Zoological Studies **56:** 1 (2017)
doi:10.6620/ZS.2017.56-01

Zoological Studies

Population Genetics of *Drosophila*: Genetic Variation and Differentiation among Indian Natural Populations of *Drosophila ananassae*

Sanjay Kumar and Arvind Kumar Singh*

Genetics laboratory, Department of Zoology, Banaras Hindu University, Varanasi- 221 005

(Received 27 February 2016; Accepted 26 December 2016; Communicated by Benny K.K. Chan)

Sanjay Kumar and Arvind Kumar Singh (2017) Genetic subdivision in natural populations of animals including *Drosophila* can be well understood by studying the role of evolutionary forces like natural selection, migration and genetic drift which are mainly responsible for the change in their genome. *Drosophila ananassae* is a cosmopolitan and domestic species and is one of the prevalently occurring *Drosophila* species in India. It occupies an important status in genus *Drosophila* due to its certain genetic peculiarities like spontaneous male meiotic crossing over, varied chromosomal polymorphism, Y-4 linkage of nucleolus organizer etc. and therefore, it seemed appropriate to investigate population structure and genetic differentiation among its natural populations. We assayed allozyme variation among 15 natural Indian populations of *D. ananassae* to evaluate its population structure and genetic differentiation. To test genetic differentiation between the populations, pairwise F_{ST} values were calculated and the results obtained clearly showed that Indian populations of *D. ananassae* are not homogeneous and exhibit moderate level of genetic differentiation. Nei's genetic distance (D) values were found to be positively correlated with geographic distance. Further, it was observed that South Indian populations of *D. ananassae* are genetically more similar to each other and showed substantial genetic variation from North Indian populations.

Key words: Allozyme, Genetic variation, Genetic differentiation, Population structure, *D. ananassae*.

BACKGROUND

Genetic variability in natural populations of organisms is of great interest in population and evolutionary genetic studies. The measure of genetic structure of populations in many animal and plant species have been done to envisage the genetic variability, population structure, degree of differentiation, demographic history and ancestral polymorphism (see reviews Avise 1994; Mitton 1994; Eanes 1999; Hoffmann and Willi 2008). *Drosophila* is one of the extensively studied genera in animal kingdom which has been utilized for its genetic characteristics especially genetic polymorphism. In various species of *Drosophila*, studies pertaining to chromosomal, allozyme and DNA polymorphisms were accomplished by a large number of population geneticists (Dobzhansky 1970; Sperlich and Pinsker 1980; Kreitman 1983; Singh and Rhomberg 1987; Morton et al. 2004; Das et al. 2004; Schug et al. 2007; Singh and Singh 2010). Adequate quantities of evidences have been provided to explain that allozyme polymorphism in natural populations of *Drosophila* is maintained by balancing selection (Ayala et al. 1972; Singh et al. 1982; Mortan et al. 2004; Mateus et al. 2010). Genetic differentiation between geographically distant populations of *D. melanogaster* was studied by Singh et al. (1982). They analyzed 26 allozyme loci in nine natural populations of this species and found only half of the total loci showing significant genetic differentiation between populations and remaining half showed identical pattern in all the populations. Mateus et al. (2010) selected two sibling species of *Drosophila*, *D. antonietae* and *D. gouveai*, to study genetic differentiation

*Correspondence: E-mail: aksbhu23@rediffmail.com

at Isocitrate dehydrogenase (*Idh*) locus. Their findings showed significant genetic differentiation at this locus, although these two species exhibit similar evolutionary history.

Genetically differentiated natural populations can be scrutinized for the role of different evolutionary forces responsible for population sub-structuring. Population sub-structuring has been studied in various animal populations especially in insects. High level of population sub-structuring has been reported in *D. ananassae* by many workers across the globe (Stephan 1989; Stephan and Langely 1989; Stephan et al. 1998; Das 2005; Schug et al. 2007; Singh and Singh 2010). This cosmopolitan species exhibits more population sub-structuring than the other species of *Drosophila* (Vogl et al. 2003; Das 2005).

Drosophila ananassae, a cosmopolitan and domestic species belongs to the *ananassae* species complex of the *melanogaster* species group (Bock and Wheeler 1972). Its extensive use in genetical and evolutionary field is due to its extraordinary characteristics. A large number of natural and laboratory populations of this species have been analyzed for its chromosomal polymorphism. More than 50 Indian natural populations of *D. ananassae* have been examined chromosomally to see the level of genetic differentiation among these populations (Singh et al. 2014; Singh 2013) and the data obtained provide ample evidence for high level of genetic differentiation. However, this species has not yet been involved to see the extent of genetic differentiation in different natural populations of India employing allozymes as markers. Recently, work on the allozyme polymorphism in natural populations of this species has been undertaken to envisage the level of genetic variation among the Indian natural populations of *D. ananassae* (Kumar and Singh 2012; Kumar and Singh 2013; Krishnamoorti and Singh 2013; Singh et al. 2013; Kumar and Singh 2014). The present study has been done with intention to see the level of genetic differentiation due to allozyme variations among Indian natural populations of *D. ananassae*. To our knowledge, it is the first exhaustive report regarding allozyme polymorphism in Indian natural populations of this species. Sampling of populations was done in such a way that north and south Indian populations could be compared for their genetic variations, as it is well acknowledged that the north Indian populations experience severe population size fluctuations during acute winter and summer conditions whereas south Indian populations (sampled for the present study) are perennial populations *i.e.*, they do not experience substantial environmental fluctuations throughout the year. In the view of this, we expect that south Indian populations would be less genetically differentiated from each other due to larger size, random mating and higher migration rate whereas, north Indian populations would be genetically more differentiated particularly due to random genetic drift and inbreeding.

MATERIALS AND METHODS

Drosophila Strains

Drosophila ananassae flies were collected from fifteen different eco-geographical localities of India (Fig. 1) by net sweeping method from fruits and vegetable markets. Place of collection, their abbreviation, latitude and time of collection are given in table 1. After bringing the flies to laboratory, naturally impregnated females were cultured in separate food vials to establish isofemale lines. Individual fly from isofemale

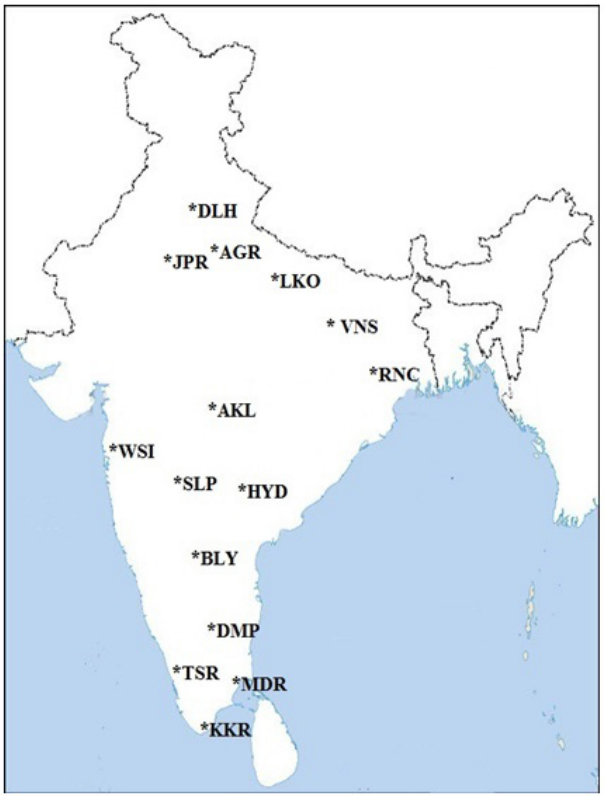

Fig. 1. Map of India showing localities from where *D. ananassae* flies were collected.

lines was used for allozyme analysis. It was tried to utilize maximum number of flies for genetic analysis, emerging in the first generation itself but in some cases, flies derived from second or third generations of isofemale lines were also used. This practice was followed only because it is not possible to analyze all the populations together. Similar methods have also been adopted by earlier workers (Ayala et al. 1974). The isofemale lines were maintained on simple yeast-agar culture medium at 24 ± 1°C with 12 hour cycle of light-dark period.

Native Gel Eletrophoresis

For this purpose, a single fly was homogenized in 50 μl 20 mM Tris buffer (pH 7.4) and the homogenate was centrifuged at 12000 rpm at 4°C for 10 minutes (Kumar and Singh 2013). Supernatant was separated into two aliquots and subjected to 8 percent native polyacrylamide gel electrophoresis in 25mM Tris and 250 mM Glycine electrode buffer (pH 8.2) at 200V for 4 hour at 4°C. In-gel staining for enzymes was done according to Shaw and Prasad (1970) and Ayala et al. (1972). The locus and allele designations were done following the standardized genetic nomenclature for enzyme coding loci (Lakovaara and Saura 1971). Genetic variability of fifteen natural populations was assessed by analyzing seven enzyme systems ACPH (EC. 3.1.3.2 Acid phosphatase), XDH (EC. 1.1.1.204 Xanthine dehydrgenase), APH (EC. 3.1.3.1 Alkaline phosphatase), AO (EC. 1.2.3.1 Aldehyde oxidase), EST (EC. 3.1.1.1 Esterase), MDH (EC. 1.1.1.37 Malate dehydrogenase) and ME (EC. 1.1.1.40 Malic enzyme) corresponding to 12 loci (*Acph1*, *Acph2*, *Xdh*, *Aph2*, *Aph3*, *Ao1*, *Ao2*, *Est2*, *Est3*, *Est4*, *Mdh* and *Me*).

Statistical Analysis

Quantitative data obtained for allozyme frequencies of all fifteen Indian natural populations of *D. ananassae* were utilized to derive genetic variability estimates, *F-statistics* and Nei's genetic identity (Nei 1972). Since, variation in the allelic or genotypic frequencies in different populations lead to population sub-structuring or subdivision, testing various parameters of *F-statistics* become imperative to study genetic differentiation among these populations. Genotype and allele frequencies were estimated using GENEPOP, version 4.2 (Rousset 2008; http://kimura.univ-montp2.fr/~rousset/Genepop.htm). Genetic variability was recorded as mean observed (H_O) and expected (H_E) heterozygosity by using software GenAlEx 6.5 (Peakall and Smouse 2012). Population inbreeding coefficient (F_{IS}) was calculated to deduce the level of inbreeding due to population sub-structuring and also the departure of H_O from Hardy-Weinberg Equilibrium (Hedrick 2005). Population structure analysis was done using traditional *F-statistics* following Wright (1951) by using GenAlEx 6.5 (Peakall and Smouse 2012). Genetic identity (I) approach was also utilized to determine the pattern

Table 1. Geographical localities and time of collection of Indian natural populations of *D. ananassae*

Populations	Abbr.	Latitude (N)	Longitude (E)	Time of collection
Kanniyakumari	KKR	8.08N	77.55E	JAN 2013
Madurai	MDR	9.93N	78.12E	JAN 2013
Thrissur	TSR	10.52N	76.22E	JAN 2013
Dharmapuri	DMP	12.13N	78.17E	JAN 2013
Bellary	BLY	15.15N	76.93E	JAN 2013
Hyderabad	HYD	17.38N	78.47E	JAN 2013
Solapur	SLP	17.68N	75.92E	OCT 2012
Washi	WSI	18.58N	72.49E	OCT 2012
Akola	AKL	20.44N	77.0E	OCT 2012
Ranchi	RNC	23.35N	85.33E	OCT 2011
Varanasi	VNS	25.20N	83.0E	JUL 2012
Lucknow	LKO	26.51N	80.55E	APR 2012
Jaipur	JPR	26.55N	74.49E	AUG 2012
Agra	AGR	27.11N	78.01E	AUG 2012
Delhi	DLH	28.40N	77.13E	AUG 2012

$^{**}P < 0.01$.

of geographic variation among Indian natural populations of *D. ananassae*. It was computed by using GenAlEx 6.5 (Peakall and Smouse 2012) according Nei (1972). To test '*isolation by distance'* effect, the values of genetic distance and geographic distance were correlated. Based on these results, an un-rooted dendrogram has been constructed by UPGMA using genetic similarity index, DendroUPGMA (Garcia-Vallve et al. 1999; http://genomes.urv.cat/UPGMA/).

RESULTS

Genetic variability

Number of individuals analyzed, estimate of genetic variability and inbreeding coefficient of all the 15 natural populations of *D. ananassae* is given in table 2. Observed heterozygosity (H_O) ranges from 0.273 (TSR) to 0.372 (KKR and JPR) with the mean observed heterozygosity of 0.334. This observation shows that these natural populations show nearly 33 per cent heterozygosity. The expected heterozygosity (H_E) ranged from 0.378 (AGR) to 0.473 (DMP) with an average value 0.43. The values of population inbreeding coefficient (F) ranged from 0.042 (VNS) to 0.387 (TSR) with average inbreeding coefficient value 0.228. To know whether these natural populations are in Hardy-Weinberg equilibrium or not, chi-square values were calculated by using values of inbreeding coefficient and number of individuals analyzed (Hedrick 2005). Statistical analysis (chi square) revealed that out of fifteen populations studied, five populations (TSR, BLY, SLP, WSI and AKL) did not show conformity with HWE.

Genetic differentiation and gene flow

As given in table 3, the values of inbreeding coefficient (F_{IS}) across populations were found to be similar at each locus that ranged from 0.172 (*Est5*) to 0.270 (*Acph2*) with an average value of 0.223. The values of fixation index (F_{ST}) across population ranged from 0.031 (*Est2*) to 0.408 (*Ao1*) with average value 0.118. The values of F_{IT}, which is the most inclusive inbreeding coefficient, ranged from 0.23 (*Est2*) to 0.548 (*Ao1*) with an average value of 0.315. This table also depicts estimate of gene flow computed on the basis of F_{ST} values across populations at each locus. It was observed that minimum gene flow across populations occurred at *Ao1* locus and maximum on *Est2* locus and the average gene flow (Nm) value was recorded to be 2.853. Table 4 incorporates pairwise F_{ST} values that can help to know the level of genetic differentiation among these populations. Pairwise F_{ST} values among populations ranged from 0.009 (KKR vs. SLP and MDR vs. SLP) to 0.156 (HYD vs. AGR), showing that Indian populations of *D. ananassae* are not

Table 2. Estimates of genetic variability (observed and expected heterozygosity) and inbreeding coefficients in Indian natural populations of *D. ananassae*

Populations	N	Ho ± SE	He ± SE	F ± SE	χ^2
KKR	48	0.372 ± 0.020	0.470 ± 0.022	0.208 ± 0.022	2.077
MDR	48	0.349 ± 0.020	0.458 ± 0.014	0.239 ± 0.034	2.742
TSR	48	0.273 ± 0.023	0.439 ± 0.018	0.387 ± 0.040	7.189**
DMP	48	0.366 ± 0.020	0.473 ± 0.010	0.229 ± 0.032	2.517
BLY	48	0.323 ± 0.017	0.463 ± 0.010	0.304 ± 0.032	4.436*
HYD	44	0.356 ± 0.039	0.410 ± 0.036	0.204 ± 0.079	1.998
SLP	48	0.292 ± 0.023	0.470 ± 0.009	0.380 ± 0.046	6.931**
WSI	36	0.299 ± 0.019	0.443 ± 0.017	0.326 ± 0.040	5.101*
AKL	34	0.289 ± 0.025	0.440 ± 0.030	0.341 ± 0.036	5.581*
RNC	48	0.358 ± 0.036	0.411 ± 0.040	0.125 ± 0.034	0.750
VNS	47	0.364 ± 0.051	0.379 ± 0.052	0.042 ± 0.017	0.085
LKO	48	0.358 ± 0.033	0.398 ± 0.025	0.121 ± 0.039	0.703
JPR	48	0.372 ± 0.018	0.422 ± 0.022	0.111 ± 0.029	0.591
AGR	48	0.295 ± 0.041	0.378 ± 0.043	0.228 ± 0.054	2.495
DLH	24	0.351 ± 0.025	0.402 ± 0.018	0.135 ± 0.042	0.875
Mean		0.334 ± 0.008	0.430 ± 0.007	0.228 ± 0.013	2.495

*$p < 0.05$, **$p < 0.01$.

homogeneous and exhibit moderate level of genetic differentiation. Higher values of F_{ST} were also recorded with VNS vs. AGR (0.144) and TSR vs. VNS (0.143). Pairwise estimates of Nm values based on F_{ST} value can be computed to envisage the frequency of gene flow between the populations and data in this regard is presented in table 5. Nm values ranged from 1.35 (HYD vs. AGR) to 27.53 (KKR vs. SLP and MDR vs. SLP). Populations like KKR vs. MDR (20.58), KKR vs. DMP (18.98), MDR vs. DMP (18.98) and DMP vs. SLP (14.46) also exhibited higher Nm values indicating that these populations experience more gene flow.

Table 6 shows pairwise genetic identity (I) values among populations. The values of 'I' ranged from 0.746 (HYD vs. AGR) to 0.985 (KKR vs. SLP). The elevated values of 'I' were also observed with KKR vs. MDR, KKR vs. DMP (0.979); MDR vs. DMP (0.976) and LKO vs. DLH (0.974). Populations showing lower values of 'I' were TSR vs. VNS (0.774) and VNS vs. AGR (0.784).

Correlation between geographic distance (Km) and Nei's genetic distance (D) was also calculated for these natural populations of *D. ananassae* and the results show that the two parameters are significantly correlated (Fig. 2; r = 0.418, $p < 0.01$). Unrooted UPGMA dendrogram using Nei's genetic identity showing relationships among fifteen natural populations of *D. ananassae* is given in figure 3. Three distinct population clusters could be seen. Seven populations which make a major cluster due to more genetic identity among them belong to Southern peninsula of India.

Table 3. Estimates of *F-statistics* in Indian natural populations of *D. ananassae*

Locus	F_{IS}	F_{IT}	F_{ST}	Nm
Acph1	0.260	0.325	0.087	2.633
Acph2	0.270	0.343	0.100	2.238
Xdh	0.243	0.277	0.045	5.305
Aph2	0.218	0.298	0.102	2.201
Aph3	0.215	0.303	0.113	1.958
Ao1	0.238	0.548	0.408	0.363
Ao2	0.176	0.299	0.150	1.420
Est2	0.205	0.230	0.031	7.834
Est3	0.227	0.282	0.070	3.305
Est5	0.172	0.284	0.136	1.590
Mdh	0.200	0.257	0.071	3.275
Me	0.252	0.331	0.106	2.119
Mean	0.223	0.315	0.118	2.853

Table 4. Pairwise estimates of F_{ST} values in Indian natural populations of *D. ananassae*

Populations	KKR	MDR	TSR	DMP	BLY	HYD	SLP	WSI	AKL	RNC	VNS	LKO	JPR	AGR	DLH
KKR	0.000														
MDR	0.012	0.000													
TSR	0.041	0.034	0.000												
DMP	0.013	0.013	0.020	0.000											
BLY	0.031	0.032	0.067	0.029	0.000										
HYD	0.060	0.066	0.098	0.076	0.084	0.000									
SLP	0.009	0.009	0.045	0.017	0.021	0.054	0.000								
WSI	0.034	0.050	0.042	0.041	0.073	0.040	0.045	0.000							
AKL	0.035	0.040	0.057	0.028	0.031	0.099	0.024	0.072	0.000						
RNC	0.073	0.084	0.103	0.077	0.075	0.026	0.068	0.048	0.092	0.000					
VNS	0.104	0.123	0.143	0.109	0.106	0.041	0.101	0.070	0.121	0.025	0.000				
LKO	0.080	0.070	0.082	0.059	0.052	0.101	0.052	0.087	0.029	0.097	0.120	0.000			
JPR	0.074	0.090	0.060	0.056	0.062	0.115	0.075	0.060	0.058	0.067	0.092	0.066	0.000		
AGR	0.102	0.105	0.081	0.069	0.077	0.156	0.091	0.101	0.041	0.116	0.144	0.036	0.035	0.000	
DLH	0.090	0.093	0.100	0.076	0.055	0.113	0.069	0.092	0.042	0.097	0.112	0.018	0.049	0.034	0.000

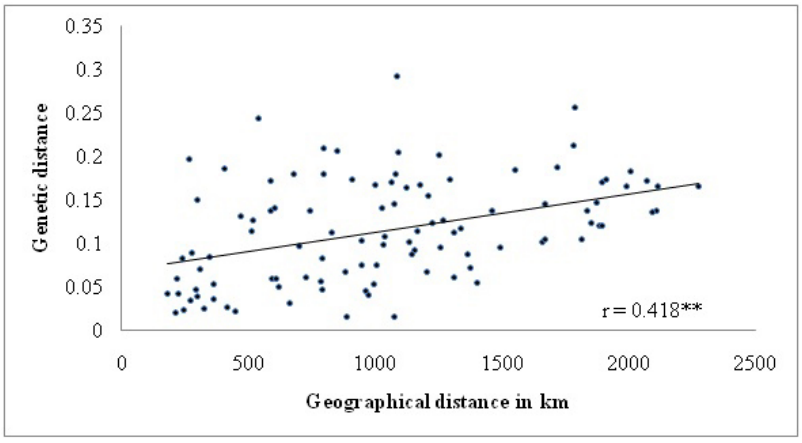

Fig. 2. Correlation between geographic distance (Km) and genetic distance in natural populations of *D. ananassae*.

Table 5. Pairwise estimate of Nm values based on F_{ST} values in Indian natural populations of *D. ananassae*

Populations	KKR	MDR	TSR	DMP	BLY	HYD	SLP	WSI	AKL	RNC	VNS	LKO	JPR	AGR	DLH
KKR	0.00														
MDR	20.58	0.00													
TSR	5.85	7.10	0.00												
DMP	18.98	18.98	12.25	0.00											
BLY	7.81	7.56	3.48	8.37	0.00										
HYD	3.92	3.54	2.30	3.04	2.73	0.00									
SLP	27.53	27.53	5.31	14.46	11.65	4.38	0.00								
WSI	7.10	4.75	5.70	5.85	3.17	6.00	5.31	0.00							
AKL	6.89	6.00	4.14	8.68	7.81	2.28	10.17	3.22	0.00						
RNC	3.17	2.73	2.18	3.00	3.08	9.37	3.43	4.96	2.47	0.00					
VNS	2.15	1.78	1.50	2.04	2.11	5.85	2.23	3.32	1.82	9.75	0.00				
LKO	2.88	3.32	2.80	3.99	4.56	2.23	4.56	2.62	8.37	2.33	1.83	0.00			
JPR	3.13	2.53	3.92	4.21	3.78	1.92	3.08	3.92	4.06	3.48	2.47	3.54	0.00		
AGR	2.20	2.13	2.84	3.37	3.00	1.35	2.50	2.23	5.85	1.91	1.49	6.69	6.89	0.00	
DLH	2.53	2.44	2.25	3.04	4.30	1.96	3.37	2.47	5.70	2.33	1.98	13.64	4.85	7.10	0.00

Table 6. Pairwise estimates of Nei's genetic identity in Indian natural populations of *D. ananassae*

Populations	KKR	MDR	TSR	DMP	BLY	HYD	SLP	WSI	AKL	RNC	VNS	LKO	JPR	AGR	DLH
KKR	1.000														
MDR	0.979	1.000													
TSR	0.931	0.942	1.000												
DMP	0.979	0.976	0.967	1.000											
BLY	0.945	0.942	0.881	0.948	1.000										
HYD	0.906	0.894	0.836	0.871	0.861	1.000									
SLP	0.985	0.984	0.920	0.969	0.961	0.914	1.000								
WSI	0.941	0.911	0.927	0.927	0.870	0.942	0.919	1.000							
AKL	0.946	0.935	0.903	0.955	0.951	0.831	0.964	0.877	1.000						
RNC	0.887	0.864	0.828	0.872	0.881	0.960	0.891	0.931	0.841	1.000					
VNS	0.848	0.808	0.774	0.832	0.841	0.948	0.848	0.910	0.811	0.976	1.000				
LKO	0.871	0.887	0.863	0.904	0.916	0.836	0.917	0.857	0.955	0.842	0.822	1.000			
JPR	0.873	0.844	0.901	0.904	0.894	0.815	0.870	0.901	0.908	0.897	0.872	0.892	1.000		
AGR	0.847	0.841	0.883	0.901	0.888	0.746	0.865	0.844	0.941	0.813	0.784	0.954	0.959	1.000	
DLH	0.848	0.842	0.833	0.871	0.909	0.818	0.884	0.846	0.935	0.845	0.835	0.974	0.920	0.958	1.000

Four populations LKO, DLH, AGR and JPR make one discrete cluster whereas three populations VNS, RNC and HYD make third cluster. WSI showed more genetic differentiation than others and therefore it did not fit with any of the three clusters. Based on the values of F_{ST}, analysis of molecular variance (AMOVA) was calculated and the values are summarized in table 7. This analysis describes the level of genetic variation within individuals (59%), among individuals (26%) and among the populations (15%). The AMOVA test indicates highly significant genetic variation ($p < 0.001$) among the populations verifying the suggestion that the Indian natural populations are genetically structured.

DISCUSSION

The amount of variation within a population is reflective of its flair, which in turn perpetually depends on the environment. When the environment is dynamic, variation is colossal, to render adaptability (ability to adapt) to the population. On the other hand, a stable environment would call for less variation entailing adaptedness (ability to remain adapted) to the population. While there are different ways of determining the variability of a population, the simplest is to measure the level of heterozygosity among the individuals of a population and also among the populations. In the present study, level of observed heterozygosity, taking into account all the loci studied, ranged from 0.273 to 0.372 with an average value of 0.334. This indicates substantial amount of genetic variation in natural populations of *D. ananassae.* However, in every population studied, the observed heterozygosity (H_O) was less than expected heterozygosity (H_E). Significant departure of observed heterozygosity from expected heterozygosity depicts the deviation of a population from Hardy- Weinberg equilibrium. Such deviations, apart from signifying the role of different evolutionary forces also reflect that mating within a population are not altogether random and there is a certain measure of inbreeding. Indeed, Hartl and Clark (2007) held that organisms in the same population often share one or more recent or remote common ancestors, and so mating between organisms in the same sub-population will often be mating between relatives. The populations of *D. ananassae* involved in the present study exhibit slight inbreeding, which is due to presence of less number of observed heterozygotes than their respective expected heterozygotes. Inbreeding coefficient is not only a reliable measure of the level of inbreeding but also speaks of the variability within a population. Values of population inbreeding coefficient range from 0.042 to 0.382, indicating that F_{IS} fall in a wide range. The average inbreeding coefficient value (0.228) describes inbreeding to be reasonable. Added to this, there is reduced gene flow among populations (N_m = 2.853). Thus, the populations of *D. ananassae* have become sub-structured. Reduction in

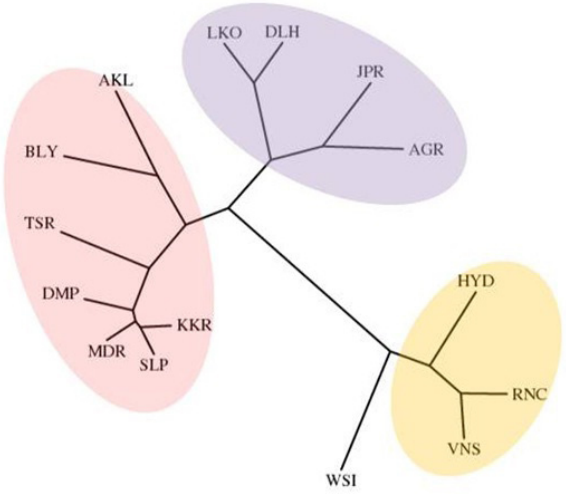

Fig. 3. Unrooted dendrogram of natural populations of *D. ananassae* based on UPGMA clustering of genetic identity values.

Table 7. Summary of AMOVA showing source of variation at different level

Source of Variation	*df*	SS	MS	F	Est. Var.	%
Among Pops.	14	677.438	48.388	25.202**	0.477	15
Among Indiv.	693	2510.13	3.622	1.886*	0.851	26
Within Indiv. (Residual)	708	1359.500	1.920	--	1.920	59
Total	1415	4547.073	--	--	3.248	100

** $p < 0.0001$. * $p < 0.01$.

heterozygosity resulting from population sub-structuring is intimately related to the reduction in heterozygosity caused by negligible migration and inbreeding. Indeed, in earlier studies conducted in this species, this phenomenon was also tested at chromosomal and DNA level and similar results have been reported (Stephan et al. 1998; Das et al. 2004; Singh and Singh 2010).

The above explanation, confirmed that allozymes can turn out to be useful markers in determining differentiation among populations, as they reflect faithfully the amount of variation within a population, the rate of migration and the level of inbreeding. It is clear that at least, at the level of the selected allozymes, there is population sub-structuring. Further, hierarchical *F-statistics*, comparing different parameters among populations also indicate the presence of inbreeding in the natural subpopulations of *D. ananassae*. F_{IS}, the estimate of inbreeding coefficient due to non-random mating in sub-populations, ranged from 0.172 (*Est5*) to 0.270 (*Acph2*), which indicates that in a given subpopulation approximately 17 to 27 percent of individuals experience inbreeding. F_{IT}, the most inclusive measure of inbreeding coefficient, ranged from 0.230 (*Est2*) to 0.548 (*Ao1*) and the mean value was found to be 0.315 which indicates that in the total population (taking all subpopulations together), 31.5 percent of individuals face inbreeding. F_{ST} values which depict the level of differentiation between different pairs of sub-populations ranged between 3 to 40 percent. The size of the sub-populations also have a major influence on the F_{ST} values as it determines the magnitude of random changes in allele frequency (Hartl and Clark 2007). Earlier studies on chromosomal polymorphism in Indian natural populations of *D. ananassae* have shown higher level of genetic differentiation *i.e.* up to 64 percent (Singh and Singh 2010). Whereas, when the present data are compared with genomic sequencing data of Das et al. (2004), there is approximately similar finding (18%). The overall population differentiation was observed by taking mean value of the F_{ST} and it was found to be 0.118. Thus, genetic differentiation is moderate between different subpopulations of India. Pairwise estimates of F_{ST} values indicate that populations situated closer from each other have lower genetic differentiation and populations separated by greater distance have higher values of F_{ST}. This could also be proven by the pairwise estimates of gene flow (Nm), as closer populations are more prone to migrants from nearby populations than the populations situated far apart. Since this cosmopolitan species is commonly found around human habitation, gene flow is possible even among populations separated through long distances and geographical barriers through transportation of fruits and vegetables by man. However, in spite of gene flow (though modest) among different geographic localities, populations have undergone substantial amount of differentiation and form structured populations due to inbreeding.

Pairwise genetic identity (I) values support *'isolation by distance'* effect (Wright 1943), and it is statistically confirmed that genetic distance and geographic distance are significantly correlated in natural populations of *D. ananassae*. The highest value of 'I' was found to be 0.985 for KKR - SLP indicating that these two populations are genetically closest populations. Conversely, genetically most distant populations are HYD- VNS (I = 0.746). Interestingly, though the geographical distance between SLP- MDR (888 km) is immense, much greater than KKR - MDR (213 km), MDR - TSR (219 km), TSR - DMP (272 km) and HYD - SLP (275 km); the two populations are genetically closer to each other, than the other population pairs. Therefore, though geographically closer populations also exhibit genetic closeness, but it is not always absolute. Most distant populations, i.e., KKR vs. VNS (I = 0.848, 1993 km) are genetically closer to each other than TSR - VNS (I = 0.771, 1786 km).

The populations sampled for the present study came from spatially separated geographical localities with varying environmental conditions. There is distinct ecological variation between Southern and Northern part of India. North Indian populations of this species experience severe population size fluctuations two times in a year, *i.e.*, during hot summers and cold winters. Hence, there is more genetic differentiation among the North Indian populations. On the other hand, South Indian populations are perennial, *i.e.*, they do not experience severe population size fluctuations as there is little fluctuation in ecological conditions throughout the year. Therefore, South Indian populations do not experience bottleneck effect, resulting in more genetic homogeneity. Owing to this, three separate population clusters can be identified for these populations. Out of nine South Indian populations, seven fall in one cluster showing similarity in their genetic constitution whereas, remaining six populations of North India form two separate groups showing that these

populations are more genetically diverged.

Acknowledgments: We are thankful to Dr. A. Das, Scientist E, National Institute of Malaria Research, New Delhi, for critically reading this manuscript and appropriate suggestions. Financial support in the form of a major research project to AKS and research fellowship in the form of project fellow to SK by University Grants Commission, New Delhi (India) is thankfully acknowledged. We thank two anonymous reviewers for their very appropriate corrections and suggestions in the manuscript.

REFERENCES

Avise JC. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York.

Ayala FJ, Powell JR, Tracey ML, Mourao CA, Perez-Salas S. 1972. Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *D. willistoni*. Genetics **70:**113-139.

Ayala FJ, Tracey ML, Barr LG, McDonald JF, Perez-Salas S. 1974. Genetic variation in natural population of five *Drosophila* species and the hypothesis of selective neutrality of protein polymorphisms. Genetics **77:**343-384.

Bock IR, Wheeler MR. 1972. The *Drosophila melanogaster* species group. Uni Texas Publ **7213:**1-102.

Das A. 2005. Population genomics and bioinformatic studies reveal evolutionary history of *Drosophila ananassae*. Cur Sci **89:**1316-1321.

Das A, Mohanty S, Stephan W. 2004. Inferring the population structure and demography of *Drosophila ananassae* from multilocus data. Genetics **168:**1975-1985.

Dobzhansky Th. 1970. Genetics of evolutionary process. Columbia University Press, New York.

Eanes WF. 1999. Analysis of selection on enzyme polymorphisms. Ann Rev Eco Syst **30:**301-326.

Garcia-Vallve S, Palau J, Romeu A. 1999. Horizontal gene transfer in glycosyl hydrolases inferred from codon usage in *Escherichia coli* and *Bacillus subtilis*. Mol Biol Evo **9:**1125-1134.

Hartl DL, Clark AG. 2007. Principles of population genetics. Sinauer Associates, Inc. Publishers, Massachusetts.

Hedrick PW. 2005. Genetics of populations. Jones and Barlett publishers, Massachusetts.

Hoffmann AA, Willi Y. 2008. Detecting genetic responses to environmental change. Nat Rev (Genetics) **9:**421-432.

Kreitman M. 1983. Nucleotide polymorphism at the *Alcohal dehydrogenase* locus of *Drosophila melanogaster*. Nature **304:**412-417.

Krishnamoorti K, Singh AK. 2013. *Esterase-4* locus comprises active and null allele in *Drosophila ananassae*. Dros Info Ser **96:**54-55.

Kumar S, Singh AK. 2012. Electrophoretic variants of Xanthine dehydrogenase enzyme in natural populations of *Drosophila ananassae*. Dros Info Ser **95:**18-20.

Kumar S, Singh AK. 2013. Intra-chromosomal association between allozyme loci in *Drosophila ananassae*. Dros Info Ser **96:**52-54.

Kumar S, Singh AK 2014. Complete absence of linkage disequilibrium between enzyme loci in natural populations of *Drosophila ananassae*. Genetika **46:**227-234.

Lakovaara S, Saura A 1971. Genetic variation in natural populations of *Drosophila obscura*. Genetics **69:**377-384.

Mateus RP, Machado LPB, Moraes EM, Sene FM. 2010. Allozyme divergence between border populations of two cryptic species of *D. buzzatii*. Cluster species (Diptera: Drosophilidi). Biochem Syst Ecol **38:**410-415.

Mitton JB. 1994. Approaches to Population Biology. Ann Rev Eco Syst **25:**45-69.

Morton RA, Choudhary M, Cariou M-L, Singh RS. 2004. A reanalysis of protein polymorphism in *Drosophila melanogaster*, *Drosophila simulans*, *Drosophila sechellia* and *Drosophila mauritiana*: Effect of population size and selection. Genetica **120:**101-114.

Nei M. 1972. Genetic distance between populations. Amer Natu **106:**283-292.

Peakall R, Smouse, PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research: an update. Bioinformatics **28:**2537-2539.

Rousset F. 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol Eco Res **8:**103-106.

Schug MD, Smith SG, Tozier-Pearce A, McEvey SF. 2007. The genetic structure of *Drosophila ananassae* populations from Asia, Australia and Samoa. Genetics **175:**1429-1440.

Shaw CR, Prasad R. 1970. Starch Gel electrophoresis of enzymes: A compilation of recipes. Biochem Gen **4:**297-320.

Singh AK, Kumar S, Bhumika. 2013. Random genetic drift affecting Alcohol dehydrogenase polymorphism in laboratory populations of *Drosophila ananassae*. J Sci Res **57:**104-108.

Singh AK, Kumar S, Ratnam D. 2014. Genetic differentiation in natural populations and their mass culture stocks of *Drosophila ananassae*. Thai J Gene **7:**123-132.

Singh BN. 2013. Genetic polymorphisms in *Drosophila*. Curr Sci **105:**461-469.

Singh P, Singh BN. 2010. Population genetics of *Drosophila ananassae*: Evidence for population sub-structuring at the level of inversion polymorphism in Indian natural populations. Inter J Biol **2:**19-25.

Singh RS, Hickey DA, David JR. 1982. Genetic differentiation between geographically distant populations of *Drosophila melanogaster*. Genetics **101:**235-256.

Singh RS, Rhomberg LR. 1987. A comprehensive study of genic variation in natural populations of *Drosophila melanogaster*. II. Estimates of heterozygosity and patterns of geographic differentiation. Genetics **117:**255-271.

Sperlich D, Pinsker W. 1980. Distribution pattern of chromosomal polymorphism in natural populations of *Drosophila*. Att'Asso Genet Ital **25:**47-60

Stephan W. 1989. Molecular genetic variation in the centromeric region of the X-chromosome in three *Drosophila ananassae* populations. II. The *Om(1D)* locus. Mol Biol Evo **6:**624-635.

Stpehan W, Langley CH. 1989. Molecular genetic variation in the centromeric region of the X-chromosome in three *Drosophila ananassae* populations. I. Contrast between the *vermilion* and *forked* loci. Mol Biol Evo **6:**89-99.

Stephan W, Xing L, Kirby DA, Braverman JM. 1998. A test of the background selection hypothesis based on nucleotide data from *Drosophila ananassae*. Proc Nat Acad Sci **95:**5649-5654.

Vogl C, Das A, Beaumont M, Mohanty S, Stephan W. 2003. Population subdivision and molecular sequence variation: theory and analysis of *Drosophila ananassae* data. Genetics **165:**1385-1395.

Wright S. 1943. Isolation by distance. Genetics **28:**114-138.

Wright S. 1951. The genetical structure of populations. Ann Eug **15:**323- 353.