A SURVEY OF ISOZYME POLYMORPHISM IN A *DROSOPHILA MELANOGASTER* NATURAL POPULATION

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Manuscript received November 11, 1974
Revised copy received February 20, 1975

ABSTRACT

A survey of biochemical polymorphism among glucose- and non-glucose-metabolizing enzymes was carried out on the June 1973 collection from the South Amherst, Mass. *Drosophila melanogaster* natural population. Polymorphic levels are among the highest recorded for this species; polymorphism among glucose-metabolizing enzymes did not differ significantly from that among non-glucose-metabolizing enzymes. Two loci, *G6Pd* on the *X* and *Est-6* on the *3rd* chromosome, displayed significant excesses of heterozygotes. *Adh* on the 2nd and *Idh*, *Odh* and *Ao* on the 3rd chromosome showed significant heterozygote deficiencies. *Idh* is ten map units to the left of *Est-6*, *Odh* twelve map units to the right and *Ao* is seven units beyond *Odh*. Temperatures in the two-week June period prior to collection were exceedingly variable. Daily high/low ranged between 76°/40° and 97°/65°F. These results support the findings of FRYDENBERG and SIMONSEN (1973) that in some populations glucose-metabolizing enzymes tend to be as polymorphic as non-glucose-metabolizing ones. They also add to the evidence obtained from other plant and animal populations that increased biochemical polymorphism is associated with more variable and/or colder climates. The increase may in part be due to increased polymorphism among glucose-metabolizing enzymes. Comparisons utilizing published data on other *D. melanogaster* populations and on *D. robusta* indicate a clinal increase in heterozygosity among glucose-metabolizing enzymes as one moves northward.

THE South Amherst, Mass. *Drosophila melanogaster* natural population is the most continuously studied *D. melanogaster* natural population to date. Work on lethals in this population was begun by IVES in 1938 (see IVES 1945) and subsequent studies have demonstrated the adaptive role of these variants in maintaining population adjustment both to short-term climatic fluctuations and longer term climatic shifts (see IVES 1970; BAND 1972a, b).

In recent years BERGER (1971) and CHARLESWORTH and CHARLESWORTH (1973) have employed this population in biochemical investigations. To date, however, no extensive survey of biochemical polymorphism in the population has been made.

[1] Work supported by AEC contract AT(04-3) to DR. FRANCISCO AYALA.

The purpose of this paper is to report a survey of 23 loci in the population, to compare heterozygosity among glucose- and non-glucose-metabolizing enzymes with estimates obtained for other *D. melanogaster* populations (KOJIMA, GILLESPIE and TOBARI 1970; GILLESPIE and LANGLEY 1974), other Drosophila species (KOJIMA, GILLESPIE and TOBARI 1970; AYALA and POWELL 1972; PRAKASH 1973) and other animal populations (FRYDENBERG and SIMONSEN 1973; GILLESPIE and LANGLEY 1974). We also compare levels of polymorphisms among isozymes in the June 1973 collection with estimates from past population studies (BERGER 1971; CHARLESWORTH and CHARLESWORTH 1973).

MATERIALS AND METHODS

One hundred and fifty F_1 strains, each descended from a single wild-caught female in a June 1973 collection made by DR. P. T. IVES at the Markert site, provided the material for the enzyme assay of the population. The assay was done in the laboratory of DR. F. A. AYALA at the University of California, Davis. Work on many Drosophila species is done by DR. AYALA's laboratory. MISS LORRAINE BARR, DR. AYALA's research associate, suggested that assay procedures used for the *D. willistoni* group would give the most satisfactory results in *D. melanogaster* and *D. simulans* species. A small sample of South American *D. simulans* from four localities that had been maintained by DR. MARTIN TRACEY provided practice material for most isozymes surveyed prior to attempting the *D. melanogaster* work.

Sample preparation, gel preparation and buffer systems used followed procedures given in AYALA *et al.* (1972, 1974). In general, the 23 enzymes studied require 5 different starch, gel buffer and bridge buffer combinations, as follows:

	I	II	III	IV	V
Starch	Sigma	Electro	Sigma	Electro	Sigma + TPN
Gel buffer	Poulik	Tris-boric	Tris-citric + EDTA	Tris-citric + EDTA	Tris-boric
Bridge buffer	Poulik	Tris-boric	Tris-citric + EDTA	Tris-citric + EDTA	Tris-boric + EDTA

Enzymes assayed in combination I are allowed to electrophorese for about 4½ hours or until the front has adavanced about 5 cm; others continue for 6 hours. Runs are at 50 mA but not in excess of 200 volts (I), 400 volts (II, V) and 150 volts (III, IV). Staining procedures are as given in AYALA *et al.* (1972, 1974).

Enzymes assayed and starch, gel and bridge buffer combinations required are: acid phosphotase (*Acph*), I; alcohol dehydrogenase (*Adh*), II; aldolase (*Aldo*), V; alkaline phosphotase (*Aph*), I; aldehyde oxidase (*Ao*), II; esterase (*Est*), I; fumarase (*Fum*), V; glucose-6-phosphate dehydrogenase (*G6Pd*), V; glutamate oxaloacetate transaminase (*Got*), V; hexokinase (*Hk*), IV; isocitrate dehydrogenase (*Idh*), IV; leucine aminopeptidase (*Lap*), I; malate dehydrogenase (*Mdh*), I; malic enzyme (*Me*), III; octonal dehydrogenase (*Odh*), II; phosphoglucomutase (*Pgm*), III; tetrazolium oxidase (*To*), III; triose phosphate isomerase (*Tpi*), IV; xanthine dehydrogenase (*Xdh*), II.

The 19 enzyme assays yield information on the genetic variation at 23 gene loci; alpha-glycerophosphate dehydrogenase (α-*Gpd*) is *Tpi-1*. Four slices can be obtained from each gel, each stained for a different enzyme according to starch, gel buffer, and bridge buffer combination used. Thus a single female from each F_1 line can be used for four enzyme assays. The split technique was not employed, whereby a single female can be used for eight enzyme assays. Thus the 19 enzyme assays required the use of up to five single females from each F_1 line.

RESULTS

When several forms of the same enzyme exist, each coded by a different gene locus, we have followed AYALA's procedure of designating the isozyme with the least anodal migration as 1, the next faster as 2, and so on (see AYALA *et al.* 1972). This presents no problem for *Got-1*, *Got-2* and *Tpi-2* not previously reported for *D. melanogaster*. However, our designations of *Hk-1*, *Hk-2* and *Hk-3* are the reverse of FOX and MEDHAVEN (1971) and SING, BREWER and THIRTLE (1973).

In Table 1 we likewise designate the slowest allele as 1, the next faster as 2, etc. Our results are thus presented comparably to those of KOJIMA, GILLESPIE and TOBARI (1970) and of earlier workers rather than to the later methods of AYALA and LEWONTIN, whereby alleles are measured against an arbitrary stan-

TABLE 1

Number of genes sampled, allelic frequencies and observed proportion of heterozygotes for polymorphisms among glucose- and non-glucose-metabolizing enzymes in an early summer population of South Amherst D. melanogaster

Enzyme	No. genes sampled	Frequency 1	2	3	4	Observed proportion heterozygotes
A. Glucose-metabolizing enzymes						
α-Gpd	292	0.2774	0.7226			0.4315
Aldo	276	1.00				0
Fum	252	1.00				0
G6Pd	268	0.4813	0.5187			0.8731
*Got-1**	172	0.9826	0.0174			0.0349
*Got-2**	200	1.00				0
Hk-1	292	0.9726	0.0205			0
Hk-2	292	0.9589	0.0411			0.0479
Hk-3	240	1.00				0
Idh	252	0.0278	0.9405	0.0079	0.0238	0.0397
Mdh	280	1.00				0
Me	240	0.9917	0.0083			0.0167
Pgm	282	0.0319	0.9539	0.0142		0.0922
Tpi	292	0.6780	0.3219			0.4520
						Mean = 0.1420 ± 0.0697
B. Non-glucose-metabolizing enzymes						
Acph	272	0.0515	0.9485			0.0735
Adh	292	0.3836	0.6164			0.3699
Aph	48	0.9583	0.0417			0.0833
Ao	250	0.8840	0.1160			0.1520
Est-6	252	0.5714	0.4127			0.7778
Lap-2	147	1.00				0
Odh	278	0.1619	0.8381			0.1223
To	250	1.00				0
Xdh	206	0.0194	0.9806			0.0388
						Mean = 0.1797 ± 0.0836
						Mean all = 0.1568 ± 0.0522

* *Got* is traditionally placed with the glucose-metabolizing enzymes.

dard, then the most common allele is designated as 100 and the slower or faster alleles designated proportionately.

The number of genes sampled and allelic frequencies for the polymorphic isozymes detected among the surveyed isozymes are shown in Table 1. Observed proportions of heterozygotes are also given.

The observed frequencies of heterozygotes indicate that a significant excess is present at two loci, *G6Pd* on the *X* and *Est-6* on the *3rd* chromosome. Heterozygote deficiencies occur at four other loci, *Adh* on the *2nd* and *Idh, Odh* and *Ao* on the *3rd* chromosome. The significance of these excesses and deficiencies is explored in Table 2; map position for each locus is also given (see O'BRIEN and MACINTYRE 1971). JOHNSON and SCHAFFER (1973) likewise found homozygote excesses more prevelant than heterozygote excesses among the nine loci they surveyed in East Coast *D. melanogaster* populations.

Four of the six loci in this survey showing homozygote or heterozygote excesses lie along the *3rd* chromosome, *Idh, Est-6, Odh* and *Ao*. Interestingly, *Idh* and *Odh* are about equidistant on either side of *Est-6*; both show a greater deficiency of heterozygotes and higher chi-square values than *Ao*, which lies 7.4 map units from *Odh* and 19.8 units from *Est-6*.

Pgm, at 43.4 map units on the *3rd* chromosome, lies between *Est-6* and *Odh*. The expected level of heterozygosity, 0.0889, is in good agreement with the observed level, 0.922, as shown in Table 1. Three alleles are found in the population (CHARLESWORTH and CHARLESWORTH 1973). However, Pgm^2 has a frequency of 0.95 in this survey, in comparison with an average frequency of 0.75 in Fall 1969 and 1970 populations studied by the CHARLESWORTHS (1973). In other species of Drosophila *Pgm* alleles have been observed to undergo cyclic variation (DOBZHANSKY and AYALA 1973; KOJIMA *et al.* 1972).

Xdh at 52 map units lies between *Odh* and *Ao* on the *3rd* chromosome. Xdh^2 has a frequency of 0.98 in this early summer population and there is good agreement between the expected level of heterozygotes, 0.038, and the observed level, 0.039. The slow allele predominated in the Fall collections analyzed by CHARLESWORTH and CHARLESWORTH (1973).

BERGER (1971) investigated the seasonal shift in isozyme frequencies at the *α-Gph* locus in the South Amherst *D. melanogaster* population. The frequency of 28% for the α-Gph^1 allele in the June 1973 population is in good agreement with his past observations on the early summer population in 1966. *Tpi-2* has not been previously investigated in this species. Whether or not isozyme frequencies at this locus display a seasonal shift remains to be determined. At neither locus is there a significant excess of heterozygotes. In fact, agreement is good between expected levels, 40% for *α-Gph* heterozygotes and 44% for *Tpi-2* heterozygotes, and observed frequencies.

As shown in Table 1, average heterozygosity for glucose-metabolizing enzymes is 0.1420 for the 14 loci surveyed. Average heterozygosity for the non-glucose-metabolizing enzymes is 0.1797 for the 9 loci surveyed. Both *Est-6* and *G6Pd* have been left in the computations since each contributes to a different group. When the proportion of polymorphic loci in the two groups is computed, the

TABLE 2

Comparison of observed and expected numbers of homozygotes and heterozygotes for G6Pd, Adh, Idh, Est-6, Odh *and* Ao *isozymes*

X (63.0)* Glucose-6-phosphate dehydrogenase							
Genotypes	1	2	1/2	T			
Observed	6	11	117	134			
Expected	31.03	36.05	66.92	134			
Deviation	—25.03	—25.05	50.08				$\chi^2_1 = 75.08$***
II (50.1)* Alcohol dehydrogenase							
Genotypes	1	2	1/2	T			
Observed	29	63	54	146			
Expected	21.48	55.47	69.95	146			
Deviation	7.52	7.53	—15.05				$\chi^2_1 = 6.94$**
III (27.1)* Isocitrate dehydrogenase							
Genotypes	1	2	3	4	hets.	T	
Observed	3	118	—	2	3	126	
Expected	0.1	112.52	0.01	0.07	13.30	126	
Deviation	2.9	5.48	—.01	1.93	—10.30		$\chi^2_1 = 146.54$***
III (36.8)* Esterase-6							
Genotypes	1	2	3	hets.	T		
Observed	23	5	0	98	126		
Expected	41.14	21.46	—	64.40	126		
Deviation	—18.14	—16.46		34.60			$\chi^2_1 = 39.51$***
III (49 2)* Octonal dehydrogenase							
Genotypes	1	2	1/2	T			
Observed	14	108	17	139			
Expected	3.64	97.63	37.73	139			
Deviation	10.36	10.37	—20.73				$\chi^2_1 = 41.98$***
III (56.6)* Aldehyde Oxidase							
Genotypes	1	2	1/2	T			
Observed	101	5	19	125			
Expected	97.63	1.68	25.64	125			
Deviation	3.32	3.32	—6.64				$\chi^2_1 = 8.39$***

* Chromosome and map position.
** $P<0.01$
*** $P<0.001$

proportion of glucose-metabolizing enzymes polymorphic is 64%; the proportion of non-glucose-metabolizing enzymes polymorphic is 78%. The level of polymorphism among the glucose-metabolizing enzymes is not significantly different from that for the non-glucose-metabolizing enzymes.

Got is technically not one of the glucose-metabolizing enzymes but has typically been placed in this enzyme set (see FRYDENBERG and SIMONSEN 1973; GILLESPIE and LANGLEY 1974). When the *Got* allozymes are transferred to the non-glucose-metabolizing group, then results for the two sets of enzymes become even more comparable. With *Got* excluded, average heterozygosity among the 12

glucose-metabolizing enzymes is 0.1628 ± 0.0580 and the proportion of polymorphic loci is 67%. With *Got* included among the now 11 non-glucose metabolizing enzymes, average heterozygosity for the group becomes 0.1502 ± 0.0704 and the proportion of polymorphic loci is 73%. Clearly, in this population the levels of polymorphism and of heterozygosity tend to be similar in the two groups of enzymes.

DISCUSSION

GILLESPIE and KOJIMA (1968) were the first to point out that both average heterozygosity and the proportion of polymorphic loci were less for glucose-metabolizing enzymes than for non-glucose-metabolizing ones. These studies on *D. ananassae* were extended by KOJIMA, GILLESPIE and TOBARI (1970) to *D. simulans*, *D. affinis*, *D. athabasca* as well as to *D. melanogaster* with similar results. Additional *D. melanogaster* studies by GILLESPIE and LANGLEY (1974) have tended to agree with past work also, while AYALA and POWELL (1972) obtained comparable results when the two groups of enzymes were compared in *D. willistoni*, *D. equinoxalis* and *D. tropicalis*, as did RICHMOND (1972) in *D. paulistorum*, another sibling species of the *D. willistoni* group. PRAKASH (1973) found similar evidence in *D. robusta* but observed that marginal populations of this species displayed both greater heterozygosity and higher levels of polymorphic loci among both groups of enzymes than did central populations.

At variance with the above results, FRYDENBERG and SIMONSEN (1973) found that glucose-metabolizing enzymes in eel pout populations along the Danish coast tended to be as polymorphic as non-glucose-metabolizing enzymes. They concluded that the hypothesis of GILLESPIE and KOJIMA (1968) may not be a general one for animal species. Our findings for the South Amherst *D. melanogaster* population support the conclusions of FRYDENBERG and SIMONSEN (1973) and indicate also that the hypothesis may not be entirely valid for Drosophila, the species from which most previous supportive evidence has been obtained.

However, in agreement with the observation of PRAKASH (1973) on *D. robusta*, marginal populations of *D. melanogaster* may be more polymorphic than those in more equable environments. From lethal-frequency climatic data studies, BAND and IVES (1961) concluded that the environment had become more marginal for the Amherst population; there was a further deterioration after 1961 (see BAND 1972a, b). As shown in Table 3, polymorphic levels in the Amherst population are higher than for North Carolina, Texas or Japanese populations of this species on which past enzyme surveys have been done. All are in warmer, perhaps more equable climates than the Amherst population.

It can be argued that the same sets of enzymes have not been analyzed in the several *D. melanogaster* populations. Hence derived arguments that Massachusetts is a more marginal location for this species than North Carolina, Texas or Japan may be inappropriate. Also lethal frequency studies in the S. Amherst population in the early 1960's came at a time of the Northeast drought, subsequently found to have had partial causation in a North Pacific disturbance (NAMAIS 1968, 1969). Furthermore, more recent studies have shown that margi-

TABLE 3

Proportion of polymorphic loci among glucose-metabolizing (group I) and non-glucose-metabolizing (group II) enzymes, average heterozygosity among group I and group II enzymes and their ratio are given for different populations of Drosophila melanogaster *and* D. robusta

A. *D. melanogaster**

Category	Enzyme group	Place: Mass.	N. C.	Japan	Texas
% polymorphic	I	64	29	36	27
loci	II	78	57	50	70
average	I	0.14	0.08	0.09	0.04
heterozygosity	II	0.18	0.20	0.16	0.24
	I/II	0.78	0.40	0.56	0.17

B. *D robusta*†

Category	Enzyme group	Region and state: North		Central		South		
		Mass.	N. J.	Nebr.	Mo.	N. C.	S. C.	Fla.
Percent	I	17	25	29	25	25	17	17
polymorphic	II	44	50	60	56	50	56	62
average	I	0.06	0.05	0.05	0.03	0.02	0.03	0.01
heterozygosity	II	0.15	0.14	0.17	0.15	0.19	0.16	0.19
	I/II	0.40	0.36	0.29	0.20	0.10	0.19	0.15

* Data on North Carolina and Japanese *D. melanogaster* populations are from KOJIMA, GILLESPIE and TOBARI (1970); the Texas *D. melanogaster* data are from GILLESPIE and LANGLEY (1974).

† The *D. robusta* data are from PRAKASH (1973), Table 11. Here the two Nebraska populations have been combined.

nal and central populations of other species tend to possess at least comparable levels of isozyme variability (SAURA *et al.* 1973; TIGERSTEDT 1973). While this enhances the possibility that isozyme polymorphisms may play a positive role in the maintenance of population-environmental adaptation, it also blurs the distinction that can be made between marginal and central populations on the basis of isozyme studies alone.

CHARLES DARWIN (1859) pointed out that it would be advantageous to a species if populations did differ in constitution over their geographical range as a consequence of climatic differences. In the *D. melanogaster* data shown in Table 3 we note that average heterozygosity among glucose-metabolizing enzymes tends to increase as one moves northward. We have retained *Got* among the glucose-metabolizing enzymes, as did GILLESPIE and LANGLEY (1974), but included *Xdh* among the group II enzymes, in keeping with KOJIMA, GILLESPIE and TOBARI (1970). As noted by GILLESPIE and LANGLEY (1974), *Xdh* is also sometimes placed in group I.

If the data of PRAKASH (1973) on *D. robusta* are reconsidered on a geographical basis and compared with *D. melanogaster* data, the same result emerges, as shown in Table 3. Average heterozygosity among glucose-metabolizing enzymes is higher in northern, lower in southern populations of the same species in the north temperate zone. Such results can only be due to selection which can favor both

clines and perhaps increased genetic polymorphism in northern localities which are subject to more striking seasonal and temperature changes. Glucose-metabolizing enzymes are associated with energy production, and alleles at many of these loci have been found to undergo seasonal or cyclic shifts (BERGER 1971; DOBZHANSKY and AYALA 1973; KOJIMA *et al.* 1972).

Other studies have also demonstrated polymorphism-environmental relations (ALLARD *et al.* 1972; BRYANT 1974; GILLESPIE and LANGLEY 1974; SCHAFFER and JOHNSON 1974). ALLARD and his group, analyzing populations of *Avena barbata* in California, found relations between levels of heterozygosity and geographical location of the populations; populations were more heterozygous in more heterogeneous intermontane regions along the coastal strip. BRYANT (1974) and GILLESPIE and LANGLEY (1974) have presented evidence that populations in more variable environments are likely to be more biochemically polymorphic. SCHAFFER and JOHNSON (1974) have shown that polymorphisms and geographical location in *D. melanogaster* cannot be exclusively attributed to migration and genetic drift.

The finding of a geographical gradient of increasing levels of heterozygosity among glucose-metabolizing enzymes as one moves northward would also seem to argue against the importance of migration and drift in maintaining polymorphisms. However, the overall increased polymorphism in this population in comparison to other *D. melanogaster* populations investigated may also be a consequence of a more northern, cooler location and the sometimes highly variable environments that are associated with more marginal habitats. In particular, the two-week period prior to collection was climatically very variable. Daily minimum and maximum temperatures ranged between 40°/76°F and 65°/97°F, a nearly 60°F spread between the lowest minimum, 40°, and the highest maximum, 97°, before the flies appeared in large numbers after the second week in June.

Thus it is doubtful if any single explanation can account for the numerous heterozygote excesses and deficiencies observed at some loci in the population at the time of population expansion. At two loci, *G6Pd* and *Est-6*, there is significant excess heterozygosity while at others, *Adh*, *Idh*, *Odh* and *Ao*, significant heterozygote deficiencies are detected. TIGERSTEDT (1973) reports that marginal populations of Norway spruce, *Picea abies*, tend to show excess esterase heterozygosity; these are populations at the northern extremes of the distribution, 68°N. Much work has been done on the *G6Pd* deficiency in man, although *Est-6* in *D. melanogaster* is suspected of being maintained by frequency-dependent selection (see TRIANTAPHYLLIDIS, CHRISTODOULOU and BECKMAN 1973; but also review LEWONTIN 1973). In the Greek *D. melanogaster* populations studied there was no persistent evidence of excess heterozygosity at the *Est-6* locus for either June or September samples (see TRIANTAPHYLLIDIS, CHRISTODOULOU and BECKMAN 1973).

Idh, *Est-6*, *Odh* and *Ao* are located on the *3rd* chromosome. Examples of linkage disequilibrium have been obtained in *D. melanogaster* for isozymes on this autosome (CHARLESWORTH and CHARLESWORTH 1973; LANGLEY, TOBARI and

KOJIMA 1974; MUKAI, WATANABE and YAMAGUCHI 1974). Year to year differences were obtained in samples from the Amherst populations (CHARLESWORTH and CHARLESWORTH 1973) and in *2nd* chromosome isozyme pairs analyzed from the Raleigh, N. C. population (MUKAI, WATANABE and YAMAGUCHI 1974). Our results indicate that both types of observations may be likely events. For instance, the CHARLESWORTHS observed significant linkage disequilibrium between alleles at the *Pgm* and *Xdh* loci in 1969, but between alleles at the *Est-6* and *Xdh* loci in 1970; however, pairwise comparisons of alleles at the *Est-6*, *Pgm* and *Ao* loci revealed no evidence of linkage disequilibrium in either year. In our observations, observed and expected numbers of genotypes are in good agreement at the *Pgm* and *Xdh* loci; the order of the six loci along the *3rd* chromosome is: *Idh*, *Est-6*, *Pgm*, *Odh*, *Xdh* and *Ao*. Thus an understanding of which results are due to selection, which to selection plus linkage and which to linkage alone will probably depend not only on continued observations of the natural population but also on experiments in controlled variable environments in the laboratory.

Though ours is the first large-scale survey of this population, BERGER (1971) reported on a seasonal survey of the 1966 population; the CHARLESWORTHS utilized 1969 and 1970 fall populations. We have analyzed a collection from the early season, June 1973, population. Where the same enzymes have been included in the four biochemical analyses, sometimes allelic frequencies are in agreement, sometimes not. Because of their observed year to year differences, the CHARLESWORTHS in fact concluded they were working with two different populations. Such conclusions are not surprising given the results of the continuing lethal frequency analyses and climatic observations in the area (see IVES 1970; BAND 1972a, b; IVES, personal communication). From a low of 16% lethal and semilethal frequency in June 1966, the proportion of these recessive variants progressed rapidly, on a year to year basis, back to 33% in 1969, to 70% in 1970. Frequencies continue to vary, in contrast to the relatively more stable results of the 1945–1961 interval.

Thus, it seems likely that continued simultaneous studies on lethal frequencies and on biochemical polymorphisms in the population may give us important clues to the adaptive role of enzyme polymorphisms. Biochemical polymorphism surveys in other northern *D. melanogaster* populations and other species may provide further evidence on the tendency of populations in this geographical region to maintain higher levels of heterozygosity among glucose-metabolizing enzymes.

Thanks are gratefully extended to DR. FRANCISCO AYALA for the opportunity to carry out the isozyme survey in his laboratory. MISSES LORRAINE BARR and CHRISTINE HOWE supplied valuable instructions, advice and assistance. DR. ROSS MACINTYRE, also visiting in the Genetics Dept., U.C., Davis, at that time, provided information on the reading of some gels. As usual, the author is deeply indebted to DR. P. T. IVES, Amherst College, Amherst, Mass. for supplying the files and more information on Amherst weather, 1836–1974, than is herein indicated. A reviewer very kindly pointed out that there were significant heterozygote deficiencies in Table 1 as well as the obvious heterozygote excesses; this has greatly improved the treatment of the data and the revision of the paper.

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