Persistence of heterozygosity owing to balancing selection at allozyme loci in laboratory populations of *Drosophila ananassae*

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ABSTRACT

Drosophila ananassae is one of the widely occurring species in Indian subcontinent. Its genetic polymorphism, at the molecular level has not been investigated substantially. The present study reveals the persistence of enzyme variants (allozymes) in the laboratory stocks of this species. Fifteen mass culture stocks of *D. ananassae* established from the flies collected from natural populations were reared in laboratory for more than 25 generations. Based on the frequency of enzyme variants of 12 enzyme loci, the different parameters of genetic variability were tested. The results obtained indicate that most of the mass culture stocks do not show conformity with HWE, at many loci. A number of mass culture stocks became monomorphic at a number of enzyme loci. Estimates of genetic variability and inbreeding coefficient clearly specified that majority populations of mass culture stocks show higher level of F value (0.315 to 0.556) indicating that the mass culture stocks experienced more inbreeding. The reduction in the level of observed heterozygosity in mass culture stocks might be due to inbreeding caused by random genetic drift. However, occurrence of heterozygotes still at considerable range (0.313 to 0.339) at some enzyme loci in mass culture stocks proves that heterozygotes are subject to selection even in the laboratory conditions. None of the populations were found to become monomorphic for all allozyme loci studied, which shows that irrespective of strong founder events, there is still heterosis, demonstrating that the populations are under substantial balancing selection. The results of genetic distance (D), compared between natural populations and their respective mass culture stocks clearly indicate that majority natural populations have undergone substantial genetic changes, when cultured as mass culture stock. This change in the genetic composition of mass culture stocks might have occurred due to random genetic drift.

Keywords: allozyme polymorphism; mass culture stocks; random genetic drift; *Drosophila ananassae*

INTRODUCTION

Population genetics deals with the genetic variation existing among the individuals of a species and the forces which affect such variation (Hedrick 2005; Hartl and Clark, 2007). The fundaments of such study equally apply in every sexually reproducing species of animals. Genetic polymorphism has been one of the essential components of population study because it helps to envisage genetic variation among the members of a species (Ayala 1974; Kreitman 1983; Eanes 1999; Morton *et al*., 2004; Schug *et al*., 2007; Hoffmann and Willi, 2008; Mateus *et al*., 2010). Measuring level of heterozygosity and inbreeding coefficient give an idea that a population is panmictic and possess enough genetic variation or it is a small one and experiencing inbreeding. Genetic differences of a population can be expressed in the form of the extent of its genetic heterozygosity and allelic diversity (Singh *et al.,* 1982; Singh and Rhomberg, 1987). Populations with substantially higher level of genetic heterogeneity are known to have better fate of survival and perpetuation than those having less genetic variation. Reduction in the size of a population leads to genetic drift that directly affects allelic diversity (Singh *et al.,* 2013; Nei 1987). Loss of genetic diversity can be slowed by stabilizing population size (Vogl *et al.,* 2003). In natural populations one can invariably see the effect of selection, its role being more profound than genetic drift, in cage populations reared in laboratory, the effect of genetic drift is more intense (Watanabe and Watanabe, 1977).

In *Drosophila ananasse*, extensive studies have been performed at chromosomal level by considering the distribution and frequency of three cosmopolitan paracentric inversions in a large number of Indian natural populations and laboratory stocks (Singh and Singh, 2008). Some studies, with respect to enzyme polymorphisms and nucleotide polymorphisms have also been undertaken by Indian workers focusing mainly on natural populations of this species (Prakash and Shamina, 1994; Das *et al.*, 2004; Kumar and Singh, 2012; 2013; 2014a; 2014b; 2017; Krishnamoorti and Singh, 2013; 2017; Singh *et al*., 2013; Singh *et al*.,

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2016). Kumar and Singh (2017) studied allozyme polymorphism in fifteen natural populations of *D. ananassae* to analyze the level of genetic variation among these populations. Their results clearly showed that there was genetic structuring among the natural populations of this species.

The present study was undertaken with the aim to answer the question that if natural populations of *D. ananassae* are reared in the laboratory conditions for a long period of time then will these populations (mass culture stocks) retain the same extent of polymorphism or likely to undergo substantial changes in their genetic constitution. A comparison between the initial populations (natural population) and final populations (mass culture stocks having completed more than 25 generations) have been done to visualize any genetic modification in the concerned pair. *D. ananassae* flies are genetically very unique due their certain unusual genetical features like spontaneous male meiotic crossing over and high level of chromosomal polymorphism (Singh *et al*., 2013). However, genetic variability due to protein polymorphism has not been studied in this species, particularly, when populations are reared as mass culture stocks. Mass culture stocks having spent several generations in laboratory conditions may undergo genetic changes mainly due to evolutionary forces, i.e., selection and random genetic drift. A comparison between the genetic status of natural populations and their mass culture stocks may help to see that to what extent populations experience genetic differences and what specific reasons lead to such variations. This study therefore reveals the dynamics of allozyme polymorphism in *D. ananassae* when the natural populations are reared in laboratory conditions for a number of generations.

MATERIALS AND METHODS

Drosophila ananassae

D. ananassae flies were collected from fifteen natural populations extending from north to extreme south of India and the population genetic parameters of these populations were analyzed (Kumar and Singh, 2017). Mass culture stocks were established from the pooled isofemale lines of each natural population of *D. ananassae*. The mass culture stocks were maintained in the laboratory on simple food medium (yeast-agar culture medium) at 24±1°C with 12 hour cycle of light-dark period for more than 25 generations.

Allozyme variants and population study

To study allozyme variants of 12 enzyme loci, electrophoretic analysis was performed. The twelve enzyme loci which were considered in the present study have been *Acph1, Acph2, Xdh, Aph2, Aph3, Ao1, Ao2, Est2, Est3, Est5, Mdh* and *Me*. For polyacrylamide gel electrophoresis, a single fly randomly taken from culture was homogenized in 50 µl, 20 mM Tris buffer (pH 7.4) solution. The homogenate was subjected to centrifugation at 12000 rpm at 4°C for 10 minutes. The supernatant obtained was then used for in gel assay in 8% native polyacrylamide gel electrophoresis in 25mM Tris and 250 mM glycine electrode buffer (pH 8.2) at 200V for 4 hours at 4°C. In-gel staining for specific enzyme was performed following the method suggested by Shaw and Prasad (1970). The locus and allele designations (nomenclature) for enzymes were adopted from Lakovaara and Saura (1971). To compare the extent of genetic modification at the level of gene frequency between natural populations and their respective mass culture stocks, genetic constitution of the natural populations were also assessed by analyzing these loci.

Statistical Analysis

Paired *student t-test* was performed to see the genetic differences between natural populations and their mass culture stocks of *D. ananassae*. GenAlEx 6.5 software (Peakall and Smouse, 2012) was employed to measure mean observed (H_O) and expected (H_E) heterozygosity for knowing genetic variability in individual mass culture stock. The inbreeding coefficient (F) can be computed by considering H_E and H_O values, as $F = H_E - H_O / H_E$. The genetic identity (I) between natural populations and their respective mass culture stocks was calculated by the formula suggested by Nei (1972) and genetic distance (D) for the same was computed as, D = 1-I.

RESULTS

Genetic variability (observed and expected heterozygosity) and inbreeding coefficients in fifteen mass culture stocks of *D. ananassae* were incorporated in Table 1. The observed heterozygosity (Ho) in mass culture stocks varied from 0.146 (MDR) to 0.339 (TSR) and inbreeding coefficient (F) ranged from 0.160 (HYD) to 0.556 (MDR). Eight mass culture stocks (MDR, DMP, BLY, WSI, AKL, RNC, LKO and DLH) showed deviation from HWE. Estimates of genetic variability and inbreeding coefficient clearly indicate that majority populations of mass culture stocks show higher level of F value (0.315 to 0.556), indicating that the mass culture stocks experience more inbreeding.

Table 1 Values showing observed and expected heterozygosity and inbreeding coefficients in fifteen mass culture stocks of *D. ananassae.*

Populations	Ho	He	F	χ^2
Kanniyakumari (KKR)	0.323±0.038	0.420±0.025	0.239±0.069	2.742
Madurai (MDR)	0.146±0.024	0.327±0.049	0.556±0.036	14.839**
Thrissur (TSR)	0.339±0.024	0.443±0.015	0.235±0.050	2.651
Dharmapuri (DMP)	0.165±0.037	0.349±0.053	0.527±0.064	13.331**
Bellary (BLY)	0.316±0.021	0.473±0.013	0.326±0.047	5.101*
Hyderabad (HYD)	0.271±0.055	0.319±0.062	0.160±0.043	1.229
Solapur (SLP)	0.299±0.046	0.377±0.054	0.210±0.040	2.117
Washi (WSI)	0.184±0.043	0.281±0.054	0.353±0.062	5.981*
Akola (AKL)	0.214±0.036	0.356±0.051	0.407±0.041	7.951**
Ranchi (RNC)	0.172±0.041	0.283±0.062	0.452±0.069	9.807**
Varanasi (VNS)	0.292±0.043	0.385±0.053	0.239±0.044	2.742
Lucknow(LKO)	0.281±0.029	0.426±0.022	0.338±0.060	5.484*
Jaipur (JPR)	0.313±0.045	0.380±0.053	0.170±0.042	1.387
Agra (AGR)	0.292±0.038	0.377±0.043	0.214±0.051	2.198
Delhi (DLH)	0.297±0.039	0.442±0.040	0.329±0.058	5.196*

*P<0.05, **P<0.01

Heterozygosity of natural (initial) populations and their mass culture stocks (final) of *D. ananassae* was observed (Table 2). When the level of heterozygosity in natural populations and mass culture stocks of all 15 populations were compared, it was found that number of heterozygotes become significantly less (P<0.001) in mass culture stocks than their respective natural populations. The change in the level of observed heterozygosity between the initial and final populations was shown in Figure 1. Observed heterozygosity of natural populations was recorded to be more than their respective mass culture stocks in 13 populations, out of total 15 populations considered. TSR and SLP mass culture stocks were the only laboratory stocks in which increase in the number of heterozygotes were recorded from their initial populations. BLY and AGR were the two mass culture stocks in which very slight decrease in observed heterozygosity was noticed. In SLP, although there was increase in observed heterozygosity in the mass culture stock, but the level of this difference was found to be meager. This comparison of observed heterozygosity between the natural populations and mass culture stocks showed that the genetic composition of a population changed in subsequent generations due to random genetic drift. In that case, if these populations have been maintained for a number of more generations, they might have undergone changes to an extent that some of the populations would have become genetically homogenous at a number of gene loci.

In order to measure the degree of genetic divergence, the populations have undergone in the laboratory environment, genetic identity and genetic distance (D) between initial populations (natural populations) and final populations (mass culture stocks) were calculated on the basis of differences in allozyme frequencies and the data are presented in Table 3. The values of D ranged from 0.0227 (AKL) to 0.224 (AGR). Besides AGR, other mass culture stocks, which showed higher values of D were HYD (0.207), JPR (0.194) and VNS (0.144). The bar diagram based on the genetic distance between initial and final populations is depicted in Figure 2. The values of D indicate that there was conspicuous variation in the degree of genetic divergence in *D. ananassae* populations when transferred to laboratory conditions.

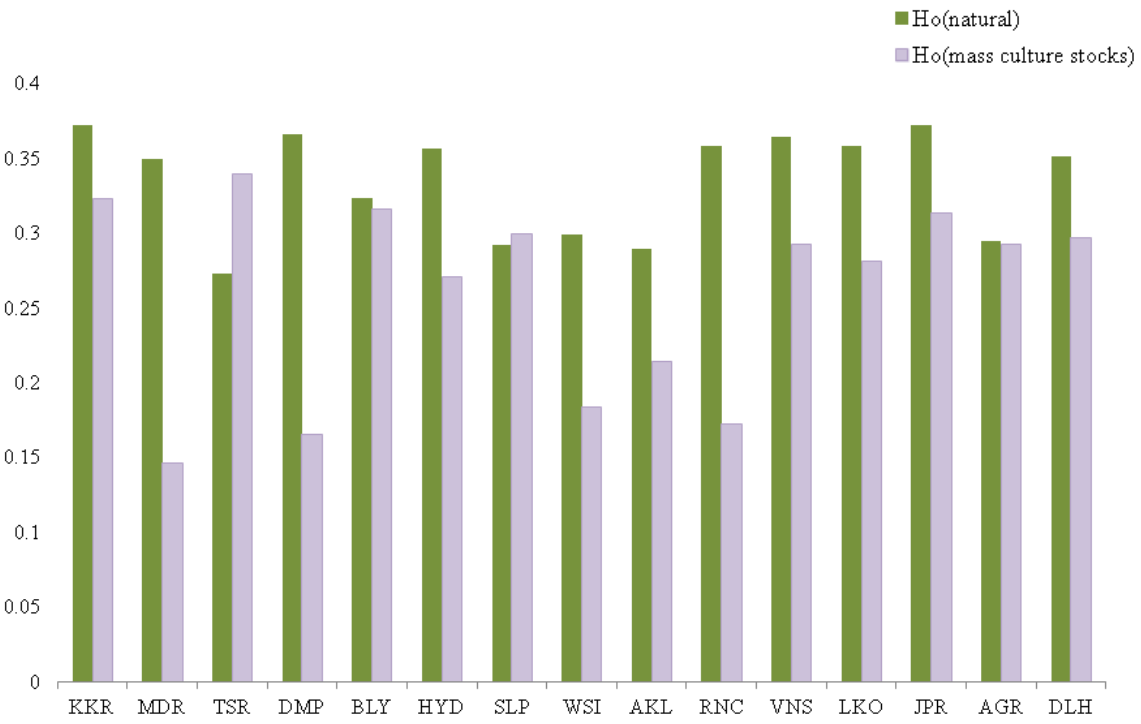

Figure 1 Histogram showing observed heterozygosity of natural (initial) populations and mass culture stocks (final) of *D. ananassae.*

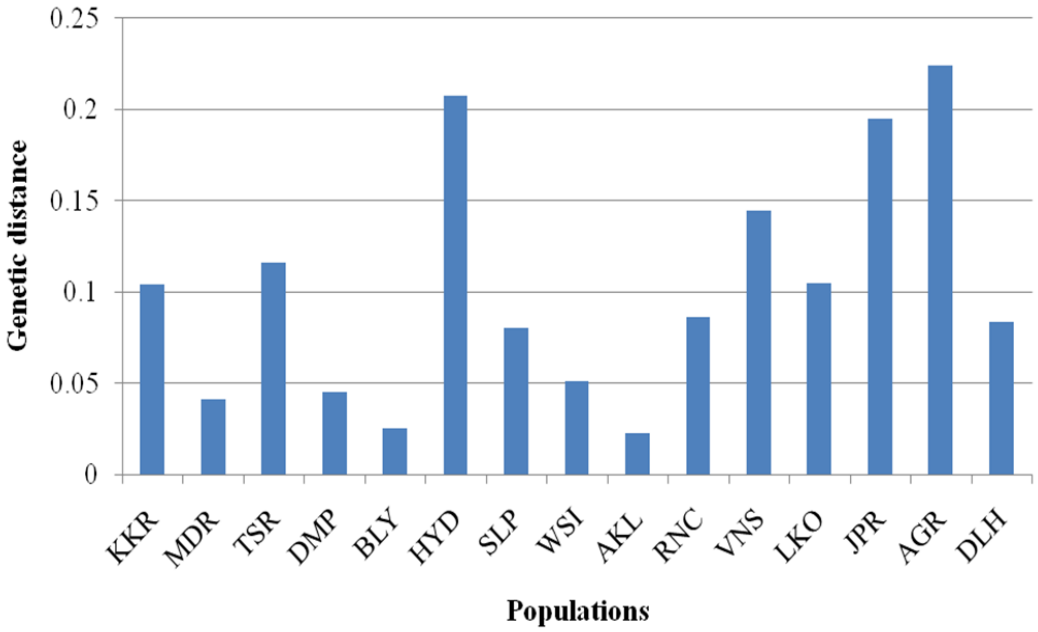

Figure 2 Bar diagram showing genetic distance between initial (natural populations) and final (mass culture stocks) populations of *D. ananassae.*

Table 2 Observed heterozygosity of natural (initial) populations and mass culture stocks (final) of *D. ananassae.*

Populations	Ho (initial)	Ho (final)
KKR	0.372	0.323
MDR	0.349	0.146
TSR	0.273	0.339
DMP	0.366	0.165
BLY	0.323	0.316
HYD	0.356	0.271
SLP	0.292	0.299
WSI	0.299	0.184
AKL	0.289	0.214
RNC	0.358	0.172
VNS	0.364	0.292
LKO	0.358	0.281
JPR	0.372	0.313
AGR	0.295	0.292
DLH	0.351	0.297

Paired t test; t stat = 3.708; d.f. = 14, $P<0.002$

Table 3 Genetic identity (I) and genetic distance (D) between natural populations and their respective mass culture stocks of *D. ananassae* based on frequencies of twelve allozyme loci.

Populations	I	D
KKR	0.8961	0.1039
MDR	0.9592	0.0408
TSR	0.8837	0.1163
DMP	0.9551	0.0449
BLY	0.9751	0.0249
HYD	0.7922	0.2078
SLP	0.9195	0.0805
WSI	0.9487	0.0513
AKL	0.9773	0.0227
RNC	0.9137	0.0863
VNS	0.8556	0.1444
LKO	0.8954	0.1046
JPR	0.8053	0.1947
AGR	0.7759	0.2241
DLH	0.9164	0.0836

DISCUSSION

Population geneticists analyze the genetic polymorphism of a species by focusing on its chromosomal, protein and nucleotide sequences. Such studies facilitate us to interpret the level of genetic variation between/among the populations of a species. *D. ananassae,* a cosmopolitan and domestic species has been given special attention due to its several unique features, one of them is spontaneous crossing over in males (Singh, 2010). This species has been studied for its chromosomal polymorphism by scrutinized its three commonly occurring paracentric inversions (Singh, 2010). However, genetic polymorphism owing to protein (enzyme) variations remained almost ignored area concerning this species. Kumar and Singh (2017) collected *D. ananassae* flies from fifteen natural populations stretched over a distance of 2800km along the length of India to study its enzyme polymorphism. Their results have indicated that Indian natural populations are structured (Kumar and Singh, 2017) and there was north-south trend in the distribution of certain enzyme variants (Kumar and Singh, 2014b). The dynamics of protein polymorphism in this species, particularly, when its natural populations are reared in laboratory conditions for several generations, have not been undertaken at all. It was therefore decided to study the genetic constitution of natural populations and their mass culture stocks (having spent more than 25 generations in laboratory environment) of *D. ananassae* to see the genetic variation between the two and the causes which may result into any variation. Through this study, it has been tried for the first time to see the effect of evolutionary forces on the level of genetic divergence, due to change in allozyme frequency, in the populations which are reared in the laboratory conditions for several generations of *D. ananassae*.

Inbreeding coefficient (F) is a measure to know the level of inbreeding in a population. Populations which are quite small and contain closely related individuals will show higher values of F than a population which is large enough and experiences random mating. A population which favors heterozygotes than homozygotes will show F value in negative. In our study, the F value ranges from 0.160 (HYD) to 0.556 (MDR). Estimates of genetic variability and inbreeding coefficient clearly indicate that majority of mass culture stocks showed higher level of F values (0.315 to 0.556) indicating that these stocks have experienced inbreeding. The reduction in the level of observed heterozygosity might be due to mating occurring among the less number (20 pairs) of founding individuals in every generation. However, persistence of heterozygotes still at higher range in some of the mass culture stocks (TSR-0.339; BLY-0.316; JPR-0.313) clearly indicate that heterozygotes were being selected even in the laboratory conditions. When the level of observed heterozygosity in natural populations (initial) and mass culture stocks (final) of all the fifteen populations were compared, it was found that number of heterozygotes became less in mass culture stocks than their respective natural populations (except for TSR and SLP). None of the laboratory stocks were found to become monomorphic for all allozyme loci studied, which shows that irrespective of strong founder effects (only twenty pairs were used as founders of each generation), there is still heterosis, demonstrating that the populations are under substantial balancing selection. Although, it is not as strong as it was observed in case of chromosomal polymorphism in *D. ananassae* (Singh and Singh, 2008).

To envisage the dynamics of allozyme polymorphism, genetic differentiation between mass culture stocks and their natural populations, genetic identity (I) and genetic distance (D) were also computed. The range of genetic distance varied from 0.023 to 0.224. Some of the stocks were found to have undergone substantial genetic changes e.g. TSR, AGR, HYD, JPR and VNS. Genetic identity (I) and genetic distance (D) between initial and final populations revealed sizeable genetic differentiation in certain populations (TSR, AGR, HYD, JPR and VNS). Populations coming from similar environmental conditions and showing initially high degree of genetic similarity have diverged to different degrees. Degree of genetic differentiation between initial and final populations was found to be different for different populations. The variation in the level of genetic divergence in *D. ananassae* populations cannot be simply explained by the process of genetic reconstruction, in view of drastic environmental change that populations suffered when they were moved to laboratory conditions. This can be clearly attributed to random genetic drift whose effect is pronounced in laboratory cultures. This seems more plausible as the populations were maintained in culture bottles by transferring only 25 pairs of flies in each generation. The event of genetic drift in laboratory populations has resulted into significant difference in frequencies of different allozyme loci in *D. ananassae*. Furthermore, a perusal on the time of establishment of the mass culture stocks and the level of genetic divergence in them, indicate

that there was random genetic divergence in the final populations irrespective of their time spent in the laboratory conditions. Among different elemental forces of evolution, natural selection and genetic drift are considered to be of immense importance that causes alterations in gene frequencies in populations. In a given environment certain alleles may be favored due to high adaptive values of their carriers by selection that will lead to gradual enhancement in the frequencies of those alleles in the populations. However, in small populations gene frequencies may fluctuate significantly by random genetic drift. The occurrence of selection and drift has been demonstrated in many cases (Andrews 2010, Singh *et al.*, 2013). Thus may be suggested that the variation in the degree of genetic differentiation in *D. ananassae* populations when transferred to laboratory conditions is likely to be due to random genetic drift.

Further, Hardy-Weinberg equilibrium was also tested in all the mass culture stocks and it was observed that out of fifteen, eight mass culture stocks showed deviation from HWE. However, only five natural populations showed significant deviation from expectation in natural populations (Kumar and Singh, 2017). Besides these comparative variations between natural populations and mass culture stocks, it was also detected that many enzyme loci showed monomorphic status in the mass culture stocks, such as *Acph2* and *Mdh* loci became monomorphic in VNS and AKL mass culture stocks respectively. Absence of polymorphism was also recorded at the *Xdh* locus in MDR and SLP. RNC and AGR mass culture stocks became monomorphic at *Aph3* locus. Monomorphic status was also observed at *Ao2* locus for WSI, RNC and JPR. Five populations, HYD, SLP, WSI, LKO and DLH became monomorphic at *Me* locus. The conversion of a polymorphic locus into monomorphic form can only be attributed to one of the evolutionary forces, i.e., random genetic drift.

A population is subject to evolutionary changes which occur due to mutation, natural selection, genetic drift and migration. These evolutionary forces do not act in isolation (Klug and Cumming, 2006). Populations being reared in laboratory conditions will not be experiencing migration at all. The mutational changes might be occurring but appearance of a new enzyme coding allele for the concerned loci is also a very remote possibility. It is therefore suggested that selection and drift can only be the factors responsible to modify the genetic constitution of mass culture populations. The results of the above study helped us to state that the enzyme variants existing in natural populations of *D. ananassae* are subject to selection because they persist even in the mass culture stocks which have already spent more than a year time span in the laboratory conditions. The different allelic forms of an enzyme persist in the mass culture stocks due to balancing selection. Reduction in the level of heterozygosity, observed in the mass culture stocks, compared to their respective natural populations, can be described due to random genetic drift occurring as a result of sampling. Therefore, populations established as mass culture stocks may not undergo abrupt alterations in the allelic frequencies, which is a common consideration that genetic drift may result the fixation of certain alleles.

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