Inversion polymorphism and extra bristles in Indian natural populations of *Drosophila ananassae*: joint variation

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Five Indian natural populations of *Drosophila ananassae* were analysed for chromosome inversions and the presence of individuals with extra scutellar bristles in the F₁ progeny of isofemale lines initiated from naturally impregnated females. Three commonly occurring inversions were found in these populations with varying frequencies as was the number of individuals with extra bristles (e.b.). Female individuals were more often found to carry extra scutellar bristles than were males. This result reveals that polygenic loci responsible for the determination of e.b. are widespread in Indian natural populations of *D. ananassae*. A significant positive correlation between the inversion frequency and the number of individuals with e.b. was detected in the isofemale lines of all the five populations. The 2L inversion, alpha, was found to be closely associated with individuals with the e.b. phenotype. The observed results are compared with earlier results obtained for *D. melanogaster*. The association of the alpha inversion with the e.b. phenotype is discussed in relation to chromosomal evolution in the *melanogaster* species group.

Keywords: *Drosophila ananassae*, extra scutellar bristles, inversion polymorphism, sex difference.

Introduction

Inversion polymorphism is clearly a part of the adaptive machinery in natural populations of *Drosophila*. Several species of *Drosophila* have been screened worldwide for this phenomenon and different situations have been found to exist. In India, two cosmopolitan species of *Drosophila*, *D. melanogaster* and *D. ananassae*, have been widely investigated for chromosome inversion polymorphism (Das, 1994 for a review). Although a considerable amount of genotypic variation is generally detected in natural populations, few differences in phenotypic characters are usually observed. It has been suggested that the genetic basis of phenotypic changes is fundamental for understanding the mechanism of evolution in natural and experimental populations (Lande, 1983) and the study of quantitative characters in wild populations could prove to be interesting for evolutionary studies (David *et al.*, 1977).

In *D. melanogaster*, it has been found that the scutellar phenotype is strongly canalized at four macrochaetae (bristles) and that the canalization is regulated by the scute locus (Rendel *et al.*, 1965). Also, it has been determined that the normal number of four scutellar bristles in *D. melanogaster* is often so tightly controlled developmentally that a change in visible phenotype occurs only after a considerable amount of gene substitution (Rendel *et al.*, 1965; Das & Singh, 1992). It also has been estimated that to alter the chaeta number from four to three or five takes about eight times as many genetic changes as to alter the number from three to one (Lee & Parsons, 1968). In natural populations of this species genetic variation for extra scutellar bristle phenotypes has been reported in India (Singh & Das, 1991), Spain (Garcia-Vazquez *et al.*, 1989) and Austria (Izquierdo *et al.*, 1991). In all these studies, chromosome inversions, especially In(3R)C, are found to be positively correlated with the extra bristle phenotypes.

Drosophila ananassae is a cosmopolitan and domestic species which occurs throughout the tropical regions of the world and is found in abundance in the Indian subcontinent. Studies on the population genetics of

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chromosome inversion polymorphism have been undertaken extensively (Singh, 1988 for a review) but less attention has been given to morphometric characters. In this paper, we examine the correlation of two genetic variables (inversions and extra bristles (e.b.)) in isofemale lines of this species in five natural populations collected in ecologically different localities in India.

Materials and methods

Drosophila ananassae flies analysed during the present study come from five different localities in the east coastal region of India: Bhubaneswar (Orissa), Cuttack (Orissa), Ratnagiri (Orissa), Balasore (Orissa) and Howrah (West Bengal). From each place, flies were collected by exposing fermented banana baits in domestic and semidomestic areas. Each wild-caught female was individually placed in a fresh food vial to initiate one isofemale line. From each line, the salivary glands of one F₁ third instar larva were dissected out and stained in lacto-aceto-orcein for polytene chromosome preparation. The breakpoints of the inversions were determined following the standard polytene chromosome map of *D. ananassae* constructed by Ray-Chaudhuri & Jha (1966). Usually, four bristles are present in the scutellum and any additional bristle in this region would be counted as extra. Twenty individuals of each sex were scored in each line for the presence of extra scutellar bristles. For statistical analysis, the percentage data were transformed by arcsine transformation (Snedecor & Cochran, 1967).

Results

Chromosomal analysis revealed the presence of three cosmopolitan inversions identified as alpha in 2L, delta in 3L and eta in 3R (Fig. 1). The frequencies of these inversions in all five populations are shown in Table 1. It is evident that the frequency of these inversions varies among populations, delta being the most frequent (47.18 per cent) in Ratnagiri and the least frequent (24.19 per cent) in Howrah. Based on the frequency of flies with extra bristles, the isofemale lines were classified for each sex in all five populations. The data are presented in Table 2.

Almost all lines were found to have individuals of either sex with e.b., except some Cuttack lines where not a single individual of either sex was found to carry extra bristles (23 of 59 lines). No more than 10 per cent of flies of e.b. phenotype were detected in any isofemale line for either sex. Generally, lines with 0–5 per cent of flies with e.b. were found to be more frequent than those with 5–10 per cent. Furthermore, more

Fig. 1 The autosomes of *Drosophila ananassae* showing the locations of the alpha (AL), delta (DE) and eta (ET) inversions in 2L, 3L and 3R, respectively.

Table 1 Frequencies (percentages) of three common inversions in Indian natural populations of *Drosophila ananassae*

Population	Total no. of chromosomes analysed	Inversions (%)		
		Alpha (2L)	Delta (3L)	Eta (3R)
Bhubaneswar	104	38.46	37.50	38.46
Cuttack	118	25.42	29.66	36.44
Ratnagiri	142	25.36	47.18	35.92
Balasore	66	34.84	28.79	39.39
Howrah	62	30.64	24.19	32.26

lines were found to have females with e.b. (Table 2). All the data were pooled and the distributions of isofemale lines in four categories are given in Table 3 which clearly indicates that the majority of lines have both females and males with e.b. Interestingly, in some lines, individuals of only one sex bearing e.b. were detected, and the lines with only females with e.b. were more frequent than the lines having only males with e.b.

In order to study the relationship between the inversion frequency and the frequency of flies with extra bristles in isofemale lines, the correlation coefficient (r) between these variables was calculated for each population (Table 4). For this computation, the frequencies of all three inversions were summed and the frequencies of e.b. in both sexes were combined in each line. In each population the two variables were found to be positively and significantly correlated (Table 4). This finding clearly indicates that inversions have a positive influence on the occurrence of individuals with e.b. On testing for independence, the alpha inversion was found to be more closely associated with the occurrence of e.b. individuals of both sexes in the populations (Table 5). Interestingly, in all populations, the majority of the lines were found to have both the alpha inversion and individuals with extra bristles and not a single line was detected in any of the populations as having the alpha inversion without e.b. A 2×2 contingency χ^2 test was carried out in all the populations independently and the values show significant results in all populations except Balasore (Table 5).

Table 2 Frequencies (percentages) of isofemale lines classified on the basis of the scutellar phenotype in both sexes of different natural populations of *Drosophila ananassae*

Population	No. of isofemale lines	Isofemale lines (%)					
		Males			Females		
		I	II	III	I	II	III
Bhubaneswar	52	51.92	34.62	13.46	25.00	42.31	32.69
Cuttack	59	77.79	11.86	10.17	61.62	20.34	18.64
Ratnagiri	71	56.34	29.58	14.08	28.17	53.52	18.31
Balasore	33	45.46	33.33	21.21	30.30	39.40	30.30
Howrah	31	41.94	32.26	25.80	25.81	41.93	32.26

I, no flies with extra bristles; II, 0–5 per cent of flies with extra bristles; III, 5–10 per cent of flies with extra bristles.

Table 3 Distribution of isofemale lines with extra bristles in different natural populations of *D. ananassae*

Population	Total no. of isofemale lines	Distribution of lines with e.b.			
		In neither	In both	In males only	In females only
		sex	sexes		
Bhubaneswar	52	0	35	7	10
Cuttack	59	23	21	7	8
Ratnagiri	71	0	59	6	6
Balasore	33	0	27	2	4
Howrah	31	0	28	0	3

Table 4 Correlation coefficients (r) between inversion frequencies (all three inversions combined) and the frequencies of flies with extra bristles (data of both sexes combined) in isofemale lines of different natural populations of *D. ananassae*

Population	r	d.f.	Probability
Bhubaneswar	+0.86	50	<0.001
Cuttack	+0.90	57	<0.001
Ratnagiri	+0.34	69	<0.01
Balasore	+0.42	31	<0.02
Howrah	+0.47	29	<0.01

Thus, the results clearly indicate that the alpha inversion and the occurrence of individuals with e.b. are positively related in natural populations of *D. ananassae*.

Discussion

It has been observed in *D. melanogaster* that the number of macrochaetae (bristles) is canalized (having little or no phenotypic effect) in natural populations and only in experimental conditions could significant changes in bristle number be seen (Singh & Das, 1991; Das & Singh, 1992). It has also been suggested that the phenotypic effect appears only when the threshold of expression that is fixed by the canalization is exceeded (Rendel, 1976; Ghysen & Richelle, 1979). The results obtained in the present study, the first of their kind for *D. ananassae*, clearly indicate that there is considerable intra- and interpopulational variation in the frequencies of three common inversions and the number of individuals carrying extra scutellar bristles. However, only a low proportion of flies of either sex (<10 per cent) was found to have e.b. in the scutellar region and above all the majority of lines had <5 per

Table 5 Association of isofemale lines with respect to the occurrence of the alpha inversion and extra bristles in different isofemale lines of *D. ananassae*

Population	Total no. of isofemale lines		Association of isofemale lines				χ^2 *	d.f.	Probability
			With alpha, with e.b.	Without alpha, with e.b.	With alpha, without e.b.	Without alpha, without e.b.			
Bhubaneswar	52	Obs.	40	8	0	4	10.13	1	<0.005
		Exp.	36.92	11.08	3.08	0.94			
Cuttack	59	Obs.	30	4	0	25	41.42	1	<0.001
		Exp.	17.30	16.70	12.70	12.30			
Ragnagiri	71	Obs.	36	25	0	10	9.73	1	<0.005
		Exp.	30.93	30.07	5.07	4.93			
Balasore	33	Obs.	23	8	0	2	2.01	1	>0.10
		Exp.	21.61	9.39	1.39	0.61			
Howrah	31	Obs.	19	8	0	4	4.61	1	<0.05
		Exp.	16.55	10.45	2.45	1.55			

*After Yates' correction.

cent of flies of the decanalized phenotype with respect to e.b. Thus it can be concluded that polygenes determining e.b. variation are widespread in Indian populations of *D. ananassae* and that phenotypic variation in natural populations of this species has a genetic basis. Similar conclusions have been drawn for Indian *D. melanogaster* both in natural samples (Singh & Das, 1991) and in selection experiments (Das & Singh, 1992).

It seems from the results that the appearance of e.b. in one sex is inversely proportional to its occurrence in the other sex, at least in natural populations of this species. In this initial stage it is difficult to discuss the cause, but certainly this situation deserves further analysis. Although more females were detected with extra dorsocentral and scutellar bristles in several natural populations of Indian *D. melanogaster* (Singh & Das, 1991), such a great difference has not been observed before.

Most importantly, inversion frequency was also found to be positively correlated with the frequency of individuals with extra scutellar bristles in *D. ananassae*. Besides detailed studies on *D. melanogaster* for such an association in different populations worldwide (Garcia-Vazquez *et al.*, 1989; Singh & Das, 1991; Izquierdo *et al.*, 1991) and similar findings in selection experiments (Garcia-Vazquez & Sanchez-Refusta, 1989; Das & Singh, 1992), this is the first report of such an association in another cosmopolitan species of *Drosophila*. In *D. melanogaster* it has been found that significant genetic activity affecting chaeta number is present in all the major chromosomes (Mather, 1941) and polygenes determining e.b. were detected in all the three

major chromosomes (Sheldon & Milton, 1972). Also, Whittle (1969) found an influence of chromosome 3 on the increase in number of extra scutellar bristles, later supported by the localization of polygenes for e.b. on chromosome 3 (Rubio & Albornoz, 1982). Unfortunately, we do not know whether such situations exist or such mechanisms occur in *D. ananassae*. Although much information on the genetics and biology of this species is now available (for details, see Tobari, 1993), our ignorance of the detailed genetics of this species prevents us from drawing any conclusions at this stage. More investigations are needed to solve this problem.

Our knowledge of chromosomal evolution in the *melanogaster* species group from the primitive karyotype to the *ananassae* subgroup (Dobzhansky, 1970) offers the opportunity to discuss an important aspect of the observed results — the close positive association of the alpha inversion and individuals with e.b. Since the third chromosome has been identified as influencing the e.b. character in *D. melanogaster* and the second chromosome of *D. ananassae* is derived from the third chromosome of *D. melanogaster*, the conserved polygenic sequences are thought to be maintained during the whole course of chromosomal evolution in the *melanogaster* species group. Thus it could be assumed that the alpha inversion acts as a complex of polygenes, including factors that favour the expression of e.b. as described for the inversion In (3R) C in *D. melanogaster* (Garcia-Vazquez *et al.*, 1989; Singh & Das, 1991; Izquierdo *et al.*, 1991). It might be true that the polygenes within the alpha inversion are in tight linkage with the inversion locus itself resulting in the observed close association between these two variables. How-

ever, a detailed study involving directional selection experiments for the e.b. character and simultaneous observations of the behaviour of the alpha inversion will provide definite conclusions. This work is currently in progress in our laboratory.

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