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Population Biology

Selection affecting enzyme polymorphisms in enclosed *Drosophila* populations maintained in a natural environment

(*Drosophila melanogaster*/population transplants/heterozygote advantage)

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ABSTRACT **Allele frequencies for the *Adh*, *Gpdh*, and *Est6* enzyme polymorphisms of *Drosophila melanogaster* show large-scale latitudinal clines, whereas those for *Pgm* do not vary systematically with latitude. To elucidate possible mechanisms of selection underlying these distributions, large collections of the species were made from five Australasian localities spanning 24° of latitude. Two replicate experimental populations were established from each collection, and each replicate was then released into an enclosure surrounding a natural habitat at a central-latitude locality. Genotype frequencies at the four loci were monitored for 15 months, covering 12 discrete generations, and selection coefficients on each polymorphism were then estimated by maximum likelihood procedures. For *Est6* no coefficients were found to be significantly different from zero. For *Pgm* some nonzero coefficients were estimated, but these were heterogeneous across experimental populations of different geographic origins. For both *Adh* and *Gpdh*, nonzero selection coefficients were estimated that were homogeneous across populations and indicated heterozygote advantage. Predicted *Adh* and *Gpdh* equilibrium allele frequencies were consistent with those found in adjacent free-living populations. It is concluded that, at such intermediate latitudes at least, selection operates on the *Adh* and *Gpdh* polymorphisms to the advantage of heterozygotes.**

A primary aim of modern population genetics has been to determine the adaptive significance of electrophoretic enzyme polymorphism (1, 2). Our approach to the detection of natural selection on such polymorphisms in *Drosophila melanogaster* has been to screen for recurrent relationships between allele frequencies and the environment at various latitudes (3–6). Of the 10 polymorphisms we studied, we found (3–5) that 6 show associations between allele frequencies and distance from the equator, which recur on all three continents for which data were available. Given the scale and repeatability of these latitudinal clines, we suggested (5, 6) that natural selection was a significant determinant of allele frequencies at these loci.

The aim of the present work was to measure directly some of the selective processes inferred from these geographic surveys. We focused on three polymorphisms showing consistent latitudinal clines, alcohol dehydrogenase (*Adh*), glycerol-3-phosphate dehydrogenase (*Gpdh*), and esterase 6 (*Est6*), and one showing no systematic variation with latitude, phosphoglucomutase (*Pgm*). Populations from a wide range of latitudes were transplanted to enclosures surrounding natural habitats at a relatively central latitude. Genotype frequencies were monitored in the transplanted populations for several generations. Selection coefficients were then estimated from these data and tested against various hypotheses concerning the modes of selection.

MATERIALS AND METHODS

Mass collections consisting of at least 100 *D. melanogaster* were made from two northern, one central, and two southern Australasian sites: Cairns (Queensland, 16.9°S, 145.7°E), Townsville (Queensland, 19.2°S, 146.8°E), Canberra (Australian Capital Territory, 35.3°S, 149.3°E), Glenvale (New Zealand, 39.5°S, 177.0°E), and Nelson (New Zealand, 41.3°S, 173.3°E). Immediately following their capture these five base populations were cultured under standard laboratory conditions for two or three generations to expand population sizes. Two cohorts of ≈1000 flies were then taken from each to form the 10 experimental populations.

The experimental populations were released into 10 1-m^3 enclosures in a substantially shaded Canberra garden. The enclosures were set in a 5 × 2 rectangular grid pattern over an area of about 10 m × 3 m, and populations were assigned to positions in the grid at random. The sides and roof of each enclosure consisted of 0.5-mm nylon gauze over a wooden frame, and the bottom was a metal tray containing ≈5 cm of sterilized soil. A heap of composting fruit and vegetables was placed on the soil each generation. The heap comprised ripe, cut apple (500 g), pear (500 g), orange (500 g), lemon (500 g), tomato (1 kg), melon (1 kg), and banana (1 kg).

The experimental populations were maintained in the enclosures for 12 discrete generations, from midsummer 1983 until midautumn 1984. At the start of each generation, adults were allowed between 3 and 7 days (depending on season and except for generation 5, see below) to lay eggs on the compost. These adults were then removed (by using a vacuum cleaner), their numbers were estimated, and, at generations 0, 4, 6, 9, and 12, samples were frozen for electrophoresis. Between 3 and 7 days after the progeny began emerging as adults, the compost and soil were replaced, and the next generation began. Details of generation times, population sizes, temperatures, and precipitation during the experiment are given in Table 1.

Generation 5, over winter 1983, lasted ≈6.5 months, from late April until early November, during which time few *Drosophila* of any life stage were apparent. However, a few hundred adults emerged in each enclosure over a period of ≈2 weeks late in October. It was uncertain whether these flies had overwintered as preadults or whether they had developed from eggs laid in the spring by a few adults that had emerged early in the winter. In any event, the allele frequency changes that occurred in this interval were excluded from the analy-

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Table 1. Generation times, population sizes, maximum and minimum temperatures, rainfall, evaporation, and relative humidities at 9:00 a.m. and 3:00 p.m.

Generation				Mean daily value					
								Rel. humid., %	
No.	Starting date, day/month	Length, days	Population size, no. $\times 10^{-3}$	Max. temp., °C	Min. temp., °C	Rainfall, mm	Evaporation, mm	9 a.m.	3 p.m.
1	19/1	19	10.5 ± 4.3	33	16	1.0	7.2	61	29
2	7/2	22	7.9 ± 3.3	31	16	0.6	6.0	65	34
3	1/3	21	6.2 ± 1.2	29	16	1.2	6.1	62	38
4	22/3	30	8.9 ± 2.0	19	8	2.7	2.7	73	48
5	21/4	205	0.3 ± 0.1	15	4	2.5	1.9	80	59
6	11/11	34	6.1 ± 1.8	22	10	5.3	4.0	75	54
7	15/12	19	4.9 ± 2.6	27	14	2.8	5.9	72	46
8	3/1	17	6.9 ± 4.2	25	13	5.5	5.2	75	49
9	20/1	17	2.9 ± 1.3	26	11	5.1	5.4	75	41
10	6/2	29	5.2 ± 1.3	25	12	1.7	4.7	77	49
11	6/3	24	2.6 ± 1.1	22	10	1.9	4.0	69	48
12	30/3	31	1.2 ± 0.8	18	8	3.3	2.5	79	52

Climatic data are taken from Bureau of Meteorology records for Canberra City, ≈5 km from the site of the experiment. Population sizes (mean ± SD) refer to progeny produced each generation. One thousand parents were used for each population at generation 1. Max. and min. temp., maximum and minimum temperatures, respectively; rel. humid., relative humidity.

sis, because the low population sizes at the time provided the opportunity for large effects of random drift.

Careful precautions were taken to avoid contamination of the experimental populations by other *D. melanogaster*. All food items were purchased before fully ripe and checked before use for signs of *Drosophila* eggs or larvae. A sample of each fruit and vegetable type was retained for several days at room temperature to confirm that no larvae developed. No food contaminated with *D. melanogaster* was detected. It is also relevant that *Drosophila simulans* was never found in the experimental populations. This species is a close sibling of *D. melanogaster* with a very similar ecology, and it generally outnumbers *D. melanogaster* by an order of magnitude in domestic Canberra composts (7). It seems unlikely that any contamination by other *D. melanogaster* would have occurred without an associated contamination by *D. simulans*.

Adh, *Gpdh*, *Est6*, and *Pgm* genotype frequencies were scored using standard electrophoretic methods (3, 4). The means ± SEM of flies scored in each experimental population at each scored generation were 198 ± 12 flies for *Adh*, 193 ± 10 flies for *Gpdh*, 207 ± 8 flies for *Est6*, and 174 ± 9 flies for *Pgm*. Initially *Adh* and *Gpdh* were each polymorphic for two electrophoretically detectable alleles in all 10 experimental populations. Initially *Est6* and *Pgm* were each polymorphic for two alleles, and in some populations three alleles, but the frequencies of the third alleles, $Est6^{1.20}$ and $Pgm^{0.70}$, were always relatively low (<5%).

Methods for the statistical analysis of the genotype frequency changes were based on Wilson and Oakeshott (8). For each polymorphism, selection coefficients were estimated for each experimental population and base population and for the total data. The four sets of two selection coefficients (s and t), which were estimated, defined relative fitnesses of

	1	: $1+t$	: $1+s$
for	Adh^S/Adh^S	: Adh^S/Adh^F	: Adh^F/Adh^F
	$Gpdh^S/Gpdh^S$	: $Gpdh^S/Gpdh^F$	: $Gpdh^F/Gpdh^F$
	$Est6^{1.00}/Est6^{1.00}$	: $Est6^{1.00}/Est6^X$	: $Est6^X/Est6^X$
	$Pgm^{1.00}/Pgm^{1.00}$	: $Pgm^{1.00}/Pgm^X$	: Pgm^X/Pgm^X,

where $Est6^X = Est6^{1.10}$ and $Est6^{1.20}$ combined and $Pgm^X = Pgm^{0.90}$ and $Pgm^{0.70}$ combined. The coefficients s and t were estimated by maximum likelihood methods using the relationships derived in Wilson and Oakeshott (8) to express subsequent genotype frequency dynamics in terms of s, t, and initial genotype frequencies.

Analyses of deviance (8) were used for significance tests of various hypotheses about the selection coefficients estimated. The deviance values obtained represent the goodness of fit of particular models of selection to the total observed genotypic data for all but the interval between generations 4 and 6 (see above). The difference in deviance between a pair of models indicates the improvement in fit due to the factor(s) by which the two models differ. The deviance difference is distributed as X^2 with degrees of freedom given by the difference in the number of separate s and t values fitted in the two models.

The simplest model was a neutral one (0,0) in which s and t were set at zero in all populations. The difference in deviance from this model provided a test of the second $(\cdot,\cdot)$, which fitted common nonzero s and t in all 10 experimental populations. The third model $(b,\cdot)$ allowed s and t to vary among the five base populations and was tested against the second. The fourth model (b,r) allowed s and t to vary among all 10 experimental populations and was tested against the third.

The magnitudes of the deviances varied with the effective population size assumed in the model fitting (because random drift is related to population size). However, effective population sizes could be estimated by Monte Carlo simulation (8) from the observed population sizes (Table 1). Separate sets of simulations for the four polymorphisms were in good agreement and indicated that the ratio f, of effective to observed population size, lay between 0.05 and 0.30 (95% confidence limits).

RESULTS

Fig. 1 shows the changes in allele frequencies over the 12 generations of the experiment. In all cases initial frequencies were within 20% of those recorded (3–6) in collections near the localities from which the base populations were obtained. The initial *Adh*, *Gpdh*, and *Est6* allele frequencies in the various base populations thus showed the latitudinal clines recorded. The largest changes during the experiment generally occurred over winter, between generations 4 and 6 but, except for *Est6*, the directions of the winter changes were seldom consistent across replicate populations and were probably due to random drift during a period of low population size. $Est6^{1.00}$ frequency increased or remained steady over winter in all but 1 of the 10 experimental populations; although such a directional trend suggests selection, the

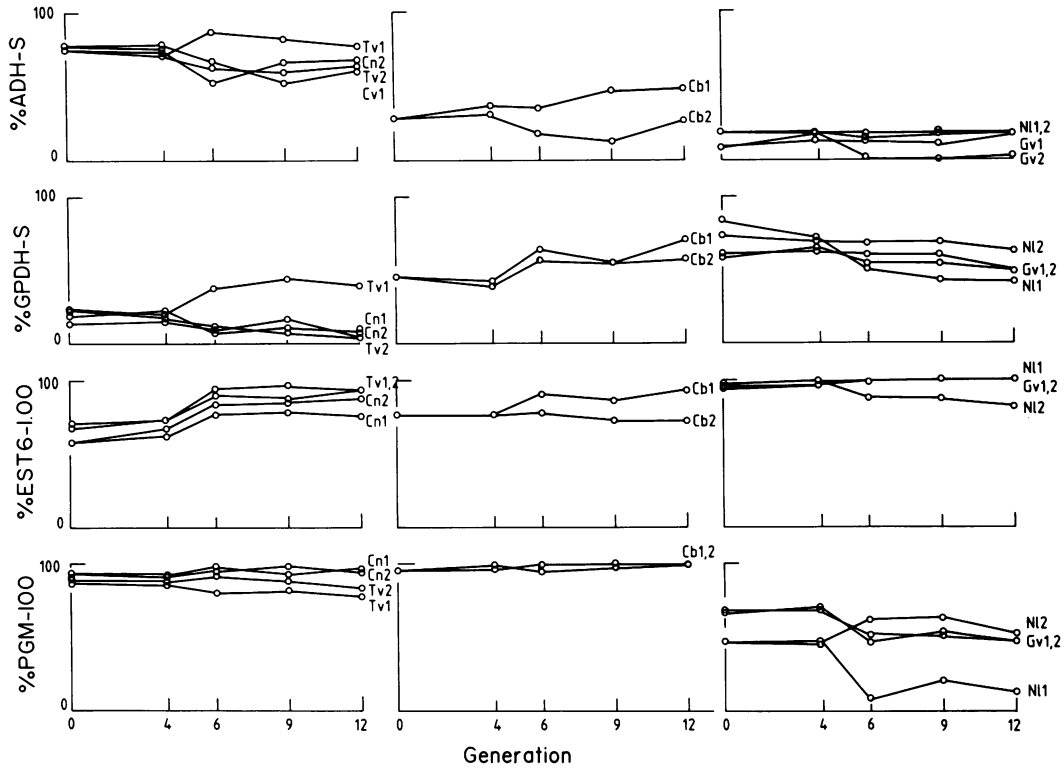

FIG. 1. Changes in *Adh*, *Gpdh*, *Est6*, and *Pgm* allele frequencies over the 12 generations in each experimental population. Data for the populations from the northern, central, and southern collections are shown on the *Left*, *Center*, and *Right*, respectively. Cn, *Cairns*; Tv, *Townsville*; Cb, *Canberra*; Gv, *Glenvale*; Nl, *Nelson*.

severe winter bottlenecks in population size precluded use of the winter data in the analyses below.

Few consistent changes in allele frequencies were observed outside winter, but there were weak trends for convergence in *Adh* and *Gpdh* allele frequencies. Although initial and final Adh^S frequencies were only trivially different in the southern populations, the northern populations showed a slight decrease (78% to 67%), and *Canberra* showed a slight increase (29% to 38%) over the 12 generations. Average $Gpdh^S$ frequency showed a negligible change in the northern populations but rose consistently in *Canberra* (45% to 64%) and fell in the southern populations (69% to 51%). $Est6^{1.00}$ frequencies were relatively stable in all populations outside winter. $Pgm^{1.00}$ frequencies, which were initially higher in the northern and *Canberra* populations, remained stable in the northern populations and went to fixation in *Canberra*. $Pgm^{1.00}$ frequencies in the southern populations were initially lower than elsewhere and remained low during the experiment.

Apart from three *Adh* and six *Gpdh* samples, which showed significant heterozygote excesses, the genotypic data were consistently in agreement with Hardy–Weinberg expectations. The heterozygote excesses that were observed for *Adh* and *Gpdh* were distributed across several populations and generations.

Table 2 shows the results of the analyses of deviance testing the goodness of fit of the genotypic frequency changes to various models of selection. For *Adh* and *Gpdh* the (·,·) models, fitting common nonzero selection coefficients to all 10 experimental populations, gave significant deviance decreases for all f values in the acceptable range, 0.05–0.30 (f = the ratio of effective to observed population size). On the other hand the (·,·) models were not significant for *Est6* or *Pgm*, even at 0.30, the upper bound for f.

Fig. 2 gives the 50%, 95%, and 99% confidence contours of the selection coefficients s and t for each polymorphism estimated under the (·,·) model. As expected from the nonsignificance of this model for *Est6* and *Pgm*, the contours for those two polymorphisms overlaid the origin, where $s = t = 0$. However, the locations of the 95% contours for *Adh* and *Gpdh* indicated significant positive values of t for both systems, negligible values of s for *Adh*, and nonsignificant but generally positive s for *Gpdh*. This suggests a degree of heterozygote advantage for both polymorphisms over all experimental populations. The best estimates of s and t predict equilibrium frequencies of ≈0.5 and 0.33 for Adh^S and $Gpdh^S$, respectively, which are consistent with the frequencies found in *Canberra* collections in this and other studies (4, 5, 9).

Table 2. Deviance differences testing the significance of three models of selection

Locus	Model	df	Deviance difference $f = 0.05$	$f = 0.1$	$f = 0.2$	$f = 0.3$
Adh	(·,·)	2	9.1*	14.9†	22.3†	26.9†
	(*b*,·)	8	11.1	19.9*	33.1†	42.2†
	(*b*,*r*)	10	11.0	19.3*	30.4†	37.7†
Gpdh	(·,·)	2	5.1‡	11.0§	17.6†	21.9†
	(*b*,·)	8	6.8	11.4	17.8*	22.3§
	(*b*,*r*)	10	8.5	14.0	21.3*	25.9§
Est6	(·,·)	2	0.8	1.2	1.8	2.0
	(*b*,·)	8	5.9	9.8	14.9‡	18.3*
	(*b*,*r*)	10	4.4	7.4	11.4	14.1
Pgm	(·,·)	2	1.7	2.4	3.1	3.4
	(*b*,·)	8	15.9*	28.5†	48.1†	62.7†
	(*b*,*r*)	10	8.3	13.1	19.1*	22.8*

f is the ratio of effective to observed population size assumed during model fitting. The following three models were tested: (·,·), common nonzero s and t over all 10 test populations; (*b*,·), s and t vary across the five base populations; (*b*,*r*), s and t vary across replicate test populations within base populations. Significance tests were based on the differences in deviance between each of these models and the next most parameterised models. df, Degrees of freedom.

*$P < 0.05$. †$P < 0.001$. ‡$P < 0.10$. §$P < 0.01$.

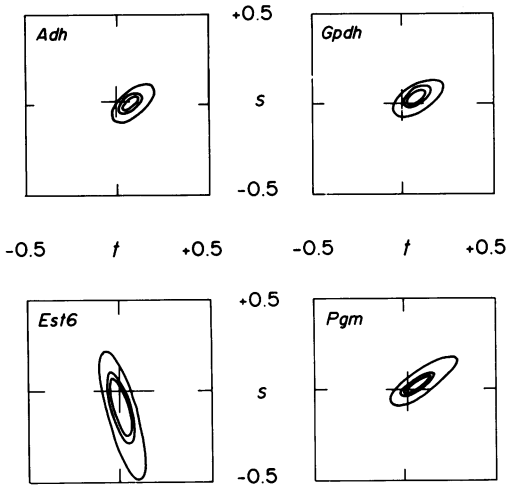

FIG. 2. The 50%, 95%, and 99% confidence contours for the maximum likelihood estimates of the selection coefficients s and t from the pooled data over all 10 experimental populations for each polymorphism. All contours are calculated at $f = 0.30$.

The $(b,\cdot)$ model, indicating differences in selection among base populations, was significant for *Pgm* at all values of f between 0.05 and 0.30 (Table 2). For *Adh* the model was not significant below $f = 0.1$; for *Gpdh* it was not significant below $f = 0.2$; for *Est6* it only became significant at f values of 0.3 or greater.

Fig. 3 shows the confidence contours of s and t for *Pgm* obtained under the $(b,\cdot)$ model for each base population. The contours for the two southern populations, *Glenvale* and *Nelson*, indicated generally positive t and negligible s. The contours for the northern populations, *Cairns* and *Townsville*, were not well defined, lying on a diagonal from negative s and t through to positive s and t and including the origin. The contour for *Canberra* resembled those for the northern populations except that it was displaced toward more positive s and negative t values and did not include the origin. The contours for the southern base populations suggested heterozygote advantage and intermediate equilibrium $Pgm^{1.00}$ frequencies. The more positive s in *Canberra* than the northern populations was consistent with the fixation of $Pgm^{1.00}$ in the Canberra populations, compared to the relative stability of high, but polymorphic $Pgm^{1.00}$ frequencies in the northern populations.

The (b,r) model, indicating differences in selection coefficients among replicate populations, was not significant at f

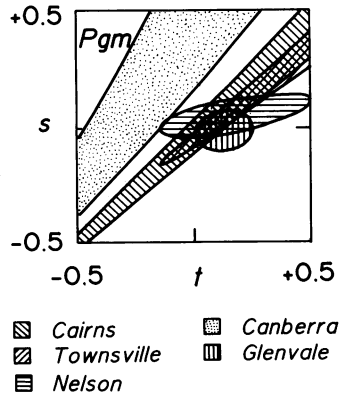

FIG. 3. The 95% confidence contours for the maximum likelihood estimates of the selection coefficients s and t for *Pgm* in each base population indicated. Format otherwise is as in Fig. 2.

values <0.1 for *Adh* or <0.2 for *Gpdh* and *Pgm*, and for *Est6* it was not even significant at $f = 0.3$.

DISCUSSION

Significant selective effects on *Adh* and *Gpdh* genotypic frequencies have been detected in enclosed populations of *D. melanogaster* living in conditions closely resembling a natural environment. The selection on these polymorphisms took the form of heterozygote advantage and was consistent across experimental populations founded from collections made over a wide range of latitudes. The results were thus also consistent with a weak trend for *Adh* and *Gpdh* allele frequencies in various experimental populations to converge to intermediate values over time, and with the observation of heterozygote frequencies significantly in excess of Hardy–Weinberg expectations in some individual samples. Moreover, the results concur with observations of stable intermediate *Adh* and *Gpdh* allele frequencies in several collections of wild *D. melanogaster* from nearby localities and others at similar latitudes (4, 5, 9). Heterozygote frequencies in excess of Hardy–Weinberg expectations have also been recorded consistently for *Adh*, albeit not for *Gpdh*, in such wild populations (10–12). We conclude that the selective forces on *Adh* and *Gpdh* in the experimental populations are to a large extent representative of those operating on the polymorphisms in unconfined populations at similar latitudes. Thus we further conclude that the selection on *Adh* and *Gpdh* in the latter populations also takes the form of heterozygote advantage.

However, one limitation of our statistical analyses that must be admitted is that they would not have detected any selective effects specific to the winter months (because population bottlenecks precluded meaningful analysis of the winter data). In addition, it must be stressed that our results do not necessarily imply that heterozygote advantage operates on *Adh* and *Gpdh* at all latitudes. The pronounced latitudinal clines for *Adh* and *Gpdh* allele frequencies (4, 5) suggest that the relative fitnesses of the homozygotes vary with latitude and at extreme latitudes may exceed the heterozygote fitnesses. The fact that *Adh* and *Gpdh* allele frequencies seldom reach fixation even at extreme latitudes might suggest that some heterozygote advantage does remain at the extremes; however, it could also be explained by directional selection at the extremes, with some migration from polymorphic populations at intermediate latitudes.

Many experiments have been carried out with laboratory populations to investigate possible physiological mechanisms of selection on *Adh* and *Gpdh*. In particular it has been established that selection between Adh^F and Adh^S occurs during detoxification of stressful concentrations of ingested ethanol; however, there is now also considerable evidence that such selection does not operate in field populations (13, 14). Another possible mechanism of selection on both *Adh* and *Gpdh* suggested by biochemical studies concerns the roles of these enzymes in reducing the cofactor NAD and providing substrates for lipid biosynthesis (15–17). These functions have fundamental effects on the availability of energy. With the developmental profiles and tissue distributions of the enzymes, this suggests that *Gpdh* is important in adult flight muscle metabolism and that both enzymes affect larval developmental rates (18, 19). Significantly, laboratory studies have indeed revealed differences among *Gpdh* genotypes in flying abilities (20) and among genotypes at both loci in developmental rates (21–25).

There is presently no direct evidence as to whether selection on flying ability or developmental rate mediates the selection on *Adh* or *Gpdh* in wild *D. melanogaster*. However, it is at least clear that selection does operate on developmental rate in wild populations; development in standard laboratory conditions is slower in populations collected from close to the equator than it is in populations from higher

latitudes (23, 26). This result suggests that mechanisms of selection on *Adh* and *Gpdh* involving developmental rate selection could explain not only the present results but also the *Adh* and *Gpdh* latitudinal clines (4, 5).

With respect to the clines, this explanation is supported by the fact that rapid development appears to select for Adh^F and $Gpdh^S$ homozygotes in the laboratory, and these genotypes are most frequent in field population collections that develop most rapidly under standard conditions (4, 5, 21–26). With respect to the present results, there is some evidence that at intermediate latitudes like Canberra intermediate developmental rates are selected (23, 26), but there is only equivocal evidence that intermediate developmental rates select for *Adh* heterozygotes and none as yet that they do so for *Gpdh* heterozygotes (21–24).

Selection was found to affect *Pgm* allele frequencies in the present study but, unlike the effects on *Adh* and *Gpdh*, was not consistent across base populations. Intermediate $Pgm^{1.00}$ frequencies had been recorded at founding in the base populations from the southern collections and the selection in those populations took the form of heterozygote advantage maintaining the intermediate frequencies. Initial $Pgm^{1.00}$ frequencies were higher in the other base populations and the selection coefficients in these populations, while not well-defined, were also generally consistent with the maintenance of those higher initial $Pgm^{1.00}$ frequencies. These results thus suggest that selection of *Pgm* in the various experimental populations was not associated with the common Canberra environment to which they were exposed, but to some factor, for example, their various genetic backgrounds, that was carried over from the original field populations.

This pattern of results stands in direct contrast to those Oakeshott *et al.* (14, 27) obtained for *Adh*, *Gpdh*, and *Pgm* in some population cages maintained in laboratory environments. These laboratory experiments indicated selection maintaining the various initial frequencies found in various base populations for *Adh* and *Gpdh* but not for *Pgm*. This is the reverse of the behaviors of the three polymorphisms in the present study. The precise reasons for the contrast are unclear but presumably they concern the very different environments to which the populations were exposed. Whereas the laboratory populations experienced defined diets and constant conditions of temperature and humidity, the present study involved diets of various rotting fruits and natural diurnal and seasonal variations in temperature and humidity. Clearly the conditions of the present study, and consequently the results, are much more relevant to wild *D. melanogaster* than studies on laboratory populations have been.

It is intriguing that of all four polymorphisms assayed in the present investigation, *Est6* is the one for which there was least evidence of selection. That selection does operate on this polymorphism in free-living *D. melanogaster* is suggested by the steep latitudinal clines for *Est6* and by the recurrence of these clines across continents and in the sibling species *D. simulans* (3, 6). One possible explanation for the inability of the present analyses to detect selection on *Est6* may relate to the necessary exclusion of winter changes in genotypic frequencies from the analyses. It may be indicative of selection that $Est6^{1.00}$ frequencies generally increased over winter and that the increases were largest in populations with initially low $Est6^{1.00}$ frequencies (Fig. 1).

A previous population transplant experiment monitoring allozyme frequencies was carried out by Barker and East (28) on *Drosophila buzzatii*. In their study the populations were not enclosed, so migration from adjacent populations into the study site could have contributed to the allele frequency changes observed. However the amounts of migration necessary to explain the observed changes varied significantly among the three enzyme polymorphisms monitored. Barker and East (28) argued that this heterogeneity could only be explained if natural selection also affected some allele frequencies. Compared to the work of Barker and East (28), the present study suffers the disadvantage that the enclosures would have excluded some aspects of the natural environment, like some parasites and competitors. On the other hand it had the advantage of excluding migration as an explanation for allele frequency change, so selection could be assessed and measured directly on individual polymorphisms. The present results thus not only confirm the conclusion from the work of Barker and East (28) and the latitudinal clines that natural selection does affect a significant proportion of enzyme polymorphisms but also show that the selection detected involves heterozygote advantage.

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