THE PATTERN OF VIABILITY CHANGES ASSOCIATED WITH GENOTYPE FREQUENCY AT THE ALCOHOL DEHYDROGENASE LOCUS IN A POPULATION OF *DROSOPHILA MELANOGASTER*[1]

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RECENT investigations on genetic variants in enzymes and proteins detected by electrophoretic mobility differences in higher organisms unveiled a large number of polymorphisms existing in these systems. Just considering Drosophila species alone, all indications are that there is a much greater number of polymorphisms in these biochemical systems than had been previously assumed by population geneticists (e.g. LEWONTIN and HUBBY 1966; JOHNSON *et al.* 1966; STONE *et al.* 1968a). The degree of polymorphism is also very high in mouse, deer mouse, butterflies, humans and other species (e.g. see STONE, KOJIMA and JOHNSON 1968b).

The natural question following is that of possible mechanisms responsible for a high degree of polymorphism. A high mutation rate per structural gene controlling enzyme and protein production is a possibility, since electrophoretic mobility differences in these molecules may result from a number of possible changes in subunits of a structural gene. A study designed to estimate a mutation rate for different electrophoretic mobilities of the products of this class of genes is in progress in our laboratory, using several loci on the third chromosome of *Drosophila melanogaster*. On the other hand, there are possibilities of selective polymorphisms shown in some cases (e.g. YARBROUGH and KOJIMA 1967). However, the mode of selection was not a simple constant heterosis in YARBROUGH and KOJIMA's study of the Esterase 6 locus in a population of *D. melanogaster*. Rather, they found that the selective advantage among the genotypes involved was dependent upon the frequency of alleles at the locus in question. More conclusive evidence on this point was given in another paper by KOJIMA and YARBROUGH (1967) in which they considered egg-to-adult viability as a measure of fitness.

The objective of this paper is to report a detailed study of frequency-dependent selection in egg-to-adult viability of a caged population of *Drosophila melanogaster* with respect to the alcohol dehydrogenase locus.

MATERIALS AND METHODS

Approximately three years ago, a mixture of several wild-type stocks of *Drosophila melano-*

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gaster was made, and the resultant population was divided into four replicated cages containing banana medium. One and a half years later, these cages were examined for the allele frequency at the alcohol dehydrogenase locus (abbreviated as *ADH* locus). Designating the allele producing fast moving isozyme in electrophoresis as *F* and that producing slow moving isozyme as *S*, two of the four cages were found to contain only *F* alleles, the third, an *F* frequency of about 0.90 and the last an *F* frequency of about 0.60. The basic material of this study came from this last cage where the *F* frequency was maintained approximately at 0.60 for the next 10 generations. This population was always kept on banana medium, and this is the culture medium used throughout this study.

From this cage, approximately 200 virgin females and the same number of males were randomly sampled, and they were pair-mated. When the cultures from individual pairs were beginning to start, the parents were taken out of the culture vials, and their genotypes were determined by the single fly electrophoretic assay technique described by JOHNSON (1966). When the paired parents were both homozygous for the same allele (either *F* or *S*) at the *ADH* locus on the second chromosome, the corresponding cultures were saved. All other cultures were discarded. Using this procedure, 16 lines of *FF* and 15 lines of *SS* were established. They were homozygous at the *ADH* locus, but represented random samples of genomes from the basic population. Each line was immediately expanded to a large culture to avoid further inbreeding.

Various sections of the study to be reported were carried out over the next eight months, and the earlier tests used flies sampled from these lines, but the later tests used flies taken from the intermix pool of the *FF* lines and that of the *SS* lines. The latter procedure was to avoid the effects of line differences on viability which supposedly became stronger as generations accumulated.

In every test, virgins and males were collected using etherization from either the lines or intermixes on one day, and they were aged for two days. On the third day, they were mated as described later for each test. This mating was accomplished by aspirating individual flies of appropriate genotypes without etherization. After two days of mating (usually in mass), females and males were separated under etherization. At the same time, necessary numbers of mated females were counted in groups. Some extra numbers of mated females were always kept besides the necessary groups of females for various tests. Mated females were allowed to recover from etherization in the next two days. On the third day after separating the two sexes, desired combinations of mated females were made as described in each test. If there were any female dead in the grouped flies, the deficient number was added by aspirating from the reserve of extras. Then, the female parents were allowed to lay eggs for a desired length of time in test culture bottles (which were always ½ pint milk bottles with the standard amount of banana medium).

When larvae were sampled at a specified developmental stage, the bottles were flooded with water for several minutes to wash larvae out from the media. Larvae in water were placed in a dish, and a random sample was taken.

The starch gel electrophoresis method for ADH was basically one described by JOHNSON and DENNISTON (1964) with some minor modifications.

RESULTS

(i) *Viability to the early 3rd instar stage under optimum density:* The objective of this test was to determine whether there was any viability difference among the *ADH* genotypes during the first 69 to 74 hours after egg-laying, without confounding this trait with female fecundity and male sexual activity associated with the *ADH* genotypes. For this purpose each experimental culture was started with 100 females of one *ADH* genotype premated by approximately 100 males of another *ADH* genotype. The optimum density refers to 150 to 200 larvae per culture. The kinds of cultures, the number of cultures of each kind and the expected progeny types are as follows:

	♀♀ parents	♂♂ parents	Number of cultures	Progeny types
I	*FS*	*FF*	2	*FF, FS*
	FF	*FS*	2	*FF, FS*
II	*FS*	*SS*	2	*FS, SS*
	SS	*FS*	2	*FS, SS*
III	*FS*	*FS*	2	*FF, FS, SS*

The expected numbers of progeny types under the assumption of no viability difference, the observed numbers of progeny types, the χ^2 value for the test of deviations between the expected and observed numbers and the χ^2 value for heterogeneity among cultures are listed in Table 1.

None of the χ^2 values is statistically significant in the data in Table 1. Thus, it is quite clear that there is no significant viability difference associated with the *ADH* genotypes during the first 72 hours of the larval stage, as far as the three conditions of genotype ratios tested in the above are concerned.

(ii) *Viability to the mid-3rd instar stage under moderately crowded condition:* Since there was no detectable viability difference in test (i), the same experimental scheme as in (i) was used to test viability differences at the mid-3rd instar stage (approximately 96 hours after oviposition) under moderately crowded larval condition. The actual degree of crowding was 600 to 1,000 larvae per culture at the time of the analysis of the larvae. The results are summarized in Table 2 according to the format of Table 1.

The data in Table 2 indicate that no viability difference associated with the *ADH* genotypes tested was detected in the mid-3rd instar larvae under moderately crowded condition.

However, under this condition the size of the larvae varied considerably. The samples used for test (ii) were collected at random with respect to the larval size. It is probable that there might have been detectable viability differences if only fast growing larvae or slow growing larvae had been collected for the analysis.

(iii) *Fecundity of* FF *and* SS *females:* Since there is no detectable selection during the early larval stage, the analysis of 3rd instar larvae in a culture started

TABLE 1

The expected and observed numbers of early 3rd instar larvae in viability tests, and the χ^2 values for the fit (F) and heterogeneity (H) among cultures

The df refers to the number of degrees of freedom

		Larval genotypes				χ^2 values	
		FF	*FS*	*SS*	Total	F	H
I	Expected	200	200	0	400	df=1	df=3
	Observed	207	193	0	400	0.49	2.43
II	Expected	0	199	199	398	df=1	df=3
	Observed	0	199	199	398	0.00	1.15
III	Expected	50	100	50	200	df=2	df=2
	Observed	47	101	52	200	0.27	1.07

TABLE 2

The expected and observed numbers of mid-3rd instar larvae in viability tests, and the χ^2 values for the fit (F) and heterogeneity (H) among cultures
The df refers to the number of degrees of freedom

		Larval genotypes				χ^2 values	
		FF	*FS*	*SS*	Total	F	H
I	Expected	200	200	0	400	df=1	df=3
	Observed	205	195	0	400	0.25	2.27
II	Expected	0	200	200	400	df=1	df=3
	Observed	0	199	201	400	0.01	1.95
III	Expected	50	100	50	200	df=2	df=2
	Observed	43	111	46	200	2.51	0.99

by premated female parents of different genotypes will give necessary information on differential fecundity of female genotypes. The optimum density of larvae as in test (i) was used for the fecundity test. Six different frequency combinations of premated females were set up as follows:

♂♂×♀♀	*FF*×*FF*	*FF*×*SS*	*SS*×*FF*	*SS*×*SS*	Total ♀♀ per culture	Number of cultures
(a)	10	30	0	60	100	2
(b)	25	50	0	25	100	3
(c)	60	30	0	10	100	3
(d)	10	0	30	60	100	3
(e)	25	0	50	25	100	3
(f)	60	0	30	10	100	4

The ratios of *FF* larvae *vs.* (*FS* + *SS*) larvae are expected to be 1:9, 1:3 and 3:2 for conditions (a), (b) and (c), respectively, and those of (*FF* + *FS*) larvae *vs.* *SS* larvae to be 2:3, 3:1 and 9:1 for conditions (d), (e) and (f), respectively, under the assumption of no fecundity difference between *FF* and *SS* females. The χ^2 test of significance on the deviations from the ratios corresponds to one degree of freedom in the pooled data under each condition, and another degree of freedom and corresponding χ^2 value orthogonal to the above can be obtained. The latter represents the test on the deviation of the observed ratios from the expected ratios of *FS* *vs.* *SS* larva counts in conditions (a), (b) and (c), and *FS* *vs.* *FF* larva counts in conditions (d), (e) and (f) under the assumption of no viability difference between the genotypes compared. The relevant data and χ^2 analyses are presented in Table 3. The value of χ^2 with one degree of freedom significant at the 0.05 level is 3.84.

None of χ^2 values for testing the fecundity differential was statistically significant, while one of the χ^2 values for the viability difference between the *FF* and *FS* larvae turned up to be slightly above 3.84 (this value is marked by a star in Table 3). So far, 12 χ^2 values, 3 from each of tests (i) and (ii) and 6 from test (iii), were computed for the test of the assumption of no viability difference during the early larva stage. 1 out of 12 χ^2 values can be significant at the 0.05

TABLE 3

Larval counts in fecundity test and χ^2 values for the test of deviations
The χ^2 values under designations F and V are those for fecundity and viability differences, respectively

Conditions	Larval genotypes *FF*	*FS*	*SS*	Total	χ^2 values F	V
(a)	26	72	151	249	0.05	0.11
(b)	85	199	90	374	1.03	0.64
(c)	209	118	48	375	2.84	1.50
(d)	48	102	225	375	0.00	3.92*
(e)	124	234	108	466	0.83	0.28
(f)	385	179	59	623	0.19	0.65

* See the text.

level due to mere chance. Thus, it was concluded that there was no fecundity difference between the *FF* and *SS* females.

(iv) *Egg-to-adult viability:* The experimental scheme used in test (iii) can be modified to test viability differences among *FF*, *FS* and *SS* genotypes observed between the egg and adult stages. The premated females of condition (a) through (f) were transferred to new culture bottles, bottle to bottle, immediately after they were used for the oviposition of test (iii). In order to produce cultures of regular crowding condition, the female parents were allowed to lay eggs for a period of two days. The mortality of these female parents during the second oviposition period was checked for each bottle, and it was mostly 0 with a few cases of 1.0% and 2.0%.

Because of no fecundity difference shown in test (iii), the pair of frequency conditions such as (a) and (d) was considered to be equivalent. Thus, the result will be treated as having three input genotype frequencies: low *F* allele frequency, 0.1, 0.3 and 0.6; medium allele frequency, 0.25, 0.50 and 0.25; high *F* allele frequency, 0.6, 0.3 and 0.1; where the preceding triplet figures refer to the frequencies of *FF*, *FS* and *SS* genotypes in that order. Further, it was decided to include more culture bottles for the two extreme frequency conditions than for the medium frequency conditions, for the expected fluctuations at the extremes would be greater. The total numbers of cultures per condition are given in Table 4.

A random sample of 200 to 400 adult progeny was taken from each culture after the adult emergence was nearly completed. The number of progeny taken was, in part, determined by the availability of electrophoretic equipment and manpower at the time of progeny analyses. The observed genotype counts for the three genotype frequency conditions are presented in Table 4 with other relevant information for this test.

The statistical test of deviations in progeny counts from the input ratios was carried out with χ^2 values with 2 degrees of freedom at each frequency level. Since the tabulated value of χ^2 with 2 degrees of freedom is 9.21 at the probability level of 0.01, all the computed χ^2 values of this kind in the table are highly significant. This indicates that there is a substantial degree of viability selection

TABLE 4

Genotype counts in adult progeny when the input frequencies are fixed at three different combinations

Inputs are given by the numbers of premated female parents providing specified genotypes in progeny

		Genotype counts				Number of cultures	χ^2 values	
		FF	*FS*	*SS*	Total		Total	D*
I	Input	10	30	60	100	7	df=2	df=1
	Observed	241	566	1090	1897		15.93	15.41
II	Input	25	50	25	100	5	df=2	df=1
	Observed	434	746	319	1499		17.68	11.06
III	Input	60	30	10	100	7	df=2	df=1
	Observed	1093	534	273	1900		40.47	40.29

* See the text.

existing in the egg-to-adult interval. A closer look at the data in I in the table suggests that there is a great excess of *FF* genotypes over the expected number; thus a one degree of freedom χ^2 value was computed which corresponds to this excess. This χ^2 value is listed under column D in the table, and it accounts for almost all of the total χ^2 value. This means that the low frequency genotype is at a considerable advantage in viability in comparison to the *FS* and *SS* genotypes. The difference, 15.93–15.41, in the χ^2 value reflects the difference in viability between the FS and SS; this difference is essentially nil.

Moving to the next frequency level, *FF* is again in excess, and this excess is measured by $\chi^2 = 11.06$ under column D. Since the tabulated value of one degree of freedom χ^2 is 6.63 at the level of 0.01, this excess is again highly significant. The difference, $17.68 - 11.06 = 6.62$, indicates that there is a significant difference in viability between *FS* and *SS* at the level of input II; *SS* is evidently poorer in viability than *FS*.

At the high *FF* input, the viability picture reverses itself from the situation under I. Now, *SS* is at a great advantage ($\chi^2 = 40.29$), while there is no difference between *FF* and *FS* genotypes.

The possibility of fitting the data with the WRIGHTIAN viability model of constant parameters (W's) was also explored. Using the input genotype frequencies and viability model of $W_{FF} = 1-s$, $W_{FS} = 1$ and $W_{SS} = 1-t$, the combination of s and t which minimizes the deviation χ^2 was sought by using a computer program. The best fit was obtained at $s = 0.00$ and $t = 0.01$ with the χ^2 value of 74.04. This χ^2 has four degrees of freedom and is highly significant. Thus it is not possible to explain the viability data in terms of constant values of s and t over the three genotype frequency conditions in Table 4.

One further point which is not given in Table 4 should be mentioned. This is the degree of consistency among the culture bottles. The sample size from individual cultures was too small to judge the over-all trend, but the analyses of individual bottles provide a measure of consistency. At the frequency level I, six

cultures out of seven showed some degree of excess in *FF*, but only three of these six cultures gave statistically significant excesses. In one culture, there was a significant excess of *FS* genotypes. Under the frequency level II, all of the five cultures bottles showed *FF* superiority over the other two genotypes, but only one culture showed a statistically significant amount of *FF* excess. Under the frequency level III, six out of seven cultures gave some excess counts of *SS* genotype, but only four of the six reached the level of statistical significance. In one culture, the three genotypes appeared in the same ratios as those used in the input frequencies.

DISCUSSION

The total effort of this study was directed to obtain the pattern of changes in egg-to-adult viability associated with genotype frequencies in populations. The other tests are those required to eliminate various factors which may affect the interpretation of the results from the egg-to-adult viability test. In tests (i) and (ii), differences in fecundity and male sexual activity were unconfounded to test viability differences which might exist among the *ADH* genotypes in very early stage larvae. Upon finding no significant viability differences among young larvae of different *ADH* genotypes, the fecundity differences of *FF* and *SS* females were tested using young larvae produced by the respective females (test iii). There was no significant fecundity difference between the two types of females when they were present at six different frequency levels. However, this fecundity test was a specialized type where there was no *FS* female among female parents. In this sense, the application of the results has to be limited, but they are directly pertinent to the interpretation of the results from test (iv).

The results of test (iv) indicate that egg-to-adult viability was dependent upon the frequencies of the three genotypes present in a given population. In this sense, the mode of selection is that of frequency-dependent selection. This type of viability changes over the range of genotype frequencies was reported by KOJIMA and YARBROUGH (1967) and YARBROUGH and KOJIMA (1967) who studied the mode of selection at the Esterase 6 locus in a *D. melanogaster* population. At a more gross level, a similar phenomenon was observed in mating behavior of Drosophila, and EHRMAN (1966) called it "minority effect."

It is interesting to note that the selective advantage in viability reverses its direction at a point between 0.5 and 0.75 in the frequency of the *F* allele. The population from which the material for this study was sampled was at the point of equilibrium with the *F* frequency of about 60% (actually the *F* frequency in this cage has been fluctuating between the upper 50's and the mid 60's in a slow rhythm over the last 20 generations). It is not possible from the present data to say whether the viability differences among the three genotypes tend to disappear as the frequency in the test material approaches the point of equilibrium. This tendency was observed in the Esterase 6 case cited above.

The magnitude of viability differences observed in test (iv) is surprisingly large when it has to be accounted for by the difference at one locus. Since the exact metabolic function of this enzyme is not known in detail, it is difficult to

make any constructive discussion on this point. The activity of this enzyme is present throughout young larval to adult life of *D. melanogaster*, although the intensity varies at different developmental stages. This indicates that this enzyme may affect the physiology of Drosophila in manifold ways. Thus, the effect of small differences at the locus level may be amplified considerably when the total viability is considered.

On the other hand, it should be remembered that the "total fitness" differences associated with the *ADH* locus might be very slight. This is suggested by the behavior of the allele frequency in the original four cages (see the beginning of MATERIALS and METHODS). It is possible that the viability differences observed in the present material may be counterbalanced with some other fitness component differences. Another possibility is that the fitness differences found with respect to a particular locus in a particular population are subject to the makeup of the genetic background in such a way that some non-allelic interactions (epistasis) influence apparent fitness differences of genotypes at a locus to a great extent.

SUMMARY

The egg-to-adult viability differences associated with the alcohol dehydrogenase locus in a cage population of *Drosophila melanogaster* were investigated. In order to obtain viability results unconfounded with other fitness component traits, several other tests were conducted to check fecundity differences of egg-laying females, segregation distortion and viability up to the 3rd instar larva. The result was that the egg-to-adult viability of different genotypes under normal culture condition depended upon the genotypic frequencies at the alcohol dehydrogenase locus. When a homozygote (either *FF* or *SS*) was present in low frequency (10%), its viability was enhanced, but when the frequency is high (60%), the viability was reduced.

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