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Original article

Latitudinal clines for alcohol dehydrogenase allozymic variation and ethanol tolerance in Indian populations of *Drosophila ananassae*

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Summary – Eight Indian geographical populations of *D ananassae*, collected along a 20°N latitudinal range, revealed significant clinal variation at the *Adh* (alcohol dehydrogenase) locus and Adh^F allelic frequency increased by about 1.5% with 1° latitude. Latitudinal increase of ethanol tolerance (1.8–3.7%) was observed in adults. Survival studies with adults showed that, in all cases, ethanol was used as a resource at low concentrations, while becoming a stress at higher concentrations. The resource/stress concentration threshold increased from 1.2 to 4% with latitude. Larval behaviour also exhibited an attraction/avoidance threshold, increasing from 1.6 to 4.4% ethanol with increasing latitude of origin. The parallel occurrence of latitudinal variation at the *Adh* locus and ethanol tolerance and utilisation in natural populations of *D ananassae* could be maintained by balancing the natural selection, which varies spatially along the north-south axis of the Indian sub-continent.

***Drosophila ananassae* / *Adh* polymorphism / ethanol utilisation / larval behaviour / latitudinal cline**

Résumé – Clines de latitude pour la variation allozymique de la déshydrogénase alcoolique (Adh) et la tolérance à l'éthanol dans des populations indiennes de *Drosophila ananassae*. *Huit populations géographiques indiennes de* D ananassae, *récoltées sur une étendue de 20 degrés de latitude nord, ont révélé une variation clinale significative au locus* Adh *(déshydrogénase alcoolique), et une augmentation de la fréquence de l'allèle* Adh^F *d'environ 1,5 point de pourcentage par degré de latitude. Une augmentation de la tolérance à l'éthanol avec la latitude (de 1,8 à 3,7%) a été observée chez les adultes.*

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Les études de survie sur adultes ont montré que, dans tous les cas, l'éthanol est utilisé comme une ressource lorsqu'il est à de faibles concentrations, alors qu'il devient un facteur de stress à des concentrations élevées. Le seuil entre ressource et stress se situait entre 1,2 et 4% selon la latitude. Le comportement larvaire a aussi montré un seuil d'attraction/évitement, augmentant de 1,6 à 4,4% d'éthanol avec l'augmentation de la latitude d'origine. Le parallélisme observé entre les variations du locus Adh *et la tolérance à l'éthanol et son utilisation dans les populations naturelles de* D ananassae *en fonction de la latitude pourrait être la conséquence d'une sélection naturelle équilibrante variant dans l'espace le long de l'axe nord-sud du sous-continent indien.*

***Drosophila ananassae* / polymorphisme de l'Adh / utilisation de l'éthanol / comportement larvaire / cline de latitude**

INTRODUCTION

Colonising species populations offer the most suitable material for microevolutionary studies (Endler, 1977; 1986). Eight *Drosophila* species are known as truly cosmopolitan while 21 *Drosophilia* species have been designated as widespread (David and Tsacas, 1981). Studies on biogeography and evolutionary history, chromosomal and allozymic polymorphism, ecological, behavioural and quantitative traits were made in the colonising populations of *D melanogaster* but such studies are lacking for most of the successful colonising and widespread drosophilids (David and Tsacas, 1981; David and Capy, 1988). *D ananassae* constitutes one of the most successful colonising and domestic species of the Indian sub-continent and was first described by Doleschall (1958) from Indonesia. Chromosomal polymorphism has been extensively studied in Indian natural populations of *D ananassae* (Singh, 1984a,b; 1989), but studies on characters such as enzyme polymorphism or physiological traits are totally lacking. To fill this gap, we have investigated *Adh* (alcohol dehydrogenase) polymorphism and ethanol tolerance in this species. *D ananassae* was found to exploit a variety of fermenting fruits in nature and larvae were observed physically immersed in fermented media. Since *Adh* is known to be involved in the utilisation and detoxification of exogenous alcohols, the present studies were made in order to analyse the extent of genic divergence at the *Adh* locus as well as ethanol tolerance in *D ananassae* populations from India.

MATERIALS AND METHODS

D ananassae, a member of the *D melanogaster* group in the Sophophora subgenus, is a successful colonising species throughout the Indian sub-continent. Isofemale lines were established from population samples of *D ananassae* from 8 Indian geographical sites (Rameswaram to Saharanpur; 9.17°N to 29.58°N, figure 1). Data on the number of isofemale lines, which were maintained for 5–6 generations in the laboratory, are given in table I. Homogenates of single individuals (one fly per isofemale line) were subjected to electrophoresis at 250 V and 25 mA at 4°C for 4 h. The gel slices were stained for the *Adh* gene–enzyme system by a standard

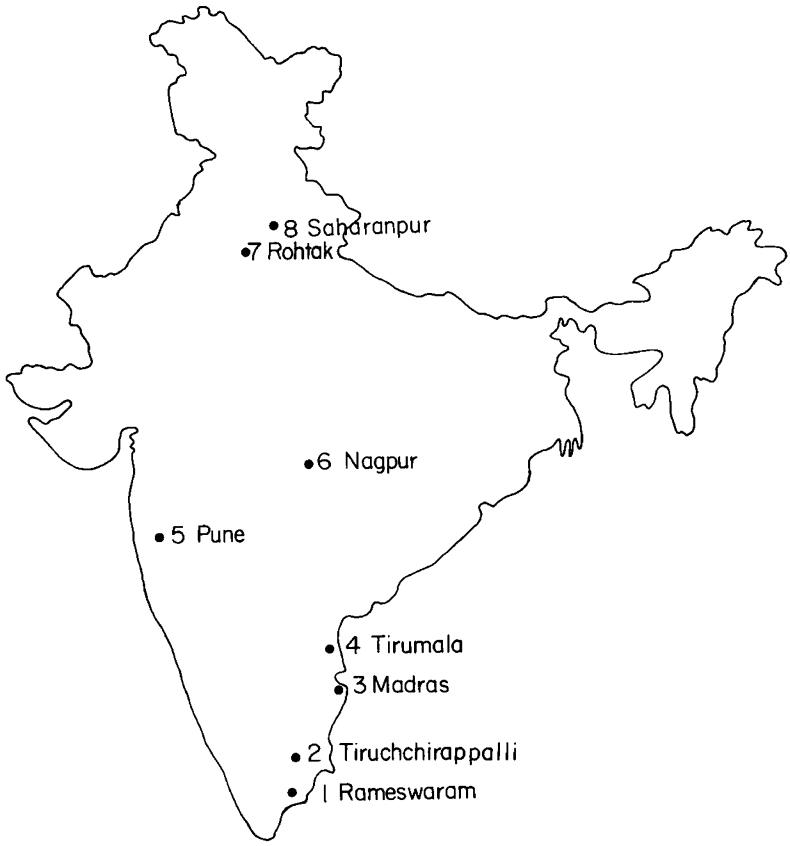

Fig 1. Map of Indian sub-continent depicting collection sites of 8 Indian populations of *D ananassae*: 1: Rameswaram (9°17′N); 2: Tiruchchirappalli (10°50′N); 3: Madras (13°04′N); 4: Tirumala (13°40′N); 5: Pune (18°31′N); 6: Nagpur (21°09′N); 7: Rohtak (28°54′N); 8: Saharanpur (29°58′N).

staining procedure (Harris and Hopkinson, 1976). 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 adult ethanol tolerance was assessed following the longevity test of Starmer *et al* (1977). In order to test ethanol utilisation, groups of 10 males or females, grown on killed yeast medium, were aged for 2 d on fresh food medium and then transferred to a set of 2 air-tight plastic vials which contained different ethanol concentrations (1–7%). All experiments were run in 5 replicates at 20°C and control experiments employed water in place of ethanol solution. Adult survivorship was monitored by daily observations of control and ethanol treatment experiments. The LT_{50} values were calculated as the number of hours at which 50% of the flies had died and were estimated by linear interpolation. The ethanol resource utilisation values were represented by the ratio LT_{50} ethanol/LT_{50} control, *ie* if this ratio was > 1, ethanol

Table I. Data on allelic frequencies at the *Adh* locus and number of isofemale lines (*N*) analysed in 8 latitudinally varying Indian natural populations of *D ananassae*.

Population	*Latitude*	*Allelic frequency*		N
		F	*S*	
Rameswaram	9°17′N	0.42	0.58	95
Tiruchchirappalli	10°50′N	0.45	0.55	80
Madras	13°04′N	0.47	0.52	74
Tirumala	13°40′N	0.50	0.50	70
Pune	18°31′N	0.52	0.48	75
Nagpur	21°09′N	0.54	0.46	78
Rohtak	28°54′N	0.66	0.34	88
Saharanpur	29°58′N	0.72	0.28	90

was utilised as a resource, but if this value was < 1, it represented stress. The ethanol threshold concentration was obtained when LT_{50} ethanol/LT_{50} control was equal to 1. The larval behaviour towards ethanol was analysed by following the method of Gelfand and McDonald (1983). The relative numbers of the larvae out of a total of 10 on the 2 sectors of agar Petri dishes (with and without ethanol) were noted after 20 min for each ethanol concentration. Five replicates were tested at each ethanol concentration at 20°C for each *D ananassae* population. The threshold values between attraction and avoidance after 20 min were then calculated.

RESULTS

Genetic basis of Adh *polymorphism*

The *Adh* enzyme in *D ananassae* revealed a single cathodal zone of activity. Segregating 2-banded patterns (of either faster or slower mobilities) and 4-banded patterns of *Adh* were observed in the individuals of *D ananassae*. Genetic crosses involving different 2-banded patterns resulted in 4-banded patterns in F_1 individuals, and 1:2:1 ratio of segregating 2-banded and 4-banded patterns in the F_2 progeny. Thus, *Adh* electrophoretic data of the parents and progeny of genetic crosses was found to be in agreement with a monogenic control of *Adh* patterns. The homozygous individuals exhibit a 2-banded pattern and the observed *Adh* electromorphs correspond to post-translational or conformational isozymes. The present observations correspond to what has been known for *Adh* for a long time in *D melanogaster* and other species.

Latitudinal Adh *allozymic variation*

The data on *Adh* allelic frequencies in 8 Indian populations are given in table I. The Adh^F frequency increased significantly with increasing latitude (1.5% with 1° latitude, $r = 0.92$). The *Adh* locus revealed significant interpopulation genotypic heterogeneity (141.07) and allelic frequency heterogeneity (33.3) on the basis of

contingency chi-squared tests among the Indian populations. The data on Wright's fixation index ($F_{ST} = 0.21$) revealed significant genic divergence at the *Adh* locus in Indian populations. Thus, the allelic frequency patterns at the *Adh* locus revealed significant clinal variation (along the south–north axis) among Indian populations.

Ethanol utilisation by adults

The *D ananassae* adults were analysed for their potential to utilise ethanol vapours in a closed system and the data from 8 geographical populations of *D ananassae* are given in figures 2 and 3. Adult longevity was found to increase in the range of 1 to 2% ethanol in south Indian populations while 1–4% ethanol revealed enhanced longevity in the north Indian populations (fig 2). The data revealed that the south Indian population of Rameswaram had a longevity of 141 h compared with the north Indian population of Saharanpur in which it was 175 h. However, the other 6 geographical populations revealed intermediate values (table II). The data on LT_{50}

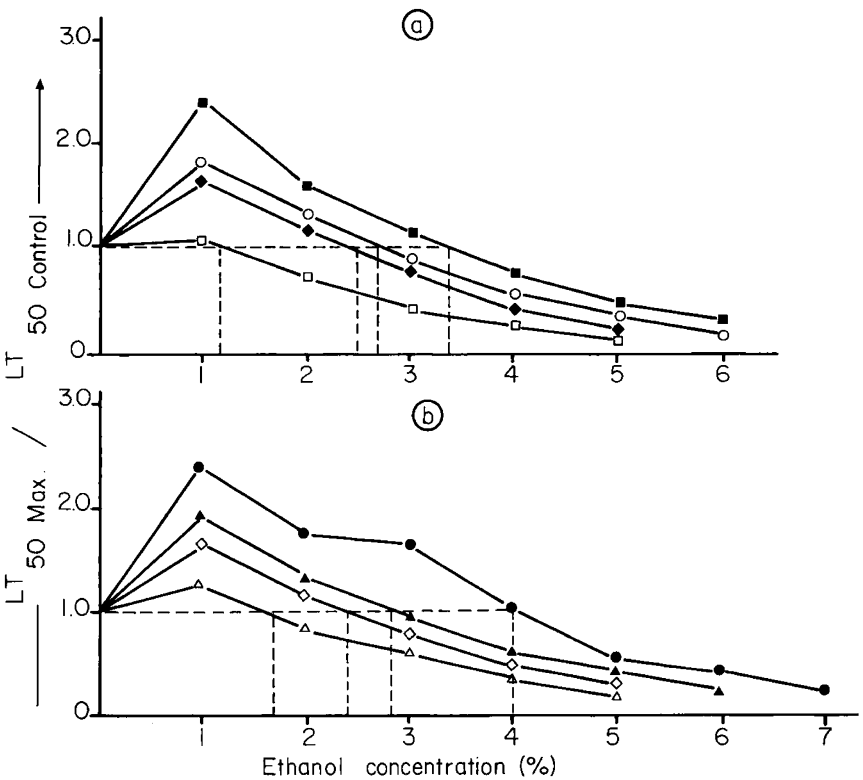

Fig 2. Comparative profiles of adult longevity at different ethanol concentrations in 8 geographical populations of *D ananassae*: (a) LT_{50} control; (b) LT_{50} max. ■—■ Rohtak; o—o Pune; ♦—♦ Tirumala; □—□ Rameswaram.

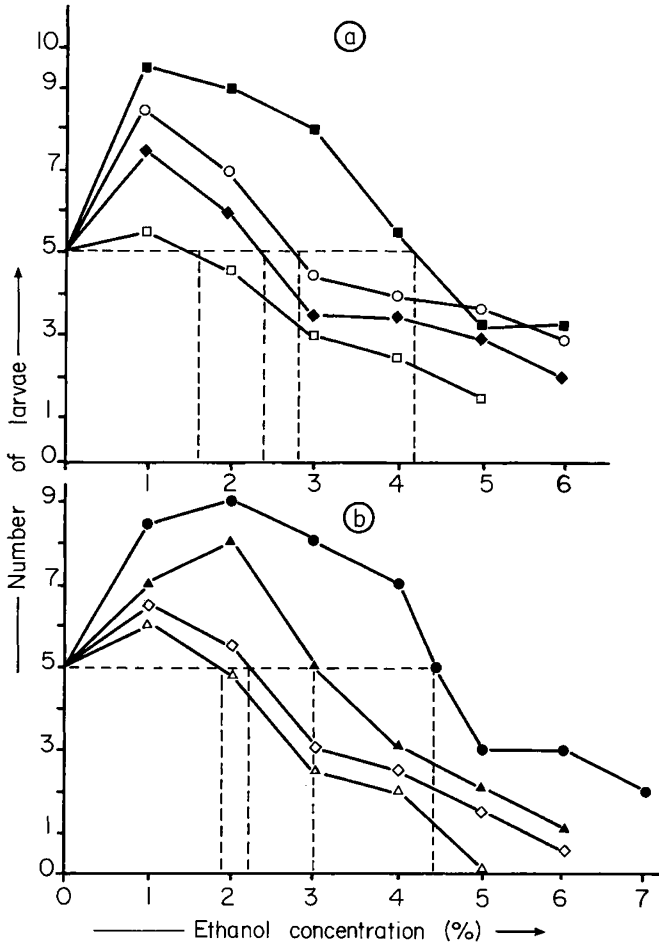

Fig 3. Mean number of larvae out of 10 preferring different ethanol concentrations in 8 Indian geographical populations of *D ananassae*. a) ■—■ Rohtak; ○—○ Pune; ♦—♦ Tirumala; □—□ Rameswaram; b) ●—● Saharanpur; ▲—▲ Nagpur; ◇—◇ Madras; △—△ Tirchirappalli.

ethanol/LT_{50} control (which constitute the measure of resource *versus* stress) are shown in figure 2. The adult ethanol threshold values were found to vary clinally in the range of 1.2 to 4.0% among the 8 populations from south to north of the Indian sub-continent (table II). Thus, ethanol concentration in the range of 3.4 to 4.0% served as a resource for north Indian populations while significantly lower ethanol concentrations (1.2–2.8%) could be utilised by south Indian populations of *D ananassae*.

Table II. Data on ethanol tolerance indices (increase in longevity, LT_{50} (h); LT_{50} ethanol/LT_{50} control; adult and larval ethanol threshold concentrations and LC_{50} values) in 8 geographical Indian populations of *D ananassae*.

Population	*LT_{50} (h)*	*LT_{50} ethanol/LT_{50} control*	*Ethanol threshold concentration*		*LC_{50} (on 4th day)*
			Larval	*Adult*	
Rameswaram	141	1.04	1.6	1.2	1.8
Tiruchchirappalli	147	1.26	1.8	1.6	2.0
Madras	150	1.67	2.2	2.4	2.2
Tirumala	150	1.72	2.4	2.5	2.4
Pune	156	1.79	2.7	2.7	3.0
Nagpur	162	1.93	3.0	2.8	3.2
Rohtak	165	2.39	4.2	3.4	3.4
Saharanpur	175	2.43	4.4	4.0	3.7

Adult ethanol tolerance

In 5 Indian populations of *D ananassae* that could utilise ethanol as a resource up to 1.5% longevities were compared at 1% ethanol and the data revealed interpopulational divergence (fig 4a). The toxic effects of ethanol concentrations were observed from mortality data on the 4th day of ethanol treatment of adults and LC_{50} values revealed clinal variation from 1.8 to 3.7%, *ie* southern populations of *D ananassae* displayed significantly lower ethanol tolerance than the north Indian populations (fig 4b).

Larval behaviour

The data on larval behaviour towards a range of concentrations of ethanol (1–6%) are represented in figure 5 and table II. The larval ethanol threshold values varied from 1.6% in the Rameswaram population to 4.4% in the Saharanpur population. The ranking order of populations is Saharanpur > Rohtak > Nagpur > Pune > Tirumala > Madras > Tiruchchirappalli > Rameswaram. The larval individuals of 8 populations of *D ananassae* revealed higher ethanol tolerance than those of adults but the pattern of clinal variation was found to be similar for both the adult and larval stages (table II). The ethanol indices in larval and adult individuals were found to vary latitudinally in all 8 populations of *D ananassae* (fig 5). The statistical correlations were found to be significantly higher among latitudinal variation *versus* larval and adult ethanol tolerance (table III). The *Adh–F* allelic frequency also revealed significant correlation with latitude. Thus, ethanol tolerance seems to be adaptively maintained by natural selection mechanisms.

Table III. Correlation coefficient (r) values between latitudes and biological variables (*Adh–F* frequency and ethanol tolerance) in 8 populations of *D ananassae*.

Parameters	r-*values*
Latitude *versus Adh–F*	+0.97*
Latitude *versus* adult ethanol tolerance	+0.94*
Latitude *versus* larval ethanol tolerance	+0.98*
Adult ethanol tolerance *versus Adh–F*	+0.92*
Larval ethanol tolerance *versus Adh–F*	+0.97*
Adult *versus* larval ethanol tolerance	+0.95*

* Significant at 5 percent level.

DISCUSSION

The present data on clinal variation at the *Adh* locus in Indian populations of *D ananassae* further supported and validated the hypothesis that occurrence of latitudinal clines among geographical populations provides strong evidence of natural selection maintaining such clinal allozymic variation (Nagylaki, 1975;

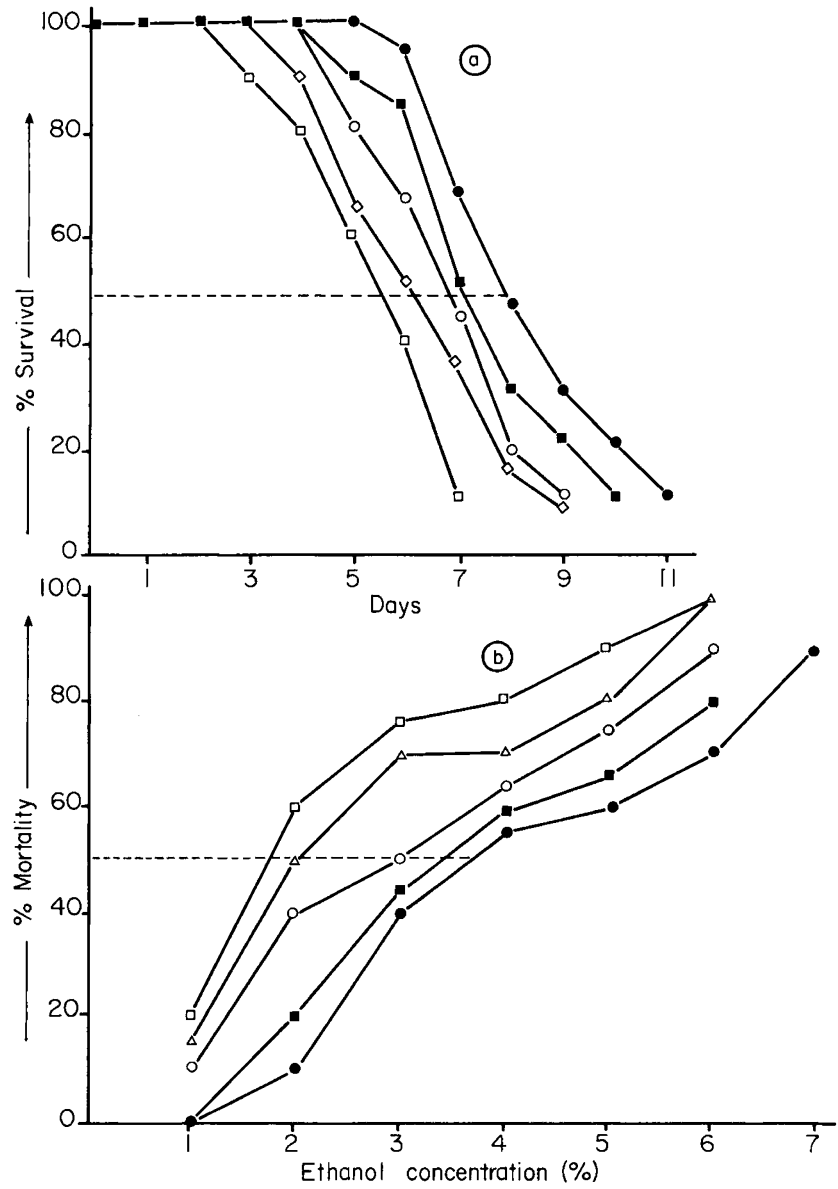

Fig 4. a) Percent adult survival at 1% ethanol; and **b)** percent mortality relationship in 5 Indian populations of *D ananassae*. •—• Saharanpur; ■—■ Rohtak; ○—○ Pune; ◊—◊ Madras; □—□ Rameswaram.

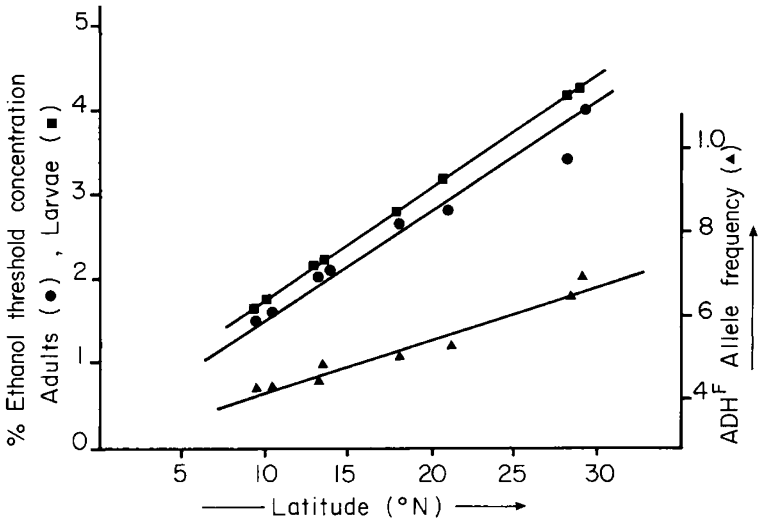

Fig 5. Latitudinal relationships of *Adh–F* allelic frequency, adult and larval ethanol threshold concentrations in 8 geographical populations of *D ananassae*.

Oakeshott *et al*, 1982). The observed latitudinal variation in *D ananassae* concurred with other reports on the populations of *D melanogaster*, *ie* US populations (Marks *et al*, 1980; Van Delden, 1982); Australian populations (Oakeshott *et al*, 1982); and European and African populations (David *et al*, 1986). The observed data on *D ananassae* could be explained on the basis of the niche-width variation hypothesis, *ie* the amount of variation in a species was proportional to the niche-width. It has been argued that a species characterized by utilisation of diverse food resources and/or climatic adaptations should possess a significantly higher amount of genic divergence compared with narrow niche-width species (Parsons, 1983; Spiess, 1989).

The Indian geographical populations of *D ananassae* revealed significant genetic divergence in their potential to utilise ethanol. Adult longevity was found to increase significantly when ethanol increases from 1 to 2% for south Indian populations and from 1 to 4% for north Indian populations of *D ananassae*. The ethanol threshold values were found to vary clinally in the range of 1.2 to 4.0% in the case of adults and 1.6 to 4.4% for larvae in geographical populations of *D ananassae* from south to north localities. The LC_{50} values revealed a clinal variation in the range of 1.8 to 3.7% ethanol, *ie* southern populations displayed lower ethanol tolerance than the northern populations. The ethanol tolerance threshold values in larval and adult individuals were found to vary latitudinally in different Indian populations of *D ananassae*. 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 of *D melanogaster* (Hickey and Mclean, 1980). Thus, both these traits have adaptive significance and are maintained by natural selection mechanisms.

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