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Original article

Parallel selection of ethanol and acetic-acid tolerance in *Drosophila melanogaster* populations from India

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Summary – Nine Indian geographical populations of *Drosophila melanogaster*, collected along the 20°N latitudinal range, revealed a significant clinal variation at the alcohol dehydrogenase (*Adh*) locus, Adh^F allelic frequency increasing significantly with latitude (0.036 ± 0.004 for 1° latitude; genetic divergence $F_{ST} = 0.25$). Patterns of ethanol and acetic-acid tolerance in adult individuals revealed significant genetic divergence. Parallel patterns of latitudinal ethanol tolerance (10 to 15%) and acetic-acid tolerance (3.7 to 13.2%) were observed in adult individuals from the 9 geographical populations. Thus, the northern and southern populations revealed divergence in the patterns of resource utilisation. The parallel latitudinal genetic divergence at the *Adh* locus and for ethanol and acetic-acid tolerance in Indian populations of *Drosophila melanogaster* could be explained by balancing natural selection varying spatially along the north–south axis of the Indian subcontinent.

ADH polymorphism / ethanol tolerance / acetic-acid tolerance / latitudinal clines / Indian populations / *Drosophila melanogaster*

Résumé – Sélection parallèle des tolérances à l'éthanol et à l'acide acétique dans des populations indiennes de *Drosophila melanogaster*. *Neuf populations géographiques indiennes de* Drosophila melanogaster, *échelonnées sur une latitude de 20° N, révèlent une variation clinale significative au locus de l'alcool déshydrogénase* (Adh), *avec un accroissement significatif de la fréquence de l'allèle* Adh^F *avec la latitude (0,036 ± 0,004 par degré de latitude) et un indice de fixation* $F_{ST} = 0,25$. *Des évolutions parallèles de la tolérance à l'éthanol (10 à 15%) et de la tolérance à l'acide acétique (3,7 à 13,2%) en fonction de la latitude sont observées chez les adultes des 9 populations géographiques, révélant ainsi des divergences dans le mode d'utilisation des ressources entre le nord et le sud. La divergence observée en fonction de la latitude à la fois au locus* Adh *et pour les*

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tolérances à l'éthanol et à l'acide acétique pourrait s'expliquer par une sélection naturelle équilibrante variant selon l'axe nord-sud du sous-continent indien.

polymorphisme de l'Adh / tolérance à l'éthanol et à l'acide acétique / clines de latitude / populations indiennes / *Drosophila melanogaster*

INTRODUCTION

The evolutionary potential of a species is a function of the amount of genetic variation it undergoes. Colonising species such as *Drosophila melanogaster* populations offer excellent material for micro-evolutionary studies (Parsons, 1983). Studies on biogeography, ecology and adaptive physiological traits in global populations of *D melanogaster* have revealed that Afrotropical populations constitute ancestral populations, which later colonised Eurasia and more recently America and Australia (David and Capy, 1988). Most studies on allozymic polymorphism have been made on American and Australian populations of *D melanogaster* while Asian populations remain unexplored (David, 1982; Oakeshott *et al*, 1982; Anderson *et al*, 1987). Gel electrophoretic analysis has helped in elucidating the genetic structure of geographical populations of diverse taxa, and it was therefore considered worthwhile characterising the extent of genic divergence at the alcohol dehydrogenase (*Adh*) locus in latitudinally varying Indian natural populations of *D melanogaster*.

D melanogaster populations exploit a wide array of fermenting and decaying fruit and vegetables, organic materials and man-made alcoholic environments. Ethanol is the end product of fermentation and ethanol vapours provide a normal energy source in *D melanogaster* (Parson, 1983). Ethanol is converted into acetic acid *via* acetaldehyde and thus concentrations of these 2 metabolites are generally found in natural habitats of the *Drosophila* species. The alcohol dehydrogenase (ADH) of *D melanogaster* converts a wide range of alcohols into aldehydes and more than 90% of the external alcohols are metabolised in a pathway initiated by this enzyme (Geer *et al*, 1989). Most studies on ethanol tolerance have been made on *D melanogaster* populations from Europe and Africa (David *et al*, 1986) and Australia (McKenzie and Parsons, 1972; Parsons, 1979, 1980a), but information on *D melanogaster* from India as well as other tropical parts of the world is still lacking. Recently, acetic acid has been found to be a parallel resource to ethanol in *D melanogaster* (Chakir *et al*, 1993, 1994). The objective of this study is to analyse acetic-acid and ethanol utilisation by *D melanogaster* populations from the Indian subcontinent.

MATERIALS AND METHODS

Isofemale lines of *D melanogaster* from 9 Indian geographical sites (Madras to Dalhousie, 13°04′N to 33°N; fig 1, table I) were established for 2–3 generations and used for measurements of ethanol and acetic-acid utilisation as well as ADH polymorphism. Homogenates of single individuals from each isofemale line were subjected to electrophoresis at 250 V and 25 mA at 4°C for 4 h and gel slices were stained for ADH (Harris and Hopkinson, 1976). The genetic control of ADH banding

patterns was interpreted from the segregation patterns of enzyme electromorphs of parents, F_1 and F_2 progeny of several single-pair matings. The genetic indices were calculated by standard statistical formulae (Ferguson, 1980).

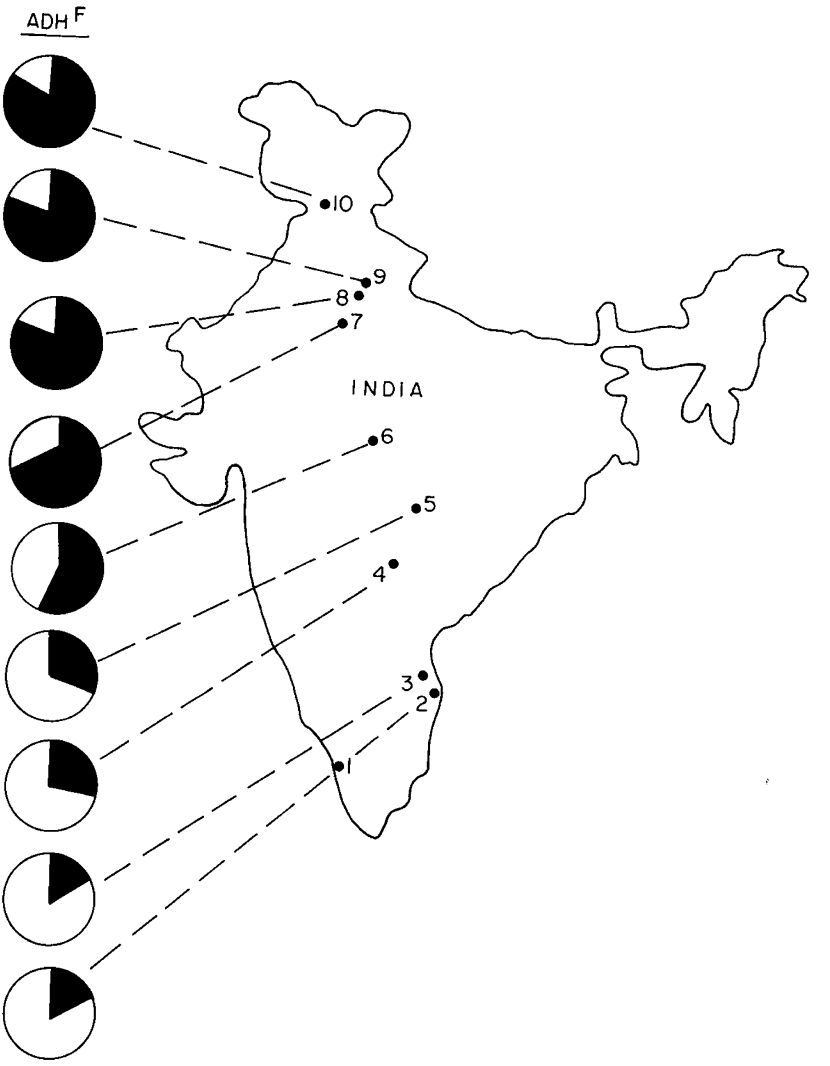

Fig 1. Map depicting collection sites of 9 Indian populations of *D melanogaster*. The frequency of Adh^F allele is shown by the black area in each pie diagram. The locations of the collections and their respective latitudes are given in table I.

Table I. Data on alcohol dehydrogenase (*Adh*) allelic frequency, percentage ethanol and acetic-acid tolerance indices (increase in longevity, LT_{50} max/LT_{50} control, adult threshold concentration and LC_{50}) in 9 Indian geographical populations of *D melanogaster*.

Population	*Latitutde*	N	Adh^F *frequency*	*Ethanol tolerance*			*Acetic-acid tolerance*		
				LT_{50} ethanol/ LT_{50} control	*Adult threshold (%)*	*LC_{50} (%)*	*LT_{50} acetic-acid/ LT_{50} control*	*Adult threshold (%)*	*LC_{50} (%)*
Madras	13° 04′N	162	0.13	1.17	10.0	9.0	1.0	3.7	5.6
Tirumala	13° 40′N	148	0.16	1.20	10.25	9.4	1.04	4.0	6.0
Hyderabad	17° 20′N	90	0.20	1.50	10.40	9.8	1.10	4.8	7.5
Pune	18° 32′N	87	0.21	1.60	11.20	9.8	1.15	5.4	8.2
Nagpur	21° 09′N	82	0.30	1.91	12.75	10.2	1.20	6.0	9.4
Bhopal	23° 16′N	54	0.56	2.60	12.40	10.2	1.28	9.0	10.0
Rohtak	28° 54′N	103	0.74	2.81	13.25	11.1	2.0	12.6	10.5
Saharanpur	29° 58′N	116	0.78	3.0	14.75	11.2	2.04	12.8	10.9
Dalhousie	33°N	125	0.82	3.48	15.0	12.0	2.38	13.2	11.7

N: number of isofemale lines; F represents fast electromorph.

Ethanol and acetic-acid tolerance patterns of mass cultures of each of 9 populations of *D melanogaster* were assessed following the procedure of Starmer *et al*, (1977). Groups of 10 males or 10 females, grown on a killed yeast medium (without any ethanol), were aged for 3 d on fresh *Drosophila* food medium and then transferred with the help of an aspirator to air-tight plastic vials (40 ml; 4 × 1 inches). The flies were admitted to the upper vial, which was separated by fine terylene cloth from the lower vial containing 10 ml of ethanol or concentrated acetic acid absorbed on 1 g cellulose wool. Such paired vials were sealed with cellophane tape and all experiments were conducted at 23°C. The alcoholic solutions were not changed during the experiment. The flies were not etherised during different experiments. The control vials contained 10 ml of distilled water absorbed on cellulose wool. Four replicates were performed for all the experiments. For each concentration, 40 males and 40 females were treated with a range of 6–8 different concentrations of ethanol or acetic acid. The male and female individuals did not reveal any significant difference in ethanol or acetic-acid tolerance and thus the data for the 2 sexes were averaged for all experiments. The effects of metabolic alcoholic vapours were assessed from the number of flies alive after various time intervals and LT_{50} values were expressed as the number of hours after which 50% of the flies had died and were estimated by linear interpolation. The ethanol and acetic-acid threshold values were used as indices, *ie* if vapours were utilised as the source then LT_{50} ethanol/LT_{50} control was found to be more than 1; if this ratio was less than 1, then it acted as stress. The threshold values were determined when LT_{50} ethanol/LT_{50} control = 1 (Parsons, 1983).

RESULTS

Populational genetic structure at the Adh *locus*

Data on the number of isofemale lines and Adh^F frequency in *D melanogaster* populations are given in table I. The clinal variation at the *Adh* locus was found to be significant (3.6% with 1° latitude; $r = 0.96$; $b = 0.036 \pm 0.004$). The data on Wright's fixation index ($F_{ST} = 0.25$) revealed significant genic divergence at *Adh* locus in Indian populations. Contingency chi-squared analysis revealed significant interpopulation genotypic heterogeneity (75.8) and allelic heterogeneity (738.4) at the *Adh* locus in Indian populations of *D melanogaster.*

Adult ethanol tolerance

The adult individuals were analysed for their potential to utilise the ethanol vapours in a closed system and the data on the ethanol and acetic-acid tolerance of 9 geographical populations are given in table I. Data on 5 geographical populations of *D melanogaster* are shown in figures 2 and 3. The intraspecific variation for ethanol tolerance was found to be significantly different along the north–south axis of the Indian subcontinent. The data on LT_{50} ethanol/LT_{50} control (which are the measures of source *versus* stress) show a latitudinal variation (table I, fig 2a). The adult ethanol threshold values were found to vary clinally in the range of 10 to 15% among 9 *D melanogaster* populations from south to north

localities (table I). The ethanol concentrations up to 15% served as a source for north Indian populations while a maximum of 10% ethanol concentration could be utilised by south Indian populations. The LC_{50} ethanol concentrations were calculated from mortality data of adults after 6 d of ethanol treatment and LC_{50} values revealed clinal variation in the range of 9.0 to 12.0%, *ie* southern populations of *D melanogaster* showed significantly lower ethanol tolerance than north Indian populations (table I, fig 3a). The longevity data on 6% ethanol revealed that northern populations under experimental conditions survived for 3 weeks (18–22 d) as compared with 2 weeks duration in southern populations (fig 3c).

Adult acetic-acid tolerance

The south Indian population of Madras revealed the minimum value of LT_{50} acetic acid/LT_{50} control (1.0) compared with higher LT_{50} acetic acid/LT_{50} control (2.38) in the Dalhousie population when adult individuals were exposed to 3% acetic acid (fig 2b). The adult acetic-acid threshold values also revealed latitudinal clines in the range of 3.7 to 13.2 (table I). The LC_{50} acetic-acid concentrations were calculated from the mortality data at 3 d and the LC_{50} values revealed a clinal variation of 5.6–11.7% (table I). The acetic-acid tolerance indices of 5 populations of *D melanogaster* are shown in figures 2b and 3b,d. The longevity periods at 3% acetic-acid were found to be more than 2 weeks in northern populations compared with about 10 d in southern populations (fig 3d).

DISCUSSION

In order to test whether the *Adh* allelic frequency changes and ethanol tolerance potential are correlated with latitude, a statistical analysis of correlation was carried out for all 9 geographical populations of *D melanogaster*. The statistical correlations were found to be significantly higher (0.96) for latitudinal variation of adult ethanol tolerance *versus* Adh^F allelic frequency. Thus, the present data on clinal variation at the *Adh* locus in Indian populations of *D melanogaster* further support and validate the hypothesis that the occurrence of parallel or complementary latitudinal clines across different continental populations provides strong evidence of natural selection maintaining such clinal allozymic variation (David, 1982; Oakeshott *et al* 1982; Anderson *et al*, 1987).

Latitudinal clines have been reported in American (Vigue and Johnson, 1973), Australian (Oakeshott *et al*, 1982), Afrotropical (David *et al*, 1986, 1989), Japanese (Watada *et al*, 1986) and Chinese populations (Jiang *et al*, 1989). The occurrence of clinal variation across diverse biogeographical regions cannot be explained on the basis of stochastic processes such as genetic drift and/or gene flow since the continental populations differ significantly in their evolutionary history as well as ecogeographical conditions. The existence of parallel clinal allelic frequency changes at the *Adh* locus provides strong evidence for the action of latitudinally related environmental gradients.

The Indian geographical populations of *D melanogaster* revealed significant genetic divergence in their potential to use acetic acid. The adult longevity periods were found to increase significantly at 1–6% for south Indian populations and

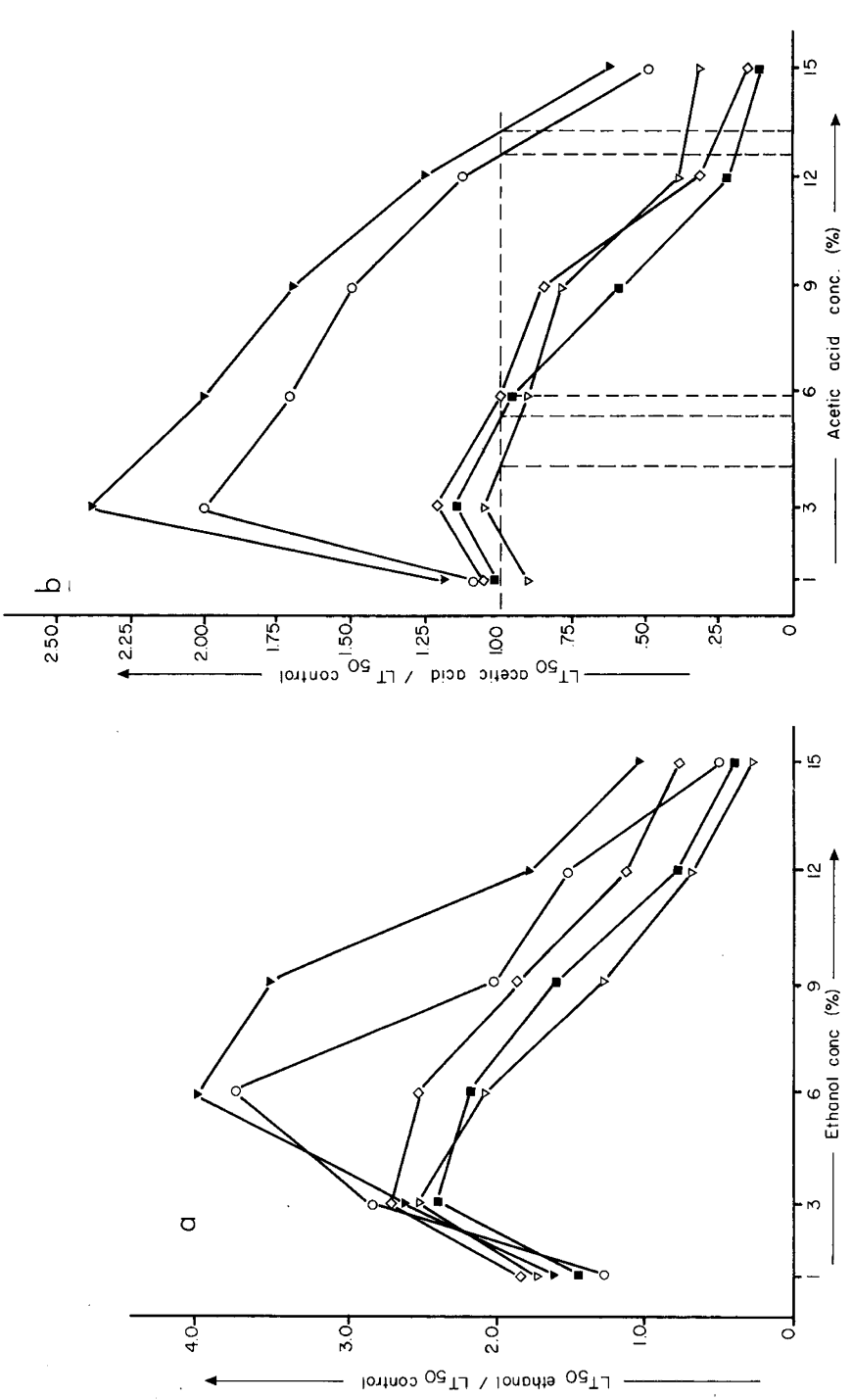

Fig 2. Adult longevity data (LT_{50} max/LT_{50} control) at different ethanol (**a**) and acetic-acid (**b**) concentrations in 5 Indian geographical populations of *D melanogaster* (▲ Dalhousie, ○ Rohtak, ◇ Nagpur, ■ Pune, △ Tirumala).

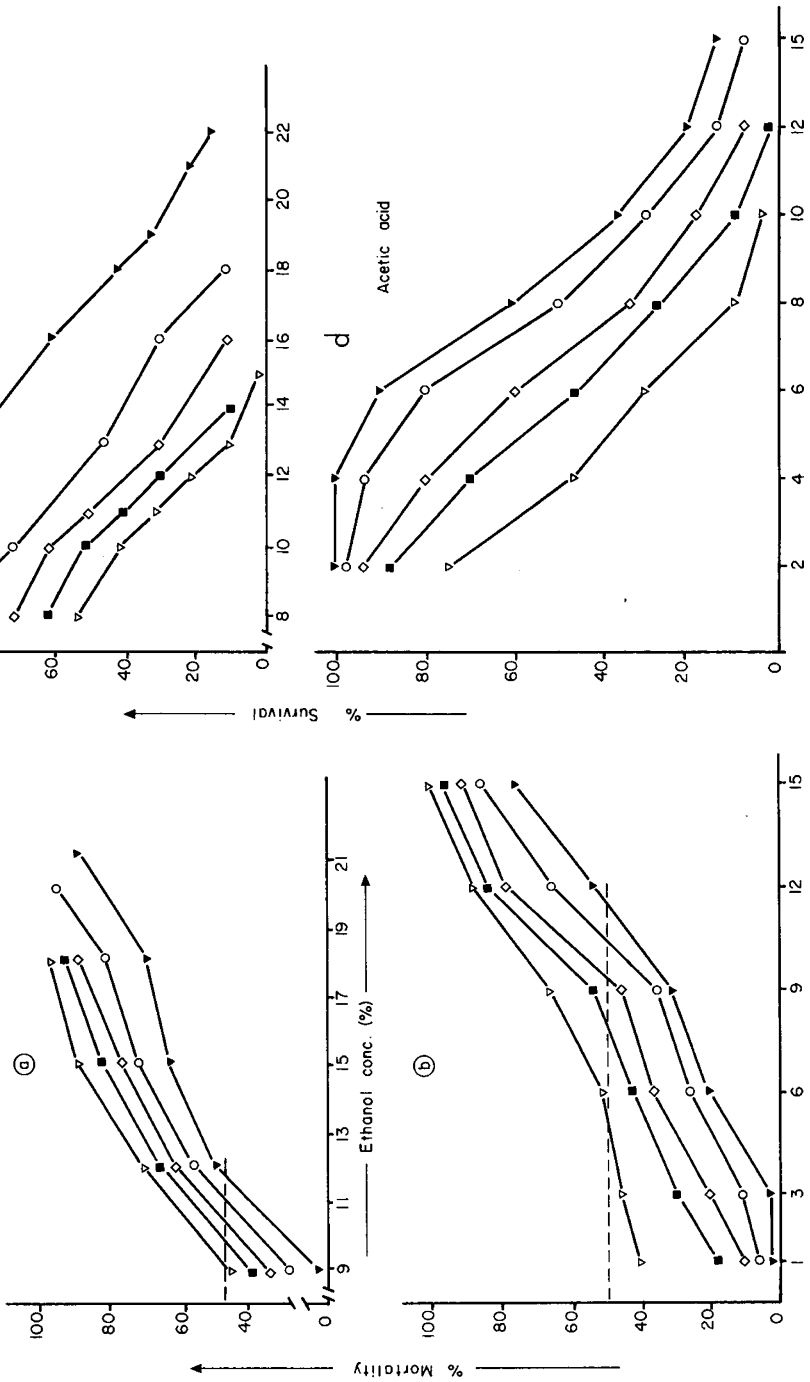

Fig 3. Comparative profiles of percentage mortality relationships for ethanol after 6 d (**a**), acetic acid after 3 d (**b**) and percentage survival on 6% ethanol (**c**) and 3% acetic-acid (**d**) in 5 geographical populations of *D melanogaster* (▲ Dalhousie, ○ Rohtak, ◇ Nagpur, ■ Pune, △ Tirumala).

1–13% for north Indian populations. The acetic-acid threshold values were found to vary clinally in the range of 3.7 to 13.2% for adults in geographical populations from south to north localities. The LC_{50} values revealed clinal variation in the range of 5.6 to 11.7% acetic acid, *ie* the southern populations had lower acetic-acid tolerance than the northern populations. Indian populations of *D melanogaster* revealed significant parallel genetic divergence in their potential to utilise ethanol and acetic acid. The parallel utilisation of ethanol and acetic acid in Indian tropical and subtropical populations of *D melanogaster* concur with such data on temperate populations of *D melanogaster* (Chakir *et al*, 1994).

High ethanol and acetic-acid-rich environments seem to be exploited by *D melanogaster*. *D melanogaster* utilises lower alcoholic concentrations but mainly detoxifies the higher ethanol concentrations occurring in its natural and man-made habitats. The observed genetic differentiation of ethanol tolerance in geographical Indian populations of *D melanogaster* concurs with other continental populations from Africa and Australia (Parsons, 1980b; David *et al*, 1986; David, 1988). The ethanol and acetic-acid tolerance threshold values in adult individuals were found to vary latitudinally in different Indian populations. The present observations are in agreement with other reports on the evidence of action of natural selection at the *Adh* locus as well as for ethanol tolerance in some allopatric populations (Hickey and McLean, 1980; Van Herrewege and David, 1980). Thus, these traits have adaptive significance and are being maintained by natural selection mechanisms.

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