

ORIGINAL ARTICLE

Mating behavior as an indicator of quality of *Drosophila subobscura* males?

Marija Savic Veselinovic¹, Sofija Pavkovic-Lucic¹, Zorana Kurbalija Novicic², Mihailo Jelic¹, Marina Stamenkovic-Radak^{1,2} and Marko Andjelkovic^{1,2,3}

¹Faculty of Biology, University of Belgrade, Belgrade, Serbia; ²Institute for Biological Research “Sinisa Stankovic” University of Belgrade, Belgrade, Serbia and ³Serbian Academy of Sciences and Arts, Belgrade, Serbia

Abstract According to current theoretical predictions, any deleterious mutations that reduce nonsexual fitness may have a negative influence on mating success. This means that sexual selection may remove deleterious mutations from the populations. Males of good genetic quality should be more successful in mating, compared to the males of lower genetic quality. As mating success is a condition dependent trait, large fractions of the genome may be a target of sexual selection and many behavioral traits are likely to be condition dependent. We manipulated the genetic quality of *Drosophila subobscura* males by inducing mutations with ionizing radiation and observed the effects of the obtained heterozygous mutations on male mating behavior: courtship occurrence, courtship latency, mating occurrence, latency to mating and duration of mating. We found possible effects of mutations. Females mated more frequently with male progeny of nonirradiated males and that these males courted females faster compared to the male progeny of irradiated males. Our findings indicate a possible important role of sexual selection in purging deleterious mutations.

Key words condition dependence; deleterious mutations; female preference; inbred lines; ionizing radiation; mutational load

Introduction

“The advantage which certain individuals have over other individuals of the same sex and species solely in respect to reproduction” is one of Darwin’s definitions of sexual selection (Darwin, 1871). One of a few proposed mechanisms of sexual selection emphasizes the role of mate choice as a driving force for sexual selection. Much of what follows will be about “female choice” and female preferences rather than “mate choice” because females are generally expected to be choosier, although male mate choice is potentially widespread and also has a distinct

role in how mating systems evolve (Edward & Chapman, 2011).

Today, almost a century and half after Darwin, we still question what the cues of female mate choice are. The existence of different reproduction strategies (monogamous and polygamous) even among species of the same genus, could be an important indicator of female mate choice. Furthermore, species with different reproductive strategies differ in morphological traits, behavior displays, pheromones or vocalizations (Andersson, 1994) which confirms the evolutionary context of chosen reproduction strategies. But which male character can act as indicator of male quality in a certain species? Is there a cost of mating with a less fit male? Female choice is a complex trait and represents a form of nonrandom mating that results from a wide variety of traits, including not only behavioral but also morphological, physiological, and perceptual features (Arnqvist & Rowe, 2005).

Correspondence: Marija Savic Veselinovic, Faculty of Biology, University of Belgrade, Studentski trg 3, 11 000 Belgrade, Serbia. Tel: +381 64 823 7859; fax: +381 11 2638 500; email: marijas@bio.bg.ac.rs

Female mate preferences describe the relationship between the phenotype of males and their probability of being accepted as a mate. Females often assess multiple traits when choosing mates, including various sexual signals and displays (Heisler *et al.*, 1987; Kirkpatrick, 1987; Jennions & Petrie, 1997; Candolin, 2003; Chenoweth & Blows, 2006). The traits of interests for mate choice are, generally, condition dependent (Andersson, 1986; Price *et al.*, 1993; Johnstone, 1995; Rowe & Houle, 1996; Griffith *et al.*, 1999; David *et al.*, 2000; Kotiaho *et al.*, 2001). About 70%–90% of variation in the mating success is not explained by the measured secondary sexual characteristics and may be in part due to general condition (Whitlock & Agrawal, 2009).

One of the proposed models of female preference evolution, and the most studied one, is the “indirect benefits” model with “good-genes” process which interpret male mating displays as indicators of high viability (Kokko *et al.*, 2003). The ability of sexual selection to target alleles with pleiotropic effects on nonsexual fitness is a central component of theoretical models of several processes, including mate choice (Whitlock & Agrawal, 2009). According to “genic capture” hypothesis (Rowe & Houle, 1996; Tomkins *et al.*, 2004), the condition of an individual is affected by a large number of loci, thus providing a “large target” for mutations. As most mutations are deleterious, females will benefit indirectly from choosing males in good condition because they are less likely to contribute germline derived mutations to offspring. In this way, sexual selection may contribute to the purging of deleterious alleles from populations.

The role of sexual selection in reducing mutational load is still controversial, with nonconsistent experimental data. The main reasons for this are the different experimental approaches and differently measured fitness components. In addition, the nature of mutational load used in experiments and diversity of species analyzed should be taken into account. Some experiments used known deleterious mutations in *Drosophila melanogaster* (Sharp & Agrawal, 2008; Hollis *et al.*, 2009; Arbuthnott & Rundle, 2012), while other used spontaneous mutation–accumulation approach (Radwan *et al.*, 2004; McGuigan *et al.*, 2011; Mallet *et al.*, 2011; Mallet *et al.*, 2012; Sharp & Agrawal, 2013), or induced mutagenesis by chemicals (Hollis & Houle, 2011) or ionizing radiation (Radwan, 2004; Pekkala *et al.*, 2009; Savic Veselinovic *et al.*, 2013). Frequencies of known deleterious mutations decreased (Hollis *et al.*, 2009) or did not change (Arbuthnott & Rundle, 2012) in sexual selection treatments. Sexual selection purged the genome by increasing fitness components measured as fecundity or productivity (Sharp & Agrawal, 2008; McGuigan *et al.*, 2011; Savic Veselinovic

et al., 2013) or viability (Radwan, 2004; Sharp & Agrawal, 2008). But such results were not obtained for all analyzed mutated alleles (Sharp & Agrawal, 2008), as well as in the experiment with the use of chemicals as mutagen (Hollis & Houle, 2011). McGuigan *et al.* (2011) observed that accumulated mutations affected mating success of males. However, in the research of Pekkala *et al.* (2009) mutations induced by ionizing radiation did not affect mating success, but affected the activity of males. Intersexual differences in the strength of selection on spontaneous mutations were confirmed for different fitness components with evidence of stronger selection on males (Mallet *et al.*, 2011; Mallet *et al.*, 2012; Sharp & Agrawal, 2013).

Drosophila is a common model organism for studying sexual selection. *D. subobscura* performs the unique wing dancing in *obscura* species group. Maynard Smith (1956) described mating ritual as a process occurring in several steps. Despite the fact that we have an extensive amount of data describing mating behavior in this species, it still represents a “living puzzle” in a context of reproductive behavior. According to Loukas *et al.* (1981) about 20% of females remate, but recent studies have shown that only 4% of females remate (Fisher *et al.*, 2013) or do not remate (Holman *et al.*, 2007; Lize *et al.*, 2011). So, this species can be considered as monandrous. There is evidence that monandry has evolved recently in *D. subobscura*, as all other members of the *obscura* clade exhibit polyandry (Holman *et al.*, 2007). If we take into account all these facts, *D. subobscura* represents an interesting species for mating choice experiments.

The main goal of this study was to investigate the influence of sexual selection on purging deleterious mutations. Male mating success was used as a measure of effectiveness of sexual selection. Latency to mating and male mating success was considered as female preference measures (Narraway *et al.*, 2010). These measures are for all traits that confer attractiveness to a mate (Head *et al.*, 2005). As genetic quality can affect mating success by its effect on general activity of males (Whitlock & Agrawal, 2009), we measured different aspects of mating behavior which include activity of males: courtship occurrence, courtship latency, latency to mating and duration of mating. We performed the experiment with highly homozygous isofemale lines (IF) of *D. subobscura* maintained in optimal laboratory conditions in order to discard the environmental influence on the phenotype. We introduced “new variability” into lines using ionizing radiation (dose of 30 Gy) on males only, with the purpose of introducing genetic variability in the quality of male progeny. The progeny of irradiated males should be heterozygous for novel mutations. It is well known that ionizing radiation produces a wide range of mutations in DNA (reviewed

in Evans & DeMarini, 1999). Introduced mutations most probably affect the condition of males, and consequently male reproductive behavior.

Materials and methods

Maintenance and manipulations of flies

Drosophila subobscura used in this study originates from laboratory stocks (10 IF lines), collected from Eastern Serbia in 2009. Randomly chosen couples of F1 progeny from each of 10 IF lines were parents of the 1st generation of full-sib (FS) mating. We performed 15 generations of full-sib matings within IF lines in order to get highly homozygous lines. To minimize the loss of IF lines, additional 2–3 individual brother–sister mating were made within each line in every generation, but progeny of only one pair was randomly chosen to continue the experiment. Although this procedure allows natural selection to operate between additional mating within lines, it was used in many inbreeding studies (Rumball, 1994; Pegueroles, 1996) to avoid excessive loss of lines and to reduce selection between lines (Rumball, 1994). The same set of 10 inbred lines (randomly chosen) was used to set up the “nonirradiated” and “irradiated” group. Ten replicates were made for each of 10 IF inbred lines with the aim to provide enough flies for further experimental procedure. All inbred IF lines were reared on standard *Drosophila* medium (water/cornmeal/yeast/sugar/agar/nipagine as fungicide), at optimal conditions for *D. subobscura* (at 19 °C, 60% of relative humidity, under 300 lux light intensity and 12 : 12 h light : dark cycles). The experiments were done in the same optimal conditions.

We manipulated the genetic quality of randomly selected 2–4 d old male flies by inducing new mutations with ionizing gamma radiation. The implemented radiation dose was 30 Gy (dose rate of 18.12 Gy/h) with the distance from the radiation source of 100 cm. All treatments were conducted under equal and constant optimal laboratory conditions. The radiation dose was chosen with the intent to induce mutations with a high probability, but not to considerably decrease fertility of males. As Pekkala et al. (2009) used a series of different radiation doses (0, 5, 10, 15, 20, 25, 30, 35, and 40 Gy) on adult *Drosophila montana*, we fixed the dose from 30 Gy in order to have enough progeny as with increasing radiation dose male fertility decreased in their experiment. Their results fitted the expectations that the number of mutations induced increases with increasing radiation dose (Edington, 1957; Evans & DeMarini, 1999).

Fifty to sixty males were treated per line, in total. The 10–15 males per line were irradiated successively, every 7–10 d in order to provide flies of same ages for the experiment over a longer time interval. Two days after irradiation, 4–6 d old irradiated males were mated with nonirradiated females of the same age, from the same lines. Those lines were labeled as “irradiated”. The progeny from these matings is potentially heterozygous for the new heritable mutations. At the same time matings between males and females were done within lines, without irradiation. Those lines were labeled as “nonirradiated.” The progeny from both types of matings was used in the experiment.

Virgin individuals were collected every 24 h. *D. subobscura* never mate in the dark (Andjelkovic & Marinkovic, 1983; Krimbas, 1993), so vials with hatching flies were kept in the dark all the time during the collection of flies. Flies were collected without anesthesia in order to avoid its influence on their behavior (Barron, 2000). Males were kept separately due to potential effects of grouping on the behavior (Ellis & Kessler, 1975). Fifteen to twenty females from the same line were kept together. All flies were 5 d old at the beginning of the experiment.

Experimental matings

In this study we attempted to determine the role of genetic quality of males by examining the effects of inherited heterozygous mutations of the “irradiated” males on several aspects of their mating behavior. This was possible by creating combinations of matings within and between “nonirradiated” lines, as well as between “nonirradiated” and “irradiated” lines. We conducted all 100 possible combinations of crosses between lines (by using females and males from each of 10 lines) in both groups. Females were always taken from the “nonirradiated” lines, but males were taken from “nonirradiated” (control group) or “irradiated” lines (treatment group). In this way, differences between treatment and control group can be assigned to new mutations.

In total we had 200 combinations (100 per group), and each combination was set up from 9 to 11 times (depending on the number of lines progeny) in order to detect the effect of mutations on male mating behavior (as each single male from line is not necessarily the carrier of mutations).

Mating trials for each combination of lines with males from “nonirradiated” and “irradiated” line were conducted simultaneously. They were observed every morning from 7 to 11 am and the experiment was conducted by placing 1 female and 1 male in individual plastic vials (“no

Table 1 Descriptive statistics for discrete variables (courtship and mating success).

Males	N (Total number of mating trials)	N_1 (Number of courting males)	% Of courting males	N_2 (Number of mated males)	% Of mated males	Number of mated males from N_1 courted	% Mated males (from courted)
“Nonirradiated”	1003	907	90.43	421	42.02	366	40.35
“Irradiated”	1003	885	88.24	383	38.22	338	38.19

N , Number of mating trials; N_1 , number of males that courted; N_2 , number of males that mated.

Table 2 Descriptive statistics for continuous variables given in seconds (courtship latency, copulation latency, and copulation duration).

Trait	“Nonirradiated”				“Irradiated”			
	Mean \pm st. error	SD	N	Range	Mean \pm st. error	SD	N	Range
Courtship latency	704.98 \pm 39.44	118 714	907	0–6942	808.27 \pm 42.44	126 331	885	12–7184
Copulation latency	1917.51 \pm 94.43	193 303	421	28–8381	1891.62 \pm 95.97	18 831	383	35–7540
Copulation duration	712.87 \pm 18.91	387 106	421	66–2584	718.08 \pm 19.20	37 678	383	63–2413

choice” design). Female was placed first, and the timing was started after the introduction of the male. Mating behavior was observed until copulation occurred, or until 2 h had elapsed. All observations were performed by the same person. Mating trials were performed for approximately 50 d, as we formed replicas of lines and successively irradiated flies from the previous generation.

Every specific contact for this species that the male initiated was scored as courtship. Latency to courtship was measured as time that elapses from the introduction of female and male in a vial and the first courtship. The majority of males courted females, but as some males did not court we scored occurrence of courtship as a discrete variable (male courted or not courted). Mate acceptance (or mating success) was recorded as a discrete variable—a male mated or not mated. Latency to mating was measured from the time of introduction of the female and male in a vial until the beginning of copulation. We recorded duration of mating as time from the beginning and the end of copulation.

Statistical analyses

The Shapiro–Wilk test was used to test row data for departures from normality. Furthermore, commonly used transformations for these traits, like log and Box cox were used, but none was successful in transforming data to normality.

The discrete variables (courtship occurring and mating success) were tested with binomial logistic regression in SPSS 17.0. Continuous data were obtained for latency

to courtship, latency to mating and duration of mating. As the data were not normally distributed, nonparametric tests were used: Mann–Whitney U for 2 group comparisons and Kruskal–Wallis for multiple comparisons. Bonferroni–Holm correction was used to adjust P values for multiple testing. The correlation between latency to courtship and latency to mating, and latency to courtship and courtship duration, were analyzed with Spearman rank nonparametric correlations.

All statistical analyses were performed in SPSS 17.0. and Past software (Hammer *et al.*, 2001).

Results

Our experimental design was fully factorial. The final sample size was limited because the number of mating trials which resulted in copulations varied per every combination of lines. Only 40% out of the total number ($n = 2006$) of mating trials successfully copulated. The number and percent of males that courted females and copulated are presented in Table 1. Descriptive statistics of other analyzed behavioral traits are presented in Table 2.

In the “nonirradiated” group, 90.43% of the males courted the female, but only 42.02% mated. In “irradiated” males, mutations did not reduce probability of courtship, but reduced the probability of mating success ($\chi^2 = 3.415$, $P = 0.048$) (Tables 1 & 3). This significance disappeared after Bonferroni–Holm correction for multiple comparisons. All mated males did not court females in both experimental groups (14.06% in “nonirradiated” and 11.75% in “irradiated” groups). Analysis of mating

Table 3 The effects of male genotype, female genotype and treatment on measured traits.

	Courtship occurrence	Mate acceptance	Mate acceptance for courting males	Latency to courtship (s)	Latency to mating (s)	Duration of mating (s)
Female genotype	$\chi^2 = 0.058$	$\chi^2 = 42.756^{***}$	$\chi^2 = 33.239^{***}$	$H = 47.72^{***}$	$H = 42.76^{***}$	$H = 10.46$
Male genotype	$\chi^2 = 1.374$	$\chi^2 = 11.955^{**}$	$\chi^2 = 10.018^*$	$H = 219.10^{***}$	$H = 52.27^{***}$	$H = 266.00^{***}$
Treatment	$\chi^2 = 1.893$	$\chi^2 = 3.415$	$\chi^2 = 2.702$	$T = 382600$	$T = 79420$	$T = 79210$

Notes: Mate acceptance and courtship occurrence were analyzed with binomial logistic regression; χ^2 is the value for Wald test. Courtship intensity, latency to courtship, latency to mating and duration of mating were analyzed with nonparametric, Mann–Whitney test for treatments, and Kruskal–Wallis test for genotypes. T is the value of Man–Whitney test. H is the values of Kruskal–Wallis test. Asterisks denote the significance of tests after correction for multiple testing: $^{***}P < 0.001$, $^{**}P < 0.01$, $^*P < 0.05$.

success of males that courted did not confirm the negative effect of mutations on mating success. Although this effect was marginally significant ($\chi^2 = 2.702$, $P = 0.068$) before correction for multiple tests, it is suggestive of a potentially significant effect that may be revealed with larger sample sizes.

Two experimental groups (“nonirradiated” and “irradiated”) differed in courtship latency (Table 3). The “irradiated” group showed extended latency to courtship compared to the “nonirradiated” group (Mann–Whitney $U = 382600$, $P = 0.046$). This difference did not remain significant after correction. Mutations had no significant effects on the other measured variables (Table 3). Latency to mating showed no significant differences between the “nonirradiated” and the “irradiated” groups. Although latency to mating did not differ between these 2 groups, latency to courtship and latency to mating were positively correlated ($r = 0.415$, $n = 702$, $P < 0.0001$). Males that started their courtship later needed longer total time to start copulation. Latency to courtship and courtship duration (time between courtship initiation and copulation) were not correlated ($r = 0.009$, $n = 702$, $P = 0.81529$).

The obtained effects of female and male genotype on mating success, courtship latency and copulation latency (Table 3) indicate the complexity of behavioral traits. All effects remained significant after correction for multiple testing and corrected levels of significance are given in the Table 3. Obtained results for latency to courtship indicate the genetic variation for this trait as the effect of male genotype was significant (Table 3; $H = 219.10$, $P < 0.00001$). Male genotypes differed in motivation to mate (Fig. 2), for example, genotypes 2, 5, and 6 courted faster, and genotypes 3 and 4 courted slower. A significant effect of female genotype on this trait (Table 3; $H = 47.72$, $P < 0.00001$) indicate that some female genotypes were courted faster (e.g., female genotypes 1, 2, and 4). They were more attractive in comparison to female genotypes 3, 7 and 9 which were courted later (Fig. 2). Neither male

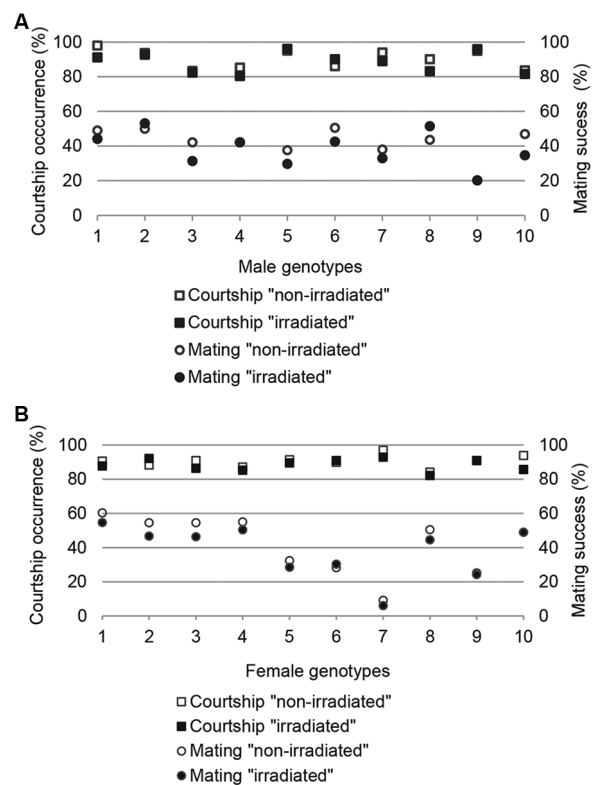


Fig. 1 The among-line differences in courtship occurrence and mating success of males (A) and females (B). The upper part of the figure presents the percent of males that courted and females that were courted, and the bottom part presents the percent of males and females that mated.

nor female genotypes differed in courtship occurrence suggesting that there was no genetic variation for this trait (Table 3, Fig. 1).

Significant effects of female genotype on latency to mating (Table 3; $H = 42.76$, $P = 0.000002$) and mate acceptance (Table 3; $\chi^2 = 33.239$, $P < 0.000001$)

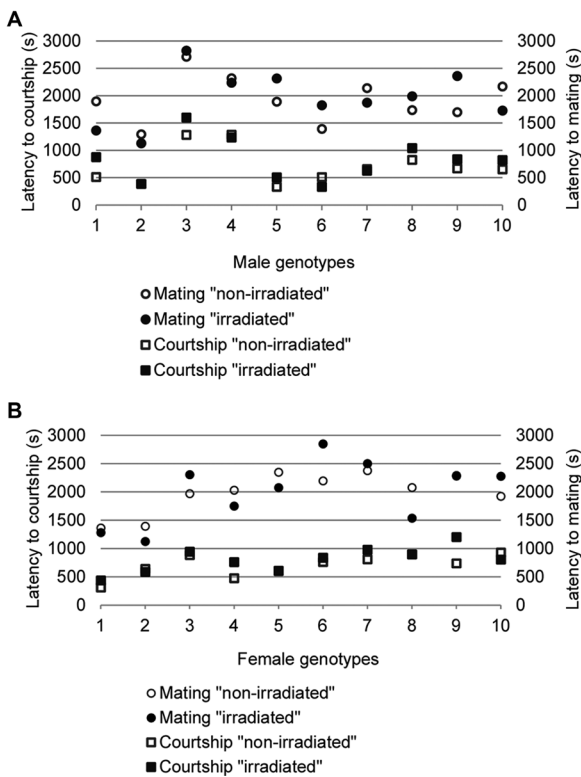


Fig. 2 The among-line differences in latency to courtship and latency to mating of males (A) and females (B). The upper part of the figure presents latency to mating, and the bottom part presents latency to courtship.

indicate a genetic basis of these female preference measures. Female genotype 7 rarely copulated unlike genotypes 1, 2 and 3 (Fig. 1). Similar pattern of latency to mating was observed as well as latency to courtship for female genotypes, with genotype 6 requiring more stimulations prior to copulation, in contrast to female genotype 2 (Fig. 2). A significant effect of male genotype on mate acceptance and latency to mating (Table 2; $\chi^2 = 10.018$, $P = 0.002$; $H = 219.10$, $P < 0.00001$) illustrate that females on average found some male genotypes more attractive than other. Some male genotypes were more successful in mating compared to others, for example, 2 and 8 unlike genotype 9 (Fig. 1). Male genotypes 2 and 6 copulated faster in contrast to genotypes 3 and 4 (Fig. 2).

Figure 4 presents individual female preferences for each isofemale line. The majority of mating trials for every combination of lines occurred, except of combination 63 (female from line 6, male from line 3) and 72, 73 (female from line 7, males from line 2 and 3). It is evident that some female genotypes are choosier from others (e.g., female from lines 6, 7, 9, and 10).

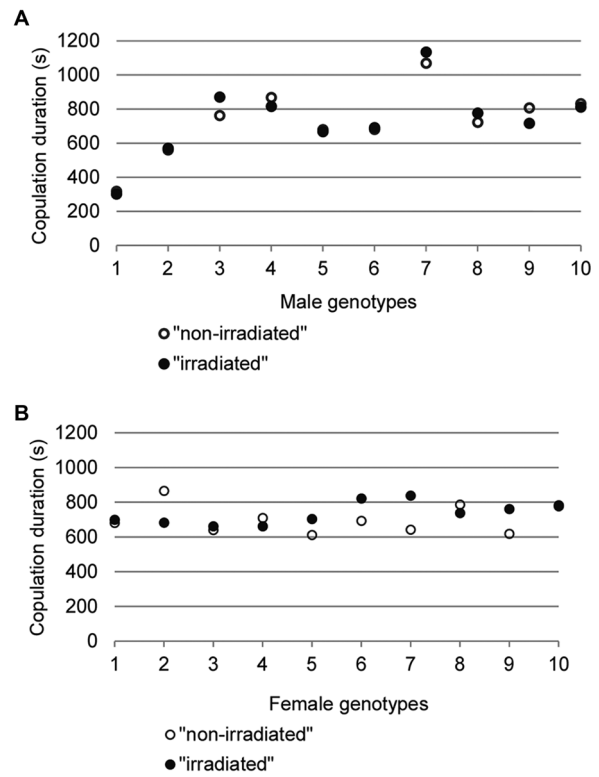


Fig. 3 The among-line differences in duration of copulation of males (A) and females (B).

These results suggest that male attractiveness has a genetic basis, as well as female preference. However, for the copulation duration was obtained only the significant effect of male genotype (Table 3; $H = 266.0$, $P < 0.000001$). Male genotype 7 copulated longer than other, and genotype 1 copulated shorter than other (Fig. 3). These results suggest that this trait is controlled only by males.

Discussion

Our study presents important findings concerning mating behavior and mating preferences in *D. subobscura*. There is no literature data about genetic variation of components of these traits in this species. Use of highly inbred lines and their comparison provides information about genetic basis of complex quantitative traits. Our experimental design has advantages for measuring mating preferences as a standard method in a “no choice” design by using highly inbred lines (Narraway *et al.*, 2010). Its purpose was to eliminate influences of male–male competition on measured behavioral traits, too. Individual level of homozygosity or heterozygosity can have significant effects on the reproductive success of an individual

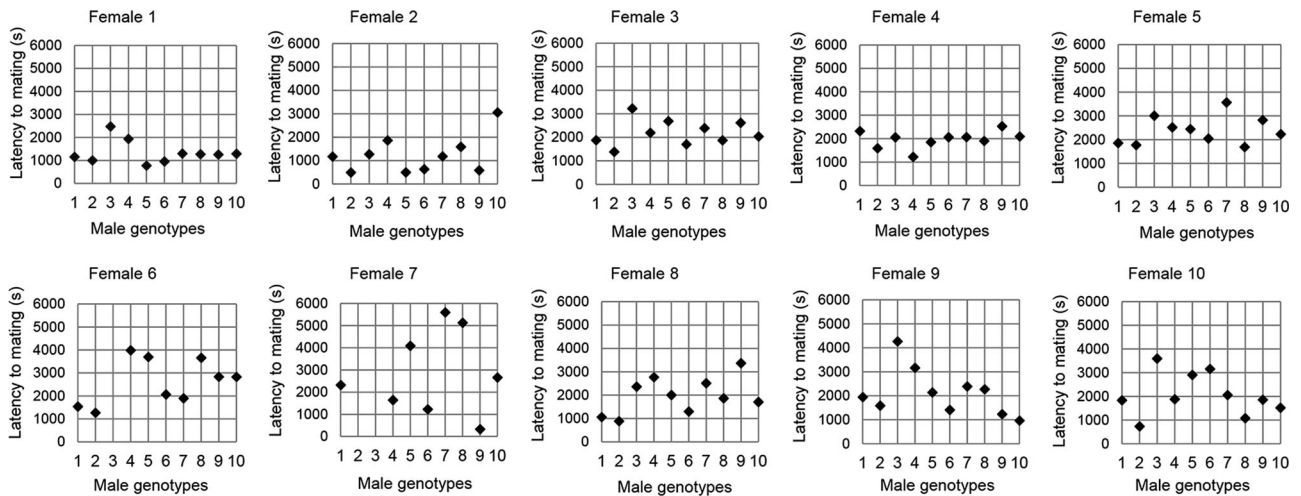


Fig. 4 Individual female preferences for each isofemale line.

(Kempenaers, 2007). There is a lack of experimental data of female preferences of inbred lines, as well. Prior study of this species demonstrated lower mating success and male performance in inbred males in comparison to outbred males (Maynard Smith, 1956), but did not research preferences of female inbred lines. So, the low total percentage of copulations in our study may be caused by the lines being highly inbred. Possibly, in *D. subobscura*, inbreeding has a large influence on the frequency of copulations, at least in limited time (2 h per replica), which is necessary for measuring female preferences (Narraway *et al.*, 2010; Liu *et al.*, 2014). But inbred females can be more choosy, more discriminating in choosing a male mate than outbred ones (Mazzi *et al.*, 2004; Immonen *et al.*, 2009). This can be important for detecting tiny differences between “irradiated” and “nonirradiated” males in our experiment.

In general, our results indicate the significant effect of both female and male genotype on latency to courtship, latency to mating and mating success, if we consider these traits regardless of irradiation treatment. Female genotypes differed in their preferences measured as latency to mating and mate acceptance, indicating the genetic basis of female preference. Genetic variation in male genotypes in latency to courtship indicate a male preference to some females. Also, males differed in their attractiveness, as some genotypes copulated faster and some copulated more frequently. Some female genotypes were more attractive as they have been courted faster. We detected significant differences between 10 isofemale lines for majority of tested traits except for duration of mating which shows that male genotype is the only source of variability in this trait, without the effect of female genotype.

These results suggest that duration of mating is a trait controlled only by males. If we consider the reproductive biology of *D. subobscura*, the particular results are not surprising. Unless *D. subobscura* provides another rare example in which monogamy benefits females, it seems more likely to be a consequence of male suppression of female remating. Among *Drosophila* species, duration of mating has been found to be largely under male control in *D. melanogaster*, *D. simulans*, *D. mojavensis*, and *D. athabasca* (Parsons & Kaul, 1966; Mac Bean & Parsons, 1967; Patty, 1975; Krebs, 1991). Females can control duration of mating and to benefit from longer copulations (Arnqvist, 1989; Schneider *et al.*, 2006), but longer copulations can be costly to females in particular conditions (Barbosa, 2011).

Some of males that copulated did not court females. This observation may suggest existence of forced copulations, without female choice. However, flies of this species mate only in the presence of light (Rendel, 1945). The behavioral pattern of visual stimulation in courtship, for this extremely light-dependent species, was only modified in a strain selected for light-independent mating where males forcibly copulated with females without a preceding courtship (Pinsker & Döschek, 1980). Further, female choice in *D. subobscura* was observed in previous studies (Maynard Smith, 1956; Steele, 1986; Immonen *et al.*, 2009). During our experiment forced copulations were not seen and females clearly showed rejection of males. Courtship in *D. subobscura* is initiated when the male approaches the female and taps her with his front legs. The male then moves around to approach female head-to-head. The female then sidesteps, but the male moves around to maintain his position facing her

(Maynard Smith, 1956). There were no sidesteps by females in the above mentioned matings, which are generally followed by male courtship in this species. It is possible that chemical stimuli had critical role in these matings, but the role of pheromones in female choice in this species was not explored so far.

In order to gain a deeper understanding of the theory of purging the genome through sexual selection, we manipulated the genetic quality of males and analyzed some aspects of mating behavior and mating success of males. Our results showed a possibly significant lower mating success in “irradiated” males compared to “non-irradiated” males suggesting that genetic quality varied and that mutations could have an effect on mating success. This means that females presumably preferred to mate with males who were not carriers of induced mutations. However, we are dealing with female choice which is a highly complex trait as females probably use multiple cues in mate choice (Jennions & Petrie, 1997; Candolin, 2003). Induced mutations showed no effect on courtship occurrence, latency to mating and duration of mating. It is well known that male attractiveness is complex and based on all the physical, chemical and morphological cues that the male offers and none of the traits is crucial for mating success individually but in their synergistic action. All of the analyzed traits in this experiment are condition dependent, and represent an important aspect in a mating behavior which is important for mating success. Our results for mating success of males that courted and not courted suggest the role of other stimuli, not only visual.

“Irradiated” males showed marginally significant extended latency to courtship compared to “nonirradiated” males which suggests a possible role of male activity in sexual selection for genetic quality in *D. subobscura*. Although this difference did not remain significant after correction, we nonetheless presume that this trend is noteworthy. The potential effect of mutations is important as initiation of courtship must be an important step in nature, where males are in competition with other males. *D. subobscura* is a monogamous species and latency to mating should be of special significance in males and their mating success (Monclus & Prevosti, 1971). Although we obtained no direct evidence that males, potential carriers of mutations, have longer latency to copulation, we detected a positive correlation between latency to courtship and latency to mating in “irradiated” males. Longer latency to mating is likely to decrease the overall mating success of the males, that is, reduce the number of mating the males realize in their entire lifetime (Shackleton *et al.*, 2005; McGhee *et al.*, 2007). However, the

beginning of the courtship did not affect the duration of courtship.

Considering a large number of mating trials at the same time for both mating groups and each combination of lines, any differences between “nonirradiated” and “irradiated group” can be assigned to mutations. Pekkala *et al.* (2009) hypothesized that their obtained results were partly an artifact of the experimental design, because females were accepting any male when they didn’t have better males to choose from. As females in our species mate mostly once, females are probably choosier and virgin female more often reject a male (Harcourt *et al.*, 1981; Chapman *et al.*, 1995). Recent study showed that male–male competition can reduce male mating success and condition of males (Lize *et al.*, 2014), so with our experimental set up this effect was not possible. Also, our results for mating success showed female preference for a certain male genotype, and if the assumption of the artifact of no choice design is true, there would be no differences between female preferences and male attractiveness, as we obtained.

If females choose to mate with males with high breeding values for fitness (good condition male), then sexual selection may be a contributor to adaptive evolutionary change. The good genes models of sexual selection and the great amount of related research emphasize the role of females choosing good quality males to mate with (Zahavi, 1975, 1977; Pomiankowski, 1988; Maynard Smith, 1991; Andersson, 1994; Kokko *et al.*, 2003). Some theoretical studies have shown that sexual selection can accelerate the spread of beneficial mutations (Proulx, 1999; Whitlock, 2000) and contribute to the shedding of genetic load (Agrawal, 2001; Siller, 2001). Presented results in this paper indicate that mutations could affect mating success, but the effect of induced mutations on nonsexual fitness of individuals used in the experiment is unknown. Savic Veselinovic *et al.* (2013) showed that sexual selection could purge mutational load for fecundity, but not for egg to adult viability. The effect of mutation depends on the type of mutation and their various epistatic effects with other genes, as well as on pleiotropic effects, which are difficult to predict (Pepin *et al.*, 2006). The recent publications indicate this complexity and importance of using different measures of fitness of males and females (Mallet *et al.*, 2011; Mallet *et al.*, 2012; Sharp & Agrawal, 2013).

In the light of the obtained results, clear cut conclusion whether sexual selection reduced mutational load could not be drawn. However, our results provide a certain evidence that sexual selection for genetic quality may operate via mating success and male mating activity.

Acknowledgments

This work was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Grant No.173012. We are grateful to Institute for Nuclear Science “Vinca,” the Laboratory for Radiation and Environmental Protection, University of Belgrade, for providing equipment for sample irradiation. We thank Cino Pertoldi and Torsten N. Kristensen for their suggestions in statistical analyses. We are grateful to the anonymous reviewers for suggestions and valuable comments which greatly improved the manuscript.

Disclosure

All authors declare no conflict of interest.

References

- Agrawal, A. (2001) Sexual selection and maintenance of sexual reproduction. *Nature*, 411, 692–695.
- Andersson, M. (1986) Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. *Evolution*, 40, 804–816.
- Andersson, M. (1994) *Sexual Selection*. Princeton University Press, Princeton, NJ.
- Andjelkovic, M. and Marinkovic, D. (1983) Selection for copulation ability of *Drosophila subobscura* in the absence of light. *Behavior Genetics*, 13, 411–419.
- Arbuthnott, D. and Rundle, H.D. (2012) Sexual selection is ineffectual or inhibits the purging of deleterious mutations in *Drosophila melanogaster*. *Evolution*, 2127–2137.
- Arnqvist, G. (1989) Multiple mating in a water strider: mutual benefits or intersexual conflict? *Animal Behaviour*, 38, 749–756.
- Arnqvist, G. and Rowe, I. (2005) *Sexual Conflict*. Princeton University Press, Princeton, NJ.
- Barbosa, F. (2011) Copulation duration in the soldier fly: the roles of cryptic male choice and sperm competition risk. *Behavioral Ecology*, 22, 1332–1336.
- Barron, A.B. (2000) Anaesthetising *Drosophila* for behavior studies. *Journal of Insect Physiology*, 46, 439–442.
- Candolin, U. (2003) The use of multiple cues in mate choice. *Biological Reviews*, 78, 575–595.
- Chapman, T., Liddle, L., Kalb, J., Wolfner, M.F. and Partridge, L. (1995) Cost of mating in *Drosophila melanogaster* is mediated by male accessory gland products. *Nature*, 373, 241–244.
- Chenoweth, S.F. and Blows, M.W. (2006) Dissecting the complex genetic basis of mate choice. *Nature Review Genetics*, 7, 681–692.
- Darwin, C. (1871) *The Descent of Man, and Selection in Relation to Sex*. J. Murray, London.
- David, P., Bjorksten, T., Fowler, K. and Pomiankowski, A. (2000) Condition-dependent signaling of genetic variation in stalk-eyed flies. *Nature*, 406, 186–188.
- Edington, C.W. (1957) The induction of recessive lethals in *Drosophila melanogaster* by radiations of different ion density. *Genetics*, 41, 814–821.
- Edward, D.A. and Chapman, T. (2011) The evolution and significance of male mate choice. *Trends in Ecology and Evolution*, 26, 647–654.
- Ellis, L.B. and Kessler, S. (1975) Differential posteclosion housing experiences and reproduction in *Drosophila*. *Animal Behaviour*, 23, 949–952.
- Evans, H.H. and DeMarini, D.M. (1999) Ionizing radiation-induced mutagenesis: radiation studies in *Neurospora* predictive for results in mammalian cells. *Mutation Research*, 437, 135–150.
- Fisher, D.N., Doff, R.J. and Price, T.A.R. (2013) True polyandry and pseudopolyandry: why does a monandrous fly remate? *BMC Evolutionary Biology*, 13, 157.
- Griffith, S.C., Owens, I.F. and Burke, T. (1999) Environmental determination of a sexually selected trait. *Nature*, 400, 358–360.
- Hammer, Ø., Harper, D. and Ryan, P.D. (2001) PAST: Paleontological statistics software package for education and data analysis. *Paleontol. Electron.* 4, 1–9.
- Harcourt, A.H., Harvey, P.H., Larsen, S.G. and Short, R.V. (1981) Testis size, body weight and breeding system in primates. *Nature*, 293, 55–57.
- Head, M.L., Hunt, J., Jennions, M.D. and Brooks, R. (2005) The indirect benefits of mating with attractive males outweigh the direct costs. *PLoS Biology*, 3, e33.
- Heisler, L., Andersson, M., Arnold, S.J., Boake, C.R., Borgia, G., Hausfater, G., Kirkpatrick, M., Lande, R., Maynard Smith, J., O’Donald, P., Thornhill, R. and Weissing, F. (1987) Evolution of mating preferences and sexually selected traits. *Sexual Selection: Testing the Alternatives* (eds J.W. Bradbury & M. Andersson), pp. 97–118. John Wiley and Sons, New York.
- Hollis, B., Fierst, J.L. and Houle, D. (2009) Sexual selection accelerates the elimination of a deleterious mutant in *Drosophila melanogaster*. *Evolution*, 63, 324–333.
- Hollis, B. and Houle, D. (2011) Populations with elevated mutation load do not benefit from the operation of sexual selection. *Journal of Evolutionary Biology*, 24, 1918–1926.
- Holman, L., Freckleton, R.P. and Snook, R.R. (2007) What use is an infertile sperm? A comparative study of sperm-heteromorphic *Drosophila*. *Evolution*, 62, 374–385.
- Immonen, P., Stundner, G., Thoß, M. and Penn, D.J. (2009) Females prefer the scent of outbred males: good-genes-as-heterozygosity? *BMC Evolutionary Biology*, 9, 104–113.

- Jennions, M.D. and Petrie, M. (1997) Variation in mate choice and mating preferences: a review of causes and consequences. *Biological Reviews*, 72, 283–327.
- Johnstone, R.A. (1995) Sexual selection, honest advertisement and the handicap principle: reviewing the evidence. *Biological Reviews of the Cambridge Philosophical Society*, 70, 1–65.
- Kempnaers, B. (2007) Mate choice and genetic quality: a review of the heterozygosity theory. *Advances in the Study of Behavior*, 37, 189–278.
- Kirkpatrick, M. (1987) Sexual selection by female choice in polygynous animals. *Animal Behaviour*, 72, 1215–1225.
- Kokko, H., Brooks, R., Jennions, M.D. and Morley, J. (2003) The evolution of mate choice and mating biases. *Proceedings of the Royal Society B*, 270, 653–664.
- Kotiaho, J.S., Simmons, L.W. and Tomkins, J.L. (2001) Towards a resolution of the lek paradox. *Nature*, 410, 684–686.
- Krebs, R.A. (1991) Function and genetics of long versus short copulations in the cactophilic fruit fly, *Drosophila mojavensis* (Diptera, Drosophilidae). *Journal of Insect Behavior*, 4, 221–233.
- Krimbas, C.B. (1993) *Drosophila Subobscura: Biology, Genetics, and Inversion Polymorphism*. Verlag Dr. Kovac, Hamburg.
- Liu, X.P., He, H.M. and Xue, F.S. (2014) The influence of female age on male mating preference and reproductive success in cabbage beetle, *Colaphellus bowringi*. *Insect Science*, 21, 515–522.
- Lizé, A., Rowan, J. D., Smaller, E.A., Lewis, Z. and Hurst, G.D.D. (2011) Perception of male–male competition influences *Drosophila* copulation behaviour even in species where females rarely remate. *Biology Letters*, 8, 35–38.
- Lizé, A., Price, T. A. R., Heys, C., Lewis, Z. and Hurst, G.D.D. (2014) The extreme cost of rivalry in a monandrous species: male–male interactions result in failure to acquire mates and reduced longevity. *Proceedings of the Royal Society of London, Series B*, 281: 20140631. 10.1098/rspb.2014.0631
- Loukas, M., Vergini, Y. and Krimbas, C.B. (1981) The genetics of *Drosophila subobscura* populations. XVIII. Multiple insemination and sperm displacement in *Drosophila subobscura*. *Genetica*, 57, 29–37.
- MacBean, I. T and Parsons, P. A. (1967) Directional selection for duration of copulation in *Drosophila melanogaster*. *Genetics*, 56, 233–239.
- Mallet, M.A., Bouchard, J.M., Kimber, C.M. and Chippindale, A.K. (2011) Experimental mutation-accumulation on the X chromosome of *Drosophila melanogaster* reveals stronger selection on males than females. *BMC Evolutionary Biology*, 11, 156.
- Mallet, M.A., Kimber, C.M. and Chippindale, A.K. (2012) Susceptibility of the male fitness phenotype to spontaneous mutation. *Biology Letters*, 8, 426–429.
- Maynard, S.J. (1956) Fertility, mating behavior and sexual selection in *Drosophila subobscura*. *Journal of Genetics*, 54, 261–279.
- Maynard, S.J. (1991) Theories of sexual selection. *Trends in Ecology and Evolution*, 6, 146–151.
- Mazzi, D., Kunzler, R., Lergiader, C.R. and Bakker, T.C.M. (2004) Inbreeding affects female preference for symmetry in computer-animated sticklebacks. *Behavior Genetics*, 34, 417–424.
- McGhee, K.E., Fuller, R.C. and Travis, J. (2007) Male competition and female choice interact to determine mating success in the bluefin killifish. *Behavioral Ecology*, 18, 822–830.
- McGuigan, K., Petfield, D. and Blows, M.W. (2011) Reducing mutational load through sexual selection on males. *Evolution*, 65, 2816–2829.
- Monclus, M. and Prevosti, A. (1971) The relationship between mating speed and wing length in *Drosophila subobscura*. *Evolution*, 25, 214–217.
- Narraway, C., Hunt, J., Wedell, N. and Hosken, D.J. (2010) Genotype-by-environment interactions for female preference. *Journal of Evolutionary Biology*, 23, 2550–2557.
- Parsons, P.A. and Kaul, D. (1966) Mating speed and duration of copulation in *Drosophila pseudoobscura*. *Heredity*, 21, 219–225.
- Patty, R.A. (1975) Investigation of genetic factors influencing duration of copulation in ‘eastern’ and ‘western’ *Drosophila athabasca*. *Animal Behaviour*, 23, 344–348.
- Pegueroles, G. (1996) Analysis of inbreeding in a colonizing population of *Drosophila subobscura*. *Genetica*, 98, 289–296.
- Pekkala, N., Puurtinen, M. and Kotiaho, J.S. (2009) Sexual selection for genetic quality: disentangling the roles of male and female behavior. *Animal Behaviour*, 78, 1357–1363.
- Pepin, K.M., Samuel, M.A. and Wichman, H.A. (2006) Variable pleiotropic effects from mutations at the same locus hamper prediction of fitness from a fitness component. *Genetics*, 172, 2047–2056.
- Pinsker, W. and Doschek, E. (1980) Courtship and rape: the mating behaviour of *Drosophila subobscura* in light and in darkness. *Zeitschrift für Tierpsychologie*, 54, 57–70.
- Pomiankowski, A. (1988) The evolution of female mating preferences for male genetic quality. *Oxford Surveys in Evolutionary Biology*, 5, 136–184.
- Price, T., Schluter, D. and Heckman, N.E. (1993) Sexual selection when the female direct benefits. *Biological Journal of the Linnean Society*, 38, 187–211.
- Proulx, S.R. (1999) Mating systems and the evolution of niche breadth. *American Naturalist*, 154, 89–98.
- Radwan, J. (2004) Effectiveness of sexual selection in removing mutations induced with ionizing radiation. *Ecology Letters*, 7, 1149–1154.
- Radwan, J., Unrug, J., Śnigórska, K. and Gawróńska, K. (2004) Effectiveness of sexual selection in preventing fitness deteri-

- oration in bulb mite populations under relaxed natural selection. *Journal of Evolutionary Biology*, 17, 94–99.
- Rendel, J.M. (1945) The genetics and cytology of *Drosophila subobscura*. II. Normal and selective matings in *Drosophila subobscura*. *Journal of Genetics*, 46, 287–302.
- Rowe, L. and Houle, D. (1996) The lek paradox and the capture of genetic variance by condition dependent trait. *Proceedings of the Royal Society B*, 263, 1415–1421.
- Rumball, W. (1994) Decline in heterozygosity under full-sib and double first-cousin inbreeding in *Drosophila melanogaster*. *Genetics*, 136, 1039–1049.
- Savic Veselinovic, M., Pavkovic-Lucic, S., Kurbalija Novicic, Z., Jelic, M. and Andjelkovic, M. (2013) Sexual selection can reduce mutational load in *Drosophila subobscura*. *Genetika*, 45, 537–552.
- Schneider, J.M., Gilberg, S., Fromhage, L. and Uhl, G. (2006) Sexual conflict over copulation duration in a cannibalistic spider. *Animal Behaviour*, 71, 781–788.
- Shackleton, M.A., Jennions, M.D. and Hunt, J. (2005) Fighting success and attractiveness as predictors of male mating success in the black field cricket, *Teleogryllus commodus*: the effectiveness of no-choice tests. *Behavioral Ecology and Sociobiology*, 58, 1–8.
- Sharp, N.P. and Agrawal, A.F. (2008) Mating density and the strength of sexual selection against deleterious alleles in *Drosophila melanogaster*. *Evolution*, 62, 857–867.
- Sharp, N.P. and Agrawal, A.F. (2013) Male-biased fitness effects of spontaneous mutations in *Drosophila melanogaster*. *Evolution*, 67, 1189–1195.
- Siller, S. (2001) Sexual selection and the maintenance of sex. *Nature*, 411, 689–692.
- Steele, R.H. (1986) Courtship feeding in *Drosophila subobscura*. II. Courtship feeding by males influences female mate choice. *Animal Behaviour*, 34, 1099–1108.
- Tomkins, J.L., Radwan, J., Kotiaho, J.S. and Tregenza, T. (2004) Genic capture and resolving the lek paradox. *Trends in Ecology and Evolution*, 19, 323–328.
- Whitlock, M.C. (2000) Fixation of new alleles and the extinction of small populations: drift load, beneficial alleles, and sexual selection. *Evolution*, 54, 1855–1861.
- Whitlock, M.C. and Agrawal, A.F. (2009) Purging the genome with sexual selection: reducing mutational load through selection on males. *Evolution*, 63, 569–582.
- Zahavi, A. (1975) Mate selection: a selection for a handicap. *Journal of Theoretical Biology*, 53, 205–214.
- Zahavi, A. (1977) Cost of honesty (further remarks on handicap principle). *Journal of Theoretical Biology*, 67, 603–605.

Accepted July 5, 2015