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ALLOZYME PHYLOGENY OF FIVE SPECIES OF *TAKAHASHII* SPECIES SUBGROUP OF *DROSOPHILA*

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ABSTRACT

Allozymic variations at eight polymorphic loci in five species belonging to *takahashii* species subgroup were analysed through horizontal starch gel electrophoresis. Phylogenetic relationships based on NEI's as well as NAIR's indices revealed two main lineages. In one of the lineage, *D. paralutea* and *D. prostipennis* constitute one group while other main lineage includes two closely related species i. e. *D. takahashii* and *D. lutescens* and one distantly related species i. e. *D. nepalensis*. Thus, observed phylogenetic relationships based on allozymic data concur with the interrelation based on interspecific hybridization tests. The extent of observed allozymic divergence among five specis of *takahashii* subgroup seem to be correlated with their allopatric and endemic geographical distribution pattern throughout the oriental region.

Key words : Gel electrophoresis, *takahashii* species subgroup, allozyme phylogeny

INTRODUCTION

The allozymes have been used as diagnostic tools for differentiating the various races, sub–species, or semi species of a particular species ; various sibling species and nonsibling species (Macintyre and Collier, 1986). The different taxa may be distinguished from each other by the presence of mutually exclusive alleles or arrays of alleles ; and sometimes by differing frequencies of some alleles. Thus gel electrophoretic technique has been used for taxonomic purposes. Electrophoretic data have been used for phylogenetic interpretation and the method used to express the degree of similarity in a complex of conspecific and heterospecific species is the construction of dendrograms (Nei, 1972).

There is no information on the extent of genetic variability in allozymic, chromosomal, ecological and adaptive physiological traits in species populations of *takahashii* species group which had originated in the oriental region (David and Tsacas, 1981: Lemeunier *et al.*, 1986). The *takahashii* species subgroup comprised of twelve described species which occurred largely in the oriental region and most of the species except *D. takahashii* were known to be endemic. Seven species of this subgroup had been reported from India and four were endemic i.' e. *D. nepalensis* and *D. kurseongensis* occuring in the northern part of the

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Indian sub−continent as well as Nepal whlie *D. giriensis* and *D. jagri* were reported from some hilly sites of southern India. The extent of genetic information on the *takahashii* species subgroup had been very much limited i. e. only few members of this species subgroup were analysed with respect to metaphase chromosomes, chromosomal inversion polymorphism and interspecific hybridization (Bock and Wheeler, 1972 : Lemeunier *et al.*,1986). The chromosomal polymorphism was analysed in Japanese populations of *D. lutescens* (Fukatami, 1976) and in some Indian population of *D. takahashii* (Dwivedi and Gupta, 1980, 1981) whlie allozymic polymorphism had been analysed only in *D. lutescens* populations so far (Fukatami, 1977). Except a single study on the salivary gland chromosomes map of *D. nepalensis* (Parshad and Gandhi, 1971), there are no reports on the genetic structure of *D. nepalensis.* Thus, the present studies were carried out to analyse allozymic polymorphism and phylogenetic relationships among five species of *takahashii* species subgroup.

MATERIALS AND METHODS

Isofemale lines of *D. takahashii* and *D. nepalensis* were characterised after Parshad and Paika (1964), Bock and Wheeler (1972) and Bock (1980) while strains of three species (*D. paralutea, D. prostipennis* and *D. lutescens*) were obtained from Dr. A. Fukatami, Japan. About 12−15 homogenates of specids spesific single individuals were loaded in each horizontal starch gel slab (15×10×1cm) and run electrophoretically at 250 V and 30 mA at 4℃ for 4 hrs and the gel slices were stained for different gene−enzyme systems (Smith, 1976 ; Harris and Hopkinson, 1976). The gene−enzyme systems include : octanol dehydrogenase (ODH, E. C. 1.1.1.73), esterases (EST, E. C. 3.1.1.1), acid phosphatases (ACPH, C. 3.1.3.2), α−glycerophosphate dehydrogenase (α−GPDH, E.C. 1.1.1.37), alcohol dehydrogenase (ADH, E.C. 1.1.1.1) and alkaline phosphatases (APH, E.C. 3.1.3.1). The genetic basis of enzyme banding patterns was interpreted from the segregation ratios of electrophoretic phenotypes of the parents and F_1 and F_2 progeny of species specific genetic crosses. The genetic interpretation of electrophoretic data and calculation of genetic indices were followed from other sources (Ferguson, 1980 ; Zar, 1984). The data on percent similarity of allozymic bands among the five species of the *takahashii* species subgroup was calculated after Nair *et al.* (1971). Genetic identity calculated by Nei's formula (1972) and a phenetic tree was constructed according to the unweighted pair group method of clustering (UPGMA) of Sokal and Sneath (1963)

RESULTS AND DISCUSSION

The electrophoretic banding patterns of different gene−enzyme systems in *D. nepalensis* and *D. takahashii* were examined. In another series of experiments, species−specific allelic isozymes (allozymes) for the polymorphic gene−enzyme systems of five species of *takahashii* species subgroup (*D. takahashii, D. nepalensis, D. lutescens, D. paralutea, D. prostipennis*) have been testes (data not shown). 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 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 species specific.

The data on allelic frequencies, the observed and expected heterozygosity, effective number of alleles (n_e), Wright's fixation index and the G−values for log−likelihood X^2 test for fit to Hardy−Weinberg expectations at eight polymorphic loci in five species of *takahashii* species subgroup (*D. takahashii, D. nepalensis, D. lutescens, D. paralutea, D. prostipennis*) were represented in tables 1−3. Two loci (*Acph* and *Aph−3*) are highly polymorphic (high allelic content in these species) while the other loci are mostly diallelic (Table 1). The patterns of genetic indices i.e. the number and frequency of alleles and amount of heterozygosity differ 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 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*. Most of the enzyme loci analysed in the five species pair are effectively polymorphic based on the criterion that the frequency of the most common allele is 〈 0.99. The low genic variation seen at the α*−Gpdh* locus concurs with the functional constraint hypothesis (Oxford and Rollinsen, 1983). 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 differentinal 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 India and Japan while *D. lutescens* is endemic to Korea and Japan (Bock, 1972,1980). The electrophoretic phenotypes of ODH, α−GPDH and EST−7 are identical in all the five *Drosophila* species while the mobility values of MDH−2, APH−1 and APH−2 in case of *D. nepalensis* are species specific. The species specific allozymic variants include $Acph^{108}$ and $Aph-3^{109}$ in *D. takahashii* while several alleles are specific to *D. nepalensis* ($Acph^{80}$, Aph^{112}, Adh^{110} and Adh^{96};$Mdh-1^{91}$ and $Mdh-1^{97}$). The distribution patterns of allozymes at four loci in *D. nepalensis* characteristically differ from all other four species of this species subgroup.

The species specific allozymic data on genetic indices such as polymorphic loci (P), average number of alleles (A), and heterozygosity for different classes of enzymes on the basis of Gillespie−Kojima's hypothesis and Johnson's hypothesis

Table 1.

Data on Allele Frequencies, Heterozygosities (H_o/H_E), Wright's Inbreeding Coefficient (F), Effective Number of Alleles (n_e) and G−Values for LOG−Likelihood x^2 Ratio test for fit to Hardy−Weinberg expectations at ACPH and APH−3 loci of A=*D. Takahashii*; B=*D. Nepalensis*; C=*D. Lutescens*: D=*D. Paralutea*; E=*D. Prostipennis.*

		Allele frequencies							Allele frequencies				
Locus	Alleles	A	B	C	D	E	Locus	Alleles	A	B	C	D	E
ACPH	108	.02	–	–	–	–	*APH−3*	112	–	.19	–	–	–
	104	.07	–	.02	–	–		109	.05	–	–	–	–
	100	.80	.91	.74	.75	.97		106	–	.45	.37	–	–
	96	–	.06	.04	–	–		105	–	–	–	.36	.32
	95	.10	–	–	.13	–		103	.33	.36	–	–	–
	93	–	–	.09	–	.02		100	.62	–	.63	.64	.68
	91	.01	–	.11	.12	.01							
	80	–	.03	–	–	–							
	N	180	150	75	60	74		N	132	110	82	70	76
	H_o	.40	.17	.44	.50	.05		H_o	.59	.53	.54	.48	.47
	H_E	.34	.17	.44	.41	.06		H_E	.50	.63	.46	.46	.43
	F	−.16	0	0	−.21	.17		F	−.16	.17	−.15	−.04	−.08
	n_e	1.53	1.20	1.75	1.69	1.06		n_e	2.02	2.71	1.86	1.85	1.76
	G− values	18.16	2.6	27.45*	10.19*	0.11		G− values	16.71*	46.46*	2.07	0.01	0.72

*Significant at 5% level; other G−values are non significant.

ACPH & APH−3 alleles have also been designated by arabic numerals in a cathodal to anodal sequence.

For ACPH alleles 80=1; 91=2; 93=3; 95=4; 96=5; 100=6; 104=7 and 108=8.

For APH−3 alleles 100=1; 103=2; 105=3; 106=4; 109=5 and 112=6.

For ACPH alleles 80=1 ; 91=2 ; 93=3 ; 95=4 ; 96=5 ; 100=6 ; 104=7 and 108=8.
For APH−3 alleles 100=1 ; 103=2 ; 105=3 ; 106=4 ; 109=5 and 112=6.

Table 2.

Data on Allele Frequencies, Observed and Expected Heterozygosities (H_o/H_E), Wright's Inbreeding Coefficient (F), Effective Number of Alleles (n_e) and G−Values For Log−Likelyhood x^2 Ratio test for fit to Hardy−Weinberg expectations at ADH and AO loci in five *Drosophila* species of takahashii species sub−group A=*D. Takahashii* ; B=*D. Nepalensis* ; C=*D. Lutescens* : D=*D. Paralutea* ; E=*D. Prostipennis.*

		Allele frequencies							Allele frequencies				
Locus	Alleles	A	B	C	D	E	Locus	Alleles	A	B	C	D	E
ADH	110	−	0.20	−	−	−	*AO*	104	.29	−	.20	.18	.28
	106	.16	−	.27	.18	.15		100	.60	.43	.39	.20	.48
	100	.84	−	.73	.82	.85		98	.11	.36	.29	.62	.24
	96	−	.80	−	−	−		95	−	.21	.12	−	−
	N	111	171	60	77	68		N	130	107	99	50	60
	H_o	.28	.27	.35	.36	.29		H_o	.46	.39	.45	.54	.65
	H_E	.27	.32	.39	.29	.25		H_E	.54	.46	.71	.55	.63
	F	−.03	.16	.11	.24	.16		F	.15	.38	.37	.02	.08
	n_e	1.36	1.47	1.66	1.43	1.35		n_e	2.19	2.78	3.44	2.22	2.77
	G− values	2.85	4.3	.44	6.31*	4.42*		G− values	26.02*	32.93*	43.90*	.18	1.66

*Significant at 5% level ; other G−values are non−significant.

Table 3.

Estimation of Allele Frequencies, heterozygosities (H_o/H_E), Wright's Inbreeding Coefficient (F), Effective Number of Alleles (n_e) and G−Values for log−likelihood x^2 Ratio test for fit to Hardy−Weinberg expectations at four Loci (EST−7, ODH, α−GPDH and MDH−1) in five *Drosophila* species of takahashii species sub−group (A : *D. Takahashii*; B : *D. Nepalensis*; C : *D. Lutescens*: D : *D. Paralutea*; E : *D. Prostipennis*).

		Allele frequencies							Allele frequencies				
Locus	Alleles	A	B	C	D	E	Locus	Alleles	A	B	C	D	E
EST−7	106	.29	.43	.37	.58	.64	*α−GPDH*	108	.91	.92	.97	.96	.98
	100	.71	.57	.63	.42	.36		104	.09	.08	.03	.04	.02
	N	130	140	50	60	66		N	164	120	76	70	84
	H_o	.40	.54	.50	.50	.42		H_o	.11	.11	.05	.08	.05
	H_E	.41	.49	.46	.48	.46		H_E	.16	.15	.06	.07	.04
	F	−.24	−.10	−.08	−.04	.09		F	.32	.27	−.17	−.14	−.25
	n_e	1.67	1.96	1.87	1.96	1.85		n_e	1.19	1.17	1.05	1.08	1.04
	G− values	.17	.74	.27	.09	.46		G− values	9.46*	−.09	.18	.27	.23
ODH	100	.71	.74	.67	.81	.79	*MDH−1*	100	.75	−	.82	.83	.72
	96	.29	.26	.33	.19	.21		97	−	.78	−	−	−
								95	.25	−	.18	.17	.28
								91	−	.22	−	−	−
	N	134	144	60	80	70		N	134	86	75	60	64
	H_o	.42	.41	.40	.37	.42		H_o	.45	.32	.20	.31	.33
	H_E	.41	.39	.44	.30	.33		H_E	.37	.35	.29	.40	.28
	F	−.02	−.05	.09	−.23	−.27		F	−.19	.13	−.32	−.18	.22
	n_e	1.67	1.64	2.26	1.45	1.49		n_e	1.60	1.53	1.42	1.39	1.7
	G− values	.93	.80	.60	6.99*	8.33*		G− values	5.03*	1.20	6.55*	4.04	3.14

*Significant at 5% level; other G−values are non−significant.

values

*Significant at 5% level ; other G−values are non−significant.

Table 4.
Comparison of genetic indices of different enzyme groups on the basis of Gillespie−Kojima's hypothesis and Johnson's hypothesis in *D. takahashii* (A), *D. nepalensis* (B), *D. lutescence* (C), *D. paralutea* (D) and *D. prostipennis.* (E).

	Species	Group Ⅰ					Group Ⅱ					Group Ⅲ				
		Loci	A	P	H_o	H_e	Loci	A	P	H_o	H_e	Loci	A	P	H_o	H_e
*Johnson's	A	3	3.33	.27	.46	.41	3	2.33	1.0	.39	.47	3	1.66	.66	.18	.17
hypothesis	B	3	2.66	.27	.41	.43	3	2.33	1.0	.36	.39	3	1.66	.66	.14	.16
	C	3	3.0	.27	.49	.45	3	2.66	1.0	.40	.51	3	1.66	.33	.08	.12
	D	3	2.33	.27	.49	.45	3	2.33	1.0	.42	.38	3	1.66	.33	.13	.16
	E	3	2.33	.27	.31	.31	3	2.33	1.0	.45	.40	3	1.66	.33	.13	.11
*Gillespie	A	3	1.66	.66	.18	.17	6	2.8	.43	.42	.41					
−Kojima's	B	3	1.66	.66	.14	.16	6	2.5	.43	.38	.41					
hypothesis	C	3	1.66	.33	.08	.12	6	2.8	.43	.44	.43					
	D	3	1.66	.33	.13	.16	6	2.3	.43	.44	.41					
	E	3	1.66	.33	.13	.11	6	2.3	.43	.38	.36					

P : Proportion of polymorphic loci ; A=mean number of alleles/locus ; Ho & He=observed & expected heterozygosity.
Functional enzyme groups based on Johnson's hypothesis ; *Group 1 : ACPH, APH−3, EST−7 (variable substrate enzymes) ; Group Ⅱ ; ADH, AO, ODH (regulatory enzymes) ; Group Ⅲ ; MDH−1 and MDH−2, −GPDH (non regulatory enzymes).
Functional enzyme groups based on Gillespie−Kojima's hypothesis ; * *Group 1 : MDH−1 & MDH−2, −GPDH (glucose−metabolising enzymes) ; Group Ⅱ ; ACPH, EST−7, APH−3, ADH, AO, ODH (other enzymes)

are in agreement with the expectations of such hypothesis (Table 4). Among the five *Drosophila* species of *takahashii* species subgroup, the extent of interspecific similarities were calculated on the basis of Nei's method as well as Nair's criterion. The calculations based on Naia's criterion of percent allozymic similsrities was preferred in the present studies since laboratory strains of three species (*D. paralutea, D. prostipennis* and *D. lutescens*) were used and their levels of allozymic polymorphism may not be representative of their respective populations. However, Nei's calculations based on allozymic frequencies data resulted in almost similar trends but the relative values of genetic similarities were found to differ to some extent (Fig. 1). The data on the genetic similarities values on all possible pairs have been presented in table 5. *D. prostipennis* and *D. paralutea* have shown maximum genetic identity or similarities because these species share more common allozymes than they do with any other species of the *takahashii* species group. Likewise, the amout of genetic aimilarities was found to be higher in the pair of *D. takahashii* and *D. lutescens*. Since the overall values of genetic similarities between *D. nepalensis* and other species pairs are quite low. This

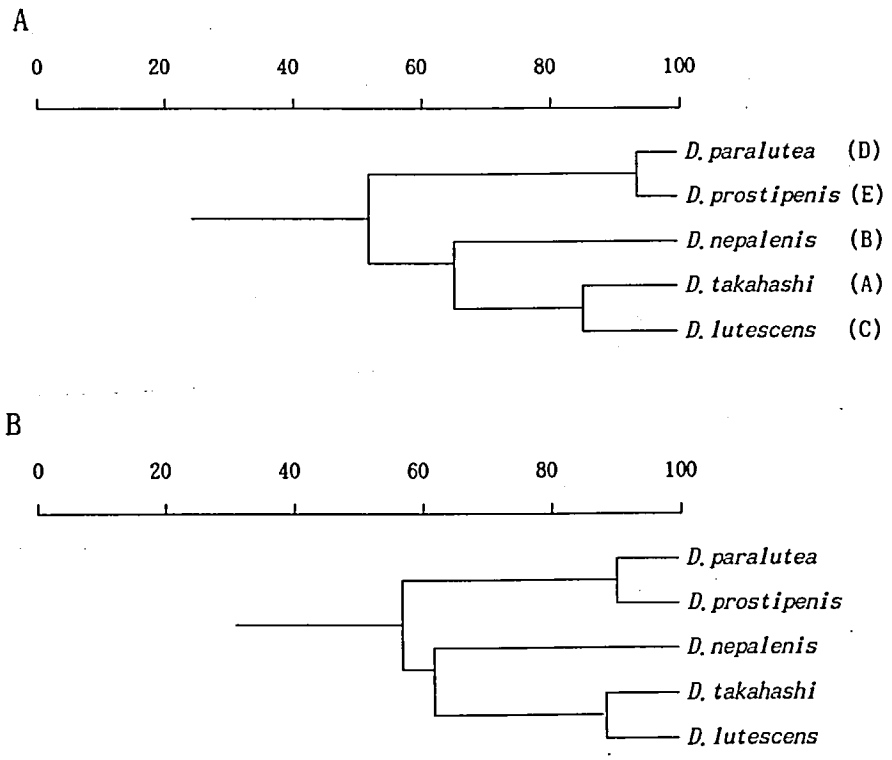

Fig. 1. Dendrograms showing interspecific relationships smong five species of *takahashii* species sub−group based on (A) percentage of allozyme/electromorphs similarity ; (B) Nei's genetic similarity values.

Table 5.
Data on species Inter−Relationships Based on percent similarity of the allozyme bands (upper diagonal); and nei's genetic similarity (I) values (Lower Diagonal) Among pairs of five species of takahashii species sub−group.

Species	*D. takahashii*	*D. nepalensis*	*D. lutescens*	*D. paralutea*	*D. prostipennis*
D. takahashii	X	57.1	86.6	85.2	83.3
D. nepalensis	.62	X	69.2	60.0	53.5
D. lutescens	.95	.65	X	83.3	83.3
D. daralutea	.85	.59	.93	X	96.3
D. prostipennis	.94	.59	.94	.96	X

indicates some amount of genetic differentiation among *D. nepalensis* and other analysed species of *takahashii* species subgroup. However, the amount of genetic differentiation seems to be very low among the other pair of closely related species i. e. *D. takahashii* and *D. lutescens*. Allozymic data has been used to express genetic similarities and genetic distances between populations belonging to same or different species (Nei, 1972). The data on the genetic similarities or distances in all pairwise combinations among a complex of conspecific and heterospecific populations or a species complex were used in the construction of dendrograms. The allozymic phylogeny of five members of *takahashii* species subgroup depicted that amongst all pairs, *D. prostipennis* and *D. paralutea* exhibited the maximum genetic similarity (I=0.96). Thus these two species are very closely related to each other and comprise one group. Out of the pairs involving the other three species (*D. takahashii, D. nepalensis, D. lutescens*), *D. takahashii* and *D. lutescens*, sibling species pair have shown the maximum genetic similarity 87). The extent of genetic similarity (I) between *D. nepalensis* and sibling species pair is 0.62 while this trio (*nepalensis, takahashii* and *lutescens*) has shown similarity values of 0.59 with the closely related species group of *D. paralutea* and *D. prostipennis*. The phenetic tree constructed in the present study largely confirms the observations based on interspecific hybridisation experiments (Sokal and Sneath, 1963). In general, the members of sibling species pairs and those depicting hybridisation potential are more closely related than other species pairs except *D. nepalensis* which is morphologically different and is completely reproductively isolated species.

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