

THE EFFECT OF LATITUDE ON GENIC VARIABILITY AT THREE LOCI IN *Drosophila melanogaster* POPULATIONS FROM INDIA

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ABSTRACT

Fourteen natural populations of *Drosophila melanogaster* from India, collected from 9.6 to 33.0°N latitude, were analysed electrophoretically and the geographical pattern of variation was compared at the polymorphic loci *Adh*, *Odh* and *Ao*. The allele frequency variation demonstrated statistically significant correlation with latitude. The populations revealed extensive clinal variation at all three polymorphic loci, which depicted moderate to higher F_{ST} values. Thus, the observed genic divergence in colonising populations of *D. melanogaster* demonstrates the effect of natural selection along the north-south axis of the Indian sub-continent.

INTRODUCTION

Colonising species populations offer suitable material for microevolutionary studies (Endler, 1986). Electromorphs (allozymes) constitute molecular markers because electrophoretic mobility variants can be genetically interpreted and have been used to characterise the genetic structure of natural populations of diverse taxa (Wills, 1981; Spiess, 1989). Studies on biogeography, ecology and adaptive physiological traits in global populations of *Drosophila melanogaster* have revealed that Afrotropical populations constitute the ancestral populations which later colonised Eurasia and more recently America and Australia (David and Capi, 1988). The levels of genetic diversity have been compared in the cosmopolitan and colonising sibling species pair, *D. melanogaster* and *Drosophila simulans* and such studies revealed extensive clinal as well as geographical genetic divergence among *D. melanogaster* populations and genetic uniformity among *D. simulans* populations (Watada *et al.*, 1986; Singh and Rhomberg, 1987). Most studies on allozymic polymorphism had been made on U.S. and Australian populations of *D. melanogaster* while

Asian populations remain unexplored (David, 1982; Oakeshott *et al.*, 1982; Anderson *et al.*, 1987).

MATERIAL AND METHODS

Isofemale lines were established from samples of *D. melanogaster* from fourteen Indian geographical sites (Cochin to Dalhousie, latitude 9°58'N to 33°0'N, Figure 1). The homogenates of single individuals were analysed electrophoretically in 12 percent starch gels at 250 V and 30 mA at 4°C for four hours. The gel slices were stained for alcohol metabolising enzymes, i.e. alcohol dehydrogenase (ADH, E.C.1.1.1.1), octanol dehydrogenase (ODH, E.C.1.1.1.73), and aldehyde oxidase (AO, E.C.1.2.3.1), respectively (Harris and Hopkinson, 1976). The genetic basis of banding patterns was interpreted from the segregation ratios of electrophoretic phenotypes of the parents as well as F₁ and F₂ progeny of several single pair matings of *D. melanogaster*. Genetic interpretation was made of electrophoretic data and calculations made of genetic indices, including allelic frequencies, contingency Chi-square analysis, observed and expected heterozygosities, simple correlation coefficient (r), Wright's fixation index (F_{ST}) (Workman and Niswander, 1970; Ferguson, 1980; Zar, 1984; Sokal and Rohlf, 1987). The log-likelihood Chi square (G-test) was used to assess whether the observed genotypes were in agreement with



Figure 1 - Map of Indian sub-continent depicting the collection sites for *Drosophila melanogaster* populations. The sites include: 1. Cochin ($9^{\circ}.58'N$); 2. Madras ($13^{\circ}.04'N$); 3. Tirumala ($13^{\circ}.40'N$); 4. Hyderabad ($17^{\circ}.20'N$); 5. Nagpur ($21^{\circ}.09'N$); 6. Bhopal ($23^{\circ}.16'N$); 7. Phuntsholing ($26^{\circ}.06'N$); 8. Rohtak ($28^{\circ}.54'N$); 9. Roorkee ($29^{\circ}.52'N$); 10. Saharanpur ($29^{\circ}.58'N$); 11. Dehradun ($30^{\circ}.19'N$); 12. Chandigarh ($30^{\circ}.42'N$); 13. Jammu ($32^{\circ}.43'N$); 14. Dalhousie ($33^{\circ}.0'N$).

those expected on the basis of Hardy-Weinberg expectations (Zar, 1984).

RESULTS AND DISCUSSION

Genetic basis

It was found that ODH and AO constitute the two anodal zones while the single cathodal zone was ADH.

The polymorphic zones of AO and ODH were represented by segregating single-band variants and triple-band patterns. The genetic basis of enzyme banding patterns was interpreted from the Mendelian segregation ratios of electrophoretic phenotypes in the progeny of genetic crosses. The single-band and triple-banded patterns represented homozygous and heterozygous genotypes respectively. The occurrence of hybrid band in heterozygotes at the *Ao* and *Odh* loci indicated that such enzymes were dimeric in nature. The ADH enzyme revealed segregating two-banded patterns (both of faster or of slower mobilities) and four-banded patterns in the individuals of *D. melanogaster*. The ADH electrophoretic data on parents and progeny of genetic crosses was found

to be in agreement with the monogenic control of ADH. Thus, homozygous individuals showing two-banded patterns represented electromorphs or allozymic variants. The present observations on ADH electrophoretic phenotypes concurred with earlier reports in *D. melanogaster* that in NAD-requiring dehydrogenases, more than one electromorph (conformational isozyme) can arise due to post-translational differential binding of co-enzyme NAD (Niesel *et al.*, 1982). Since all the enzyme banding patterns were found to be identical in both the sexes, the loci coding these enzyme systems were autosomal.

Population genetic structure

The heterozygosity levels revealed significant differences at all the three loci among the different geographical populations (Table I). The patterns of deviations from Hardy-Weinberg equilibrium were found to be different for the three loci. This indicated that the genetic structures of the geographical populations was consistently heterogeneous and could be subjected to natural selection mechanisms. The populations revealed allelic differentiation at all the three loci (*Adh*, *Odh* and *Ao*) and significant genotypic heterogeneity was also observed at these loci (Table II).

The data on interpopulational heterozygosity also revealed marked differences. The heterozygosity was partitioned into its within-population and between population components and the proportion of heterozygosity assigned to inter-population genetic differentiation was measured as Wright's Fixation Index (F_{ST}). The observed F_{ST} value indicated significantly higher genic differentiation at *Adh* locus; moderate at *Odh* locus and lower genetic differentiation was observed at *Ao* locus (Table II). Allele frequencies of Adh^F , Odh^F and AoS were found to be significantly and positively correlated with latitude (Table II, Figure 2).

The statistical comparison of *Adh* and *Odh* allelic frequency data in Indian populations of *D. melanogaster* with those of other allopatric populations (Afrotropical, Chinese, Japanese and European) revealed (a) consistency of the direction of latitudinal clines on the different continents; (b) the extent of latitudinally related variation of allelic frequencies differed significantly for the *Adh* locus among Indian versus Afrotropical populations as well as Indian versus European populations and for the *Odh* locus among Indian versus Chinese, Japanese, European and Afrotropical populations (Table III). Thus, the direction of latitudinal cline was found to be similar among different allopatric populations but the allelic frequencies differed significantly (Table III).

The existence of parallel clinal allelic frequency changes at *Adh*, *Odh* and *Ao* loci provide strong evidence

Table I - Data on distribution of allelic frequencies, heterozygosity (observed/expected), and G-values for log-likelihood chi-square test for fit to Hardy-Weinberg expectations at three polymorphic loci (*Adh*, *Odh* and *Ao*) in fourteen natural populations of *Drosophila melanogaster*.

Locus	Genetic indices	CON	MDS	TRM	HYD	NGR	BPL	PTS	RTK	RUK	SRP	DUN	CHD	JMU	DLH
<i>Adh</i>	F	.11	.13	.16	.21	.30	.56	.74	.74	.77	.78	.80	.80	.82	.82
	S	.89	.87	.84	.79	.70	.44	.26	.26	.23	.22	.20	.20	.18	.18
	H.obs.	.15	.16	.15	.20	.21	.33	.26	.27	.21	.22	.22	.23	.20	.20
	H.exp.	.20	.23	.26	.33	.42	.49	.38	.38	.35	.34	.32	.32	.30	.29
	G-values	5.78	10.40*	20.30*	13.20*	18.51*	5.83*	9.53*	8.54*	18.69*	11.72*	8.75*	7.13*	9.57*	10.92*
<i>Odh</i>	F	.18	.20	.25	.35	.55	.58	.60	.62	.64	.64	.64	.65	.66	.67
	S	.82	.80	.75	.65	.45	.42	.40	.38	.36	.36	.35	.34	.33	.33
	H.obs.	.29	.33	.38	.33	.36	.33	.30	.31	.30	.30	.24	.22	.23	.23
	H.exp.	.30	.32	.37	.46	.49	.49	.48	.47	.46	.46	.46	.46	.44	.44
	G-values	.03	4.06	.09	10.94*	8.57*	11.53*	34.19*	10.35*	11.32*	11.32*	37.51*	20.55*	21.45*	21.45*
<i>Ao</i>	F	.84	.81	.80	.78	.79	.75	.72	.70	.69	.66	.65	.64	.62	.62
	S	.16	.19	.20	.22	.21	.25	.28	.30	.31	.34	.35	.36	.38	.38
	H.obs.	.25	.23	.22	.23	.23	.30	.29	.14	.14	.16	.16	.16	.21	.22
	H.exp.	.27	.31	.32	.34	.33	.38	.40	.42	.43	.45	.46	.46	.47	.47
	G-values	.87	4.09	8.26*	2.46	8.33*	7.63*	5.76*	47.64*	46.60*	41.10*	39.92*	35.26*	41.95*	35.55*

CON - Cochin; MDS - Madras; TRM - Tirumala; HYD - Hyderabad; NGR - Nagpur; BPL - Bhopal; PTS - Phuntsholing; RTK - Rohtak; RUK - Roorkee; SRP - Saharanpur; DUN - Dehradun; CHD - Chandigarh; JMU - Jammu; DLH - Delhousie.

*Significant at 5 percent level.

Table II - Statistical analysis of allozymic variability at three polymorphic loci (*Adh*, *Odh* and *Ao*) in fourteen geographical populations of *Drosophila melanogaster*.

Locus	Genetic indices						
	Interpopulation heterogeneity of		Wright F_{ST} analysis			Correlation coefficient (r)	
	Allelic freq. (d.f.)	Genotypic freq. (d.f.)	H_S	H_T	F_{ST}	Latitudinal	Longitudinal
<i>Adh</i> ^F	1172.9* (13)	158.87* (14)	.33	.50	.34*	+.98*	-.09
<i>Odh</i> ^F	365.55* (13)	203.36* (14)	.43	.50	.14*	+.96*	-.04
<i>Ao</i> ^S	58.92* (13)	325.42* (14)	.39	.72	.46*	+.98*	-.28

*Significant at 5 percent level. d.f. = degree of freedom.

for the action of latitudinally related environmental gradients. The biochemical properties of *Adh* allozymes have suggested that temperature or cooler places could favour *Adh*^F while tropical or warm places would select *Adh*^S allelic variants (Oakeshott, 1976). The observed clinal pattern at *Adh* locus in Indian populations is in agreement with the known higher thermostability of *Adh*^S variant.

Latitudinal clines have also been reported in Australian populations (Oakeshott *et al.*, 1982).

Afrotropical populations (David *et al.*, 1986, 1989). Japanese populations (Watada *et al.*, 1986), Chinese populations (Jiang *et al.*, 1989) and diverse continental populations (Singh *et al.*, 1982; Singh and Rhomberg, 1987). The occurrence of clinal variation across diverse biogeographical regions can not be explained on the basis of stochastic processes such as genetic drift and/or gene flow since the continental populations differed significantly in their evolutionary history as well as ecogeographical conditions.

Table III - Statistical comparison of *Adh* and *Odh* allelic frequencies of Indian versus other continental populations of *Drosophila melanogaster*.

Populations		<i>Adh</i> ^F			<i>Odh</i> ^F			References
		Range	Mean	t	Range	Mean	t	
India	(6)	.16-.82	.56	-	.25-.67	.52	-	Present study
China	(6)	.57-.80	.75	1.62	1.0-1.0	1.0	7.32*	Jiang <i>et al.</i> , 1989
Japan	(6)	.50-.93	.73	1.43	1.0-1.0	1.0	7.32*	Watada <i>et al.</i> , 1986
Europe	(6)	.92-.94	.93	3.33*	.92-.99	.97	6.25*	David, 1982
Afrotropical	(6)	.01-.38	.09	3.73*	.88-.10	.91	4.9*	David, 1982

Number of populations are given in parentheses; allelic frequencies ranges include minimum and maximum values; F denotes faster allozymic variant; t - Student's 't' test. Indian populations include Tirumala (TRM), Nagpur (NGR), Bhopal (BPL), Rohtak (RTK), Saharanpur (SRP) and Dalhousie (DLH).

*Significant at 5 percent level.

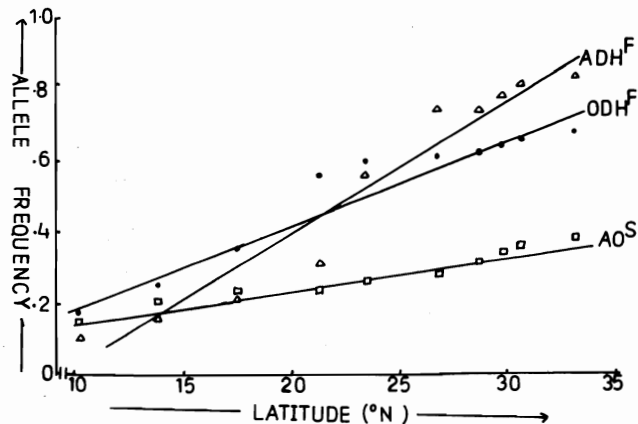


Figure 2 - Patterns of changes in allelic frequencies at three polymorphic loci (*Adh*, *Odh* and *Ao*) in fourteen latitudinally varying natural populations of *Drosophila melanogaster*.

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RESUMO

Foram analisadas electroforeticamente, 14 populações naturais de *Drosophila melanogaster* da Índia, coletadas de 9,6 a 33° de latitude N e o padrão de variação geográfica foi comparado nos loci polimórficos *Adh*, *Odh* e *Ao*. A variação da frequência alélica revelou correlação estatisticamente significativa com a latitude. As populações mostraram extensa variação "clinal" em todos os três locus polimórficos os quais tiveram moderado a alto valores de F_{ST} . Então, a divergência gênica em populações colonizadas de *D. melanogaster*

mostrou o efeito da seleção natural ao longo dos extremos norte-sul do sub-continente da Índia.

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