

Genetic differentiation in natural populations and their mass culture stocks of *Drosophila ananassae*

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ABSTRACT

Natural populations and their mass culture stocks of *Drosophila ananassae* were analyzed for the frequencies of three cosmopolitan inversions. The frequency of ST gene rearrangement was recorded to be high in both natural populations as well as in the mass culture stocks. Furthermore, out of three cosmopolitan inversions, the AL and DE had higher occurrence showing their adaptive significance to this species. ET inversion that persists in all populations, was thinly represented than the other two. These natural populations, coming from different geographical localities, showed very little genetic variation. The mass culture stocks also showed almost similar genetic composition as their respective natural populations. Various parameters of population genetics were computed in order to find out genetic structure of the natural populations. The results indicate that genetic changes in the natural populations have scarcely undergone, and no significant genetic differentiation could be observed when natural populations were compared with the respective mass culture stocks.

Keywords: *Drosophila ananassae*; inversion polymorphism; genetic identity; genetic differentiation

INTRODUCTION

In *Drosophila*, chromosomal polymorphism owing to inversions is common occurrence and is

often maintained by balancing selection (Dobzhansky, 1970; Sperlich, 1973). Chromosomal polymorphism is very common in various species of *Drosophila* and constitutes an adaptive trait (da Cunha, 1960; Dobzhansky, 1970; Parson, 1973; Sperlich and Pfriem, 1986). It has been reported that the geographically wide spread species of *Drosophila* show higher level of chromosomal polymorphism as they are believed to be ecologically versatile. In *D. willistoni*, a good correspondence between the mean number of heterozygous inversions and an index expressing environmental heterogeneity in natural populations have been recorded by da Cunha and Dobzhansky (1954). They suggested that chromosomal polymorphism is a device to cope with the diversity of the environment. Genetic distance indexes have been used to quantify the amount of genetic differentiation at the level of chromosomal polymorphism in natural populations of several species of *Drosophila* (Powell *et al.*, 1972; Prevosti *et al.*, 1975; Fukatami, 1976; Pinsker and Sperlich, 1979).

Studies on different species of *Drosophila* have been done in order to know the genetic mechanism of maintenance of inversion polymorphism when populations were transferred from natural to laboratory conditions. In some cases, decline in frequency or total elimination of polymorphic inversions was detected like in *D. pseudoobscura* (Dobzhansky and Pavlosky, 1957;

Dobzhansky and Spasky, 1962; Anderson *et al.*, 1967; Watanabe *et al.*, 1970), *D. subobscura* (de Frutos, 1978), *D. poulistorum* (Powell and Richmond, 1974), and *D. melanogaster* (Alahiotis *et al.*, 1976; Inque, 1979; Singh and Das, 1992).

D. ananassae, a cosmopolitan and domestic species, presents a high degree of inversion polymorphism and is frequent occurrence in India. In this species three paracentric inversions are coexistent and are referred as cosmopolitan inversions. These inversions have their wide geographic distributions and they also persist in the laboratory conditions due to their heterotic buffering. The different natural populations of *D. ananassae* showed geographic differentiation of inversion polymorphism (Singh and Singh, 2008). The present communication reports the results of chromosomal analysis of seven natural populations and their mass culture stocks of *D. ananassae*. The frequency of standard (ST) and inversion rearrangements have been obtained, and based on their frequency genetic identity among the natural populations have been computed. The natural populations have also been raised as mass culture stocks, therefore, we have

also been able to see the deviation in the frequency of inversions when natural populations are transferred to laboratory conditions.

MATERIALS AND METHODS

Seven natural populations of *D. ananassae* were sampled and their mass culture stocks were established. Table 1 shows the places (with their abbreviations) from where the flies were collected and a line diagram (Figure 1) depicts the site map of these populations. Naturally collected flies from a particular place were used for raising isofemale lines and also to establish mass culture stock. Polytene chromosome analysis for three cosmopolitan paracentric inversions (AL in 2L, DL in 3L, and ET in 3R) of this species was made by squashing 3rd instar larvae with usual lacto-aceto-orecin method, and the frequency of different chromosome banded pattern was recorded. The chromosomal analysis of F₁ larvae was done from isofemale lines to get data of natural populations. Mass culture populations established from seven natural populations were maintained for 5 generations and then the chromosomal analysis of the larvae was done to get data for mass culture stocks.

Table 1 Name of collection places and their abbreviation and number of individuals analyzed (N) in natural populations and their mass culture stocks (in parentheses).

Serial No.	Populations	Abbreviation	N
1	Om Nagar Sultanpur	ONS	199 (304)
2	Kamala Neharu Institute	KNI	200 (315)
3	Kadipur	KDP	77 (100)
4	Pratapgarh	PTG	93 (150)
5	Faizabad	FZD	65 (125)
6	Bikapur	BKP	42 (60)
7	Lucknow	LKO	15 (92)

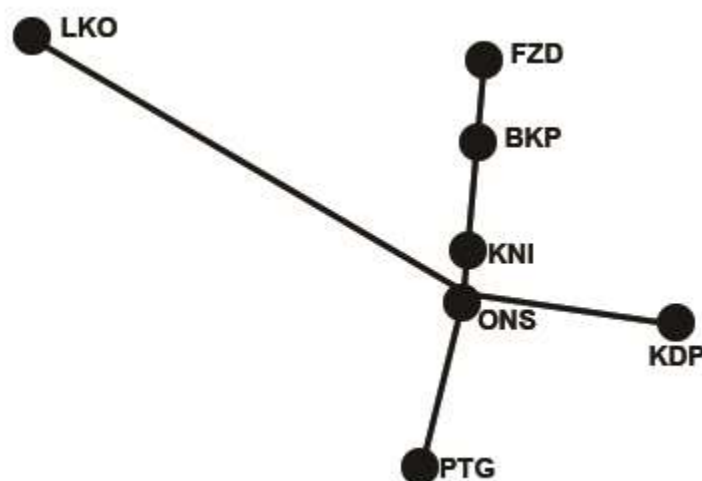


Figure 1 Line diagram showing geographical locations and direction of seven natural populations of *D. ananassae*.

Chromosomal rearrangements have been interpreted as alleles, and therefore respective genotypes were scored at all three loci (AL, DE and ET). For these polymorphic loci, genotype classes were tested for conformity with Hardy-Weinberg expectations by using chi-square test and observed genotype classes were used to calculate allele frequencies. In analyzing chromosomal rearrangement data, we have used several measures of genetic variation. As a measure of possible inter-population differentiation, genetic distances were calculated using the formula proposed by Nei (1972). Test for population differentiation was computed using *F*-statistics i.e., F_{ST} , F_{IT} , and F_{IS} (Weir and Cockerham, 1984; Wright, 1951, 1978) for each of the loci to deduce that to what extent these populations have undergone genetic differentiation.

RESULTS

Polytene chromosome analysis of natural populations of *D. ananassae* revealed that these populations are polymorphic for the three cosmopolitan inversions. Due to the presence of three inversions, i.e., alpha (AL) in 2L, delta (DE) in 3L and eta (ET) in 3R, we were able to record three

classes for each inversion locus (i.e., ST/ST, ST/AL, AL/AL in 2L; ST/ST, ST/DE, DE/DE in 3L and ST/ST, ST/ET, ET/ET in 3R). The frequency of inversion rearrangements and observed heterozygotes (H_o) found in the seven natural populations as well as their mass culture stocks have been shown in Table 2. By using Hardy-Weinberg equation, the expected frequency of heterozygotes for each class was calculated. Based on the data of observed and expected frequency of heterozygotes, inbreeding coefficient (F) was also obtained. Hardy-Weinberg equilibrium was tested for natural populations and their mass culture stocks to find out the deviation from expectation.

The data on natural populations show that the frequency of AL ranges from 0.06 to 0.30, DE ranges from 0.06 to 0.20 and ET ranges from 0.02 to 0.16. Out of three inversions, it was AL that shows more frequency in occurrence than the others. In mass culture stocks similar analysis of data revealed that the range of occurrence of these rearrangements, i.e., AL, DE and ET was 0.09 to 0.17, 0.08 to 0.12 and 0.02 to 0.15 respectively. The data on mass culture stocks indicate that all the three inversions persist when the flies were maintained in the laboratory conditions.

Table 2. Frequency of AL, DE and ET gene arrangements, observed and expected heterozygosity, inbreeding coefficient, χ^2 values in natural populations and mass culture stocks of *D. ananassae*.

Populations	AL	H _O	H _E	F	χ^2	DE	H _O	H _E	F	χ^2	ET	H _O	H _E	F	χ^2
natural populations															
ONS	0.09	0.11	0.16	0.31	14.95*	0.13	0.20	0.23	0.13	4.77*	0.05	0.06	0.17	0.64	38.89*
KNI	0.11	0.11	0.19	0.41	37.44*	0.11	0.16	0.20	0.20	10.00*	0.08	0.11	0.15	0.25	7.79*
KDP	0.06	0.07	0.11	0.36	11.91*	0.06	0.06	0.11	0.45	12.69*	0.04	0.08	0.08	0.0	0.25
PTG	0.10	0.096	0.18	0.46	13.99*	0.11	0.11	0.19	0.43	17.29*	0.02	0.03	0.04	0.25	0.152
FZD	0.08	0.076	0.147	0.48	18.24*	0.14	0.18	0.24	0.23	3.28	0.04	0.076	0.076	0.04	0.00
BKP	0.20	0.16	0.32	0.48	10.0*	0.14	0.14	0.24	0.40	7.58*	0.16	0.30	0.26	-0.26	1.459
LKO	0.30	0.33	0.42	0.21	0.635	0.20	0.26	0.32	0.18	0.406	0.13	0.27	0.23	-0.17	0.369
mass culture stocks															
ONS	0.09	0.12	0.16	0.23	15.41*	0.11	0.10	0.19	0.44	54.06*	0.04	0.06	0.07	0.22	6.087*
KNI	0.10	0.12	0.18	0.33	20.88*	0.12	0.16	0.21	0.23	13.19*	0.09	0.12	0.16	0.26	14.95*
KDP	0.10	0.10	0.18	0.44	19.52*	0.12	0.16	0.21	0.23	5.87*	0.15	0.19	0.25	0.24	5.20*
PTG	0.17	0.20	0.28	0.28	11.4*	0.10	0.13	0.18	0.27	10.07*	0.08	0.08	0.147	0.45	31.26*
FZD	0.14	0.15	0.24	0.36	13.12*	0.12	0.13	0.21	0.39	19.38*	0.08	0.12	0.15	0.18	2.46
BKP	0.17	0.15	0.28	0.46	14.56*	0.08	0.10	0.15	0.32	7.829*	0.02	0.05	0.04	-0.28	0.209
LKO	0.11	0.13	0.19	0.33	9.65*	0.10	0.10	0.18	0.46	14.02*	0.08	0.10	0.15	0.32	4.343

* $P < 0.05$

Furthermore, a drastic reduction in the frequency of ET rearrangement was recorded in two populations (BKP and LKO) and only in KDP population unexpected increase could be observed.

The χ^2 analyses for natural populations showed that out of 21 combinations tested, 8 showed insignificant deviation from expectation. The populations which showed significant deviation ($P < 0.05$) from expectation also showed higher F values. This, in fact, happened only because in such combinations, observed heterozygotes were found to be much less than expected heterozygotes. Similar trend was also found in mass culture stocks. Table 3 incorporates average observed and expected heterozygosity and inbreeding coefficient (F) in natural populations and their mass culture stocks. The inbreeding coefficient varies from 0.10 (LKO) to 0.43 (PTG) in natural populations and 0.27 (KNI) to 0.37 (LKO) in mass culture stocks. We could note that the inbreeding occurs almost at a similar rate in mass culture stocks than in natural populations where varying level of F values was recorded. Table 4 shows locus wise mean heterozygosity of all the seven populations and their mass culture stocks. The different values of F-statistics have also been calculated (Wright, 1951; Wier and Cockerham, 1984). F_{ST} values indicate that

there is higher genetic differentiation with respect to AL inversion than the other two loci (DE and ET). To quantify the degree of genetic differentiation undergone by polymorphic systems of each of the populations in the course of microevolutionary process, the genetic identity and genetic distance have been measured following the formula proposed by Nei (1972). Genetic identity between different pairs of natural populations and their mass culture stocks have been depicted in Table 5. The natural populations like KDP and PTG; ONS and FZD do not show any genetic differentiation ($I = 1$). ONS and KNI populations come from very close places (separated by only 5 km distance) and these two natural populations are also genetically very close ($I = 0.9990$). In all these populations, LKO comes from maximum distance. It shows more genetic distance from BKP than others. However, PTG and ONS separated by a distance of 40 km show even higher genetic identity (0.9995) than ONS and KNI populations. Furthermore, populations like FZD and BKP (distance 20 km) show less genetic identity value (0.9527) than LKO and KDP (0.9736), which are separated by a distance of 140 km. The overall genetic analyses done with respect to genetic identity of these seven natural populations help us to infer that these populations do not show isolation by distance.

Table 3 Average observed and expected heterozygosity and inbreeding coefficient (F) in natural populations and mass culture stocks (in parentheses) of *D. ananassae*.

Populations	H_o	H_E	F
ONS	0.12 (0.10)	0.18 (0.14)	0.33 (0.30)
KNI	0.128 (0.13)	0.18 (0.18)	0.29 (0.27)
KDP	0.07 (0.15)	0.10 (0.21)	0.28 (0.28)
PTG	0.08 (0.13)	0.14 (0.20)	0.43 (0.32)
FZD	0.11 (0.13)	0.15 (0.20)	0.25 (0.35)
BKP	0.20 (0.10)	0.27 (0.15)	0.26 (0.35)
LKO	0.28 (0.11)	0.32 (0.17)	0.10 (0.37)

Table 4 Locus wise mean heterozygosity of all natural populations and their mass culture stocks (in parentheses), and values of F-statistics.

Loci	H _O	H _S	H _T	F _{IS}	F _{ST}	F _{IT}
AL	0.13 (0.14)	0.21 (0.21)	0.23 (0.22)	0.40 (0.34)	0.06 (0.036)	0.43 (0.36)
DE	0.16 (0.13)	0.22 (0.19)	0.22 (0.19)	0.27 (0.336)	0.009 (0.005)	0.28 (0.34)
ET	0.13 (0.10)	0.14 (0.14)	0.14 (0.14)	0.07 (0.26)	-0.036 (0.028)	0.036 (0.28)

Table 5 Genetic identity between different pairs of natural populations and their mass culture stocks (in parentheses) of *D. ananassae*.

	ONS	KNI	KDP	PTG	FZD	BKP	LKO
ONS	-						
KNI	0.9990 (0.9991)	-					
KDP	0.9983 (0.9985)	0.9986 (0.9987)	-				
PTG	0.9995 (0.9974)	0.9989 (0.9978)	1.000 (0.9957)	-			
FZD	1.000 (0.9984)	0.9987 (0.9993)	0.9978 (0.9973)	0.9994 (0.9994)	-		
BKP	0.9543 (0.9970)	0.9571 (0.9956)	0.9513 (0.9911)	0.9529 (0.9989)	0.9527 (0.9979)	-	
LKO	0.9811 (0.9783)	0.9837 (0.9786)	0.9736 (0.9770)	0.9810 (0.9775)	0.9795 (0.9785)	0.9555 (0.9760)	-

Genetic identity and genetic distance between natural population and its respective mass culture stock was also computed to envisage whether the populations undergo substantial genetic alteration when transferred to laboratory conditions. Table 6 shows genetic identities (I) and genetic distance (D) between natural populations and their mass culture stocks. Based on R X C contingency table, the deviation between natural populations and their mass

culture stocks was also calculated and the χ^2 values were shown in the same table. Complete genetic identity or very close genetic identity was found in KNI, ONS, PTG and KDP populations. In other three populations, the higher level of genetic similarity was also recorded. Due to such genetic closeness between the natural populations and their mass culture stocks, no significant deviations could be obtained for all these populations

Table 6 Genetic identity (I) and genetic distance (D) between natural populations and mass culture stocks of *Drosophila ananassae* and χ^2 values.

Serial No.	Populations	I	D	χ^2
1	ONS	0.9998	0.0002	9.57
2	KNI	1.00	0.00	3.079
3	KDP	0.9954	0.0046	14.184
4	PTG	0.9973	0.0027	12.73
5	FZD	0.9702	0.0298	5.57
6	BKP	0.9547	0.0453	14.03
7	LKO	0.9625	0.0375	13.39

DISCUSSION

Population dynamics of three cosmopolitan inversions, which have been established as an integral part of genetic endowment of *D. ananassae*, have been extensively studied (Singh 2013). Indian natural populations of *D. ananassae* show geographic differentiation with respect to three cosmopolitan inversions and in general the populations from the south India, including Andaman and Nicobar Islands maintain the inversion in high frequency as compared to the populations from the North. The values of Nei's identities suggest that Indian populations of *D. ananassae* have undergone considerable amount of genetic divergence as a consequence of their adaptation in varying environments (Singh, 1998). Chromosomal polymorphism in natural population of *D. ananassae* derived from Sultanpur was studied (Singh, 2000; Ratnam and Singh, 2008) and it was observed that the mean heterozygous inversions per individual varies from 0.30 to 0.41 in natural population and 0.23 to 0.33 in mass culture stock.

In the present study, seven populations, which came from the radius of 150 km were analyzed. These populations were sampled at the

same month of the year. The observations clearly indicate that ST rearrangement is always higher in frequency than their respective inversion rearrangements in all the natural populations studied. Furthermore, out of three inversions, the AL and DE occur at higher frequency than the ET, which showed its less representation although it persisted in all populations. Its existence at the lower frequency may be due to balancing selection. Similar analysis was also performed with mass culture stocks. The aim to establish mass culture stocks was to observe whether natural populations, which were transferred to laboratory conditions underwent the process of genetic modification and the adaptive nature of these inversions persisted in laboratory conditions. We could not find fixation of any rearrangement, ST or inversion for the three inversion loci in any of the mass culture stocks. Table 6 shows no significant difference in genetic makeup of natural populations and mass culture stocks in all the populations studied. Thus, *D. ananassae* maintains its genetic endowment even in laboratory conditions, provided it has not spent long time in laboratory with the influence of genetic drift.

Genetic identity and genetic distance indexes have been used by many authors to quantify the degree of genetic divergence at the level of chromosomal and allozyme polymorphism in several species of genus *Drosophila* (Powell *et al.*, 1972; Powell, 1973; Ayala *et al.*, 1974; Prevosti *et al.*, 1975; Fukatami, 1976; Pinsker and Sperlich, 1979; Fontdevila *et al.*, 1981; Larruga *et al.*, 1983; Singh *et al.*, 1982; Singh and Singh, 2007, 2008). The differences between populations are lower in allozyme system than in chromosomal polymorphism (Powell, 1973). Fukatami (1976) reported positive correlation between genetic and geographic distance in *D. lutescens*. In *D. subobscura*, Prevosti *et al.* (1975) reported that geographical barriers have a sharp effect in increasing the degree of genetic differentiation. They noted more genetic similarity between the island populations than those between island and the neighboring mainland populations. Pinsker and Sperlich (1979) made a comparison of allozyme polymorphism of *D. subobscura* with its inversion polymorphism and found some correlations between frequency changes in inversions and those of alleles at enzyme loci on the same chromosome. They reported genetic divergence between the populations due to inhibition of gene flow. In *D. melanogaster* genetic differentiation between geographically distant populations was studied, using allozyme polymorphism by Singh *et al.* (1982) and according to them genetic differentiation between the populations is not a function of distance between them.

Persistence of commonly occurring inversions in the mass culture stocks established from flies collected from the natural populations of *D. ananassae* is only due to adaptive superiority of the heterozygotes. Futch (1966) validated the forces of gene exchange in *D. ananassae* populations, even among those populations, which remain separated by major geographical barriers. The population

statistics F_{ST} , the fixation index, measures the amount of inbreeding due to population sub-division or the reduction in heterozygosity of a subdivision due to genetic drift and as such has been used as a measure of differentiation between subpopulations (Hartl, 1981; Hadrick, 1983). Wright (1978) suggested that the range of F_{ST} values from 0.00 to 0.14 show least genetic differentiation. In this study the different parameters of population genetics were undertaken to see genetic differentiation among the natural populations and their mass culture stocks. F_{ST} values obtained in the present case clearly indicate that natural populations have not undergone substantial genetic differentiation, which may be due to spatial closeness occurrence of gene flow among these populations. A similar trend was also recorded with their mass culture stocks indicating that these stocks could not experience the effect of random genetic drift as they had spent only five generations in the laboratory conditions. Furthermore, it is obvious to observe that natural populations show less inbreeding than the mass culture stocks (average F_{IS} value for natural populations is 0.246 and for the mass culture stocks is 0.313).

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