

MATING DISCRIMINATION AND WING SIZE DIFFERENCES AFTER SELECTION FOR MATING SPEED ON BOTH SEXES OF *D. MELANOGASTER*

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This paper gives the analysis of the mating discrimination and wing size changes in *D. melanogaster* lines obtained after selection for mating speed that was performed on both sexes. The degree of reproductive isolation among the selected lines and the control population is significantly higher after selection but generally decreases later on. However there is a non significant degree of reproductive isolation among the lines selected for mating speed. Selection regime on different sexes mating speed seems to produce only changes in the wing size among the selected lines.

KEY WORDS: *D. melanogaster*, selection, mating speed, reproductive isolation, wing size.

INTRODUCTION

Studying the mechanisms which explain the genetic processes of speciation is of considerable interest to evolutionary biology. While the concept of allopatric speciation is widely accepted, there is not much empirical evidence that sympatric speciation occurs (MAYNARD SMITH, 1966; MAYR, 1982). RICE (1987) and RICE and SALT (1990) showed that reproductive isolation can evolve as a correlated character under sympatric conditions. THODAY and GIBSON (1962) give evidence that disruptive selection on sternopleural chaeta in *D. melanogaster* could induce mating discrimination between selected lines. DIJKEN and SCHARLOO (1979) tested for sexual isolation between lines selected for locomotor activity and found sexual isolation between high- and low-activity lines. The mating preference was

accompanied by fertility differences between the selected lines, so both the prezygotic and postzygotic isolation occurred.

The difference in courtship behaviour in *Drosophila* prevents members of different species to copulate and represents premating ethological isolation. Intraspecific variability also exists in mating behavior components (the phases of courtship and copulation process) and can lead to qualitative changes in the reproductive behavior and prevent reproduction. Thus, sexual selection appears as a result of differences in the mating success and can evolve into reproductive isolation among subpopulations (LANDE, 1981; MAYNARD SMITH, 1989). The chance of divergence may be higher if selection is performed on a behavioral trait, such as mating speed.

In an earlier paper (STAMENKOVIĆ-RADAK et al., 1992) we investigated genetic correlations between the sexes in *D. melanogaster* for one reproductive character (time to the first mating) under bidirectional selection. The present paper gives results of further analysis in the selected lines. When offspring of the lines selected under different regimes for mating speed is tested for mating in an environment, a reproductive barrier via mate choice can appear. We analyze the degree of reproductive isolation in both sexes among selected lines, with reference to the control population.

Wing size is a morphometric character whose change could also be a byproduct of the selection performed. Although the wing length has been widely accepted as an indicator of the body size (ROBERTSON and REEVE, 1952; SOKOLOF, 1965), some recent results (CATCHPOLE, 1994) suggest that this may not always be acceptable. As far as mating success is concerned, we think that wing size is a relevant parameter to be examined, without necessarily correlating it with body mass, because of the important role of the male wing in *D. melanogaster* courtship (EWING, 1961). The amount of wing vibration represents the amount of courtship that is related to female requirements for a reception of mating.

In this paper, an analysis of the wing size after selection for mating speed on both sexes is presented, and a possible role of the changes in this parameter during selection in an evolution of a reproductive isolation among the selected lines is discussed.

MATERIAL AND METHODS

The lines of *Drosophila melanogaster* used in the experiments were obtained through an artificial selection design used to alter the mating speed in both sexes independently, in the fast and the slow direction (*for details of the selection and its result see, STAMENKOVIĆ-RADAK et al., 1992*). The time to the first mating in

virgin males and females was chosen as the selected character. The selection was carried out over nine generations, and four sets of lines were established: fast females (FF), slow females (SF); fast males (FM), and slow males (SM). After this, the selected lines were kept without selection, but under moderate inbreeding, by transferring 20 pairs, per bottle, per generation, at random. These lines are analyzed in the present paper. The control population is maintained in 12 bottles (200 mL each) throughout the selection and later, by transferring 20 pairs at random per bottle. In this way, the effect of inbreeding is averaged between the selected and control samples, which enables the observed differences to be related to the selection.

Measuring reproductive isolation

After nine generations the selection regime was relaxed and the lines were maintained by transferring 20 random pairs within each of the lines onto fresh media. Occasionally, the mating speed was tested and we noticed that within one hour of observation more flies remained unmated with tester flies from the control population. This may be caused by inbreeding in the selected lines or some other changes in flies' morphometry and/or behavior due to the previous selection, which results in a certain degree of reproductive isolation between lines.

Two experiments were done in separate generations (28th and 34th). Each experiment consisted of 6 groups with 10 vials. Twenty flies were put in each vial, 5 virgin males and 5 virgin females from two samples observed. To distinguish the flies of the same sex between two samples, males and females from one line were dusted with UV-fluorescent coloured dust, 15 hours before testing (TERZIĆ *et al.*, 1994). This method does not harm the wings like wing clipping, etc. Flies were observed for one hour and mated pairs aspirated individually as they mate. Their phenotype (according to the line of origin) was determined the same day, under a UV lamp. The frequencies of homomatings (within the members of the same line) and heteromatings (between the flies from different lines) were scored.

The degree of reproductive isolation among the selected lines and the control was estimated by deviations from random mating (chi-square) and reproductive isolation index given by MALOGOLOWKIN-COHEN *et al.* (1965). Index (I) = $(x_{11}+x_{22})-(x_{12}+x_{21})/N$, where x_{11} and x_{22} are homogamic and x_{12} and x_{21} heterogamic matings. N is the total number of matings observed.

Wing size

After the ninth generation of selection, samples of flies of both sexes, from all lines and the control are analyzed for wing size. The wing length is measured as a distance between anterior cross vein to the tip of the third longitudinal vein (Partridge

et al., 1987). One wing per fly is measured with an ocular micrometer fitted to binocular microscope.

RESULTS

The reproductive isolation measurement (Table I) shows that there is no significant deviation from random mating between flies from the female selected lines. Lines generally perform significantly more homogamic matings in 28th generation of the experiment, but that decreases or even disappears later on. Reproductive isolation is high between the FF line and the control after selection, but decreases with time. It remains high between the male selected lines and the control and between SF and the control. Isolation also decreases among the male selected lines themselves, although they both differ significantly from the control.

Table 1.
Degree of reproductive isolation between selected lines and control population FF = fast females;
SF = slow females; FM = fast males; SM = slow males; C = control

generation	lines studied	isolation index (I)	hi-square test
28	SFC	-.031**.177	21.174, p<0.01
34		.143**.187	7.999, p<0.05
28	FF/SF	-.053**.229	7.618
34		-.250**.242	7.500
28	SM/C	.19**.137	38.396, p<0.001
34		.368**.151	7.894
28	FM/SM	.222**.230	13.556, p<0.001
34		-.167**.201	1.999
28	FM/C	.320**.134	35.486, p<0.001
34		.385**.148	10.128
28	FF/C	.866**.075	90.100
34		-.130**.206	5.522

There is no significant difference in wing size between the flies of the opposite sex other than one on which the selection regime is performed (Table II). Only

females from the „female” selected lines and males from the „male” selected lines show significant difference in the wing size. In the fast mating females (FF) the wings are significantly the smallest among all the samples. The result on the wing size measurement in males from the male selected lines show that the wings of the males selected for slow mating are significantly bigger than the ones from the fast lines, but they do not differ from the controls. There is no influence on the female wing size both in the male selected lines and the control. Only females from SM line have significantly larger wings than the females from FF lines. It seems that the selection for different mating speed performed on the females influences highly the change in the female wing size only in the fast line, but it strongly influences the male wing sizes both in the fast and slow female selected lines. The directions of these male wing changes are interesting: male wings from both female selected lines are significantly smaller than the control ones but they do not differ among themselves. It seems that selection on female mating speed produces a decrease in the male wing size compared to the control.

Table 2.

Wing size comparisons between the flies of both sexes from the lines selected for fast and slow mating speed in males and females. FF = fast females; SF = slow females; FM = fast males; SM = slow males; C = control. The wing sizes are given in mm.

lines compared	wing size		probabilities from t-test comparisons of wing size	
	males	females	males	females
FM/C	1.147/1.168	1.277/1.270	0.0752	0.401
FM/FF	1.147/1.130	1.277/1.241	0.0862	0.0001
FM/SM	1.147/1.180	1.277/1.278	2.438×10^{-7}	0.791
FM/SF	1.147/1.128	1.277/1.276	0.014	0.962
FF/C	1.130/1.168	1.241/1.269	0.007	0.015
FF/SM	1.130/1.180	1.241/1.278	7.999×10^{-8}	6.4003×10^{-6}
FF/SF	1.130/1.128	1.241/1.276	0.811	0.0004
SM/C	1.180/1.168	1.278/1.269	0.285	0.265
SM/SF	1.180/1.128	1.278/1.276	1.63×10^{-11}	0.782
SF/C	1.128/1.168	1.276/1.269	0.001	0.482

DISCUSSION

Mating success is related to the rate of courtship time through fly's general activity which is related to body size. Large flies are found to run faster, move more, make louder sound (PARTRIDGE *et al* 1978; SANTOS *et al.*, 1992). The amount of male wing vibration is under genetic control and its role is important in courtship (McDONALD, 1979). While number of published data show that larger flies have mating advantage and mate more successfully (MONCLUS and PREVOSTI, 1971; PARTRIDGE *et al.* 1987), in our experiment, selection regimes, according on different sexes seem to produce changes in wing sizes in different directions. One explanation may be the difference in experimental design between our and other studies. In our experiment single males are allowed to mate single females during selection, thus the effects of male fighting and competition are eliminated. After the selection is relaxed, flies are inbred and the interlined variability may remain high. However, a reproductive isolation experiment brings flies from oppositely selected lines together and multiple matings are observed. The obtained differences compared to the control should then be attributable to the selection performed on mating speed. The results clearly show that selection caused the increased reproductive isolation among the selected lines and the control, but the direction of the selection had no significant influence on it.

Males similar in size (from FF and SF lines) show apparently no deviations from random mating, although they differ from the control. It is possible that the amount of wing vibrations in males is important and not the body size. Smaller male wings possibly produce higher vibration and display better courtship than large ones. EWING (1961) shows that in his lines selected for large body size the amount of wing vibration increases in „small” lines. The increase of wing vibration in males with smaller wings in our experiment could be caused by higher specific hexokinase (HK) activity detected in those males (unpublished data). This enzyme is known to be involved in flight metabolism in flies (LAURIE-AHLBERG *et al.*, 1985).

In selection on the „escape” behaviour of *D. melanogaster*, GRANT and METTLER (1969) obtain a small but significant degree of mating discrimination as a correlated response to selection. Our selection for high or low mating speed in both sexes possibly produces selective covariation. It is difficult to separate the effects of selection from other effects. Selection performed on females may have influence on the male wing size, as they became smaller with respect to the control but, as reproductive isolation has not occurred between these lines it obviously has no effect on reproductive behavior, such as to prevent them to copulate with certain phenotypes. Differences in selection pressures on the sexes exist, so the genetic correlations between the sexes can constrain the evolution of sexual dimorphism (FALCONER,

1989). Male reproductive potential is determined by the number of eggs laid. Perhaps the results on fecundity of females from these lines would give some interesting data of reproductive potential of those females as a sort of „compensation effect”. It seems that characters involved in the rate of female receptivity divergence (more proper term when „mating speed” is measured in females) do not lead to reproductive isolation. Also, heteromatings are high between males from FM and SM lines after selection, thus the characteristics that cause divergence in male mating speed are not the most important ones in reproductive isolation occurrence among those phenotypes.

As our previous paper shows, mating speed seems to be correlated between the sexes in *D. melanogaster*. The existence of correlation with other characters such as the wing size can be confused with various side effects of selection under a certain regime. Distinguishing a correlative response from direct selection is important in behaviour studies and many behaviours make sense only if considered as a part of the whole phenotype (PRICE and LANGERN, 1992). For the problem of reproductive isolation in sympatric conditions it is of importance which characters are relevant for females to choose their mates. To answer this, experiments which would include female preferences for certain male genotypes correlated with fitness, would be valuable to explain the evolution of certain behavioural traits.

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ДИСКРИМИНАЦИЈА У ПАРЕЊУ И РАЗЛИКЕ У ВЕЛИЧИНИ
КРИЛА НАКОН СЕЛЕКЦИЈЕ ЗА БРЗИНУ ПАРЕЊА ОБА ПОЛА
D. MELANOGASTER

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Извод

У раду је анализиран степен дискриминације у парењу и промене величине крила у линијама *D. melanogaster* добијених селекцијом за брзину парења оба пола. Степен репродуктивне изолације између селекционисаних линија и контроле статистички је значајан иако опада кроз генерације након селекције. Међутим, нема значајног смањења учесталости хетерогаметних парења међу самим селекционисаним линијама. Селекциони режим који је имао утицаја на брзину парења оба пола вероватно узрокује само промене у величини крила међу селекционисаним линијама.

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