not exposed to ELF-MF. Moreover, exposure of just eclosed adults to ELF-MF also alters locomotor activity. According to all these results we can propose that the effect of the applied magnetic field might be on the serotonergic transmission. In the case of exposure of egg-first larvae developmental stage to ELF-MF, changes expressed at adult stage indicate that egg and the first instar larvae developmental stage are very sensitive to ELF-MF.

In conclusion, observed significant reduction of locomotor activity during recording in males and females of *D. subobscura* which were exposed to ELF-MF for 48 h at egg-first instar larvae and just eclosed adult developmental stage could point out the consequences of exposure to ELF-MF (50 Hz, 0.5 mT) which might be extrapolated to humans.

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Mating frequency of *Drosophila subobscura* from two populations.

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Mating is a fundamental process for animals with sexual reproduction. Mating success and sexual selection are poorly investigated in *Drosophila subobscura*, a species with complex courtship behavior represented by: orientation, “wing dance”, jump (attempting copulation), forelegs posture, vibration, rowing, and copulation (Milani, 1956; Brown, 1965). The specific nature of behavior during courtship and mating, as well as mate choice, are genetically and environmentally determined (Terzić *et al*., 1996; Jennions and Petrie, 1997; O’Dell, 2003). Signals for mating, which are reciprocally exchanged between the sexes, allow identification of pairs. Mating choice largely depends on sexual traits and preferences for them (Servedio and Saetre, 2003). Adaptation to different environmental conditions can lead to modification of these signals and recognition systems (Coyne and Orr, 1998).

The aim of this study was to examine the intra- and interspecific variability in the number of achived matings of both sexes, as well as mate choice in *D. subobscura* sampled from two populations from ecologically different habitats. Considering that significant chromosomal inversion polymorphism between these two populations exists (Andjelković *et al*., 2003), as well as phenotypic variances in body size (Stamenković-Radak *et al*., 2008), which are at some degree related to the variable environmental factors, possible sexual behavior divergence between these populations is expected.

Mating success of *D. subobscura* flies was estimated in the "multiple choice" test. Flies were sampled on the mountain Goč (Serbia), from oak and beech forests, which are located at a distance of approximately 6.9 km and at an altitude of 787 m and 875 m, respectively. The oak forest with E exposure (*Fraxineto-Quercetum*, N 5 43°32'57.38" and E 20°40'2.32") is a light and changeable habitat, while beech forest on exposure NE (*Abieto-Fagetum*, N 43° 33' 28.43" and E 20°45'10.96") is dark forest with small daily and intraseasonal fluctuations of environmental factors. Flies used in the experiment were maintained on standard cornmeal substrate for *Drosophila* in full-sib lines through four generations under optimal laboratory condition for this species (temperature ~ 19°C, relative humidity of 60%, under 300 lux of light and 12 h: 12 h light: dark regime).

"Multiple choice" test was done in eighteen replicates. Flies were placed in the vials containing standard cornmeal substrate according to the following scheme: 5 males from oak (O_m) + 5 females from oak (O_f) + 5 males from beech (B_m) + 5 females from beech (B_f). Flies from different populations were alternatively marked with fluorescent dust 24 hours before mating observation. Mating was observed during 90 minutes *per* replica, and flies of both sexes and populations were scored for number of matings during each of three separate, consecutive 30 minute periods. After copulation started, mated individuals were transferred into separate vials, and their population origin identified later under the UV lamp. Using UV dust does not influence mate choice in *Drosophila* (Terzić *et al*., 1994). Differences in percents of realized matings were tested with Z - test (Zar, 1984).

During 90 minutes of observation, 40 out of possible 180 matings were realized (22.2%). Within the first hour of observation 31 copulations occurred (82.5%). During the first 30 minutes 19 matings were observed (47.5%), while 14 matings (35.0%) were observed during the second 30 minutes, and 7 matings (17.5%) within the last 30 minutes.

In relation to the total number of realized matings, the males from oak forest realized 38.46% of matings during the first 30 minutes, 46.15% within the next 30 minutes, and in the last period O_m achieved 15.38% of matings. Females from oak forest achieved 50% of matings during the first period, 35% in the next 30 minutes and 15% of matings within the last period. Males from beech forest realized 51.85% of the total number of matings during the first 30 minutes, 29.63% during the second period, and 18.52% within the last 30 minutes. Within the first period of observation B_f realized 45% of the total number of matings, 35% during the second and 20% of matings in the last 30 minutes. Males from beech forest were more successful in mating (67.50%) than males from oak forest (32.50%). During the first 30 minutes, B_m realized significantly more matings then O_m ($Z = -4.129$, $p < 0.01$), while in the second period, O_m and B_m achieved approximately equal number of matings ($Z = -1.069$, $p > 0.05$). Within the last 30 minutes, B_m realized significantly more matings than O_m ($Z = -2.268$, $p < 0.05$) (Figure 1). If data for 60 and 90 minutes of observation periods are taken cumulatively (Figure 2), B_m out-performed O_m ($Z = -3.830$, $p < 0.01$; $Z = -4.427$, $p < 0.01$, respectively). However, females from both populations were equally successful in mating if each observation period is compared, as well as cumulatively (Figure 1 and Figure 2).

Number of realized heterogamic matings ($O_m \times B_f$ and $B_m \times O_f$) was significantly higher (62.50%) than the number of realized homogamic matings ($O_m \times O_f$ and $B_m \times B_f$) ($Z = -3.162$, $p < 0.01$). Individuals from the oak forest realized more heterogamic (86.21%) than homogamic matings

(13.79%). Flies from the beech forest also realized more heterogamic than homogamic matings, but with smaller ratio (69.44% and 30.56%, respectively).

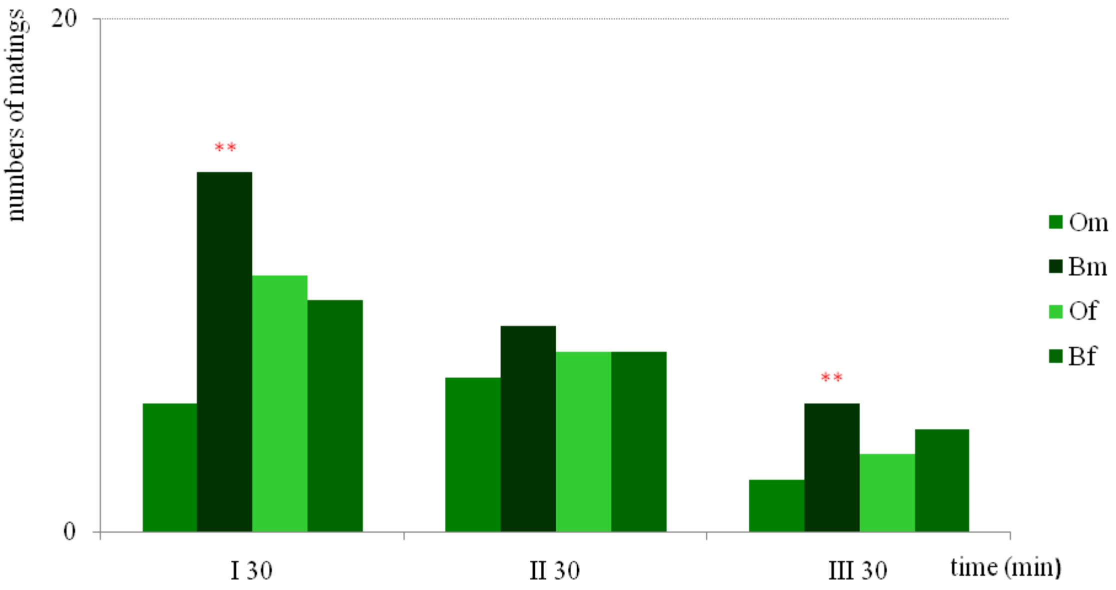

Figure 1. Number of matings achieved by both sexes in *D. subobscura* from two populations during every 30 minutes of the observation (I, II, and III). Abbreviations: O – flies from oak forest, B - flies from beech forest, m – males, f - females. **$p < 0.01$

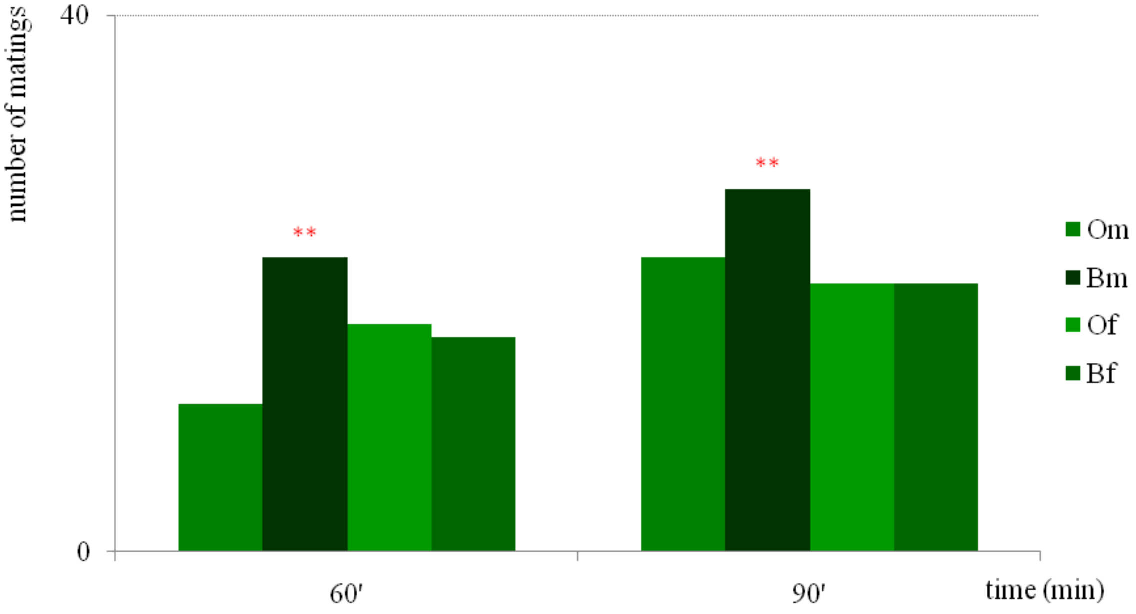

Figure 2. Cumulative number of matings within 60 and 90 minutes observing periods achieved by both sexes in *D. subobscura* from two populations. Abbreviations as in Figure 1.

A significant difference in the number of homogamic matings of B flies compared to the O flies was observed during the first and last 30 minutes of observation period, as well as during 60 minutes and the whole observation period of 90 minutes. The O_m mated more often with B_f than with females from the same population (O_f) during the last 30 minutes, and in the whole 60 and 90 minutes observation periods. The O_f achieved more heterogamic matings with B_m during each observation period, as well as in cumulative instance. The B_f mated equally with both B and O males in each period of observation, except during the first 30 minutes when more homogamic matings were realized. Results showed that no difference was observed in number of homo- and heterogamic matings in which B_m took part. The O_f realized more heterogamic matings than B_f during the first 30 minutes, and cumulatively, during 60 and 90 minutes. Results of Z - test for achieved homogamic and heterogamic matings are presented in Table 1.

Table 1. The comparison of the obtained homogamic and heterogamic matings; Z-test values. Abbreviations: as in Figure 1.

	I 30	II 30	III 30	60'	90'
$O_m \times O_f / B_m \times B_f$	-2.828 **	-0.894	-2.828 **	-4.221 **	-3.614 **
$O_m \times O_f / O_m \times B_f$	-0.894	-1.633	-2.828 **	-3.333 **	-2.774 **
$O_m \times O_f / B_m \times O_f$	-3.795 **	-2.268 *	-3.464 **	-5.680 **	-5.367 **
$B_m \times B_f / O_m \times B_f$	2 *	-0.756	0	1	0.894
$B_m \times B_f / B_m \times O_f$	-1.069	-1.414	-0.894	-1.705	-1.925
$O_m \times B_f / B_m \times O_f$	-3.015 **	-0.667	-0.894	-2.683 **	-2.8 **

*$p < 0.05$; **$p < 0.01$

In this experiment, "multiple choice" design was used, where sex ratio was equal (F: M = 1:1), so competitive interactions were less pronounced. Prolonged time of mating observation led to the greater number of recorded copulations. Still, the most of copulations occurred within one hour observing period.

Higher number of heterogamic compared to homogamic matings indicated that there was negative assortative mating, and flies did not discriminate each other. Mating preference, propensity to mate with a certain phenotype, is variable even in females from the same population, which may be the result of genetic differences, developmental trajectories, or due to environmental factors (Jennions and Petrie, 1997). Traits that are sexually selected can be reliable indicators of mating benefits, and females can have substantial benefits from choosing males with larger values of these characteristics in different environments (Kokko, 2003; Schwartz and Hendry, 2006).

However, according to our results, males from beech forest achieved significantly more matings than those from the oak. It seems that some traits (morphological, physiological, and/or behavioral) contributed to higher mating success of B_m which will be the subject of further research. *D. subobscura* may be a reliable model system for mating experiments, including studying of different effects of environmental conditions on this behavior.

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Effects of fungicide Dithane M 45 in *Drosophila melanogaster* on courtship behavior.

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Introduction

The dithiocarbamate pesticide Dithane M 45 is being used extensively to kill fungi. Although its primary function is to increase crop yield and food production, researchers showed that it has toxic effects and effect on genetic recombination in *Drosophila melanogaster* (Vasudev and Krishnamurthy, 1976, 1979, 1982), chromotoxic effects in plants (Pandey *et al.*, 1994), toxic effects on alga, *Stichococcus bacillaris* (Marton, 1974), embryo mortality in chickens (Keseru *et al.*, 2003), and non-clastogenic in mice (Vasudev and Krishnamurthy, 1994).

D. melanogaster has been proved beyond doubt as the best available sub-mammalian test system to screen genotoxic effects of environmental pollutants (Vogel and Sobels 1976; Sobels 1974; Vasudev, 1980; Wurgler *et al.*, 1985; Siddique *et al.*, 2005). Even though protocols of *D. melanogaster* have been validated for genotoxicity studies, time and again attempts are being made to introduce inexpensive, short duration and efficient parameters. Hence, in this direction, we presume that courtship behavior may be used as a parameter to understand the effects of environmental pollutants. It is pertinent to mention here that *D. melanogaster* with its well established series of sequential stereotyped elements of courtship behavior (Spieth, 1974, 1983; Bastock and Manning, 1955; Guruprasad *et al.*, 2010), an attempt has been made to use this protocol to understand the genetic effects of environmental pollutants. Nonetheless, Yamamoto and Koganezawa (2013), Dauwalder (2011), and Latham *et al.* (2013) have demonstrated that *fruitless* and *doublesex* genes are involved in courtship behavior. Furthermore, until now as far as we are aware there are no reports on the effects of environmental pollutants on courtship behavior. Therefore, the present work has been undertaken to understand the effects of a fungicide Dithane M 45 on courtship behavior of *D. melanogaster* and to authenticate this protocol for genotoxicity studies.

Materials and Methods

Dithane M 45, a zinc ion manganese ethylene bisdithiocarbamate, where 2% zinc, 16% manganese, and 62% ethylene bisdithiocarbamate obtained from Indofil chemicals Ltd, Mumbai, India and *D. melanogaster* Oregon-K strain were used for the present studies.