

Are larger and/or more symmetrical *Drosophila melanogaster* (Diptera, Drosophilidae) males more successful in matings in nature?

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ABSTRACT. Are larger and/or more symmetrical *Drosophila melanogaster* (Diptera, Drosophilidae) males more successful in matings in nature? Sexual selection in *Drosophila melanogaster*, related to body size and fluctuating asymmetry in wing length and number of sex comb teeth in males, was tested in natural conditions. Males collected *in copula* were significantly larger than those collected as a single, while no difference in mean number of sex comb teeth between copulating and single males was observed. On the other hand, single males had greater asymmetry both for wing length and number of sex comb teeth than their mating counterparts. It looks like that symmetry of these bilateral traits also may play a role in sexual selection in this dipteran species in nature.

KEYWORDS. Body size; *Drosophila melanogaster*; fluctuating asymmetry; mating success; sex combs.

RESUMO. São maiores e/ou mais simétricos os machos de *Drosophila melanogaster* (Diptera, Drosophilidae) com mais sucesso nos acasalamentos na natureza? A seleção sexual em *Drosophila melanogaster* foi testada em condições naturais. Os machos coletados em cópula foram significativamente maiores do que na amostra controle, enquanto que diferenças no número médio de dentes do pente sexual não foram estatisticamente significativas. Por outro lado, os machos que não estavam copulando no momento da coleta foram mais assimétricos, tanto em relação ao comprimento das asas como em relação ao número de dentes do pente sexual. Parece que a simetria dos traços bilaterais pode ter um papel na seleção sexual desta espécie na natureza.

PALAVRAS-CHAVE. Assimetria flutuante; *Drosophila melanogaster*; pente sexual; sucesso reprodutivo; tamanho do corpo.

The concept of sexual selection was introduced by Darwin (Darwin 1871), who defined it as the effects of the “struggle between the individuals of one sex, generally the males, for the possession of the other sex”. This definition has undergone some modifications over time; nowadays, sexual selection is usually defined as selection that arises from differences in mating success (Arnold 1994). Two forms of sexual selection, intra- and intersexual selection, act on those traits that increase mating success. Intrasexual selection implies competition between members of the same sex for access to mates, while intersexual selection means that members of one sex choose members of the opposite sex (*i.e.* mate choice). Sexual selection may take place at a number of different stages in reproduction (Moller 1994): in *Drosophila*, it can occur before mating takes place (pre-copulatory sexual selection, Taylor *et al.* 2008), as well as after mating (*e.g.* sperm competition) (Singh *et al.* 2002).

Drosophila is a frequent model organism for studying the phenomenon of sexual selection. Body size, as a trait that provides an advantage in attracting mates, was the most used morphological feature in such studies (Markow *et al.* 1996), whether the fitness consequences of *Drosophila* sexual selection is under much debate (see for review Taylor *et al.* 2008). Although the relationship between success in mating and body size was confirmed in some species as, for example, *D. ananassae* (Singh & Singh 2003), *D. buzzatii* (Santos *et al.* 1988), *D. melanogaster* (Bangham *et al.* 2002; Partridge *et al.* 1987a; Partridge *et al.* 1987b; Pavković-Lučić *et al.* 2009; Tay-

lor & Kekić 1988), *D. mojavensis* (Markow & Ricker 1992), *D. nigrospiracula* (Markow 1988), *D. simulans* (Markow & Ricker 1992; Taylor *et al.* 2008), *D. testacea* (James & Jaenike 1992), in others the body size and (male) mating success was uncorrelated as in *D. hydei* (Pavković-Lučić & Kekić 2007), *D. immigrans* (Pavković-Lučić & Kekić 2009), *D. littoralis* (Aspi & Hoikkala 1992), *D. pseudoobscura* (Markow *et al.* 1996), *D. willistoni* (Basso da Silva & Valente 2001) or even negatively correlated as in *D. subobscura* (Steele & Partridge 1988).

Testing the relationship between developmental stability and sexual selection was one more approach proposed in sexual selection studies in *Drosophila* (Markow *et al.* 1996), as well as in other animal species (Moller & Swaddle 1997). This approach looked at the consequences of developmental instability by searching associations between asymmetries in bilateral morphological characteristics and mating success. The most common measure used to observe and describe the magnitude of developmental instability is fluctuating asymmetry (FA). FA is defined as a small, random departure from anticipated bilateral symmetry, such that a plot of the differences between sides generates a normal distribution (Markow 1995); it refers about subtle departures from identical expression of a trait across an axis of symmetry (Ludwig 1932) and, in many taxa is under stabilizing selection for reduced asymmetry (Polak & Starmer 2001). The fundamental basis for the study of FA is an *a priori* expectation that symmetry is the ideal state of bilaterally paired traits (Tomkins & Kotiaho 2001).

Many papers regarding relationship between developmental instability and sexual selection in different animal taxa showed negative associations between FA of certain traits and mating success; also, a large body of papers demonstrated no associations between FA and success in mating or reverse associations have been found (for detailed review see Appendix A to the paper by Simmons *et al.* 1999). When different *Drosophila* species were used for testing associations between mating success and symmetry of bilateral traits, also, no consistent pattern was observed (Bourguet 2000; Markow & Ricker 1992; Markow *et al.* 1996; Norry *et al.* 1998; Polak *et al.* 2004; Santos 2001; Vishalakshi & Singh 2008). In the first laboratory study in which relationship between male *D. melanogaster* mating success and FA was examined, it was concluded, that, when wild type males were competed for single females, successful males were larger and more symmetrical for sternopleural bristle numbers (Markow 1987). In another study, there was no relationship between the symmetry of sternopleural bristle number and mating success in *D. melanogaster* (Bourguet 2000).

As each behavior has its morphological, physiological and biochemical correlates, very small differences in morphology between the bilaterally symmetrical traits may be important in the expression of behavioral phenotypes, e.g. during the complex process of *Drosophila* courtship. *Vice versa*, small deviations detected in the sexual behavior (for instance, in repeatable performance of behavioral trait) may reflect the very small deviations in morphology.

Working with field *D. melanogaster* samples, we wanted to examine if male wing length and number of sex comb teeth (secondary sexual male character), as well as FA of those traits are related to male mating success and sexual selection. Courtship behavior in *Drosophila melanogaster* is very complex, consisting of a stereotyped ritual (Hall 1994) and engages multiple sensory systems (Griffith & Ejima 2009). It consists of several, well defined phases during which visual, olfactory, acoustic, tactile, and gustatory stimuli are exchanged (Hall 1994; Greenspan & Ferveur 2000). Because of the possible importance of wing size and symmetry during courtship (in producing “love song”, since approximately 80 per cent of the sexual stimulation in *D. melanogaster* is provided by wing vibration, Ewing 1964), as well as size and symmetry of sex combs (which are involved in grasping the female’s abdomen, Spieth 1952; Ng & Kopp 2008), it was supposed that larger and more symmetrical males could be more successful in mating.

MATERIAL AND METHODS

Field study was conducted in a weekend settlement on the banks of Lake Stara Tisa, near the village of Bačko Gradište, Serbia. To attract fruit flies, a mash of seasonal fruits (about 10 kg of mixed apples, plums, and grapes, with small amount of sugar to stimulate fermentation) was put in a 15-liter barrel. The barrel was located near a weekend house below an extension of the roof, where it was protected from sunshine and

rain. This specific habitat turns out to be very attractive for domestic *Drosophila* species, particularly for *D. melanogaster* and *D. hydei* (Kekić 2002; Pavković-Lučić & Kekić 2007).

Flies were collected by aspirator in the early morning, during their maximal sexual activity, on the 7th August 2004. Altogether, we collected 93 *D. melanogaster* mating pairs. Also, we captured the other, unmated flies, which were present in the barrel. We used them as “non-mating” flies (control group): in total, we collected 107 nonmated (single) males and 106 single females. It is important to collect copulating and single males at the same time and from the same location on the substrate, because local groups of males compete for receptive females and the single male was likely to have been out-competed by the mating one (Markow 1995). Both mating and non-mating flies were collected by aspirator, as significant difference in wing length between groups of flies collected by different sampling methods was previously observed (Kekić *et al.* 1995).

Mating pairs, as well as control groups of flies were preserved in separate eppendorfs filled with 70% ethanol and were taken into laboratory, where their body size was measured and number of sex comb teeth counted.

Wing length was used as the measure of body size (Partridge *et al.* 1987a, 1987b): it was measured using the length of the third longitudinal vein, from the anterior crossvein to the distal edge (Fig. 1). All measurements were performed by single person, using binocular microscope fitted with an ocular scale, at the ocular magnitude 10x and objective magnitude 8x (1mm = 62 measurement units).

Number of sex comb teeth was counted under microscope at a magnification of x 120, after mounting each male’s leg on a glass slide in a drop of glycerol (Fig. 2).

Numerical values were calculated using statistical software Statistica 5.0; *t-test* was used to determine if there is significant difference in mean wing length and mean number of sex comb teeth between mating and non-mating individuals. The coefficient of phenotypic correlation (Pearson, *r*) was used to estimate phenotypic likeness of the individuals and thus the degree of assortative mating; theoretically, if $r = 1$, then individuals are mating completely assortatively.

The intensity of sexual selection for longer wings was calculated as $i = SD/\phi$, where *SD* = selection differential, and ϕ = standard deviation of a control sample (Falconer 1981).

Asymmetry of an individual was measured as the left (L) minus the right (R) of the bilaterally paired traits. When comparing levels of fluctuating asymmetry between copulating and non-copulating males, we followed the guidelines of Palmer (1994) and Palmer & Strobeck (2003). This statistical procedure includes several steps: testing for departure of normality, for directional asymmetry (departure of the mean of (L – R) from an expected mean of zero using *one-sample t test*), for a relationship between asymmetry and trait magnitude (*i. e.* size dependence of FA) and, finally, for measurement error. We used Palmer index FA 6 as a measure of fluctuating asymmetry, as it was recommended when clear evidence exists of a size dependence of $|L - R|$ among indi-



Figs. 1–2. 1, *Drosophila melanogaster* wing with marked wing length; 2, sex comb teeth in *D. melanogaster*.

viduals within a sample, which was the case with our data. FA 6 is computed as $\text{var}[(L-R)/(L+R)/2]$ and lends itself to the most powerful test for differences between two samples (F-test); it is very efficient for estimating the between-sides variation (Palmer 1994).

In studies of FA it is important to establish that true FA exists, since measurement error also creates random differences between L and R. For wing length, measurement error was estimated on the basis of repeated measurements in a random sample of 50 males. Variances of two (L-R) measurements were not significantly different ($F = 1.1705$, $P > 0.05$).

Contrary to the wing length, which is metric trait, number of sex comb teeth is meristic feature and its phenotype is defined by counting teeth in sex combs in both male legs. As in this case there is not intermediate or diminished expression of the trait (Markow *et al.* 1996), we counted number of sex comb teeth three times to avoid the measurement error.

RESULTS

Males *D. melanogaster* collected *in copula* were significantly larger than males from a control sample, *i. e.* mating males had significantly longer wings than those stayed unpaired (Table I). This was a case for females, too, *i. e.* mating females had significantly longer wings than control group. For 93 females captured during copulation, the mean wing length was $\bar{x} \pm \text{S.E.} = 102.38 \pm 0.45$ (in terms of measurement units, 1mm = 62 measurement units). A random sample of 106 non-copulating females had a mean wing length of $\bar{x} \pm \text{S.E.} = 100.90 \pm 0.50$. This difference in mean wing length between mating and control group of females was statistically significant ($t = 3.29$, $df = 197$, $P < 0.01$).

Mating was random with respect to wing size, *i. e.* assortative mating for wing length was not observed. Pearson's coefficient of correlation (r) in wing length of flies collected *in copula* was 0.07; it was not statistically significant and implies random mating according to this phenotypic trait. Intensity of selection for longer wings was $i = 0.443$ and was very similar to those previously observed in this species in field conditions (Taylor & Kekić 1988).

Contrary to the wing length, statistically significant difference in mean number of sex comb teeth between copulating and non-copulating males was not observed (Table I).

Table I. Mean wing length and number of sex comb teeth in copulating and non-copulating *D. melanogaster* males. 1 mm = 62 measurement units. Abbreviation: n.s. – non-significant.

Male phenotypic trait	Mating status	N	$\bar{x} \pm \text{S.E.}$	t-test	df	P
Wing length	"in copula"	93	89.06 ± 0.58	3.03	198	<0.01
	control group	107	86.64 ± 0.56			
Number of sex comb teeth	"in copula"	91	10.50 ± 0.09	0.91	186	n.s.
	control group	97	10.40 ± 0.08			

After calculating FA 6 indices, it was observed that single, non-mated males had greater asymmetry both for wing length and number of sex comb teeth than their mating counterparts (Table II), *i. e.* mated males were more symmetrical in those morphological traits.

Table II. Comparisons of FA in wing length and number of sex comb teeth between mating and non-mating *D. melanogaster* males; F-test.

Levels of comparisons of FA	N	df	F	P
Wing length of mated males	93	92	1.549	< 0.05
Wing length of non-mated males	107	106		
Number of sex comb teeth in mated males	91	90	1.455	< 0.05
Number of sex comb teeth in non-mated males	97	96		

DISCUSSION

In *Drosophila*, body size is closely related with courtship pattern, mating success, locomotor activity, flight capacity, competitive interactions and different fitness components (Bangham *et al.* 2002; Partridge *et al.* 1987b; Santos *et al.* 1988; Santos *et al.* 1992; Singh & Singh 2003). The significance of the role of *Drosophila* male body size in mating success varies in different species and different conditions: however, in *Drosophila melanogaster*, larger male body size was often associated with mating success both in natural and laboratory conditions (Bangham *et al.* 2002; Partridge *et al.* 1987a; Partridge *et al.* 1987b; Pavković-Lučić *et al.* 2009; Taylor & Kekić 1988). The possible explanations for greater mating success of larger males include delivering more courtship (Partridge *et al.* 1987a; Partridge *et al.* 1987b), producing more courtship song (Partridge *et al.* 1987b) or their

winning in fights (Partridge & Farquhar 1983). Larger *D. melanogaster* males also mated earlier than smaller males (Pavković-Lučić *et al.* 2009) and had higher postcopulatory success than smaller ones (Bangham *et al.* 2002). Our result also revealed that males that possessed longer wings were more successful in achieving copulations. It is consistent with our previous field (Taylor & Kekić 1988) and laboratory investigations (Pavković-Lučić *et al.* 2009) concerning this species.

As *Drosophila* species differ significantly according to age of their sexual maturity, components of mating behavior and reproductive strategies (Markow 2002), as well as with respect to ecological context in which they realize their lifetime functions, it is not surprising that males of various species differ in the significance of the same (morphological or behavioral) trait in the context of sexual selection. In *D. melanogaster* both sexes mature simultaneously and sexual size dimorphism is expressed. In our sample, sexual size dimorphism, defined as F/M ratio was about 1.15, which means that females had 15% longer wings than males. Females of *D. melanogaster* are usually courted by more than one male at a time, mostly three or four or even more (Taylor & Kekić 1988). Also, females of this species belong to slow remating category (Markow 2002), being unreceptive for almost a week (McRobert *et al.* 1997). For these reasons, in this species, operational sex-ratio (OSR) is biased toward males (Markow 2002), thus possible promoting body-size dependent sexual selection in natural conditions. This was not a case in *Drosophila hydei*, another *Drosophila* species under our field investigation during August 2004. *D. hydei* is monomorphic species and possesses many different morphological, physiological, developmental and behavioral traits that make it very different from *D. melanogaster*. Contrary to *D. melanogaster*, *D. hydei* is characterized with female biased operational sex ratio (Markow 2002), which may influence that body size is not a crucial parameter in sexual selection in this species (Pavković-Lučić & Kekić 2007).

Because fluctuating asymmetry (FA) is assumed to signal the phenotypic quality, as determined by the developmental environment of prospective mates, it is also the focus of studies of sexual selection (Markow 1995). It is assumed that individuals tend to optimize the size and symmetry of traits involved in sexual selection, *i.e.*, a high quality individuals will be able to develop large and/or adequately expressed traits, which will also be symmetric. In the context of sexual selection, FA could be the measure of the quality of individuals that are in competition to achieve mating, as well as criteria used in decisions concerning the quality of partners. However, there is a question if FA is a sensitive index of the overall genetic quality, since different traits may react differently on causes of FA. Also, trait size may be more sensitive to developmental conditions than FA. For example, in *D. ananassae*, the level of FA was similar in mated and unmated males (including wing length and sex comb teeth), while the size of all traits under investigation was higher in mated than in unmated flies (Vishalakshi & Singh 2008).

Whether wings' plasticity is adaptive, it could be expected that their development may be less sensitive to the action of genetic and environmental factors, because of their exceptional importance in flight, courtship sound production, rapid escape from predators, etc. From this point of view, low FA of such important morphological trait could be expected in mated males. On the other hand, the wings could be exposed to intensive directional sexual selection, which may reduce the effectiveness of control mechanisms during development and, consequently, lead to increased FA. Our results showed that males with symmetrical wings were more successful in mating in nature; it is possible that there is influence of minor FA on courtship performance or competitive interactions among rivals.

The sex combs, another bilateral feature under our study, is secondary sexual male trait that is represent as an array of specialized mechanosensory bristles on the forelegs. Their morphology as well as function varies greatly among *Drosophila* species (Ng & Kopp 2008). In *D. melanogaster*, males use sex combs for grasping of extruded female genitalia before mounting (Spieth 1952); it was confirmed by different techniques that this morphological structure contributes to their mating success (Ng & Kopp 2008). According to our results, low FA in secondary sexual trait in *D. melanogaster* provided reliable indicator of male quality in natural conditions, *i. e.* greater symmetry in number of sex comb teeth was associated with male mating success and/or females detected the higher level of FA through their mechanosensory organs, and rejected males in which FA in this trait persist. However, by comparing our and literature data (Ahuja & Singh 2008; Markow *et al.* 1996; Ng & Kopp 2008; Polak *et al.* 2004; Vishalakshi & Singh 2008), we could support the previous records that the number of sex comb teeth, as well as their FA may affect mating success in opposite directions in different *Drosophila* species (Ahuja & Singh 2008; Ng & Kopp 2008).

In conclusions, mating *D. melanogaster* males displayed larger body size and were more symmetrical both in wing length and number of sex comb teeth. It looks like, that, in nature, FA reflect phenotypic state that influences males' ability to mate and/or females may use FA of those traits to assess male quality. It is possible that morphological asymmetries in traits involved in mating (wing or sex combs) may lead to some kind of "behavioral asymmetries" (*i.e.* "asymmetrical song" or "asymmetrical tactile stimuli", respectively), if symmetrical signals produced by two examined traits used in sexual selection can give important information about the quality of the phenotype. However, we have no direct information about females ability, limited or not, to detect the small differences in number of sex comb teeth or wing symmetry.

After all, since many genetic and environmental factors may cause developmental instability (Moller & Swaddle 1997) and, since different data were obtained in different traits, conditions and species analyzed (see for review Vishalakshi 2011), a general conclusion based on our results

cannot be drawn, because data were obtained from a single sampling. The more complete conclusions considering this species should be supported by additional field work, by the analysis originating from different time-points and from different sampling sites. Furthermore, calculating FA for one or two traits may not be sufficient for evaluation of the stability of the development of the whole body, so the larger number of bilateral traits which are of different importance for the organism may be required for further and more complete analysis.

It is also important to note that we have no information about the relative ages of males in both mating and non-mating categories, as well as information about possible different larval or pupal mortality among individuals with higher FA. It is also possible that some other traits, which we did not observe in experiment, were more sensitive to genetic and environmental factors during development making their carriers developmentally-unstable. Furthermore, males may have some other traits that are not exposed to FA, on which female choice may be made (for example, different behavioral or morphological, non-bilateral traits). Females, especially in nature, are exposed to great number of courting males, which further complicates their possibility of perception between different stimuli provided by rivals.

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