

## COMPARATIVE BEHAVIOURAL ANALYSIS OF MATING BETWEEN YELLOW AND WILD TYPE *DROSOPHILA* OF *MELANOGASTER* SPECIES GROUP

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### Abstract

During the present study we have analyzed the effect of *yellow* (*y*) gene mutation on behavioural isolation and mating activity using male-choice method in two species of *melanogaster* group, *D. melanogaster* and *D. ananassae*. The results demonstrate that there is no behavioural isolation due to *yellow* gene mutation in both the species. Comparative analysis of mating activity results depict that *D. melanogaster* mutant females have increased receptivity in contrast to the *D. ananassae* mutant females suggesting the putative role of 'y' gene in the mate discrimination in *D. melanogaster*.

**Keywords:** Behavioural isolation, *melanogaster* species group, *yellow* gene, male-choice, mating activity.

### Introduction

The evolution of reproductive isolation is the first step in speciation. Evolutionary studies in different species of *Drosophila* have made major and remarkable contributions to our understanding of the concept of speciation (Powell, 1997; Singh, 1997; Coyne and Orr, 2004). Among the different means of reproductive isolation, sexual or ethological isolation is the most important mode of reproductive isolation as it is the primary step of isolation in speciation.

The genetic basis of behaviour cannot be understood unless one can demonstrate the existence of genetic variations in behaviour upon which selection could act. Several different categories of mutants have been identified influencing mating behaviour of *Drosophila* like mutants having general decrements in courtship vigor and male mating ability, abnormalities of female receptivity, courtship song, learning and memory, vision and olfaction, sex-determination variants etc (Hall, 1994). Mating in *Drosophila* depends on complex interactions between the sexes consisting of the interchange of visual, acoustic, and chemical stimuli (Spieth and Ringo, 1983; Ritchie, 2007). The role of visual stimuli has been detected in mate recognition which provides a basis for sexual isolation within and between the species of *Drosophila* (Spieth, 1966; Ewing, 1983). For successful mating, male mating activity and female receptivity are the key responsible factors (Bateman, 1948). Effects of various gene mutations in different species of *Drosophila* have been studied. In *D. melanogaster*

effect of X- linked genes e.g. Bar eye, yellow body colour, white eye colour, cut wing and raspberry have been found to diminish the mating propensity of males (Reed and Reed, 1950; Rendel, 1951; Petit, 1958; Geer and Green, 1962). In *D. subobscura*, Rendel (1945) found non random mating between *yellow* mutant and wild type. Tan (1946) performed similar experiments in *D. pseudoobscura* and found similar results as reported by Rendel. In *D. ananassae* white eyed males are less successful in mating than wild males (Singh et al, 1985). Chatterjee and Singh (1987) observed mating success between Beadex mutant and wild type of *D. ananassae* and they did not find any evidence of selective mating between Beadex mutant and wild type flies. However, a comparison of the mating activity of the two strains showed that Beadex gene diminishes the mating propensity of the males.

Present study deals with comparative behavioural analysis of two species belonging to the *melanogaster* species group: *D. melanogaster* and *D. ananassae* (Bock and Wheeler, 1972) for the effect of *yellow* gene mutation. The *melanogaster* species group consists of five subgroups. *D. melanogaster* and *D. ananassae* are the members of *melanogaster* and *ananassae* subgroups respectively. Both the species are cosmopolitan in distribution and morphologically distinct from each other. The *yellow* (*y*) gene is a recessive X-linked gene in both the species. The mutants are clearly distinguished from wild type flies because of their yellow body colour. Till now, the effect of '*y*' gene mutation in mating of *D. ananassae* flies has not been reported.

### **Materials and Methods**

Behavioural isolation and mating propensity of both the sexes have been investigated between yellow mutant and wild type flies of *D. melanogaster* and *D. ananassae* employing male-choice method. The mutant and wild strains of both the species of *Drosophila* are being maintained in our laboratory from several years. Before the experimentation, mutant stocks were crossed with wild stocks up to six generations, so that mutant stocks become isogenic to wild except at the *yellow* gene locus. After the mutants were made isogenic to wild type, the two stocks (mutant and wild type) were cultured separately in food bottles. Virgin males and females were collected from these stocks and aged for seven days. In the male-choice method, 15 males of one type i.e. either mutant or wild and 15 females of both types (15 wild type + 15 mutants) were introduced in Elens-Wattiaux mating chamber. The total number of flies in a chamber was 45 and sex-ratio was 1 male: 2 females. When a pair commenced mating it was aspirated out and kept in a separate empty vials to identify the type of mating. Mating was observed for 1 hr. The experiments were conducted in five replicates. All the experiments were carried out between 7.00 to 11.00 A.M. in a temperature controlled room at approximately 24<sup>0</sup>C and maintained in 12hour light/dark cycle.

To measure the degree of sexual isolation between mutants and wild type, isolation index was calculated using the formula proposed by Stalker (1942).

$$\text{Isolation index (I.I.)} = \frac{\% \text{ homogamic matings} - \% \text{ heterogamic matings}}{\% \text{ homogamic matings} + \% \text{ heterogamic matings}}$$

Isolation indices vary between -1 to +1. When isolation index is zero, there is no isolation and when it is one, there is complete isolation. The significance of isolation indices were calculated by standard error (Zouros and Entremont, 1980).

$$\text{S.E.} = \sqrt{1 - I^2/N}$$

N is the number of crosses analyzed. Chi-square values were calculated under the assumption of random mating using RXC contingency table. Also, chi-square values were calculated to check the mating propensities of males and females.

## Results

The results of male-choice experiments involving yellow mutant and wild type for both the species are presented in table 1. In the cross involving yellow males and yellow + wild females of *D. melanogaster*, 61.33% homogamic matings and 33.33% heterogamic matings occurred. However, the difference between homo- and heterogamic matings was found to be insignificant. In the cross involving wild males and yellow + wild females of *D. melanogaster*, the number of heterogamic matings was found to be slightly more than homogamic ones and as a result isolation index was found to be -0.0313. Similar experiments were performed between yellow mutant and wild type of *D. ananassae* and the results depicted that there is random mating. When yellow males were allowed to mate with both types of females, isolation index was found to be -0.172. While, wild males when kept with yellow and wild females, homo- and heterogamic matings were found to be 41.33% and 29.33% respectively and the isolation index was 0.170. Chi-square values and isolation indices were found to be insignificant in both the crosses for both the species indicating the occurrence of random mating.

In table 2 Chi-square on marginal totals were presented to assess the relative sexual activity of yellow and wild type flies of both the sexes in the two species. In *D. melanogaster*, it was found that there is significant difference for the female receptivity between 'y' mutant and wild type; however, difference in the male mating activity between mutant and wild type was found to be insignificant. However, the experiments involving 'y' mutant and wild type of *D. ananassae* gave insignificant difference both for female or male sexual activity.

**Table 1****Results of Male-choice experiments between yellow and wild-type flies of two *Drosophila* species of *melanogaster* species group**

<i>Drosophila</i> species	Crosses		Homogamic matings		Heterogamic matings		$\chi^2$	I. I. $\pm$ S.E.
	Females	Male	N	%	N	%		
<i>D. melanogaster</i>	yellow + Wild	yellow	46	61.33	25	33.33	2.358 <sup>NS</sup>	0.295 $\pm$ 0.348
	yellow + Wild	Wild	31	41.33	33	44.00	0.0218 <sup>NS</sup>	-0.0313 $\pm$ 0.115
<i>D. ananassae</i>	yellow + Wild	yellow	24	32.00	34	45.33	0.562 <sup>NS</sup>	-0.172 $\pm$ 0.114
	yellow + Wild	Wild	31	41.33	22	29.33	0.473 <sup>NS</sup>	0.170 $\pm$ 0.114

*N* is the number of females mated.

NS = not significant.

**Table 2** **$\chi^2$  for 1:1 ratio on marginal totals to test the relative sexual activity of yellow and wild type flies of both the sexes in male-choice experiments of two *Drosophila* species of *melanogaster* species group**

<i>Drosophila</i> species	♂	Wild	yellow	Total
<i>D. melanogaster</i> ♀	Wild	31	25	56
	yellow	33	46	79
	Total	64	71	-
	$\chi^2$ wild, yellow ♀, 3.92 $P < 0.05^*$ $\chi^2$ wild, yellow ♂, 0.362 $P > 0.5$			
<i>D. ananassae</i> ♀	Wild	31	34	65
	yellow	22	24	46
	Total	53	58	-
	$\chi^2$ wild, yellow ♀, 3.252 $P > 0.05$ $\chi^2$ wild, yellow ♂, 0.225 $P > 0.05$			

\* Significant.

## Discussion

The *yellow* gene causes yellow body pigmentation in *Drosophila*. Beside its phenotypic expression, it has some pleiotropic effect which might be reflected in the behaviour of this fly. Sturtevant (1915) showed that yellow males are usually unsuccessful in competition with wild type with respect to mating in *D. melanogaster*. In another study conducted by Bastock (1956) in *D. melanogaster*, it was suggested that 'y' mutation causes reduced male mating success. She also recorded change in courtship pattern due to alteration in wing vibration. During the study she noticed enhanced female receptivity in yellow females.

Our results support Bastock's finding for the enhanced receptivity of yellow female than wild type in *D. melanogaster* but we haven't found difference in male mating success between yellow and wild type. The enhanced receptivity of yellow females might be due to their inability to exercise choice upon courting males. Evolutionary model of Watanabe and Kawanishi (1979) explains that rigidity of the mate recognition system is the most important attribute and helps in retaining the identity of a new species. Thus, enhanced receptivity of yellow females of *D. melanogaster* exposes them to the risk of contamination of the gene pool from other strains. Based on the existing results it may be inferred that 'y' gene is influencing mate recognition system in *D. melanogaster*.

*D. ananassae* which is another species of the *melanogaster* species group shows no difference in female receptivity or male mating success between yellow mutant and wild type. This behavioural difference between *D. melanogaster* and *D. ananassae* due to 'y' mutation could be due to the positional difference of this gene. In *D. melanogaster*, 'y' gene is located at the tip of the X-chromosome, in a region with strong reduction in recombination rate while in *D. ananassae*; this gene is located in a region with normal recombination rate. It is suggested that, this change in the recombinational environment affected synonymous divergence in the 'y' gene coding region (Munte et al, 2001). Here comes the importance of comparative evolutionary research as in the present study 'y' mutation enhances female receptivity in *D. melanogaster*, while it has no effect in *D. ananassae*.

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