No effect of marking procedures and choice-situations on the pattern of matings in *Drosophila ananassae*.

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Species maintain their entity through isolating mechanisms, which act as reproductive barriers and restrict the intermingling of genomes from two different species (Mishra and Singh, 2005). Dobzhansky (1937) coined the term 'isolating mechanisms' for genetically conditioned barriers that prevent gene flow between Mendelian populations. These mechanisms are characterized as premating barriers, postmating-prezygotic and postzygotic barriers (Coyne and Orr, 2004). The phenomenon of sexual isolation has been extensively studied in the genus *Drosophila* and has been found to be widespread (Singh, 1997). It is included in the category of premating barriers which causes rapid evolution of mating signals and responses (Mendelson, 2003).

Drosophila is the most studied genus regarding the development of premating isolation through the modification of sexual behaviour in natural or genetically manipulated populations (Casares *et al*., 2005).

Different patterns of sexual isolation exist in interspecific and intraspecific populations of *Drosophila*. Incipient reproductive isolation occurring between geographic strains of same species has been reported in many species of *Drosophila* which originated as a result of genetic divergence (for references see Singh and Sisodia, 1996) that affect mate recognition systems. Evidence for incipient sexual isolation has been found in different geographical strains of *Drosophila malerkotliana* and *D. parabipectinata* (Singh and Chatterjee, 1992). Evidence of positive assortative mating has been reported in *D. bipectinata* (Singh and Sisodia, 1996). Symmetrical and asymmetrical sexual isolation has been reported among laboratory strains of *Drosophila ananassae* (Singh and Chatterjee, 1985). Sexual isolation is also found in two sibling species of *Drosophila: D. ananassae and D. pallidosa* (Vishalakshi and Singh, 2006).

Drosophila ananassae is a cosmopolitan and domestic species. It occupies a unique status among the genus *Drosophila* due to certain peculiarities in its genetic behaviour. The most unusual feature of this species is spontaneous male meiotic recombination in appreciable frequency. The other unusual features are varied chromosomal polymorphism, high mutability, Y-4 linkage of nucleolus organizer, segregation distortion, parthenogenesis, absence of genetic coadaptation in different geographical populations and extra chromosomal inheritance (Singh, 2000).

Pattern of matings can be characterized as random and assortative mating. In the former, individuals of a given sex have an equal chance of mating with other individuals of the opposite sex, while in the latter individuals choose their mates according to their phenotype (Hartl and Clark, 2007). Mating patterns are identified by scoring homogamic and heterogamic matings which can be quantified by using marking procedures in which flies of one strain are marked. These procedures are used as the flies of two strains are indistinguishable. Som and Singh (1998) found no effect of marking procedures on mating success, which suggested that the performance of flies or the outcome of mating preference test remain similar irrespective of marking procedures. Different methods of marking procedures have been used by various investigators which include wing clipping (Ehrman, 1966, 1968), thorax marking (Singh and Chatterjee, 1985), placing a small drop of enamel paint on mesonotum (Arita and Kaneshiro, 1979), marking both the wings using ink (Zouros and D'Entremont, 1980), colouring the flies with fluorescent dust (Markow, 1980) and feeding the flies with red and green colored food (Wu *et al*., 1995). In the present study, wing clipping and thorax

marking procedures are used for marking the flies. Choice-situations include multiple-choice, male-choice, female-choice and no-choice methods. In the present communication, it has been found that these methods and marking procedures have no effect on pattern of matings.

In order to test the effect of different marking procedures and choice situations on pattern of matings in *D. ananassae*, two mass culture stocks established from flies collected from different geographic localities were used: (i) PJ-Panjim, established in 2001. (ii) QL-Quilon, established in 1985.

These stocks were initiated from flies collected from natural populations and had spent varying number of generations in laboratory. They were maintained on simple culture medium at approximately 24ºC by transferring about 50 flies (females and males in equal number) to fresh food bottles in each generation.

The two marking procedures were used to distinguish the flies of two strains. These procedures are :

1. Wing clipping – In this procedure, distal part of right wing is clipped in each fly of a particular strain (Ehrman, 1966, 1968).
2. Thorax marking – In this procedure, thorax is marked by a spot of quickly drying nail polish in each fly of a particular strain (Singh and Chatterjee, 1985).

In order to test the effect of choice-situations on the pattern of matings, four experimental methods were used in the present study. These are:

- **Male-choice:** Males of one type were kept with females of both types, *i.e.*, 15 males of one type with 15 females of each of the two types. The total number of flies in each trial was 45 and sex ratio was 1 male : 2 females.
- **Female-choice:** Females of one type were kept with males of both types, *i.e.*, 15 females of one type with 15 males of each of the two types. The total number of flies in each trial was 45 and sex ratio was 1 female : 2 males.
- **Multiple-choice:** Females and males of both types were used in equal ratio,15 flies of each type and of each sex. The total number of flies in each replicate was 60 and sex ratio was 1 female : 1 male.
- **No-choice:** The flies were not given a choice and one type of male was confined with one type of female. Thus a total of four different combinations were carried out in no-choice experiments. The total number of flies in each replicate was 30 and sex ratio was 1 female : 1 male.

In each stock, virgin females and males were collected and aged for 7 days. To identify the females and males of two strains, the flies of one strain were marked. Then flies were introduced into Elens–Wattiaux (1964) mating chambers and matings were observed for 60 min. at approximately 24ºC under normal light conditions during 0700 – 1100 hrs. In all the experiments, 5 trials were run for each experimental set. When a pair commenced mating then it was aspirated out and the types of individuals mated were recorded. Then homogamic and heterogamic matings were scored. χ^2 values were calculated under the assumption of random mating to test the difference between homogamic and heterogamic matings. Sexual isolation was tested by calculating the Isolation Estimate (Merrell, 1950).

$$\text{Isolation Estimate (I.E.)} = \frac{\text{Number of heterogamic matings}}{\text{Number of homogamic matings}}$$

Table 1. Results of four experimental methods using wing clipping marking procedure between two wild laboratory strains of *D. ananassae*

	PJ female x PJ male	PJ female x QL male	QL female x QL male	QL female x PJ male	I.E.	χ^{2*}
Multiple-choice	27	34	25	22	1.07	0.148
Female-choice PJ female	31	24	-	-		
Female-choice QL female	-	-	25	28	0.93	0.148
Male-choice PJ male	43	-	-	25		
Male-choice QL male	-	39	22	-	0.98	.008
No-choice						
PJ female x PJ male	56	-	-	-		
PJ female x QL male	-	56	-	-	0.88	0.875
PJ male x QL female	-	-	-	49		
QL female x QL male	-	-	63	-		

*d.f. = 1, P>.05

Table 2. Results of four experimental methods using thorax marking procedure between two wild laboratory strains of *D. ananassae.*

	PJ female x PJ male	PJ female x QL male	QL female x QL male	QL female x PJ male	I.E.	χ^{2*}
Multiple-choice	28	38	21	18	1.14	0.47
Female-choice PJ female	29	27	-	-		
Female-choice QL female	-	-	36	22	0.75	2.24
Male-choice PJ male	36	-	-	24		
Male-choice QL male	-	45	16	-	1.33	0.42
No-choice						
PJ female x PJ male	55	-	-	-		
PJ female x QL male	-	55	-	-	0.91	0.46
PJ male x QL female	-	-	-	48		

*d.f. = 1, P>.05

Results of the experiments conducted during the present study are presented in Tables 1 and 2. In all the crosses, the isolation estimate is close to one and deviation from randomness is not statistically significant, which indicates random mating and there is no isolation between these two strains. From the data, mating propensities were also calculated (data not shown), and it was found that both the strains have equal mating propensity. So, we may infer that the pattern of matings remains similar with all the four choice-situations and two marking procedures. Since the flies of two strains are indistinguishable, marking procedures are used such that damage does not occur to flies as it may affect courtship behavior. Wing clipping marking is better than thorax marking as it is less laborious and does not cause damage to flies. Among the four choice-situations, male-choice and multiple-choice are widely used. Despite the common use of multiple-choice as it provides natural conditions but due to differences in sexual vigor erroneous interpretation of obtained data can be misinterpreted as isolation. Casares *et al.* (1998) have shown that due to difference in mating propensities, the number of matings are close to 100%, and there is high risk of obtaining significant

results that do not correspond to true choice. This factor is eliminated in male-choice as single type of males are present so this experimental design is preferred. Pattern of matings can be affected by mating propensity and factor of discrimination (Spieth and Ringo, 1983). Difference in mating propensities affects number of matings in a specified time. If mating propensity is more then it may give rise to erroneous interpretation. This factor is eliminated by using male-choice method.

With regard to choice-situations and marking procedures, patterns of matings are not affected. So, it may be concluded that marking procedures and choice-situations have no affect on pattern of matings in *D. ananassae*.

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References: Arita, L.H., and K.Y. Kaneshiro 1979, Proc. Hawaiian Entomol. Soc. 13: 31-34; Casares, P., M.C. Carracedo, B. Del Rio, R. Pineiro, L. Garcia-Florez, and A.R. Barros 1998, Evolution 52: 126-133; Casares, P., R.Pineiro, and M.C. Carracedo 2005, J. Genet. 84(3): 259-264; Coyne, J.A., and H.A. Orr 2004, *Speciation*, Sinauer Associates Inc. Publs., USA; Ehrman, L., 1966, Anim. Behav. 14: 332-339; Ehrman, L., 1968, Genet. Res. 11: 135-140; Elens, A.A., and J.M. Wattiaux 1964, Dros. Inf. Serv. 39: 118; Hartl, D.L., and A.G. Clark 2007, *Principles of Population Genetics*, Sinauer Associates Inc. Publs., USA; Markow, T.A., 1980, Behav. Genet. 10: 553-556; Menelson, T.C., 2003, Evolution 57: 317-327; Merrell, D.J., 1950, Evolution 4: 326-331; Mishra, P., and B.N. Singh 2005, Current Science 89: 1813-1819; Singh, B.N., 1997, Ind. J. Exp. Biol. 35: 111-119; Singh, B.N., and S. Chatterjee 1985, Can. J. Genet. Cytol. 27: 405-409; Singh, B.N., and S. Chatterjee 1992, Indian J. Exp. Biol. 30: 260-263; Singh, B.N., and S. Sisodia 1996, Current Science 71: 517-518; Singh, B.N., 2000, Current Science 78: 391-398; Som, A., and B.N. Singh 1998, Dros. Inf. Serv. 81: 202-203; Spieth, H.T., and J.M. Ringo 1983, In: *The Genetics and Biology of* Drosophila. (Ashburner, M., H.L. Carson, and J.N. Thompson jr., eds.), Academic Press, New York, 3c: 1-97; Vishalakshi, C., and B.N. Singh 2006, Current Science 90: 1003-1006; Wu, C.-I., H. Hollocher, D.J. Begun, C.F. Aquadro, Y. Xu, and M.-L. Wu 1995, Proc. Natl. Acad. Sci. USA 92: 2519-2523; Zouros, E., and C.J. D'Entremont 1980, Evolution 34: 421-430.

A preliminary report on chromosomal polymorphism in Mexican populations of *Drosophila nebulosa.*

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Introduction

Drosophila nebulosa (Sturtevant) exhibits a moderate degree of chromosomal polymorphism with up to 17 different paracentric inversions in chromosome III plus two other inversions in the left arm of the sex chromosome (XL) and already described (Pavan, 1946). This neotropical species has broad geographical distribution extending from Buenos Aires, Argentina, in South America up to Central Mexico and Texas (Patterson and Wagner, 1943), and it is considered the most common member of the *willistoni* group found in Mexico and inhabiting eastern areas in this country (Patterson and Mainland, 1944). With respect to its inversion or chromosomal polymorphism in this species are few reports; examples of them are that of Pavan (1946), da Cunha *et al.* (1953), Bonorino