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Genetic Diversity and Linkage Disequilibrium in *Drosophila melanogaster* With Different Rates of Development

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ABSTRACT

We have examined eight enzyme polymorphisms in groups of *Drosophila melanogaster* flies with fast, intermediate and slow development. The allelic frequencies are similar in all three developmental classes, but the distribution of the genotypes among the classes is significantly heterogenous for the three loci on the second chromosome. When the total sample of 300 individuals is examined, significant gametic disequilibrium appears in 3 out of 13 pairs of genes located on the same chromosome and in 4 out of 15 pairs of genes located on different chromosomes. This 25% incidence of disequilibrium between pairs of genes is larger than previously observed in other natural populations (but similar to the incidence observed in laboratory populations). The greater frequency of significant gametic disequilibrium in our study is probably due to the larger number of genomes sampled.—Some models specifically predict that individuals with faster rates of development (*i.e.*, greater fitness) should be more heterozygous (and exhibit more linkage disequilibrium) than individuals with slower development. This hypothesis is not supported by our results.

RATE of development is an important fitness component, notorious for its small phenotypic variance and for the difficulty of modifying it by artificial selection. In *Drosophila*, selection for faster or slower rates of development is often unsuccessful, although a response is usually obtained when the selection is sustained for very many generations (*e.g.*, TIGERSTADT 1969). This eventual success indicates that genetic variation for developmental rate is present in the populations, which is also supported by other sources of evidence (ROBERTSON 1963, 1964; MARINKOVIĆ and AYALA 1986a,b).

If there is genetic variance for the trait, one might expect that individuals with diverse developmental rates might be genetically heterogeneous. In the present experiments we investigate this hypothesis by examining the allele and genotypic frequencies of genes coding for enzymes in groups of *Drosophila melanogaster* individuals with different rates of development. It has also been proposed, more specifically, that levels of heterozygosity may be related to rate of development in such a way that more heterozygous individuals will develop at a faster rate (reviewed in MITTON and GRANT 1984). We test this particular hypothesis as well, which is relevant to the general problem of how genetic variation is maintained in natural populations, given that individuals with faster rate of development have higher fitness than those that develop more slowly (COLE 1954; LEWONTIN 1965; PARSONS 1983). We use allozymes as indicators of genetic divergence because they are polymorphic in natural populations and, hence, they may be markers of genetic diversity. It is also possible, of course, that allozymes (particularly those modifying metabolic rates) may themselves be partially responsible for differences in developmental rate (MITTON and GRANT 1984).

MATERIALS AND METHODS

About 200 *D. melanogaster* females collected from a natural population in Furnace Creek (Mojave Desert, California) were brought to the laboratory and distributed into several dozen separate cultures (half-pint bottles) with standard *Drosophila* medium made of cornmeal and molasses. These cultures were subcultured by transferring 20–30 F_1 individuals from each culture to a fresh one. Groups of 10–20 pairs from the F_2 generation (collected from several cultures in such a way that males and females always came from different cultures) were placed in culture bottles without food on the bottom. Medium for egg laying was provided in a small Petri dish snugly fitted to the top of the bottles and sealed to them with masking tape. The Petri dishes were replaced at 1-hr intervals. Groups of eggs collected from a single Petri dish were placed in culture bottles with food, in uncrowded conditions. Emerging adults were collected at 6–8-hr intervals. Three developmental groups were differentiated: fast (flies developed from egg to adult in less than 10 days), intermediate (developed in 12 days), and slow (developed in 20–22 days). The experiments were conducted at 23°.

One hundred males from each developmental class (sampled in approximately equal numbers from no less than 20 cultures) were each assayed by starch gel electrophoresis for 28 gene loci coding for enzymes. The crude homogenate of each fly was absorbed with three separate filter-paper wicks, which were then placed into three different gels. Four horizontal slices were made of each gel after electrophoresis,

and each slice was assayed for one or more enzymes. The methods for gel preparation, electrophoresis, and enzyme staining were as described by AYALA *et al.* (1972).

Only 9 out of 28 loci studied were moderately to highly polymorphic. One polymorphic locus, aldehyde oxidase, is, however, problematic. The five electromorphs distinguishable in our gels differ very little in electrophoretic migration, so that we had difficulty identifying some genotypes, mainly heterozygotes. Therefore, we report data only for the other eight polymorphic loci. The symbols used for them in this paper, and their map location (DOANE and TREAT-CLEMONS 1982) are as follows (see also Table 1): *chromosome II*—α-glycerophosphate dehydrogenase ($\alpha Gpdh$, 20.5), alcohol dehydrogenase (*Adh*, 50.1), and hexokinase-C (*hex*, 73.5); *chromosome III*—superoxide dismutase (*Sod*, 34.6), phosphoglucomutase (*Pgm*, 43.4), esterase-C (*Est*, 47.7), octanol dehydrogenase (*Odh*, 49.2), and acid phosphatase-1 (*Acph*, 101.1).

Statistical tests for nonrandom association of the allele variation within the three developmental classes follow the methods of COCKERHAM (1969, 1973). The components of the variance of gene frequencies are: σ_w^2, the variance due to allelic differences within individuals; σ_b^2, variation among individuals within the same subpopulation, *i.e.*, developmental class; and σ_a^2, variation due to differences among the developmental classes. Gene correlations are measured by three parameters: f, the correlation between genes within individuals within developmental classes; Θ, the correlation between genes of different individuals in the same developmental class; and F, the correlation between genes within individuals in the whole population. These three parameters precisely correspond to the more familiar F-statistics in hierarchic populations (WRIGHT 1969, p. 294*ff.*): $f = F_{IS}$, $\Theta = F_{ST}$, and $F = F_{IT}$.

We test for linkage disequilibrium among the allozymes following two methods. First, we use a method proposed by P. M. BURROWS, which yields a correlation coefficient that incorporates the departures from Hardy-Weinberg equilibrium for the sample frequencies at each locus (COCKERHAM and WEIR 1977; WEIR 1979; WEIR and COCKERHAM 1979). This method does not require the assumption of random mating. The normalized parameter, R, which we use may be interpreted as the average correlation between nonallelic genes in zygotes (LANGLEY, SMITH and JOHNSON 1978); $4NR^2$ (where N is the number of individuals sampled) has an approximate chi-square (χ^2) distribution with one degree of freedom. We also use the maximum likelihood (ML) method proposed by HILL (1974, 1975): ML estimates of the gametic frequencies are obtained and then a succession of models are tested that assume independence of gene frequencies. This method assumes random mating, and hence the expected frequencies of zygotes are the products of the frequencies of the two gametic combinations involved. Some allele frequencies are low, so that multilocus associations have missing genotypic classes. We have alleviated this problem in two steps. First, we have combined the frequencies of the two rarer alleles at the two loci (*Pgm* and *Acph*) that have three alleles. Next, we have assumed a frequency of $1/2N$ for any missing genotypic class ($N = 100$ for each developmental class and 300 for the total sample), which has a negligible effect on the test but overcomes the computational handicap.

RESULTS

The allelic frequencies at eight gene loci coding for enzymes are given in Table 1, separately for each developmental class. The three loci on the second chromosome ($\alpha Gpdh$, *Adh* and *Hex*) are all highly polymorphic. Two loci on the third chromosome are also substantially polymorphic (*Pgm* and *Est*), the other three loci only moderately so (*Sod*, *Odh* and *Acph*). The agreement between the observed and the expected frequency of heterozygotes is fairly good at any one locus. There is only one statistically significant difference (*Acph*, fast developmental class) at the five percent level among the 24 comparisons—just what would be expected by chance assuming that there are no differences between the observed and expected heterozygosities, except those due to sampling variation. A small average deficiency of heterozygotes is apparent, however, when the observed and expected frequencies are compared at each locus and the differences averaged over all loci (Table 2). The deficiency is statistically significant for the three developmental classes combined, as well as for the fast and intermediate classes separately. Table 3 gives the mean differences in heterozygosity between each pair of developmental classes. None of the differences is statistically significant. The allelic frequencies in all three classes also are very similar on the whole: the genetic identity (*I*; NEI 1972) between the classes is very high (last column of Table 3).

The possibility of other genetic differences among the developmental classes is first explored by comparing the genotypic frequencies by a chi-square heterogeneity test. The results are given in Table 4. The numbers of individuals with some of the rarer genotypes have been combined for simplicity in the table; some have also been combined, as indicated in the table, in order to calculate the χ^2. The distribution of the genotypes among the classes is significantly heterogeneous for each of the three loci on the second chromosome, which (together with *Est* on the third chromosome) are also the most polymorphic of the eight loci in Table 4. None of the five loci on the third chromosome gives significant genotypic heterogeneity among the developmental classes.

In addition, we have used the method of COCKERHAM (1969, 1973) in order to test the gene frequency correlations in a hierarchical fashion. The results are given in Table 5. The parameter Θ is a measure of the genetic divergence among the developmental classes (it is the ratio of the variance due to differences between the populations divided by the total variance: $\sigma_a^2/[\sigma_a^2 + \sigma_b^2 + \sigma_w^2]$, see MATERIALS AND METHODS). Θ is statistically significant for all three loci on the second chromosome, where the previous χ^2 test had detected genotypic heterogeneity among the classes. In addition, Θ is statistically significant for *Acph* on the third chromosomes. *Acph* and *Sod* give also statistically significant values of the two other correlation parameters, f and F.

TABLE 1

Allele frequencies at nine gene loci in three developmental classes of *D. melanogaster*

Gene (chromosomal locus)	Developmental class	Allele frequencies				Heterozygosity		
		US	S	F	UF	Observed	Expected	χ^2
αGpdh (*II*, 20.5)	Fast		0.300	0.700		0.400	0.420	0.16
	Intermediate		0.275	0.725		0.330	0.399	1.98
	Slow		0.405	0.595		0.530	0.482	0.92
Adh (*II*, 50.1)	Fast		0.305	0.695		0.410	0.424	0.08
	Intermediate		0.240	0.760		0.340	0.365	0.27
	Slow		0.390	0.610		0.500	0.476	0.23
Hex (*II*, 73.5)	Fast		0.875	0.125		0.150	0.219	2.78
	Intermediate		0.838	0.162		0.263	0.271	0.04
	Slow		0.775	0.225		0.370	0.349	0.19
Sod (*III*, 34.6)	Fast		0.010	0.990		0.020	0.019	0
	Intermediate		0.045	0.955		0.070	0.086	0.33
	Slow		0.035	0.965		0.050	0.068	0.51
Pgm (*III*, 43.4)	Fast		0.035	0.855	0.010	0.230	0.251	0.24
	Intermediate		0.110	0.880	0.010	0.220	0.205	0.03
	Slow		0.085	0.910	0.005	0.140	0.165	0.45
Est (*III*, 47.7)	Fast		0.695	0.305		0.390	0.424	0.47
	Intermediate		0.745	0.255		0.330	0.380	1.06
	Slow		0.755	0.245		0.370	0.370	0
Odh (*III*, 49.9)	Fast		0.046	0.954		0.093	0.088	0.03
	Intermediate		0.030	0.970		0.040	0.058	0.59
	Slow		0.037	0.963		0.074	0.071	0.01
Acph (*III*, 101.1)	Fast	0.035	0.040	0.925		0.060	0.142	5.52*
	Intermediate	0.005	0.010	0.985		0.030	0.030	0
	Slow	0.010	0.005	0.985		0.030	0.030	0

The two most common alleles at each locus are identified as S ("slow") and F ("fast"); additional alleles are labeled, according to their mobility, as US ("ultraslow") and UF ("ultrafast').
* $P < 0.05$.

TABLE 2

Mean of the observed and expected heterozygosities and of their difference in three developmental classes, separately and combined

Developmental class	Heterozygosity				
	Observed	Expected	Difference	t	d.f.
Fast	0.219 ± 0.057	0.248 ± 0.056	−0.029 ± 0.011	2.65*	7
Intermediate	0.203 ± 0.048	0.225 ± 0.054	−0.022 ± 0.009	2.50*	7
Slow	0.258 ± 0.073	0.251 ± 0.067	0.007 ± 0.009	0.85	7
Total	0.227 ± 0.034	0.241 ± 0.033	−0.015 ± 0.006	2.39**	23

* Statistically significant $P < 0.05$; ** $P < 0.01$

TABLE 3

Mean heterozygosity difference and genetic identity (*I*) between pairs of three developmental classes of *D. melanogaster*

Developmental classes compared[a]	Heterozygosity difference					
	Observed	t	Expected	t	d.f.	I
F-I	0.016 ± 0.023	0.70	0.030 ± 0.020	1.11	7	0.999
F-S	−0.039 ± 0.036	1.08	−0.003 ± 0.029	0.09	7	0.996
I-S	−0.055 ± 0.033	1.65	−0.025 ± 0.020	1.29	7	0.995

[a] F, fast; I, intermediate; S, slow.

The three loci on the second chromosome are fairly evenly distributed over approximately one-half (from 20.5 to 73.5) of the total length of this chromosome. The other five loci span about 60% (from 34.6 to 101.1) of the third chromosome: three of them (*Pgm*, *Est* and *Odh*) lie somewhat close to each other (from

TABLE 4

Number of individuals with a given genotype in each of three developmental classes of *D. melanogaster*

Gene locus	Developmental class	Genotypic numbers S/S	S/F	F/F	χ^2	d.f.
αGpdh	Fast	10	40	50	11.8*	4
	Intermediate	11	33	56		
	Slow	14	53	33		
Adh	Fast	10	41	49	13.0*	4
	Intermediate	7	34	59		
	Slow	14	50	36		
Hex	Fast	80	15	5	12.9*	4
	Intermediate	70	26	3		
	Slow	59	37	4		
Sod	Fast		2	98	3.7	2
	Intermediate	1[a]	7[a]	92		
	Slow	1[a]	5[a]	94		
Pgm	Fast	5[b]	21	74	3.5	4
	Intermediate	3[b]	20	77		
	Slow	3[b]	13	84		
Est	Fast	50	39	11	2.5	4
	Intermediate	58	33	9		
	Slow	52	37	6		
Odh	Fast		9	88	1.4	2
	Intermediate	1[a]	4[a]	95		
	Slow		7	88		
Acph	Fast	9[a,b]	0[a]	91	5.0	2
	Intermediate	2[a,b]	1[a]	96		
	Slow	1[a,b]	2[a]	97		

* Statistically significant, $P < 0.02$.

The χ^2 is for the genetic heterogeneity among the three classes.

[a] These numbers have been combined with each other (within a given developmental class and gene locus) before calculating the heterogeneity χ^2. Yates' correction for continuity has been used whenever the number of columns is 2 (the number of rows is always 3).

[b] Includes *F/UF* heterozygotes (*Pgm*); or *US* homozygotes and heterozygotes (*Acph*).

43.4 to 49.9). We have tested for gametic disequilibrium among the nearly 300 individuals sampled at all loci; and also among the 100 individuals in each developmental class. The tests are based on the zygotic frequencies, given that we have no information about the gametes. Table 6 gives the value of R, a parameter that corresponds to one-half the correlation between non-allelic genes in gametes and that does not assume random mating (LANGLEY, SMITH and JOHNSON 1978; COCKERHAM and WEIR 1977; WEIR 1979). For genes located on the same chromosome, there are three significant correlations out of 13 pairwise comparisons in the total sample; none of them involves two closely linked genes. When pairs of genes located on different chromosomes are analyzed, there are four significant correlations out of 15 comparisons in the total sample.

The number of significant correlations is smaller within any one developmental class than in the total sample, but this is due in part to the smaller samples involved. (Indeed the average correlations are not

TABLE 5

Gene frequency correlations between genes within individuals within a given developmental class (f), between different individuals within a given developmental class (θ), and between genes within individuals in the whole population (F)

Gene locus	f	θ	F
αGpdh	0.0363	0.0164*	0.0521
Adh	0.0129	0.0179*	0.0832
Hex	0.0719	0.0122*	0.0598
Sod	0.1971**	0.0052	0.2012**
Pgm	0.0579	0.0019	0.0598
Est	0.0763	−0.0001	0.0764
Odh	0.0590	−0.0036	0.0557
Acph	0.5501**	0.0215*	0.5597**

* Statistically significant, $P < 0.05$; ** $P < 0.01$.

smaller in absolute value for the developmental classes than for the total sample. The means of the absolute value of R are 0.048 and 0.059 for the linked and unlinked genes in the developmental classes, but 0.030 and 0.043 in the total sample.) For genes on the same chromosome, there are four significant correlations out of 39 (only one involves two very closely linked loci, *Est* at 47.7 and *Odh* at 49.9 on the third chromosome, in the "intermediate" developmental class). There are, in addition, eight significant correlations out of 45 between genes on different chromosomes. *αGpdh* shows significant correlation in two out of the three developmental classes with *Sod* and also with *Pgm*; the other four correlations appear each in only one developmental class.

If we consider jointly all comparisons between linked or unlinked genes, seven out of 28 correlations are statistically significant in the total sample (25%) and 12 out of 84 (14.3%) in the samples of the separate developmental classes.

We have made additional tests for gametic (linkage) disequilibrium using the maximum likelihood (ML) method of HILL (1974, 1975). This method assumes random mating, which is not required by the correlation estimation given in Table 6. The ML method uses a succession of models that assume gene frequency independence and make possible to examine triple gene associations. This method may also be more efficient than the correlation method, particularly when the linkage disequilibrium is large. We have tested for nonrandom associations between all three gene loci on the second chromosome and between the three more closely linked loci on the third chromosome. The results are reported in Tables 7 and 8. Among the two-locus associations, two are statistically significant (*αGpdh-Hex* and *Pgm-Odh*), both in the total sample. These are the same two significant associations (and the only two for the associations now considered) detected by the correlation method (Table 6). In addition, there are two significant three-

TABLE 6

Average correlation, *R*, between nonallelic genes in individuals from three developmental classes

Gene association	Developmental class			Total
	Fast	Intermediate	Slow	
Linked genes:				
αGpdh × *Adh*	−0.0671	0.0431	0.0471	0.0275
αGpdh × *Hex*	0.0833	0.0165	0.0253	0.0607*
Adh × *Hex*	−0.0621	−0.0294	−0.0508	−0.0308
Sod × *Pgm*	0.1024*	−0.0123	−0.0605	−0.0054
Sod × *Est*	0.0429	0.0953	−0.0457	0.0259
Sod × *Odh*	−0.0227	−0.0386	−0.0335	−0.0341
Sod × *Acph*	−0.0289	0.0202	−0.0137	−0.0067
Pgm × *Est*	−0.0138	−0.0043	−0.0578	−0.0184
Pgm × *Odh*	−0.0872	−0.0200	−0.0648	−0.0581*
Pgm × *Acph*	0.0446	−0.0498	−0.0392	0.0202
Est × *Odh*	−0.0089	−0.1039*	0.0147	−0.0168
Est × *Acph*	0.0802	0.0217	0.0256	0.0586*
Odh × *Acph*	−0.1274*	−0.1312**	−0.0244	−0.0331
Unliked genes:				
αGpdh × *Sod*	0.0998*	−0.1077*	−0.0747	−0.0289
αGpdh × *Pgm*	0.1033*	0.0661	0.1154*	0.0864**
αGpdh × *Est*	0.0185	−0.0396	−0.0503	−0.0252
αGpdh × *Odh*	0.0129	−0.1094*	−0.0192	−0.0332
αGpdh × *Acph*	−0.0628	−0.0311	0.0241	−0.0369
Adh × *Sod*	0.0303	0.0194	−0.0129	0.0045
Adh × *Pgm*	−0.0263	−0.0640	−0.0912	−0.0629*
Adh × *Est*	0.0332	0.0472	−0.0239	−0.0119
Adh × *Odh*	0.0769	−0.0998*	0.0739	0.0254
Adh × *Acph*	0.2884***	0.0267	0.0707	0.1559***
Hex × *Sod*	−0.0384	−0.0301	−0.0707	−0.0412
Hex × *Pgm*	−0.0660	−0.0957	−0.0021	−0.0597*
Hex × *Est*	−0.0042	−0.0659	0.0701	0.0401
Hex × *Odh*	−0.0112	−0.0722	0.1257*	0.0204
Hex × *Acph*	0.0232	−0.0021	−0.0672	−0.0185

* Statistically significant, $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 7

Maximum likelihood tests (χ^2 values) for the association between three loci on the second chromosome of *D. melanogaster*

Model Source		d.f.	Developmental class			Total
			Fast	Intermediate	Slow	
1. Association of:						
αGpdh and *Adh*		1	1.59	1.84	2.74	2.74
αGpdh and *Hex*		1	2.84	1.06	0.09	4.03*
αAdh and *Hex*		1	1.98	1.06	0.74	1.89
2. Association of:	Conditional on:					
αGpdh and *Adh*	*Hex*	1	3.52	1.22	1.62	3.50
αGpdh and *Hex*	*Adh*	1	2.26	2.00	1.03	2.21
Adh and *Hex*	*αGpdh*	1	3.12	2.00	0.38	4.35*
3. All two-locus associations		1	3.90*	0.04	1.55	2.74
4. All two locus and three-locus associations		4	5.21	0.86	4.00	5.16

The genotypic frequencies at all three loci jointly considered fit the Hardy-Weinberg expectations, except for the fast developmental class ($\chi^2 = 30.57$, 19 d.f., $P \cong 0.05$).
* $P < 0.05$.

locus associations, one in each chromosome, both also in the total sample. When the three two-locus associations in each chromosome are jointly considered, only one (out of eight values) is statistically significant (Table 7).

We have examined two other sets of three loci from the third chromosome (*Sod*, *Pgm*, *Est*; and *Sod*, *Est*, *Acph*). The number of significant cases of disequilibrium is not greater than in Tables 7 and 8. Summarizing the data for the four three-locus sets, the in-

TABLE 8

Maximum likelihood test (χ^2 values) for the association between three loci on the third chromosome of *D. melanogaster*

Model	Source	d.f.	Developmental class Fast	Intermediate	Slow	Total
1. Association of:						
Pgm and *Est*		1	0.12	0.00	1.40	0.32
Pgm and *Odh*		1	2.27	0.01	0.99	4.03*
Est and *Odh*		1	0.24	2.51	0.12	0.23
2. Association of:	Conditional on:					
Pgm and *Est*	*Odh*	1	0.07	0.03	1.27	0.40
Pgm and *Odh*	*Est*	1	2.23	0.02	0.86	4.10*
Est and *Odh*	*Pgm*	1	0.19	2.48	0.01	0.30
3. All two-locus associations		1	0.01	0.10	0.15	0.01
4. All two-locus and three-locus associations		4	2.60	2.59	2.53	4.66

The genotypic frequencies at all three loci jointly considered fit the Hardy-Weinberg expectations in the total sample as well as in each of the three developmental classes.
* $P < 0.05$.

stances of significant disequilibrium are 2 of 48 for model 1, 4 of 48 for model 2, 1 of 16 for model 3, and 0 of 16 for model 4. For our data, the ML method does not seem to be more efficient in detecting linkage disequilibrium than the correlation method.

DISCUSSION

Genes coding for enzymes are obvious targets to test whether individuals with different rates of development may also differ in their genetic constitution. This is because some allozyme variants affect metabolic rates and, hence, could conceivably affect rate of development (KOEHN and SHUMWAY 1982; MITTON and GRANT 1984). A more general reason, however, is that genes coding for enzymes are often polymorphic in natural populations. They make it possible to ascertain whether groups of individuals with different developmental rates might also differ in their allelic frequencies. This could be because these genes themselves affect the rate of development, or simply because they serve as markers for the small blocks of genes within which they are located. CAVENER (1983) monitored the allelic frequencies at four enzyme polymorphisms in lines of *D. melanogaster* flies selected for divergent developmental rates: significant changes in allelic frequencies occurred at two loci (*αGpdh* and *6Pgd*) in the lines selected for fast developmental rate.

We have examined eight enzyme polymorphisms in groups of *D. melanogaster* individuals with divergent rates of development. Individuals with fast rates of development have higher fitness, other things being equal, than those with slow development (COLE 1954; LEWONTIN 1965; PARSONS 1983). Increased heterozygosity has been associated with higher fitness (LERNER 1954; WRIGHT 1977). If rate of development were one of the fitness components affected by heterozygosity levels, one would expect greater heterozygosity in fast than in slow-developing individuals (MITTON and GRANT 1984). This hypothesis is not, however, supported by our results. No significant differences in average heterozygosity exist between the fast, intermediate, or slow developing individuals. Our data show an unanticipated small deficiency of heterozygotes at several loci that is significant for the fast and intermediate developmental classes when averaged over all loci. The parents of the assayed flies were groups of 10–20 F_2 pairs collected from several cultures, different ones for the males and the females. The assayed individuals of each developmental class were F_3 flies collected a few from each of 20 or more cultures. It is possible that the F_2 parents differed somewhat in gene frequencies from culture to culture so that a small Wahlund effect has been created when sampling from many cultures. In any case Table 3 shows that no significant differences exist between the developmental classes either for the observed or the expected heterozygosities.

Nevertheless, we have obtained evidence of genetic differentiation among the developmental classes. The genotypic frequencies are significantly heterogeneous in each of the three loci on the second chromosome. None of the five third-chromosome loci shows significant heterogeneity by a simple χ^2 heterogeneity test (Table 4); and only one, *Acph*, by a correlation test (Table 5). Enzyme polymorphisms have been earlier implicated in the developmental rate of *D. melanogaster* (*e.g.*, CAVENER 1983), as well as in *D.* pseudo-*obscura* (MARINKOVIĆ and AYALA 1975a,b). It may be that these loci themselves affect the developmental rate of the flies. But it is, of course, possible that other loci, closely linked with these examined, may actually be the ones directly involved in rate of development. Given the small number of loci examined, we cannot tell the relevance of the fact that all three second-chromosome loci, but none (if we use the χ^2 hetero-

geneity test) or only one (if we use the correlation test) of the five third-chromosome loci, gives significant heterogeneity among the developmental classes. This difference could be a sampling accident, or it could be that rate of development depends on more loci located on the second than on the third chromosome.

Gene interactions affecting fitness have been repeatedly observed in *Drosophila*. Visible mutants (WILSON 1968, 1972) as well as genes coding for enzymes (MARINKOVIĆ and AYALA 1975a,b) have been implicated, although most studies involve interactions between full chromosomes (*e.g.*, SPASSKY, DOBZHANSKY and ANDERSON 1965; TEMIN *et al.* 1969; SEAGER, AYALA and MARKS 1982). Selective interactions between specific alleles at different loci may yield linkage disequilibrium, more so when the loci are more closely linked (LEWONTIN 1974). One problem, however, is that almost any pattern of linkage disequilibrium can be accounted for by a number of causes besides natural selection, and alternative explanations are usually difficult or impossible to exclude. Nevertheless, some light might be shed on this question by examining patterns of linkage disequilibrium in adaptively different subgroups of individuals descended from the same set of parents, such as are the three developmental classes in our experiments.

Linkage disequilibrium between allozymes in animal populations has been the subject of a number of studies during the last decade. Disequilibrium has been detected in several organisms, such as the salamander *Plethodon cinereus* (WEBSTER 1973), the blue mussel *Mytilus edulis* (MITTON and KOEHN 1973), the fish *Fundulus heteroclitus* (MITTON and KOEHN 1975), and humans (SMOUSE and NEEL 1977; HEDRICK and THOMSON 1986). Most studies, however, have been with several species of *Drosophila*, particularly *D. melanogaster* (see, *e.g.*, LANGLEY, SMITH and JOHNSON 1978; and LAURIE-AHLBERG and WEIR 1979 and references therein). The evidence suggests that there is little linkage disequilibrium among allozymes, in the absence of inversions, in natural populations; but much more exists in laboratory populations (often attributable to small population sizes).

An extensive study of linkage disequilibrium in natural populations of *D. melanogaster* is that of LANGLEY, SMITH and JOHNSON (1978). Eight enzyme loci were surveyed in some 100 samples from natural populations and in two laboratory populations. Genotypic, rather than gametic data, were analyzed by the correlation method also used in our study (Table 6). The frequency of cases of significant linkage disequilibrium between pairs of loci in the natural populations was 5.1% for linked loci and 6.7% for loci on different chromosomes. In the two laboratory populations, the frequency of significant disequilibria was much greater: 37.5% for linked pairs and 10.3% for unlinked loci. LAURIE-AHLBERG and WEIR (1979) studied 17 enzyme loci in 9 laboratory populations of *D. melanogaster* and found significant associations at frequences fairly similar to those of LANGLEY *et al.* in laboratory populations: 34.5% and 8.9% for linked and unlinked pairs of loci, respectively, when a correlation method was used; and 44.8% and 10.1% when a maximum likelihood method was used.

The frequency of significant cases of linkage disequilibrium in our data is nearly as large as in the laboratory populations just reported, when we consider the total samples: 23% (3 of 13) between pairs of linked loci, and 27% (4 of 15) for unlinked loci (see Table 6). Our flies are the F_3 generation from flies collected in nature. It is possible that linkage disequilibrium may have built up in those few generations, and that this accounts for the larger number of cases of disequilibrium in our study than in the natural populations of LANGLEY, SMITH and JOHNSON (1979). But the flies are randomized samples from numerous cultures with several thousand individuals *in toto*. The difference may also be due in good part to differences in sample size. LANGLEY *et al.* included in their analysis all the samples for which at least 50 individuals were assayed at each pair of loci; most samples seem to have been between 100 and 150 individuals. In our total samples, the number of individuals is 300. The influence of sample size becomes apparent when we look separately at the developmental classes of our experiment, for which the frequencies of significant instances of linkage disequilibrium is only 10% (4 of 39) for linked loci and 18% (8 of 45) for unlinked loci. These values are much closer to the 5.1% and 6.7% obtained by LANGLEY, SMITH and JOHNSON (1979) in the natural populations.

The expectation that more instances of linkage disequilibrium would appear among the individuals with faster rate of development (=higher fitness) is not, at least not unambiguously, realized. There are five cases of significant disequilibrium among the fast-developing individuals, five also among those with intermediate rate of development, and only two among the slow-developing individuals (Table 6). But this deficiency of cases of significant disequilibrium in the slow developmental class is not large enough to be meaningful. The means of the absolute values of R are: 0.060 ± 0.010, 0.052 ± 0.007, and 0.050 ± 0.006, for the fast, intermediate, and slow developmental class, respectively. This gradual decrease in the average linkage disequilibrium from the fast to the slow class is as one would expect; however, an analysis of variance does not indicate significant heterogeneity among the three developmental classes in this respect ($F = 0.39$, with 2, 81 d.f., $P > 0.50$).

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