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Mating frequency of *Drosophila melanogaster* strains reared on carrot and banana diets.

Pavković-Lučić, Sofija[1], Jelena Trajković [2], and Tatjana Savić [2]. [1]University of Belgrade, Faculty of Biology, Studentski trg 16, 11000 Belgrade, Serbia; [2]University of Belgrade, Institute for Biological Research "Siniša Stanković", Despot Stefan Blvd. 142, Belgrade, Serbia.

Mating in *Drosophila* is probably one of the most studied behaviors. Different aspects of courtship, quality, and quantity of stimuli exchanged, as well as various components of reproductive behavior (mating latency, mating speed, mating success, copulation duration, sexual rejection, remating time, and frequency, female benefits of remating, sperm competition, and so forth) were studied in many *Drosophila* species, using diverse experimental designs and techniques (for reviews see Hall, 1994; Greenspan and Ferveur, 2000; Markow, 2002; Singh *et al*., 2002; Markow and O' Grady, 2005; Lasbleiz *et al*., 2006). The complex genetic basis involved in determination of mating behavior was thoroughly studied over decades, starting from using mutant strains and gynandromorphs (for review see Yamamoto *et al*., 1997), up to modern researches of underlying neural, neurochemical, and molecular mechanisms (Mackay *et al*., 2005; Zhou *et al*., 2012). Beside genetic influences, environmental conditions may also contribute to some components of mating behavior *via* their impact on traits involved in sexual selection. Among them, nutrition is one of the most important factors that may influence both morphological and/or physiological characteristics, and, indirectly, behavioral ones.

In this work, number of matings of two *Drosophila melanogaster* strains maintained for twelve years on different substrates was estimated. These substrates are modified standard *Drosophila* laboratory food, prepared by adding fruit (banana, flies designated as "B strain") and vegetable (carrot, flies designated as "C strain"), without sugar and yeast (Kekić and Pavković-Lučić, 2003). Two diets differ in chemical composition, odor, taste, and color.

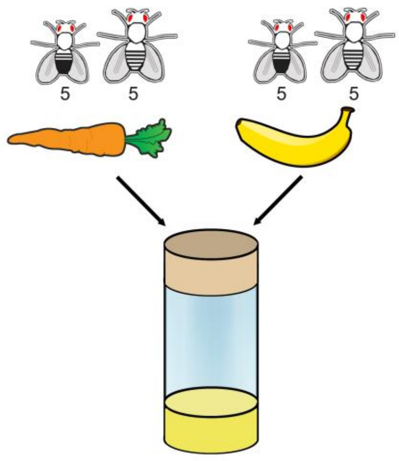

Figure 1. Mating scheme *per* replica.

"Multiple choice" behavioral assays were composed of replicates in which twenty flies (*per* replicate) were introduced into a mating vial containing standard cornmeal substrate. Flies were crossed following mating scheme (Figure 1): 5 males reared on carrot substrate (C males) + 5 females reared on carrot substrate (C females) + 5 males reared on banana substrate (B males) + 5 females reared on banana substrate (B females). Thirty-seven replicates were run, *i.e.*, a total of 740 flies participated. Mating observations lasted 90 minutes and every single pair was removed into the new separate vial. Flies of both sexes and strains were scored for number of matings during each of three separate, consecutive 30 minutes periods. Flies of different strains were later identified using UV lamp, since they were alternatively marked with fluorescent powder 24 hours before mating. Using UV dust does not influence mating frequency in this species (Terzić *et al*., 1994). Significant differences in number of matings achieved by flies of both sexes and strains were tested using Z-test.

Out of a possible 370 matings, 205 matings occurred (55.4%). During the first 30 minutes, 56 matings were observed (27.3%); during the next 30 minutes, 105 matings were observed (51.2%), and within the last 30 minutes, 44 matings were recorded (21.5%). Most of the copulations occurred within one hour observing period (78.5%).

Carrot-fed males were more successful in mating (58.54%) than males developed on banana diet (41.46%). During the first 30 minutes, C males realized 51.79% matings, while B males took part in 48.21% of the matings, and this difference is not significant ($Z = 0.535$, $p > 0.05$). Over the next 30 minutes, C males achieved 20% more matings than B males ($Z = 4.099$, $p < 0.01$). The same situation was observed in the last 30 minutes when C males realized 63.64% matings, which is significantly more ($Z = 3.618$, $p < 0.01$) than B males (36.36 %) (Figure 2). When cumulative data were used (Figure 3), C males outperformed B males during 60 minutes observing period ($Z = 3.625$, $p < 0.01$), as well as within 90 minutes observing period ($Z = 4.889$, $p < 0.01$).

Females developed on carrot diet were also more successful in mating. They achieved significantly more matings (78.57% and 56.19%) than banana-fed females (21.43% and 43.81%) during the first 30 minutes ($Z = 8.552$, $p < 0.01$), as well as during the next 30 minutes ($Z = 2.537$, $p < 0.05$), respectively. C and B females achieved the same number of matings in the last 30 minutes (Figure 2). Using cumulative data (Figure 3), C females were more successful in mating during 1 hour observing period ($Z = 7.093$, $p < 0.01$), as well as within whole 90 minutes ($Z = 6.286$, $p < 0.01$).

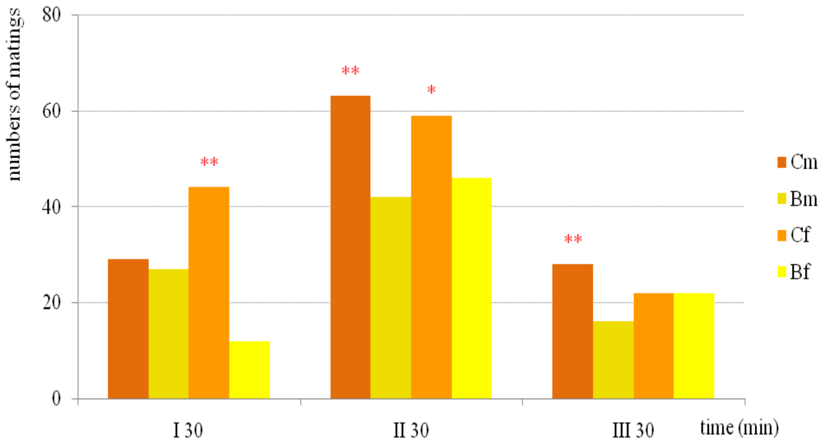

Figure 2. Number of matings achieved by both sexes of two *D. melanogaster* strains during every 30 minutes of observing period (I, II, and III). Abbreviations: B – flies reared on banana substrate, C - flies reared on carrot substrate, m – males, f - females.

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

Comparing to some other experimental designs, when sex ratio is biased toward one sex, in "multiple choice" design, which was used in this experiment, sex ratio was equal (F:M = 1:1). Therefore, competitive interactions were less pronounced, since every single fly had a chance to find a mate. Additionally, prolongation of the time of mating observations led to a greater number of recorded copulations. According to results presented here, carrot-fed flies achieved significantly more matings than B flies. Subsequent morphometric analysis showed that B flies were significantly larger than C flies, but body size *per se* did not provide them higher success in mating (Trajković *et al*., 2012). It seems that some other traits (physiological and/or behavioral) contributed to higher mating success of C flies.

Fruit flies use their olfactory and gustatory systems for evaluating smell and taste of food as well as odor and taste stimuli exchanged with mates (Ferveur, 2005; Laissue and Vosshall, 2008; Ruebenbauer *et al*., 2008; Everaerts *et al*., 2010; Lebreton *et al*., 2012; Rohwedder *et al*., 2012).

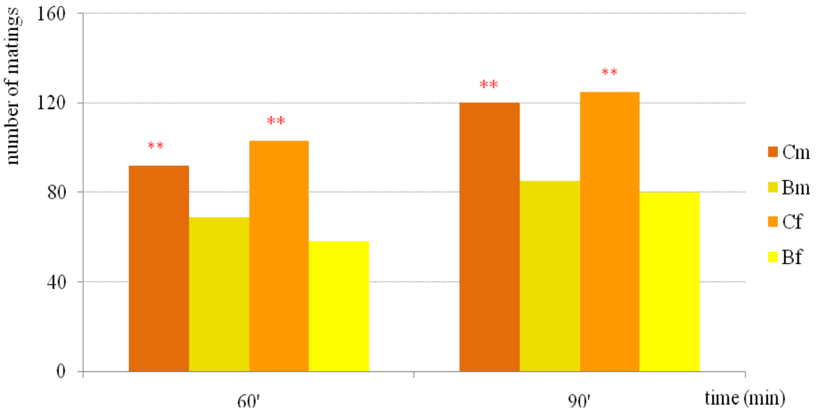

Figure 3. Cumulative number of matings achieved by both sexes of two *D. melanogaster* strains within 60 minutes and 90 minutes observing period. Abbreviations: as in Figure 1.

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

For *D. melanogaster*, banana is one of the favorite diets (Shorrocks, 1974), and their larvae were less-stressed during development on banana than on standard laboratory food (Carsten *et al*., 2005). However, when competed with carrot-fed flies, they were less successful in mating. Contrary to information available for banana as a *Drosophila* feeding substrate, less is known about the effect of carrot diet, especially long-lasting, on fruit fly mating performances. It is possible that carotenoids may have some impact on flies' physiological state, through their influence on vision and production of mating signals, as precursors to pheromones, which was observed for some insects (Heath *et al*., 2013). Precise roles of carotenoids in diet on insect behavior, including *Drosophila* mating performances, are insufficiently known and further researches are demanded in this area.

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