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Population genetics of *Drosophila ananassae*: genetic differentiation among Indian natural populations at the level of inversion polymorphism

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Summary

The present study, which is one of the longest temporal (two decades) and largest spatial (different parts of India covered) investigations on inversion polymorphism in natural populations of *D. ananassae*, was undertaken to understand the dynamics of inversion polymorphism in a broad and comprehensive manner. Forty-five natural populations from different ecogeographic regions of the country (covering the regions from Kashmir to Kanniyakumari and Gujarat to Nagaland) were analysed for chromosomal inversions. All the populations show the presence of the three cosmopolitan inversions, frequencies of which vary among the populations analysed. Simple correlations between frequencies of different inversions and regression analysis of inversion frequencies with latitude, longitude and altitude were insignificant. This reinforces the concept of rigid polymorphism in *D. ananassae*. Genetic divergence (spatial and temporal) at the level of chromosomal polymorphism among natural populations was calculated. Results show spatial divergence but no temporal divergence. Rigid polymorphic systems of *D. ananassae* did not show long-term directional trends. On the basis of the present study, and after including comparisons with the studies conducted more than two decades ago, the most important conclusion to be drawn is that the three cosmopolitan inversions in *D. ananassae* segregate within populations at fairly similar frequencies, and the general geographic pattern has remained constant.

1. Introduction

Chromosomal inversion polymorphisms are one of the best-studied systems in population genetical studies. Chromosomal analyses can be utilized as genetic markers, for which chromosome inversions are considered as alleles, and are used to examine various population genetic parameters (Powell, 1997). Inversions have also been used to study geographical clines, temporal cycles, meiotic drive and natural selection (McAllister, 2002; Ananina *et al*., 2004).

In natural populations of *Drosophila*, chromosomal polymorphism due to inversions is common and is an adaptive trait. Overdominance, frequency dependent selection, or variable selection in time or space can contribute to the adaptive character of chromosomal polymorphism. However, chromosomal polymorphism may be maintained by selection in a heterogeneous environment rather than by overdominance (Da Cunha, 1960; Dobzhansky, 1970; Sperlich & Pfriem, 1986; Krimbas & Powell, 1992; Iriarte & Hasson, 2000; Muntè *et al*., 2005; Kennington *et al*., 2006). The geographically widespread species of *Drosophila* are expected to be chromosomally more polymorphic because they are ecologically versatile (Da Cunha & Dobzhansky, 1954).

Drosophila ananassae, a member of the *ananassae* species complex of the *ananassae* subgroup of the *melanogaster* species group (Bock & Wheeler, 1972), is a cosmopolitan and domestic species. It is largely circumtropical in distribution and shows a high degree of chromosomal polymorphism (Singh, 1996). It occupies a unique status in the genus *Drosophila* due to certain peculiarities in its genetical behaviour (Singh, 2000). *D. ananassae* harbours a large number of inversions in its natural populations. Among these,

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Table 1. *Details of collections of* D. ananassae

Name of locality	State	Time of collection	No. of females analysed
Jammu (JU)	Jammu and Kashmir	Oct 2006	130
Dharamshala (DH)	Himachal Pradesh	Oct 2006	46
Kangra (KG)	Himachal Pradesh	Oct 2006	65
Dehradun (DN)	Uttaranchal	Oct 2005	54
Haridwar (HD)	Uttaranchal	Oct 2005	45
Mansa Devi (MD)	Uttaranchal	Oct 2005	30
Gangtok (GT)	Sikkim	June 2006	34
Lucknow (LK)	Uttar Pradesh	Aug 2005	48
Guwahati (GU)	Assam	June 2006	101
Raidopur (RP)	Uttar Pradesh	Sept 2005	25
Chowk (CW)	Uttar Pradesh	Sept 2005	71
Deemapur (DM)	Nagaland	Sept 2006	211
Shillong (SH)	Meghalaya	June 2006	47
Patna (PN)	Bihar	Oct 2006	211
Allahabad (AB)	Uttar Pradesh	Sept 2005	51
Imphal (IM)	Manipur	Sept 2006	119
Gaya (GY)	Bihar	Oct 2006	79
Ujjain (UJ)	Madhya Pradesh	Nov 2005	30
Bhopal (BP)	Madhya Pradesh	Nov 2005	58
Indore (IN)	Madhya Pradesh	Nov 2005	101
Jamnagar (JM)	Gujarat	Dec 2005	52
Howarah (HW)	West Bengal	June 2005	35
Sealdah (SD)	West Bengal	June 2005	11
Kolkata (KL)	West Bengal	June 2005	61
Rajkot (RJ)	Gujarat	Dec 2005	52
Dwarka (DW)	Gujarat	Dec 2005	90
Ahemdabad (AD)	Gujarat	Dec 2005	21
Paradeep (PA)	Orissa	May 2005	33
Bhubneswar (BN)	Orissa	May 2005	09
Puri (PU)	Orissa	May 2005	16
Shirdi (SI)	Maharashtra	June 2006	103
Nashik (NA)	Maharashtra	June 2006	134
Mumbai (MU)	Maharashtra	Jan 2006	99
Visakhapatnam (VP)	Andhra Pradesh	June 2005	33
Vijaywada (VD)	Andhra Pradesh	June 2005	26
Panaji (PJ)	Goa	Feb 2006	33
Madgaon (MA)	Goa	Feb 2006	78
Gokarna (GK)	Karnataka	Feb 2006	80
Manglore (ML)	Karnataka	Feb 2006	118
Banglore (BL)	Karnataka	Apr 2005	36
Yesvantpur (YS)	Karnataka	Apr 2005	15
Pondicherry (PC)	Tamil Nadu	Apr 2005	21
Ernakulam (ER)	Kerala	Apr 2006	58
Thiruvananthapuram (TR)	Kerala	Apr 2006	54
Kanniyakumari (KR)	Tamil Nadu	Apr 2006	56

only three are cosmopolitan in distribution (Singh, 1998). The population genetics of chromosomal polymorphism in Indian natural populations of *D. ananassae* have been studied (Singh, 1998), showing that there is geographic differentiation of inversion polymorphism in Indian natural populations. In recent years, molecular studies have focused on the effect of population subdivision on genetic variation (Stephan, 1989; Stephan & Langely, 1989; Stephan & Mitchell, 1992; Stephan *et al*., 1998; Aulard *et al*., 2002; Vogl *et al*., 2003; Das *et al*., 2004; Pfeiler *et al*., 2007; Schug *et al*., 2007).

The intention of the present study was to explore the role of natural selection and genetic drift on the degree of inversion polymorphism. A country such as India, with its wide range of diversity in geo-climatic conditions, provides a very good platform for conducting such studies. By combining our new data with those from earlier surveys (done about two decades ago), we have generated a time series that enables us to explore the evolutionary dynamics of inversion polymorphism. Such long time series are rare but nonetheless crucial for studying the evolutionary dynamics of inversion polymorphism.

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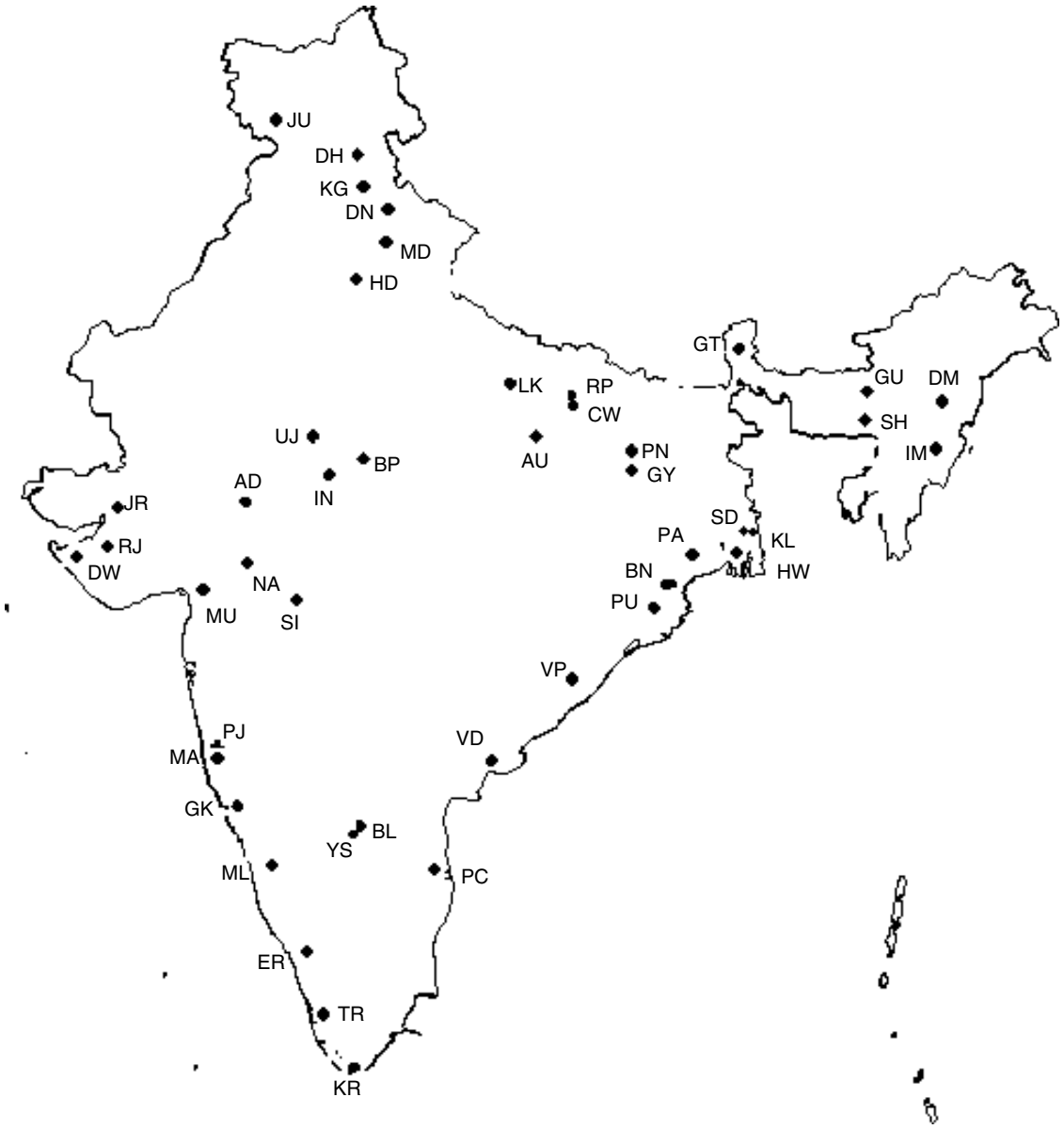

Fig. 1. Map of India showing the localities from where *Drosophila ananassae* flies were collected. JU, Jammu; DH, Dharamshala; KG, Kangra; DN, Dehradun; HD, Haridwar; MD, Mansa Devi; GT, Gangtok; LK, Lucknow; GU, Guwahati; RP, Raidopur; CW, Chowk; DM, Dimapur; SH, Shillong; PN, Patna; AB, Allahabad; IM, Imphal; GY, Gaya; UJ, Ujjain; BP, Bhopal; IN, Indore; JR, Jamnagar; HW, Howarah; SD, Sealdah; KL, Kolkata; RJ, Rajkot; DW, Dwarka; AD, Ahemdabad; PA, Paradeep; BN, Bhubneswar; PU, Puri; SI, Shirdi; NA, Nashik; MU, Mumbai; VP, Visakhapatnam; VD, Vijaywada; PJ, Panaji; MA, Madgaon; GK, Gokarna; ML, Manglore; BL, Banglore; YS, Yeswantpur; PC, Pondicherry; ER, Ernakulam; TR, Thiruvananthapuram; KR, Kanniyakumari.

2. Materials and methods

(i) D. ananassae *populations*

D. ananassae flies were collected from 45 different ecogeographical localities of India ranging from Jammu in the north to Kanniyakumari in the south, and Dwarka in the west to Deemapur in the east (Table 1). Collections of flies were planned in such a way as to include regions with apparent differences in ecogeographical conditions, to determine the effect of geo-climatic factors on the dynamics of inversion polymorphism. For instance, in states on the coastline (all south Indian states, Orissa and West Bengal) collection was done from coastal regions and mainland regions, while for those states with no coastlines, collections were done from different altitudes (North east, Uttaranchal and Himachal Pradesh). For instance, in Haridwar (Uttaranchal) flies were collected from the periphery of the city and also from the Mansa Devi shrine located at a height of 3500 m in the heart of the city. In other locations such as Uttar Pradesh and Madhya Pradesh with no such geographical differences, collection was done from places spatially separated (about 200 km or more) from each

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Table 2. *Frequencies* (*in per cent*) *of three inversions, namely AL* (*2L*), *DE* (*3L*) *and ET* (*3R*), *and the level of inversion heterozygosity in natural populations of* D. ananassae

Population	Latitude (°N)	No.	AL	DE	ET	Mean no. of heterozygous inversions per individual
JU	34·08	260	61·6	16·2	15·4	0·92
DH	32·22	92	59·8	27·2	4·4	0·95
KG	32·10	130	58·5	39·3	3·1	0·87
DN	30·19	108	63·9	39·9	8·4	0·94
HD	29·98	90	48·9	35·6	6·7	0·84
MD	29·58	60	63·4	38·4	16·7	1·10
GT	27·20	68	95·6	14·8	38·3	0·70
LK	26·50	96	69·8	6·3	20·9	0·72
GU	26·17	202	92·6	11·4	36·2	0·78
RP	26·00	50	60·0	8·0	14·0	0·76
CW	26·00	142	49·3	11·3	16·2	0·88
DM	25·92	422	92·7	20·0	27·3	0·81
SH	25·36	94	97·6	20·8	28·1	0·73
PN	25·35	422	96·5	8·8	22·1	0·57
AB	25·28	102	63·8	18·7	14·8	1·07
IM	24·81	238	84·9	27·4	31·9	0·96
GY	24·75	158	96·3	16·5	23·5	0·74
UJ	23·25	158	68·4	35·0	16·7	0·86
BP	23·16	116	67·3	24·2	5·2	0·75
IN	23·05	202	65·9	38·2	13·4	1·17
JR	22·47	104	89·5	26·0	18·3	0·71
HW	22·45	70	75·8	28·6	5·8	0·77
SD	22·43	22	81·9	27·3	18·2	0·18
KL	22·32	122	84·5	31·2	21·4	0·93
RJ	22·30	104	85·6	24·1	19·3	0·88
DW	22·23	180	92·8	19·5	17·3	0·63
AD	22·03	42	95·3	16·7	16·7	0·47
PA	20·55	66	77·3	28·8	25·8	0·75
BN	20·27	18	88·9	38·9	16·7	0·66
PU	19·50	32	84·4	28·2	28·2	0·56
SI	19·45	206	85·5	18·5	6·8	0·58
NA	19·00	268	82·1	16·8	4·2	0·64
MU	18·96	198	84·9	10·7	20·3	0·65
VP	17·42	66	67·0	25·8	19·7	0·78
VD	16·31	52	67·4	46·2	36·6	0·76
PJ	15·25	66	92·5	45·5	15·2	0·81
MA	15·18	156	87·2	35·9	17·4	0·80
GK	14·48	160	91·3	60·0	17·5	0·82
ML	12·85	236	87·9	8·5	7·3	0·72
BL	12·58	72	68·1	45·9	25·0	1·38
YS	12·58	30	60·0	46·7	13·4	1·46
PC	11·93	42	59·6	50·0	31·0	1·85
ER	10·00	116	80·2	61·3	19·9	0·84
TR	8·53	108	85·2	58·4	14·9	0·90
KR	8·07	112	79·5	77·7	26·8	0·82

other. In each case flies were collected from fruit and vegetable markets by the 'net sweeping' method. The geographical positions of the set of 45 localities are shown in Fig. 1.

(ii) *Chromosomal analyses*

To estimate the inversion frequencies, wild females collected from natural populations were cultured individually in food vials and chromosomal analysis of F_1 larvae was done using the lacto-aceto-orcein method. The present quantitative analysis is based on the identification of the karyotypes of only one F_1 larva from each wild female. Breakpoints were determined by comparison with the standard map of polytene chromosomes of *D. ananassae* constructed by Ray-Chaudhuri & Jha (1966).

(iii) *Statistical analyses*

Simple correlations between frequencies of different inversions and correlation and multiple regression of angularly transformed inversion frequencies on latitude, longitude and altitude were analysed.

(iv) *Genetic divergence*

Nei's (1973) gene diversity formulae (H_T, H_S, G_{ST} and D_M) and Nei's (1972) genetic identity (I) were used to evaluate the distribution of genetic diversity within and among populations and also the broad geographic trends in genetic diversity. Genetic distance was also estimated to determine the temporal divergence between the same populations studied two decades earlier and analysed during the present study (Singh, 1984*a*, *b*, 1989*a*, *b*, 1991).

3. Results

(i) *Chromosome inversions*

All the populations showed the presence of the three cosmopolitan inversions, with alpha ranging from 48·9% (Haridwar) to 97·6% (Shillong), delta from 6·3% (Lucknow) to 77·7% (Kanniyakumari), and eta from 3·1% (Kangra) to 38·3% (Gangtok). Data on inversion frequencies are presented in Table 2. In general, inversions are more prevalent in the south and north-eastern parts of India while standard gene arrangements are more common in north Indian populations, thus showing a north–south trend in inversion frequencies. However, populations from the similar ecogeographic regions, i.e. from the same state, show more or less similar trends in inversion frequencies. The same is true for the level of inversion heterozygosity, which ranges from 0·18 in Sealdah to 1·85 in Pondicherry (Table 2). Quantitative data on the frequencies of the three cosmopolitan inversions in Indian natural populations of *D. ananassae* show that there are significant variations in the frequencies of these inversions and the level of inversion heterozygosity among the populations, and that the natural populations are geographically differentiated at the level of inversion polymorphism.

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Table 3. *Pearson correlation coefficients* (*r*) *and regression analysis of inversion frequencies with latitude, longitude and altitude*

	Simple correlation (*r*)			Multiple regression (*b*)			
Inversions	Latitude	Longitude	Altitude	Latitude	Longitude	Altitude	R^2
AL (2L)	−0·237	0·067	−0·296	−0·138	0·378	−0·002	0·097
DE (3L)	−0·004	−0·238	0·031	−1·018	−0·065	0·004	0·323
ET (3R)	−0·039	0·170	−0·144	−0·444	0·687	0·0003	0·441

($P>0·05$).

Table 4. *Pearson correlation coefficients* (*r*) *between frequencies of different inversions*

	AL (2L)	DE (3L)	ET (3R)
AL (2L)		0·014	0·367
DE (3L)			0·042
ET (3R)			

($P>0·05$).

(ii) *Statistical analyses*

For the cosmopolitan inversions, a significant correlation with latitude, longitude and altitude was not found even after multiple regression analysis (Table 3). Correlations between frequencies of different inversions were positive but insignificant (Table 4).

(iii) *Genetic differentiation* (*temporal and spatial*) *at the level of inversion polymorphism*

Genetic distance was estimated to determine the temporal divergence between the same populations studied two decades earlier and analysed during the present study. Time of collection of initial populations (collected and analysed two decades earlier) and same populations (final populations) analysed in the present study along with D values and chi-square (χ^2) values are given in Table 5. Among 12 such populations, D came closer to zero in each comparison, which shows no divergence on the temporal scale. Also, a $2\times$ n χ^2 test to measure differences in karyotypic (2L, 3L and 3R) frequencies was statistically insignificant in all comparisons. Estimates of Nei's gene diversity (Table 6) showed that total gene diversity (H_T) values varied between 0·255 (GY) and 0·506 (JR) with an average of 0·456. Within-population diversity (H_S) values ranged from 0·160 (ML) to 0·461 (PC) with an average of 0·308, while diversity among populations (G_{ST}) ranged from 0·054 (GY) to 0·638 (ML) with an average of 0·333. To determine broad geographic trends in genetic diversity, populations from the same state or province have been grouped together (Table 7). This gives a value for total diversity (H_T) of 0·453, for within-population diversity (H_S) of 0·315) and for among-population diversity (G_{ST}) of 0·311), while the magnitude of absolute gene differentiation (D_M) is 0·220. The analysis showed that 31·1% of genetic differentiation could be attributable to the geographic location of the population. Genetic identity was also calculated among 45 natural populations to determine spatial divergence. Genetic identity values (given as supplementary data in Table S1) range from 0·564 (LK vs GK) to the maximum of 1·0 (DN vs UJ; KL vs SD and UJ vs IN). The $2\times$ n χ^2 values to measure the differences in karyotype frequencies (2L, 3L and 3R) among natural populations were calculated but the χ^2 values and associated probabilities are not given here due to the size of the table. In an overall comparison, it is evident that most statistically significant differences in karyotypic frequencies are found between populations from different ecogeographic regions. However, in most of the comparisons between populations coming from similar regions, differences are statistically not significant. For instance, differences are highly significant ($P<0·001$) for LK vs GK but insignificant differences ($P>0·05$) are found for DN vs UJ; UJ vs IN pairs, etc. The comparisons by calculating $2\times$ n χ^2 values between populations corroborate the results obtained from the I values.

A dendrogram was constructed by UPGMA clustering of I values, among the 45 natural populations (see Fig. S1 in the supplementary material). As shown in the dendrogram, no definite trends could be revealed, barring a few cases where populations that are geographically separated and with entirely different climatic conditions show little genetic similarity, such as LK vs GK; CW vs ER and CW vs MA, etc. Here, LK and CW (in Uttar Pradesh) are inland regions while MA, GK and ER are coastal regions. The maximum similarity between KL and SD is reasonable as these are separated geographically by less than 10 km. In other cases, similarity and dissimilarity among the populations have nothing to do with either

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Table 5. *Values of genetic distance (D) and 2× n χ^2 analysis between populations of* D. ananassae *analysed in the present study and the similar populations analysed previously*

Initial population	Time of collection	Final population	Time of collection	Genetic distance (D)	χ^2*	d.f.
LK[a]	Aug 1982	LK[f]	Aug 2005	0·045	7·710	7
ER[b]	Oct 1983	ER	Apr 2006	0·013	9·590	8
TR	Oct 1983	TR	Apr 2006	0·030	10·260	8
BN[c]	Oct 1984	BN	May 2005	0·051	7·930	8
PU	Oct 1984	PU	May 2005	0·046	10·110	8
MU	Mar 1985	MU	Jan 2006	0·023	13·780	8
PJ	Mar 1985	PJ	Feb 2006	0·019	10·660	8
KL	Oct 1985	KL	June 2005	0·020	15·490	8
JU[d]	Oct 1987	JU	Oct 2006	0·045	13·710	8
GU[e]	Nov 1989	GU	June 2006	0·038	13·970	8
SH	Nov 1989	SH	June 2006	0·030	11·590	7
KR	Nov 1989	KR	Apr 2006	0·044	12·990	8

*$P > 0·05$.
Abbreviations: Refer to localities listed in Table 1.
References: [a]Singh (1984*a*); [b]Singh (1984*b*); [c]Singh (1989*a*); [d]Singh (1989*b*); [e]Singh (1991); [f]present study.

the geographic distances or geo-climatic factors. Also, there is no trend showing a positive relationship between genetic distance and geographic distance. KR shows the least genetic identity with the rest of the populations.

In all the pairwise comparisons, south Indian populations show a high level of genetic identity amongst themselves as well as with north Indian populations and north-eastern populations except PC and BL in some instances. Populations from West Bengal (KL, SD, HW), Gujarat (AD, JR, DW, RJ), Andhra Pradesh (VD, VP), Orissa (PU, PA, BN) and Maharashtra (MU, NA, SI) show higher identity with each other, which could be due to similarity in geo-climatic conditions as most of the populations from these regions were collected from coastal regions and adjoining areas. Surprisingly, ML shows little genetic identity with the KR, ER and TR populations, though these regions are geographically close and lie along the same coast (west coast of India). Bihar populations show a higher genetic identity with north-eastern populations. Thus, broadly all the population pairs from similar states that were collected at the same time show a higher genetic similarity with each other.

4. Discussion

The results of the present study confirm previous findings in a new way because of the total geographical areas covered, the time period spanned and the number of populations analysed from different parts of the country.

(i) *Geographic pattern of inversion frequencies and level of inversion heterozygosity*

Populations from similar geographic regions show similar patterns with respect to the frequency of three cosmopolitan inversions and the level of inversion heterozygosity. This could be due to identical habitat and similar geo-climatic conditions. There are, however, spatial differences, which could be due to inter-habitat differences. When populations were grouped by region, most genetic variation was found among populations between different regions rather than populations within regions. Changes in inversion frequencies in space provide strong evidence that inversion polymorphism is maintained by selection (Mettler *et al.*, 1977; Stalker, 1980; Kennington *et al.*, 2006).

(ii) *Temporal divergence*

In one of the longest temporal studies conducted (nearly two decades), none of the populations showed temporal changes, i.e. there were no long-term directional changes and hence temporal constancy.

(iii) *Spatial* (*geographical*) *divergence*

It is evident from the present analysis (after including estimates of Nei's G_{ST} and I) that Indian natural populations of *D. ananassae* have undergone a considerable degree of genetic divergence at the level of inversion polymorphism. The results presented here indicate spatial changes, i.e. inter-habitat differences

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Table 6. *Nei's gene diversity statistics and population differentiation parameters across 45 Indian natural populations of* D. ananassae

Populations	H_T	H_S	G_{ST}
JU	0·431	0·340	0·211
DH	0·427	0·326	0·236
KG	0·446	0·344	0·228
DN	0·446	0·349	0·217
HD	0·400	0·342	0·145
MD	0·468	0·390	0·166
GT	0·490	0·259	0·471
LK	0·415	0·275	0·337
GU	0·487	0·258	0·470
RP	0·388	0·286	0·262
CW	0·365	0·312	0·145
DM	0·490	0·279	0·430
SH	0·488	0·250	0·487
PN	0·490	0·192	0·608
AB	0·415	0·322	0·224
IM	0·484	0·330	0·318
GY	0·255	0·241	0·054
UJ	0·472	0·380	0·194
BP	0·428	0·294	0·313
IN	0·465	0·376	0·191
JR	0·506	0·284	0·438
HW	0·453	0·279	0·384
SD	0·474	0·312	0·341
KL	0·485	0·329	0·321
RJ	0·479	0·300	0·373
DW	0·288	0·236	0·180
AD	0·478	0·202	0·577
PA	0·480	0·362	0·245
BN	0·481	0·300	0·376
PU	0·484	0·341	0·295
SI	0·454	0·213	0·530
NA	0·441	0·206	0·532
MU	0·463	0·248	0·464
VP	0·459	0·369	0·196
VD	0·488	0·448	0·081
PJ	0·488	0·288	0·409
MA	0·487	0·311	0·361
GK	0·484	0·301	0·378
ML	0·442	0·160	0·638
BL	0·493	0·431	0·125
YS	0·472	0·343	0·273
PC	0·493	0·461	0·064
ER	0·486	0·356	0·267
TR	0·486	0·316	0·349
KR	0·464	0·343	0·260
Mean	0·454	0·308	0·333

Abbreviations: H_T, total diversity; H_S, diversity within populations; G_{ST}, diversity among populations; D_M, absolute population differentiation.

Table 7. *Nei's gene diversity statistics and population differentiation parameters when 45 Indian natural populations of* D. ananassae *were grouped by regions*

State/province	No. of populations	H_T	H_S	G_{ST}	D_M
Jammu and Kashmir	1	0·431	0·340	0·211	–
Himachal Pradesh	2	0·436	0·335	0·231	0·101
Uttaranchal	3	0·438	0·360	0·178	0·117
Uttar Pradesh	4	0·395	0·298	0·245	0·129
North-east	5	0·487	0·275	0·435	0·265
Bihar	2	0·372	0·216	0·419	0·312
West Bengal	3	0·470	0·306	0·348	0·246
Madhya Pradesh	3	0·455	0·350	0·230	0·157
Orissa	3	0·481	0·334	0·305	0·220
Andhra Pradesh	2	0·473	0·408	0·137	0·130
Gujarat	4	0·437	0·255	0·416	0·242
Maharashtra	3	0·452	0·222	0·508	0·345
Goa	2	0·487	0·299	0·386	0·376
Karnataka	4	0·472	0·308	0·347	0·218
Kerala	2	0·486	0·336	0·308	0·300
Tamil Nadu	2	0·478	0·402	0·158	0·152
Mean		0·453	0·315	0·311	0·220

Abbreviations: H_T, total diversity; H_S, diversity within populations; G_{ST}, diversity among populations; D_M, absolute population differentiation.

without temporal changes (inter-decadal differences) in inversion polymorphism of *D. ananassae*. Whereas spatial changes in the present study reflect flexibility, the lack of temporal changes reveals rigidity in the polymorphic system of *D. ananassae* – though, the terms flexible and rigid are not strictly fixed in their meaning (Dobzhansky, 1962).

Studies of molecular genetic variation in Indian natural populations of *D. ananassae* to date with respect to the level of genetic differentiation show (F_{ST} estimates and NJ approach based on F_{ST}) that F_{ST} values of the order of 0·1 (much lower than our G_{ST} estimates) apply to Indian populations (Vogl *et al*., 2003; Das *et al*., 2004; Schug *et al*., 2007). Schug *et al*. (2007) have concluded that adaptive mutations have had a significant influence on molecular variation across broad regions of the genome. Even considering enzyme polymorphism studies, the main conclusion is that populations of *D. ananassae* have a moderate level of genetic variation and appear to be weakly differentiated in spite of their worldwide distribution (see Tobari, 1993, for references). If chromosome data are considered, *D. ananassae* populations typically show a high level of differentiation (see Singh, 1998, for references).Thus, compared with allozymes and molecular markers, the picture of geographic differentiation appears to be different for chromosome rearrangements, which are more variable and more differentiated even over short distances. This could be partly due to the fact that allozymes and molecular markers are in general more 'neutral' than chromosome rearrangements.

The most important conclusion of the two-decade study, after comparing the present work with the

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previous study (Singh, 1984*a*, *b*, 1989*a*, *b*, 1991), is that the three cosmopolitan inversions have continued to segregate within populations at fairly similar frequencies. The general geographic pattern has also remained similar, which could be due to similarity in geo-climatic conditions, hence pointing towards the role of natural selection. It could therefore be said that natural populations of *D. ananassae* are geographically differentiated due to their adaptation to varying environments, and natural selection operates to maintain the three cosmopolitan inversions.

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