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Electrophoretic and Cryptic Genic Variability in Natural Populations of *Zaprionus indianus*

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Eight population samples of *Zaprionus indianus*, collected along 20° latitudinal range, were analysed electrophoretically as well as with heat denaturation technique for allozymic variation of four gene-enzyme systems. Adh^F and $Est\text{-}1^F$ allelic frequencies were found to be positively and significantly correlated with latitude while $\alpha\text{-}Gpdh^S$ and $Est\text{-}2^F$ were negatively correlated with latitude. Widespread cryptic allozymic variation was observed at *Adh*, *Est*-1 and *Est*-2 loci while no additional genic variation could be detected at α-*Gpdh* locus. The geographical differentiation of electrophoretic and cryptic variation at three polymorphic loci concur with nich-width variation hypothesis.

Key Words: Starel gel electrophoresis, Allozymic variants, Cryptic variation, Zaprionus indianus

Introduction

Colonising species populations offer suitable material for microevolutionary studies (Endler 1986). Electromorphs (allozymes) constitute molecular markers which can be genetically interpreted and have been used to characterise the genetic structure of natural populations of diverse taxa (Wills 1981, Spiess 1989). The levels of genetic diversity have been compared in the cosmopolitan and colonising sibling species pair, *D. melanogaster* and *D. simulans* and such studies revealed extensive clinal as well as geographical genetic divergence among *D. melanogaster* populations as compared to genetic uniformity among *D. simulans* populations (Watada et al. 1986, Singh & Rhomberg 1987). Thus, electrophoretic analysis of genetic structure of some colonising species has helped in elucidating the genetic potential for colonisation as well as in understanding biogeographical origin of such species. However, such studies have not been attempted so far on colonising drosophilids of the Indian sub-continent. Thus, it was considered worthwhile to examine electrophoretic and cryptic patterns of allozymic variation in populations of *Z. indianus*.

Materials and Methods

The population samples of *Zaprionus indianus* were bait-trapped from eight latitudinally varying sites (10°N to 32.19°N). Electrophoretic allozymic variants as well as isoelectrophoretic thermostability variants of *Adh*, α-*Gpdh* and *Est* loci were analysed following the standard methods (Harris & Hopkinson 1976, Trippa et al. 1978). Homogenates of single individuals were applied to 12% starch slab gels and were analysed electrophoretically employing 250 V and 30 mA at 4°C for 4 hrs. Each gel was

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sliced into three and slices were stained for *ADH*, *α-GPDH* and EST gene-enzyme systems.

Isoelectrophoretic variants (having similar electrophoretic mobility but differing in thermostability) were screened by following the technique of Trippa et al (1978). Temperature as well as time for the heat treatment were selected on the basis of several preliminary experiments for the three gene-enzyme systems (ADH, α-GPDH and EST). The isoelectrophoretic thermo-resistant (tr) and thermosensitive (ts) variants were examined in the species individuals by heat treating the enzymes *in situ* in the starch gel slices for 12 minutes at 42°C for ADH and at 56°C for 12 minutes in case of esterase and α-GPDH. The electromorph patterns in control as well as treated gel slices were compared so as to identify isoelectrophoretic variants (tr & ts). Genetic control of electromorphs or banding patterns in each species was interpreted from the segregation patterns of electromorphs of parents, and F_1 and F_2 progeny of several species specific crosses. The genetic interpretation of banding patterns and calculation of genetic indices such as allelic frequency, heterozygosity and effective number of alleles were followed from standard sources (Ferguson 1980). The loglikelihood X^2 test (G-test) was used to assess whether the observed genotypes were in agreement with those expected on the basis of Hardy-Weinberg equilibrium (Zar 1984).

Results

Genetic Basis of Enzyme Polymorphism

The electrophoretic phenotypes for three gene-enzyme systems in *Z. indianus* have been represented in figure 1. The two cathodal zones (EST-1 and EST-2) are represented by segregating single-band variants and triple-band patterns. Genetic crosses between individuals having triple-banded and single-band patterns at EST-1 as well as EST-2 produced about equal proportions of offsprings (1:1) with electrophoretic phenotypes like the parents. The genetic crosses between individuals having triple-banded patterns at EST-1 and EST-2 result in 1:2:1 proportions of offsprings with alternating single-band variants and triple-band patterns. The occurrence of hybrid band in heterozygotes indicates that *Est*-1 and *Est*-2 are dimeric in nature. The ADH and α-GPDH enzymes have revealed a single zone of activity and their electrophoretic mobility patterns have been shown in the zymogram (figure 1). The segregating two-banded patterns (of faster or slower mobilities) and three-banded patterns of ADH were observed in the individuals of *Z. indianus*. However, α-GPDH band patterns are represented by two-banded and four-banded patterns..The data on the segregation patterns of ADH and α-GPDH banding patterns in the progeny of species specific genetic crosses are in agreement with monogenic Mendelian inheritance. Thus, the two-banded and

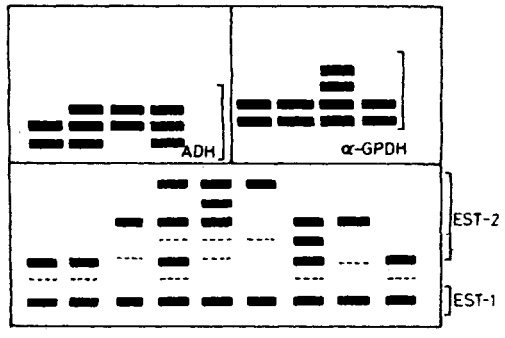

Figure 1 Schematic representation of starch gel electrophoretic patterns for three gene enzyme systems in single individual homogenates of *Z. indianus*. Single band variants and triple-band patterns for EST-1 and EST-2 represent homozygous and heterozygous genotypes, respectively. The occurrence of inter zone hybrid bands between EST-1 and EST-2 has been shown by dotted bands. ADH and α-GPDH show two banded patterns in homozygotes while three banded/four banded patterns represent heterozygotes. Arrow indicates the direction of currnt flow

three-banded/four-banded patterns at the *Adh* and α-*Gpdh* locus represent homozygous and heterozygous individuals respectively.

Populational Genetic Structure

The data on allelic frequencies at four polymorphyic loci have been compared in latitudinally varying geographical populations along the North-South transect of the Indian subcontinent. Most loci have shown diallelic variation patterns represented by three genotypes (FF, SS and FS) while genotypic distribution patterns at *Est*-2 locus are characteristically large due to occurrence of rare alleles. The data on the comparative distribution of allelic frequencies in all the eight natural populations of *Z. indianus* are shown in table 1. The *Est*-2 locus has revealed two common alleles in all the populations but some populations have shown rare alleles. However, two rare alleles were found in Dehradun population and three rare alleles in Rohtak population. α-*Gpdh* locus has revealed one common and one rare allele while *Adh* locus revealed two common alleles in all the wild caught populations. All the population samples have shown high heterozygosity values at most of polymorphic loci. The changes at four loci are significant and indicate regular trends of increase or decrease in allelic frequency in latitudinally varying populations of *Z. indianus* (table 1). Since the North-South transect of the Indian subcontinent represents an array of latitudinally as well as climatically variable habitats, the clinal variation at different polymorphic loci could be adaptively matainied in geographical population of *Z. indianus* from India. The

Table 1 *Data on the distribution of allelic frequencies and application of log-likelihood x^2 test (G-test) for Hardy-Weinberg expectations at four polymorphic loci in eight natural populations of Zaprionus indianus*

Locus	Alleles	Allelic frequency data in populations							
		Ernakulam	Bangalore	Tirumala	Hyderabad	Nagpur	Bhopal	Rohtak	Dehra Dun
Adh	F	.25	.34	.36	.40	.43	.45	.49	.48
	S	.75	.66	.64	.60	.57	.55	.51	.52
	N	52	132	64	88	43	42	95	92
	G-values	0.30	11.04*	0.04	1.87	0.84	0.32	0.01	0.88
α-*Gpdh*	F	0	.03	.04	.06	.04	.03	.12	.15
	S	1.0	.97	.96	.94	.96	.97	.88	.85
	N	60	85	70	80	98	92	68	102
	G-values	0	0.14	0.29	0.69	0.34	0.15	2.47	0.06
Est-1	F	.71	.77	.79	.76	.74	.78	.90	.93
	S	.29	.23	.21	.24	.26	.22	.10	.07
	N	57	55	63	72	96	72	68	86
	G-values	0.63	0.45	2.28	0.47	0.61	0.11	0.14	0.90
Est-2	F'	—	—	—	—	—	—	.02	.03
	F	.61	.57	.63	.67	.64	.66	.48	.49
	M	—	.07	.11	.04	.05	—	.06	.05
	S	.39	.36	.26	.29	.31	.34	.42	.43
	S'	—	—	—	—	—	—	.02	—
	N	57	86	69	76	90	57	113	86
	G-values	0.02	2.07	26.96*	12.55*	10.48*	0.16	37.26*	41.86*

F', F, M, S and S' represent faster, fast, medium, slow and slower electromorphs respectively. N = Sample size.
* Significant at 5% level.

Table 2 *Distribution patterns of allelic frequencies at two polymorphic loci (Est-1 and Est-2) on the basis of standard gel electrophoresis plus post-electrophoretic heat denaturation technique in five population samples of Z. indianus*

Locus	Alleles	Ernakulam		Bangalore		Hyderabad		Rohtak		Dehra Dun	
		tr	ts	tr	ts	tr	ts	tr	ts	tr	ts
Est-1	F	.69	.02	.73	.04	.70	.06	.85	.05	.86	.07
	S	.26	.03	.20	.03	.20	.04	.08	.02	.06	.003
	Total tr & ts	.95	.05	.93	.07	.90	.10	.93	.07	.927	.073
	H & H′	.41	.45	.35	.42	.36	.46	.18	.27	.13	.25
	n_e & n_e'	1.69	1.82	1.54	1.72	1.56	1.85	1.22	1.37	1.15	1.33
	n_e'/n_e	1.08		1.12		1.19		1.12		1.16	
Est-2	F′	—	—	—	—	—	—	—	.02	—	.03
	F	.56	.05	.51	.06	.60	.07	.39	.09	.40	.09
	M	—	—	.07	—	.04	—	.06	—	.045	.005
	S	.34	.05	.32	.04	.22	.07	.34	.08	.33	.10
	S′	—	—	—	—	—	—	—	.02	—	—
	Total tr & ts	.90	.10	.90	.10	.86	.14	.79	.21	.775	.225
	H & H′	.48	.57	.54	.63	.47	.58	.59	.71	.57	.71
	n_e & n_e'	1.92	2.32	2.17	2.70	1.89	2.38	2.44	3.45	2.32	3.45
	n_e'/n_e	1.21		1.24		1.26		1.41		1.49	

F′, F, M, S and S′ represent faster, fast, medium slow and slower electromorphs, respectively. H and n_e = heterozygosity and effective number of alleles on the basis of electrophoresis alone; and H′ & n_e' = heterozygosity and effective number of alleles on the basis of post-electrophoretic heat denaturation technique; n_e'/n_e = increase in effective number of alleles; tr and ts refer to temperature resistant and temperature sensitive variant allozyme respectively.

electrophoretic data revealed that the frequency of Adh^F, α-$Gpdh^F$ and *Est*-1^F correlated positively with the latitude while the allele frequency of *Est*-2^F, was found to be negatively correlated with latitude (table 1).

Heat Stability Polymorphism

The data on distribution of tr and ts allelic frequencies at *Est*-1 and *Est*-2 loci in the five populations of *Z. indianus* are shown in table 2. The application of heat denaturation technique has revealed that the most common electromorphs at *Est*-1 and *Est*-2 loci comprise of tr and ts variants which occur with polymorphic frequencies in all the populations. The cryptic variants have resulted in a significant increase in heterozygosity at such loci. Howevere, the patterns of allelic frequencies of isoelectrophoretic thermostability variants have shown clinal variation at both the loci in all the eight populations of this species (table 2).

The data on the heat stability polymorphism at the polymorphic *Adh* and α-*Gpdh* loci in eight geographical populations from different parts of India have been represented in table 3. The *Adh* locus is represented by one frequent and other less frequent allele on the basis of standard electrophoresis alone. However, the fast *Adh* allele in almost all the natural populations have revealed isoelectrophoretic variants while the frequent slow allele did not reveal any cryptic variation at this locus in all the populations. The patterns of cryptic variability patterns were found to be varying latitudinally in all the populations analysed. The heat stability polymorphism at the *Adh* locus has resulted in 10 to 20 percent increase in the effective number of

Table 3 *Data on the distribution patterns of allelic frequencies at two polymorphic loci (Adh and α-Gpdh) on the basis of standard gel electrophoresis plus post-electrophoretic heat denaturation technique in eight populations of* ***Zaprionus indianus***

Locus	Electromorphs	Ernakulam		Bangalore		Tirumala		Hyderabad		Nagpur		Bhopal		Rohtak		Dehra Dun	
		tr	ts	tr	ts	tr	ts	tr	ts	tr	ts	tr	ts	tr	ts	tr	ts
Adh	F	.25	—	.27	.07	.30	.06	.30	.10	.33	.10	.34	.11	.40	.09	.33	.15
	S	—	.75	—	.66	—	.64	—	.60	—	.57	—	.55	—	.51	—	.52
	Total tr & ts	.25	.75	.27	.73	.30	.70	.30	.70	.33	.67	.34	.66	.40	.60	.33	.67
	H & H′	.37	.37	.45	.49	.46	.50	.48	.54	.49	.56	.49	.57	.50	.57	.50	.60
	n_e & n_e'	1.59	1.59	1.82	1.96	1.85	2.0	1.92	2.17	1.96	2.27	1.96	2.33	2.0	2.33	2.0	2.50
	n_e'/n_e	1.0		1.08		1.08		1.13		1.16		1.19		1.17		1.25	
α-*Gpdh*	F	—	—	.03	—	.04	—	.06	—	.04	—	.03	—	.12	—	.15	—
	S	1.0	—	.97	—	.96	—	.94	—	.96	—	.97	—	.88	—	.85	—
	Total tr & ts	1.0	—	1.0	—	1.0	—	1.0	—	1.0	—	1.0	—	1.0	—	1.0	—
	H & H′	—	—	.06	.06	.08	.08	.11	.11	.08	.08	.06	.06	.21	.21	.25	.25
	n_e & n_e'	1.0	1.0	1.06	1.06	1.08	1.08	1.12	1.12	1.08	1.08	1.06	1.06	1.3	1.3	1.33	1.33
	n_e'/n_e	1.0		1.0		1.0		1.0		1.0		1.0		1.0		1.0	

F and S represent fast and slow electromorphs. H & n_e = heterozygosity and effective number of alleles on the basis of electrophoresis alone; H′ and n_e' = heterozygosity and effective number of alleles on the basis of post electrophoretic heat denaturation technique; n_e'/n_e = increase in effrective number of alleles. tr and ts refer to temperature resistant and temperature sensitive variant allozyme. Populations are arranged in the increasing order of latitude.

alleles at this locus. The present observations revealed genic differentiation with respect to isoelectrophoretic variation at the *Adh* locus (table 3). The present study has revealed the occurrence of clinal patterns of heat stability polymorphism (Cryptic variation) in addition to electrophoretic variability at *Est*-1, *Est*-2 & *Adh* loci in natural populations of *Z. indianus*. However, no additional cryptic genic variation was observed at α-*Gpdh* locus through the heat denaturation technique.

Discussion

The present data on clinal variation at *Est*-1, *Est*-2, *Adh* and α-*Gpdh* loci in Indian populations of *Z. indianus* further support and validate the hypothesis that occurrence of latitudinal clines among geographical populations provide strong evidence of natural selection maintaining such clinal allozymic variation (Anderson & Oakeshott 1984, Singh & Rhomberg 1987). The occurrence of parallel clinal allelic frequencies divergence at *Adh* and *Est* loci in a colonising species such as *Z. indianus* could be due to variable climatic gradient along North-South axis of the Indian subcontinent. The observed latitudinal variation concur with other reports on *Adh* polymorphism from different continental popualtions of *D. melanogaster* (David 1982, David et al. 1989, Oakeshott et al. 1982, Watada et al. 1986).

Different population samples of *Z. indianus* have revealed clinal patterns of tr and ts isoelectrophoretic variants of *Est*-1, *Est*-2 and *Adh* loci. The cryptic isoelectrophoretic variation significantly increased the heterozygosity and effective number of alleles at three out of four polymorphic loci. However, the common occurrence of heat stability polymorphism in all the populations suggest that the natural selection might be responsible for the maintenance of such cryptic genic variation. Temperature constitutes an important component of the environment and empirical data exist on the adaptive correlation of biochemical properties of allozymic (allelic isozymes) variants with habitat temperature in some organisms (Wills 1981). Thus, it can be suggested that in clinally varying heterogeneous environments of the Indian subcontinent, tr and ts variants might confer adaptive advantage to *Z. indianus* individuals which occur in its natural habitat during all seasons of the year and that the species has colonised all along the Indian subcontinent. Present observations on the widespread occurrence of heat stability polymorphism at three loci in *Z. indianus* concur with other reports in *D. melanogaster* (Sampsell 1977, Bewley 1978, Trippa et al. 1978).

Indian populations of *Z. indianus* revealed genetic divergence among geographical populations and this could be due to species specific adaptive capacity for climatic conditions as well as food resources which are characteristics of a broader niche-width species. Thus, the present observations on the extent and patterns of genic divergence in geographical populations of *Z. indianus* are in agreement with niche-width variation hypothesis.

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