

LONG-TERM DIETARY EFFECTS ON FRUIT FLY “LOVE STORY”: SIZE AND SYMMETRY OF SEX COMBS AND MALE MATING SUCCESS

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ABSTRACT

The influence of three types of diets on the number and symmetry of sex comb teeth (male secondary sexual character) as well as on behavioral trait (mating success) was tested in fruit fly in laboratory conditions. Mating experiments were conducted with three *Drosophila melanogaster* strains reared more than one year on different food. The quality of the diet itself had no influence on the number of sex comb teeth, neither on their symmetry. The size of sex comb and levels of fluctuating asymmetry were similar in mated and unmated males. However, males developed on different substrates showed differences in mating success: males of the “banana strain” and of the “cornmeal strain” were more successful in achieving copulations than males of the “tomato strain”. It seems that some other traits (morphological, physiological, and behavioral) were more important for male mating success than tactile stimuli provided by sex combs during the courtship.

Key words: *Drosophila*, nutrition, sex combs, mating behavior.

INTRODUCTION

Courtship behavior in fruit fly, *Drosophila melanogaster* is complex and consists of a series of well-defined steps, including male orienting, tapping, “singing” (i.e. producing “love song” by wing vibration), licking, attempting copulation and copulation itself, if the female is receptive (Hall, 1994). During those behavioral steps, flies exchange different types of stimuli: visual, olfactory, acoustic, gustatory, and tactile (Greenspan and Ferveur, 2000; Markow and O’Grady, 2005). All sensory domains are important in perception, but some of them either singly or in combinations, may be of higher importance for some behavioral aspects, periods of life, specific daily and/or seasonal activities, etc. During the courtship, an intensive sexual selection occurs, through competition among mates, and/or mate (female) choice.

A variety of traits, which are presumed to be involved in sexual selection have been tested in both the laboratory and field. Investigations related to body size-dependent inter- or intrasexual interactions have been the subject of much research on *Drosophila* species, including *D. melanogaster* (for a review see Pavkovi -Lu i and Keki , 2013). Furthermore, symmetry of bilaterally paired morphological traits and its role in sexual selection has been extensively debated in *Drosophila* species (Markow *et al.*, 1996; Polak and Taylor, 2007; Polak, 2008; Vishalakshi, 2011). The main focus of these studies were to establish whether females found more symmetrical males more attractive, and/or whether symmetrical males would have a mating advantage. In such studies, it is thought the development of a stable phenotype is of major importance for the

optimal performance of individual organisms (Møller, 1999) involving an *a priori* expectation that symmetry is the ideal state of bilaterally-paired traits (Tomkins and Kotiaho, 2001).

Studies concerning the impact of nutrition have often assessed the morphological and physiological responses of individuals exposed to different quality and quantity of nutrients (for a review see Kristensen *et al.*, 2011), but less is known about the long-term consequences of different diets, especially at the behavioral level. When considering drosophilids, such a nutritional investigation was conducted in the Hawaiian species *Drosophila grimshawi* by Dronev (1998), who established that the nutritional quality of the adult diet (primarily protein content) had a major influence on courtship vigour. In *D. melanogaster*, males reared on low protein diets had lower premating success (Fricke *et al.*, 2008). In cactophilic species, rearing cacti could affect some components of the mating behavior in *D. mojavensis* (Brazner and Etges, 1993) as well as male mating success in *D. buzzatii* and *D. koepferae* (Hurtado *et al.*, 2012).

As symmetry may be related to the morphological, physiological or behavioral state of an organism, here we have tested the relationship between male mating success and size and symmetry of its secondary sexual trait (sex comb, see Fig. 1) after more than a year of maintaining of flies on three types of diet. As sex comb is a secondary sexual trait, it may be more sensitive than “ordinary” morphological traits to environmental changes (here expressed as nutritional variability), due to the increased demands involved in its “expensive” production (see Møller and Pomiankowski, 1993). Furthermore, females would be able to use the size

and symmetry of this trait as ideal phenotypic cues in mate choice. In this respect, we presumed that 1) long-lasting diets could influence both the size and symmetry of sex combs, and 2) that more symmetrical males should be more successful in mating.

MATERIALS AND METHODS

Fly stocks: *D. melanogaster* flies maintained for more than one year (over 35 generations) on three types of diet: standard laboratory food (cornmeal-sugar-agar-yeast medium, designated as C strain), banana (B strain) and tomato substrates (T strain) were used (for recipes see Keki and Pavkovi -Lu i , 2003). Banana and tomato substrates were made without sugar and yeast. These diets differed in water, protein, lipid and carbohydrate content, also in aroma, flavor and color. Before the experiment started, flies were maintained in optimal laboratory conditions: without competition, at room temperature, relative humidity of about 50-60% and 12h:12h light/dark cycle.

Behavioral assays: After eclosion, virgin flies were kept separately according to sex and strain for 3-5 days in food vials. Mating assays were performed in the morning (from 08:00 to 12:00) in vials with standard cornmeal medium. Females maintained on standard laboratory food (C strain) were offered to choose between two male types ("female choice" experiments) belonging to different "nutritional strains" (C and B, C and T and B and T). Virgin flies in adequate proportions were placed in mating vials and left for one hour (*per* replica) to mate. Three experimental groups were made, designated as FC 1 (female choice 1), FC 2 (female choice 2) and FC 3 (female choice 3). Flies were crossed as follows:

FC 1: 10 females (C) + 10 males (C) + 10 males (B),

FC 2: 10 females (C) + 10 males (C) + 10 males (T),

FC 3: 10 females (C) + 10 males (B) + 10 males (T).

Ten replicates were run for each experimental set, i.e. 100 females and 200 males were tested *per* experimental group. Mating pairs were removed with the help of an aspirator; the flies that did not copulate within the 1 h test period were designated as unmated (i.e. "unsuccessful"). After mating, males from different strains were identified using a UV lamp, as they were marked with fluorescent powder 24 h before testing. Mating pairs, as well as unmated flies were then preserved in separate eppendorffs filled with 70% ethanol until their number of sex comb teeth was counted.

Measurements: Front legs were carefully removed and the number of sex comb teeth were counted on both sides (legs) of the fly under a microscope at a magnification of x 120. Mating success was scored as the number of copulations achieved by males that belonged to the different strains.

Statistical analysis: Statistical analyses were performed with the Statistica 5.0 package. Chi square test (χ^2) was used to test the difference in number of copulations achieved by males from different food regimes (calculated on the basis of 1:1 null hypothesis). Sex comb size (i. e. sex comb teeth number) was measured by the average value of left and right sides $(L+R)/2$. The means of a trait were compared using the t-test. As a measure of developmental stability, fluctuating asymmetry (FA) was used. This considers variation of differences in left (L) minus right (R) sides of a bilateral character. FA was used to measure: I) the developmental instability levels in mated and unmated males and II) the developmental instability levels in males reared for more than one year on different diets (independently of their mating status, mated or not). In the FA analysis, 50 males *per* nutritional strain, and 50 males *per* mating status (copulating and non-copulating) were used. Asymmetry of an individual was scored as the left (L) minus the right (R) of the number of sex comb bristles. Statistical procedure for the FA analyses included the following steps: testing for departure of normality, for directional asymmetry (one sample t-test), and for a relationship between asymmetry and trait magnitude (i.e. size dependence of FA) (Palmer, 1994; Palmer and Strobeck, 2003). As we found trait asymmetry to be significantly correlated with trait size, FA 6 was computed as $\text{var}[(L-R)/(L+R)/2]$. Differences between variances were tested by the F-test. Since measurement error in tooth counts is negligible (Polak and Taylor, 2007) and tooth counts are highly repeatable, we counted the number of sex comb teeth three times to avoid the measurement error.

RESULTS AND DISCUSSION

The number of copulations achieved by males developed on different food in the three experimental groups (FC 1, FC 2 and FC 3) is presented in Table 1 and Figure 2. Chi square (χ^2) analyses showed differences in the type of mating observed (Table 1). B and C males were equally successful in mating, since difference in number of copulations achieved was not significant. Males from C and B strains were significantly more successful in mating than those reared on tomato. The order of males from different nutritional strains that competed for females according to number of copulations achieved could be designated as $B = C > T$.

Males reared on different food did not differ significantly in average number of sex comb teeth (Table 2). In addition, no significant differences were found between mated and unmated males in mean number of sex comb teeth (Table 2). FA of sex comb teeth number was also not significantly different, i.e. the larval rearing substrate influenced neither size, nor FA of sex comb teeth (Tables 2 and 3). Furthermore, independently of the substrate in which males completed their development,

mated and unmated males did not differ in the FA of sex comb bristles (Table 3).

In *Drosophila*, adaptation of feeding behavior to nutrient composition has an important ecological role in the wild (Carvalho, 2010). In field conditions, flies may face periods of nutritional stress, so they must continuously alter their “nutritional strategies”. Genetic variation certainly contributes to this plasticity and, within a species, individuals may exhibit polymorphic and/or plastic responses to fluctuating food availability (Kaun *et al.*, 2007). The fact that individuals can survive using different nutritional resources can be considered as an example of a flexible, adaptive metabolism.

The literature concerning relation between nutrition (quality and quantity) and FA is controversial. Some studies found that food stress were related with higher FA, while, in some other studies, FA remains unaffected by nutritional stress (for review see Vishalakshi, 2011; Vijendravarma *et al.*, 2011). Sometimes, an effect was found on FA for some traits but not for others (Vishalakshi, 2011), or, trait size was more sensitive than trait symmetry (Vishalakshi and Singh, 2008). In recent study with *D. melanogaster*, no changes in the levels of FA of several wing measurements as either a plastic or an evolutionary response to malnutrition were detected, while nutritional stress had a significant effect on wing trait sizes at both plastic and evolutionary level (Vijendravarma *et al.*, 2011). Furthermore, sex comb bristle number and length significantly differed between *D. melanogaster* males reared on poor and rich diets - both sex comb components were significantly larger on a rich diet (Ahuja *et al.*, 2011).

In our experiment, males reared on different foods did not significantly differ either in average teeth number, or in their symmetry. Our results demonstrate a plastic response when *D. melanogaster* was exposed to different nutritional environments. Flies that had been reared for more than one year under described laboratory conditions possible undergone adaptive change to laboratory food and other laboratory conditions. In this respect, stable development of flies from our strains may be explained as a result of phenotypic plasticity of biochemical pathways and adaptive metabolism, which are important for surviving in ecologically different habitats. Furthermore, sex combs are canalized traits and much heritable variation is not expected. Highly canalised development (of the trait) reduces the effects of the genes and environment on developmental pathways (Møller and Swaddle, 1997).

Another question under our study was if mated and unmated males differed in size and symmetry of this phenotypic trait. According to the literature, the importance of male sex combs for mating success and other behavioral performances may vary among *Drosophila* species (see Vishalakshi, 2011 for a review).

It is known from earlier studies that male sex combs are involved in the exchange of tactile stimuli during courtship and mounting (Spieth, 1952), and, in *D. melanogaster*, their loss (by either mechanical or genetic means) strongly reduced the ability of males to copulate (Ng and Kopp, 2008). In our study, significant difference in average number of sex comb teeth, as well as in their symmetry between mated and unmated males were not observed. It seems that they were not used as sensitive phenotypic cues for choosy females. Rather, the action of stabilizing selection on sex comb teeth number may be involved: unadequate teeth number may influence female choice in a way that females avoid copulation with males possessing them, because of an inappropriate tactile stimulation (see Markow *et al.*, 1996).

Males belonging to different nutritional strains showed differences in mating success. Banana-fed males significantly exceeded the number of copulations achieved by males from T strain, while males from B and C strains were equally successful. This was contrary to our expectation that males from the yeasted diet (C strain) would be more attractive for females, as they were significantly larger, on average, than those reared on banana and tomato, probably because of adding sugar and yeast as an important resources used during development (for food/body size relation see Pavkovi -Lu i and Keki, 2010). Even if C males were physically dominant over smaller males, B males were equally successful. For *D. melanogaster*, the high quality of banana diet was previously observed. In their extensive study, Carsten *et al.* (2005) suggested that differences in transcript levels of stress-related genes implied that larvae were less stressed on a banana as opposed to a cornmeal medium. Thus, behavioral performance of banana-fed males in our experiment may be related to larval nutrition during development. This would also be in agreement with the proposition that insects reared on the species' preferred host would be expected to exhibit higher mating success (see Hurtado *et al.*, 2012), as banana is one of the most preferable natural feeding substrates for *D. melanogaster* (Shorrocks, 1974). Contrary to B and C males, males developed on tomato had rather poor mating success: they were insufficiently attractive for females, possible because of their weak courtship vigour.

As the better male mating performance was neither due to effects mediated solely through body size, nor through effects mediated through size and symmetry of a secondary sexual trait, alternative explanations for this observation are required. Long-term rearing of flies on different diets may cause physiological shifts in other traits involved in courting activities that are highly correlated with type of nutrition, but not examined here. Also, it is possible that long-term maintenance of strains on different diets may influence their olfactory profiles, and indirectly, their "chemical attractiveness". Having in mind that differences in male mating success among

nutritional strains could reflect differences in chemical attractiveness, our future studies should include chemical analyses of olfactory profiles in both sexes of all strains.

Since female preference may be also plastic (Dolgin *et al.*, 2006), females of all strains (C, B and T) should be tested for mating preferences.

Table 1. Male mating success in three experimental groups (FC 1, FC 2, FC 3). Abbreviations: C - cornmeal-sugar-agar-yeast medium; B – banana medium; T – tomato medium.

Experimental group	Male types	Number of copulations	Chi square (³)	d.f.	P
FC 1	males C	35	3.32	1	0.0684
	males B	52			
FC 2	males C	61	10.56	1	0.0011
	males T	30			
FC 3	males B	59	8.71	1	0.0032
	males T	31			

Table 2. Mean number of sex comb teeth ($\bar{X} \pm SE$) in males reared on different food and between mated and unmated males. Abbreviations: L – left size of a trait; R – right size of a trait; t – t test. Developmental substrate type and mating status are given in the brackets.

Levels of comparison	N	$\bar{X} \pm SE$	t	d.f.	P
(L+R)/2 (cornmeal)	50	10.26 ± 0.10	1.808	98	0.074
(L+R)/2 (banana)	50	9.93 ± 0.15			
(L+R)/2 (cornmeal)	50	10.26 ± 0.10	0.964	98	0.337
(L+R)/2 (tomato)	50	10.11 ± 0.12			
(L+R)/2 (banana)	50	9.93 ± 0.15	0.936	98	0.352
(L+R)/2 (tomato)	50	10.11 ± 0.12			
(L+R)/2 (mated males)	50	10.29 ± 0.14	1.654	98	0.101
(L+R)/2 (unmated males)	50	9.98 ± 0.12			

Table 3. Comparisons of FA 6 indices in sex comb teeth among males maintained on different food and between mated and unmated males; F-test. Developmental substrate type and mating status are given in the brackets.

Levels of comparison of FA	N	FA 6	F	P
sex comb teeth (cornmeal)	50	0.004104	1.02485	n.s.
sex comb teeth (banana)	50	0.004206		
sex comb teeth (cornmeal)	50	0.004104	1.16033	n.s.
sex comb teeth (tomato)	50	0.004762		
sex comb teeth (banana)	50	0.004206	1.13219	n.s.
sex comb teeth (tomato)	50	0.004762		
sex comb teeth (mated males)	50	0.003850	1.07093	n.s.
sex comb teeth (unmated males)	50	0.003595		

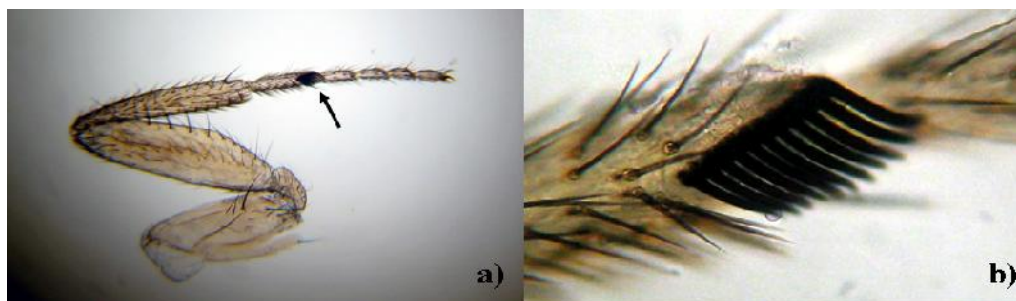


Fig. 1. Sex comb in *Drosophila melanogaster* male: a) front leg with sex comb marked with black arrow; b) sex comb bristles (photographed with a Canon Power Shot camera attached to a Leica stereomicroscope).

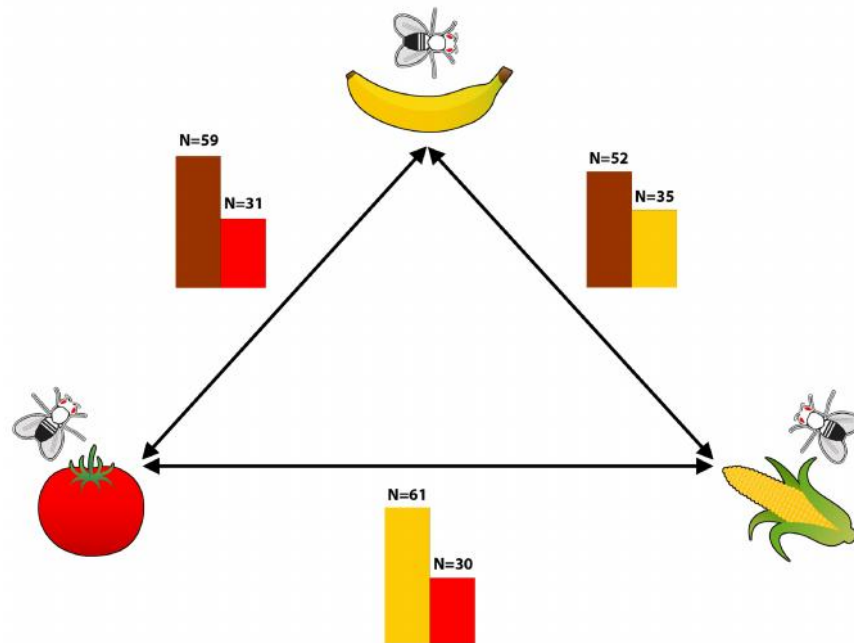


Fig. 2. Number of copulations achieved by males reared for more than one year on different food.

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