



RESEARCH ARTICLE

Genetic structuring of *Drosophila bipectinata* in Indian natural populations based on the distribution of cosmopolitan inversions

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Abstract. Genetic differentiation among different natural populations of a species depends upon the environmental factors and the evolutionary forces that operate on them. In this study, seven Indian natural populations of *D. bipectinata*, two from north and five from south India, have been studied for their chromosomal inversion polymorphism. A total of nine paracentric autosomal inversions were recorded from these seven places but only three of them, present on the 2L, 2R and 3L were found to be cosmopolitan in distribution. In all the populations, the frequency of standard gene arrangement was found to be high than their respective cosmopolitan inversion gene arrangement. The average heterozygosity (H_o) of cosmopolitan inversions increases from north to south. There is a latitudinal cline in the distribution of three cosmopolitan inversion arrangements because their frequency increases with the decreasing latitude, i.e. from north to south India. A comparison of the genetic profile of two north Indian and five south Indian natural populations of *D. bipectinata* reveals the role of natural selection as well as bottleneck effect in the genetic structuring of these populations which may be due to their varying ecological conditions to which they are constantly encountered. Further, the presence of all kinds of paracentric inversions in individual populations was analysed following Poisson distribution to see whether these inversions occur randomly in natural populations or not and the results indicate that north Indian populations show the random occurrence of these inversions than the populations derived from the south.

Keywords. chromosomal polymorphism; cosmopolitan inversions; genetic differentiation; latitudinal clines; *Drosophila bipectinata*.

Introduction

Every population of sexually reproducing species is characterized by substantial variation among its members due to a number of genetic phenomena. Such genetic variation among the members of a population can be studied at their chromosomal, protein and DNA levels by analysing their polymorphic status. Chromosomal polymorphism can be efficiently studied in genus *Drosophila* due to presence of polytene chromosomes in them. Structural changes, particularly paracentric inversions are very common in a number of species of this genus and are known to persist in natural as well as laboratory populations due to their adaptive significance. Population genetical studies by using chromosomal polymorphism in different species of *Drosophila* have been performed by a number of researchers (Bock 1971a; Ranganath and Krishnamurthy 1975; Sperlich and Pfriem 1986; Singh 2001). Certain paracentric chromosome inversions persist as cosmopolitan in the distribution in certain species

of this genus and can be utilized as genetic markers to analyse genetic variability in their different populations.

Taxonomists have recorded more than 155 species of *Drosophila* from the Indian subcontinent, of which, four species of the *D. bipectinata* species complex are much talked due to their recent origin and propagation (Bock 1971b; Bock and Wheeler 1972; Gupta and Panigrahy 1990; Das and Singh 1992; Koop and Barmina 2005; Mishra and Singh 2006). It was Bock (1971b) who initiated working on the distribution, genetic polymorphism and phylogenetic relationships among the four species of the *D. bipectinata* species complex. The four species of this species complex, *D. bipectinata*, *D. parabipectinata*, *D. malerkotliana* and *D. pseudoanassae* are sympatric and morphologically very similar. *D. bipectinata* being an ancestral species to other three has become important for its genetic legacy (Singh and Singh 2001; Mishra and Singh 2006; Banerjee and Singh 2012, 2017). Koop and Barmina (2005) studied mitochondrial and nuclear gene loci to establish

phylogenetic relationships among the four species of this complex and opined that *D. bipectinata* and *D. parabipetinata* are the two most closely related species, whereas *D. malerkotliana* and *D. pseudoananassae* are relatively distantly related.

D. bipectinata is one of the abundantly occurring species in India, particularly, in its southern peninsula. This species did not find enough attention for its genetic uniqueness, although it has been considered primogenitor of the other three species of the *bipectinata* species complex (Tomimura *et al.* 2005; Mishra and Singh 2006; Banerjee and Singh 2017). There are sporadic reports regarding chromosomal polymorphism in natural and laboratory populations of *D. bipectinata*, mainly with regard to the distribution of three commonly occurring inversions, In(D)2L, In(C)2R, and In(H)3L (Banerjee and Singh 1996; Koop and Barmina 2005). In the present study, we analysed inversion polymorphism in *D. bipectinata* to decipher genetic variation among its natural populations, spread in a range of nearly 2900 km. Flies were collected from the northern part of India during August to October months because it is the only appropriate time when this species is substantially seen on the fruit bait or vegetable markets while it can be collected almost in any period of the year from southern parts due to almost perennial environmental condition, especially due to less fluctuation in the surrounding temperature. Based on the frequency of cosmopolitan inversions and the level of heterozygosity in these populations of *D. bipectinata*, an idea of genetic structuring has been conceived.

Materials and methods

D. bipectinata flies were sampled from seven geographic localities falling at the north–south range of India (figure 1). Two populations, MBD and VNS came from the northern part, whereas the remaining five (MDR, RMM, TVM, NGC and KKR) were collected from the southern peninsula of India. Naturally inseminated females were individually placed in food vials to get their larvae for chromosomal analysis. For the preparation of polytene chromosomes, third instar larva randomly selected from a vial was dissected in insect saline (0.67%) under the stereo-binocular to get its salivary glands. The salivary glands were transferred onto a clean glass slide, treated with fixative (aceto-methanol) for about 30 s and then subjected to LWA (lactic acid-1: water-2: acetic acid-3) for about 10 s. The glands were then stained in 2% lacto-aceto-orcein for half an hour. Finally, the stained glands were washed (in 45% acetic acid) and squashed in mountant (lactic acid and 60% acetic acid in 1:1 ratio) by pressing the glands covered with glass cover. The identification of different chromosome arms and inversion breakpoints were done on the basis of polytene chromosome map provided by Panigrahy (1984).

Computational analysis

The number of individuals with a particular karyotype was observed for each locus showing the cosmopolitan distribution of an inversion. Thus, ST/ST, ST/IN and IN/IN were three karyotypes for each polymorphic locus, where ST represented normal gene arrangement and IN showed inversion arrangement. The frequency of ST and IN arrangements for a specific inversion locus was computed based on Hardy–Weinberg (HW) formula. To test HW equilibrium (HWE) for individual locus, the expected frequency of different karyotypes was calculated by considering the frequency of ST and IN of respective gene locus. Based on the observed and expected number of karyotypes, chi square (χ^2) test was performed to see whether there is a significant difference between observation and expectation.

Since, we observed the presence of inversions in every arm, the appearance of rarely occurring heterozygous inversions were also recorded. To test whether the distribution of heterozygous inversions in the larvae is a random event, Poisson distribution method was employed. Inbreeding coefficient (F) that indicates the level of inbreeding is an important population genetical parameter. This can be calculated by the equation $F = H_e - H_o/H_e$, where H_e and H_o are expected and observed heterozygosity respectively. We calculated this parameter by considering all the three cosmopolitan inversion loci and taking average value for each population.

The parameter of genetic identities (I) between different pairs of populations help us to know which pair of populations is genetically closer and this can be calculated by using the formula given by Nei (1972). We adopted this formula to compute the value of 'I' for 21 comparisons.

Results

In the present study, a total of nine paracentric inversions, distributed in different autosomal arms were observed from the seven populations, but only three of them were found to be cosmopolitan in occurrence. The location of all these inversions has been depicted in figure 2, where the asterisk mark specifies cosmopolitan inversions.

The commonly occurring inversions, In(D)2L is subterminal in 2L and occupies nearly 30% area of this arm. In(C)2R is median in 2R which covers ~35% area of this arm and In(H)3L, subterminal in 3L and occupies 25% area of 3L. The other inversions which were spotted in heterozygous forms but had very meager frequency are as follows: (i) In(T)2R, present in MBD and VNS populations. (ii) In(B)2L, present in MBD and VNS populations. (iii) In(M)3R, present in all populations. (iv) In(B)3R, present only in MBD population. (v) In(B)3L, present in all population except RMM. (vi) In(I)3L, present only in MBD populations.

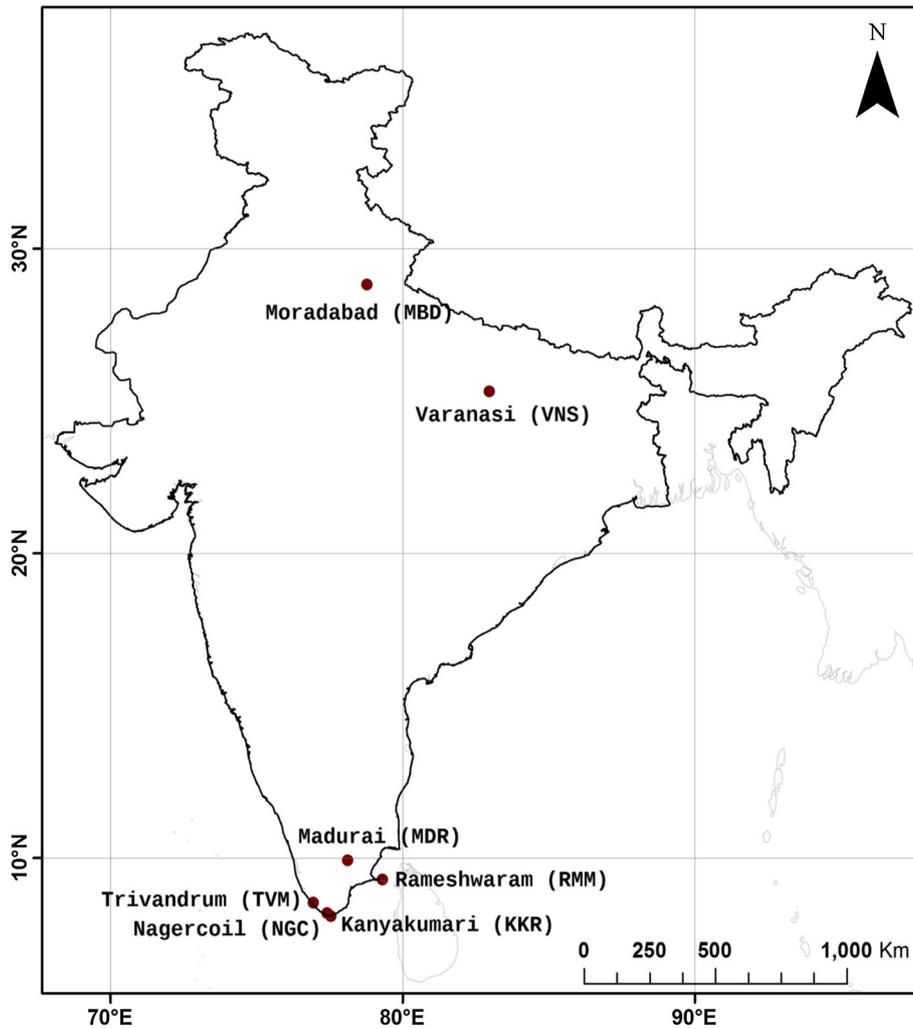


Figure 1. Map of India depicting seven places from where *D. bipectinata* flies were sampled.

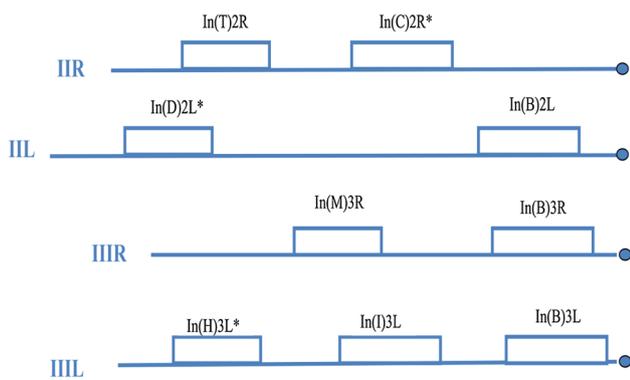


Figure 2. Location of nine paracentric inversions in autosomal chromosome arms of *D. bipectinata*. *Cosmopolitan inversions.

The number of all the three karyotypes of each cosmopolitan inversion was noted in the third instar larvae of all the seven populations. Data regarding the ST (standard gene arrangement) and their respective inversion arrangement for the seven natural populations have been presented in table 1.

The frequencies of ST and inversion types was calculated from the observed number of different inversion karyotypes like ST/ST, ST/In and In/In for the three cosmopolitan inversions loci. There exists a uniform trend for the higher frequency of ST arrangement in the two north Indian populations (MBD and VNS) when compared to the remaining five populations sampled from south Indian localities for all the three inversion loci. Thus, the south Indian populations have higher frequency of cosmopolitan inversions than north Indian populations. HWE analysis revealed that of the 21 cases, only four showed significant deviations from expectation ($P < 0.05$). Thus these natural populations are mainly in HWE.

Data regarding all the three observed and expected karyotypes of In(D)2L locus for the seven natural populations of *D. bipectinata* is presented in figure 3. Although the number of ST/ST karyotypes exceeds other two karyotypes (i.e., ST/In(D)2L and In(D)2L/In(D)2L), however due to higher frequency of heterozygotes in south Indian populations, the frequency of inversion arrangements is maintained at higher

Table 1. Frequencies of ST and their inversion arrangements (In(D)2L, In(C)2R and In(H)3L) in seven Indian natural populations of *D. bipunctinata*. Chi square (χ^2) values have been computed based on HWE analysis.

Inversion	Gene arrangements	Populations						
		MBD	VNS	MDR	RMM Frequency	TVM	NGC	KKR
In(D)2L	ST	0.93	0.92	0.90	0.90	0.88	0.86	0.87
	In(D)2L	0.07	0.08	0.10	0.10	0.12	0.14	0.13
χ^2		3.12	2.05	1.73	7.40**	3.67	9.26**	3.01
In(C) 2R	ST	0.91	0.93	0.87	0.87	0.87	0.89	0.89
	In(C) 2R	0.09	0.07	0.13	0.13	0.13	0.11	0.11
χ^2		1.81	3.34	3.63	0.16	2.61	2.35	0.70
In(H)3L	ST	0.95	0.94	0.92	0.92	0.94	0.93	0.93
	In(H)3L	0.05	0.06	0.08	0.08	0.06	0.07	0.07
χ^2		8.96**	5.50*	0.28	2.13	2.4	1.61	3.36

* $P < 0.05$, ** $P < 0.01$.

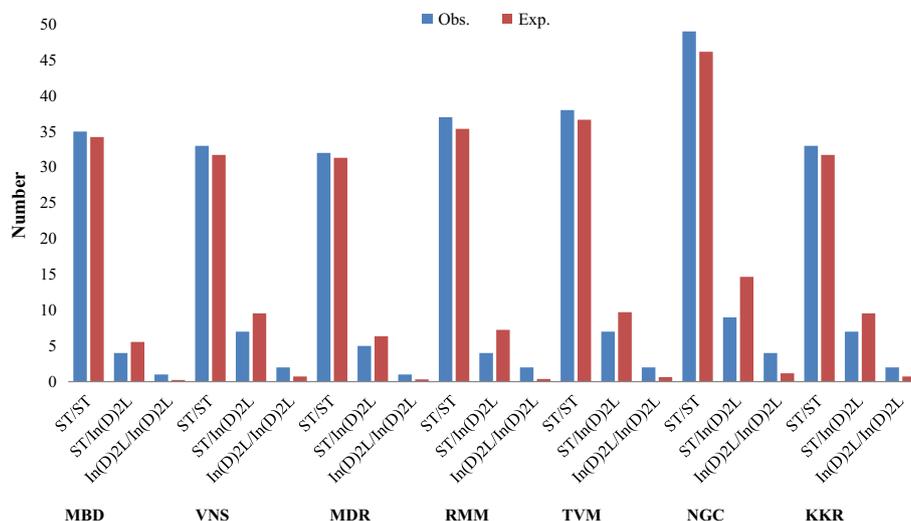


Figure 3. Bar diagram showing observed and expected numbers of different karyotypes in 2L due to ST/In(D)2L inversion in seven natural populations of *D. bipunctinata*.

level in them. The significant difference ($P < 0.01$) between observation and expectation for different karyotypes were found only in two populations, i.e. RMM and NGC. The number of observed and expected karyotypes of In(C)2R inversion locus for the seven populations has been depicted in figure 4. There is an insignificant difference between observation and expectation in all the three karyotypes combination of seven populations, a clear indication that this locus exists in HWE. Figure 5 shows the number of observed and expected karyotypes for In(H)3L inversion in all the natural populations of this species. Of the seven populations analysed, only two i.e., MBD and VNS did not show conformity with HWE indicating that some evolutionary force(s) do influence the karyotypic distribution in these two populations, however, the remaining five populations of south Indian parts showed an insignificant difference between observation and expectation.

Figure 6 presents the bar diagrams delineating frequency of ST and inversion gene arrangements, In(D)2L, In(C)2R, and In(H)3L in seven sampled populations of *D. bipunctinata*. The frequency of ST remained high (more than 0.85) for all the three commonly occurring inversion loci of all the populations, however, inversion gene arrangements are seen to be elevated in five south Indian populations.

Table 2 shows the average observed heterozygosity (H_o), average expected heterozygosity (H_e) and inbreeding coefficient (F) of the populations. The value of H_o ranges from 0.091 to 0.148 being lowest for MBD and highest for MDR. A perusal on H_o values indicate that south Indian populations harbour higher level of heterozygosity than the two north populations. The value of F was found to be highest for MBD (0.311) followed by VNS (0.269) indicating that *D. bipunctinata* flies do remain as a small populations in north India and experience inbreeding than the populations found

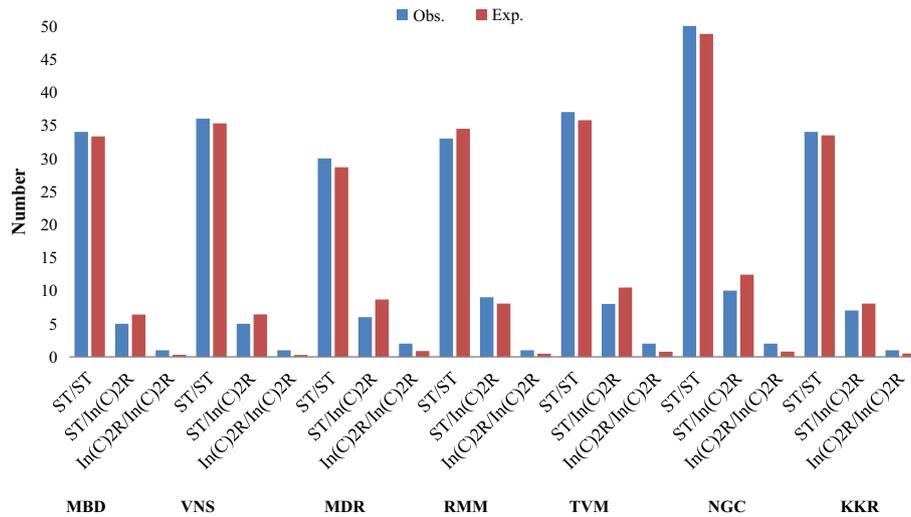


Figure 4. Bar diagram showing observed and expected numbers of different karyotypes in 2R due to In(C)2R inversion in seven natural populations of *D. bipectinata*.

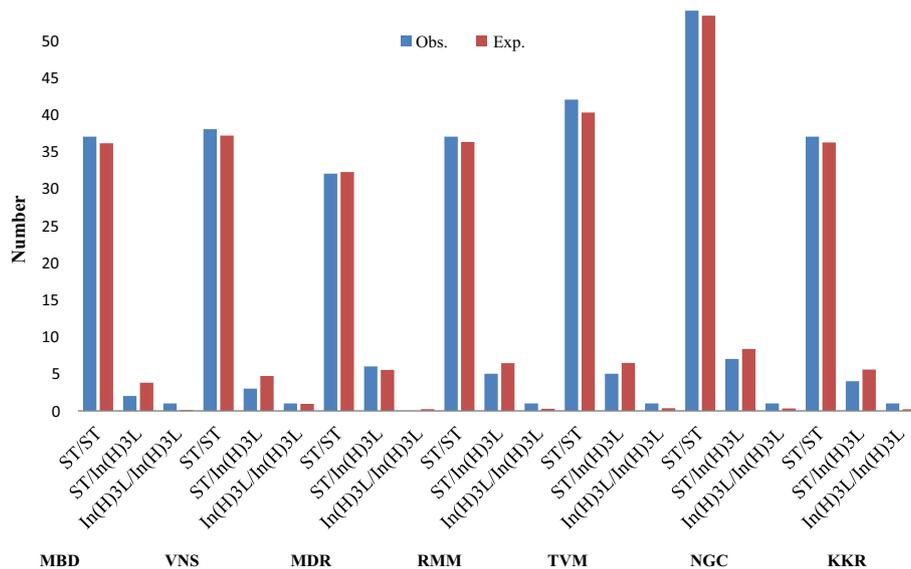


Figure 5. Bar diagram showing observed and expected numbers of different karyotypes in 3L due to In(H)3L inversion in seven natural populations of *D. bipectinata*.

at the south where F values remain comparatively lesser. Figure 7 depicts the values of F , H_e and H_o through a line diagram.

Genetic identity

Genetic identity (I) was calculated by adopting the method published by Nei (1972). This calculation, based on three cosmopolitan inversion loci provided pairwise values of ' I ' for all the 21 comparisons possible from the seven populations (figure 8). The value of I was found to range between 0.9952 and 1. The populations showing the highest level of genetic differences were MDR vs MBD and RMM vs MBD.

A perusal of these comparisons reveal that the majority of south Indian populations have genetic similarities among them. Even higher genetic similarity was recorded between MBD and VNS. Populations like NGC and KKR being located a closest distance of 20 km are genetically very similar and fall in the same group. Remaining three populations, RMM, MDR and TVM fall in one cluster in which more similarity could be found between RMM and MDR.

The dendrogram prepared on the values of genetic identity (I) using UPGMA clustering method is shown in figure 9 that depicts genetic similarity among the natural populations of this species. The south Indian populations make a compact cluster where KKR and NGC, RMM and MDR do not

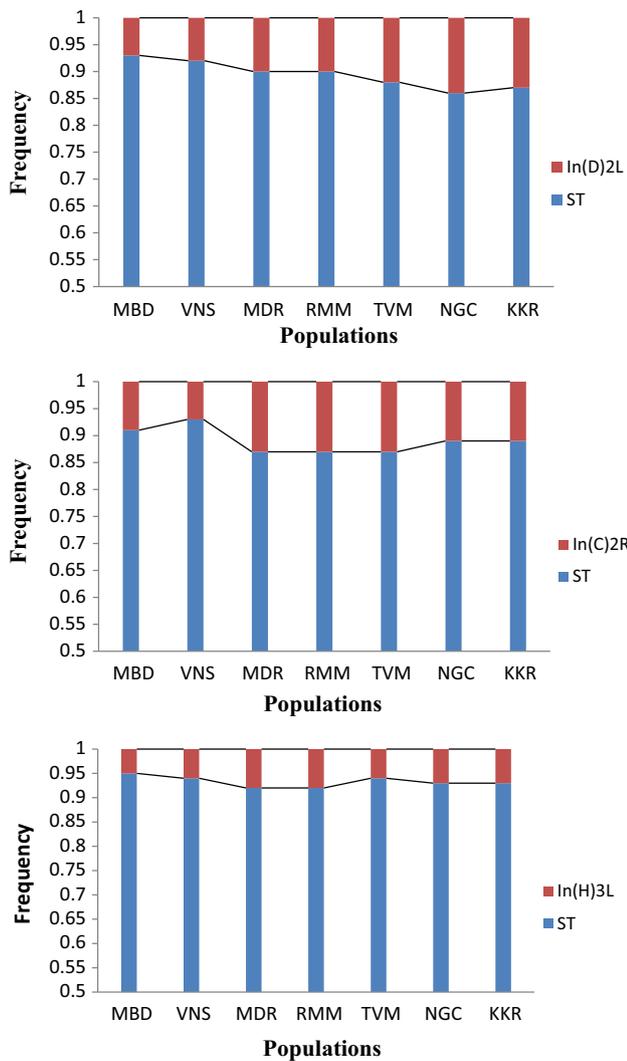


Figure 6. Frequency of ST and inversion gene arrangements for three commonly occurring inversion loci in seven natural populations of *D. bipectinata*.

Table 2. Estimates of average observed and expected heterozygosity and inbreeding coefficient in seven natural populations of *D. bipectinata*.

Population	Avg. H_o	Avg. H_e	F
MBD	0.091±0.022	0.132±0.020	0.311±0.020
VNS	0.095±0.018	0.130±0.010	0.269±0.018
MDR	0.148±0.009	0.186±0.022	0.204±0.019
RMM	0.139±0.035	0.185±0.023	0.248±0.023
TVM	0.142±0.019	0.189±0.029	0.248±0.024
NGC	0.140±0.014	0.190±0.032	0.263±0.025
KKR	0.143±0.024	0.184±0.028	0.223±0.020

show any genetic variation. MDR and TVM are also genetically very close to each other. Similar genetic similarity was expected between VNS and MBD as these are the

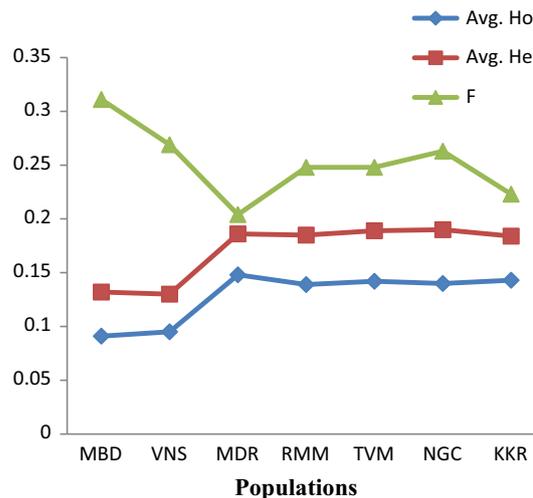


Figure 7. Graphical presentation of average frequency of observed and expected heterozygosity and inbreeding coefficient in seven natural populations of *D. bipectinata*.

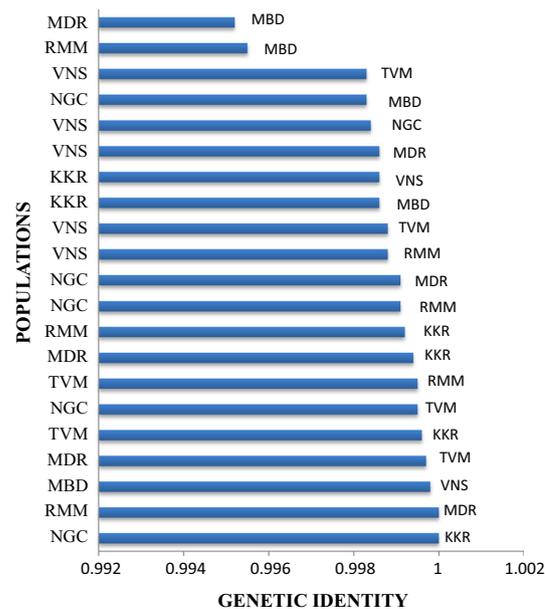


Figure 8. Pairwise comparison of genetic identity (I) in Indian natural populations of *D. bipectinata*.

two north Indian populations that experience similar ecological exposures, however, both VNS and MBD deviate and make an independent identity. This may be due to the acute variations in the temperature during summer and winter seasons, due to which, the dynamics of inversion polymorphisms in these two north Indian populations get influenced independently as a result of random genetic drift. Five south Indian populations, i.e. MDR, RMM, TVM, NGC and KKR form one major cluster due to substantial genetic identity.

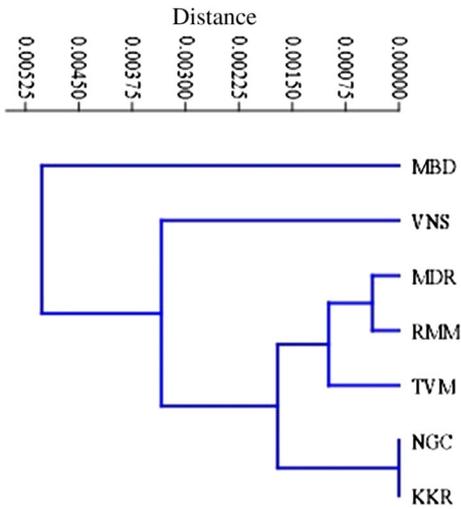


Figure 9. Construction of a dendrogram based on UPGMA clustering showing genetic affinities among the seven natural populations of *D. bipectinata*.

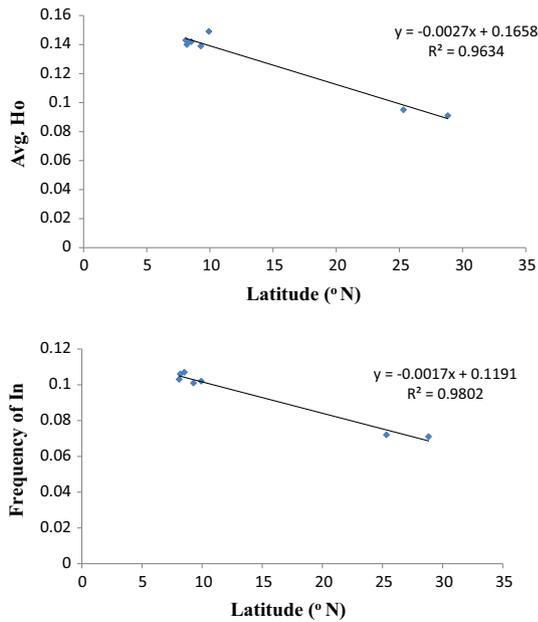


Figure 10. Graphs showing the correlation between observed heterozygosity and frequencies of inversions with latitude in natural populations of *D. bipectinata*.

Correlation

To test relationships between average heterozygosity versus latitude and inversion gene arrangement frequency versus latitude, correlation coefficient (r) was computed. There exists a negative correlation between average heterozygosity and latitude ($r = 0.98$), that is with increasing latitude; there is a decrease in the level of heterozygosity. Likewise, the average frequency of commonly occurring inversion arrangements was found to be increasing with decreasing latitudes ($r = 0.99$), indicating a graded or clinal difference

Table 3. Number of heterozygous inversions present in the larvae of seven different natural populations of *D. bipectinata*.

Population	No. of heterozygous inversions present in the larvae				χ^2
	0	1	2	3	
MBD	21 (21.50)	17 (14.41)	4 (4.83)	1 (1.08)	0.72
VNS	24 (22.60)	12 (14.01)	4 (4.48)	2 (0.96)	1.55
MDR	18 (21.92)	19 (12.05)	1 (3.31)	–	6.31*
RMM	27 (25.80)	13 (13.16)	0 (3.36)	3 (0.57)	13.78*
TVM	24 (28.20)	22 (14.38)	1 (3.67)	–	6.61*
NGC	34 (37.63)	26 (18.81)	1 (4.70)	1 (0.75)	6.09*
KKR	27 (25.99)	12 (12.47)	1 (2.99)	2 (0.47)	6.36*

* $P < 0.05$.

of inversion arrangement along the north–south stretch of India (figure 10).

Distribution of heterozygous inversions in the populations

Since some other inversions were also recorded other than commonly occurring inversions, a frequency distribution pertaining to observed and expected number of heterozygous inversions in all the seven populations of this species was also analysed and the data is presented in table 3. Poisson distribution was done to find out the expected number of individuals bearing 0, 1, 2, or 3 heterozygous inversions. This calculation was performed by using the formula (Gupta 2009) $p = e^{-m} \cdot m^x / x!$. The mean number of heterozygous inversions per individual was found to be 0.48 (KKR) to 0.67 (MBD). Based on the number of observed and expected heterozygotes, chi square analysis was done to see whether the distribution of inversions in the populations is random. The results of this analysis reveal that there is a nonrandom occurrence of heterozygous inversions in south Indian populations of *D. bipectinata*.

Discussion

Chromosome arrangements may be normal or inverted (being reversed 180° at its position) at certain regions of the chromosome. Such inversion locus may therefore be expressed either straight (in case of ST/ST and In/In) or making a loop at its location when heterozygous (ST/In). The frequency of standard gene arrangement and inversion gene arrangement of an inversion locus can be computed

based on the number of three inversion karyotypes in *Drosophila*.

Although several species of *Drosophila* have been subjected to chromosomal polymorphism from all across the globe to test genetic variability in their natural populations, however, *D. bipectinata*, being one of the precursors of *D. bipectinata* species complex has not been focussed considerably for such investigation. Cosmopolitan inversions confer adaptive significance to the species and undergo variation in their distribution, owing to environmental fluctuations (Choi 1977; Aulard *et al.* 2002; Hoffmann *et al.* 2004; Singh and Singh 2007, 2010; Singh 2017, 2019; Kapun and Flat 2019). This study enables us to explain that members of a species settled geographically at long distances do undergo genetic variation and the genetic difference among them may be more obvious if the period of their settlement to such places has a longer time span. *D. bipectinata* is believed to be originated recently in Indian subcontinent and is considered to give rise to other three species of the *bipectinata* species complex (Bock 1971b; Yang *et al.* 1972; Sperlich and Pfriem 1986; Gupta and Panigrahy 1990; Singh and Singh 2001; Matsuda *et al.* 2005; Tomimura *et al.* 2005; Mishra and Singh 2006; Banerjee and Singh 2012, 2017). It has not expanded its territorial distribution to a major area of the world and thus studying its genetic profile becomes an interesting aspect. There is ample evidence that the frequency of ubiquitous inversions change due to geographical differences and temporal variations (Brncic 1972; Knibb *et al.* 1981; Ananina *et al.* 2004; Kennington and Hoffmann 2013).

In the present study, genetic variations have been estimated between north and south Indian natural populations of *D. bipectinata* by considering their inversion polymorphism. Chromosomal polymorphism of commonly occurring (cosmopolitan) inversions was undertaken with the intention that the natural populations of this species occupying two extreme edges of India bear the same genetic constitution or show substantial variation. Two places, Moradabad and Varanasi are separated by a distance of 683 km and come under the northern part of India (between 20°N to 30°N and at 80°E), whereas the remaining five places belong to the southern parts of India. The distance between Varanasi and its nearest south Indian city Madurai from where the collection was done is of 2270 km. The ecological conditions of the north and south India distinctly differ, as north Indian places of sampled flies experience an environmental temperature fluctuation between 5 and 45°C, whereas in the south, such a wide range of temperature variation does not occur and it may range between 20 and 35°C.

Correlation study in the present study on the latitudinal position and average heterozygosity reveals a steep decline in the heterozygosity with increasing latitude. Thus, the south Indian populations have a high frequency of heterozygotes than north Indian populations. This result verifies that the acute temperature fluctuations in north Indian places reduce *D. bipectinata* populations to an extent that the

remaining founder members have lesser heterozygosity and being a small population, they undergo inbreeding, which further reduces the number of heterozygotes in the population. Whereas, populations sampled from south India are coastal populations, except Madurai, and remain to be always large populations due to the least variation in the overall environment. The correlation analysis for the latitudinal position and inversion frequency also reveals a negative correlation between these two parameters, indicating that there is a clinal variation of inversion frequency from north to south. HWE tested for the three cosmopolitan inversion loci; 2L, 2R, and 3L revealed that most of the populations exist in the equilibrium (out of 21 cases, only four showed deviation from HWE).

Although, frequency of ST gene arrangement was found to be distinctly high than inversion arrangement for all the three commonly occurring inversions in all the seven populations, however, there is comparatively high frequency of inversions in south Indian populations. A graded increase in the frequency of In(D)2L can be seen from north to south with little difference between two extreme south populations, i.e. NGC and KKR. An almost similar condition exists with In(C)2R, where a steep increase in its frequency occurs in MDR when compared to VNS and remains static in other south Indian populations. The third commonly occurring inversion, In(H)3L showed marginal variation among the north and south populations, however, the extreme north populations, MBD showed a minimum frequency of this inversion gene arrangement. The narrow range fluctuation of inversion arrangements indicates the role of natural selection acting on these three inversion loci. Had there been laboratory stocks raised with a fewer members of parents, the role of genetic drift could have been seen due to severe changes in the gene arrangements of some of the populations. The effect of genetic drift in shaping genetic endowment of north Indian populations, MBD and VNS, is also obvious as the observed heterozygosity is less in these two populations which face flush and crash situations due to ecological fluctuations. The south Indian populations, however, showed a higher range of heterozygosity (0.139–0.148) giving a reflection that south Indian populations face almost similar ecological conditions throughout the year and experience relatively similar evolutionary events to shape their genetic constitution. One of the studies conducted by considering a dozen allozyme gene loci in *D. ananassae*, reiterated that there is a distinct impact of environmental conditions due to closeness in a geographical area on the genetic profile of its natural populations (Kumar and Singh 2017, 2019).

Earlier researchers who worked on chromosomal polymorphism of this species complex found that *D. bipectinata* possesses comparatively higher polymorphism than other members of this complex (Bock 1971a; Gupta and Panigrahy 1990; Banerjee and Singh 2016, 2017). Our study on a population sampled from a small town located 8 km from Moradabad has revealed very high degree of chromosomal polymorphism in this population of *D. bipectinata* (Singh and Singh 2018) for which ecological condition

of this area was considered to be the main cause as this place is located near to a sugarcane factory whose sludge inundates the larger part of this area. The distribution of this species in and across the Indian subcontinent is an indication of its good adaptability, however, it is found to be very sparsely distributed in the northern parts whereas, in south India, its presence is very enormous. The genetic constitution of this species also shows variation from north and south which could be attributed to distance and ecological condition experienced by the flies sampled from the long stretch of India.

The results of this study help to surmise that natural populations of *D. bipectinata* spread through a distance of nearly 2900 km experience the effect of natural selection in one part, i.e. southern peninsula of India, whereas, it mainly passes through bottleneck effect in its northern part giving rise to distinct genetic variation, particularly among its north and south populations. Based on the results of the distribution of heterozygous inversions in these seven populations, we hypothesize that south Indian populations harbour cosmopolitan as well as rarely occurring inversions nonrandomly due to major effect of natural selection, whereas, their random distribution in the north is a chance event owing to severe environmental fluctuations.

While analysing these populations, besides commonly occurring inversions, those with very little and sporadic presence could also be noted down, however, their homozygous arrangements could not be ascertained. This data helped us to compare with the earlier observation conducted by previous workers. Bock (1971b) identified a total of six paracentric autosomal inversions in the laboratory population of *D. bipectinata* from Borneo, out of which four were distributed on the second chromosome arms and remaining two were present on both arms of the third chromosome. The highest number of chromosome inversions in this species has been reported by Gupta and Panigrahy (1990) who observed nine such inversions in the 2nd and 3rd chromosome arms. In this study also a total of nine inversions have been identified and their distribution in the natural populations has been tested by assuming that they exist randomly. The statistical analysis indicates that they occur randomly in north Indian populations whereas, non-randomly in south populations.

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