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Terahertz Radiation Improves Adaptation Characteristics in *Drosophila melanogaster*

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Abstract—An investigation into the effect of nonionizing terahertz radiation (0.1–10 THz) on living organisms is urgent due to the recent development of modern technologies employing such radiation. The aim of this study was to establish the impact of terahertz radiation on successive generations of fruit flies. The effects of terahertz radiation on the survival ability and lifespan of the Oregon-R strain of *Drosophila melanogaster* proved to be diverse: they were negative or neutral at early life stages and positive at late stages. The female flies exposed to the radiation showed increased survival rate during the second half of the life of imago. The males demonstrated low sensitivity to the radiation. There were no significant differences noted in the dynamics of maturation and total number of offspring between the female flies that were exposed to the radiation and those that were not. The results of the study practically did not depend on the sex and maturity stage of the oocytes irradiated.

Keywords: biological effects of terahertz radiation, drosophila, lifespan, survival rate, sexual dimorphism, oocyte

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INTRODUCTION

In the modern fundamental science, interest has arisen toward the study of the interaction of nonionizing terahertz radiation (0.1–10 THz) and living matter due to the possible biological danger of technologies developing on its basis recent years. In a number of studies, data on different effects of terahertz radiation on biological systems of different levels of organization were obtained. The experimental data are determined not only by the source characteristics and radiation dose, but also by the properties of the biological objects: isolated biological macromolecules, bacteria, cell cultures, and whole organisms (Kirichuk et al., 2008; Wilmink and Grundt, 2011).

It was demonstrated that the radiation induced conformational transitions in DNA and protein molecules. In experiments on fresh red blood cells, it was found that the radiation decreased the osmotic resistance of the cell membrane and increased the spontaneous release of hemoglobin. After the irradiation of isolated neurons of the supraesophageal ganglion of a pond snail and cultures of different human cell lines, a change in the structure of the cell membrane was observed (Zalyubovskaya, 1970; Fedorov, 2011).

The radiation affects the stability and proliferative activity of human lymphocytes in a cell culture; induces multidirectional changes in gene expression in the cell culture, bacteria, and many-celled animals; and reprograms differentiation of stem cells of mammalians. A change in expression could be observed several successive generations after the exposure (Fedorov, 2011; Alexandrov et al., 2013; Bock et al., 2010; Demidova et al., 2013; Wilmink et al., 2010). High-intensity radiation could induce cell death in a culture, sterility, and recessive lethal mutations in the first generation of drosophilas. In the successive generations of the animals irradiated, the spectrum of mutational changes broadens. Low intensity radiation has a positive effect, induces an increase in the fertility of *Drosophila*, initiates a decrease in the number of somatic mutations in the wings of adult flies induced by γ irradiation in the larval period, enhances reparation in the region of burns in humans by epithelization, and decreases microbial insemination (Zalyubovskaya et al., 1970; Fedorov et al., 2001; Wilmink and Grundt, 2011). It is of interest that terahertz radiation affects behavioral responses in male mice, inducing a change in motor activity and increased anxiety in animals (Bondar et al., 2008).

In our study we observed the effects of terahertz radiation in successive generations of fruit flies, parents, and their offspring F1. We analyzed changes in important adaptive traits, survival and lifespan (LS) in males and females of a model object, *Drosophila*. We studied the effect of the radiation on the numbers of imago, sex ratio, and peculiarities of the dynamics of development of offspring F1 having originated from irradiated drosophila females and males of the initial strain. The sensitivity of oocytes at different developmental stages to different external effects is not similar (Weisman and Golubovsky, 2008). In connection with this, in the present study we evaluated the data in offspring F1 having originated from oocytes mature or immature at the moment of irradiation of females of *Drosophila*.

MATERIALS AND METHODS

In the experiment we used a classical normal inbred line, the Oregon R strain, of *Drosophila melanogaster* from the resources of the Laboratory of Genetics of Populations of the Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences. To perform the experiment, the line was bred at a normal temperature of 25°C. Then, using a binocular loupe, virgin males and females were taken immediately after the emergence of the imago during the day. During selection, the animals were anaesthetized with ether. First, *Drosophila* were put into similar vials with a standard nutrient medium, females being kept separate from the males.

Then, flies of both sexes at an age of 2–3 days were put into the restricted space of containers (standard plastic Eppendorf tubes with a height of 50 mm and diameter of 5.5 mm), 20–30 individuals in each container, without food. The tube openings were covered by parafilm films with openings for air supply made using a needle. In a test experiment, it was found that the stretched film absorbs no more than 1% of the irradiation. The half of the tubes with flies was irradiated sequentially, with each being irradiated for 30 min. The total time that the flies were in the tubes was about 2.5–3 h.

The study of the dynamics of survival rate and LS. For this experiment, 480 flies were put into containers, 240 of them being subjected to terahertz radiation. An analysis of the LS was performed under standard conditions, placing *Drosophila* that had been subjected to the irradiation and those that had not into vials with fresh food into thermostats at a constant temperature of 25°C (30 individuals per vial). Every 3 days the flies were transferred to fresh food and the mortality was recorded through the end of the life cycle of all flies of the line in this experiment variant. The flies that were not irradiated under stress (120 flies of each sex) were considered an inner control group (control 1). The outer control group was represented by *Drosophila* that in the experiment were under conventional conditions for line cultivation using standard food (control 2).

The study of peculiarities of the dynamics of development of offspring F1. The irradiated and nonirradiated females were placed into standard laboratory conditions on fresh feed at a temperature of 25°C (20 individuals each in separate glass) and immediately males were planted from the outer control group. Every 2 days the parental flies were transplanted into other glasses to reveal the effects of influence of radiation on mature and immature oocytes by the moment of irradiation. From consecutive 2-day ovipositions we obtained offspring. It is known also that oogenesis in females of *Drosophila* proceeds ceaselessly, on the strength of which at every moment of time the ovaries of *Drosophila* contain oocytes of different degrees of maturity. Ovipositions from females in the first 2 days after irradiation correspond to oocytes mature by the moment of irradiation. Offspring obtained from eggs laid 9–10 days after irradiation developed from oocytes immature by the moment of irradiation, inasmuch as one cycle of oogenesis, starting from the stage of sexual stem cells, occupies no more than 10 days (Ogienko et al., 2007; Horne–Badovinac and Bilder, 2005). Beginning from the first day of emergence of imago, in offspring F1 of all experiment and control groups each day we registered the numbers of emerged flies and their sex and evaluated the dynamics of emergence of imago to the last day of emergence.

Terahertz radiation source. A system made at the Institute of Automation and Electrometry, Siberian Branch, Russian Academy of Sciences was used. The system generates broadband pulse terahertz radiation within a range of 0.1–2.2 THz with a pulse duration of 1 ps and pulse power of 8.5 mW. The pulse repetition frequency was 76 MHz. The irradiation was modulated with a frequency of 10 kHz. A detailed description of the system is given in (Antsygin et al., 2010). The diameter of the Eppendorf tube corresponds to that of the terahertz ray (5 mm).

Statistical methods. Specific effects of influence of terahertz radiation on the studied traits were evaluated comparing the data of the experimental and outer and inner control groups. To determine the reliability of distinctions between the curves of survivability in experiment and control samples of *Drosophila*, we applied a logrank test (Bland and Altman, 2004). The correspondence of experimental splittings on the basis of sex to the theoretically expected ones was evaluated with the aid of a χ^2 criterion (Rokitskii, 1973). To determine the significance of the differences between the LS values, a Student's *t*-test was performed.

RESULTS

It is shown that maintenance of *Drosophila* in the course of several hours even on a depleted nutrient medium (Raushenbakh et al., 2004) or at elevated density in limited space (Mosse et al., 2006) causes a nonspecific stress reaction. In the given study, the flies put into tubes were subjected to a combination of stressor factors: space limitation, crowding, and food deprivation. In connection with this, the results of terahertz radiation are considered against the background of this combined stress.

Dynamics of mortality and LS. The mean lifespan of the Oregon R females of the outer control group was 34.3 ± 1.36 days; that of the males was 32.5 ± 1.2 days, the LS being lower than was observed before. The sex differences in LS indices and profiles of survival curves typical for the line with a sharp increase in the mortality at the last stage of life were preserved. The figure gives curves of the decrease in the number of the control males and females over the entire period of observation (Fig. 1a), which, on the whole, differ significantly ($\chi^2 = 13.79$; $p < 0.05$).

The survival rate of females under short combined stress (space limitation, crowding, and food deprivation) throughout the entire experiment did not change when compared to the outer control. There were no significant differences found between the curves of these groups of females ($\chi^2 = 0.57$; $p < 0.05$). The survival of females of the experimental group in the first half of life did not differ from that of the outer control group, but a positive effect on the survival of females was observed in the second half of the life. The females irradiated had their "slope point" shifted 9 days later when compared to the outer control group. The survival curves, on the whole, for the females irradiated and those of the outer control group significantly differed ($\chi^2 = 9.98$; $p < 0.05$). The irradiation of females significantly increased the survival of the flies under stress, with the survival curves for the flies under stress and those for the animals irradiated differing significantly ($\chi^2 = 5.98$; $p < 0.05$) (Fig. 1b).

For most of their lives, the effect of stress on the survival in the males was negative and their mortality was higher than in the control. In the last third of their lives, they were observed to have a significant increase in the survival rate when compared to the outer control. On the whole, the survival curves for the males of this group and those of the outer control group significantly differed ($\chi^2 = 5.97$; $p < 0.05$) (Fig. 1c). Terahertz radiation after short-term stress almost did not affect the survival of the males. The survival curves of the animals of the two groups, inner control and irradiated, did not differ ($\chi^2 = 1.21$; $p < 0.05$). As in the flies under stress, the profile of the dynamics of mortality of the animals irradiated significantly differed from that of the outer control ($\chi^2 = 9.39$; $p < 0.05$). The mean lifespan of males and females in all the groups studied did not significantly differ.

Peculiarities of the dynamics of F1 development. Offspring of mature oocytes. The object of our study was the Oregon R strain of *Drosophila melanogaster*. At normal temperature of 25°C and standard nutrient medium, the life cycle of F1 offspring up to the imago stage constitutes about 10 days. Under standard conditions, in different variants of the control and experimental groups, the expanse of periods of emergence and the dynamics of the development of the animals could slightly vary. The natural development of fruit flies and time characteristics of other physiological and ontogenetic processes depend on a complex dynamic set of factors of the environment. Many factors cannot be taken into account in the experiment. Special research is required to reveal their origin.

In generation $F1_{oc}$ with parents from the outer control group developed from oocytes mature at the moment of irradiation of the females under study, the

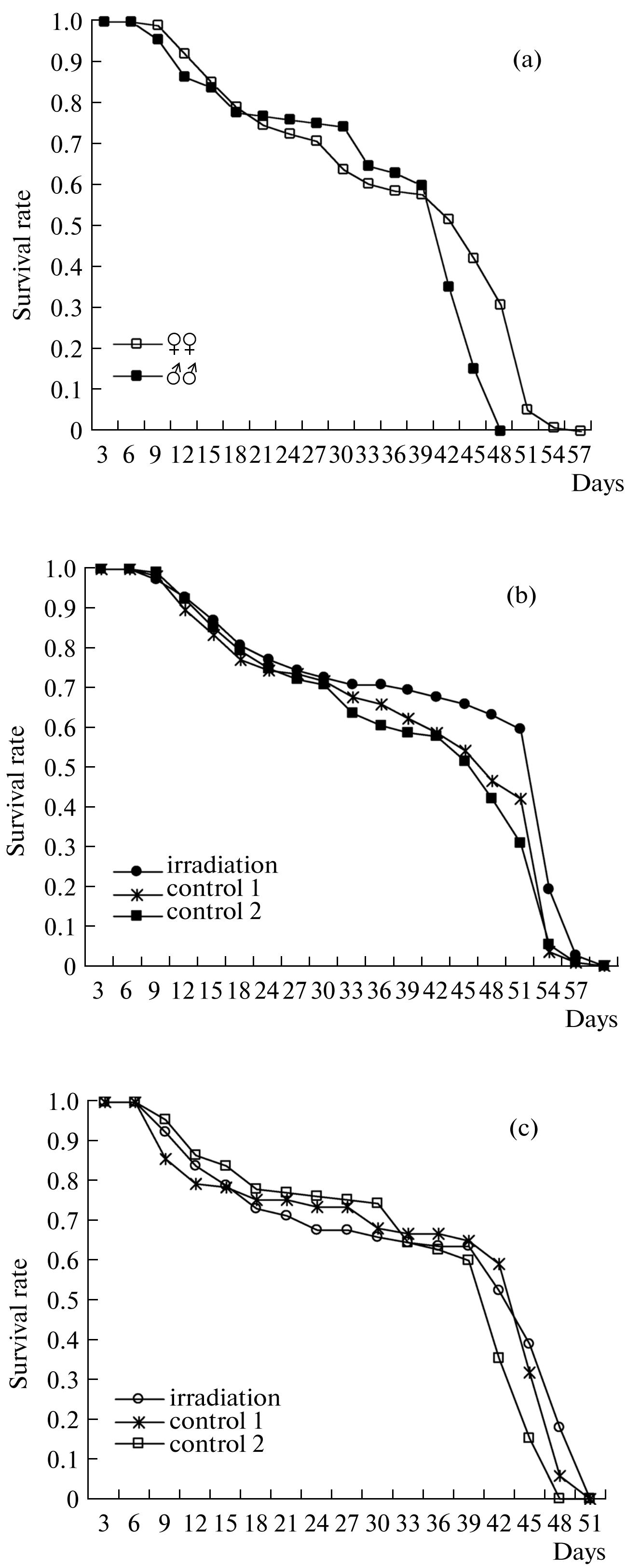

Fig. 1. Survival rate of imago of Oregon R *Drosophila melanogaster* at 25°C in control 2 (a), in females (b), and in males (c).

dynamics of maturation up to the imago stage in males and females differed ($\chi^2 = 7.06$; $p < 0.05$). The dynamics of emergence of female and male imago in generation $F1_{sc}$ from stressed nonirradiated females and intact males (the outer control group) significantly changed when compared to generation $F1_{oc}$ ($\chi^2 = 16.72$, $\chi^2 = 6.7$, respectively; $p < 0.05$) and differed in females and males ($\chi^2 = 15.02$; $p < 0.05$). The total number of females and males emerged in $F1_{oc}$ and $F1_{sc}$ constituted 714, 710 and 742, 727 specimens, respectively. Short-term stress almost did not affect the number of imagoes in generation $F1_{sc}$ and sex ratio.

In generation $F1_{tr}$ from the females subjected to terahertz radiation and intact males, significant differences in the dynamics of emergence between females and males were found ($\chi^2 = 13.29$; $p < 0.05$). The dynamic of emergence of females and males in this offspring significantly differed from that in group $F1_{oc}$ ($\chi^2 = 10.47$, $\chi^2 = 3.9$, respectively; $p < 0.05$). Observable differences between the dynamics of emergence of females and males in the offspring of the females irradiated and stressed upon pair statistical comparison were found to be insignificant ($\chi^2 = 1.21$, $\chi^2 = 0.31$, respectively; $p > 0.05$). The total number of offspring $F1_{tr}$ constituted 683 males and 724 females. Statistical estimates demonstrated that, in the all generations ($F1_{oc}$, $F1_{sc}$, and $F1_{tr}$), the deviations from the theoretically expected sex ratio 1 : 1 were unreliable ($\chi^2 = 00.01$, $\chi^2 = 0.15$, $\chi^2 = 1.19$, respectively; $p > 0.05$) (Fig. 2a).

Offspring of immature oocytes. The dynamics of development of males and females up to the imago stage in generation $F1_{oc}$ from intact females and males having originated from oocytes immature at the moment of irradiation of the experimental females differed insignificantly ($\chi^2 = 0.02$, $p > 0.05$). In group $F1_{sc}$ (offspring of females under stress and intact males), the dynamics of emergence of females and males differed reliably ($\chi^2 = 9.24$, $p > 0.05$) and varied significantly from offspring $F1_{oc}$ (females, $\chi^2 = 6.66$; males, $\chi^2 = 23.92$). The number of females and males in $F1_{oc}$ and $F1_{sc}$ constituted 711, 767 and 784, 834 specimens, respectively. The splitting in regards to sex in the both cases approached the theoretically expected splitting 1 : 1 ($\chi^2 = 2.12$, $\chi^2 = 1.55$, respectively; $p > 0.05$).

In generation $F1_{tr}$ from females subjected to terahertz radiation from eggs laid 9–10 days after irradiation (which corresponds to oocytes immature at the moment of irradiation), the dynamics of maturation of females and males almost coincided with the dynamics typical for the animals in the group $F1_{sc}$ ($\chi^2 = 0.92$, $\chi^2 = 0.12$, respectively; $p < 0.05$) but was completely different when compared to the data for $F1_{oc}$ ($\chi^2 = 11.34$, $\chi^2 = 20.94$, respectively; $p < 0.05$). The total number of males that emerged was found to be smaller than that of females (747 and 799 specimens, respectively), which did not lead to significant deviation from the theoretically expected splitting in regards to sex 1 : 1 ($\chi^2 = 1.75$, $p < 0.05$) (Fig. 2b).

DISCUSSION

Model experiments on *Drosophila* make it possible to follow the dynamics of aging and mortality in populations of individuals of the same age and to assess the effects on these traits of gender and environment. A comparison of model data with those of humans makes it possible to predict demographics and their dependence on environmental impacts.

The effects of terahertz radiation on survival and LS under stress in our experiment with *Drosophila* of the Oregon R line were multidirectional, depending on animal age. The effect on survival in early periods of the life of *Drosophila* was negative or neutral; in the later periods, it was positive. The phenomenon of the duality of vital structures and processes, antagonistic pleiotropy, is widespread in nature. Age-specific antagonistic pleiotropy is peculiar to the LS genes of *Drosophila* (Nuzhdin et al., 1997). Gender differences were manifested in the response of *Drosophila* to terahertz radiation. The males appeared to be less susceptible to radiation. In irradiated females, survival significantly increased by the second half of imago life. The irradiation of *Drosophila* did not significantly affect the values of the mean and maximal LS when compared to the control, but it increased the gap between the values of the mean LS of the males and females in this animal group. A gender specificity of the response to terahertz radiation was also found in experiments with rats, with females being more susceptible to this exposure (Kirichuk et al., 2008).

To find remote effects of terahertz irradiation of female of *Drosophila*, the number of imago, sex ratio, and peculiarities of the dynamics of the development in generation F1 were analyzed. Despite the fact that there were differences observed at individual time intervals, the indices of the dynamics of maturation between the offspring of irradiated and nonirradiated flies under stress, on the whole, did not differ. The results almost did not depend on the maturation stage of the oocytes irradiated. The total number of the specimens having reached the imago stage differed insignificantly. There was a tendency observed toward a decrease in the total number of males in the offspring of the females irradiated corresponding to the mature oocytes when compared to the inner control, though the ratio between sexes did not differ from the theoretically expected splitting 1 : 1. It could be suggested that mature oocytes are sensitive to the effect of terahertz radiation. The response could be determined by frequency and power of the radiation, exposure, dose, etc. At other experimental parameters with other genetic lines of *Drosophila*, other effects of terahertz radiation on the offspring of the females irradiated could be found.

As for certain mechanisms of the effect of terahertz radiation on living objects, it could be connected with a change in the functional activity of cells and gene expression. The results of the experiments of N.P. Zalyubovskaya (1970) in vitro in lines of human cell cultures made it possible for the author to suggest

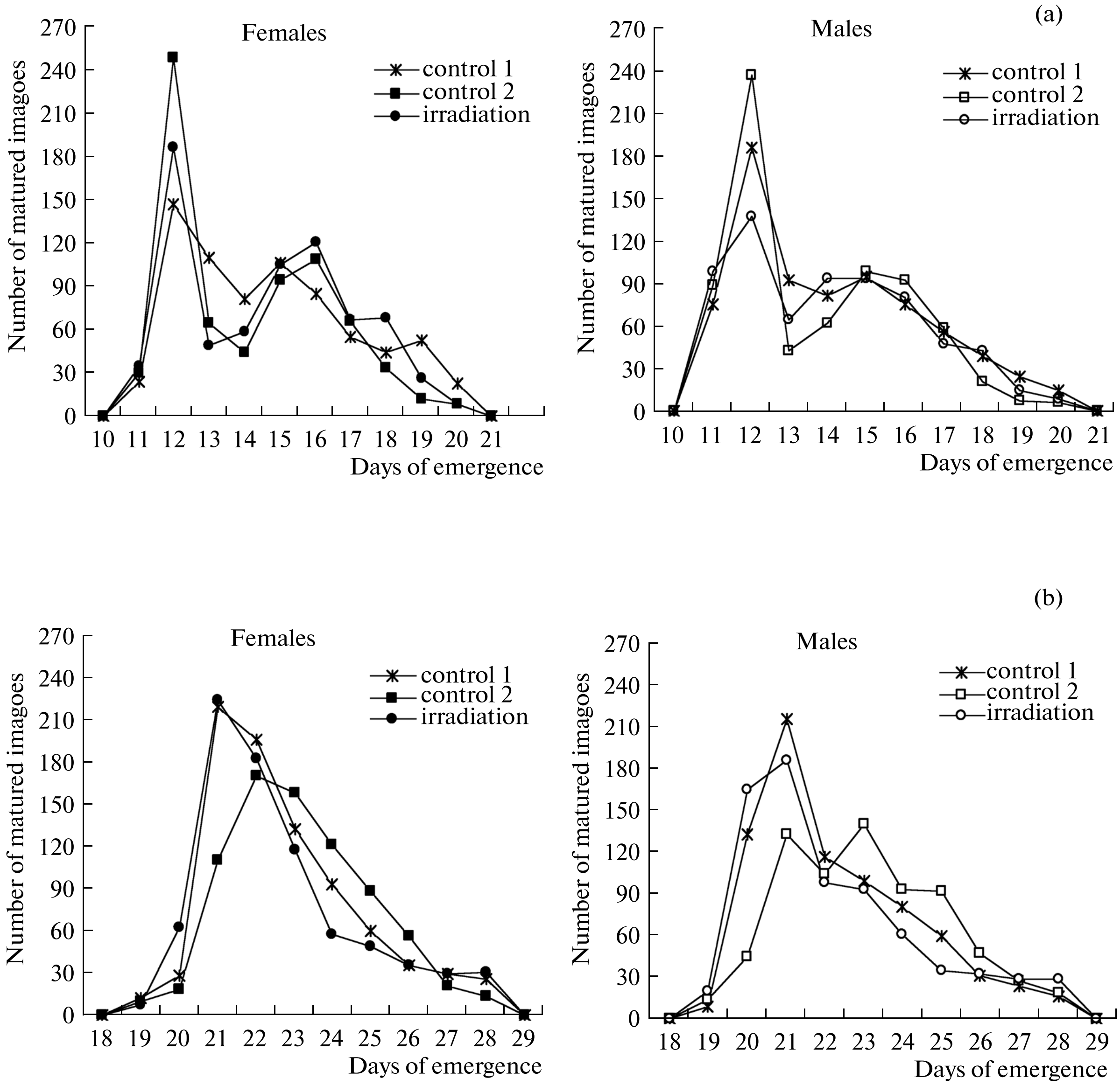

Fig. 2. Dynamics of maturation of males and females in offspring of control and experimental flies of the Oregon R line. Oocytes mature (a) and immature (b) at the moment of irradiation.

that terahertz radiation could directly affect the DNA function. Later, the experimental data indicating the induction of changes by terahertz radiation at the molecular level were obtained. It was found that it could induce the partial or complete destruction of hydrogen bonds between complementary strands of DNA, significantly change the degree of condensation of DNA in the chromosome, and lead to a change in the regulation of gene expression and DNA replication (Alexandrov et al., 2010). In response to the terahertz irradiation of a culture of mesenchymal mouse stem cells, expression of part of the genes is suppressed or activated as a result of direct partial activation or suppression of transcription factors (Bock et al., 2010). Similar data on a change in the gene expression were obtained in cells of a number of human lines (Wilmink et al., 2010).

A direct connection between the effect of terahertz radiation and a change in the expression of the *katG* and *copA* genes was found in the experiment with *E. coli*. A stable induction of the expression of the GFP reporter gene with the KatG promoter was found for a prolonged time after terahertz irradiation. The expression change was preserved for the following eight generations after irradiation (Demidova et al., 2013). The mechanisms for the effect of terahertz irradiation on survival and LS in our study might be connected with changes of the cellular membrane, gene expression, and signaling pathways governing these features.

CONCLUSIONS

The effects of terahertz radiation on the survival and LS under stress in our experiment *Drosophila* of the

Oregon R line were multidirectional depending on animal age. The effect on the survival in early periods of the life of *Drosophila* was negative or neutral; in the later periods, it was positive. Gender differences were manifested in the response of *Drosophila* to terahertz radiation. The males appeared to be less susceptible to radiation. In the irradiated females, survival significantly increased by the second half of imago life. The irradiation of *Drosophila* did not significantly affect the values of the mean and maximal LS when compared to the control, but it increased the gap between the values of the mean LS of the males and females in this animal group. Significant remote effects of terahertz radiation on the female of *Drosophila* in regards to the total number of imago, sex ratio, and peculiarities of the dynamics of the development in offspring F1 were not observed. Despite the fact that there were differences observed at individual time intervals, the indices of the dynamics of maturation between offspring of irradiated and nonirradiated flies under stress, on the whole, did not differ. The results almost did not depend on the maturation stage of the oocytes irradiated.

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