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# БАЯНДАМАЛАРЫ

# ДОКЛАДЫ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
РЕСПУБЛИКИ КАЗАХСТАН

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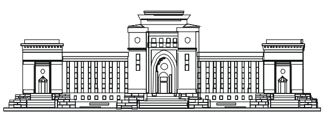

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# MUTAGENIC EFFECTS OF ALPHA RADIATION IN DROSOPHILA TEST-SYSTEM

**Abstract.** There is a long period of time between an agent intervention on a living organism and biological consequences. For this reason, methods for determining a potential mutagenic activity of individual environmental components and natural complexes are required. We used a traditional Muller-5 (Basc) test based on Drosophila melanogaster for testing the influence of ecological factors. Due to this test system, we analyzed genetic effects of α-radiation, which is formed during the radioactive decay of radon daughter products. The test system was used to detect mutations with an autonomous manifestation. Now days, it is also used for detecting conditional mutations with non-autonomous manifestation, which form special features related to an invariant part of species appearance of a living organism. The most striking property of conditional mutations is morphoses formation. In our research, the morphoses appeared in the second generation and were due to the presence of conditioned mutations in parents taken from the $F_1$. The primary inducer of the conditional mutations emergence was ionizing α-radiation, and in the next generation they were supplemented by the genetic characteristics of the parents being the inversions. The revealed morphoses formed a characteristic group of deformities: blackspots (melanomas) or white spots on a body; curled, curved, or undirected wings; blister on the wings, without one wing, with deformation of the head, thorax and abdomen, mutation of sterility. Sterility was tested in several generations of flies. A characteristic feature of all morphoses is asymmetry and it is defined as a genetically unstable variation of individual morphogenesis associated with changes in the environment. A statistical analysis of experimental data in the Muller-5 test system (Basc) showed that α-radiation has a mutagenic effect with a probability of not less than 95%.

**Key words:** radon, emanation, α-radiation, inversion, Basc, Drosophila, morphoses.

## Introduction

Almaty is a city with the highest natural radiation in Kazakhstan, which rich in such natural resources as minerals, metal ores and natural gas and oil reserves. Kazakhstan has 12 % of the world's uranium resources and may be exposed to a variety of hazardous materials including radon, a radioactive gas occurring naturally as an indirect decay product of uranium. Radon gets out of the earth surface through 5 tectonic faults crossing the city territory. Radon and its decay products are sources of α-radiation - a stream of heavy positively charged particles [1]. In nature, alpha particles occur as a result decay of heavy elements atoms, such as uranium, radium and thorium. Emanation (a release of radon into the air pores) happens when the radium decay took place near the soil surface and it was mainly carried out by recoil energy produced by a radon nucleus in the process of radium nucleus disintegration.

Most of radiation is produced not so much from radon but its decay daughter products. Radon emissions are supposed to be dangerous for living organisms and can cause oncological diseases in humans. In human body, radon facilitates some processes also leading to lung cancer. The decay of radon nuclei and its daughter isotopes in the lung tissue causes a micro-burn, as the whole alpha particles energy is absorbed at its decay point. Combination of radon and smoking is especially hazardous and increases the disease risk. According to the US Department of Health, radon had regarded to be the second factor (after smoking) that causes lung cancer, mostly, of bronchogenic (central) type. Lung cancer caused by radon irradiation is the sixth most frequent reason causing death from cancer [2]. Radon radionuclides cause more than a half radiation dose, which a human body receives from natural and technogenic environmental radionuclides [3-4].

For this reason, the aim of present work is α-radiation mutagenic activity by Drosophila melanogaster test-system based on Meller-5 or Basc method.

**Materials and methods**

The uranium isotope – $U^{238}$ isotope was used as source of α-radiation used. Alpha-rays is one of the ionizing radiation types performing a stream of rapidly moving, positively charged particles (alpha-particles). The main source of this radiation is the radioactive isotopes and daughter products of a natural radon gas. One of the peculiarities of alpha-radiation is its low penetrating power. Alpha particles range in matter (that is a path where ionization is producing) is very short (hundreds of millimeter in biological media, 2.5-8 cm in air). However, along a short path, alpha particles create a great number of ions. That provides a relative biological efficiency, 10 times greater than when exposing the X-ray and gamma radiation.

Testing of α-radiation genetic activity was carried out using the fruit fly Drosophila melanogaster. So, some tests based on incidence of different mutations types have been developed for drosophila. The processes occurring in the Drosophila melanogaster are extremely interesting for the community of researches engaged in developmental genetics [5]. This fly is chosen as an object in the variety genetic schemes, as it is one of the highly researched and well characterized higher organisms in genetics. Approximately 2/3 of genes that are responsible for a human disease are homologous to genes in Drosophila melanogaster genome. The main biochemical processes in Drosophila melanogaster and mammalian cells are identical. Also, one of the Drosophila melanogaster main advantages lies in the fact that in metabolism process a microsomal activation of substances occurs, when promutagens can be converted into mutagens. This makes it possible to find invisible mutagens, which acquire genotoxicity in metabolism process. Tests based on Drosophila melanogaster are recommended by WHO for studying the mutagenic and toxic activity of anthropogenic xenobiotics and pharmacological agents [6].

A traditional Muller-5 (Basc) test based on Drosophila melanogaster was used for testing mutagenic activity of α-radiation. Muller-5 method allows to identify lethal and morphological mutations in the $F_2$ (second generation) X-chromosome. The body of Drosophila, like those of all other insects, is divided into segments having certain morphological differences [7]. All flies were identified by their eyes, wings and bristles, because they contained yellow and white genes [8]. We divided males of Oregon wild-type into two samples: the first sample with the males was irradiated by the $U^{238}$ isotope at the exposure of 20-24 hours, and the second control sample was placed nearby, which was not exposed to α-radiation. To obtain $F_1$ in the Muller-5 (Basc) test system, we used parents different in body and eyes color as well as the shape, because it greatly facilitates females and males identification for parents crossing and second generation analysis.

Presence or absence of males in the population can be determined in a tube without anesthesia. Flies crossing for getting $F_1$ was carried out massively and individually for $F_2$ females. The scheme provides an opportunity for $F_2$ flies crossing without selecting the virgin females. One female from the first generation and two or three M-5 males from the original tube or from the M-5 line have been places in the test tube. For females, such crossing was individual, and the number of tubes was corresponded to half of the analyzed X-chromosomes [9-10]. Sterility was tested using several generations of flies.

The test scheme and a line of flies in the experiment is known as Muller-5 method. This method was developed by H. J. Muller for identifying and recording recessive, sex-linked lethal mutations in drosophila. In the X-chromosome of this line there are 2 inversions - $sc^8$ and – $sc^{49}$ (δ 49), which impede a crossing-over between sex chromosomes. The $sc^8$ inversion captures a major part of X-chromosomes. Since crossing occurs in long inversions, another, shorter inversion δ 49, is introduced into the $sc^8$ inversion. So, the δ 49 inversion suppresses the cross in the middle region of the X-chromosome. The genes order in $sc^8$ δ 49 chromosome is violated twice, therefore the cross in it is completely excluded. As a result, both inversions are not associated with a recessive lethal effect, and females homozygous for the Muller-5 chromosome and the same hemizygotic males are viable [6].

A recessive mutation $w^{a}$-apricot eyes and dominant mutation Bar-striped eyes serve as phenotypic markers (Figure 1). We obtained two phenotypic classes of females and in the second generation ($F_2$). In the first generation ($F_1$) we received B / + females, carrying in the heterozygote X-chromosome of Muller-5 and irradiated male's X-chromosome, and males bearing the Muller-5 X – chromosome in a hemizygot. The recessive lethal cannot move from the irradiated chromosome to the Meller-5 chromosome, because of locking crossing-over between the irradiated normal chromosome and the Muller-5 chromosome. This makes it possible to bring the irradiated chromosome to a hemizygous state [11].

Figure 1 – Muller-5 line flies: on the left male (smaller) and female on the right

The cultures of the genetic lines and all crosses were kept and propagated on a standard medium [9].

**Results and their discussion**

Genetic analysis of α-radiation mutagenic activity was carried out according to the scheme shown in Figure 2. Every culture is analyzed visually for revealing morphological mutations after the second-generation flying-out. The conditional mutations are regulatory gene mutations, which are responsible for forming interspecies of similar characteristics [13]. The morphosis formation is a considerable phenomenon of conditional mutations. "Morphosis" means non-inherited morphological disturbances, caused by parent genetic peculiarities. The morphosis, appearing in conditioned mutants, is considered to be different manifestation rates of developmental disturbance. In the case of alpha-radiation induction the offspring were characterized by mutant phenotype of body (white and black plaques, asymmetrical body) and wing: moderate, medium, pronounced, extreme (reduction and even complete disappearance of wing) (Figure 3) [8].

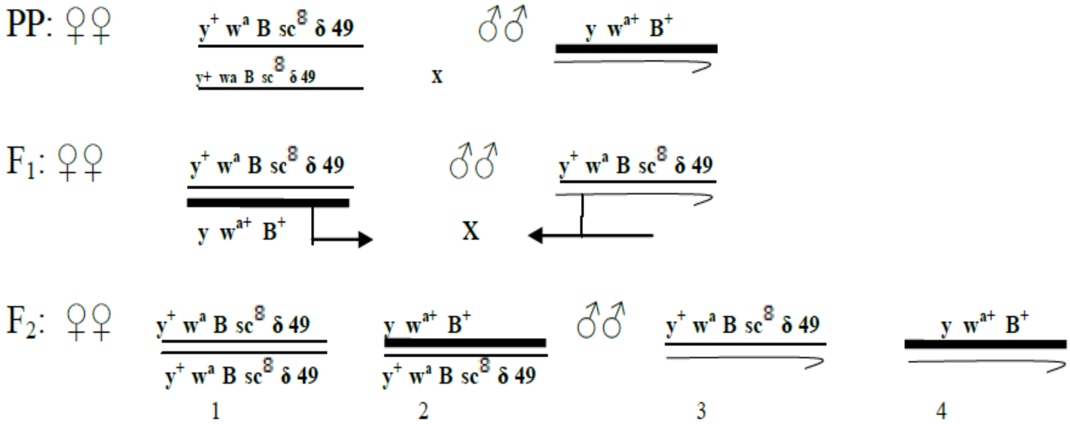


Figure 2 – The scheme of flies crossing according to Muller-5 method for revealing lethal and morphological mutations in X-chromosome: 1 – Muller-5 females; 2 – females with a gray body, with narrow apricot eyes, heterozygote by inducing lethal mutations in X – chromosome;3 – gray males with narrow apricot eyes; 4 – yellow males with a lethal mutation in X-chromosome are not develop. Irradiated X-chromosome is shown as a bold line[11]

$F_2$ analysis did not reveal recessive sex-linked mutations. Nevertheless, to evaluate the possible genotoxic effect of α-radiation on individual development of flies, the frequency of occurrence of morphoses was estimated (Table 1).

Table 1– Frequency of morphoses in flies irradiated with α-particles and without it

| Index / Sample | Number of flies analyzed (absolute) | Absolute frequency of morphoses | Relative frequency of morphoses, % |
|---|---|---|---|
| With α-radiation (expirement) | 3848 | 28 | 0.73±0.04 |
| Withoutα-radiation (control) | 3700 | 10 | 0.27±0.01 |

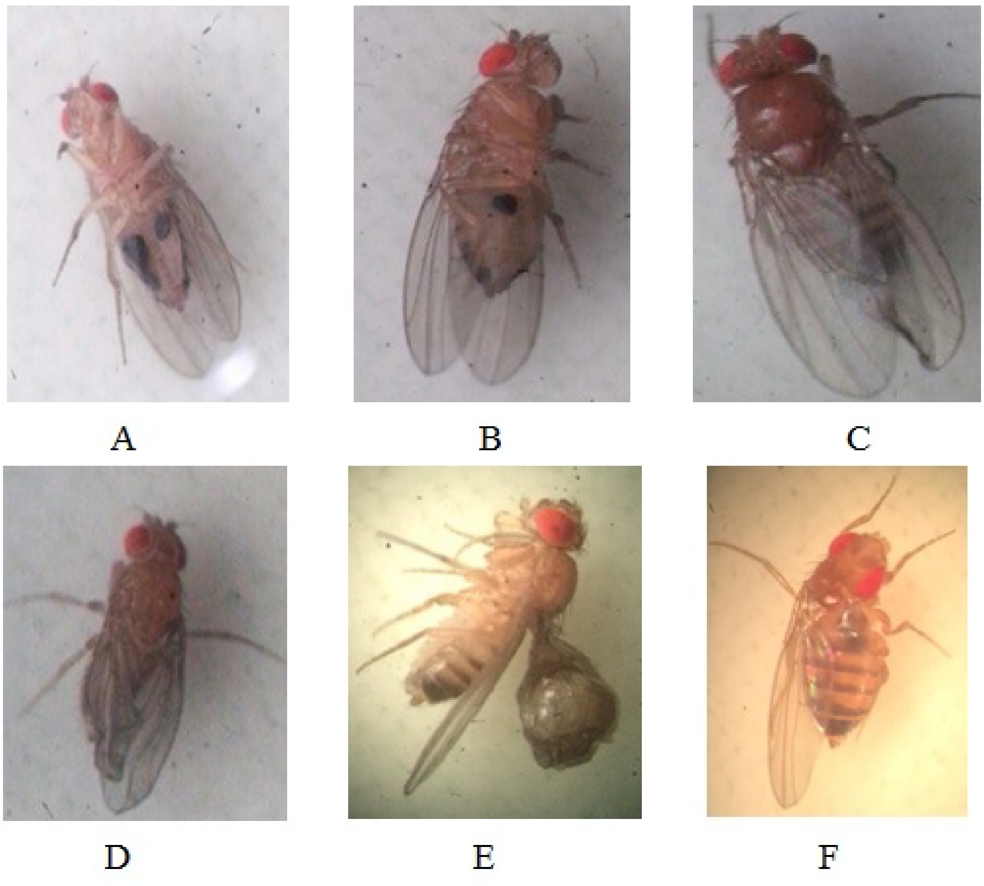

Figure 3 –Morphosis of the second generation according to Muller-5 test-system:
A, B –black plaques on a body; C – moderate mutant wing phenotype (improperly outspreaded wing); D – pronounced mutant wing phenotype (improperly outspreaded wing); E –extreme mutant wing phenotype – a right wing in the form of an unstructured bubble; F – morphoses combination - extreme mutant wing phenotype(without a wing), deformation of the head, thorax and abdomen

Classical genetics is built on mutations with an autonomous manifestation, based on inheritance signs laws. The Muller-5 (Basc) test system used was designed to evaluate just such mutations. At the present time we began studying mutations with non-autonomous manifestation, which form special features related to an invariant part of species in a living organism appearance. The researchers named such mutations – conditional mutations and received them in Drosophila under the influence of ionizing radiation (X-ray irradiation) [13].

One of the main conditions for manifesting a conditional mutation was the presence of chromosome rearrangement in the genotype. At the time being, a conditional mutation is called a local DNA damage, its manifestation being depending on the structure of other genome regions. The most striking property of conditional mutations is the morphoses formation. The term "morphosis" is used to determine non-inherited morphological disorders (deformities) caused by the exposure of extreme environmental factors.

In the genetic literature, morphosis is defined as a non-adaptive and unstable variation of individual morphogenesis, associated with the changes in the external environment. In our experiment, the morphoses were manifested in the second generation ($F_2$) and were due to the presence of conditioned mutations in parents taken from $F_1$. A primary inducer of conditional mutations emergence was α-radiation, and in the second generation they were joined by genetic characteristics of the parents - these are inversions. The morphoses in our experiment formed a very specific group of deformities (Figure 3).

Most morphoses do not prevent flies from hatching out of pupa, existing, mating, and even giving offspring. The researchers also encounter with cases of morphoses forming, but occurs not so often [13].

As seen in Figure 3, a peculiar feature of all morphoses is asymmetry. They can be distributed over all parts of the body and affect the shape of the head, eyes, chest, legs and wings. Dark spots (or melanomas) similar to necrotic spots that contain conglomerates of dark tissue can appear on all parts of the body.

There can be a single or several individuals containing ugliness may arise. Thus, we found up to 6 morphoses in one $F_2$ tube. All of them had an individual appearance. The morphoses appeared in $F_3$, but unlike modifications, they did not reveal phenotypic invariance. So a wing morphosis in $F_2$could be revealed as melanoma in $F_3$ and vice versa. You can say that the type of morphosis is not inherited.

Experimental and control comparison of results was carried out by the Yates' chi-squared test (Table 2) [12].

Table 2 – Experiment and control results in the 2x2 table [14]

| | | | |
|---|---|---|---|
| Experiment | *a* (the number of flies without mutation and morphoses) | *b* (the number of flies with mutation and morphoses) | ∑ |
| | 3820 | 28 | 3848 |
| Control | *c*(the number of flies without mutation and morphoses) | *d* (the number of flies with mutation and morphoses) | |
| | 3690 | 10 | 3700 |
| ∑ | 7510 | 38 | 7548 |

A statistical experimental data processing in the Muller-5 test-system showed that $\chi^2_{exp}$ =6,99, a $\chi^2_{table}$=3,8at*k* =1 and P≤ 0,05. Therefore at P≤ 0,05$\chi^2_{exp}$> $\chi^2_{table}$. For this reason we can affirm that alpha-radiation possesses a mutagenic effect.

**Conclusion**

Recessive, sex-linked lethal mutations, modifications and morphoses as the main criterion of α-radiation mutagenic effect evaluation in drosophila have been chosen. Classical genetics is based on mutations with an autonomous manifestation and in our case they are recessive lethal. Mutations with non-autonomous manifestation have been studied quite recently. Due to this, it stands to the reason that the genes, which are responsible for such mutations, form special signs. Basically, these are modifications and morphoses that touch on invariable part of organism's morphology. A common method of mutations evaluation based on Drosophila melanogaster test-system has been used in the experiment. The RK Committee for the mutagenicity evaluation of pharmacological preparations recommends this test.

According to the results obtained, a statistically significant difference in the incidence of recessive lethal mutations and conditional mutations induced in the X - chromosome of the drosophila's Oregon line males with alpha irradiation and without it has been shown. The nonparametric chi-square test demonstrated that the frequency distribution control is statistically different at 95% probability level in the experiment and control. Thus, mutagenic activity is revealed in drosophila by alpha-rays irradiation.

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**ДРОЗОФИЛАНЫҢ ТЕСТ-ЖҮЙЕСІНДЕГІ α–СӘУЛЕЛЕНУДІҢ МУТАГЕНДІ ӘСЕРІН ТАЛДАУ**

**Аннотация.** Агенттің тірі ағзаға әсер етуі және биологиялық нәтижесі көріну арасында үлкен уақыт айырмашылығы болады, сондықтан қоршаған ортаның жеке компоненттері, комплекстері секілді потенциалды мутагенді белсенділікті анықтау үшін әдістер қажет. Экологиялық стресс-факторлардың генетикалық активтілігін тексеру үшін біз дәстүрлі Меллер-5 (Basc) тест-жүйесін *Drosophila melanogaster*-ге пайдалана отырып жүргіздік. Радонның ыдырау өнімдері кезіндегі негізгі пайда болатын α-сәулеленудің генетикалық эффектісін талдадық. Қолданған тест-жүйе классикалық генетикада мутациялардың автономды көріну жағдайына аңықтауға қолданылған. Қазіргі кезде автономды емес шартты мутациялардың көріну жағдайында қолданатын болды. Шартты мутациялар тірі организмнің түр бейнесінің өзгермейтін ерекше белгілерді қамтамасыз етеді. Шартты мутациялардың анық қасиеті – ол морфоздардың пайда болуы. Біздің зерттеуімізде морфоздар екінші ұрпақта пайда болды және бірінші ұрпақтан алынған аталықтардағы шартты мутациялармен негізделінген. Шартты мутациялардың пайда болуына біріншілік индуктор болып иондаушы α-сәулелер болды. Келесі ұрпақта оларды аталықтардың генетикалық ерекшеліктері – инверсиялары толтырды. Табылған морфоздар кемтарлықтардың сипатталған тобын құрастырды: дене бетіндегі қара (меланомалар), немесе ақ дақтар; оралған, майысқан, немесе жайылмаған қанаттар; қанаттардағы көпіршіктер, бір қанатсыз, бастың, көздің, торакстың және қарынның деформациясы, ұрықсыздықтың мутациясы. Ұрықсыздық шыбындардың бірнеше ұрпақтарда тексерілді. Барлық морфоздардың сипатталған белгісі ассиметрия болды және ол қоршаған ортаның өзгерістерімен байланысты жеке морфогенездің генетикалық тұрақты емес вариациясы болып саналады. Меллер-5 (Basc) тест-жүйе статистикалық талдауы α-сәуленің 95%-дан кем емес сенімділікпен мутагенді әсерің көрсетті.

**Тірек сөздер**: радон, эманация, α-сәулелену, инверсия, Basc, дрозофила, морфоздар.

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## АНАЛИЗ МУТАГЕННОГО ЭФФЕКТА α-ИЗЛУЧЕНИЯ В ТЕСТ-СИСТЕМЕ ДРОЗОФИЛЫ

**Аннотация.** Между воздействием агента на живой организм и проявлением биологических последствий проходит часто большой промежуток времени, поэтому необходимы методики определения потенциальной мутагенной активности как отдельных компонентов окружающей среды, так и комплексов. Для проверки генетических эффектов факторов окружающей среды мы использовали традиционный тест Меллер-5 (Basc) на Drosophilamelanogaster.С помощью этой тест-ситемы мы проанализировали генетические эффекты α-излучения, которое образуется при радиоактивном распаде дочерних продуктов радона. Данная тест-система в классической генетике использовалась для детекции мутаций с автономным проявлением. В настоящее время ее применяют и для обнаружения условных мутаций с неавтономным проявлением, которые формируют особые признаки, относящиеся к инвариантной части видового облика живого организма. Самое яркое свойство условных мутаций – это образование морфозов. В наших исследованиях морфозы проявились во втором поколении и были обусловлены наличием условных мутаций у родителей, взятых из первого поколения ($F_1$). Первичным индуктором возникновения условных мутаций являлось ионизирующее α-излучение, а в следующем поколении их дополняли генетические особенности родителей – это инверсии. Обнаруженные морфозы составили характерную группу уродств: черные пятна на теле (или меланомы); закрученные, изогнутые, или нерасправленные крылья; белые пятна на теле, пузыри на крыльях, безодного крыла, с деформацией головы, глаз, торакса и брюшка, мутации стерильности. Стерильность проверялась в нескольких поколениях мух. Характерной чертой всех морфозов является ассиметрия и определяется она как генетически не стабильная вариация индивидуального морфогенеза, связанная с изменениями окружающей среды. Статистичекий анализ данных эксперимента в тест-системе Меллер-5 (Basc) показала, что α-излучение обладает мутагенным эффектом с вероятностью не менее 95%.

**Ключевые слова:** радон, эманация, α-излучение, инверсия, Basc, дрозофила, морфозы.

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