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MUTATION OF THE *DROSOPHILA MELANOGASTER* L. UNDER THE INFLUENCE OF THE ELECTROMAGNETIC RADIATION

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Abstract. Brief results of the previous studies of the effect of electromagnetic radiation on the fruit flies are quoted. The influence of electromagnetic radiation of industrial frequency on the living organisms has been investigated. Correlative dependence between phenotype *Drosophila melanogaster* L., duration and intensity of harmful factors has been established. Phenotypic manifestations have been fixed and *Drosophila melanogaster* L. mutation under the constant influence of the magnetic field induction from 2,25 to 20 μT in three generations of test objects have been characterized. The dependence of the increased frequency of mutations occurrence and the increase of their diversity caused by the increase of magnetic field induction has been revealed.

Key words: electromagnetic radiation, electromagnetic pollution, test objects, mutation.

1. Introduction

Drosophila melanogaster L. was among the first organisms used for genetic analysis, and today it is one of the most widely used and genetically best-known of all eukaryotic organisms. All organisms use common genetic systems; therefore, decoding such processes as transcription and replication in fruit flies helps to understand these processes in other eukaryotes, including humans. Charles W. Woodworth is considered to be the first to breed *Drosophila* in great quantity and suggest using them for genetic research during his time at Harvard University. Thomas Hunt Morgan began using fruit flies in experimental studies of heredity at Columbia University in 1910.

About 75 % of the known human disease genes have a recognizable match in the genome of fruit flies, and 50 % of fly protein sequences have mammalian homologs. An online database called Homophila is available to search for human disease gene homologues in flies and vice versa. *Drosophila melanogaster* L. is used as a genetic model for several human diseases including the neurodegenerative Parkinson's and Huntington's disorders, spinocerebellar ataxia and Alzheimer's disease. The fly is also used to study mechanisms underlying aging and oxidative stress, immunity, diabetes, and cancer, as well as drug abuse [1].

(Drosophila melanogaster L. is one of the most studied organisms in biological research, in particular in genetics and developmental biology. There are several reasons:

- This culture requires little equipment and little space is needed even for large cultures and, in general, the overall cost is low.
- It is small and easy to grow in the laboratory and its morphology is easy to identify once it is anesthetized (usually with ether, carbon dioxide gas, by cooling them, or with products like FlyNap).
- It has a short generation time (about 10 days at room temperature) so several generations can be studied within a few weeks.
- It has high fecundity (females lay up to 100 eggs per day, and about 2000 in a lifetime) [2].
- Males and females could be readily distinguished and virgin females could be easily isolated, therefore, facilitating genetic crossing.
- The mature larvae show giant chromosomes in the salivary glands called polytene chromosomes – «puffs» indicate regions of transcription and hence gene activity.
- It has only four pairs of chromosomes: three autosomes, and one sex chromosome.
- Males do not show meiotic recombination, consequently, facilitating genetic studies.
- Its recessive lethal «balancer chromosomes» carrying visible genetic markers can be used to keep stocks of lethal alleles in a heterozygous state without recombination due to multiple inversions in the balancer.
- Genetic transformation techniques have been available since 1987.
- Its complete genome was sequenced and first published in 2000 [3].

2. Material and methods

At the beginning of the XX century Carpentero used *Drosophila melanogaster* L. for certain biological problems solution. Medvedev studied the influence of family crossing on the objects [4]. Stervant started the first research on the behavior genetics of *Drosophila melanogaster* L. He compared sexual activity and mating selectivity of normal and mutant animals. He took recessive mutations that change eye pigmentation (white), the body pigmentation (yellow) and influence the shape of the wings (curved) and females with yellow colour of the body. Ginter had found out that interspecies hybridization of *Drosophila melanogaster* L. and *Drosophila* simulans were more successful providing that yellow females were taken for it [5].

In 1971, Ron Konopka and Seymour Benzer published «Clock mutants of *Drosophila melanogaster*», a paper describing the first mutations that affected the animal's behaviour. Wild-type flies show an activity rhythm with a frequency of about a day (24 hours). Researchers found mutants with faster and slower rhythms as well as broken rhythms – flies that move and rest in random spurts. Work over the next 30 years has shown that these mutations (and others like them) affect a group of genes and their products that comprise a biochemical or biological clock. This clock is found in a wide range of fly cells, but the clock-bearing cells that control activity are several dozen neurons in the fly's central brain [6].

The definition of “mutagen sensitivity” of Drosophila after its processing with methyl-methane sulphonate (MMC) or ultraviolet rays at a larval stage, made it possible to conclude that the studied mutation determines experimentally high sensitivity of early and late larvae of fruit flies to lethal action, and 4-5 fold reduction of enzyme activity was recorded in the mutant cells.

In 1985 scientists studied fertility and frequency of dominant lethal mutations of the radiosensitive line of *Drosophila* rad (2) 201G1 after irradiation of females by γ-rays. It has been determined that the doses of γ-rays, which frequency is more than 10 Hz, have strong sterilizing effect on mutant females and contribute to increased mortality of the flies after the irradiation [7]. Scientists Moss I. B. and Savchenko V. K studied the impact of the x-ray radiation and melanin pigment on fertility and vitality of experimental *Drosophila melanogaster* L. populations for 55 generations [8]. The results of this research showed that the viability of the individuals in irradiated populations, on average, is lower than that in the reference population. The fertility at the irradiation first decreases and then increases exceeding the reference level. Addition of melanin in nutrient medium is beneficial for both indicators in irradiated and reference populations.

Genotoxic impact of carcinogenic aromatic compounds on mus-mutants of *Drosophila melanogaster* L. was studied in 1991 by Shpigelman and others [9]. Larvae of homozygotes of all studied mus-lines were sensitive to the carcinogenic aromatic compounds, in comparison with the reference line. In the period from 1993 to 1995 the research group (Ratner V. A., Bubenshikova E. V., Vasileva L. A., and others) determined the doses of γ-radiation that will cause a number of mutations in isogenic strain of *Drosophila melanogaster* L [10].

On the basis of the biotesting method Kniazeva I. R. examined the impact of electromagnetic radiation with 460 MHz frequency and powerful electromagnetic pulses (EMP) on the organism of the maturating fruit fly [11]. In 2002 Chernova G. V. and Vorsobina N. V. studied the effect of low-intensity pulsed laser radiation (LPLR) on life-span of *Drosophila melanogaster* L. [12]. Assessment of the LPLR effectiveness was conducted on the basis of the analysis of the main parameters of aging. The effects of increasing and shortening of the life-span were discovered. In fact, recent studies show that there are over 7000 scientists studying this bug worldwide as a fulltime job.

Regular *Drosophila melanogaster* L. have red eyes and their bodies are generally a mixture between brown and yellow [13]. Their general length is about 0.3 cm. The male fruit flies usually have a slightly darker body than the females. Another characteristic of the male *Drosophila* is a larger black spot on the abdomen. The male fruit flies are also slightly smaller than the females which is rather unusual in nature. While mating, the male fruit fly attaches himself to the female with very small hair like bristles before inseminating his target [14].

The development period for *Drosophila melanogaster* L., as many ectothermic species, varies with temperature. The shortest development time (from egg to adult) is 7 days and it is achieved at 28 ^{o}C. Development time is increasing at higher temperatures (11 days at 30 ^{o}C) due to heat stress. Under ideal conditions, the development time at 25 ^{o}C is 8.5 days, at 18 ^{o}C it takes 19 days and at 12 ^{o}C over 50 days. Females lay some 400 eggs (embryos), about five at a time, into rotting fruit or other suitable material such as decaying mushrooms and sap fluxes. The eggs, which are about 0.5 millimeters long, hatch after 12–15 h (at 25 ^{o}C) [15]. The resulting larvae grow for about 4 days (at 25 ^{o}C) while molting twice (into 2nd- and 3rd-instar larvae), at about 24 and 48 h after enclosion. During this time, they feed on the microorganisms that decompose the fruit, as well as on the sugar of the fruit itself. Then the larvae encapsulate in the puparium and undergo a four-day-long metamorphosis (at 25 ^{o}C), after which the adults enclose (emerge) [16].

3. Results

The determination of the negative impact of magnetic field on the test objects is based on the system including the following elements: the activity level, mortality, fertility, occurrence of teratology. The influence of the electromagnetic pollution on the

organisms was studied on the basis of changing reaction of the test-objects in the result of different levels of magnetic field induction [17]. During the experiments the normal conditions were maintained, and the effect of noise excess was excluded. The first mutations appearance was recorded during the constant effect of electromagnetic field of industrial frequency with induction 2,25 μT and more. In the first generation of *Drosophila melanogaster* L. the following mutations were found in females: deformation of the left wing (fig. 1 A) and deformation of the female's body (fig. 1 B). None of the females did give the posterity F_2 during the breeding.

The teratologies discovered in the second generation (the abortive rudimentary wing (fig. 1 C) and deformation of female wings (fig. 1 D)) – had the genetic nature and recessive pattern of the inheritance, which caused mortality. Flies with rudimentary wings cannot fly: they have a defect in their "vestigial gene", on the second chromosome. These flies have a recessive mutation. Due to this both vestigial genes carried by each fly (one from each parent) have to be altered to produce the abnormal wing shape. If only one is mutated, the healthy version can override the defect. The largest number and variety of the mutations were recorded in F_3. In the third generation of Drosophila the abnormalities of the proportions and body sizes were revealed (figs 1 D–F), which proves that the electromagnetic field impact can cause the atypical structures.

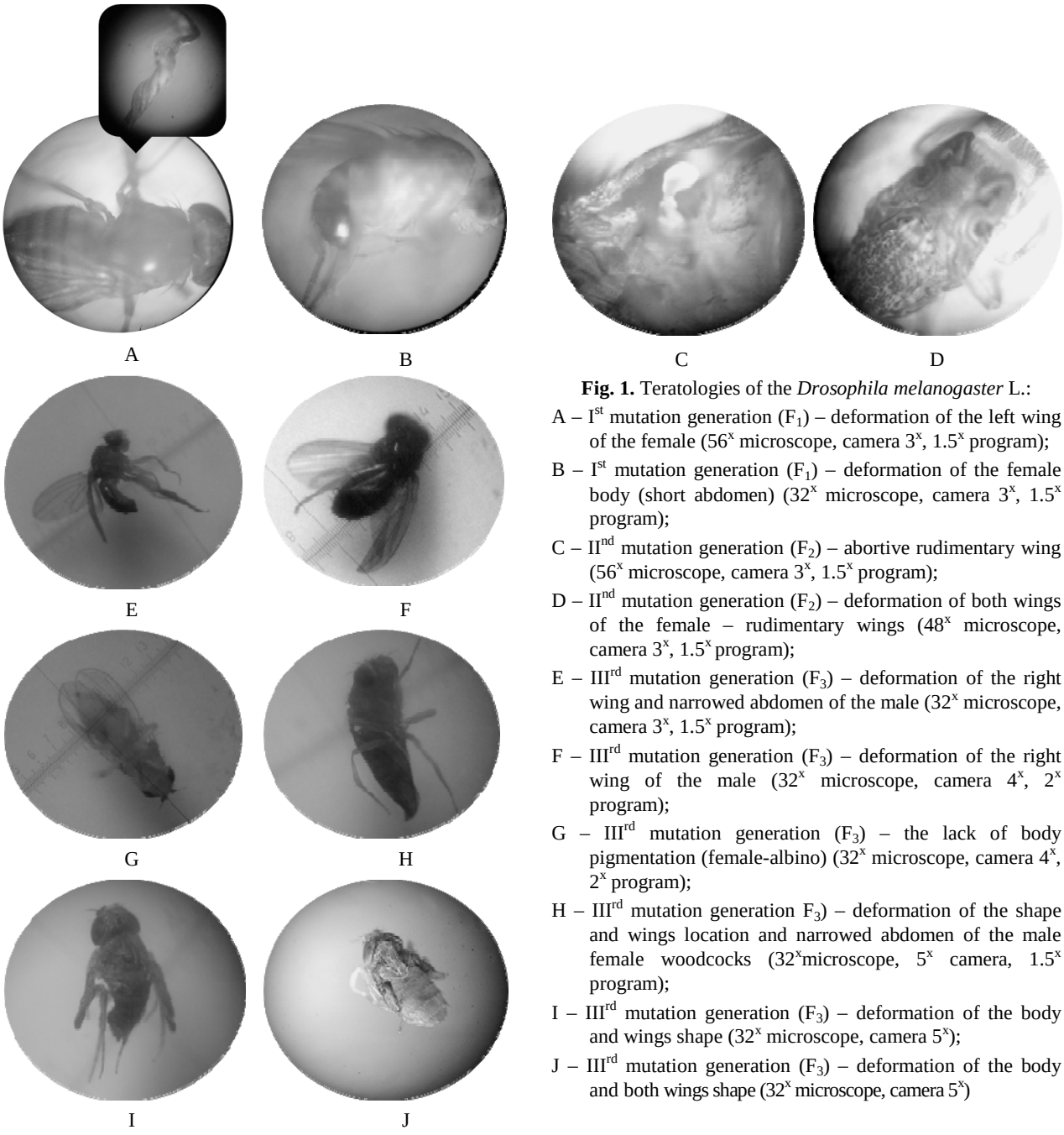

Fig. 1. Teratologies of the *Drosophila melanogaster* L.:

A – Ist mutation generation (F_1) – deformation of the left wing of the female (56^x microscope, camera 3^x, 1.5^x program);

B – Ist mutation generation (F_1) – deformation of the female body (short abdomen) (32^x microscope, camera 3^x, 1.5^x program);

C – IInd mutation generation (F_2) – abortive rudimentary wing (56^x microscope, camera 3^x, 1.5^x program);

D – IInd mutation generation (F_2) – deformation of both wings of the female – rudimentary wings (48^x microscope, camera 3^x, 1.5^x program);

E – IIIrd mutation generation (F_3) – deformation of the right wing and narrowed abdomen of the male (32^x microscope, camera 3^x, 1.5^x program);

F – IIIrd mutation generation (F_3) – deformation of the right wing of the male (32^x microscope, camera 4^x, 2^x program);

G – IIIrd mutation generation (F_3) – the lack of body pigmentation (female-albino) (32^x microscope, camera 4^x, 2^x program);

H – IIIrd mutation generation F_3) – deformation of the shape and wings location and narrowed abdomen of the male female woodcocks (32^{x}microscope, 5^x camera, 1.5^x program);

I – IIIrd mutation generation (F_3) – deformation of the body and wings shape (32^x microscope, camera 5^x);

J – IIIrd mutation generation (F_3) – deformation of the body and both wings shape (32^x microscope, camera 5^x)

The range of the mutant signs or features that are affected by mutations is very wide. There are no signs and features that could not mutate in different levels. All the morphological, physiological, biochemical, behavioral characteristics and properties are affected by genetic variation. These variations are expressed by qualitative and quantitative differences, or in other words, by average values of varying features. Mutations can occur in both directions: towards increasing and decreasing of the intensity of a particular feature or property. Mutations can be either very sharply expressed (up to mortality), or presented as non-significant deviations from the original form (the so-called "small" mutations).

At magnetic field induction about 2,25–5 μT frequency of mutations in *Drosophila melanogaster* L. within three generations varies in the range of 0,2–1 %, and the most prominent are the deformation of the abdomen and the modification of the wings (fig. 2). When the EMP induction is from 5 to 20 μT the frequency of mutations is increasing (up to 3 %) and the mortality is high (>15 %). New forms of mutations occur (fig. 1 G), or deformation of body parts increases (figs 1 H, I, J). It is established that the higher magnetic field induction is, the bigger the number of the mutated animals and a variety of mutations are (from slight distortion of body parts to the albinism and lethal gene mutations).

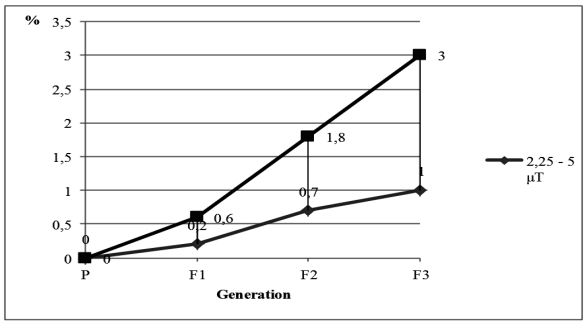

Fig. 2. Frequency of mutations in *Drosophila melanogaster* L. induced by electromagnetic radiation

4. Conclusions

Constant impact of the electromagnetic radiation with the induction over 2,25 μT predetermines the following phenotype subsequent mutations in three generations of *Drosophila melanogaster* L.:

- the deformation of body parts;
- the change in body pigmentation;
- the change of the size of the whole body or components;
- the replacement of one physiological organ by another one.

The level of the mutation changes in the phenotype does not depend on the intensity and duration of the mutagenic factors. So, a weak mutagen, which acts for a short time, sometimes is able to cause more considerable changes in the phenotype than the stronger one. However, with the increasing intensity of the mutagenic factor the frequency of mutations occurrence increases only up to a certain limit.

All of the mutagenic factors do not have the lowest limit of their action, it means that there is no such a limit of their intensity, under which they cannot cause mutations. This property of the mutagens has important theoretical and practical significance, as it proves that the genotype must be protected from all mutagenic factors, whatever low the intensity of their action is. Though, in the early stages of the body development the sensitivity to the mutagenic factors is higher than in adults.

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