *white* includes 4 introns (the main one is of 3 kb and three smaller ones) and 5 exons. As a result of processing of the primary transcript of *white* locus the polyadenylated RNA of 2.6 kb is formed, which is only 0,0005% of the total polyadenylated RNA in *Drosophila*. Transcriptional study of the gene confirms the assumption that the *white* locus consists of two parts: the transcribed region and a regulatory complex. The physical length of the transcribed region is 5,8 kb. The regulatory region includes at least 1,8 kb.

The results of our experiments have shown that the level of heterozygosity has no effect on the conversion rate in *D. melanogaster white* locus in the region between alleles [1] and [a], but there are more chances for conversion at this site if allele [a] comes from mother.

There is a growing evidence base of facts that genomic and environmental stressors are capable to induce significant levels of developmental instability that can serve as an early signal for monitoring of population stress effects (Leary et al., 1985). Developmental stability is the ability of the body "to mitigate" environmental and genetic disorders affecting the developmental capabilities of a certain phenotype. Inability to correct occasional violations during the development can manifest itself in the form of fluctuating asymmetry, which is defined as a small deviation from perfect bilateral symmetry of morphological structures (Leary et al., 1985). The genetic basis of developmental stability is actively discussed in the last five decades. Increase or decrease of the level of developmental genomic instability due to stress is explained by two hypotheses. The first argues that the level of stability is a reflection of underlying genomic heterozygosity, and the second argues that it reflects the stability of the overall level of genomic coadaptation (Dobzhansky, 1950).

Coadaptive gene complexes are formed during the evolution history of the genome by natural selection, and defined as a specific balance of loci in the genome. Such coordination in the genome is to protect individuals from the emergence of defects that can be caused both by environmental and genetic factors (Palmer, 1996). Destruction of coadaptation can manifest itself in individuals as the reduced likelihood of optimum phenotype development due to the increase in developmental instability (Leary et al., 1985).

Heterozygosity theory suggests that heterozygosity correlates with the level of developmental instability so that heterozygous individuals are better adapted to the genomic and environmental changes, if compared to the homozygous due to a greater developmental stability. The theory also postulates that a single locus encoded enzymes related to metabolism efficiency influence the developmental stability of various morphometric features (Palmer, Strobeck, 1986), predicting that the level of heterozygosity at loci

encoding functional proteins, will vary inversely with the level of developmental instability (Leary, Allendorf, 1989). However, an important assumption is that heterozygosity for different genetic markers clearly reflects the level of heterozygosity of the entire genome, or at least heterozygous loci that contribute to the formation of morphological phenotype (Marshall, Muller, 1917).

Do heterozygosity and / or genomic coadaptation influence the parameters of developmental stability is still not clear (Parsons, 1990). Available data point out the trend of fluctuating asymmetry increase in inbreeding (Palmer, Strobeck, 1986), interspecific or inter-population hybridization, although some studies have reported exceptions to this model (Clarke et al., 1992).

The consensus seems to have to prove that any stress will increase the phenotypic variance of most quantitative traits. And it is well known among Drosophila species: phenotypic variance of individuals collected "in the field" is higher compared to those bred in vitro (Coyne, Beecham, 1987). Increased phenotypic variability of flies "from nature" is usually associated with higher environmental variance, which leads to a decrease in heritability. It has been suggested that some of the mechanisms may change the phenotypic variance components (The Genome Sequence of *Drosophila melanogaster*, 2000).

Summarizing our results we can say that the variability of *Drosophila melanogaster* locus *white* compound heterozygotes under varying degrees of genetic background heterozygosity manifests itself in deviation of the sex ratio in the offspring of first and second generations, in reduced viability of the second generation individuals, in the emergence of the irregular offspring of different nature. The data obtained partly confirm both theories discussed. The sex ratio shift in F2 is registered both in cases of higher and lower heterozygosity of genetic background, but in the case of higher level it is more pronounced. Aneuploids also were found among offspring with higher level of genetic background heterozygosity. These facts confirm the theory of coadaptive gene complexes destruction. The gene conversion was registered in the cases of both higher and lower level of heterozygosity, but as far as in our experiment we can assume the effect of gene conversion as positive one (eye pigmentation and supporting metabolisms normalization) the result obtained partly confirms the theory of heterozygosity (i.e. among the offspring of the individual who is heterozygous for different mutant alleles there is always a higher chance for normal individual contrary to the individual who is homozygous for the same mutant allele).

Conclusions

In this work we have experimentally studied and analyzed the characteristics of variability of *Drosophila melanogaster* locus *white* compound heterozygotes at different degrees of genetic background heterozygosity. The variability of *Drosophila melanogaster* locus *white* compound heterozygotes under varying degrees of genetic background heterozygosity appears as a deviation of the sex ratio in the offspring of first and second generation, reduced viability of the second generation, and as the emergence of the irregular offspring. The sex ratio in all cases of deviation is shifted in favor of females, indicating that X-linked recessive lethal mutations are present in genotypes of individuals of base stocks. Reduction of viability in the second generation is the result of the genotype acting as a system. Irregular offspring of the first generation is represented by aneuploid individuals; of the second one – by individuals with gene conversion and white mosaics. The incidence of the event depends on the type of the event.

References

Allele Dmel \ w[1]. The report. Drosophila genome database. Electronic resource. http://flybase.org/reports/FBal0018074.html

Allele Dmel \ w[a]. The report. Drosophila genome database. Electronic resource. http://flybase.org/reports/FBal0018195.html

Baklanova-Yelkina Ye.Y., Ivanov V.I., Kaurov B.A., Mglinets V.A. Study of the variability of the manifestation of *Nasobemia* mutation in *Drosophila melanogaster* // Tsitol. Genet. – 1987. – Vol.21, No3. – P. 195–199. (in Russian).

Blair S.S. Genetic mosaic techniques for studying *Drosophila* development // Development. – 2003. – Vol.130. – P. 5065–5072.

Boyko Ye.A., Chepel L.M., Sukhanov S.V. Influence of the degree of inbreeding of parental components of silkworm interline hybrids on heterosis effect manifestation and on reaction to laser radiation // The Journal of V.N.Karazin Kharkiv National University. Series: biology. – 2008. – Iss.8, No 828. – P. 24–29. (in Russian).

Boyko Ye.A., Sukhanov S.V., Shakhbasov V.G. Genetic differences in resistance of silkworm (*Bombyx mori* L.) to electromagnetic field // Materials of The 8-th International Scientific Ecological Conference «Actual problems of the stability of living systems preservation». – Belgorod, 2004. – P. 23–24. (in Russian).
Chen J.-M., Cooper D.N., Chuzhanova N. et al. Gene conversion: mechanisms, evolution and human disease // Nature Reviews Genetics. – 2007. – Vol.8. – P. 762–775.
Clarke C.L., Sandle J., Jones A.A. et al. Mapping loss of heterozygosity in normal human breast cells from BRCA1/2 carriers // British Journal of Cancer. – 2006. – №95. – P. 515–519.
Clarke G.M., Oldroyd B.P., Hunt P. The genetic basis of developmental stability in *Apis mellifera*: Heterozygosity versus genetic balance // Evolution. – 1992. – Vol.46. – P. 753–762.
Coyne J., Beecham E. Heritability of two morphological characters within and among natural populations of *Drosophila melanogaster* // Genetics. – 1987. – Vol.117. – P. 727–737.
Dobzhansky T. Genetics of natural populations. XIX. Origin of heterosis through natural selection in populations of *Drosophila pseudoobscura* // Genetics. – 1950. – Vol.35. – P. 288–302.
Dreesen T.D., Johnson D.H., Henikoff S. The brown protein of *Drosophila melanogaster* is similar to the white protein and to components of active transport complexes // Mol. Cell. Biol. – 1988. – Vol.8. – P. 5206–5215.
Epstein Ch.J. The consequences of chromosome imbalance: principles, mechanisms, and models. – Cambridge: Cambridge University Press, 2007. – 487p.
Erhardt S., Schwieler L., Nilsson L. et al. The kynurenic acid hypothesis of schizophrenia // Physiol. Behav. – 2007. – Vol.92, Iss. 1–2. – P. 203–209.
Frank J., van Geel M., van Steensel M.A.M. Loss of heterozygosity studies on chromosome 12q in disseminated superficial actinic porokeratosis: lessons to be learned // Journal of Investigative Dermatology. – 2007. – №127. – P. 2058–2059.
Frank S.A. Somatic evolutionary genomics: Mutations during development cause highly variable genetic mosaicism with risk of cancer and neurodegeneration // PNAS. – 2010. – Vol.107, suppl. 1. – P. 1725–1730.
Garcia A.M., Calder R.B., Dollé M.E.T. et al. Age- and temperature-dependent somatic mutation accumulation in *Drosophila melanogaster* // PLOS Genetics. – 2010. – Electronic resource. http://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1000950
Garcia A.M., Derventzi A., Busuttil R. et al. A model system for analyzing somatic mutations in *Drosophila melanogaster* // Nature Methods. – 2007. – Vol.4, Iss.5. – P. 401–403.
Gene Dmel \ w. The report. Drosophila genome database. Electronic resource. http://flybase.org/reports/FBgn0003996.html.
Gregg T.G., Day J.W. Nondisjunction of the X chromosomes in females of *Drosophila hydei* // Genetica. – 1965. – Vol.36. – P. 172–182.
Kahn A., Sick K. Gene conversion in the *shibire^{ts}* locus of *Drosophila melanogaster* // Hereditas. – 1982. – Vol.97. – P. 59–63.
Kaidanov L.Z. An analysis of genetic after-effects of selection and inbreeding in *Drosophila melanogaster* // Biology Bulletin Reviews. – 1979. – Vol.40, Iss.6. – P. 834–850. (in Russian).
Kirpichenko T.V., Strashnyuk V.Yu., Vorobjova L.I., Shakhbazov V.G. The effect of the genotype on the expressiveness of character *vestigial* and polyteny of giant chromosomes in *Drosophila melanogaster* Meig. // Russian Journal of Genetics. – 2002. – Vol.38, Iss.12. – P. 1371–1375.
Koehler K.E., Hawley R.S., Sherman S., Hassold T. Recombination and nondisjunction in humans and flies // Hum. Mol. Genet. – 1996. – Vol.5. – P. 1495–1504.
Leary R.F., Allendorf F.W., Knudsen K.L. Developmental instability and high meristic counts in interspecific hybrids of salmonide fishes // Evolution. – 1985. – Vol.39. – P. 1318–1326.
Leary R.F., Allendorf F.W. Fluctuating asymmetry as an indicator of stress: implications for conservation biology // Trends in Ecology and Evolution. – 1989. – Vol.4. – P. 214–217.
Levchuk L.V., Tots'kyy V.M. Chromosome substitution and genotype adaptation in *Drosophila melanogaster* // Tsitol. Genet. – 1998. – Vol.32, Iss.2. – P. 42–48. (in Russian).
Marshall W.W., Muller H.G. The effect of long-continued heterozygosis on a variable character in Drosophila // J. Exp. Zool. – 1917. – N 22. – P. 457–470.
McDonald J.H. Handbook of biological statistics (3rd ed.). – Baltimore, Maryland: Sparky House Publishing, 2014. – 296p.
Mount S.M. Sequence similarity // Nature. – 1987. – Vol.325. – P.487.

O'Malley D.P., Orazi A., Dunphy C.H. et al. Loss of heterozygosity identifies genetic changes in chronic myeloid disorders, including myeloproliferative disorders, myelodysplastic syndromes and chronic myelomonocytic leukemia // Modern Pathology. – 2007. – №20. – P. 1166–1171.
Oxenkrug G.F. Interferon-gamma-inducible kynurenines / pteridines inflammation cascade: implications for aging and aging-associated psychiatric and medical disorders // J. Neural Transm. – 2011. – Vol.118, Iss.1. – P. 75–85.
Palmer A.R. Waltzing with asymmetry: is fluctuating asymmetry a powerful new tool for biologists or just an alluring new dance step? // BioScience. – 1996. – Vol.46. – P. 518–532.
Palmer A.R., Strobeck C. Fluctuating asymmetry: measurement, analysis, patterns // Annual Review of Ecology and Systematics. – 1986. – Vol.17. – P. 391–421.
Parsons P.A. Fluctuating asymmetry: an epigenetic measure of stress // Biological Review. – 1990. – Vol.65. – P. 131–145.
Rihito M., Shuhei N., Atsuhiro Sh. et al. Molecular mechanisms of the whole DNA repair system: a comparison of bacterial and eukaryotic systems // Journal of Nucleic Acids. – 2010. – Article ID 179594, 32p. http://www.hindawi.com/journals/jna/2010/179594/
Savary S., Denizot F., Luciani M.-F. et al. Molecular cloning of a mammalian ABC transporter homologous to *Drosophila white* gene // Mammalian Genome. – 1996. – №7. – P. 673–676.
Shakhbazov V.G. Ecological and biophysical genetics. – Kh.: Shtrikh, 2001. – 436p. (in Russian)
Shakhbazov V.G., Shereshevska C.M., Shestopalova N.G. About heat resistance and reparation ability of heterosis plants // Questions of genetics, breeding and heterosis in animals. – K: Naukova Dumka, 1971. – 239p. (in Russian).
Sullivan D.T., Sullivan M.C. Transport defects as the physiological basis for eye color mutants of *Drosophila melanogaster* // Biochem. Genet. – 1975. – Vol.13. – P. 603–613.
Takeuchi S., Tsukasaki K., Bartram C.R. et al. Long-term study of the clinical significance of loss of heterozygosity in childhood acute lymphoblastic leukemia // Leukemia. – 2003. – №17. – P. 149–154.
Tearle R.G., Belote J.M., McKeown M. et al. Cloning and characterization of the *scarlet* gene of *Drosophila melanogaster* // Genetics. – 1989. – Vol.122. – P. 595–606.
Tearle R. Tissue specific effects of ommochrome pathway mutations in *Drosophila melanogaster* // Genetical Research. – 1991. – Vol.57. – P. 257–266.
The Genome Sequence of *Drosophila melanogaster* // Science. – 2000. – Vol.24, Is.287, No 5461. – P. 2185–2195.
Vasilyeva L.A., Ratner V.A. Effect of genotypic environment on the phenotypic expression of mutation *radius incompletes* in *Drosophila melanogaster* // Russian Journal of Genetics. – 2000. – Vol.36, Iss.2. – P. 126–137.
Vorobyova L.I. The dependence of the heterosis effect on the level of baselines heterozygosity. Thesis, PhD: 03.00.15 / Kharkov State University – Kh., 1988. – 17p. (in Russian).
Yurchenko N.N., Golubovsky M.D. Contemporary genetics of the white locus of *Drosophila melanogaster* // Russian Journal of Genetics. – 1988. – Vol.24, Iss.4. – P. 581–591.
Zeng Y., Li H., Schweppe N.M. et al. Statistical analysis of nondisjunction assays in *Drosophila* // Genetics. – 2010. – Vol.186. – P. 505–513.
Zhuravlyova L., Strashnyuk V., Shakhbazov V. Influence of culture density on heterosis effect in *Drosophila melanogaster* // Visnyk of L'viv Univ. Biology Series. – 2004. – Iss.5. – P. 102–109. (in Ukrainian).
Zolotyh I., Nekrasova A. The role of genotype and aging in changing of expressivity of gene *Bar Drosophila melanogaster* // Visnyk of L'viv Univ. Biology Series. – 2004. – Iss.38. – P. 88–91. (in Ukrainian).
Zolotyh I., Nekrasova A., Shakhbazov V. The role of genotype system and some visible mutations in definition of life-span of *Drosophila melanogaster* // Visnyk of L'viv Univ. Biology Series. – 2004. – Iss.35. – P. 115–120. (in Ukrainian).

Представлено: **Т.В.Тижненко / Presented by: T.V.Tyzhnenko**
Рецензент: В.В.Навроцька / Reviewer: V.V.Navrotskaya
Подано до редакції / *Received: 06.05.2015*