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PATTERNS OF ALLOZYMIC VARIATION IN INDIAN NATURAL POPULATIONS OF TWO COLONISING DROSOPHILIDS

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ABSTRACT

Isofemale lines derived from yearly population samples of *Drosophila immigrans* from Hasimara (W.B.) and *Zaprionus indianus* from Dehradun (U.P.) were analysed for allozymic variability patterns through standard starch gel electrophoresis as well as post-electrophoretic heat denaturation techniques. Five polymorphic loci revealed marked constancy in temporal allelic frequency patterns in both the colonising drosophilids. The thermostability isoelectrophoretic variation at the Acph and Est-2 loci in *D. immigrans* and at Est-1 and Adh loci in *Z. indianus* was also found to be characteristically uniform and persistent. Such patterns of allozymic homogeneity could possibly result from natural selection mechanisms.

Key words: Temporal allozymic variation, gel electrophoresis, colonising drosophilids, cryptic variation.

Evolutionary potential of a species is a function of the amount of genetic variation occurring in it. The experimental population genetic studies include analysis of inherited variation and its modulation in time and space [1–4]. Allozymes (genetic variants of enzymes) revealed by gel electrophoretic technique constitute useful markers to examine the role played by microevolutionary processes in modifying the genetic architecture of a species population [5–8]. There is lack of information on genic variation patterns in the Indian natural populations of the various colonising drosophilids. The present paper reports temporal patterns of electrophoretic and cryptic variations in the natural population samples of *D. immigrans* from Hasimara (West Bengal) and of *Z. indianus* from Dehradun.

MATERIALS AND METHODS

Individual flies of *D. immigrans* were bait-trapped from Hasimara (W.B.) in January, 1988 and in January, 1990, and were maintained as isofemale lines. *Z. indianus* individuals were bait-trapped from Dehradun (U.P.). Homogenates of single individuals from the

isofemale lines were applied to 12% starch gels and electrophoresed at 250 V and 30 mA at 4°C for 4 h. Each starch gel slab accommodated 12 samples and the gel slices were stained for acid phosphatase (ACPH), esterase (EST), alcohol dehydrogenase (ADH), octonol dehydrogenase (ODH), aldehyde oxidase (AO), malate dehydrogenase (MDH), and α-glycerophosphate dehydrogenase (α-GPDH) following the methods of Brewer [9], and Harris and Hopkinson [10]. The genetic basis of polymorphic loci was interpreted from the segregation pattern of parents and progenies (F_1, F_2 and BC) of species-specific genetic crosses. The genetic indices were calculated following the standard method [11]. The application of heat denaturation technique [12] involved post-electrophoretic thermal treatment of the enzyme in situ in the starch gel slice (at 52°C for 12 ± 1 min in case of acid phosphatase; at 56°C for15 ± 1 min for esterase; and at 48°C for 15 ± 1 min for alcohol dehydrogenase). The respective control gel slices were pretreated at 37°C. The isoelectrophoretic cryptic variants had similar electrophoretic mobility but differed in their thermostability.

RESULTS AND DISCUSSION

Allozymic variation in D. immigrans. Data on electrophoretic variation for five gene–enzyme systems analysed in the yearly samples of *D. immigrans* are given in Table 1. Each of the two gene–enzyme systems (ADH and α-GPDH) revealed a monomorphic zone of activity represented by nonsegregating two-band phenotypes in all the individuals. However, one polymorphic zone each for ACPH, MDH-1, AO and ODH is represented by the segregating single-band variants (fast or slow) and triple-band patterns. The Est-2 zone was represented by segregating single-band variants (fast or slow) and two-band patterns, while the other five esterase zones were monomorphic. The data on the genetic basis of enzyme phenotypes revealed that the single-band variants (fast or slow) represent allelic variants/allozymes or homozygous genotypes while the 2-band (in case of monomeric enzymes) or 3-band patterns (for dimeric enzymes) indicate heterozygous genotypes. The allelic variants at all the polymorphic loci had codominant expression and the banding patterns were identical in both sexes.

The data on distribution patterns of genotypes, allele frequencies, observed and expected heterozygosity, and G-values of the log-likelihood χ^2 test for Hardy–Weinberg equilibrium for the five polymorphic loci are given in Table 1. The patterns of genic variation include occurrence of a common allele and few rare alleles at Acph locus while four loci (Est-2, Mdh-1, Ao and Odh) are represented by two frequent alleles. Thus, the range of heterozygosities observed at various polymorphic loci correlates well with the number of alleles and their frequencies. The patterns of allele frequencies at various loci (samples collected in 1990) have been found to be very similar to those of the population sampled in 1988. The yearly data on allele frequency patterns revealed no deviation on the basis of

Table 1. Data on temporal distribution patterns of allelic frequencies, observed and expected heterozygosity and G-values for log-likelihood χ^2 test for fit to Hardy–Weinberg equilibrium in yearly population samples of *Drosophila immigrans* from Hasimara (W. B.) and *Zaprionus indianus* from Dehradun (U.P.)

D. immigrans								*Z. indianus*							
locus	year	allelic frequency			N	het. obs./exp.	G value	locus	year	allelic frequency			N	het. obs./exp.	G value
		F′	F	S						F	S	**			
Acph	1988	0.04	0.03	0.93	111	0.15/0.13	0.93	Acph-1	1989	0.47	0.48	0.05	82	0.66/0.55	21.33*
	1990	0.035	0.075	0.89	102	0.21/0.20	2.68		1990	0.49	0.46	0.05	108	0.59/0.55	27.62*
		F		S						F		S			
Est-2	1988	0.72		0.28	115	0.36/0.40	0.92	Acph-2	1989	0.80		0.20	66	0.27/0.32	1.16
	1990	0.67		0.33	88	0.30/0.44	9.46*		1990	0.78		0.22	102	0.23/0.34	10.72*
Mdh-1	1988	0.18		0.82	102	0.31/0.30	4.66*	α-Gpdh	1989	0.13		0.87	116	0.16/0.23	8.45*
	1990	0.16		0.84	95	0.26/0.27	0.14		1990	0.15		0.85	102	0.25/0.25	0.06
Ao	1988	0.59		0.41	119	0.57/0.49	3.90*	Adh	1989	0.51		0.49	80	0.47/0.50	0.20
	1990	0.61		0.39	87	0.44/0.48	0.61		1990	0.49		0.51	95	0.49/0.50	0.01
Odh	1988	0.75		0.25	107	0.41/0.38	0.90	Mdh-1	1989	0.83		0.17	160	0.24/0.28	3.36
	1990	0.80		0.20	84	0.32/0.32	0.40		1990	0.84		0.16	107	0.28/0.27	0.28

*Significant at 5% level; other G-values are nonsignificant.

**Cumulative gene frequency of three rare alleles (F′ = 0.02, M = 0.01 and S′ = 0.02).

F′, F, M, S & S′ represent faster, fast, medium, slow and slower electromorphs, respectively.

N—sample size.

Students' t test. The yearly population samples revealed homogeneity patterns in allele frequencies and heterozygosity (Table 1). The yearly population samples revealed deficiency of heterozygotes at the Est-2 locus and excess of heterozygotes at Ao locus. However, three loci (Est-2, Mdh-1 and Ao) revealed significant deviations from the Hardy–Weinberg equilibrium.

Allozymic variations in Z. indianus. The polymorphic zones of ACPH-1, ACPH-2 and MDH-1 are represented by segregation, single-band variants, and triple-band patterns. The genetic basis of species-specific enzyme banding patterns was interpreted from the segregation ratios of electrophoretic phenotypes of the parents and progeny of the species-specific genetic crosses. Genetic crosses between individuals having triple-banded patterns (ACPH-1, ACPH-2, MDH-1) revealed 1:2:1 ratio of alternating single-banded and triple-band patterns; and are thus in agreement with monogenic Mendelian inheritance. However, the segregating two-band patterns (of faster or slower mobilities) and three-band patterns of ADH were observed in the individuals of *Z. indianus*. Genetic crosses involving segregating two-band patterns resulted in three-band patterns in F_1 individuals and 1:2:1 ratio of segregating two- and three-band patterns in F_2 progeny. The ADH electrophoretic data of the parents and progeny of genetic crosses were in agreement with the monogenic control of ADH patterns. The α-GPDH banding patterns are represented by two- and four-band patterns. The data on the segregation patterns of α-GPDH banding patterns in the progeny of species-specific crosses are in agreement with monogenic Mendelian inheritance. The present observations on ADH and α-GPDH electrophoretic phenotypes concur with earlier reports in *D. melanogaster* that in NAD requiring dehydrogenases, more than one electromorphs (conformational isozymes) may arise due to post-translational differential binding of coenzyme NAD.

The patterns of distribution of allele frequencies include the occurrence of two common alleles and two rare alleles at the Acph-1 locus, two common alleles at Adh locus, and one more and one less frequent alleles at the other three loci (Acph-2, α-Gpdh, Mdh-1). The electrophoretic analysis at such five polymorphic loci in natural population samples taken in two consecutive years from two different sites have revealed persistence of common, less frequent, as well as rare alleles in the natural populations sampled. However, the temporally analysed population samples for two loci (Acph-2 and α-Gpdh) showed differential patterns in terms of deviation from the Hardy–Weinberg expectations (Table 2). Thus, the yearly population samples of *Z. indianus* have revealed patterns of genetic homogeneity.

The data on the distribution of the thermoresistant (tr) and thermosensitive (ts) alleles, allele frequencies, heterozygosity, and effective number of alleles (n_e') at two polymorphic loci in the yearly population samples of *D. immigrans* as well as *Z. indianus* are presented in Table 2. The single frequent allele at the Acph and two frequent alleles at Est-2 loci have revealed two isoelectrophoretic variants (tr and ts) which occur with polymorphic

frequencies in the yearly population samples. The occurrence of cryptic variation resulted in significant increase in heterozygosity as well as effective number of alleles (Table 2). However, there is no difference in the distribution patterns of tr and ts alleles in the yearly population samples.

Different population samples of *D. immigrans* and *Z. indianus* have depicted homogeneous patterns of distribution of the thermoresistant (tr) and thermosensitive (ts) isoelectrophoretic variants for common alleles at the polymorphic loci. The cryptic isoelectrophoretic variation has not changed the overall pattern of allelic variation at such loci. The common occurrence of heat stability polymorphism in the yearly populations suggests that natural selection may be responsible for maintenance of such cryptic genic variation. Temperature constitutes an important component of the environment, and empirical data show adaptive correlation between the bio-chemical properties of allozymic (allelic isozymes) variants and the habitat temperature in some organisms [18]. Thus, it can be suggested that in heterogeneous environments, the tr and ts variants of

Table 2. Data on temporal patterns of cryptic/isoelectrophoretic thermostability polymorphism on the basis of postelectrophoretic heat denaturation test at two polymorphic loci in yearly population samples of *D. immigrans* from Hasimara (W.B.) and *Z. indianus* from Dehradun (U.P.)

Parameter	*D. immigrans*								*Z. indianus*							
	ACPH				EST-2				EST-1				ADH			
	1988		1990		1988		1990		1989		1990		1989		1990	
	tr	ts	tr	ts	tr	ts	tr	ts	tr	ts	tr	ts	tr	ts	tr	ts
F′	—	0.04	—	0.035	—	—	—	—	—	—	—	—	—	—	—	—
F	—	0.03	—	0.75	0.54	0.17	0.52	0.15	0.85	0.05	0.86	0.07	0.40	0.09	0.33	0.15
S	0.70	0.23	0.67	0.22	0.22	0.07	0.25	0.08	0.08	0.02	0.067	0.003	—	0.51	—	0.52
Total freq.	0.70	0.30	0.67	0.33	0.76	0.24	0.77	0.23	0.93	0.07	0.927	0.073	0.40	0.60	0.33	0.67
H & H′	0.13	0.45	0.20	0.49	0.40	0.63	0.44	0.64	0.18	0.27	0.13	0.25	0.50	0.57	0.50	0.60
n_e & né	1.15	1.83	1.24	1.96	1.66	2.70	1.79	2.77	1.22	1.37	1.15	1.33	2.0	2.33	2.0	2.50
né/n_e		1.59		1.58		1.62		1.55		1.12		1.16		1.17		1.25

H and né—heterozygosity and effective number of alleles on the basis of electrophoresis alone; H′ and ne′—heterozygosity and effective number of alleles on the basis of postelectrophoretic heat denaturation technique; né/n_e—increase in effective number of alleles. tr and ts refer to temperature resistance and temperature sensitive allozyme variants.

acid phosphatases, esterases, and alcohol dehydrogenases may confer adaptive advantage to the individuals of a population which occur in its natural habitat during all seasons of the year.

The present investigation has revealed temporal stability of allelic frequencies at five polymorphic loci in *D. immigrans* and *Z. indianus* populations. Our results agree with the earlier reports on temporal constancy at phosphoglucomutase (Pgm) locus in *D. melanogaster* [13–15]. The present observations on the temporal similarity of thermostability variants at the Acph and Est-2 loci in *D. immigrans* and at Est-1 and Adh loci in *Z. indianus* are in agreement with the earlier reports on persistence of cryptic variation for alcohol dehydrogenase (ADH) in *D. melanogaster* [16]. On the contrary, temporal changes in allele frequency have also been reported in higher vertebrates, such as, birds which are characterized by overlapping generations [17]. The mechanisms of maintenance of genetic polymorphism are currently being argued on the basis of "Selectionist" and "Neutralist" hypotheses [18]. According to selectionists, occurrence of uniformity may be taken as an evidence of operation of some kind of natural selection at the polymorphic loci while the neutralists predict random genetic differences. The results of our study reveal homogeneous genic variability patterns (electrophoretic and cryptic) at the polymorphic loci in yearly populations of *D. immigrans* as well as *Z. indianus*. This may be attributed to the action of some forces of natural selection.

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