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Latitudinal variation in octanol dehydrogenase and acid phosphatase allele frequencies in *Drosophila melanogaster*

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Summary. The octanol dehydrogenase (*Odh*) and acid phosphatase (*Acph*) loci of *Drosophila melanogaster* are each polymorphic for two electrophoretically detectable alleles. The frequencies of the *Odh* and *Acph* alleles have been analysed in populations sampled from up to a 40° latitudinal range in each of Australasia, North America and Europe/Asia. Odh^S frequency is found to be significantly negatively associated with distance from the equator in all three zones. The relationship of $Acph^S$ frequency to distance from the equator is significant and negative in Australasia but neither significant nor consistent in sign in North America and Europe/Asia.

Key words: *Drosophila* – Enzyme polymorphisms – Latitudinal clines

Introduction

Over the past two decades geographic surveys of allele frequencies have been undertaken for many polymorphic enzyme loci in numerous plant and animal species (see Nevo 1978 for a review). Yet the results have not produced any general solution to the selectionist-neutralist debate because most geographic patterns of allele frequencies can be explained by either selective or stochastic processes (Ayala et al. 1974; Lewontin 1974 for discussions).

One pattern which has recently been interpreted as convincing evidence for selection is a consistent association between allele frequency and distance from the equator over a large latitudinal range in both hemispheres and across different zoogeographic zones. This pattern has been found for five out of the first six polymorphic enzyme loci systematically scored (*Adh, Gpdh, G6pd, Pgd* and *Est-6*, but not *Pgm*) in populations of *Drosophila melanogaster* sampled over about 40° of latitude in each of Australasia, North America and Europe/Asia (Wilks et al. 1980; Oakeshott et al. 1981, 1982, 1983). Since such a high proportion of these few polymorphisms demonstrate evidence of selection, it is important to establish the generality of this latitudinal pattern over additional polymorphic systems. This paper reports the results for octanol dehydrogenase (*Odh*) and acid phosphatase (*Acph*) in *D. melanogaster.*

Odh and *Acph* are each polymorphic in this species for two electrophoretic variants. Johnson and Schaffer (1973) and Voelker et al. (1978) have already reported a significant latitudinal cline in the frequencies of the *Odh* but not *Acph* alleles in eastern USA. They found Odh^S systematically decreased in frequency from about 16% between 25° and 30° latitude to about 8% between 40° and 45°, while $Acph^S$ remained at around 5% frequency over the 18° latitudinal range sampled. This paper tests whether the negative relationship between Odh^S frequency and latitude and the geographic homogeneity for $Acph^S$ found in eastern USA recur in Australasia and Europe/Asia. It also describes analyses testing for associations of *Odh* and *Acph* allele frequencies with some climatic variables which might underlie the geographic patterns.

Materials and methods

Odh allele frequencies were scored in 22 collections and *Acph* allele frequencies in 41 collections after electrophoresis on gradient polyacrylamide gels (High Purity GG-1A "Gradipore": Sydney, Australia) and staining according to Courtright et al. (1966) and MacIntyre (1966). An additional 50 *Odh* allele frequency scores and 39 *Acph* allele frequency scores were obtained from 16 previously published reports; Anxolabehere et al. (1973), Band (1975), Cavener and Clegg (1978,

1981), Franklin (1981), Girard (1976), Girard et al. (1977), Johnson and Schaffer (1973), Kojima et al. (1970), Langley et al. (1974, 1977), Mukai et al. (1974), Mukai and Voelker (1977), Smith et al. (1978), Triantaphyllidis (1973), Triantaphyllidis et al. (1980) and Voelker et al. (1978).

The Australasian samples were from Australia, New Zealand and Papua-New Guinea and the North American samples were from Canada and the USA. The European and Asian samples were collected from Belgium, Finland, France, Greece, Hungary, Italy, Japan, The Netherlands, The Seychelle Islands, Spain, West Germany and Yugoslavia. The breakdown of the number of collections analysed by zoogeographic zone, with our data first followed by those from other reports was:

	Australasia	North America	Europe/Asia
Odh	19+5	0+32	3+13
Acph	24+2	0+23	17+14

Seven of the 32 *Odh* scores and three of the 23 *Acph* scores in North America had not been included in the analyses of Johnson and Schaffer (1973) and Voelker et al. (1978). Fifty-two of the total of 100 collections were scored for both *Odh* and *Acph*. All 100 populations had been collected less than 12 months prior to scoring and the mean and standard error of the number of genes scored per collection were 563 ± 158 for *Odh* and 383 ± 135 for *Acph*. Only data based on more than 50 genes were included in the analysis.

Sources of data on latitude, longitude, altitude and four climatic variables were the same as in Wilks et al. (1980) and Oakeshott et al. (1981, 1982, 1983). The four climatic variables (20 year averages) were T_{max} (average daily maximum temperature in °C for the hottest calendar month), T_{min} (average daily minimum temperature in °C for the coldest calendar month), R_{max} (total rainfall in mm for the wettest calendar month) and R_{min} (total rainfall in mm for the driest calendar month). Climatic data were not available for three collections from Europe and Asia. Northern and southern latitudes were all analysed as positive values to represent distances from the equator but longitude was signed, with eastern values positive and western negative. As was explained in Oakeshott et al. (1981, 1982), values of altitude (metres) required logarithmic transformation, both rainfall variables needed transformation into square roots, and allele frequencies were angularly transformed.

Results

Octanol dehydrogenase

Odh^F and Odh^S were the only electrophoretically detectable alleles found among the 72 collections analysed and Odh^S was always the less common allele, ranging from 0% in 12 samples to 37% in one (Fig. 1). Partial correlation and regression analyses (Table 1) indicated that Odh^S frequency was significantly negatively related to latitude in each of the three zoogeographic zones. (Most of the data for Europe/Asia was from Europe and the relationship remained significant and negative when only the European data were included in the analysis.)

Analysis of covariance confirmed that the differences between zones in the slope of the latitudinal relationship were not significant ($F_{(2,66)} = 1.23$, $P > 0.05$) and explained only 2% of the total variance in Odh^S frequency between all 72 samples: the latitudinal relationship common to all three zones was highly significant ($F_{(1,66)} = 22.13$, $P < 0.001$) and explained 15% of total variance; mean differences in Odh^S frequency between zones were also highly significant, even after adjusting for the overall latitudinal relationship ($F_{(2,66)} = 27.87$, $P < 0.001$), when they explained 38% of total variance. As the following means and standard errors show, the greatest differences in mean Odh^S frequency were between North America and Europe/Asia, although the North American collections were generally at intermediate latitudes:

	Australasia	North America	Europe/Asia
latitude	28.3 ± 1.9	34.0 ± 1.0	45.3 ± 1.4
$\%Odh^S$	4.6 ± 1.6	10.9 ± 0.8	2.6 ± 1.3

There were no consistent associations between Odh^S frequency and longitude, altitude, T_{min}, R_{max} and

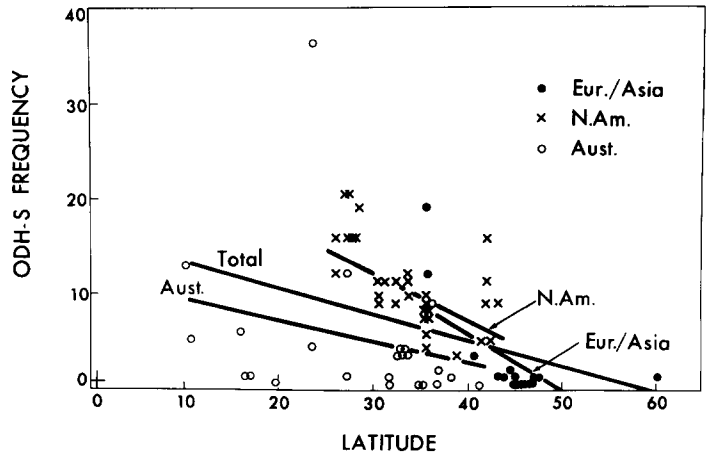

Fig. 1. Odh^S frequency with latitude in Australasia, North America and Europe/Asia. The regression lines relating the untransformed Odh^S frequencies to latitude are shown within each zone and across all three. (The analyses in Tables 1 and 2 were carried out on angularly transformed allele frequencies)

Table 1. Partial correlation (r) and multiple regression (b) coefficients of Odh^S frequencies on location and climatic variables. Only significant b values are given. There were insufficient climatic data for useful analyses of the European/Asian *Odh* scores

	r			b		
	lat	long	alt	lat	long	alt
Australasia	−0.59**	0.04	0.69***	−0.57**		8.40***
N. America	−0.54**	−0.30	−0.41*	−0.36**		−2.23**
Eur./Asia	−0.66**	0.89***	0.48	−0.31*	0.18***	

	r				b			
	T_{max}	T_{min}	R_{max}	R_{min}	T_{max}	T_{min}	R_{max}	R_{min}
Australasia	0.66**	0.25	−0.35	0.29	1.81***			
N. America	0.18	−0.39*	0.62***	−0.62***			1.44***	−0.97**

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ in two-tailed tests

R_{min}. However Odh^S frequency was positively related to T_{max} in both Australasia and North America. Although the correlation with T_{max} in North America was not significantly different from zero, it was not significantly different from the Australasian correlation (which was significantly different from zero) either ($P > 0.05$). And when the data from all three zones were pooled, the partial correlation and the regression coefficient between Odh^S frequency and T_{max} were both positive and highly significant ($r = 0.64$, $b = 1.61$, $P < 0.001$).

Acid phosphatase

No electrophoretically detectable *Acph* alleles other than $Acph^F$ and $Acph^S$ were recorded in the 80 collections scored and $Acph^S$ frequency ranged from 0% in 29 samples to 18% in one (Fig. 2). There was a negative association between $Acph^S$ frequency and latitude in Australasia which was significant at the 5% level (Table 2). However this relationship was not apparent in either of the other two zones where, despite similar sample sizes, the associations were small and inconsistent in sign.

An analysis of covariance indicated that the negative relationship with latitude remained significant at the 5% level when the data for all three zones were pooled ($F_{(1,74)} = 5.74$, $P < 0.05$). However this only accounted for 4% of the total variance among all 80 samples. The zonal differences in the slope of this weak association were not significant ($F_{(2,74)} = 2.38$, $P > 0.05$) and accounted for 3% of total variance. Differences between zones in $Acph^S$ frequency were highly significant even after adjusting for overall latitudinal effects ($F_{(1,74)} = 26.89$, $P < 0.001$) and these differences accounted for 39% of total variance. As with *Odh*, (and indeed *Adh*, *Gpdh*, *G6pd* and *Pgd*; Oakeshott et al. 1981, 1983) the greatest difference in average $Acph^S$ frequency was between North America and Europe/

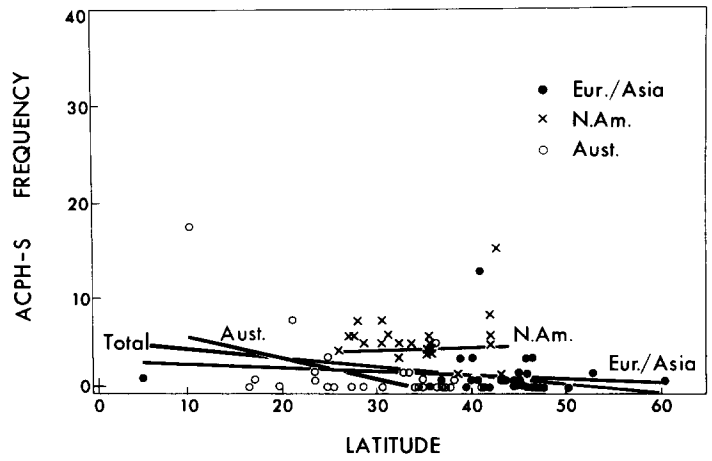

Fig. 2. $Acph^S$ frequency with latitude in Australasia, North America and Europe/Asia. Regression lines are calculated as in Fig. 1

Table 2. Partial correlation (r) and multiple regression (b) coefficients of $Acph^S$ frequencies on location and climatic variables. Only significant b values are given

	r			b		
	lat	long	alt	lat	long	alt
Australasia	−0.49*	−0.04	0.28	−0.36*		
N. America	0.04	−0.23	0.02			
Eur./Asia	−0.16	−0.17	−0.06			

	r				b			
	T_{max}	T_{min}	R_{max}	R_{min}	T_{max}	T_{min}	R_{max}	R_{min}
Australasia	0.46*	0.57**	−0.52	0.15	0.81*	0.98**	−1.24*	
N. America	−0.07	0.01	−0.05	−0.11				
Eur./Asia	0.06	−0.05	−0.01	−0.23				

* $P < 0.05$, ** $P < 0.01$ on two-tailed tests

Asia, although these were not the most differentiated zones in terms of the average latitude of collecting sites:

	Australasia	North America	Europe/Asia
latitude	29.3 ± 1.6	34.6 ± 1.2	43.5 ± 1.6
$\%Acph^S$	1.7 ± 0.7	5.4 ± 0.5	1.3 ± 0.4

$Acph^S$ frequency was not consistently associated with longitude, altitude or any of the four climatic variables across the three zones. The only significant climatic associations were in Australasia, where $Acph^S$ frequency was significantly positively correlated with T_{max} and T_{min} and significantly negatively correlated with R_{max}.

Discussion

These analyses have revealed associations between Odh^S frequency and distance from the equator which are consistent in sign and slope and which extend over about 40° of latitude on either side of the equator and in three different zoogeographic zones. This strongly suggests that the *Odh* alleles are subject to natural selection and that a stable and pervasive latitudinal gradient exists in the selective differences between the *Odh* genotypes.

Associations between Odh^S frequency and the four climatic variables were investigated to provide information on the environmental agent(s) which might contribute to the selection gradients. A consistent positive correlation was indeed found between Odh^S frequencies and T_{max} but this need not reflect direct selective effects of high temperatures. It could also be explained by the selective action of other, undefined, ecological variables correlated with T_{max}.

It is interesting in this respect that neither Franklin (1981) nor Cavener and Clegg (1981) could detect consistent seasonal variation in Odh^S frequencies, despite very intensive sampling of particular Australian and North American populations. This argues against direct selective effects of high temperatures and in favour of the action of ecological variables (e.g. food, competitor and predator species) which might correlate with temperature maxima across localities spanning vast latitudinal ranges, but not across seasons within localities. However, it must be admitted that climatic differences between years and the few generations per season would hinder the detection of any systematic seasonal changes in allele frequency which did occur, unless the underlying selection coefficients were relatively large.

The inconsistency of the relationship between Odh^S frequency and altitude (Table 1) is also worth noting here. Some similarity might be expected between the climatic effects of changing latitude and altitude. However, in the present samples, altitude correlated with

Table 3. Partial correlations of latitude and altitude with the four climatic variables in the samples analysed in each zoogeographic zone

	Australasia	North America	Europe/Asia
lat-T_{max}	−0.65***	−0.78***	−0.60**
lat-T_{min}	−0.52***	−0.70***	−0.33
lat-R_{max}	−0.46***	−0.10	−0.79***
lat-R_{min}	−0.20	−0.34*	0.52***
alt-T_{max}	0.33**	0.31*	0.07
alt-T_{min}	−0.29*	−0.27*	−0.47**
alt-R_{max}	−0.16	−0.06	0.02
alt-R_{min}	0.15	−0.02	−0.26

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ in two-tailed tests

the climatic variables far less strongly and consistently than did latitude (Table 3).

There is little other evidence in the literature to suggest the agents or mechanisms of selection which might underlie the *Odh* clines. Consistent and sometimes statistically significant excesses of *Odh* heterozygotes have been found in collections from wild (Cavener and Clegg 1981) and laboratory populations (Laurie-Ahlberg and Weir 1979). And *Odh* allele frequencies appear to be stable over years within Australian (Franklin 1981) and North American (Smith et al. 1978; Cavener and Clegg 1981) wild populations. These data are consistent with heterozygote advantage and balancing selection but they do not explain latitudinal clines.

Evidence is needed of different selective modes in different environments. Clark (1982) has demonstrated that the ODH-S allozyme has greater in vitro enzymic activity than ODH-F. However, there is as yet no evidence that such biochemical differences between the allozymes are reflected in environment-dependent fitness differences between genotypes. Minawa and Birley (1978) found Odh^S frequencies had not diverged significantly among laboratory populations kept for over a year in three environments differing with respect to temperature and components of the food media.

It should also be noted that the selection gradients giving rise to the *Odh* clines might not act directly on the locus, but on linked genes in gametic disequilibria with *Odh* alleles. This possibility cannot be ruled out by the available data but consistent gametic disequilibria have not been reported in wild populations between *Odh* and the linked chromosome inversion *In(3R)P* (Voelker et al. 1978), or between *Odh* and alleles of the closely linked (0.2 cM) *Est-C* locus (Mukai et al. 1974; Mukai and Voelker 1977).

No relationship between $Acph^S$ frequency and latitude is apparent in North America or Europe/Asia, but a weak negative association is found in Australasia. The Australasian relationship is only significant at the 5% level, but it is supported by the climatic associations of $Acph^S$ frequency in this zone, and by other Australasian data showing higher $Acph^S$ frequencies in summer than other seasons. In his five year survey of six Hunter Valley populations (on the central eastern seaboard of Australia), Franklin (1981) found an average $Acph^S$ frequency of $3.1 \pm 0.3\%$ in summer collections and only $2.1 \pm 0.1\%$ at other times.

No information has been published on biochemical differences between the ACPH-F and ACPH-S allozymes. The only report of fitness differences between *Acph* genotypes was that of Nassar (1980), who used a laboratory population derived from Kansas, USA and found frequency dependent selection operating to maintain $Acph^S$ frequencies similar to those originally observed in the wild population.

Many environmental and genetic effects might contribute to the difference between the latitudinal associations of $Acph^S$ frequency in Australasia versus North America and Europe/Asia. For example, gametic disequilibrium between *Acph* alleles and the linked inversion *In(3R)P* has only been investigated in North America, where it is found to be small (Voelker et al. 1978); in Australasia it may be sufficiently large that the *Acph* cline can be explained by the latitudinal cline known to exist for *In(3R)P* (Knibb et al. 1981). A precedent for such continental differences is the recent finding by Knibb (1983) that disequilibria with the linked inversion *In(2L)t* can account for the *Gpdh* latitudinal clines in Australasian but not North American populations of this species.

Nor are the inversions the only components of the genetic background which will differ between zoogeographic zones. Several other allozyme loci in addition to *Acph* and *Odh* also show substantial mean differences in frequency between zones (Oakeshott et al. 1981, 1982, 1983); this suggests substantial differentiation in genetic backgrounds between regions. To the extent that the genetic background influences the selection on an individual locus, the differences in background may account for the inconsistency of the *Acph* latitudinal cline (and indeed its climatic associations) across zones.

To conclude, the *Odh* and *Acph* results can be collated with analogous analyses for *Adh, Gpdh, G6pd, Pgd, Est-6* and *Pgm* in *D. melanogaster* (Oakeshott et al. 1981, 1982, 1983). Of these eight polymorphisms, one (*Pgm*) shows no latitudinal associations on any of the zoogeographic zones, two (*Acph* and, after considering *In(2L)t, Gpdh*) show some latitudinal associations which are inconsistent across zones, and five (*Odh, Adh, G6pd, Pgd* and *Est-6*) show large-scale complementary latitudinal clines inexplicable by inversions in all three zones. The results indicate that the last five enzyme polymorphisms are subject to natural selection, but of course do not constitute evidence that the first three are not.

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