Heredity (1983) **50**, (1), 67–72 0018-067X/83/05810067$02.00

GEOGRAPHIC VARIATION IN *G6pd* AND *Pgd* ALLELE FREQUENCIES IN *DROSOPHILA MELANOGASTER*

J. G. OAKESHOTT*, G. K. CHAMBERS†, J. B. GIBSON*,
W. F. EANES‡ AND D. A. WILLCOCKS*

** Department of Population Biology, Research School of Biological Sciences, Australian National University, P.O. Box 475, Canberra City, A.C.T. 2601; Australia. †Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138, U.S.A..*
‡ Department of Ecology and Evolution Division, of Biological Sciences, State University of New York at Stony Brook, Stony Brook, New York 11794, U.S.A.

Received 14.ix.82

1. INTRODUCTION

Large-scale and complementary latitudinal clines have recently been found in Australasia, North America and Europe/Asia for allele frequencies at three out of four enzyme loci studied in *Drosophila melanogaster* (*Adh*, *Gpdh* and *Est*-6 but not *Pgm*; Oakeshott *et al.*, 1981, 1982). This paper tests for similar patterns for alleles of glucose-6-phosphate dehydrogenase ($G6pd^F$ and $G6pd^S$) and 6-phosphogluconate dehydrogenase (Pgd^F and Pgd^S) in this species. These two loci are unlinked to each other on the inversion-free X chromosome (*G6pd* at 1-63, *Pgd* at 1-0·9; Cavener and Clegg, 1981*a*).

2. MATERIALS AND METHODS

G6pd allele frequencies were scored in 48 collections from Australasia (including 7 from New Zealand), 35 from North America, 22 from Europe and one from Asia. *Pgd* allele frequencies were obtained from all these collections except seven from North America. Both loci were scored by electrophoresis of adult males (*Pgd* was sometimes difficult to score in females) in 7·5 per cent polyacrylamide gels (Tris-borate-EDTA, pH 8·5) and staining as in Bijlsma and van Delden (1977). In addition, *G6pd* allele frequencies for eight North American collections, one from Europe and two from Asia were available from Kojima *et al.* (1970), Berger (1971), Band (1975), Cavener and Clegg (1981*a*), Hori and Tanda (1981) and C. H. Langley (unpublished). *Pgd* allele frequencies were also available for one of the Asian and six of the North American collections. All 117 populations analysed were kept in the laboratory for less than 12 months prior to scoring. The number of genes scored per sample ($\bar{x} \pm$S.E.) was 247 ± 48 for *G6pd* and 225 ± 52 for *Pgd*.

Sources of data on latitude, longitude, altitude and four climatic variables were the same as in Oakeshott *et al.* (1981). The climatic variables (20-year averages) were Tmax (average daily maximum temperature in °C for the hottest month), Tmin (average daily minimum temperature in °C for the coldest month), Rmax (total rainfall in mm for the wettest month) and Rmin (total rainfall in mm for the driest month). Northern and southern latitudes were all given positive values to represent distance from the equator but longitude was signed (western values negative). Altitude (metres) was logarithmically transformed, rainfall variables were transformed into square roots, and allele frequencies were angularly transformed.

3. RESULTS

G6PD-F and G6PD-S were the only electrophoretic variants of G6PD detected. $G6pd^F$ frequency ranged from 0 per cent in two samples to 100 per cent in 13 others (fig. 1). Much of this variation occurred across the three zoogeographic zones and was unexplained by mean zonal differences in latitude. The differentiation was greatest between North America and Europe/Asia, as these means and standard errors show:

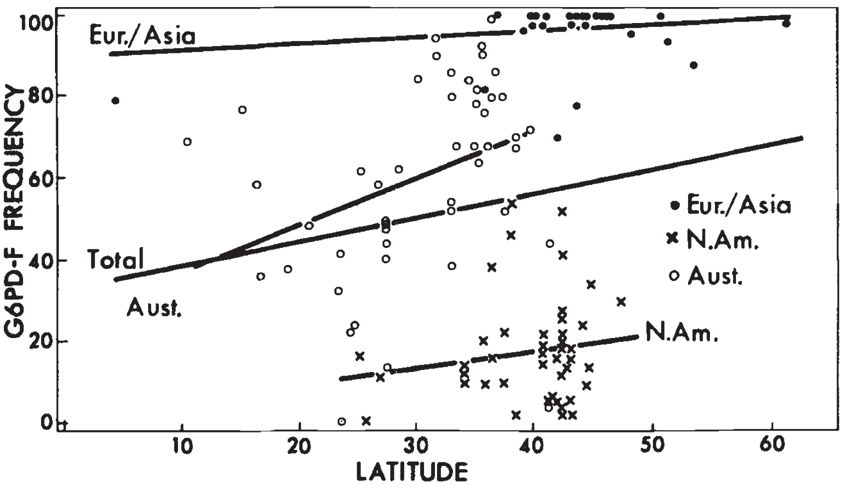

FIG. 1.—$G6pd^F$ frequencies (%) and their relations to latitude (regressions of untransformed values).

	Australasia	North America	Europe/Asia
latitude	31±1	40±1	43±2
%$G6pd^F$	59±3	18±2	95±2

Within each zone $G6pd^F$ frequency was significantly positively related to latitude (table 1). Furthermore, analysis of covariance indicated that differences between the slopes of the regressions on latitude in the three zones were not significant ($F^2_{111} = 0{\cdot}71$, $P > 0{\cdot}05$) and explained much less than 1 per cent of total variance in $G6pd^F$ frequency between all 117 samples: the overall regression across all three zones was highly significant ($F^1_{111} = 14{\cdot}52$, $P < 0{\cdot}001$) and explained 3 per cent of total variance: the mean differences in $G6pd^F$ frequency between zones were also highly significant, even after adjusting for the latitudinal regression ($F^2_{111} = 169{\cdot}52$, $P < 0{\cdot}001$), when they explained 73 per cent of total variance.

TABLE 1

Partial correlation (r) *and multiple regression* (b) *coefficients of* $G6pd^{F}$ *frequencies on location and climatic variables. Only significant* b *values are given*

	r			*b*		
	lat	long	alt	lat	long	alt
Austral.	0·44**	−0·31*	−0·19	1·00***	−0·37*	
N. Amer.	0·32*	−0·44**	−0·18		−0·25*	
Eur./Asia	0·47*	−0·60**	0·58**	0·46*	−0·15**	8·96**

	r				*b*			
	*T*max	*T*min	*R*max	*R*min	*T*max	*T*min	*R*max	*R*min
Austral.	−0·21	−0·08	0·02	−0·06				
N. Amer.	−0·29	0·06	−0·23	−0·34*				−1·97**
Eur./Asia	0·29	0·43*	−0·33	0·38				

* $P<0{\cdot}05$, ** $P<0{\cdot}01$, *** $P<0{\cdot}001$ on two-tailed tests.

There were also significant associations between $G6pd^{F}$ frequency and longitude, with $G6pd^{F}$ frequency decreasing eastward within each zone. No association between $G6pd^{F}$ frequency and altitude or any climatic variable was consistent across the three zones.

In addition to PGD-F and PGD-S, a third PGD variant was occasionally found, PGD-UF, which had a mobility of 1·06 relative to PGD-F and 1·20 relative to PGD-S. Pgd^{UF} frequency was not associated with latitude although it was significantly higher in Australasia (2·2 ± 0·7 per cent) than North America (0·6 ± 0·3 per cent) or Europe/Asia (0·4 ± 0·2 per cent).

Pgd^{F} frequencies ranged from 12 per cent in one population to 100 per cent in 29 (fig. 2). Zonal differences in Pgd^{F} frequency occurred but these were less marked than for $G6pd^{F}$ and were associated to a larger

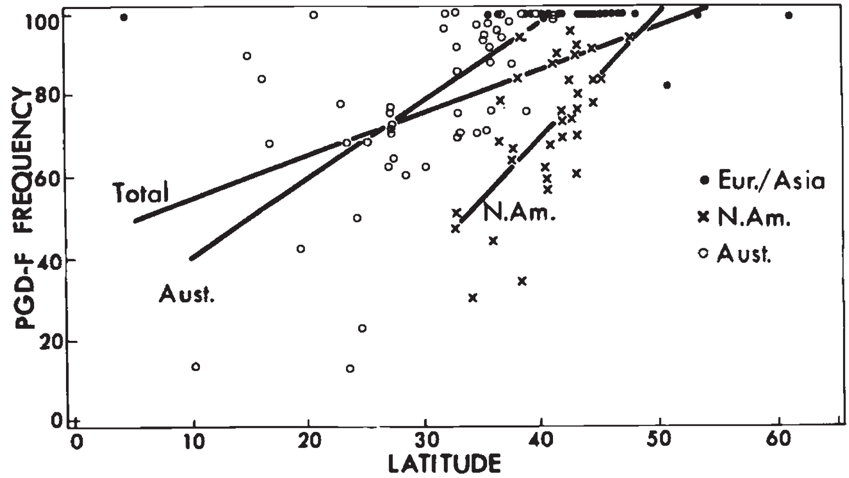

FIG. 2.—Pgd^{F} frequencies (%) and their relations to latitude (regressions of untransformed values).

degree with a positive correlation with latitude:

	Australasia	North America	Europe/Asia
latitude	31 ± 1	40 ± 1	43 ± 2
%Pgd^F	77 ± 3	72 ± 3	99 ± 1

Indeed, of the 24 collections from Europe/Asia, which on average over the whole sample were furthest from the equator, 22 were fixed for Pgd^F.

The positive relation of Pgd^F frequency to latitude was also highly significant within both Australasia and North America (table 2) but the low level of *Pgd* polymorphism in Europe/Asia precluded any meaningful test for clines within this zone. Analysis of covariance indicated that differences in the slope of the regression on latitude in North America and Australasia were not significant ($F^{1}_{100} = 0·84$, $P > 0·05$) and explained less than 1 per cent of the total Pgd^F frequency variance among samples: the overall regression on latitude across all three zones was highly significant ($F^{1}_{100} = 46·32$, $P < 0·001$) and explained 18 per cent: the mean differences in Pgd^F frequency among all three zones were also highly significant ($F^{2}_{100} = 44·36$, $P < 0·001$) even after adjusting for latitude, when they explained 34 per cent.

TABLE 2

Partial correlation (r) *and multiple regression* (b) *coefficients of* Pgd^F *frequencies on location and climatic variables. Only significant* b *values are given*

	r			*b*		
	lat	long	alt	lat	long	alt
Austral.	0·63***	−0·12	−0·31*	1·53***		−5·61*
N. Amer.	0·84***	−0·68*	−0·77*	1·65***	−0·31***	−11·09***

	r				*b*			
	*T*max	*T*min	*R*max	*R*min	*T*max	*T*min	*R*max	*R*min
Austral.	−0·30*	−0·02	−0·10	0·04	−2·21***			
N. Amer.	−0·39*	−0·13	−0·14	−0·41*	−1·59**		−2·15*	−2·05*

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$ on two-tailed tests.

In both Australasia and North America Pgd^F frequency was significantly negatively related to altitude and *T*max. The *T*max relation was also found when data for Europe/Asia were included in a pooled analysis over all three zones (partial $r = -0·51$, $P < 0·001$). Pgd^F frequency was not consistently significantly related to longitude, *T*min, *R*max or *R*min in Australasia and North America.

4. DISCUSSION

The consistency and extent of the associations with distance from the equator in the three zones strongly suggest that natural selection affects the frequencies of the common *G6pd* and *Pgd* alleles. This brings to five the number of enzyme polymorphisms in *D. melanogaster* for which large-scale latitudinal clines have been found with similar directions in different zones (Oakeshott *et al.*, 1981, 1982). However, compared to the patterns for *Pgd* and the other three loci (*Adh*, *Gpdh* and *Est*-6), the slopes of the *G6pd* clines are uniformly shallow and a much higher proportion of allele frequency variance among and within zones is unexplained by latitude. Noteworthy here is the report by Cavener and Clegg (1981*a*) of greater temporal stability in *G6pd* than *Pgd* frequencies in natural populations. The latitudinal clines are presumably caused by selective gradients in climatic variables and these gradients may not be sufficiently sharp for *G6pd* to enable parallel temporal variation to be expressed.

One biochemical property which apparently does not underlie the *G6pd* and *Pgd* clines in any simple way is *in vitro* thermostability. G6PD-F is much less thermostable than G6PD-S while no differences have been detected between the two PGD allozymes in this property (Bijlsma and van der Meulen-Bruijns, 1979; Cavener and Clegg, 1981*b*). Yet $G6pd^F$ frequency is not consistently negatively correlated with Tmax in the three zones while Pgd^F frequency is significantly negatively associated with Tmax in both Australasia and North America.

Another property which might be relevant is *in vitro* enzymic activity. G6PD catalyses the initial step and PGD the third step in the pentose phosphate pathway. At each locus the *F* variant produces less enzymic activity than *S* and comparisons of the fitnesses of laboratory stocks fixed for different alleles indicate that either the low activity $G6pd^F - Pgd^F$, or the high activity $G6pd^S - Pgd^S$ combination of alleles is favoured, depending on the sugar and fatty acid levels in the food (Bijlsma and van Delden, 1977; Bijlsma, 1978; Cavener and Clegg, 1981*b*). There is also evidence for positively correlated effects of modifier genes on G6PD and PGD activities (Hori and Tanda, 1981; Cochrane and Lucchesi, 1981; Laurie-Ahlberg *et al.*, 1981) and positively correlated changes in G6PD and PGD activities in selection experiments on fatty acid enriched food (Bijlsma, 1980). As the nutrient composition of the natural substrates of wild *D. melanogaster* is likely to vary with latitude, the allozymic activity differences could be implicated in the latitudinal *G6pd* and *Pgd* clines. In particular the enzymes' close metabolic relationships would predict similar directions for the $G6pd^F$ and Pgd^F clines, as indeed is observed.

Acknowledgements.—We are indebted to over 40 *Drosophila* workers who collected for us in Europe and North America. In particular we thank Prof R. C. Lewontin, Michelle Smith and Jody Hey for coordinating the North American collections. We are grateful to Prof C. H. Langley for giving us *G6pd* data for London, England and Raleigh, North Carolina. G.K.C. was supported by NIH Grant No. GM21179 to R. C. Lewontin.

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