

Nutrition Quality, Body Size and Two Components of Mating Behavior in *Drosophila melanogaster**

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Two components of mating behavior, mating latency and duration of copulation, were investigated in *Drosophila melanogaster* males from three different "nutritional" strains, reared for more than 35 generations on banana, tomato and cornmeal-agar-yeast substrates. Males from different strains did not differ according to mating latency and duration of copulation. Also, the sizes of males from different strains did not contribute to these behavioral traits.

Key words: *Drosophila melanogaster*, nutrition quality, mating latency, duration of copulation, body size.

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In *Drosophila*, as in many other animal species, copulation is preceded by a very complex process of species-specific courtship; in this process, males play a more active role than the females (EHRMAN 1973; HALL 1994; GREENSPAN & FERVEUR 2000). During courtship, males and females exchange different types of stimuli which inform them about belonging to the same species, about their readiness to mate, as well as about the qualities of potential mates (YAMADA *et al.* 2002; BLOWS & HIGGIE 2002).

During this process, sexual selection occurs in *Drosophila*, as well as in many other animals (see eg. ARNOLD 1983). For example, in both field and laboratory studies with *Drosophila melanogaster*, the body size of males was positively correlated with mating success, whether it was achieved through inter- or intrasexual interactions (EWING 1964; PARTRIDGE & FARQUHAR 1983; PARTRIDGE *et al.* 1987a, b; WILKINSON 1987; MARKOW 1987, 1988; TAYLOR & KEKIĆ 1988; PITNICK 1991; BANGHAM *et al.* 2002). A similar situation was observed in many other insect taxa (THORNHILL & ALCOCK 1983) and in some other *Drosophila* species (see eg. SANTOS *et al.* 1988; HEGDE & KRISHNA 1997; SISODIA & SINGH 2001), but not

in all (for a review see MARKOW *et al.* 1996; PAVKOVIĆ-LUČIĆ & KEKIĆ 2007).

On the other hand, body size in *Drosophila* is strongly influenced not only by genetic makeup (ROBERTSON 1957; WILKINSON 1987; STANIĆ & MARINKOVIĆ 1999), but also by environmental conditions (e.g. larval density, nutrition, temperature) experienced during development (ROBERTSON 1963; DAVID *et al.* 1983; CAVICCHI *et al.* 1985; HILLESHEIM & STEARNS 1991).

The available evidence indicates that the determinates of variation in phenotypic size can greatly affect how size is correlated with male and female fitness components in *Drosophila* (JOSHI 2004). For instance, when size variation was induced by substantial variation in nutrition quality or larval density, size was positively correlated with male mating success (SANTOS 1996) and female fecundity (ROBERTSON 1957; HOULE & ROWE 2003) nevertheless, if size variation was induced by variation in growth temperature during the larval period (DAVID *et al.* 1983), it did not appear to be correlated with male and female reproductive success (PARTRIDGE *et al.* 1995; ZAMUDIO *et al.* 1995).

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However, there is insufficient data concerning the influence of different environmental factors contributing to body size on other components of mating behavior. In this paper two components of mating behavior, mating latency and duration of copulation, were tested in relation to different nutritional resources to which *Drosophila melanogaster* strains were exposed for more than 35 generations.

Mating latency is an important component of fitness in *Drosophila* and is correlated with different fitness components such as fecundity, fertility and longevity (HEGDE & KRISHNA 1999). Duration of copulation is a species-specific trait and is primarily under genetic control (see MARKOW & O'GRADY 2006), but may be affected by different factors, including previous mating experience (SINGH & SINGH 2004; PAVKOVIĆ-LUČIĆ & KEKIĆ 2006).

Material and Methods

Fly strains. In this experiment, three "nutritional" strains of *Drosophila melanogaster* were used which originated from a natural population collected in New Belgrade. After collection, flies were reared for more than 35 generations on three different substrates: standard cornmeal-agar-yeast medium (C strain), banana (B strain) and tomato medium (T strain). Banana and tomato substrates represent modified standard laboratory food and were made without yeast or sugar; these diets permitted normal growth of *D. melanogaster* flies (KEKIĆ & PAVKOVIĆ-LUČIĆ 2003). Flies were reared on these substrates from generation to generation in 250 ml glass bottles, without competition (about 100 individuals per bottle), in 6 to 8 bottles per strain, at room temperature (from 24°C to 26°C), relative humidity about 50-60% and 12h:12h light:dark cycle.

Experimental procedures. Virgin flies (from F₃₆-F₃₈ laboratory generations) were sexed without anesthesia every few hours after emergence and maintained separately according to sex and strain for 3-5 days in food vials until they were used in experiments. All experiments were performed in the morning, from 8:00AM to 12:00AM.

Mating latency and duration of copulation were scored in female choice experiments. Females of the C strain were mated with males from two different "nutritional" strains. Three experimental groups were formed and flies were crossed as follows:

- I group: 10 females (C)+10 males (C)+10 males (B)
- II group: 10 females (C)+10 males (C)+10 males (T)
- III group: 10 females (C)+10 males (B)+10 males (T)

Ten trials were run for each experimental group, meaning that 300 flies were tested per every experimental group, i.e. a total of 900 flies participated.

Mating latency and duration of copulation were recorded for each mating pair during a 1h observation period (per replica). Mating latency was scored as the time between the introduction of females and males into a mating vial until inception of copulation, while duration of copulation was measured as the time from inception to the termination of copulation. The number of pairs scored for duration of copulation was smaller than those scored for mating latency because in several cases mating pairs were discarded and subsequently eliminated from statistical analysis. Males of different strains were then identified using a UV lamp (as they were marked with different colours of fluorescent dust, red and green, 24h before testing) and left in eppendorf tubes filled with 70% ethanol for further morphological analyses. Males were scored for wing length (approximated as the length of the third longitudinal vein, PARTRIDGE *et al.* 1987a), which is an index of body size, i. e. larger *Drosophila* flies have longer wings (ROBERTSON & REEVE 1952). Wing lengths were measured by a single person, using a binocular microscope fitted with an ocular scale (1mm = 62 measurement units).

Statistics. Mean wing lengths (body sizes) of mated males from three nutritional strains were compared using *t*-tests. *T*-tests were also used for comparisons of mean mating latency (*log* transformed data) and mean duration of copulation in the three experimental groups.

Results and Discussion

Mean mating latencies of the three experimental groups are presented in Table 1. No significant differences were recorded in mean mating latency between any of the experimental groups (Table 1), i. e. females from the C strain did not mate earlier with males of either strain. The mean duration of copulation for the three experimental groups is given in Table 2. Likewise, no significant difference in mean duration of copulation between homogamic and/or heterogamic crosses was observed (Table 2). Copulation duration was very uniform among all mating types and endured, on average, between 19 and 21 minutes at room temperature.

In this experiment, mating latency and duration of copulation was indirectly studied in relation to male body size, as different diets contributed to this phenotypic trait. The results of comparisons of mean body sizes of mated males belonging to the three nutritional strains in all experimental groups are presented in Table 3 (compare with Table 1).

Table 1

Mean mating latency (log transformed) in three experimental groups. Abbreviations: C – cornmeal-agar-yeast strain; B – banana strain, T – tomato strain, t – t-test, df – degrees of freedom

Experimental group	Type of mating	N	$\bar{X} \pm SE$	t	df	P
I	(♀ C x ♂ C)	35	2.81 ± 0.07	0.72	85	0.48
	(♀ C x ♂ B)	52	2.88 ± 0.06			
II	(♀ C x ♂ C)	61	2.73 ± 0.06	0.87	89	0.39
	(♀ C x ♂ T)	30	2.83 ± 0.11			
III	(♀ C x ♂ B)	58	2.70 ± 0.05	1.26	86	0.21
	(♀ C x ♂ T)	30	2.82 ± 0.08			

Table 2

Mean duration of copulation (in seconds) in three experimental groups. Abbreviations as in Table 1

Experimental group	Type of mating	N	$\bar{X} \pm SE$	t	df	P
I	(♀ C x ♂ C)	34	1241.21 ± 27.46	1.76	83	0.08
	(♀ C x ♂ B)	51	1191.86 ± 13.89			
II	(♀ C x ♂ C)	58	1288.36 ± 23.77	1.34	86	0.18
	(♀ C x ♂ T)	30	1236.17 ± 28.29			
III	(♀ C x ♂ B)	58	1259.79 ± 22.39	1.11	85	0.27
	(♀ C x ♂ T)	29	1213.41 ± 36.94			

Table 3

Mean body size (wing length) of males from three nutritional strains in all experimental groups. Abbreviations as in Table 1

Experimental group	Nutritional strain	N	$\bar{X} \pm SE$	t	df	P
I	C	35	82.84 ± 0.68	4.88	85	<0.001
	B	52	79.16 ± 0.42			
II	C	61	92.31 ± 0.30	7.32	89	<0.001
	T	30	87.97 ± 0.58			
III	B	58	84.33 ± 0.51	0.03	86	0.98
	T	30	84.35 ± 0.46			

Males from the C strain were significantly larger than males from B and T strains, probably because the C medium contains higher concentrations of yeast and sugar, which are important protein and energetic resources. For example, in experimental group II, the mean wing length of C males was $\bar{X} \pm SE = 92.31 \pm 0.30$, N = 61, while mean wing length of T males was $\bar{X} \pm SE = 87.97 \pm 0.58$, N = 30, and this difference was significant (t = 7.32, df = 89, P < 0.001). On the other hand, in experimental group III, no significant difference was observed between mean wing lengths of males from B and T strains (B males: $\bar{X} \pm SE = 84.33 \pm 0.51$, N = 58; T

males: $\bar{X} \pm SE = 84.35 \pm 0.46$, N = 30; t = 0.03, df = 86, P = 0.98).

We observed great variability in male wing length between different samples from the same nutritional strain used in different experimental groups (Table 3). These differences are not unexpected, since samples used in experimental groups were formed by collecting individuals from different bottles by chance; environmental conditions in every bottle may be specific and different from the others. Also, these differences may be partly in-

duced by variability among generations, since flies from F₃₆-F₃₈ were used.

When male body size was significantly induced by nutritional variation, no influence of size on the examined components of mating behavior was found. For example, larger males (C males) did not mate earlier than smaller "banana" and "tomato" males, neither did their size influence copulation duration (Tables 1 & 2). Similarly, when male body size was experimentally modified by different growth temperatures, no significant difference in mating latency and duration of copulation was found when comparing matings in which larger (development at 18°C) vs. smaller (development at 25°C) males took part (KEKIĆ *et al.* 2007).

It is possible that female body size has a more important role in determining copulation duration. For example, when body size variation was induced by varying the degree of crowding among larvae from an inbred strain of *D. melanogaster*, copulation duration was shown to depend on female body size, but not (or much less so) on male body size (LEFRANC & BUNDGAARD 2000). In our experiment, only females that had developed on cornmeal-agar-yeast medium were used: it is possible that variation in female body size determined by different diets has more of an influence on the two examined components of mating behavior. Our previous results also suggested a much more important role for females in controlling the duration of copulation, at least when concerning their mating experience (PAVKOVIĆ-LUČIĆ & KEKIĆ 2006). Females also might be expected to be in control of mating speed (the time from the beginning of courtship until copulation), because rape is mechanically impossible in mature *Drosophila melanogaster* (STAMENKOVIĆ-RADAK *et al.* 1992), but was observed in immature females (MARKOW 2000).

References

- ARNOLD S. J. 1983. Sexual selection: the interface of theory and empiricism. (In: *Mate Choice*. P. Bateson, ed. Cambridge University Press, Cambridge): 67-107.
- BANGHAM J., CHAPMAN T., PARTRIDGE L. 2002. Effects of body size, accessory gland and testis size on pre- and post-copulatory success in *Drosophila melanogaster*. *Anim. Behav.* **64**: 915-921.
- BLOWS M. W., HIGGIE M. 2002. Evolutionary experiments on mate recognition in the *Drosophila serrata* complex. *Genetica* **116**: 239-250.
- DAVID J. R., ALLEMAND R., VAN HEREWEGE J., COHET Y. 1983. Ecophysiology: abiotic factors. (In: *The Genetics and Biology of Drosophila*. Ashburner M., Carson H. L., Thompson, J. N. eds. Academic Press, London): 105-170.
- DAVICCHI S., GUERRA D., GIORGI G., PEZZOLI C. 1985. Temperature-related divergence in experimental populations of *Drosophila melanogaster*. I. Genetic and developmental basis of wing size and shape variation. *Genetics* **109**: 665-689.
- EHRMAN L. 1973. Sexual behavior. (In: *The Genetics and Biology of Drosophila* (Ashburner M., Wright, T. R. F. eds. Academic Press, London.): 127-180.
- EWING A. W. 1964. The influence of wing area on the courtship behaviour of *Drosophila melanogaster*. *Anim. Behav.* **12**: 316-320.
- GREENSPAN R. J., FERVEUR J.-F. 2000. Courtship in *Drosophila*. *Ann. Rev. Genet.* **34**: 205-232.
- HALL J. C. 1994. The mating of fly. *Science* **264**: 1702-1714.
- HEGDE S. N., KRISHNA M. S. 1997. Size-assortative mating in *Drosophila malerkotliana*. *Anim. Behav.* **54**: 419-426.
- HEGDE S. N., KRISHNA M. S. 1999. Body size and fitness characters in *Drosophila malerkotliana*. *Curr. Sci.* **77**: 178-179.
- HILLESHEIM E., STEARNS S. C. 1991. The responses of *Drosophila melanogaster* to artificial selection on body weight and its phenotypic plasticity in two larval food environments. *Evolution* **45**: 1909-1923.
- HOULE D., ROWE L. 2003. Natural selection in a bottle. *Am. Nat.* **161**: 50-67.
- JOSHI A. 2004. Are bigger flies always better: the role of genes and environment. *J. Genet.* **83**: 13-15.
- KEKIĆ V., PAVKOVIĆ-LUČIĆ S. 2003. Fruit and vegetable food media suitable for maintaining *Drosophila melanogaster* flies. *Dros. Inf. Serv.* **86**: 147.
- KEKIĆ V., OBRADOVIĆ T., PAVKOVIĆ-LUČIĆ, S. 2007. Growth temperature, mating latency and duration of copulation in *Drosophila melanogaster*. *Dros. Inf. Serv.* **90**: 111-113.
- LEFRANC A., BUNDGAARD J. 2000. The influence of male and female body size on copulation duration and fecundity in *Drosophila melanogaster*. *Hereditas* **132**: 243-247.
- MARKOW T. A. 1987. Genetic and sensory basis of sexual selection in *Drosophila*. (In: *Evolutionary Genetics of Invertebrate Behavior*. M. Huettel ed. Plenum Press, New York): 89-96.
- MARKOW T. A. 1988. Reproductive behavior of *Drosophila* in the laboratory and in the field. *J. Comp. Physiol.* **102**: 169-174.
- MARKOW T. A. 2000. Forced matings in natural populations of *Drosophila*. *Am. Nat.* **156**: 100-103.
- MARKOW T. A., O'GRADY P. M. 2006. *Drosophila*: A guide to species identification and use. Academic Press, London, 259 pp.
- MARKOW T. A., BUSTOZ D., PITNICK S. 1996. Sexual selection and a secondary sexual character in two *Drosophila* species. *Anim. Behav.* **52**: 759-766.
- PARTRIDGE L., FARQUHAR M. 1983. Lifetime mating success of male fruitflies (*Drosophila melanogaster*) is related to their size. *Anim. Behav.* **31**: 871-877.
- PARTRIDGE L., HOFFMANN A., JONES J. S. 1987a. Male size and mating success in *Drosophila melanogaster* and *Drosophila pseudoobscura* under field conditions. *Anim. Behav.* **35**: 468-476.
- PARTRIDGE L., EWING E., CHANDLER A. 1987b. Male size and mating success in *Drosophila melanogaster*: the role of female and male behaviour. *Anim. Behav.* **35**: 555-562.
- PARTRIDGE L., BARRIE B., BARTON N. H., FOWLER K., FRENCH V. 1995. Rapid laboratory evolution of adult life-history traits in *Drosophila melanogaster* in response to temperature. *Evolution* **49**: 538-544.
- PAVKOVIĆ-LUČIĆ S., KEKIĆ V. 2006. Duration of copulation in first and second matings in *Drosophila melanogaster* females. *Period. Biol.* **108**: 81-84.
- PAVKOVIĆ-LUČIĆ S., KEKIĆ V. 2007. Is body size sexually-selected trait in *Drosophila hydei* males? *Arch. Biol. Sci. (Belgrade)* **59**: 21P-22P.
- PITNICK S. 1991. Male size influences mate fecundity and remating interval in *Drosophila melanogaster*. *Anim. Behav.* **41**: 735-745.

- ROBERTSON F. W. 1957. Studies in quantitative inheritance. XI. Genetic and environmental correlation between body size and egg production in *Drosophila melanogaster*. *J. Genet.* **55**: 428-443.
- ROBERTSON F. W. 1963. The ecological genetics of growth in *Drosophila*. VI. The genetic correlation between the duration of the larval period and body size in relation to larval diet. *Genet. Res.* **4**: 74-92.
- ROBERTSON F. W., REEVE C. R. 1952. Studies in quantitative inheritance. I. The effects of selection of wing and thorax length in *Drosophila melanogaster*. *J. Genet.* **50**: 416-448.
- SANTOS M. 1996. Apparent directional selection of body size in *Drosophila buzzatii*: larval crowding and male mating success. *Evolution* **50**: 2530-2535.
- SANTOS M., RUIZ A., BARBADILLA A., QUEZADA-DIAZ J. E., HASSON E., FONTDEVILLA A. 1988. The evolutionary history of *Drosophila buzzatii*. XIV. Larger flies mate more often in nature. *Heredity* **61**: 255-262.
- SINGH S. R., SINGH B. N. 2004. Female remating in *Drosophila*: comparison of duration of copulation between first and second matings in six species. *Curr. Sci.* **86**: 465-470.
- SISODIA S., SINGH B. N. 2001. Mating success and morphometric traits in *Drosophila ananassae*. *Curr. Sci.* **80**: 1444-1447.
- STAMENKOVIĆ-RADAK M., PARTRIDGE L., ANDJELKOVIĆ M. 1992. A genetic correlation between the sexes for mating speed in *Drosophila melanogaster*. *Anim. Behav.* **43**: 389-396.
- STANIĆ S., MARINKOVIĆ D. 1999. Differences between heritabilities for the wing length in males and females of *Drosophila melanogaster*. *Arch. Biol. Sci. (Belgrade)* **51**: 37P-38P.
- TAYLOR C. E., KEKIĆ V. 1988. Sexual selection in natural population of *Drosophila melanogaster*. *Evolution* **42**: 197-199.
- THORNHILL R., ALCOCK J. 1983. *The Evolution of Insect Mating Systems*. Harvard University Press, Cambridge, Massachusetts.
- YAMADA H., MATSUDA M., OGUMA Y. 2002. Genetics of sexual isolation based on courtship song between two sympatric species: *Drosophila ananassae* and *D. pallidosa*. *Genetica* **116**: 225-237.
- ZAMUDIO K. R., HUEY R. B., CRILL W. E. 1995. Bigger isn't always better: body size, developmental and parental temperature and male territorial success in *Drosophila melanogaster*. *Anim. Behav.* **49**: 571-677.
- WILKINSON G. S. 1987. Equilibrium analysis of sexual selection in *Drosophila melanogaster*. *Evolution* **41**: 11-21.