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Influence of extremely low frequency magnetic field (50 Hz, 0.5 mT) exposure on fitness components of *Drosophila subobscura*.

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Summary

In addition to naturally occurring radiation, magnetic fields which are introduced by a man with the advanced development of industry and technology present an additional factor in environment that could have a significant influence on living systems. Impact of magnetic fields at different developmental stages of biological systems might induce changes on different organizational levels. In this study are presented developmental time, developmental dynamics, and viability of *Drosophila subobscura* after exposure of egg-first instar larvae developmental stage to extremely low frequency magnetic field (50 Hz, 0.5 mT). Exposure for 48 h at egg-first instar larvae developmental stage significantly shortens developmental time and increases viability of *D. subobscura*.

Introduction

Extremely low frequency magnetic fields (ELF-MFs, ≤ 300 Hz) derived from power lines as well as from the majority of household electrical appliances, represent one of the most important scopes for researching in magneto biology (Gandhi *et al.*, 2001; Gauger, 1985). ELF-MFs are in interaction with biological systems' tissues inducing electric fields and currents in them (Mathie *et al.*, 2003). Mostly, studies about effects of ELF-MFs on *Drosophila* are dealing with influence on reproductive behavior and fitness components. Ramirez *et al.* (1983) show decreased oviposition after exposure to pulsated ELF (100 Hz, 1.76 mT) and sinusoidal fields (50 Hz, 1 mT). In addition, there are found increased egg mortality and diminished adults' viability. *D. melanogaster* females and progeny exposed to ELF-MF show weakened oviposition in their subsequent generations (Gonet *et al.*, 2009). Also, Mirabolghasemi and Azarnia (2002) show a significant increase in the number of

abnormal adult flies from the exposed larvae at different stages of development, contrary to groups raised from the exposed eggs. If exposure to ELF-MF at any developmental stage makes changes on biological systems, it might be expected that the duration of ELF-MF exposure could evoke different levels of influence and, therefore, also a different response of the biological system. The aim of this research was to determine effects of exposure for 48 h of ELF-MF (50 Hz, 0.5 mT) on the following components of fitness: developmental time, developmental dynamics as a number of eclosed flies in every successive day, and viability of *Drosophila subobscura*.

Materials and Methods

Drosophila stock

D. subobscura was collected from a beech forest on Serbian mountain Goč and formed isofemale (IF) lines which were maintained in five full-sib inbreeding generations at temperature $19 \pm 1^\circ\text{C}$, humidity 60%, in a 12h:12h light:dark cycle and at 300 lux illumination on standard corn meal medium (9% sugar, 10% cornmeal, 2% agar, 2% yeast, nipagin dissolved in 96% ethanol).



Figure 1. ELF-MF apparatus.

ELF-MF apparatus

The ELF-MF was obtained by an electromagnet that consisted of three circular coils (37 cm in diameter) of insulated copper wire (0.75 mm in diameter). The coils were set at 23 cm distance from each other, which produced homogeneous magnetic field in a horizontal direction (Figure 1). The 50 Hz current was taken from local 220 V power network via an adjustable transformer. The electromagnet was supplied by a current of 2.8 A, producing uniform 50 Hz magnetic field without any observable temperature fluctuation or vibrations. Within the coils where the samples were placed, magnetic field was 0.5 ± 0.01 mT. Magnetic field was measured using a Hirst GM05 Gaussmeter (probe PT 2837, Hirst Magnetic Instruments LTD, Cornwall, UK).

Experimental procedure

Randomly collected 30 non-virgin 3-8 days old females were put in a 200 cm^3 vials, covered and pasted with Petri dish which contains standard corn meal medium. Vials were placed upside-down (Figure 2) and maintained for 24 h to lay eggs on standard corn meal medium for *Drosophila*. The following three groups of eggs were made:

1. intact (20 replicas) – egg-first instar larvae were out of ELF-MF apparatus,
2. *sham* (15 replicas) – egg-first instar larvae were in ELF-MF apparatus turned off, and
3. ELF-MF (35 replicas) – egg-first instar larvae were in ELF-MF apparatus turned on.

Since intact and *sham* group did not significantly differ they were cumulated and expressed as one control group.

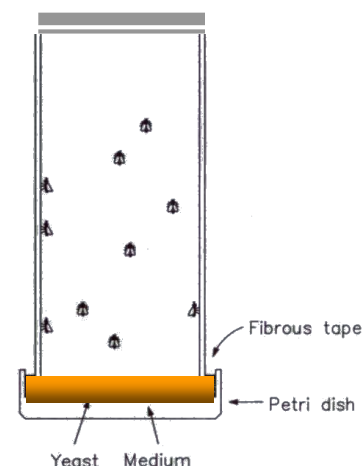


Figure 2. Vials with females laying eggs.

The following components of fitness were analyzed: developmental time, developmental dynamics as a number of eclosed flies in every successive day, and viability.

Collected eggs (75 per vial) were transferred to a thin film of standard corn meal medium in Petri dishes and exposed, or not, to the ELF magnetic field. After 48 h thin films were placed in 60 cm³ vials with standard corn meal medium for completing egg-adult development and maintained at optimal laboratory conditions ($19 \pm 1^\circ\text{C}$, 60% humidity, 300 lux illumination and 12h day/12h night regime). In order to measure fitness components, eclosed flies were counted for each replica, every day at the same time.

Statistical analysis

The Kolmogorov–Smirnov test was used to confirm data normality. The normality of data was found in all treatments. Z test was used for testing intact and *sham* groups. The mean values of developmental time and viability were analyzed by one-way ANOVA. Embryonic and post-embryonic developmental time was measured in days once all the adults had emerged. Egg-to-adult viability was calculated as the ratio of the emerged adults to the number of collected eggs. All analyses were performed using Statistica 6.0 for Windows (StatSoft Inc., Aurora, CO, USA).

Results and Discussion

It is well known that insects have the ability to perceive external magnetic fields. There are many literature data concerning magnetic field influences on genetic, development, growth, viability, reproduction, and orientation in insects (Mirabolghasemi and Azarnia, 2002; Graham *et al.*, 2000). Also, proliferating and less differentiated cells are more sensitive and vulnerable to electromagnetic radiation than non-proliferating and more differentiated ones (Prasad, 1995). One of the goals of this study was to show how exposure to ELF-MF (50 Hz, 0.5 mT) affects embryonic and post-embryonic developmental time of *D. subobscura*. Results are presented in Table 1. One-way ANOVA showed significantly shorter developmental time in flies from ELF-MF exposed groups ($F = 11.45$; $p < 0.01$).

Table 1. Embryonic and post-embryonic developmental time of *D. subobscura* in control conditions and after exposure to ELF-MF (50 Hz, 0.5 mT). Each value represents mean \pm SEM. Significance was tested with one-way ANOVA.

	Developmental time
Control	20.329 ± 0.035
ELF-MF	$19.845 \pm 0.041^{**}$

$^{**}p < 0.01$

In terms of the developmental dynamics of the formation of adults, hatching in control group started the 18th day. Eclosing of adults from ELF-MF exposed groups started earlier, between 17th and 18th day (Figure 3). Peak of eclosing in ELF-MF exposed groups was a day earlier (the 19th day, 39.81 %) comparing to control groups (the 20th day, 45.56 %). Likewise, adults from ELF-MF exposed groups finished eclosing one day earlier (the 25th day) comparing to control groups.

In groups exposed to the ELF-MF, 50% of individuals were eclosed by about the 19th. Eclosion of *D. subobscura* adults in control group was started after time point of 19 days.

Viability of *D. subobscura* flies was higher after the ELF-MF exposure compared to control (Figure 4). Mean values of viability of control flies was 51.2 ± 2.3 % and 57.2 ± 2.9 % for ELF-MF exposed flies. One-way ANOVA showed significantly higher viability of flies from ELF-MF exposed groups ($F = 4.191$; $p < 0.05$).

Results obtained for exposure of egg and the first instar larvae developmental stages of *D. subobscura* to ELF-MF show significant influence on developmental time, developmental dynamics, and viability. Results in this study revealed that exposure to ELF-MF (50 Hz, 0.5 mT) at egg-first

instar larvae developmental stage shortens developmental time and increases viability of *D. subobscura*. Due to literature data in which it is shown that insects are more resistant to different types of electromagnetic radiation than mammals (Koval *et al.*, 1977, 1979; Koval and Kazmar, 1988), it should be considered that effects of ELF-MF obtained on *Drosophila* can be significant in human protection.

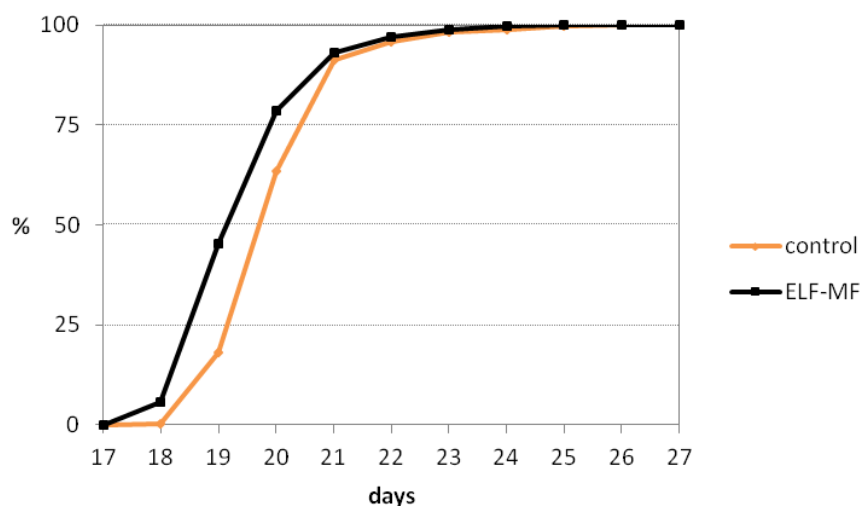


Figure 3. Developmental dynamics of *D. subobscura* after exposure of egg-first instar larvae developmental stage to ELF-MF (50 Hz, 0.5 mT).

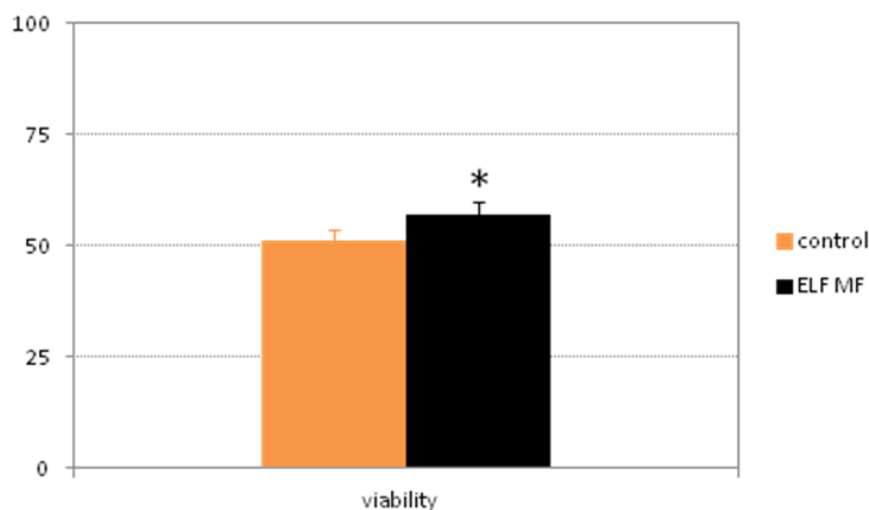


Figure 4. Viability of *D. subobscura* after exposure of egg-first instar larvae developmental stage to ELF-MF (50 Hz, 0.5 mT). Significance was tested with one-way ANOVA (* $p < 0.05$).

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Check-list of drosophilid species so far described and recorded from Uttarakhand state, India.

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Systematic position:

PHYLUM: ARTHROPODA

CLASS: INSECTA

SUBCLASS: PTERYGOTA

DIVISION: ENDOPTERYGOTA

ORDER: DIPTERA

SUBORDER: BRACHYCERA

SUPER FAMILY: EPHYDROIDEA

FAMILY: DROSOPHILIDAE

Subfamily Steganinae

1. Genus *Gitona* Meigen

1. *Gitona distigma* Meigen, 1830

2. Genus *Phortica* Schiner

Subgenus *Phortica*

2. *Phortica (Phortica) bandes* (Singh and Negi, 1992)

3. *Phortica (Phortica) biprotrusa* (Chen and Toda, 1998)

4. *Phortica (Phortica) pseudotau* (Toda and Peng, 1990)

3. Genus *Leucophenga* Mik

5. *Leucophenga albiceps* (de Meijere, 1914)