LATITUDINAL RELATIONSHIPS OF ESTERASE-6 AND PHOSPHOGLUCOMUTASE GENE FREQUENCIES IN *DROSOPHILA MELANOGASTER*

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Received 9.vii.81

SUMMARY

Geographic variation in Esterase-6 (*Est-6*) and Phosphoglucumutase (*Pgm*) gene frequencies in Australasian populations of *Drosophila melanogaster* are compared with analogous data collated from 16 previous reports for North America and Europe/Asia. A large-scale latitudinal cline is found on all three zoogeographic zones for *Est-6* and overall, *Est-6*^{1.00} frequency increases from about 20 per cent around 20° latitude to about 80 per cent approaching 50° latitude. In contrast, there is no consistent evidence for a latitudinal cline in *Pgm* gene frequencies in any of the three zones, with *Pgm*^{1.00} frequency generally about 85 per cent and *Pgm*^{1.20} and *Pgm*^{0.70} frequencies each between 5 per cent and 10 per cent. The consistent *Est-6* clines are attributed to latitudinal selection gradients but no consistent correlations are found between *Est-6* gene frequencies and maximum or minimum temperature or rainfall which might be associated with these gradients. The directions of the *Est-6* clines in fact run counter to expectations based on the *in vitro* thermostabilities of the respective allozymes.

1. INTRODUCTION

IN two previous papers we have described geographic variation in the frequencies of four common cosmopolitan chromosome inversions (*In(2L)t*, *In(2R)NS*, *In(3L)P* and *In(3R)P*) and alleles at two polymorphic enzyme loci (Alcohol dehydrogenase and Glycerol-3-phosphate dehydrogenase, *i.e.*, *Adh* and *Gpdh*) in populations of *D. melanogaster* in three zoogeographic zones, Australasia, North America and Europe/Asia (Knibb *et al.*, 1981; Oakeshott *et al.*, 1981). For each of the six polymorphisms in each zone there was a statistically significant latitudinal cline covering between 25° and 40° latitude. Furthermore, for each polymorphism the direction of the latitudinal relationship was the same on the different continents. Since these continents have had very different histories of colonization and migration of *D. melanogaster* (Anderson, 1981) it was concluded that to some extent at least natural selection underlay the clines for all six polymorphisms.

This paper examines the latitudinal relationships of allelic frequencies for the Esterase-6 (*Est-6*) and Phosphoglucumutase (*Pgm*) polymorphisms in Australasia, North America and Europe/Asia. Preliminary evidence (Johnson and Schaffer, 1973) suggests the existence of a latitudinal cline for *Est-6*, but not for *Pgm*, in eastern U.S.A. (9 localities for *Est-6*

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and 15 for *Pgm* over 18° latitude). We test here whether the cline for *Est-6* and the lack of it for *Pgm* recur in additional collections in North America and, more importantly, on other continents and in the Southern Hemisphere.

A second set of hypotheses examined here concerns possible climatic associations of *Est-6* and *Pgm* gene frequencies. *In vitro* thermostability differences exist between allozymes of both *Est-6* and *Pgm* (Danford and Beardmore, 1979; Fucci *et al.*, 1979) and we test whether these differences are reflected in associations between gene frequencies and temperature variables in wild populations.

2. MATERIALS AND METHODS

The Australasian collections were scored for *Est-6* and *Pgm* after electrophoresis on cellulose-acetate sheets, using methods based on those of Trippa *et al.* (1978a). The *Est-6* and *Pgm* gene frequencies from North America and Europe/Asia were collated from 16 previous reports; Band (1975), Charlesworth and Charlesworth (1973), Cochrane and Richmond (1979), Danieli and Costa (1977), Girard *et al.* (1977), Hollis (1972), Kojima *et al.* (1970), Langley *et al.* (1974, 1977), Pipkin *et al.* (1976), Triantaphyllidis *et al.* (1973), Trippa (1971), Trippa *et al.* (1974, 1978a, 1978b) and Voelker *et al.* (1978).

Collections of less than 20 genes *per locus* were excluded from the analysis. The number of collections analysed and the mean and standard error of the number of genes sampled *per collection* were 66 and 106 ± 7 in Australasia, 43 and 295 ± 31 in North America and 27 and 925 ± 209 in Europe/Asia for *Est-6*, and 66 and 90 ± 1 in Australasia, 18 and 90 ± 2 in North America and 18 and 86 ± 1 in Europe/Asia for *Pgm*. The Australasian and North American data covered a wide range of latitudes and longitudes but with the exception of one *Est-6* and two *Pgm* scores in Japan, the data for Europe/Asia were all obtained within about 10° latitude and 20° longitude in Europe.

The latitudes, longitudes and altitudes of collection sites were obtained from *The Times Atlas of the World, Comprehensive Edition* (The Times; London, 1967) and climatic data for adjacent weather stations from *World Weather* (Her Majesty's Stationery Office; London, 1967) and *Climatic Averages Australia* (Australian Government Publishing Service; Canberra, 1975). As in Wilks *et al.* (1980), Oakeshott *et al.* (1981) and Knibb *et al.* (1981), the four climatic variables used were maximum temperature ($T_{\max}$ —average daily maximum in °C for the hottest calendar month), minimum temperature ($T_{\min}$ —average daily minimum in °C for the coldest calendar month), maximum rainfall ($R_{\max}$ —total rainfall in mm for the wettest calendar month) and minimum rainfall ($R_{\min}$ —total rainfall in mm for the driest calendar month). Twenty year averages were used for all four climatic variables. For *Est-6* only 125 of the total of 136 collections were close and at a comparable altitude, to a suitable weather station, while for *Pgm* the corresponding figures were 94 of 102 collections. For sites where a suitable weather station was found the average distance from collection site to weather station was 17 ± 2 miles.

Northern and southern latitudes were both analysed as positive values in order to represent distances from the equator. However, longitude was

signed, with eastern values positive and western negative; longitude does not affect climate *per se* so it was just used as a general distance measure. Both rainfall variables were transformed into square roots while gene frequencies were angularly transformed and standardised for sample size.

3. RESULTS

In terms of the alleles present in the polymorphisms, the Australasian data confirm the previous results for *Est-6* and *Pgm* in the Northern Hemisphere. The previous work indicated two common electrophoretically defined alleles, *Est-6*^{1.10} and *Est-6*^{1.00} (map position 3-36.8); the combined frequencies of minor electrophoretic variants and occasional null alleles seldom exceed 5 per cent (e.g., Langley *et al.*, 1974; Cochrane and Richmond, 1979). Both *Est-6*^{1.10} and *Est-6*^{1.00} occurred at polymorphic frequencies in all 66 Australasian samples and the only other electrophoretically defined allele we detected was *Est-6*^{1.25} (Franklin, 1981), which occurred sporadically at frequencies generally below 5 per cent.

Previously seven electrophoretically defined *Pgm* alleles (map position 3-43.4) have been reported from Northern Hemisphere populations, although only three, *Pgm*^{1.20}, *Pgm*^{1.00} and *Pgm*^{0.70} are regularly polymorphic and one, *Pgm*^{1.00}, generally exceeds frequencies of 80 per cent (Trippa *et al.*, 1970; Langley *et al.*, 1974). Only *Pgm*^{1.20}, *Pgm*^{1.00} and *Pgm*^{0.70} were consistently polymorphic in Australasia. The other electrophoretically defined alleles detected were *Pgm*^{1.50} and *Pgm*^{0.50} (Franklin, 1981) but their combined frequency nowhere exceeded 5 per cent.

Figures 1 and 2 summarise the North American, European and Australasian data in terms of the frequencies of *Est*^{1.00}, *Pgm*^{1.20} and *Pgm*^{1.00};

FIG. 1.—*Est-6*^{1.00} frequencies (white areas of Pie diagrams) in North America, Europe and Australasia. The frequency in a Japanese population not shown in the figure is 83 per cent at 42.9°N, 141.8°E.

FIG. 1.—(cont.)

rare *Est-6* alleles are pooled with *Est-6*^{1.10} and rare *Pgm* alleles are pooled with *Pgm*^{0.70}. Neither of the pooled frequency classes are analysed separately since they are not independent of the frequencies of *Est*^{1.00}, or *Pgm*^{1.20} and *Pgm*^{1.00}. (The full Australasian data are available from J.G.O. on request.)

The North American *Est-6*^{1.00} cline originally found by Johnson and Schaffer (1973) in eastern U.S.A. is substantiated by the additional data for that continent, particularly by the more southerly Mexican data of

Pipkin *et al.* (1976); *Est-6*^{1.00} frequency increases from an average of about 20 per cent around 20° latitude to about 60 per cent around 40° latitude. Furthermore, this positive relationship between *Est-6*^{1.00} frequency and latitude extends through the higher latitudes represented by the European samples, in which *Est-6*^{1.00} frequency increases still further, from about 60 per cent at 40° latitude to about 80 per cent approaching 50° latitude. Finally, the Australasian data indicate that a similar positive relationship also occurs in the southern hemisphere, where *Est-6*^{1.00} frequency increases from about 40 per cent below 15° latitude to about 70 per cent above 35° latitude.

TABLE 1

Partial correlation (*r*) and standardised multiple regression (*b*) coefficients of *Est-6*^{1.00}, *Pgm*^{1.20} and *Pgm*^{1.00} frequencies on latitude, longitude and altitude. Only significant *b* values are presented, or considered in the proportions of variance explained by the regressions (Multiple *R*²)

	<i>r</i>			<i>b</i>			Multiple <i>R</i> ²
	lat	long	alt	lat	long	alt	
<i>Est-6</i> ^{1.00} in							
N. Amer	0.31*	0.22	0.19	0.48**			0.23
Eur/Asia	0.53**	0.21	-0.24	0.48*			0.23
Aust	0.28*	-0.09	0.40***	0.28*		0.40***	0.35
<i>Pgm</i> ^{1.20} in†							
Eur/Asia	-0.20	-0.02	0.11				
Aust	-0.07	0.17	-0.01				
<i>Pgm</i> ^{1.00} in							
N. Amer	0.27	-0.33	-0.42				
Eur/Asia	-0.35	-0.85***	-0.50		-0.70***	-0.38**	0.79
Aust	-0.15	0.09	-0.15	-0.25*			0.06

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† Insufficient data for *Pgm*^{1.20} in North America (fig. 2).

Table 1 describes the clines statistically, in terms of the partial correlation and multiple regression coefficients of *Est-6*^{1.00} frequency on latitude for each zoogeographic zone; all these coefficients are positive and statistically significant. There is not, however, any consistent relationship of *Est-6*^{1.00} frequency with longitude or altitude. Although there is a significant positive relationship with altitude in Australasia, this is not consistent in sign or statistically significant on the other two continents.

Table 2 describes statistically the overall *Est-6*^{1.00} frequency differences between continents, and the role of the latitudinal relationship of *Est-6*^{1.00} frequency in producing these differences. In fact about half the continental differences can be explained in terms of the latitudinal effects.

No consistent latitudinal relationships are obvious in fig. 2 for either *Pgm*^{1.20} or *Pgm*^{1.00}, and the only association significant as both a partial correlation and multiple regression in table 1 is between *Pgm*^{1.00} frequency and longitude in Europe/Asia, where *Pgm*^{1.00} frequency declines eastward. (Even this longitudinal effect is largely just a reflection of a difference between Japan and Europe, for there was no collection in between to establish a regular cline.) Table 2 shows no overall continental or latitudinal

TABLE 2

Analyses of variance for overall latitudinal and continental effects on $Est-6^{1.00}$, $Pgm^{1.20}$ and $Pgm^{1.00}$ frequencies. The continental differences are assessed before and after removal of the latitudinal effects as covariates. Degrees of freedom are in parentheses

	<i>F</i> ratio for latitude	<i>F</i> ratio for continents		% continental differences due to latitude
		before latitudinal correction	after latitudinal correction	
<i>Est-6</i> ^{1.00}	50.5*** (1, 132)	27.7*** (2, 132)	16.3*** (2, 132)	41%
<i>Pgm</i> ^{1.20}	2.1 (1, 87)	44.7*** (1, 87)	43.8*** (1, 87)	2%
<i>Pgm</i> ^{1.00}	1.6 (1, 99)	1.9 (2, 99)	2.2 (2, 99)	-18%†

*** *P* < 0.001.

† i.e., the latitudinal effects, albeit non-significant, actually work against expression of the continental differences in $Pgm^{1.00}$ frequency.

effects on $Pgm^{1.00}$ frequency, although there are continental, but not latitudinal, effects on $Pgm^{1.20}$ frequency (lower $Pgm^{1.20}$ frequencies occur in Europe/Asia, than in the other continents).

Table 3 examines the associations of $Est-6^{1.00}$, $Pgm^{1.20}$ and $Pgm^{1.00}$ frequencies with *T*_{max}, *T*_{min}, *R*_{max} and *R*_{min}. Most of the significant

FIG. 2.— $Pgm^{1.20}$ and $Pgm^{1.00}$ frequencies (white and hatched areas respectively of the Pie diagrams) in North America, Europe and Australasia. Asterisked values indicate collections in which $Pgm^{1.20}$ was not reported separately from $Pgm^{0.70}$ and other rarer alleles. The frequencies in two Japanese populations not shown in the figure are 0 per cent $Pgm^{1.20}$, 67 per cent $Pgm^{1.00}$ and 0 per cent $Pgm^{1.20}$, 76 per cent $Pgm^{1.00}$, both at 42.9°N, 141.8°E.

FIG. 2.—(cont.)

climatic associations which are found involve $Est-6^{1.00}$ and $Pgm^{1.00}$ in Europe/Asia, but only one of these involves T_{max} and none recurs on the other continents.

4. DISCUSSION

Although only two of the 136 collections analysed were fixed for $Est-6^{1.10}$ and none was fixed for $Est-6^{1.00}$, a pronounced latitudinal cline in $Est-6$ gene frequencies has been found in North America, Europe/Asia and Australasia. Overall $Est-6^{1.00}$ frequency increases from about 20 per

TABLE 3

Partial correlation (*r*) and standardised multiple regression (*b*) coefficients of Est-6^{1.00}, Pgm^{1.20} and Pgm^{1.00} frequencies on maximum and minimum temperature and rainfall. Only significant *b* values are presented, or considered in the proportions of variance explained by the regressions (Multiple R²)

	<i>r</i>				<i>b</i>				Multiple R^2
	Tmax	Tmin	Rmax	Rmin	Tmax	Tmin	Rmax	Rmin	
<i>Est-6</i> ^{1.00} in									
N. Amer	0.09	0.11	-0.25	0.23				0.55***	0.31
Eur/Asia	0.09	0.17	0.50	0.49*			0.43***	0.41*	0.36
Aust	0.14	-0.08	-0.22	0.20			-0.50***		0.25
<i>Pgm</i> ^{1.20} in									
Eur/Asia	-0.03	-0.09	-0.08	-0.13					
Aust	-0.22	0.13	-0.01	0.04					
<i>Pgm</i> ^{1.00} in									
N. Amer	-0.30	0.08	0.04	0.39					
Eur/Asia	-0.80***	0.92***	-0.70**	0.69**	-0.57***	1.19***	-0.36***	0.44***	0.89
Aust	0.31*	0.00	0.06	0.07	0.34*				0.12

* *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001.

cent around 20° latitude to about 80 per cent around 50° latitude and it seems likely that this clinal pattern is produced at least in part by natural selection. Non-selectionist explanations seem incompatible with the consistency of the cline across immense geographic zones with very different histories of colonisation and migration of *D. melanogaster*.

For similar reasons it is doubtful that the *Est-6* cline represents a transient state in the selective replacement of one *Est-6* gene by another. It could be argued for example that *Est-6*^{1.10} had a recent equatorial origin but is advantageous at all latitudes and is slowly spreading away from the equator. While this argument cannot be disproved, it seems unlikely that the spread would have reached similar stages at all longitudes covered in the analyses.

It is therefore suggested that the *Est-6* cline is maintained by stable latitude-dependent selection pressures, although the precise selection pressures involved cannot be deduced simply from the distributional data (Endler, 1973). It also must be admitted that the selection may not be acting directly on the locus; *Est-6* may simply mark a larger chromosome region which is selected. It is worth noting in this respect that *Est-6* is consistently in linkage disequilibrium with *In(3L)P*, a common cosmopolitan inversion with breakpoints straddling the locus. Although *In(3L)P* decreases in frequency with increasing latitude on all three zoogeographic zones, the direction of the disequilibria (*Est-6*^{1.00}, *In(3L)P* gametes in excess) in fact works against the expression of the *Est-6* cline (Voelker *et al.*, 1978).

Of the selection mechanisms which might be acting directly on the *Est-6* locus, some evidence for both temperature- and frequency-dependent selection has been obtained from laboratory studies. In the inbred population Danford and Beardmore (1979) studied, the thermostability of the enzymes produced by *Est-6*^{1.00} was greater than that from *Est-6*^{1.10}. This agrees with the evidence from laboratory perturbation experiments and fitness component analyses carried out between 25° and 30°C which indicate higher equilibrium *Est-6*^{1.00} frequencies than at temperatures between 18° and 25° (MacIntyre and Wright, 1966; Beardmore, 1970; Minawa and Birley, 1978; Oakeshott, 1979; Abedin *et al.*, 1980). The selection at all the temperatures tested is frequency-dependent (Morgan, 1976; Birley and Beardmore, 1977 and references therein) and may involve mating behaviour (Aslund and Rasmuson, 1976) and the deposition of different products of ester metabolism in the media by different *Est-6* genotypes (Birley and Beardmore, 1977).

Whether the temperature- or frequency-dependence is relevant to the selection in wild populations however remains doubtful. We have found no consistent Tmax-*Est-6*^{1.00} frequency correlations underlying the latitudinal clines. And in fact the directions of the clines, with *Est-6*^{1.00} at lower frequencies at the warmer, low latitudes, is opposite to that expected from the temperature effects in the laboratory studies. Furthermore, Franklin (1981), who monitored six populations over five years in the Hunter Valley, Australia, found *Est-6*^{1.00} frequency consistently declined in the hot summer months and rose again in winter. This seasonality is consistent with the directions of the clines is opposite to the pattern expected from the laboratory work.

Part of the explanation for this paradox may be the existence of electrophoretically cryptic thermostability variants within *Est-6*^{1.10} and *Est-6*^{1.00} in wild populations. A recent survey of four wild populations from eastern U.S.A. has revealed one common and two much rarer thermostability alleles within *Est-6*^{1.00} and four relatively common thermostability alleles within *Est-6*^{1.10} (Cochrane and Richmond, 1979). One high priority for future research would seem to be a wider geographic and temporal survey of these variants.

Although *Pgm* was polymorphic in all but five of the 102 collections analysed here, there was little geographic variation in *Pgm* gene frequencies. *Pgm*^{1.00} was the most common allele in every collection and its frequency was generally greater than 85 per cent. Nor was there any evidence, either within or across continents, for a cline in *Pgm*^{1.20} or *Pgm*^{1.00} frequencies. This marked contrast with the *Est-6* variation is again paralleled by Franklin's (1981) seasonal data. Just as he found consistent seasonal variation in the frequencies of the *Est-6* genes for which there are consistent latitudinal clines, he failed to find consistent seasonal variation in the frequencies of the *Pgm* genes for which there is no evidence for consistent latitudinal clines.

Geographic homogeneity of frequencies has been taken as firm evidence for selection by authors examining allozyme variation in *Drosophila* species with much more restricted geographic ranges than *D. melanogaster* (e.g., Ayala *et al.*, 1974 and references therein). Therefore the large geographic scale encompassed by our analyses might suggest that selection was responsible for the observed consistency of *Pgm* gene frequencies. However, only relatively low levels of random migration are required for neutralist theory to account for geographic homogeneity of gene frequencies and, as in most geographic surveys of electrophoretic variation, the levels of migration between the populations analysed here have not been measured.

Despite this ambiguity, the postulated selection on *Pgm* concurs with evidence from laboratory perturbation experiments in which selection establishes equilibrium *Pgm*^{1.00} frequencies around 80 per cent. The fact that this equilibrium applies at 18°C, 25°C and 29°C (Oakeshott, 1979; Carfagna *et al.*, 1980) is also consistent with the geographic homogeneity of *Pgm* gene frequencies and their lack of consistent association with *T*_{max}, but it contrasts with biochemical evidence indicating differences between the *in vitro* thermostabilities of respective *Pgm* allozymes. The thermostabilities of the *Pgm*^{1.20} and *Pgm*^{1.00} products appear to be greater than those of *Pgm*^{0.70}, although there are also indications of electrophoretically cryptic thermostability variants within *Pgm*^{1.00} (Trippa *et al.*, 1978*b*; Fucci *et al.*, 1979).

Thus the results of the latitudinal and climatic analyses for *Pgm*, like those for *Est-6*, and indeed for *Adh* and *Gpdh* (Wilks *et al.*, 1980; Oakeshott *et al.*, 1981) seem unrelated to the *in vitro* thermostabilities of the relevant allozymes. The thermostability differences may be valuable biochemical tools but their adaptive value for flies in wild populations remains to be established.

Whatever the doubts about the mechanisms of selection involved however, overall an impressive picture emerges when the *Est-6* and *Pgm* distributions are collated with the two previous papers in this series. The frequencies of alleles at four enzyme loci and four common cosmopolitan

inversions have now been examined in North America, Europe/Asia and Australasia. Seven of the eight systems show consistent and complementary large-scale latitudinal clines on all three zoogeographic zones, and the other system, *Pgm*, shows a consistent lack of relation to latitude on all three zones. Overall the results suggest that a substantial proportion of the electrophoretic and chromosomal variation in natural populations of *D. melanogaster* may be selectively maintained.

Acknowledgements.—We thank Dr M. A. Adena, Mr P. R. Anderson and Mr W. R. Knibb for valuable discussions and Mrs W. Eddey and Ms A. V. Wilks for excellent technical assistance. We are also grateful to Mr Keith Winters and Mr Ken Hall of the A.C.T. Regional Office of the Australian Bureau of Meteorology for advice and assistance in compiling the climatic data.

5. REFERENCES

- ABEDIN, K., MCNAMARA, S., OSTERBUR, D., AND STEINER, W. W. M. 1980. Studies on the effect of temperature on fitness and fecundity at the Esterase-6 locus in *D. melanogaster*. *Drosophila Inform. Serv.*, 55, 10-11.
- ANDERSON, P. R. 1981. Geographic clines and climatic associations of *Adh* and *Gpdh* gene frequencies in *Drosophila melanogaster*. In *Genetic Studies of Drosophila Populations*, eds. J. B. Gibson and J. G. Oakeshott, pp. 237-250. Australian National University, Canberra.
- ASLUND, S., AND RASMUSON, M. 1976. Mating behaviour as a fitness component in maintaining allozyme polymorphism in *Drosophila melanogaster*. *Hereditas*, 82, 175-178.
- AYALA, F. J., TRACEY, M. L., BARR, L. G., MCDONALD, J. F., AND PEREZ-SALAS, S. 1974. Genetic variation in natural populations of five *Drosophila* species and the hypothesis of the selective neutrality of protein polymorphisms. *Genetics*, 77, 343-384.
- BAND, H. T. 1975. A survey of isozyme polymorphism in a *Drosophila melanogaster* natural population. *Genetics*, 80, 761-771.
- BEARDMORE, J. A. 1970. Ecological factors and the variability of gene pools in *Drosophila*. In *Essays in Evolution and Genetics in Honour of Theodosius Dobzhansky*, eds. M. K. Hecht and W. C. Steere, pp. 299-314. Appleton-Century-Crofts, New York.
- BIRLEY, A. J., AND BEARDMORE, J. A. 1977. Genetical composition, temperature, density and selection in an enzyme polymorphism. *Heredity*, 39, 133-144.
- CARFAGNA, M., FUCCI, L., GAUDIO, L., PONTECORVO, G., AND RUBINO, R. 1980. Adaptive value of *Pgm* polymorphism in laboratory populations of *Drosophila melanogaster*. *Genet. Res.*, 36, 265-276.
- CHARLESWORTH, B., AND CHARLESWORTH, D. 1973. A study of linkage disequilibrium in natural populations of *Drosophila melanogaster*. *Genetics*, 73, 351-359.
- COCHRANE, B. J., AND RICHMOND, R. C. 1979. Studies of Esterase-6 in *Drosophila melanogaster*. II. The genetics and frequency distributions of naturally occurring variants studied by electrophoretic and heat stability criteria. *Genetics*, 93, 461-478.
- DANFORD, N. D., AND BEARDMORE, J. A. 1979. Biochemical properties of Esterase-6 in *Drosophila melanogaster*. *Biochem. Genet.*, 17, 1-22.
- DANIELI, G. A., AND COSTA, R. 1977. Transient equilibrium at the *Est-6* locus in wild populations of *Drosophila melanogaster*. *Genetica*, 47, 37-41.
- ENDLER, J. A. 1973. Gene flow and population differentiation. *Science*, 179, 243-250.
- FRANKLIN, I. R. 1981. An analysis of temporal variation at isozyme loci in *Drosophila melanogaster*. In *Genetic Studies of Drosophila Populations*, eds. J. B. Gibson and J. G. Oakeshott, pp. 217-236. Australian National University, Canberra.
- FUCCI, L., GAUDIO, L., RAO, R., SPANO, A., AND CARFAGNA, M. 1979. Properties of two common electrophoretic variants of Phosphoglucosmutase in *Drosophila melanogaster*. *Biochem. Genet.*, 17, 825-836.
- GIRARD, P., PALABOST, L., AND PETIT, C. 1977. Enzymatic variation at seven loci in nine natural populations of *Drosophila melanogaster*. *Biochem. Genet.*, 15, 589-599.
- HOLLIS, R. J. 1972. Allozymic variation in *D. melanogaster* from Virginia. *Drosophila Inform. Serv.*, 49, 116.

- JOHNSON, F. M., AND SCHAFFER, H. E. 1973. Isozyme variability in species of the genus *Drosophila*. VII. Genotype-environment relationships in populations of *D. melanogaster* from the eastern United States. *Biochem. Genet.*, 10, 149-163.
- KNIBB, W. R., OAKESHOTT, J. G., AND GIBSON, J. B. 1981. Chromosome inversion polymorphisms in *Drosophila melanogaster*. I. Latitudinal clines and associations between inversions in Australasian populations. *Genetics*, in press.
- KOJIMA, K., GILLESPIE, J., AND TOBARI, Y. N. 1970. A profile of *Drosophila* species' enzymes assayed by electrophoresis. I. Number of alleles, heterozygosities and linkage disequilibrium in glucose metabolising systems and some other enzymes. *Biochem. Genet.*, 4, 627-637.
- LANGLEY, C. H., ITO, K., AND VOELKER, R. A. 1977. Linkage disequilibrium in natural populations of *Drosophila melanogaster*: seasonal variation. *Genetics*, 86, 447-454.
- LANGLEY, C. H., TOBARI, Y. N., AND KOJIMA, K. 1974. Linkage disequilibrium in natural populations of *Drosophila melanogaster*. *Genetics*, 78, 921-936.
- MACINTYRE, R. J., AND WRIGHT, T. R. F. 1966. Responses of Esterase-6 alleles of *Drosophila melanogaster* and *D. simulans* to selection in experimental populations. *Genetics*, 53, 371-387.
- MINAWA, A., AND BIRLEY, A. J. 1978. The genetical response to natural selection by varied environments. I. Short-term observations. *Heredity*, 40, 39-50.
- MORGAN, P. 1976. Frequency-dependent selection at two enzyme loci in *Drosophila melanogaster*. *Nature*, 263, 765-766.
- OAKESHOTT, J. G. 1979. Selection affecting enzyme polymorphisms in laboratory populations of *Drosophila melanogaster*. *Oecologia*, 43, 341-354.
- OAKESHOTT, J. G., GIBSON, J. B., ANDERSON, P. R., KNIBB, W. R., ANDERSON, D. G., AND CHAMBERS, G. K. 1981. Alcohol dehydrogenase and glycerol-3-phosphate dehydrogenase clines in *Drosophila melanogaster* on different continents. *Evolution*, in press.
- PIPKIN, S. B., FRANKLIN-SPRINGER, E., LAW, S., AND LUBEGA, S. 1976. New studies of the alcohol dehydrogenase cline in *D. melanogaster* from Mexico. *J. Hered.*, 67, 258-266.
- TRIANAPHYLLIDIS, C. D., CHRISTODOU, C., AND BECKMAN, L. 1973. Polymorphism at two esterase loci in *Drosophila melanogaster* from northern Greece. *Hereditas*, 74, 25-30.
- TRIPPA, G. 1971. New mutants of *D. melanogaster*: *Pgm*^A and *Pgm*^B. *Drosophila Inform. Serv.*, 46, 42.
- TRIPPA, G., CATANO, A., LOMBARDOZZI, A., AND CICCETTI, R. 1978a. A simple approach for discovering common non-electrophoretic enzyme variability: a heat denaturation study in *Drosophila melanogaster*. *Biochem. Genet.*, 16, 299-305.
- TRIPPA, G., DANIELI, G. A., COSTA, R., AND SCOZZARI, R. 1978b. Phosphoglucumutase (*Pgm*) and Esterase-6 (*Est-6*) alleles in *Drosophila melanogaster*: an attempt to measure linkage disequilibrium. *Genetica*, 49, 225-227.
- TRIPPA, G., LOVERRE, A., BARBERIO, C., ULIZZA, L., AND SCOZZARI, R. 1974. Studies on the Phosphoglucumutase (PGM) polymorphism in two successive years in natural populations of *Drosophila melanogaster*. *Acad. Naz. Lincei Rend. Cl. Sci. Fis. Mat. Nat. Serv.*, VIII 56, 89-93.
- TRIPPA, G., SANTOLAMAZZA, C., AND SCOZZARI, R. 1970. Phosphoglucumutase locus in *Drosophila melanogaster*: linkage and population data. *Biochem. Genet.*, 4, 665-669.
- VOELKER, R. A., COCKERHAM, C. C., JOHNSON, F. M., SCHAFFER, H. E., MUKAI, T., AND METTLER, L. E. 1978. Inversions fail to account for allozyme clines. *Genetics*, 88, 515-527.
- WILKS, A. V., GIBSON, J. B., OAKESHOTT, J. G., AND CHAMBERS, G. K. 1980. An electrophoretically cryptic alcohol dehydrogenase variant in *Drosophila melanogaster*. II. Post-electrophoresis heat-treatment screening of natural populations. *Aust. J. Biol. Sci.*, 33, 575-585.