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ESTIMATION OF THE GENETIC ACTIVITY OF HEAVY METALS SALTS ON CHANGING OF RECOMBINATION PARAMETERS IN DROSOPHILA

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The effect of various concentrations of heavy metals salts (lead and mercury nitrates) on recombination parameters in drosophila is studied. It was demonstrated that these compounds have genetic activity and increase the crossing-over frequency in zone *y-v* of chromosome 1 (X) of drosophila, reduce its variability, as well as induce a double crossing-over.

Keywords: Drosophila, genetic activity, heavy metals, lead and mercury nitrate, recombination parameters, crossing-over frequency.

Environmental pollution is one of the global ecological problems of modern times [3]. Among the most common and dangerous pollutants are heavy metals and their compounds [8]. Most toxic of them are mercury and lead, released in large quantities in the environment with waste production of ferrous and non-ferrous metallurgy, machinery manufacturing as well as the work of road transport [13]. Biological activity of mercury and lead compounds on human body is thoroughly studied. Its main manifestations are structural and functional changes in nervous system, metabolic disorders, increase in the frequency of malformations [17]. In the meantime, the issues of genetic activity of heavy metals and their compounds have been underexplored. There are only a few reports of their mutagenic activity, discovered when studying the frequency of chromosomal rearrangement in individuals living in areas contaminated by heavy metals [5, 19]. However, it is the genetic activity that is the most important indicator in assessing long-term effects of pollution [6]. Traditionally, by genetic activity of the factor we understand its impact on the mutations frequency. At the same time, the indicator of genetic activity of the experimental factor is not only a mutagenic, but also a recombinogenic activity [2, 11, 12]. Moreover, in recent years there is a tendency to believe that the decisive role in the formation of genotypic variability of higher organisms does not belong to mutations, but to meiotic recombination [4, 9, 15]. Since the level of recombination variability, emerged in the course of evolutionary process appears to be optimal, any significant deviation of it (upwards as well as downwards) can be considered undesirable [10]. In general, the study of the influence of anthropogenic pollutants on the recombination indicators is essential to forecast long-term effects of ecological degradation of the biosphere.

The aim of this research was to evaluate the genetic activity of the most common and dangerous heavy metals salts – lead and mercury nitrates – on changing of recombination parameters in drosophila. In this research we studied the effect of these compounds on the crossing-over frequency in drosophila chromosome 1, its variability, as well as the interference of crossing-over exchanges. While interpreting the results we used perceptions of the recombination role in the adaptation of organisms to changing environmental conditions.

Materials and methods

In the research we used two laboratory lines *Drosophila melanogaster* from genetic collection of the Department of Zoology and Genetics of Brest State University named after A.S. Pushkin:

1) *y, cut, v* – mutant line carrying three recessive linked genes in chromosome 1 (X): *y (yellow)* – yellow body, locus 0; *cut* – cut wings, locus 20,0; *v (vermillion)* – bright red eyes, locus 33,0;

2) *Berlin* – wild-type line carrying dominant alleles of the above mentioned genes: *y* + – gray body; *cut* + – normal wings; *v* + – red eyes.

To calculate the crossing-over frequency between linked genes *y, cut,* and *v* we conducted the breeding of these lines in the direction *y, cut, v* x *Berlin*. The F_1 hybrids received were grown in standard nutrient medium of the following composition: water – 350 ml, yeast – 40 g, manna-croup – 13 g, sugar – 13 g, agar-agar – 4,5 g [1] in penicillin bottles with the medium volume of 5 ml. The experiment was conducted in five replications.

In the course of the experiment, heavy metals salts (mercury, lead) were added directly to the nutrient medium for the cultivation of drosophila in amounts providing their particular concentration. To conduct experimental explorations we used lead and mercury nitrates as salts readily soluble in water. As a starting point for selection of reactant concentrations we took maximum permissible concentration (MPC) of mercury and lead nitrates, comprising 0,005 and 0.1 mg/L, respectively [16]. We also studied genetic activity of mercury and lead nitrates in concentrations exceeding 10, 100 and 1000 times MPC, namely, for $Hg(NO_3)_2$ – 0,05, 0,5 and 5 mg/L, for $Pb(NO_3)_2$ – 1, 10 and 100 mg/L (during a concentration 100 mg/L posterity did not develop, therefore data for this concentration are absent). Reactants were added directly to the nutrient medium in which F_1 hybrids were grown. With that, at first we prepared mercury (or lead) nitrate solution with the concentration 5 times exceeding the estimated. Then 1 ml of this solution was thoroughly mixed with 4 ml of nutrient medium. In the control 1 ml of distilled water was mixed with 4 ml of nutrient medium.

After the nutrient medium with the given mercury and lead nitrate concentrations set solid, its surface was lubricated with yeast solution, and 3 pairs of parental individuals were planted in each penicillin bottle according to the crossing scheme. Virgin females were selected beforehand from the line *y, cut, v* used as maternal component of crossing. After hybridization, the females lay eggs on nutrient medium with high content of mercury (lead) nitrate, the whole cycle of F_1 hybrids development took place in this nutrient medium and reactants were easily admitted in their body. Therefore meiosis in hybrid individuals, the crossing-over process, precedent events, as well as postmeiotic period were developing associated with elevated concentrations of mercury and lead nitrates, their action we link to all of the observed effects.

Test crossing was performed for F_1 females, grown on nutrient medium supplemented with $Hg(NO_3)_2$ and $Pb(NO_3)_2$. After the breeding (F_A), which was developed on a pure nutrient medium without the addition of mercury and lead nitrates, complete quantitative account of all 8 phenotypic classes (non-crossover individuals; crossover individuals in genes *y* and *cut*; crossover individuals in genes *cut* and *v*, double crossovers – all classes by two) was carried out. Crossing-over frequency (*rf*) and their standard errors (*m*) were calculated by standard formulas [14].

Results and discussion

It is known that crossing-over frequency is largely dependent on the effect of environmental factors [10]. In this regard, the experimental part of the research included setting of crossings to determine the crossing-over frequency between linked genes of chromosome I (X) of drosophila and assessment of the impact of lead and mercury nitrates on the crossing-over process. The results obtained are shown in tables 1 and 2 (table 1 presents the crossing-over frequency in different segments of zones *y*-*v* of chromosome 1 (X) of drosophila under the influence of $Pb(NO_3)_2$, table 2 presents it under the influence of $Pb(NO_3)_2$).

Analysis of data represented in table 1 shows that by adding lead nitrate to the nutrient medium for F_1 hybrids growing, the crossing-over frequency (*rf*) increases at meiosis in these hybrids in all segments of the studied chromosome 1 zone. The greatest increase which is statistically significant, is registered in segment *y-cut* at a concentration of $Pb(NO_3)_2$ 0,1 mg/L and at a concentration of 10 mg/L in the segments *cut-v, y-v.* In other cases, the observed increase in the crossing-over frequency is not statistically significant, therefore more correct it would be to talk about a tendency to *rf* increase in zone *y-v* of chromosome 1 (X) of drosophila under the influence of lead nitrate.

Table 1

Crossing-over frequency (%) in zone *y-v* of chromosome 1 of drosophila and its changes under the influence of lead nitrate

Variant of experiment	Number of individuals	Crossing-over frequency in segments						Double crossing-over frequency M±m
		y – cut		*cut – v*		*y-v*		
		M±m	t	M±m	t	M±m	t	
Control	1085	13,92±1,05	–	14,84±1,08	–	28,76±1,37	–	0
0,1 mg/l	1148	17,62±1,12*	2,40	14,67±1,04	0,12	31,11±1,37	1,21	0,84±0,27
1 mg/l	1145	14,59±1,04	0,45	14,76±1,05	0,11	28,30±1,33	0,24	0,52±0,21
10 mg/l	877	16,31±1,25	1,47	20,07±1,35**	3,02	33,87±1,59*	2,43	1,25±0,38

Comment. *,** – difference from control is significant when P<0,05; 0,01 correspondingly.

An important result indicating some genetic activity of $Pb(NO_3)_2$ is the induction of double crossing-over. So, if in control, despite a rather large sampling, double crossing-over was not registered, under the influence of $Pb(NO_3)_2$ its frequency is 0,84% at the lowest concentration and 1,25% at its highest.

Table 2

Crossing-over frequency (%) in zone *y-v* of chromosome 1 of drosophila and its changes under the influence of mercury nitrate

Variant of experiment	Number of individuals	Crossing-over frequency in segments						Double crossing-over frequency M±m
		y – cut		cut - v		y-v		
		M±m	t	M±m	t	M±m	t	
Control	1361	14,76±0,96	–	11,84±0,88	–	25,83±1,19	–	0,31±0,15
0,005 mg/l	1373	14,93±0,97	0,13	13,47±0,92	1,28	27,68±1,21	1,09	0,36±0,16
0,05 mg/l	1395	14,48±0,94	0,15	12,69±0,89	0,68	26,59±1,18	0,45	0,29±0,14
0,5 mg/l	1415	15,48±0,96	0,53	12,44±0,88	0,48	27,07±1,18	0,73	0,42±0,17
5 mg/l	1330	13,38±0,93	1,03	13,06±0,92	0,96	25,69±1,20	0,08	0,23±0,13

When analyzing data in table 2 we can see that in the experiment with mercury nitrate similar results were obtained, which shows identical genetic activity of lead and mercury compounds. Thus, the crossing-over frequency at meiosis in F_1 hybrids in general tends to increase as differences between empirical and control values are not significance. However, at the highest concentration of $Hg(NO_3)_2$ in nutrient medium (5 mg/L) the crossing-over frequency tends to reduce. The frequency of double crossing-over during the action of mercury nitrate does not change significantly.

An additional indicator of the influence of environmental factors on biological processes may be variability. Variability of this or that characteristic and its changes under the influence of environmental factors could be more sensitive criteria for assessing the degree of such influence.

For example, if under the influence of any factor an equal increase in the number of individuals with extreme expressions of signs (max and min) occurs, the average value of the characteristic remains unchanged, but its variability is greatly increased. And alternatively, if the effect of the factor leads to a decrease in the number of individuals with extreme expressions of characteristics, variability will decrease. Thus, environmental factors can have a significant effect on the body without changing the sign of the average value, but increasing or decreasing their variability.

In our experiment, we assessed the variability of crossing-over frequency within experimental variants and chromosome segments under study. As a unit of variability we considered F_A family of drosophila. With this aim in view the range of variability was determined and *rf* variation coefficients as well as their standard errors were calculated. The results are shown in tables 3–6.

Table 3

Range of variability of crossing-over frequencies under the influence of lead nitrate (max-min)

Variant of the experiment	Values of the range of rf variability in segments			
	y – cut	*cut – v*	*y-v*	double
Control	15,59	13,26	17,13	0,0
0,1 mg/l	9,19	8,59	5,39	2,16
1 mg/l	9,85	8,32	14,77	0,35
10 mg/l	10,03	17,70	16,07	1,35

Table 4

Range of variability of crossing-over frequencies under the influence of mercury nitrate (max-min)

Variant of the experiment	Values of the range of rf variability in segments			
	y - cut	*cut – v*	*y-v*	double
Control	6,18	7,83	10,06	0,91
0,005 mg/l	11,30	6,00	9,85	1,30
0,05 mg/l	8,26	7,61	12,02	0,86
0,5 mg/l	8,35	9,60	10,87	1,47
5 mg/l	5,79	12,56	15,72	0,80

Table 5

Variation coefficients of crossing-over frequencies under the influence of $Pb(NO_3)_2$

Variant of the experiment	Values of the variation coefficients for segments V(%)±S_v			
	y – cut	*cut – v*	*y-v*	double
Control	29,26±6,54	26,22±5,86	16,89±3,78	0,00
0,1 mg/l	16,07±3,59	16,41±3,67	7,52±1,68*	87,18±19,49
1 mg/l	20,13±4,50	18,51±4,14	14,13±3,16	83,15±18,59
10 mg/l	17,22±3,85	25,32±5,66	13,87±3,10	76,08±17,01

Note: * – difference from control is significant when P<0,05.

Table 6

Variation coefficients of crossing-over frequencies under the influence of $Hg(NO_3)_2$

Variant of the experiment	Values of the variation coefficients for segments V(%)±S_v			
	y - cut	*cut – v*	*y-v*	double
Control	10,92±2,46	22,08±4,94	11,56±2,58	124,53±27,83
0,005 mg/l	21,41±4,78	16,38±3,66	10,88±2,43	131,21±28,74
0,05 mg/l	15,85±3,55	17,28±3,86	12,49±2,79	124,64±28,03
0,5 mg/l	16,18±3,76	20,34±4,55	13,24±2,96	111,32±24,73
5 mg/l	12,95±2,90	26,84±6,00	14,94±3,34	153,22±34,14

It is obvious from the data presented in tables 3–6 that the crossing-over frequency is characterized by considerable variability, and some differences in the range of variability and values of the variation coefficients are observed both for different variants of the experiment, and for different segments of the investigated zone of chromosome 1. In most cases, there are no significant diversity between the variants of the experiment; therefore the observed changes are of a trend nature.

Thus, under the influence of lead nitrate we can observe the decrease of the crossing-over frequency variation in comparison with the control in all investigated segments of chromosome 1. With that, the concentration of $Pb(NO_3)_2$ 0,1 mg/L results in a significant reduction in *rf* variability in segment *y*-*v*. Analysis shows that most probably the reason for this is the reduction of the range of variability (table 3). An exception is a segment *cut*-*v*, within which the range of variation and the variation coefficient in all experimental variants decreases and increases by concentration of 10 mg/L.

There are several different patterns for the crossing-over frequency variability under the influence of $Hg(NO_3)_2$. The general tendency of the variation coefficient changes is so that initially, with the concentration increasing, their values are reduced and then increased, and at the highest concentration (5 mg/L) exceeds the control level. The only one segment *y*-*cut* is not subject to this consistency, in which the variation coefficient increases (its maximum value is achieved at a concentration of 0,005 mg/L) with the increase of the concentration, and decreases at the highest concentration (here reliable effects are absent). A similar pattern is observed while analyzing the range of variability in this segment (table 4). In segments *cut*-*v* and *y*-*v* the smallest range of variability is recorded at a concentration of $Hg(NO_3)_2$ 0,005 mg/L, and the greatest range of variation is achieved at a concentration of 5 mg/L.

In general, analysis of the data received shows that the phenomenon of segment specificity is peculiar for changes of the characteristics of the crossing-over frequency variation, in accordance to which various segments of the zones studied differently respond to treatment.

The effect of reducing the variability of features under the influence of various factors is observed while treating organisms with low doses of chemical mutagens, however no assumptions about the mechanisms of such a reduction were expressed [7]. Reduced range of variability, being the cause of a smaller variation coefficient, may be stipulated by a similar targeted reaction of all individuals studied on the effect of lead and mercury compounds.

Before making assumptions about the mechanisms of lead and mercury nitrates influences on recombination parameters, it should be noted that the crossing-over is a complex process that involves many successive steps [10]. Changes taking place at any of these steps, whether they are stipulated by the influence of environmental factors or simply by accidental causes, will lead, ultimately, to changes of crossing-over frequency.

Crossing-over is a set of processes involving homologous chromosome pairing, formation of breaks in DNA strands, cross reunion of strands, DNA breaks reparation, correction of molecular heterozygosity sites, etc. Many of these processes are enzymatic. Homologous chromosome pairing is seen as a necessary condition for crossing-over passing. If it goes well, in the future crossing-over frequency depends on the efficiency of the enzymes that primarily cause DNA breaks, and then all the other processes necessary for its realization. Environmental factors often cause conjugation disorders of homologous chromosomes, resulting in the reduction of the crossing-over frequency. On the other hand, they may increase the number of DNA breaks due to direct or indirect effect on the process, which leads to the increase of the crossing-over frequency. The final result is determined by cooperation of the above two processes. In our case, there is a tendency to the crossing-over frequency increase. And the most significant effect is characteristic

of the proximal zone of chromosome 1 (segment *cut-v*), especially when the action of lead nitrate. This is confirmed by data obtained in other studies according to which other factors, such as temperature, cause the greatest increase in the crossing-over frequency in segments close to the centromere, and to a lesser extent affect the crossing-over frequency in other chromosomal zones (so called centromeric effect) [18]. Centromeric effect is explained by the fact that in centromeric zones the crossing-over is normally suppressed because they are rich in heterochromatin.

From the obtained data it follows that low concentrations of substances lead to an increase of the gene recombination degree. However, higher concentrations (in case of mercury nitrate it is 5 mg/L) lead to a decrease in the crossing-over frequency, and perhaps even to the oppression of gametes and zygotes viability.

Increase of the crossing-over frequency under the influence of lead and mercury nitrates may have adaptive significance, since crossing-over creates new combinations of genes and some of them can provide a great adaptation of organisms to unfavorable environmental factors, including elevated concentrations of heavy metals. At the same time, increase in the crossing-over frequency may reduce a direct adaptation by the breakup of adaptation gene complexes, united by natural or artificial selection. In this case, the majority of new gene combinations result in "genetic death" for their carriers.

Reducing the recombination frequency under the influence of chemicals used in the experiment may have a negative value for the organism itself in the perspective. In the changing environmental conditions, variability reduction may lead to adaptation reduction. Such reduction is apparently caused by a lower probability of occurrence of individuals with favorable gene combinations providing higher adaptability to new conditions [15].

It seems that a described tendency of the crossing-over frequency increasing under the influence of environmental factors (in this case, lead and mercury nitrates) should be considered as a manifestation of a feedback genotype-environment principle, according to which a body responds appropriately to changes of environmental factors, in a certain way adapting to this action [10]. In most cases, an adequate reaction takes place within the modification variability, but in our case it goes about genetic variability.

Despite the fact that in the course of this research using recombination tests we established the fact of genetic activity of lead and mercury nitrates, it is very problematic to evaluate if changes caused by genetic program of development will be beneficial or detrimental to organisms. It is possible that in different situations, in which organisms appear to be in the course of their life, this issue will be solved in different ways.

Thus, the F_1 hybrids breeding of drosophila on nutrient medium containing lead nitrate at concentrations of 0,1; 1; 10 mg/L leads to an increase in the crossing-over frequency in meiosis, and with the increase of concentration the effect will be enhanced. The effect of mercuric nitrate at concentrations of 0,005; 0,05; 0,5 is accompanied by a similar effect, but its higher concentration (5 mg/L) causes a decrease in the crossing-over frequency. The influence of lead and mercury nitrates is to increase the crossing-over frequency mainly in the proximal zone of chromosome 1 (X) of drosophila. Adding lead and mercury nitrate to the nutrient medium for the development of drosophila leads to the induction of double crossing-over exchanges. The effect of lead and mercury salts is accompanied by a decrease in the crossing-over frequency variability in chromosome 1 (X) of drosophila. At the same time, most of the observed effects were not statistically significant and are in the nature of trends.

The results of investigations of the genetic activity of lead and mercury compounds with the help of using recombination tests on drosophila can be extrapolated to other biological objects (including humans) and used in the prediction of genetic effects of environmental pollution.

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ОЦІНКА ГЕНЕТИЧНОЇ АКТИВНОСТІ СОЛЕЙ ВАЖКИХ МЕТАЛІВ ЗА ЗМІНОЮ РЕКОМБІНАЦІЙНИХ ПОКАЗНИКІВ У ДРОЗОФІЛИ

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Вивчено вплив різних концентрацій солей важких металів (нітратів свинцю і ртуті) на показники рекомбінативної мінливості у дрозофіли. Показано, що ці сполуки мають генетичну активність і збільшують частоту кросинговеру в ділянці *y-v* хромосоми 1 (X) дрозофіли, знижують її мінливість, а також індукують подвійний кросинговер. Обговорюються ймовірні генетичні наслідки досліджуваних ефектів.

Ключові слова: Drosophila, генетична активність, важкі метали, свинець і нітрат ртуті, параметри рекомбінації, частота кросинговеру.

ОЦЕНКА ГЕНЕТИЧЕСКОЙ АКТИВНОСТИ СОЛЕЙ ТЯЖЁЛЫХ МЕТАЛЛОВ ПО ИЗМЕНЕНИЮ РЕКОМБИНАЦИОННЫХ ПОКАЗАТЕЛЕЙ У ДРОЗОФИЛЫ

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Изучено влияние различных концентраций солей тяжёлых металлов (нитратов свинца и ртути) на рекомбинационные показатели у дрозофилы. Показано, что данные соединения обладают генетической активностью и увеличивают частоту кроссинговера в зоне *y-v* хромосомы 1 (X) дрозофилы, снижают её изменчивость, а также индуцируют двойной кроссинговер. Обсуждаются вероятные генетические последствия наблюдаемых эффектов.

Ключевые слова: Drosophila, генетическая активность, тяжелые металлы, свинец и нитрат ртути, параметры рекомбинации, частота кроссинговера.