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Climatic adaptations of life-history traits in *Drosophila melanogaster*: analysis of genetic and plastic effects.

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Abstract

Five Indian geographical populations of *Drosophila melanogaster* were analyzed for their genetic divergence of life history traits. Clinal variations were observed for body weight, ovariole number, and four pre-life history traits (*i.e.*, fecundity, hatchability, viability, and duration of development). Rearing populations at different growth temperatures shows that the variations among wild flies are likely to reflect variations in the environmental conditions under which they developed. The results of cold assay suggest that the northern populations were cold resistant as compared to southern populations. Significant correlations of the mean monthly coefficients of variation of temperature with these fitness related traits can best explain the observed clinal variations under natural selection. Key words: *Drosophila melanogaster*, body weight, ovariole number, fecundity, hatchability, viability, duration of development, cold assay.

Introduction

Along geographical gradients, variations in fitness related traits reflect evolutionary response to selection pressures imposed by changing climatic conditions (Endler, 1986; Powell, 1997; Hedrick, 2005). Latitudinal clines for ovariole number in *Drosophila melanogaster* populations from the European-African transect (Lemeunier *et al.*, 1986) and for developmental time in Australian populations (James *et al.*, 1995; James and Partrige, 1995) suggest the role of natural selection.

Extreme environmental conditions and stresses can have negative significant effects on physiological as well as life history traits of organisms. Analysis of such stressful conditions is a major focus in the development of the understanding of ecological adaptations and biogeographical distribution of a species (Karan and David, 2001). Genetic studies of life history traits and fitness characters are generally made in the laboratory (Delpuech *et al.*, 1995), and phenotypic plasticity is often considered as an unwelcome noise (Coyne and Beecham, 1987; Falconer, 1989). When investigations are made in different environments, however, a significant genotype-environment interaction is often seen (Gibert *et al.*, 1998).

Latitudinal clines for ovariole number in *Drosophila melanogaster* populations from the Europian-African transect (Lemeunier *et al.*, 1986) and for developmental time in Australian populations (James *et al.*, 1995; James and Partrige, 1995) suggest the role of natural selection.

Partridge *et al.* (1994) studied the pre-adult life history of the laboratory populations for evidence of adaptation to thermal regime by measuring the pre-adult viability at two different temperatures, and they suggested that pre-adult traits might contribute to the differences in pre-adult survival and adult body size. Since only a few populations have been investigated from different parts of the globe, there is need to analyze several other continental populations of colonizing species. Thus, it was considered pertinent to check fecundity, viability, and duration of development in *Drosophila melanogaster* populations of colonizing species from different latitudinal sites differing in ecological parameters.

Many ectothermic species have to face cold stress to survive in temperate climates (Gibert *et al.* 2001). Chill coma (the temperature at which coma is observed) is considered as an appropriate way for the estimation of cold tolerance of a species (Schenker, 1984; Leather *et al.* 1993; Convey, 1997). Measuring recovery from chill coma appears to be a convenient and novel means for revealing the thermal adaptations of a species and its probable climatic origin. Gibert *et al.* (2001) compared several *Drosophila* species and found a considerable variability of chill-coma recovery time. In the present study, we compared chill-coma recovery time for five geographical populations of *D. melanogaster* and found that northern populations were cold resistant and southern populations were cold sensitive. A negative cline for recovery from chill-coma was observed for *D. melanogaster* populations.

In the present study, we analyzed five Indian geographical populations of *Drosophila melanogaster* and tried to find adaptive variations in life history traits by three complementary kinds of investigations: (i) by comparing different geographic populations and their climatic adaptation, (ii) by comparing recovery time from chill-coma, and (iii) by comparing adults grown at different temperatures. An attempt has been made to address the following questions: (i) To what extent geographical populations of *D. melanogaster* vary in their life history traits? (ii) Do life history patterns reveal genetic as well as plastic differences? (iii) Can geographical differences in life history traits be explained on the basis of climatic variables of the site of origin of populations?

Materials and Methods

Wild living adults of *D. melanogaster* were collected from five latitudinal sites in a single trip in the month of October. From each collection site, about 40 to 60 individuals were obtained which were used to initiate 10 isofemale lines and one mass culture (initiated with 30 pairs of wild-caught flies). The cultures were maintained at a constant growth temperature of 24±1°C for more than 10 generations prior to the experiments. For all cultures, transfers were made with randomly selected forty pairs (female and males in equal numbers) each generation.

Body weight of groups of ten females (3 days old) was measured with electronic balance with a precision of 0.01 mg. For all the populations, mean values per fly (mg × 100) were based on ten replicates of groups of ten males or females.

Female fecundity was measured by placing a pair of virgin but 6 days old female and male in a breeding chamber for 12 h mating and thereafter the male was removed. The eggs laid on the food placed at the bottom of the oviposition chamber were counted daily. The flies were transferred to fresh food vials every day, and the number of eggs laid by each female after 24 h was recorded. The fecundity of each site was observed for 15 successive days (from 7^{th} to 21^{st} day) as this period coincided with highest egg production. After the 21^{st} day, the ovaries were dissected from the female flies and the total ovariole number (for both ovaries) was obtained by fixing the dissected ovaries in a saturated solution of potassium dichromate. For each population, four replicates of five isofemale lines were used to obtain population means.

For estimating hatchability, four replicates of 25 eggs were kept on a small squarish black paper moistened with 70% alcohol on food in such a way that eggs faced upwards. The numbers of hatched eggs were counted after 24 hours. From the hatched eggs, first instar larvae moved towards the food leaving behind the chorion on black paper along with unhatched eggs.

Egg-to-adult development time and viability were measured at exact densities of 60 eggs per vial, using the technique described in Chippindale *et al.* (1994). All the five populations were assayed simultaneously. The racks were randomly distributed on the incubator shelves, and repositioned and rotated several times daily to minimize the effects of temperature and lighting gradients that may exist in incubators. Checks for emerging adults were made every six hours after pupal darkening at 0100, 0700, 1300 and 1900 h. Checks were terminated when no adults had emerged in the assay for three days. Newly emerged adults from puparium were transferred to temporary holding vials containing food every 6 hourly. After the estimated peak eclosion period, for a given population, the conditioning period was setup; four males and four females were sorted into each vial, provisioned with standard medium. Pupal counts were then performed on each vial.

To evaluate chill coma recovery, females (3 days old, five females per culture bottle) were placed individually in 40 ml glass vials, which were immersed in ice cubes. The vials were removed after 8 h and recovery time was scored. Flies were considered recovered when they stood up. There were four groups of flies per population each obtained from a different culture bottle.

Effects of seven different growth temperatures (13°, 15°, 17°, 21°, 25°, 28°, and 31°C) were analyzed in three populations (one each from low, mid, and high latitude) for fecundity, hatchability, viability, and duration of development of both sexes. These assays were done on mass cultures maintained at 24°C in the laboratory. Five replicates were used for such type of studies.

Correlation and linear regression analysis was attempted for various life history traits with respect to latitude of origin or body weight. The slope values (b) indicate latitudinal increase in trait value while a (intercept) represents possible trait value at equatorial sites.

Table 1. Female body weight, ovariole number and four pre-life history traits in five geographical populations of *D. melanogaster*. Correlations with CV of $T_{average}$ of sites of origin are also given.

Population	Latitude (°N)	Body weight (mg)	Ovariole number	Fecundity	Hatchability	Viability	Duration of Development (hours)	
							Male	Female
Vellore	12.55	0.70 ± 0.013	34.40 ± 0.13	29.80 ± 0.17	55.00 ± 0.15	53.40 ± 0.23	221.0 ± 1.09	262.50 ± 1.20
Hyderabad	17.27	0.76 ± 0.010	37.60 ± 0.21	34.60 ± 0.23	58.30 ± 0.19	59.80 ± 0.15	236.4 ± 1.25	276.50 ± 1.13
Nagpur	21.06	0.83 ± 0.007	41.70 ± 0.11	42.00 ± 0.14	66.70 ± 0.10	61.50 ± 0.20	257.2 ± 1.13	291.00 ± 1.17
Gwalior	26.14	0.97 ± 0.011	45.30 ± 0.15	47.40 ± 0.19	71.00 ± 0.21	68.30 ± 0.22	274.5 ± 1.20	311.00 ± 1.11
Chandigarh	30.44	1.04 ± 0.008	49.00 ± 0.19	52.60 ± 0.21	78.50 ± 0.17	73.40 ± 0.17	290.0 ± 1.16	328.00 ± 1.19
r with T_{cv}	--	0.98	0.98	0.98	0.98	0.97	0.94	0.97

Results

Basic data for female body weight, ovariole number, and four pre-life history traits in five geographical populations of *D. melanogaster* are given in Table 1. Positive relationship of ovariole number and body weight is illustrated in Figure 1. Furthermore, correlations between female body size (body weight) and all the pre-life history traits and also for ovariole number are highly significant (r = 0.96 to 0.99). This suggested that there is correlated natural selection pressure for life history traits and body size changes in *D. melanogaster*. Regression coefficient is highly significant for maximum fecundity (1.71 ± 0.09), hatchability (1.39 ± 0.10), and viability (1.16 ± 0.05). This is also evident from the magnitude of differences in trait values across geographical transact. For two

traits, ovariole (b = 0.55) and for duration of development (0.63 for female and 0.72 for males), slope values are lower but significantly higher than zero. R^2 explains genetic determination of trait variability, and all the values are highly significant (Table 2). Regression analysis of life history traits with body weight exhibited higher slope values (0.84 from max. fecundity and 0.63 for hatchability), while slope values are lower for ovariole no. (b = 0.25), viability (h = 0.48), and duration of development (0.36 to 0.39). Statistical analysis further revealed that there exists a strong positive correlation (r = 0.98) between ovariole number and fecundity.

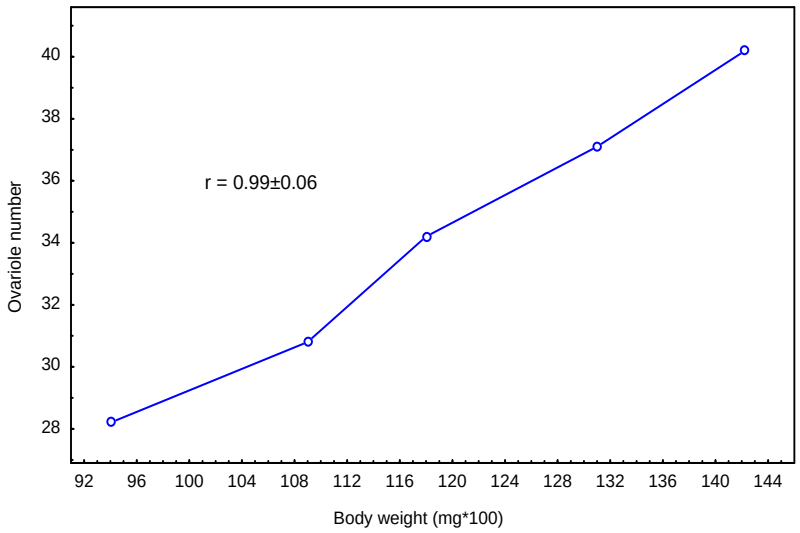

Figure 1. Correlation of body weight with ovariole number for five geographical populations of *D. melanogaster*. Each point represents the mean of 30 values.

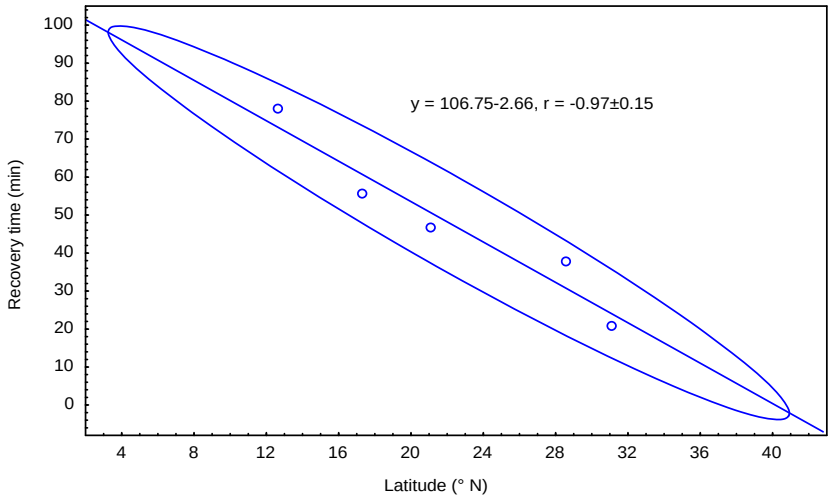

Figure 2. Regression analysis and negative correlation of recovery from cold with the latitude of origin of the populations of *D. melanogaster.*

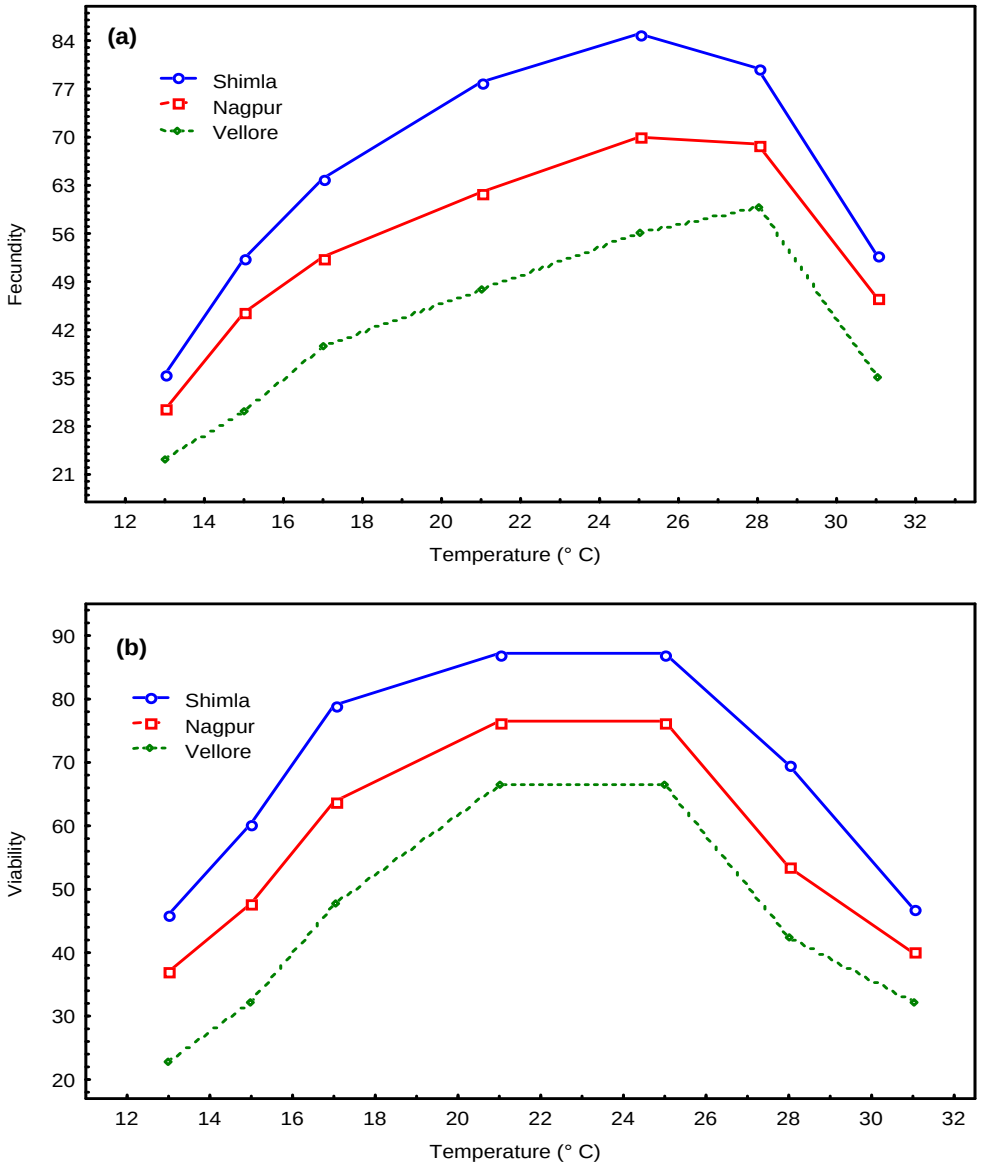

Figure 3. Comparison of changes in (a) mean fecundity (number of eggs laid) and (b) percent viability as a function of growth temperature (12 to 31°C) for three Indian geographical populations of *D. melanogaster*.

To compare the field conditions, a cold assay was conducted in the laboratory. A stress of 0ºC was given for 14 hours and the recovery time was observed. Northern populations were found to be more cold resistant as compared to southern population. Figure 2 illustrates the linear regression and negative correlation of recovery time with the latitude of origin.

Three out of five populations of *D. melanogaster* were further analyzed for life history traits across full thermal range. Figure 3 (a and b) demonstrates the reaction norms for fecundity and viability, respectively, at different growth temperatures. Both traits show significantly lower values at 13°C and 31°C in all the three population. For both the traits, optimum values were in the range of 21 to 25°C, while lower and higher temperatures evidenced stressful effects. The values were

significantly lower for southern populations across full thermal range as compared with northern and central Indian populations. For both the life history traits, across full thermal range, correlation values (r) with coefficient of variation of mean monthly temperatures (T_{cv}) of the site of origin were highly significant (0.94 to 0.98). Thus, a gradual increase in life history characteristics with latitude as well as with T_{cv} suggests their adaptive significance. Between populations, differences were significant and were supported by ANOVA. For two traits (*i.e.*, hatchability and viability) the percent variation due to geographical populations were accounted 13.40 and 10%, respectively, while for fecundity, such variation was lower (5%). Temperature showed to account for 85 to 87% of trait variability, whereas interactions of populations with temperatures (*i.e.*, P * T) resulted in 2 to3% of total variability.

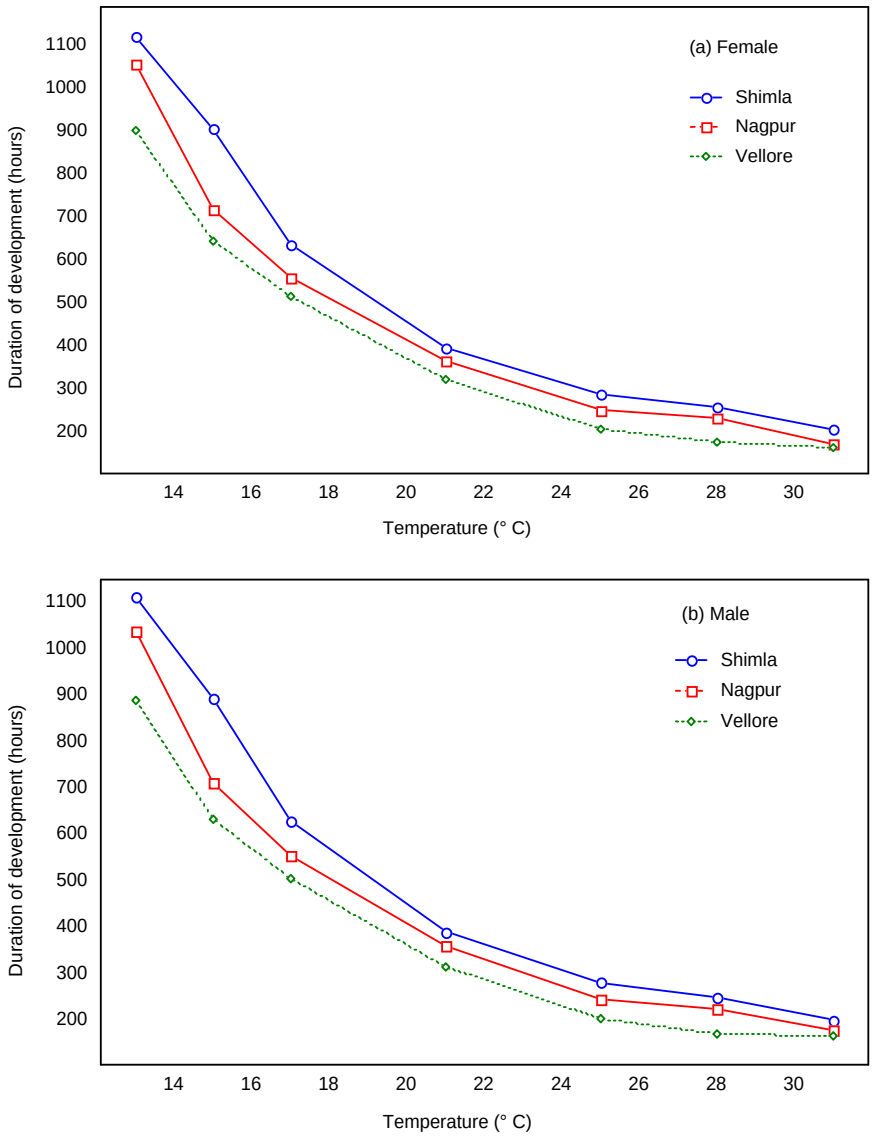

Figure 4. Comparison of changes in duration of development (hours) for both sexes, (a) female and (b) male, at seven growth temperatures for three latitudinal populations of *D. melanogaster*.

Growth temperature is a significant parameter for duration of development of both sexes of *Drosophila melanogaster* (Figure 4 a and b) and 92% of total variation in this trait, on the basis of ANOVA, can be accounted by the range of developmental temperature. Geographical populations differed slightly (~ 6%) across a covering range of 12.58 to 31.30° latitude. The difference in duration of development among the three geographical populations was more at their lower growth temperature as compared with their higher growth temperature, and it decreased with increasing growth temperature. At the optimal growth temperature (*i.e*., 25°C), the differences in duration of development were of the order of 2-5 days and were more among the southern and northern populations (*i.e.*, Shimla versus Bangloare). This suggests that the geographical populations of *D. melanogaster* are genetically adapted with respect to the ecological conditions of the site of origin (*i.e*., tropical versus subtropical) on the Indian subcontinent.

Discussion

In *Drosophila*, like other animal species, different habitats and resources are likely to impose different selective pressure on natural populations. According to local conditions, various environments may result in increased polymorphism in *D. melanogaster*. Indian populations differing in latitudinal habitats exhibit divergence of life history traits. Such characteristics could be subjected to balanced polymorphism during seasonal cycles and to adaptive modifications during the process of colonization of different regions. Southern populations have shown lower number of ovarioles than northern populations, *i.e*., oviposition capacity is significantly different between latitudinal regions. Higher number of ovarioles in northern localities as compared to low number of ovarioles in southern regions could be adaptive traits as they allow higher fecundity under seasonally varying and colder environmental conditions. Lower ovariole number in southern population could be a trade off in favour of lower body weight and greater dispersal ability under tropical conditions.

At higher developmental temperature, the fly acquires low number of ovarioles; hence, developmental temperature may favor phenotypes with lower oviposition capacity of the population. These differences in various life history traits may be explained on the basis of differences in ecological and environmental conditions along the latitudinal axis of Indian subcontinent. *Drosophila* populations in India experience almost homogenous hot and humid environmental conditions in the south as compared to northern part, where seasonal variability exists (natural populations experience progressively colder winter and hotter summer in the months of May, June, and July). Thus, during winter, colder conditions impose greater selection pressure on abundant populations.

In summary, the occurrence of latitudinal clines for different body size traits and pre life-history traits are itself a strong argument in favor of the adaptive significance of genetic variations since environmental parameters vary regularly according to latitude. By comparing recovery time from chill-coma, northern populations were found to be more cold resistant as compared to southern population. Further, the negative correlation of recovery time with the latitude of origin suggests the genetic adaptive significance of the trait. In laboratory-grown flies, between population differences were highly significant, and temperature played a major role to shape the reaction norms. Thus, the present investigations on life history traits evidence adaptive genetic divergence due to climatic selection.

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Quantitative courtship acts of 2LA inversion homo- and heterokaryotypes of *Drosophila ananassae.*

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Relationship between inversions, morphological traits, and fitness characters has been well documented in *Drosophila*. The relationship between inversions and behavioral traits, however, has not been studied. The present study has, therefore, been carried out to find out the effect of inversions on courtship acts in *Drosophila ananassae*. Homozygous 2LA inversion stock of *D. ananassae* was established from the females collected at semi domestic locality of Dharwad, India. This stock was maintained at 21 ±1ºC and relative humidity of 70% for ten generations. Before starting the experiments the inversion heterokaryotypes were generated by crossing males with homokaryotic inversion with normal female or vice versa. When the progeny appeared, the virgin females and bachelor males were isolated, kept separately, aged for five days, and used for observation of courtship behavior. The courtship behavior of males and females was observed by confining a bachelor male and a virgin female of a given type in an Elens-Wattiaux mating chamber. A total of twenty five pairs of the following combinations were studied: a. both male and female heterokaryotypic, b. male homokaryotypic and female heterokaryotypic, c. both male and female homokaryotypic, and d. male heterokaryotypic and female homokaryotypic. The courtship elements were quantified following the procedure of Hegde and Krishna (1997). Following courtship elements such as tapping, scissoring, vibration, circling, licking, ignoring, extruding, and decamping were analyzed. The data gathered were subjected to one way ANOVA.

In the present study (Table 1), it is noticed that male courtship acts such as tapping, wing vibration, scissoring, circling, and licking were less in crosses involving homokaryotypic male and female than in crosses involving heterokaryotypic male and females with 2LA inversion. The female courtship activities ignoring, extruding, and decamping were higher in crosses involving both homokaryotypic male and homokaryotypic female than in crosses involving both male and female heterokaryotypic to 2LA inversion. The courtship activities of crosses involving a homokaryotypic male with another homokaryotypic female were lower than the crosses involving heterokaryotypes. Thus the male's activity was higher in pairs where both male and female are heterokaryotypes or else one of the two sexes is heterokaryotypic. This agrees with the findings of Spiess *et al* (1966), who