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Effects of microwaves on the puffing pattern of *D. melanogaster*

Research Article

Lyubov A. Shakina, Vladimir N. Pasiuga, Olexandr M. Dumin, Yuriy G. Shckorbatov*

Institute of Biology, Kharkiv National University, 61077 Kharkiv,Ukraine

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Abstract: The influence of electromagnetic field exposure on puffing pattern of salivary gland polythene chromosomes, viability and fertility of *Drosophila melanogaster* of the wild type Canton-S line was studied. *Experimental conditions:* Electromagnetic field characteristics: frequency – 36.64 GHz, power density – 0.4 W/m^2, exposure time -10 seconds. Electromagnetic field exposure was conducted on the egg stage. *Results*: in larvae developed from the exposed eggs 3 of 8 chromosomal puffs tested (71CE, 82EF, and 83E) had significantly smaller dimensions than these in control at the prepupal stage. Viability of *Drosophila* estimated by the number of adult flies hatched from exposed eggs decreased, while the number of dominant lethal mutations increased. *Conclusion*: the exposure to a low-level microwave irradiation suppressed puffing activity at ecdysone-inducible loci of *Drosophila* polythene chromosomes, increased frequency of dominant lethal mutations and decreased *Drosophila* viability but did not influence *Drosophila* fertility.

Keywords: *Electromagnetic field • Non-ionizing radiation • Polythene chromosome • Puff • Salivary gland chromosome*

1. Introduction

The effects of electromagnetic fields on biological objects are intensively studied in connection with problems of so-called "electromagnetic pollution" and possible medical applications of electromagnetic fields. The investigation of such effects, especially on the DNA and chromosomal level is of great interest. In our previous work it was demonstrated that microwave radiation induced a significant decrease in viability of *Drosophila* assessed by counting the number of progeny from previously exposed flies [1-4]. In scientific literature there are different views on the possibility of induction of mutations in *Drosophila* by microwaves. No significant mutagenic effects of microwaves (12 MHz, SAR up to 20 W/kg) on the genes controlling eye pigmentation in *Drosophila melanogaster* were detected by Hamnerius *et al.* [5]. Marec *et al.* [6] revealed no significant mutagenic effect of microwave radiation (frequency 29, 98.5, 146.36 and 2375 MHz, exposure time – 5 min, intensity of irradiation in the range from 15 to 25 W/cm^2) on sex-linked recessive lethal mutation of *Drosophila melanogaster*. But the other research groups have registered a considerable mutagenic effect of microwaves [4,7,8]. Shckorbatov *et al.* [4] showed an increasing rate of dominant lethal mutations induced by microwave radiation (35 GHz, intensity from 30 to 265 μW/cm^2, exposure time 10 sec). The increased puffing activity and increased mutation rate in *Drosophila melanogaster* induced by 2.45 GHz microwave radiation (30-60 sec.) were demonstrated by Tonomura *et al.* [7,8]. It should be noted, however, that the parameters of the latter experiments raised some concerns because a microwave oven has been used as a source of electromagnetic radiation. Effects of microwaves on *Drosophila* fertility has been demonstrated by Atli &Unlü [9,10]. The maximum decrease in fertility of *Drosophila* under microwave irradiation was observed by Zalyubovskaya [11] when the flies were irradiated by microwaves with a wavelength of about 7 mm, and the irradiation with 5,5 and 8 mm waves was less effective. No differences were found in viability at larva-pupa and pupa-imago stages, however, it was found that the mean pupation time was delayed linearly with increasing exposure time to electromagnetic field with frequency 10 GHz. In the

* *E-mail: yury.g.shckorbatov@univer.kharkov.ua*

exposed group (3 h exposure + 30 min interval + 3 h exposure) the mean offspring number was significantly less than that of the control [9]. The same 10 GHz EMF exposure (with SAR approximately 9,8 mW/kg) decreases fertility of *Drosophila melanogaster* causing the decrease in the egg production [10]. Exposure of the flies to the carrier frequency 900 MHz "modulated" by human voice, (speaking emission), for 6 min per day during the first 2-5 days of their adult lives, decreased the reproductive capacity of *Drosophila melanogaster* by 50-60%, whereas the corresponding "non-modulated" field decreased the reproductive capacity by 15-20% as shown by Panagopoulos *et al.* [12]. In our opinion, the described "modulation" effect may be caused by an energy saving process in cellular phone in silence mode. In other work by Panagopoulos *et al.* [13] *Drosophila* flies were exposed in vivo to either GSM 900-MHz (Global System for Mobile telecommunications) or DCS 1800-MHz (Digital Cellular System) radiation from a common digital mobile phone, for a few minutes per day during the first 6 days of their adult life. The authors reported that decease in fertility was due to a degeneration of a large number of egg chambers (follicle cells, nurse cells and the oocyte). Microwave-induced DNA fragmentation in the cells of the exposed flies was found at all the stages of an early and mid-oogenesis [13].

As it was shown by Lee *et al*., the microwave-induced changes in *Drosophila* are connected with changes in protein kinase regulation systems namely, electromagnetic field of frequency 835 MHz activated extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) signaling, but not p38 kinase [14]. At SAR 1.6 W/kg mainly ERK signaling was activated, whereas at SAR 4.0 W/kg JNK signaling was strongly activated. In addition, SAR 4.0 W/kg increased the number of apoptotic cells in the fly brain [14]. Exposure to microwaves (1.900 MHz; SAR – 1.4 W/kg) of *Drosophila melanogaster,* during the 10-day developmental period (60 min at 11 AM and 60 min at 4 PM daily) from egg laying through pupation increased numbers of offspring, elevated hsp70 levels, increased serum response element (SRE) DNA-binding and induced the phosphorylation of the nuclear transcription factor, ELK-1 as demonstrated Weisbrot *et al.* [15]. Showing an increase in fertility [15] is in some contradiction to the data presented in [9-14]. In our opinion, the experimental conditions in this work [15] were different from those in [9-14]. Specifically, it is feasible that the microwave-exposed larvae of *Drosophila* were exposed to microwaves in cultural medium with high water content that led to intensive absorption of microwave energy by the media. As a result, the exposed larvae received only a small part of total microwave energy.

Polythene chromosomes, typical to many of the larval tissues of *Diptera,* are uniquely suited for analysis of structural and functional activity of interphase chromosomes. At the sites of active transcription so-called puffs – regions of decondensation of chromosomes – are observed. By directly measuring puff dimensions one may assess the level of transcription [16]. The aim of our work was to study the effects of microwave radiation on the puffing activity of polythene chromosomes of *Drosophila melanogaser*.

2. Experimental Procedures

As an experimental object, the inbred wild-type line of *Drosophila melanogaster* Canton-S (CS) was used. This stock was obtained from *Drosophila* collection of the Department of Genetics and Cytology of the Kharkov National University by courtesy of the members of the Department of Genetics and Cytology. Flies were grown on a standard sugar-yeast medium at 24 ± 0.5°C. The synchronous hatches of *Drosophila* were obtained by the following procedure: eggs were laid during 3 hours by 30 4-days old *Drosophila* females.

The eggs of *Drosophila* were exposed to microwaves (frequency 36.64±0.05 GHz; power density at the surface of exposed object – 0.4 W/m^2). As a source of microwave radiation we used a semi-conductor device constructed on the base of Gunn diode. Irradiation was realized by almost plane wave (15 cm from horn antenna edge); exposure time in all experiments was 10 seconds. To estimate SAR for eggs at the above mentioned radiation exposure the numerical simulation of the field distribution in eggs by FDTD method [17] was carried out. We took into account the influence of the sugar-yeast medium and glass test-tube. Known data about the form of *Drosophila* eggs [18] was used to estimate their dielectric properties. *Drosophila* egg may be described as an ellipsoid of rotation with greater diameter 450 mm and smaller diameter 150 mm. Its shell consists of five layers [19,20]. Overall water contents is 76% [21]. The simulation shows that SAR distribution inside eggs is homogeneous enough; the heating in different points differs no more than twice. The surface of the sugar-yeast medium under an egg is heated three times more than eggs. The average SAR of eggs equals to 0.24 W/kg that is twice less than the maximum SAR for one egg. The mean increase of egg temperature under irradiation is less than 0.0008 grad, the maximum increase in temperature is less than 0.0016 grad. It is evident that there is no significant thermal effect in microwave exposed eggs in our experiment as well as there is no need to account for the heat transfer from the sugar-yeast medium.

Puffing activity in *Drosophila* salivary gland giant chromosomes was investigated by orcein staining using the standard methodology [22] with magnification of x 800. The puffing activity in polythene chromosomes was evaluated in females at the 0-hour prepupal stage. Estimation of puffs dimensions was carried out in the chromosomes with degree of polytheny: 1024. Puffs were localized with the aid of chromosome maps by Briges [23]. Eight puffs were studied: 21F, 22C, 23E (chromosome 2L), 63F, 71CE, 72CD (chromosome 3L); 82EF, 83E (chromosome 3R). The photos of these puffs are presented in Figure 1 Dimensions (width) of puffs were measured by the ocular micrometer and compared with the width of adjacent chromosomal disc not involved in the process of puffing. Puff size ratio 21F/22B, 22C/22B, 23E/24C, 63F/64B, 71CE/73A, 72CD/73A, 82EF/84A, 83E/84A was measured. The average puff size ratio was determined in 25-40 nuclei in each variant of the experiment, in not more than in five nuclei in each preparation. For each experiment 5-17 larvae were used.

The *Drosophila* fertility was assessed by the mean number of eggs laid by 10 *Drosophila* females during an 8-hour period, calculated per one fly. The experiments were done in triplets (3 series of 10 flies each).

The frequency of dominant lethal mutations (DLM) was determined by the standard methodology [23] by counting eggs that ceased development. Some of the eggs ceased their development during the first 9 hours. The frequency of such events was considered the frequency of early dominant lethal mutations [24]. Some eggs ceased their development in the period of 9-72 h. The frequency of such events was considered the frequency of late dominant lethal mutations [24]. The frequency of mutations in non-exposed *Drosophila* hatchings was used as a control.

The viability of *Drosophila melanogaster* after egg exposure to microwaves was evaluated by the quantity of adult insects developed from the exposed eggs in one synchronous hatching; the experiment was repeated 3 times and each experiment consisted of triplicates. In the control variants eggs in the synchronous hatchings were sham-exposed.

The experimental data were processed by the Student method. Statistical significance was assumed for $P<0.05$.

3. Results

The results of the study of the influence of microwave radiation on the puffing activity in polythene chromosomes are presented in Figure 2.

As one can see from the Figure 2, in larvae that developed from the exposed eggs (experiment) the size of puffs in polythene chromosomes was reduced as compared to unexposed (control) larvae. These microwave-induced changes were significant in loci: 71CE (25.1% reduction of puff width, $P<0.05$), in puffs 82EF, and 83E, where reduction of puff width was,

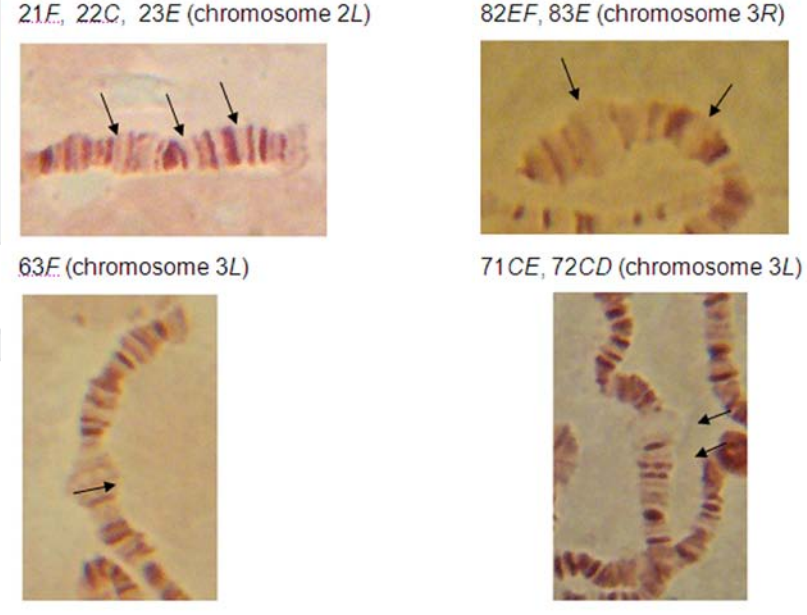

Figure 1. Puffs in polythene chromosomes of *D. melanogaster*: 21F, 22C, 23E (chromosome 2L), 63F, 71CE, 72CD (chromosome 3L); 82EF, 83E (chromosome 3R), at magnification × 1800.

respectively 16.5% (P<0.05) and 15.5% (P<0.01). In loci 21F, 22C, 23E, 63F, and 72CD the puff dimensions were not changed significantly but, nevertheless, the tendency for decreasing the puff dimensions was observed in puffs 21F, 23E, 63F, and 72CD. Thus, the results obtained in our study show mainly negative influence of microwave radiation on the process of transcription in polythene chromosomes of *Drosophila*. In this series of experiments no significant effects produced by microwave exposure on *Drosophila* fertility was shown. In the control group the number of eggs laid by one female during 8 h was 18.1±0.7, in the microwave-exposed group – 20.1±0.7. The results of assessment of the frequency of dominant lethal mutations are presented in Figure 3. The experimental data about frequency of early dominant lethal mutations is presented in Figure 3.

As one can see from Figure 3a, the flies developed from the exposed eggs in experiments 2 and 3 revealed a significantly elevated level of early dominant lethal mutations (P<0.05). In the first experiment, a tendency for an increase in the frequency of dominant lethal mutations was discovered. The frequency of late dominant lethal mutations was significantly increased in flies developed from the exposed eggs only in experiment 2 (Figure 3b). As a result, the overall frequency of dominant lethal mutations in *Drosophila* exposed to microwaves was significantly increased in two of three experiments (experiments 2 and 3 in Figure 3c).

The effect of microwave radiation on the viability of *Drosophila* was studied by means of counting the number of adult flies developed from exposed hatchings, as it is described in Materials and Methods section. This characteristic is integral and comprises several components: viability at the egg, larval and pupal stages. The number of male flies developed from synchronous hatchings is presented in Figure 4a.

As it is shown in Figure 4a, the number of male offspring is significantly less if eggs were exposed to microwaves in two of three experiments. The number of female offspring developed from the eggs exposed to microwaves is also significantly less (p<0.05) than from control hatchings (Figure 4b).

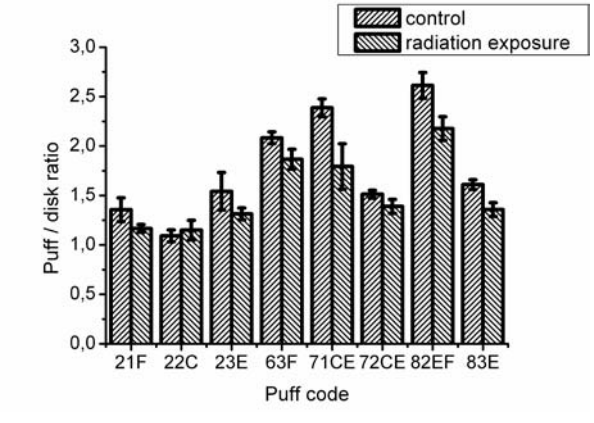

Figure 2. Effect of microwave radiation exposure on puffing pattern in polythene chromosomes of *Drosophila melanogaster.*

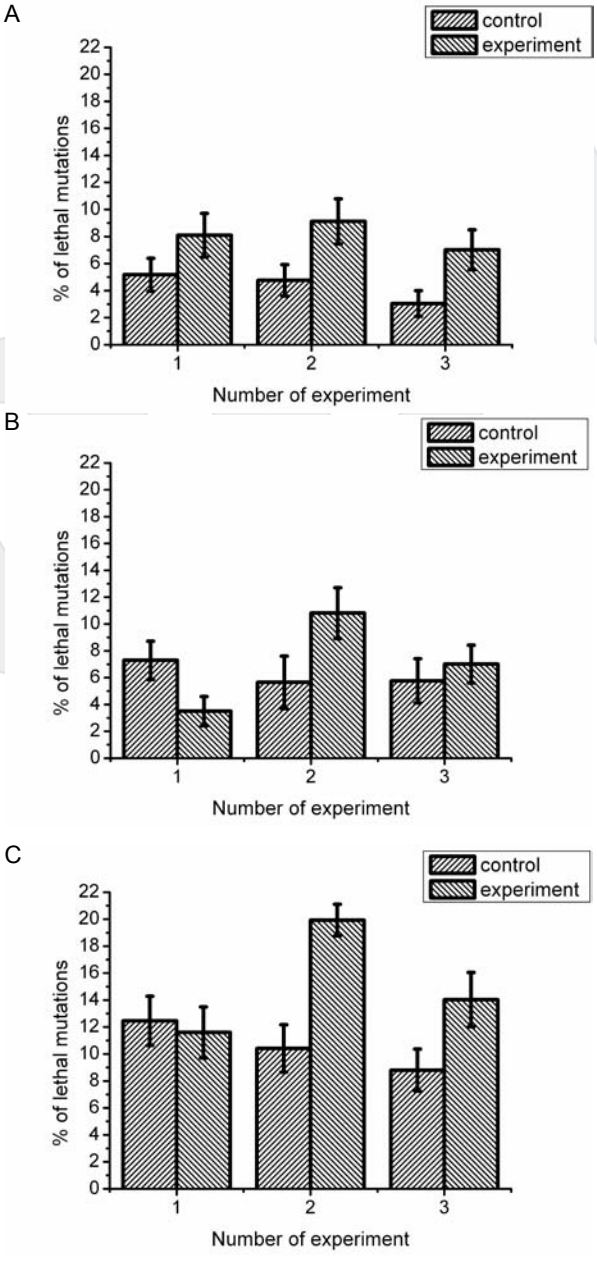

Figure 3. Effect of the microwave radiation exposure on the frequency of dominant lethal mutations in *Drosophila melanogaster* (a – early dominant lethal mutations, b – late dominant lethal mutations, c – sum of early and late dominant lethal mutations).

As a result, the overall number of offspring in exposed hatchings was less than in the control ones in two of three experiments (Figure 4a).

As it was mentioned above, the data presented in Figure 4 represent an overall *Drosophila* viability on the stages from egg to imago (adult fly). The data on viability of *Drosophila* in control and experimental (exposed) groups at the critical to *Drosophila* stages of metamorphosis (larva-pupa and pupa-imago) is presented in Figure 5.

The data in Figure 5 show that the microwave exposure even increase the percentage of *Drosophila* passed through larva-pupa and pupa-imago metamorphosis.

4. Discussion

The experimental data presented in Figure 2 indicate a significant decrease of transcriptional activity in puffs 71CE, 82EF, and 83E. These puffs are ecdysone-inducible and their activity changes in connection with the stage of *Drosophila* development [25]. The decrease of activity of developmental puffs may be related to the microwave-induced developmental retardation shown by the other authors [8,9]. On the other hand, our results are somewhat concomitant with the results in [7,6], that demonstrate an existence of microwave-induced changes in the pattern of puffing of *Drosophila* polythene chromosomes. However, in the present work no microwave-induced puffs were observed and the decrease of puffing activity was demonstrated. We found no microwave-induced decrease in *Drosophila* fertility which contradicts the results in [10]. This may be due to the difference in the experimental scheme – in [10] *Drosophila* females were exposed to microwaves for 4 and 5 hours at intensity of 0.0156 W/m^2.

The microwave-induced increase of the frequency of dominant lethal mutations shown here is in a good agreement with the data from some laboratories [4,7,8] and contradicts with results of other laboratories [5,6]. Such discrepancy may be caused by the difference in the experimental approaches used in different laboratories.

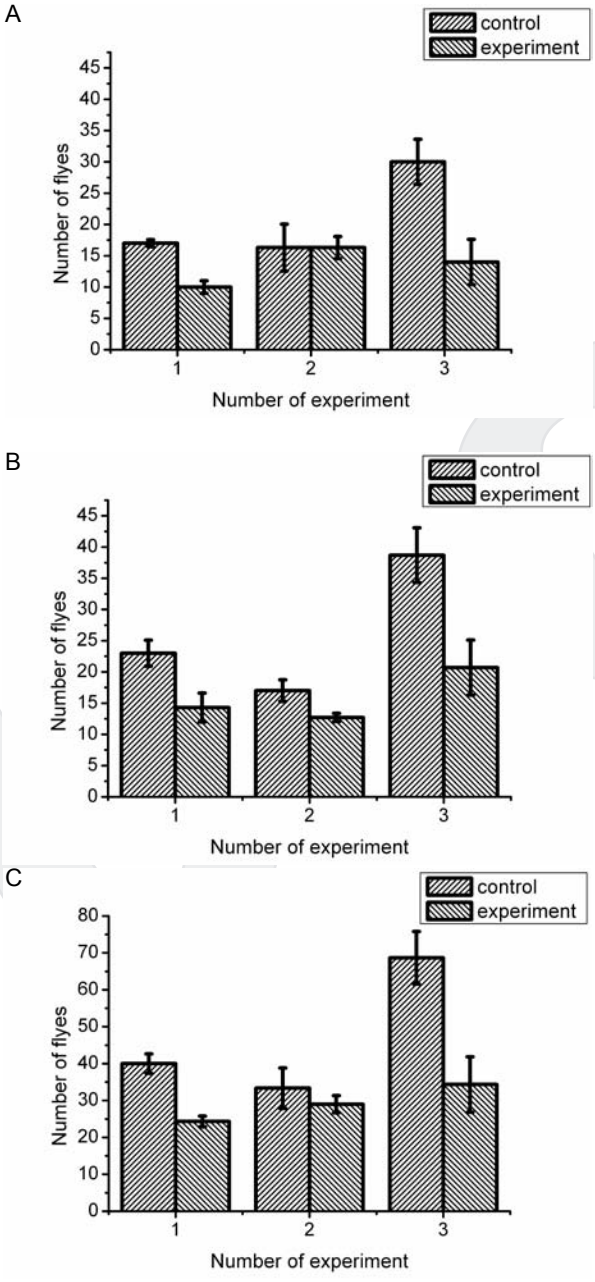

Figure 4. Effect of the microwave radiation exposure on quantity of the offspring developed from synchronous hatchings of *Drosophila melanogaster* (a – male offspring, b – female offspring, c – sum of male and female offspring).

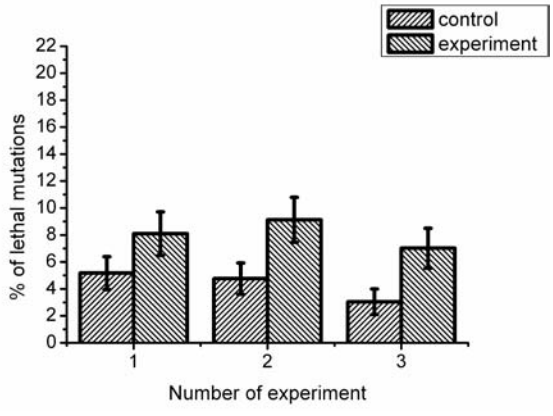

Figure 5. Effect of the microwave radiation exposure on viability of the offspring developed from synchronous hatchings of *Drosophila melanogaster* during metamorphosis.

The discovered decrease of *Drosophila* viability is in a good agreement with our previous results [1-4]. Because we do not observe any decrease in viability at the stages of larva-pupa and pupa-imago, it is possible that such decrease in viability is caused by the decrease in viability at the egg and larva stages.

The microwave fields applied in this article (0.4 W/m^2) are relatively high power in relation to natural levels of electromagnetic radiation (0.02 mW/m^2) for urban areas [26]. In this case the influence of natural levels of electromagnetic radiation may be neglected.

5. Conclusions

This work demonstrates suppression in puffing activity at ecdysone-inducible loci on *Drosophila* chromosomes induced by the low-level microwave exposure. The microwave radiation was also found to induce a decrease in *Drosophila* viability, presumably at the egg and larva stages, to increase frequency of dominant lethal mutations but did not influence *Drosophila* fertility.

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