tetes sp.; 6. *Waagenoconcha* sp.

The faunal assemblage indicates late Lower Permian (Artinskian) age for the Upper Member, favouring a quiet water marine environment. The lithological character of this Member suggests a reducing environment facilitating the formation of carbonaceous shale and pyrite. A partial or semi-isolation of the basin such as that found in the lagoonal environment could deposit coarser clastics during higher energy and fine clays at tranquility. The rocks of the Middle Member represent deposit of an unstable shelf environment characterized by extensive slumping while at the time of deposition of the Lower Member, the basin must have remained very shallow as indicated by sedimentary structures. Bioturbation, burrows and clastic dykes occur frequently in the lower and middle members.

The present find has established a chain of Permian horizons all along the foothills from Arunachal Pradesh in the east to Jammu and Kashmir in the west. This find may help in revision of chronostratigraphy of Bhutan Lesser Himalaya as these rocks (Seti Khola Formation) occur immediately to the north of Siwalik all along the foothills except for a stretch of about 30 km between Sarbhang and Geylegphug. Close proximity of marine Permian sequence, Gondwana and Siwalik exposed along foothills suggests possibilities of existence of marine Permian horizon underneath the Siwalik.

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Electrophoretic variation in laboratory strains of two sibling *Drosophila* species

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Genetic variation at eight gene–enzyme systems has been studied in laboratory strains of *Drosophila takahashii* and *Drosophila lutescens* which constitute a pair of sibling species. Comparison of the genetic structure of *D. takahashii* and *D. lutescens* reveals that ADH, ODH, α-GPDH and EST-7 constitute non-differentiating loci since both the electrophoretic phenotypes and allelic frequencies are similar for these loci. However, MDH-1, AO and APH-3 loci constitute species-discriminating loci because except for a few shared alleles, the allelic frequency patterns are differential in the two sibling species. The data on the indices of genetic diversity patterns in the sibling species pair are discussed.

THE use of allelic isozymes (allozymes) as probes of genetic structure of populations and species has revolutionized the status of population, ecological and evolutionary genetics[1]. The gel electrophoretic analysis of gene–enzyme systems has made it possible to infer genotypes from electrophoretic phenotypes due to codominance of allelic expression[2]. Thus, the data on electrophoretic mobility differences in species individuals have been used to infer genetic differences at enzyme loci in various drosophilids but there is little information on the allopatrically distributed sibling species populations of *D. takahashii* and *D. lutescens*[3,4]. The present paper reports the patterns of electrophoretic variability for eight gene–enzyme systems in laboratory strains of these two sibling *Drosophila* species.

Isofemale lines of *D. takahashii* and laboratory strains of *D. lutescens* were characterized after Bock[5]. About 12–14 homogenates of single individuals of both species were loaded in each horizontal starch gel slab (15 × 10 × 1 cm) and run electrophoretically at 250 V and 30 mA at 4°C for 4 h and the gel slices were stained for different gene–enzyme systems[6]. Enzymes include octanol dehydrogenase (ODH, E.C.1.1.1.73); esterase (EST, E.C.3.1.1.1); acid phosphatase (ACPH, E.C.3.1.3.2); α-glycerophosphate dehydrogenase (α-GPDH, E.C.1.1.1.8); alkaline phosphatase (APH, E.C.3.1.3.1); malate dehydrogenase (MDH, E.C.1.1.1.37); aldehyde oxidase (AO, E.C. 1.2.3.1) and alcohol dehydrogenase (ADH, E.C.1.1.1.1). The genetic basis of enzyme-banding patterns was interpreted from the segregation ratios of electrophoretic phenotypes of the parents and progeny (F_1/F_2/backcross) of species-specific genetic crosses (Table 1). The segregation ratios (1:1 or 1:2:1) of electrophoretic phenotypes for all the enzymes in both species agree with monogenic Mendelian inheritance. The population genetic structure of each species was described in terms of genetic indices[7,8].

Figure 1 depicts the electrophoretic banding patterns of different gene–enzyme systems in *D. takahashii* while the species-specific allelic isozymes (allozymes) for the polymorphic gene enzyme systems have been represented in Figure 2. The polymorphic zones of ODH, ACPH, APH-3, MDH-1 and AO are represented by segregating single-band variants (fast or slow) and triple-banded patterns. On the contrary, segregating two-banded patterns (conformational isozymes) for ADH and α-GPDH represent allelic isozymes. The

Table 1. Genetic control of electrophoretic phenotypes of five different enzymes in laboratory populations of *Drosophila* sibling species.

Zone	Genetic crosses	Enzyme phenotypes FF	FS	SS	Sample size	Testing the Mendelian ratios	χ^{2*}
ADH	FF × FF	12	—	—	12	—	—
	FF × FS	9	11	—	20	1:1	0.20
	FS × FS	6	13	5	24	1:2:1	0.25
	SS × SS	—	—	13	13	—	—
	FS × SS	—	8	12	20	1:1	0.80
ODH	FF × SS	—	18	—	18	—	—
	FS × FF	8	10	—	18	1:1	0.22
	SS × SS	—	—	16	16	—	—
MDH-1	FS × FF	10	14	—	24	1:1	0.66
	FS × FS	8	14	10	32	1:2:1	0.75
	FS × SS	—	6	10	16	1:1	1.0
	FF × FS	18	14	—	32	1:1	0.50
α-GPDH	FF × FF	12	—	—	12	—	—
	FS × FS	5	13	6	24	1:1	0.25
EST-7	SS × SS	—	—	26	26	—	—
	FS × SS	—	20	24	44	1:1	0.36
	FS × FS	10	18	8	36	1:2:1	0.22
	FF × FF	10	—	—	10	—	—

F and S represent fast and slow segregating electrophoretic variants (allozymes); *non-significant at 5% level.

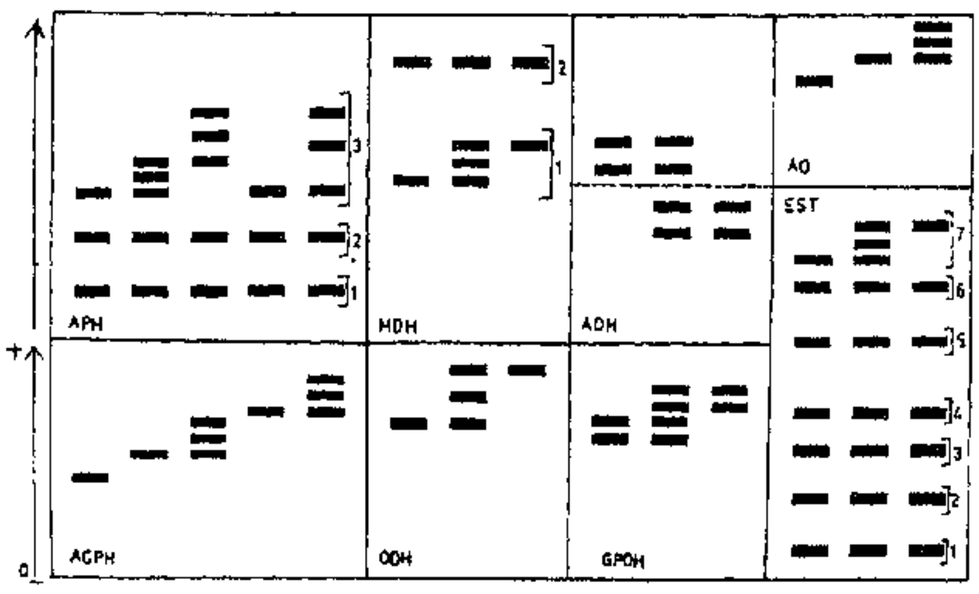

Figure 1. Representation of starch gel electrophoretic patterns for eight gene-enzyme systems in single individual homogenates of *D. takahashii*. Arrow indicates the direction of current flow.

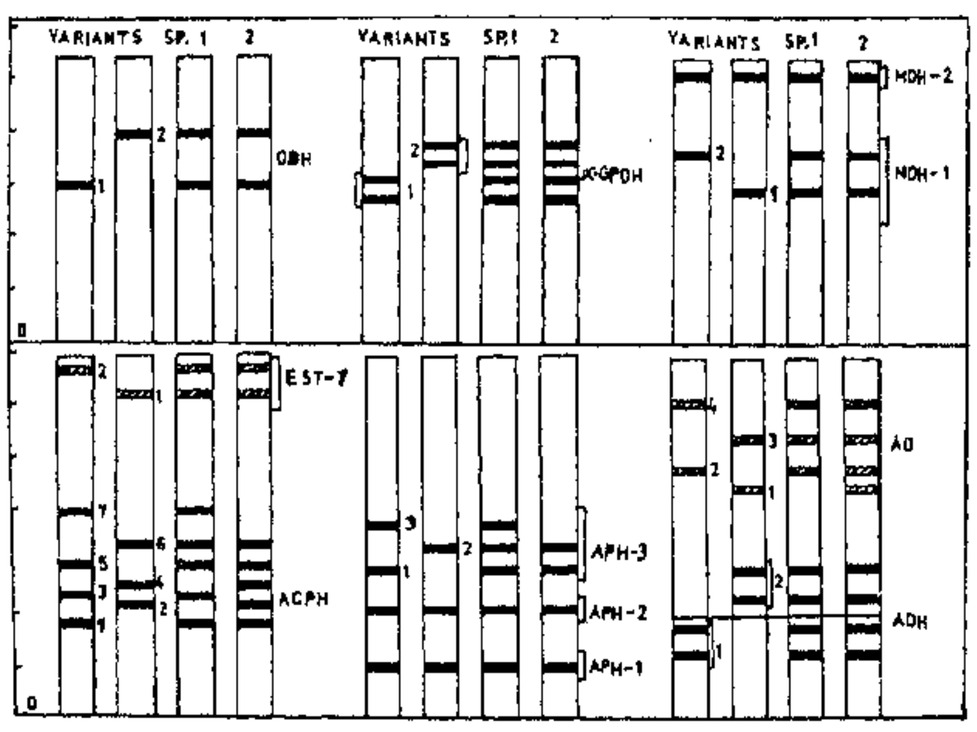

Figure 2. Schematic comparative representation of allelic isozymes (allozymes) of eight gene–enzyme systems in two sibling species—*D. takahashii* and *D. lutescens*. Each enzyme has been shown in four bar-type diagrams. Allelic variants (electromorphs) for each enzyme are shown in first two bars while species-specific allozymes (*D. takahashii* and *D. lutescens*) are shown in bars 3 and 4 respectively. The bands have been drawn according to their electrophoretic mobilities. AO and EST-7 band patterns have been superimposed.

banding patterns of heterozygous individuals depict subunit structure of allozymes and accordingly monomeric proteins (two-banded heterozygotes) include esterases; dimeric proteins (triple-banded heterozygotes) include ODH, ACPH, APH, MDH and AO. The monomorphic zones include EST-1 to EST-6, APH-1 and 2 and MDH-2 and the electrophoretic mobilities values of all such zones are identical in both the sibling species. The identical electrophoretic phenotypes occur at most of the polymorphic zones except ACPH, APH-3 and AO (Figure 2).

The data on allelic frequencies, the observed and expected heterozygosity, Wright's fixation index and the G-values for log-likelihood χ^2 test for fit to Hardy–Weinberg expectations at eight polymorphic loci in sibling species (*D. takahashii* and *D. lutescens*) have been represented in Table 2. The two loci (ACPH and AO) are highly polymorphic (high allelic content) in the two sibling species while the other loci are mostly diallelic (Table 2). The patterns of genetic indices i.e. the number and frequency of alleles and the amount of heterozygosity are almost identical at four loci (ADH, ODH, α-GPDH and EST-7). However, the two sibling species have revealed differential patterns of genetic indices at another four loci (MDH-1, AO, APH-3 and ACPH). The range of heterozygosities observed at various polymorphic loci correlates well with the number of alleles and allelic frequencies in both species. Significant deviations

Table 2. Data on distribution of allelic frequencies, heterozygosities, Wright's fixation index (f) and G-values for log likelihood χ^2 test for fit to Hardy–Weinberg expectations at 8 loci in *Drosophila takahashii* and *D. lutescens*.

	D. takahashii							D. lutescens						
Locus	N	F	S	H_o/H_e	f	G-values	($d.f.$)	N	F	S	H_oH_e	f	G-values	(d.f.)
ADH	114	0.16	0.84	0.28/0.27	−0.03	2.85	1	60	0.27	0.73	0.35/0.39	0.11	0.44	1
ODH	104	0.71	0.29	0.42/0.41	−0.02	0.93	1	60	0.67	0.33	0.40/0.44	0.09	0.60	1
MDH-1	134	0.75	0.25	0.45/0.37	−0.19	5.03*	1	75	0.18	0.82	0.20/0.29	0.32	6.55*	1
α-GPDH	164	0.91	0.09	0.11/0.16	0.12	9.46*	1	76	0.97	0.03	0.05/0.06	0.17	0.18	1
EST-7	130	0.29	0.71	0.40/0.41	0.02	0.17	1	50	0.37	0.63	0.50/0.46	−0.08	0.27	1
		1	2	3	4				1	2	3	4		
AO	130	0.29	0.60	0.11	—	0.46/0.54	0.15	26.02*	3	99	0.20	0.39	0.29	0.12	0.45/0.71	−0.37	43.9*	6
APH	132	0.05	—	0.33	0.62	0.59/0.51	−0.16	16.17*	3	82	—	0.37	—	0.63	0.54/0.46	−0.15	2.07	1
ACPH	180	0.07	0.82	0.10	0.01	0.40/0.34	−0.16	18.2*	6	75	0.02	0.78	0.09	0.11	0.44/0.44	0	27.95*	6

N, Sample size; Fast (F) and slow (S) represent diallelic loci while 1, 2, 3 and 4 refer to allelic variants at tetra-allelic loci. H_o and H_e, observed and expected heterozygosity.
The +ve and −ve values of Wright's fixation index (f = 1 − obs.het/exp.het.) indicate deficiency of heterozygotes and excess of heterozygotes respectively. Log-likelihood χ^2 test (G-test) was used to obtain statistical significance of the difference between obs. and exp. genotypes[11,12]. *G-values significant at 5% level.

from Hardy–Weinberg expectations have been observed at AO, APH-3, α-GPDH and ODH in *D. takahashii* and at ACPH, AO and MDH-1 in *D. lutescens*. The data on Wright's fixation index (f) in *D. lutescens* indicate deficiency of heterozygotes at MDH locus and excess of heterozygotes at AO locus.

Most of the enzyme loci analysed in the sibling species pair are effectively polymorphic based on the criterion that the frequency of the most common allele is <0·99. The lack of genic variation seen at the α-GPDH locus concurs with the functional constraint hypothesis[9]. Also, the occurrence of two-banded electrophoretic phenotypes in ADH and α-GPDH allelic variants concurs with the earlier reports that in NAD-requiring dehydrogenases, more than one electromorph (conformational isozyme) may arise due to post-translational differential binding of coenzyme NAD.

The sibling species pair of *D. takahashii* and *D. lutescens* are indistinguishable morphologically and are known to differ in their geographical distribution, i.e. *D. takahashii* occurs in South-east Asia as well as from India and Japan while *D. lutescens* is endemic to Korea and Japan[5]. The present observations reveal some genetic differentiation at 50% of the loci examined in the two sibling species. The results on allelic frequencies and heterozygosities at polymorphic loci in *D. lutescens* concur with the data reported earlier[4]. Comparison of the genetic structures of *D. takahashii* and *D. lutescens* reveal that ADH, ODH, α-GPDH and EST-7 constitute non-differentiating loci since both the electrophoretic phenotypes as well as allelic frequencies are similar for these loci. However, MDH-1, AO and APH-3 constitute species-discriminating loci because except a few shared alleles, the allelic frequency patterns are differential in the two sibling species. The present observations do not reveal a diagnostic locus with distinct mobilities and/or nonsharing of major alleles in the two sibling species. It is proposed to extend the present studies to analyse patterns of genic variation at several gene–enzyme systems in ecogeographical populations of *D. takahashii* and *D. lutescens* to assess the occurrence of species diagnostic loci as well as the role played by evolutionary processes in the maintenance of genetic variability[9,10].

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