Genotype-by-Environment and Epistatic Interactions in
Drosophila melanogaster: The Effects of Gpdh Allozymes,
Genetic Background and Rearing Temperature on Larval
Developmental Time and Viability

Phillip T. Barnes, Barbara Holland and Valerie Courreges

Zoology Department, Connecticut College, New London, Connecticut 06320

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ABSTRACT

The possible role of temperature as a component of natural selection generating the latitudinal clines in Gpdh allele frequencies in natural populations of Drosophila melanogaster was examined. Effects of rearing temperature (16°, 22° and 29°) and of Gpdh allozymes (S and F) on larval developmental time and viability were measured. Eight genetic backgrounds from each of three populations (continents) were used to assess the generality of any effects. Analyses of variance indicated significant temperature effects and allozyme-by-genetic background interactions for both characters. Viability showed significant genetic background effects, as well as significant temperature-by-allozyme and temperature-by-allozyme-by-population interactions. In general, the S/S genotype was significantly lower in viability than the F/F and F/S genotypes at extreme temperatures (16° and 29°), with no significant differences at 22°. However, each population had a slightly different pattern of viability associated with temperature, and only the Australian population showed a pattern that could contribute to the observed cline formation. Although the same two interactions were not significant for developmental time, examination of the means showed that the S/S genotype had a slightly faster rate of development at 16° than the F/F genotype in all populations (by an average of 0.25 day or 1.1%). The low temperature effect on developmental time is consistent with the clines observed in nature, with the S allele increasing in frequency with higher latitudes. The results for both viability and developmental time are consistent with the interpretation of Gpdh as a minor polygene affecting physiological phenotypes, as indicated by previous work with adult flight metabolism. Finally, it is proposed that the temperature-dependent antagonistic effects of the allozymes on viability vs. developmental time and flight metabolism may be the underlying force giving rise to the worldwide polymorphism.

The sn-glycerol-3-phosphate dehydrogenase (GPDH, EC 1.1.1.8) gene-enzyme system has been extensively studied at the molecular and biochemical to population levels. It represents an interesting case for investigating the importance of variation in structural and regulatory gene expression in evolution (Laurie-Ahlberg 1985). In Drosophila melanogaster, the structural gene is polymorphic for the GpdhF and GpdhS allozyme alleles in virtually every natural population surveyed (Oakeshott et al. 1982). These two alleles show a latitudinal cline in frequency that is similar in direction on three continents, where GpdhS increases in frequency as latitude increases (Oakeshott et al. 1982; Oakeshott, McKechnie and Chambers 1984). Such consistent latitudinal clines have been interpreted as the result of natural selection (Oakeshott, McKechnie and Chambers 1984). However, other studies have given conflicting evidence about the potential role of natural selection in affecting this polymorphism. For example, Berger (1971) observed seasonal changes in Gpdh allele frequencies in natural populations in the northeastern United States. The seasonal changes involved an increase in the frequency of GpdhS in the fall. This observation would be compatible with the geographic clines if temperature was an underlying environmental factor affecting fitness. In agreement with this hypothesis, Miller, Pearcy and Berger (1975) reported temperature-dependent differences in several kinetic properties associated with the homo- and heterodimeric forms of the two allozymes. On the other hand, Cavener and Clegg (1981) found no evidence for seasonal variation in allele frequencies in a southeastern U.S. population, and Bewley, Niesel and Wilkins (1984) reported a lack of temperature-dependent kinetic differences between the allozymes.

The results of Miller, Pearcy and Berger's (1975) kinetic study could provide an explanation for the seasonal and clinal variation, if the differences in kinetic properties actually have some effect on a fitness-related phenotypic character. Barnes and Laurie-Ahlberg (1986) reported such a connection be-
tween the allozyme genotypes and adult flight metabolism. The effect was small and depended on the temperature at which the flies were reared and on the ambient temperature during flight. They concluded that \textit{Gpdh} acted as a minor polygene affecting flight metabolism.

Although GPDH has a well known and key role in flight in insects (Sacktor 1975), where the absence of the enzyme inhibits flight [at least in \textit{D. melanogaster} (O'Brien and Maclntyre 1972; O'Brien and Shimada 1974; Kotarski et al. 1983]), it probably also has an additional role in providing a metabolic precursor for lipid biosynthesis (Gilbert 1967; Geer, McKechnie and Langevin 1983; Geer, Langevin and McKechnie 1985). In fact, the importance of this dual role is suggested by the existence of two major isozyme forms of the enzyme in \textit{D. melanogaster}, GPDH-1 and GPDH-3. The isozymes are tissue and developmental-stage specific in expression and have different physiochemical and kinetic properties (reviewed in Bewley and Miller 1979). GPDH-1 is found almost entirely in adult flight muscle (O'Brien and Maclntyre 1972). GPDH-3 is the only form found in larvae, but it is also found to some extent in the adult; in both stages it is localized primarily in the fat body tissue (reviewed in Bewley and Miller 1979). GPDH-1 and GPDH-3 are produced by the same structural gene, with GPDH-3 being interpreted as a postranslational modification of the GPDH-1 primary sequence (Nie\-sel et al. 1982).

Another important aspect of the GPDH gene-enzyme system is that, of over 200 drosophilid species examined, only eight have shown within species heterogeneity and only two of the eight are considered polymorphic \cite{D. melanogaster and \textit{Drosophila subarctica} (Lakovaara, Saura and Lankinen 1977; Collier 1977; Coyne et al. 1979; Lakovaara and Keranen 1980]). Because of such within species homogeneity it has been argued that the \textit{Gpdh} gene is subject to strong purifying selection acting on flight ability \cite{Zera, Koehn and Hall 1985}. On the other hand, studies on the frequencies of null alleles at the \textit{Gpdh} locus, as well as at numerous other allozyme loci, in natural populations of \textit{D. melanogaster} have suggested that strong purifying selection may not operate against these null alleles \cite{Voelker et al. 1980; Langley et al. 1981; Freeth and Gibson 1985}. It was proposed that the null alleles are maintained by mutation-selection balance, with an average estimated reduction in fitness of the heterozygotes of only 0.0015. Thus, \textit{Gpdh} null alleles reached frequencies of approximately 0.01 in the populations studied, which is about 30 times higher than expected if the nulls were recessive lethal, according to the estimates of Langley et al. (1981).

Nevertheless, the exceptional nature of the \textit{Gpdh} allozyme polymorphism in \textit{D. melanogaster} suggests that natural selection might be involved in its maintenance, and the existence of the two isozyme forms implies that the effect of selection could occur at either or both the larval or adult stages. Barnes and Laurie-Ahlberg (1986) have examined the role of the allozymes and environmental temperature on adult flight metabolism. Alternatively, Cavenner (1983) has shown a change in the allozyme frequencies during larval developmental rate selection, with \textit{Gpdh} being increasing in frequency in the fast selected lines and \textit{Gpdh} increasing in the slow selected lines. If one assumes that selection favors a faster rate of development at cooler summer temperatures in order to complete one or more generations during the season, then Cavenner's (1983) results suggest the opposite of what one might expect based on the latitudinal clines. However, Cavenner's (1983) results were obtained at near-optimal rearing temperatures of \textit{D. melanogaster}, while Barnes and Laurie-Ahlberg's (1986) results with flight metabolism suggested that genotype-by-environment interactions can modify the expression of such physiological traits. The study presented here specifically tests the effects of the \textit{Gpdh} allozymes, genetic backgrounds and environmental temperature on larval developmental time and viability.

\section*{MATERIALS AND METHODS}

\textbf{Experimental stocks:} Pairs of homozygous \textit{F} and \textit{S} sublines were derived from originally polymorphic isofemale lines through a specific inbreeding scheme described in Barnes and Laurie-Ahlberg (1986). Sixteen pairs of sublines (from 16 isofemale lines) were obtained from each of three populations (continents): Sydney, New South Wales, Australia; Groningen, The Netherlands, Europe; and Raleigh, North Carolina, North America. These pairs of sublines were a subset of those used by Barnes and Laurie-Ahlberg (1986) and were randomly chosen from among those sets that contained only the standard gene arrangement on the left arm of chromosome two, where the \textit{Gpdh} locus is found \cite{O'Brien and Maclntyre 1978}. Within each population, isofemale lines were randomly paired and sublines were crossed between isofemale lines to generate the experimental genotypes (Table 1). The value of this scheme is threefold. First, it controls for hidden intralocus variation by assuring that all copies of a particular allele in a homozygous subline are identical by descent (autozygous). Second, it controls for dominance heterosis among genotypes by generating all experimental genotypes from crosses between two inbred lines. And third, the crosses approximately regenerate the natural heterogeneity in genetic background. As a result, the three \textit{Gpdh} genotypes (allozymes) are superimposed on eight genetic backgrounds within each of three populations, giving 72 unique combinations of allozymes and genetic backgrounds.

\textbf{Rearing conditions:} Groups of 15 pairs of adults from each \textit{F} and \textit{S} subline were placed separately in large (16 dram) vials with fresh food and allowed to lay eggs for 3 days. Virgin females and males were collected from the offspring in these vials and aged separately for five days on
Gpdh Allozymes and Development

TABLE 1

Number of Gpdh genotypes from each location used in the experiment and their origin from the isofemale lines

<table>
<thead>
<tr>
<th>Location</th>
<th>Day</th>
<th>Australia</th>
<th>Europe</th>
<th>North America</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>F9F12F12</td>
<td>F12F9F12</td>
<td>F12F9F12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S9S12S12</td>
<td>S12S9S12</td>
<td>S12S9S12</td>
</tr>
<tr>
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<td></td>
<td>F9F12F12</td>
<td>F12F9F12</td>
<td>F12F9F12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S9S12S12</td>
<td>S12S9S12</td>
<td>S12S9S12</td>
</tr>
<tr>
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<td>F9F10F10</td>
<td>F10F9F10</td>
<td>F10F9F10</td>
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<tr>
<td></td>
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<td>S9S10S10</td>
<td>S10S9S10</td>
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<tr>
<td></td>
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<td>S9S12S12</td>
<td>S12S9S12</td>
<td>S12S9S12</td>
</tr>
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</table>

For example, autozygous allozyme sublines F14 and S14 from isofemale line A1 were crossed with F12 from A2 to give the F12F14F12 subline. Nesting within location should also be nested within setup day. However, preliminary evidence indicated that setup day was an insignificant source of variation, so the effect of day was ignored in the analysis. In addition, to minimize any spurious “day” effect caused by differences among genetic backgrounds assigned to the different days, the isofemale line crosses were randomly assigned to each day in each of the three blocks. However, the original pairing of isofemale lines for the crosses, as described under “Experimental stocks,” was maintained throughout the experiment.

The whole experiment was repeated three times (blocks), once every other week. There were 1296 vials or “observations” in the experiment, 432 in each of the three blocks. As pointed out in BARNES and LAURIE-ALBREG (1986), each autozygous subline potentially represents a unique allele at the Gpdh locus. Thus, the number of independent alleles in the experiment is the number of sublines, 48 S and 48 F. Each genotype in the experiment (say F12F12; see Table 1) is not only a unique combination of genetic background and allozyme, but also may be unique at the Gpdh locus itself if there is within-allele heterogeneity in the population. In the analysis of variance (ANOVA) model, the allozyme-by-genetic background-within-location effect \( A \times G(L) \) represents both the true interaction between allozyme and genetic background, as well as within-allele variation due to factors within or closely linked to the Gpdh locus (i.e., the region made autozygous). Thus, significance of this interaction effect in the experiment could arise from either of these sources of variation separately or from both in combination.

The specific ANOVA model can be deduced in a straightforward manner from the sources given in Table 2 and from the following consideration of fixed vs. random effects. Random effects are block (B) and genetic background (G). Fixed effects are rearing temperature (R), geographic location (L) and Gpdh allozyme (A). Because the ANOVA involves a mixed effects model, error mean squares and Satterthwaite’s F-tests were calculated according to methods described by Searle (1971, p. 401) and by NETER and WASSERMAN (1974, p. 664). Differences among means for specified sources were tested for significance using either the least significant difference (LSD) method (SNEDECOR and COCHRAN 1967) or Tukey’s multiple comparison method (NETER and WASSERMAN 1974), depending on whether the F-test was or was not significant, respectively (SNEDECOR and COCHRAN 1967). For tests of hypotheses involving specific
and 8.33/8.17 days at 29°.

There was a significant difference between sexes in any of the other treatments in the experiment. Therefore, only an analysis of the developmental time for treated as replicates in the analyses reported here.

There were no significant interactions between sex and rearing temperature-by-allotype interaction with rearing temperature-by-allotype interaction for egg-to-adult developmental time and viability were both highly significant and together contributed to more than 50% of the variation in viability. The allotype main effect (A) was marginally significant. Although variation among locations (L) was not significant, its interaction with rearing temperature (R × L) was. Other significant interactions of note for viability were rearing temperature-by-allotype (R × A), location-by-

The ANOVAs and variance component estimates for egg-to-adult developmental time and viability are given in Table 2. For developmental time, the environmental sources of variation, particularly rearing temperature (R), were the major contributors to most of the variation in the experiment. For comparison, the contributions of the three main genetic effects, population or continental location (L), allotype (A) and genetic background (G).

\[ \text{Variance components} \]

\begin{tabular}{|c|c|c|c|c|}
\hline
Source & Estimate & \% of total variance & Estimate & \% of total variance \\
\hline
B & 10.92*** & 0.1668 & 0.27 & 0.64 & 0.0577 & 0.27 \\
R & 217.75*** & 60.7059 & 97.41 & 5.46*** & 1.7757 & 8.31 \\
B × R & 37.72*** & 0.7524 & 1.21 & 2.18(*) & 0.1631 & 0.76 \\
L & 0.82 & 0 & 0 & 0.74 & 0 & 0 \\
B × L & 1.15 & 0.0073 & 0.01 & 1.34 & 0.3806 & 1.78 \\
R × L & 0.82 & 0 & 0 & 2.23** & 0.8165 & 3.82 \\
B × R × L & 1.31 & 0.0192 & 0.03 & 1.14 & 0.0577 & 0.27 \\
A & 0.97 & 0 & 0 & 1.93(*) & 0.6208 & 2.90 \\
B × A & 0.10 & 0 & 0 & 0.76 & 0 & 0 \\
R × A & 0.74 & 0 & 0 & 1.79* & 0.3589 & 1.68 \\
B × R × A & 0.29 & 0 & 0 & 0.68 & 0 & 0 \\
L × A & 0.51 & 0 & 0 & 1.85(*) & 1.2730 & 5.96 \\
B × L × A & 1.69 & 0.0164 & 0.03 & 0.89 & 0 & 0 \\
R × L × A & 0.62 & 0 & 0 & 2.18*** & 0.8004 & 3.74 \\
B × K × R × L × A & 1.75* & 0.0374 & 0.06 & 0.23 & 0 & 0 \\
G(L) & 0.82 & 0 & 0 & 2.91*** & 5.7752 & 27.02 \\
B × G(L) & 3.91*** & 0.1315 & 0.21 & 1.91*** & 2.4856 & 11.63 \\
R × G(L) & 1.12 & 0.0196 & 0.03 & 2.03*** & 1.1361 & 5.32 \\
B × R × G(L) & 3.62*** & 0.3561 & 0.57 & 0.41 & 0 & 0 \\
A × G(L) & 1.75* & 0.0470 & 0.08 & 2.99*** & 5.5772 & 25.91 \\
B × A × G(L) & 1.39* & 0.0531 & 0.09 & 1.02 & 0.1917 & 0.90 \\
R × A × G(L) & 1.06 & 0.0084 & 0.01 & 0.41 & 0 & 0 \\
\hline
\text{Error} & 816 & \hline
Total & 1295 & \hline
\end{tabular}

\( ^* \text{Letter codes refer to: block (B), rearing temperature (R), continental location (L), allotype (A) and genetic background (G).} \)

\( ^{\dagger} \text{SATTERTHWAITE's approximation for a mixed effects model.} \)

\( ^{\ddagger} \text{Negative variance component estimates were assumed to equal zero.} \)

\( ^{\S} \text{Error equals pooled residual [B × R × A × G(L)] and within cells errors for SATTERTHWAITE's approximation.} \)

\( ^{*} 0.05 < P < 0.10, ^{*} P < 0.05, ^{**} P < 0.01, ^{***} P < 0.001. \)

measures for which the direction of the difference had been predicted, LSDs using one-tailed t-values were employed.

RESULTS

Preliminary ANOVAs showed no significant effect due to reciprocal crosses for either developmental time or viability. Therefore, reciprocal crosses were treated as replicates in the analyses reported here. There was a significant difference between sexes in developmental time, with females averaging a faster rate of development than males at all three temperatures. The male/female development times were 23.57/22.81 days at 16°, 11.95/11.58 days at 22° and 8.33/8.17 days at 29° (LSD = 0.15 day). However, there were no significant interactions between sex and any of the other treatments in the experiment. Therefore, only an analysis of the developmental time for all flies, sexes combined, is reported here.

The ANOVAs and variance component estimates for egg-to-adult developmental time and viability are given in Table 2. For developmental time, the environmental sources of variation, particularly rearing temperature (R), were the major contributors to most of the variation in the experiment. For comparison, the contributions of the three main genetic effects, population or continental location (L), allotype (A) and genetic backgrounds nested within locations (G(L)), were essentially zero. The only genetic effect of significance was the allotype-by-genetic background interaction [A × G(L)]. These results suggest that there is little measurable genetic variation affecting developmental time within and among these populations.

On the other hand, the ANOVA for viability shows significant contributions from both environmental and genetic sources. Rearing temperature (R) again was a major source of environmental variation. The genetic backgrounds [G(L)] and the allotype-by-genetic background interaction [A × G(L)] were both highly significant and together contributed to more than 50% of the variation in viability. The allotype main effect (A) was marginally significant. Although variation among locations (L) was not significant, its interaction with rearing temperature (R × L) was. Other significant interactions of note for viability were rearing temperature-by-allotype (R × A), location-by-
**Figure 1.**—Effect of rearing temperature on developmental time and viability. (A) Developmental time (in days). (B) Viability (in number of emergent adults). N = 432 per mean. The 95% confidence intervals (C.I.) are shown.

**Figure 2.**—Effects of allotype and of the rearing temperature-allotype interaction on viability. (A) Allotype main effect; N = 432 per mean. (B) Rearing temperature-by-allotype interaction; N = 144 per mean. The 95% C.I. are shown.

**Figure 3.**—Effects of interactions involving continental location on viability. (A) Rearing temperature-by-location interaction. (B) Location-by-allotype interaction. N = 144 per mean. The 95% C.I. are shown. Location symbols: A = Australia, E = Europe and N = North America.

The Pdph allozymes and development study examines the effects of rearing temperature and allotype on developmental time and viability. Figure 1 shows the effect of rearing temperature on developmental time and viability. Figure 2 illustrates the effects of allotype and rearing temperature on viability. Figure 3 displays the effects of interactions involving continental location on viability. The study finds that viability is highest at the intermediate temperature (22°), with S/S having slightly lower viability than both F/F and F/S at the extreme temperatures (16° and 29°), but not at the intermediate temperature (22°). The population from Europe has significantly higher viability than both Australia and North America at 16° and 22°, but not at 29°, suggesting that the Groningen population is adapted to cooler annual temperatures than the other two populations. The effect of the allotypes on viability is also dependent to some extent on the continental location (L × A interaction), as seen in Figure 3B. The means for this interaction effect show that there is no difference among allotypes from Australia and also probably no difference among those from North America. However, in Europe S/S is significantly inferior to F/F and F/S, reflecting perhaps a different epistatic relationship between allotypes and genetic background in this population. The effect of rearing temperature on these epistatic interactions (R × L × A) is shown in Figure 4. In Australia there is a slight, but nonsignificant, tendency for S/S to be superior to F/F and F/S at 16° and 22°. In Europe S/S is always significantly inferior to F/F and F/S, regardless of temperature. The pattern for North America is complicated, with S/S showing significantly lower viability than F/F and F/S at 16°, but with apparent heterozygote advantage occurring at 29°. As mentioned previously, developmental time was most strongly affected by the environmental sources of variation. The lability of developmental time to environmental variation is seen not only in the rearing temperature effect (R), but also in the significant block (B) and block-by-rearing temperature interaction (B × R × L).
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FIGURE 4.—Three-way interaction effect of rearing temperature-by-location-by-allotype on viability. \( N = 48 \) per mean. The 95% C.I. are shown. (A) Australia, (E) Europe and (N) North America.

FIGURE 5.—Block-by-rearing temperature interaction effect on developmental time. \( N = 144 \) per mean. The 95% C.I. are shown.

\( \times R \) effects (Table 2). Figure 5 shows the means for the block-by-rearing temperature interaction, where the source of the significant variation is seen at 16° only. Perhaps low temperature amplifies the effect of other minor sources of environmental variation on developmental time that were not explicitly controlled in the experiment (e.g., amount of live yeast added to each vial, exact ratio of dry medium to water in food preparation, fluctuation in humidity from 40 to 60%, etc.). Indeed, except for the rearing temperature main effect \( R \) and the allotype-by-genetic background interaction \( [A \times G(L)] \), all other significant sources of variation in the ANOVA for developmental time (Table 2) involve the block term.

Although the allotypes and their interactions with rearing temperature were not significant in the ANOVA for developmental time, the means for the rearing temperature-by-location-by-allotype interaction \( (R \times L \times A) \) are given in Table 3. At 16° in all three locations, the \( S/S \) genotype shows a slightly faster rate of development than \( F/F \), averaging approximately 0.25 day (or 1.1%). At 22° and 29° the differences among genotypes are very slight (less than 0.1 day). The LSD (one-tailed \( t \)) equals 0.43 day. Within each block, however, this relationship between \( F/F \) vs. \( S/S \) at low temperature is not strictly maintained. Of the nine possible comparisons of \( F/F \) vs. \( S/S \) at 16° (three blocks \( \times \) three locations), two show \( F/F \) with a faster rate of development. A Wilcoxon signed rank test (Snedecor and Cochran 1967, p. 128) gives a rank sum value of 7 (a value of 6 or less would be considered significant, \( P = 0.055 \)). Although not significant, these results suggest that \( S/S \) individuals may possibly have a slightly faster rate of development than \( F/F \) individuals at low temperature, which could be a source of selective advantage favoring the \( S \) allele at low temperatures.

The \( F/S \) heterozygotes vary in rate of development at 16°, from the slowest in Australia through intermediate in North America to the fastest in Europe (Table 3). Again, these difference are not significant, but the pattern does complicate the issue. These results can be interpreted as arising from a changing dominance relationship for developmental rate between the two alleles due to different epistatic interactions between the \( Gpdh \) locus and the genetic content of the three different populations (locations). Thus, the \( F \) allele may be dominant in Australia but recessive in Europe, with no dominance occurring in North America. Epistasis was invoked previously to explain the variation in viability seen in Figures 3B and 4. However, if the viability of the three allotypes

### Table 3

Means for developmental time (in days) associated with the rearing-temperature-by-location-by-allotype interaction

<table>
<thead>
<tr>
<th>Rearing temperature</th>
<th>Allotype</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Australia</td>
</tr>
<tr>
<td>16°</td>
<td>( FF )</td>
<td>23.45</td>
</tr>
<tr>
<td></td>
<td>( FS )</td>
<td>23.69</td>
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<tr>
<td></td>
<td>( SS )</td>
<td>8.26</td>
</tr>
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</table>

\( N = 48 \) for each mean value.
at 16° is examined for each population (Figure 4), it appears that the F allele is always dominant over S. If these results do reflect different epistatic interactions for the two separate traits, then two different, but not mutually exclusive, sets of genes may be involved, with the Gpdh locus as one genetic element in common.

**DISCUSSION**

Viability is strongly affected by both genetic and environmental temperature sources of variation, as well as by significant interactions among genetic sources and between genetic and environmental temperature sources. However, only in the Australian population (R × L × A interaction; Figure 4) is the pattern of viability such that it can help explain clinal seasonal variation in the Gpdh allele frequencies.

Developmental time is strongly affected by the environmental sources of variation [blocks (B) and rearing temperature (R)], but shows little effect due to genetic sources. Nevertheless, the means for the three-way interaction among rearing temperatures, locations and alleles (R × L × A; Table 3) suggest that the alleles may contribute toward variation in developmental time that is compatible with seasonal and clinal variation data. This trend is apparent in all three populations and is expressed as a slightly higher, though not significantly so, rate of development for the S/S allele at low temperature. Such results are in agreement with the hypotheses of CAVERNER (1983) and KNIBB, OAKESHOTT and WILSON (1987) that allozyme variation at the Gpdh and related loci (Adh and Gpd) affects the rate of development, perhaps through their role in lipid biosynthesis. However, the effect is small, subject to modification by the genetic background or within-allele heterogeneity [A × G(L)] and is temperature dependent (Table 3), suggesting that Gpdh is a minor polygene affecting such physiological phenotypes, as BARNES and LAURIE-AHLBERG (1986) pointed out for flight metabolism.

Previous studies have shown that there is significant quantitative genetic variation in D. melanogaster for both viability (e.g., MUKAI and NAGANO 1983) and developmental time (e.g., ROBERTSON 1963, 1964). It might also be expected that there would be a certain degree of covariance between these two traits because the genes contributing to the two characters would not be mutually exclusive. The results obtained in the present study show that Gpdh may be considered to be one of the polygenes affecting both characters.

It was surprising that no measurable genetic variation among populations (L) or among genetic backgrounds within populations [G(L)] was found for developmental rate (Table 2). This result does not indicate that no genetic variation was present, as pointed out by MARINKOVIĆ and AYALA (1986a, b). In their case they were unable to change the rate of embryogenesis (time from egg-laying to egg-hatching) by artificial selection in D. melanogaster but were able to show that substantial amounts of genetic variation existed for both rate of embryogenesis and egg-to-adult developmental time by extracting second and third chromosomes and making them homozygous. Other studies with Drosophila have shown that there is a significant lag time between the initiation of selection for change in egg-to-adult developmental time and response to the selection (SANG and CLAYTON 1957; MARIEN 1958; CLARKE, MAYNARD SMITH and SODHI 1961; PROUT 1962). The usual interpretations for these phenomena involve strong physiological constraints, canalization and/or homeostatic mechanisms that restrict the expression of genetic variation for the trait or because stabilizing natural selection strongly opposes any change in the character. The results reported here, where both environmental and genetic components were examined simultaneously, seem to suggest that the effect of environmental variation on developmental time may overwhelm the effect due to genetic variation present, with the result that additive genetic variation (and, hence, narrow sense heritability) would appear to be very small in comparison to variation induced by the environment.

In fact, these selection experiments result in changes in developmental time that are sometimes modest at best. For example, CAVERNER (1983) was able to generate an average difference of approximately 1.25 days between his most extreme pairs of fast and slow selected lines after 18 generations (average developmental times of 15.22 vs. 16.44 days at 23–24°C) and KNIBB, OAKESHOTT and WILSON (1987) were only able to cause a change of approximately 0.7 day between fast and slow selected lines after 36 generations (actual developmental times at 22°C not reported). In the current study the nonsignificant differences between the S/S and F/F alleles at 16° averaged approximately 0.25 day for the three locations (average developmental time of 23.18 days). Considering the fact that selection of CAVERNER (1983) and by KNIBB, OAKESHOTT and WILSON (1987) affected the entire gene pool of their respective populations, not just Gpdh, then the difference of 0.25 day reported here could make an important contribution to an overall change in developmental time in a population over many generations and/or in unison with other polygenes.

Although the evidence seems to be mounting that thecline in Gpdh allele frequencies is maintained by selection generated through temperature dependent effects, clear-cut conclusions are not yet warranted. For instance, although the experimental design that is used here and in BARNES and LAURIE-AHLBERG.
(1986) attempts to reduce spurious effects due to linkage disequilibrium between Gpdh and other genetic elements (by sampling many chromosomes from different populations and by looking for average effects of Gpdh alleles over this heterogeneity in genetic background), one still cannot completely dismiss the possible effects due to linkage to the Gpdh locus. DNA sequence and restriction site variation data at the alcohol dehydrogenase (Adh) locus have shown that linkage disequilibrium in D. melanogaster can exist on a worldwide basis in the region around a single locus (Kreitman 1983; Aquadro et al. 1986). Thus, effects attributable to allozymes in this study could still be due to elements closely linked to the Gpdh locus. In addition, the fewer the number of second chromosomes sampled within each location, the more likely linkage disequilibrium will extend over larger regions of the chromosome. This study sampled 52 chromosomes per geographic location [Barnes and Laurie-Ahlberg (1986) sampled 60 per location]. Thus, there is some potential for linkage disequilibrium to extend beyond the immediate vicinity of the Gpdh locus in the current study. As pointed out in Materials and Methods (under "Experimental design"), the allotype-by-genetic background interactions $A \times G(L)$ found for both developmental time and viability may actually confound the interaction between the region around the Gpdh locus and the genetic background with any true allotype-by-genetic background interaction.

Also, Cavener (1983) showed that the Gpdh$^5$ allele decreased in frequency in lines selected for a fast rate of development and increased in those selected for a slow rate of development (see his Table 3; these results were transposed in his discussion of the significance of these data for the latitudinal clines). Similarly, Marinković et al. (1987) assayed electrophoretically the offspring of recently collected wild D. melanogaster and found the F/F genotype to occur at a higher frequency in their "fast" and "intermediate" developmental time categories than in their "slow" developmental time category. Thus, the results of these two studies are incompatible with an explanation of the latitudinal clines based on developmental rate and contradict the conclusion reached in this study, that Gpdh$^5$ is associated with a slight increase in developmental rate at low temperature. These contradictory results could arise from different allotype-by-genetic background interactions (a significant component found in this study) or to potentially different linkage disequilibria established in the three studies between Gpdh and other parts of the genome. Indeed, one of Cavener's (1983) populations showed an initial increase in Gpdh$^5$ frequency in the fast selected lines, which subsequently was reversed in direction. He hypothesized that this pattern possibly was due to linkage disequilibrium between Gpdh$^5$ and In(2L)T, where Gpdh$^5$ initially decreased in frequency as In(2L)T was presumably lost from the population, whereupon Gpdh$^5$ then began to increase. These problems might find some resolution in selection experiments for developmental rate at different temperatures with widely differing genetic backgrounds, such as the populations initially sampled for this study.

The contradictory results for seasonal variation in Gpdh allele frequencies reported by Berger (1971) vs. Cavener and Clegg (1981), as well as the contradictory results for temperature-dependent differences in enzyme kinetics reported by Miller, Pearce and Berger (1975) vs. Bewley, Niesel and Wilkins (1984), also cannot be dismissed. A more recent study on seasonal variation in enzyme polymorphism in Spain does little to help resolve the contradictory evidence for Gpdh allozymes (Muñoz-Serrano, Alonso-Moraga and Rodero 1985). In their study, one field "subpopulation" seemed to show an annual cycle opposite to Berger's (1971), while a second appeared to fluctuate randomly. However, sample sizes were relatively small (40 flies per location per sample date), so it is hard to make conclusions about differences between the subpopulations. Currently, an intensive weekly survey of potential seasonal variation in allele frequencies at the Gpdh and Adh loci, along with some potentially relevant environmental variables, is underway in a natural population in a southeastern Connecticut orchard (P. T. Barnes and S. M. Sasic, unpublished results). Additional intensive studies on seasonal variation from different geographic locations and enzyme kinetics with an emphasis on statistical power may help in resolving these contradictions.

Finally, the low temperature effects of the Gpdh alleles on adult flight metabolism (Barnes and Laurie-Ahlberg 1986) and developmental time, as reported here, can only explain the cline in allele frequencies, not the existence of the polymorphism. However, the antagonistic temperature-dependent effects of the Gpdh alleles on viability vs. developmental time and flight metabolism, where Gpdh$^5$ is favored at extreme temperatures for viability and Gpdh$^5$ is favored at lower temperature for developmental time and flight, may provide a starting point for explaining the world-wide Gpdh polymorphism in terms of balancing selection.

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