With compliments

EVIDENCE FOR INCIPIENT SEXUAL ISOLATION WITHIN *DROSOPHILA BIPECTINATA*

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ABSTRACT

Sexual isolation among six strains of *Drosophila bipectinata* derived from distant geographic populations was examined by multiple choice method in Elens-Wattiaux mating chamber. In all the crosses, homogamic matings are more frequent than heterogamic ones. The isolation estimate ranges from 0.54 to 0.92. In six crosses, the isolation estimate remains low and the deviation from randomness is significant statistically which shows non-random (preferential or positive assortative) mating. This provides evidence for incipient sexual isolation within *D. bipectinata*.

INTRODUCTION

The importance of isolation has been recognized for a long time. During the process of speciation, the diverging populations must acquire some means of isolation so that the genes from one gene pool are prevented from dispersing freely into a foreign gene pool. Thus without isolation evolution is impossible. The term 'isolating mechanisms' was coined by Dobzhansky in 1937 (cited in Dobzhansky 1951) for genetically conditioned barriers which prevent gene exchange between Mendelian populations. Sexual (or ethological) isolation constitutes the most important class among the different means of reproductive isolation in animal species. Thus it plays an important role in evolution. Sexual isolation is a premating barrier to gene exchange in which the opposite sexes of different populations fail to mate due to behavioural incompatibility. The phenomenon of sexual isolation has been extensively studied in the genus Drosophila and has been found to be widespread (Patterson & Stone, 1952; Dobzhansky, 1951; Mayr 1966; Parsons, 1973; Spieth & Ringo, 1983; Chatterjee & Singh, 1989). It has been found that complete isolation exists between some species and incomplete between others. The degree of isolation and pattern of mating preference are often used to indicate the phylogenetic relationship between the species and also their evolutionary sequence (Kaneshiro, 1976; Watanabe & Kawanishi, 1979; Singh *et al.*, 1981; Dwivedi *et al.*, 1982).

The differentiation of races in sexual and cross-breeding organisms is due to modification of gene frequencies in allopatric populations by natural selection and/or genetic drift (Dobzhansky 1951). Races attain the status of species if they develop reproductive isolation. Incipient reproductive isolation occurring between the geographic strains of the same species has been widely demonstrated in a number of species in the genus *Drosophila* (Dobzhansky, 1944; Patterson & Wheeler, 1947; Miller, 1958; Miller & Westphal, 1967; Dobzhansky & Mather, 1961; Ayala, 1965; Malogolowkin-Cohen *et al.*, 1965; Baimai, 1970; Bicudo, 1978; Ahearn, 1980; Ehrman & Parsons, 1980; Singh & Chatterjee, 1985 a,b) which corroborates the hypothesis that incipient isolation originates as a side effect of genetic divergence. However, some species of *Drosophila* such as *D. pseudoobscura* and *D. melanogaster* do not show significant deviation from random mating among natural populations in spite of genetic divergence in their natural populations (Anderson & Ehrman, 1969; Petit *et al.*, 1976; Henderson & Lambert, 1982).

Drosophila bipectinata originally described by Duda in 1923 from Darjeeling, India belongs to the *bipectinata* species complex of the *ananassae* subgroup of the *melanogaster* species group (Bock & Wheeler, 1972). It is known to have a wide geographical distribution ranging from India through southeast Asia and New Guinea to Fiji and Samoa in the Pacific (Bock & Wheeler, 1972). It is characterised by chromosomal and genic polymorphism in its natural populations (Bock, 1971; Yang *et al.*, 1972; Panigrahy, 1984) and is of common occurrence in Indian subcontinent. *D.bipectinata* shows incomplete sexual isolation with other closely related members of the *bipectinata* species complex (Bock, 1978; Singh *et al.*, 1981, 1982). During the present study sexual isolation was examined among six geographic strains of *D.bipectinata* and the results are reported in this paper.

MATERIALS AND METHODS

In the experiments described here, six geographic strains of *D.bipectinata* were employed. All the strains were initiated from mass culture. The strains and their place of origin are mentioned below:

1. BHU Campus, Varanasi, Uttar Pradesh
2. Unchawa Lodge (UL) near U.P. College, Varanasi, Uttar Pradesh
3. Patan, Nepal (Np)
4. Townsville (Tv), Australia
5. Baripada (Br), Orissa
6. Mysore (My), Karnataka

To study sexual isolation among different strains, the multiple-choice method was used and mating was observed in Elens-Wattiaux mating chamber. Virgin females and males were collected and aged for 7 days in small batches. In all the experiments, 15 flies of each sex were used and 5 trials were run for each experimental set. Fifteen females of each of the two strains were introduced into the mating chamber with 15 males of each of the two strains and were observed for 60 minutes. When a pair commenced mating, it was aspirated out and the type of individuals mated was recorded. All the crosses were run at 24 ± 1°C temperature and between 7-11 AM. In order to identify the flies of different strains in each cross, the flies of one strain were marked with a small spot of fast drying nail polish on the scutellum. Before making the crosses, the effect of marking was tested and the results indicated that marking has no effect either on the performance of flies or outcome of sexual isolation tests. The experiment was repeated by marking the flies of other strain. The results of both experiments were pooled.

To measure the degree of sexual isolation among different strains, the isolation estimate was calculated using the formula of Merrell (1950):

$$\text{Isolation Estimate} = \frac{\text{Number of heterogamic matings}}{\text{Number of homogamic matings}}$$

The isolation estimate ranges from zero to infinity. If isolation estimate is one, there is no sexual isolation between the strains tested. If it is zero, then isolation is complete. Since the strains of the same species were tested, the x^2 values were calculated to measure the differences between homogamic and heterogamic matings under the assumption of random mating. Any significant deviation from randomness would indicate non-random or preferential mating.

RESULTS

The results of multiple-choice experiments involving six geographic strains of *D. bipectinata* are given in Table 1. In total 15 crosses were made. In all the crosses, the homogamic matings outnumber heterogamic ones and the deviation from randomness is statistically significant in six crosses which shows positive assortative mating within *D. bipectinata*. These six crosses showing non-random (preferential) mating are: BHU Campus x Townsville; BHU Campus x Mysore; Nepal x Townsville; Nepal x Baripada; Townsville x Baripada; and Townsville x Mysore. In these crosses the females of one strain prefer their own males and discriminate against the alien males. The strains showing sexual isolation are derived from distant geographic populations such as Varanasi (Uttar Pradesh), Mysore (Karnataka), Baripada (Orissa), Nepal and Townsville (Australia). The value of isolation estimate ranges from 0.54 to 0.92. When there is no isolation (random mating) the numbers of homogamic and heterogamic matings should be nearly equal and the value of isolation estimate will be one. In all the crosses, the values of isolation estimate remain below one and in six crosses there is considerable decrease in isolation estimate due to less number of heterogamic matings which indicates the existence of incipient sexual isolation within *D. bipectinata*

DISCUSSION

Bock (1978) studied sexual isolation between two strains of *D. bipectinata* originating from Borneo and Thailand and did not find evidence for sexual isolation between them as isolation indices were clo-

Table 1 Numbers of matings in 60 minutes in mating chamber between different strains of *Drosophila bipectinata* in multiple-choice experiments

Strains	Numbers of homogamic and heterogamic matings				Isolation	
	Homo.	Het.	Homo.	Het.	Estimate	χ^{2+}
BHU Campus x Unchawa Lodge	BHU♀ x BHU♂ 44	BHU♀ x UL ♂ 32	UL♀ x UL ♂ 41	UL♀ x BHU♂ 34	0.78	2.39
BHU Campus x Nepal	BHU♀ x BHU♂ 47	BHU♀ x Np ♂ 30	Np♀ x Np ♂ 38	Np♀ x BHU♂ 35	0.76	2.67
BHU Campus x Townsville	BHU♀ x BHU♂ 52	BHU♀ x Tv ♂ 27	Tv ♀ x Tv♂ 46	Tv♀ x BHU♂ 26	0.54	13.41*
BHU Campus x Baripada	BHU♀ x BHU♂ 40	BHU♀ x Br ♂ 36	Br ♀ x Br ♂ 44	Br ♀ x BHU♂ 31	0.80	1.91
BHU Campus x Mysore	BHU♀ x BHU♂ 44	BHU♀ x My♂ 28	My♀ x My♂ 51	My♀ x BHU♂ 26	0.57	11.28*
Unchawa Lodge x Nepal	UL ♀ x UL ♂ 44	UL ♀ x Np ♂ 32	Np ♀ x Np ♂ 34	Np♀ x UL ♂ 39	0.92	0.33
Unchawa Lodge x Townsville	UL ♀ x UL ♂ 39	UL ♀ x Tv ♂ 42	Tv♀ x Tv ♂ 43	Tv♀ x UL ♂ 27	0.84	1.12
Unchawa Lodge x Baripada	UL ♀ x UL ♂ 44	UL ♀ x Br ♂ 36	Br ♀ x Br ♂ 41	Br ♀ x UL ♂ 30	0.78	2.39
Unchawa Lodge x Mysore	UL ♀ x UL ♂ 41	UL ♀ x My ♂ 41	My♀ x My♂ 42	My♀ x UL ♂ 28	0.83	1.29
Nepal x Townsville	Np ♀ x Np ♂ 42	Np ♀ x Tv ♂ 33	Tv ♀ x Tv ♂ 48	Tv ♀ x Np ♂ 29	0.68	5.16*
Nepal x Mysore	Np ♀ x Np ♂ 43	Np ♀ x My ♂ 33	My♀ x My ♂ 39	My♀ x Np ♂ 34	0.82	1.51
Nepal x Baripada	Np ♀ x Np ♂ 39	Np ♀ x Br ♂ 33	Br ♀ x Br ♂ 55	Br ♀ x Np ♂ 23	0.60	9.62*
Townsville x Baripada	Tv ♀ x Tv ♂ 49	Tv ♀ x Br ♂ 19	Br ♀ x Br ♂ 43	Br ♀ x Tv ♂ 39	0.63	7.71*
Townsville x Mysore	Tv ♀ x Tv ♂ 50	Tv ♀ x My♂ 28	My ♀ x My ♂ 43	My♀ x Tv♂ 27	0.59	9.76*
Baripada x Mysore	Br ♀ x Br ♂ 37	Br ♀ x My ♂ 39	My ♀ x My ♂ 42	My♀ x Br ♂ 31	0.89	0.54

*Significant
+ d.f.=1

se to zero and difference between homogamic and heterogamic mating was insignificant. During the present study six geographic strains of *D. bipectinata* were used to detect whether sexual isolation exist within *D. bipectinata*. The strains coming from distant localities in India, Nepal and Australia were tested. Mating was observed directly in Elens- Wattiaux mating chamber. From the results it is evident that homogamic matings are more frequent than heterogamic ones in all the crosses. Interestingly, there is significant deviation from randomness indicating positive assortative mating in six crosses viz. BHU Campus x Townsville; BHU Campus x Mysore; Nepal x Townsville; Nepal x Baripada; Townsville x Baripada; and Townsville x Mysore. Isolation estimate also remains low in these crosses. Thus the females of one strain prefer their own males and discriminate against alien males. The strain from Australia shows incipient sexual isolation with four strains (Varanasi, Nepal, Orissa and Mysore) coming from other continent. The non-random (a preferential) mating observed between these strains clearly suggests that *D. bipectinata* is characterised by incipient sexual isolation. Thus the populations of *D. bipectinata* may have developed premating isolation as a by-product of genetic divergence resulting from the action of natural selection and genetic drift, operating in different populations.

Significant deviation from random mating is common in interpopulation crosses involving several *Drosophila* species. Incipient sexual isolation among different geographic strains of the same species have been reported in *D. paulistorum* (Dobzhansky & Spassky, 1959; Malogolowkin- Cohen *et al.*, 1965), *D. athabasca* (Miller, 1958; Miller & Westphal, 1967), *D. birchii* (Ayala, 1965; Baimai, 1970), *D. prosaltans* ((Bicudo, 1978), *D. silvestris* (Ahearn, 1980), *D. immigrans* (Ehrman & Parsons, 1980) and *D. ananassae* (Singh & Chatterjee, 1985 a,b). The results obtained in these species of *Drosophila* provide evidence that different populations of the same species may develop reproductive isolation as a result of genetic divergence which is important step leading to the creation of new species. However, populations of *D. pseudoobscura* characterised by genetic divergence do not show divergence in mating behavior (Anderson & Ehrman, 1969). Similarly, the widely geographically separated populations of *D. melanogaster* do not show mating isolation in spite of substancial amount of genetic divergence in these populations (Petit *et al.*, 1976; Henderson & Lambert, 1982) which suggests that there is considerable stability in mate recognition system of *D. melanogaster*. The mate recognition system

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