

A STUDY OF FREQUENCY-DEPENDENT SELECTION OBSERVED IN
THE ESTERASE-6 LOCUS OF *DROSOPHILA MELANOGASTER*
USING A CONDITIONED MEDIA METHOD¹

S. L. HUANG, MADHO SINGH² AND KEN-ICHI KOJIMA

Department of Zoology, University of Texas at Austin, Austin, Texas 78712

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IN an earlier paper, KOJIMA and YARBROUGH (1967) reported that the relative viabilities between the fertilized egg stage and the adult stage were not constant for the three genotypes of esterase-6, when the gene frequency was deviating from its equilibrium frequency in a laboratory population of *Drosophila melanogaster*, which had not changed genotype frequencies at several loci for more than 45 generations. Furthermore, the intensity of selection appeared to depend upon the degree of deviation from the equilibrium gene frequency. The greater the deviation, the more intense was the selection pressure toward the equilibrium frequency. A similar but more carefully designed experiment, using *Drosophila melanogaster*'s alcohol dehydrogenase (*ADH*) locus with two alleles, showed a similar result for viability as those of the *Est-6* experiment cited above (KOJIMA and TOBARI 1969a). This mode of selection was called frequency-dependent selection by these authors. In this paper, alleles esterase-6^F and -6^S are abbreviated as *F* and *S*, respectively.

YARBROUGH and KOJIMA (1967) also carried out a separate study by setting up a series of cage populations using the same *Est-6* lines, in which the frequency of the *F* allele was approximately 90% in four cages, and approximately 10% in another four cages at the initial generation. The *F* frequency converged rapidly to the 0.40–0.50 range in the cages with the high initial *F* frequency, and to the 0.30–0.35 range in the cages with the low initial frequency. Thereafter, it approached very slowly to the original equilibrium value of the *F* frequency. From this information, they also concluded that the fitness values of the three genotypes (*FF*, *FS*, *SS*) were frequency dependent.

The major objective of this paper is to propose a possible mechanism which explains the observed results reported by the papers mentioned above, by examining a major component of fitness associated with this small region of chromosome (interval about 2.5% recombination long) marked by the esterase-6 locus.

MATERIALS AND METHODS

From the original base population, new homozygous *FF* and *SS* lines, 15 of each, were obtained by pair matings using the procedure described by KOJIMA and TOBARI (1969b). The

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² Present address: Department of Biology, State University College, Oneonta, New York 13820.

main concern was to minimize the changes in the genetic background of the already equilibrated population in the cage. The readers should refer to the two papers dealing with *Est-6* and *ADH* mentioned above for the detailed methods of setting up the initial materials. These lines were used for the conditioned media experiment.

The estimation of viability in the conditioned media: Crosses of *FF* × *FF*, *FF* × *SS*, *SS* × *FF*, and *SS* × *SS* were made using the lines to obtain *FF*, *FS*, and *SS* first instar larvae. 5 ml of KALMUS (1943) medium was poured into vials of size 12.5 × 94 mm, and 2 ml of 5% brewers' yeast suspension (about 100 mg) was injected into the solidified medium in each vial. Fifty first-instar larvae of each genotype were carefully transferred into separate vials. The vials were kept at 24°–25°C for 6–7 days until most larvae reached the pupal stage. All visible pupae from the vials were removed. The vials were then kept on dry ice for about an hour to kill the remaining larvae and pupae if any were left in the media. Three types of conditioned medium, one by each genotype were thus obtained.

After bringing the cold media to room temperature in air, 150 first-instar larvae of each genotype were transferred into each vial of the three conditioned media. One replication consisted of nine vials, i.e., three genotypes × three conditioned media. There were eight replications of nine such cultures. The vials were kept at 24°–25°C for about 17–18 days. During this time, adults from each vial were counted as they emerged. Counting was continued until all flies emerged. Counting was usually finished by the 18th day from the 150 larvae transfer time. The number of adults of a genotype *i* that emerged from a vial with the medium conditioned by genotype *j*, divided by 150 (the input number) gave an estimate of X_{ij} (viability). The subscripts *i* and *j* take values of 2, 1, 0, for *FF*, *FS*, and *SS*, respectively.

RESULTS

The viability coefficients estimated by using the conditioned media are expressed relative to X_{22} in Table 1. The values x_{ij} in the table are the averages of eight replications of the conditioned media experiment. The standard errors for the relative viabilities are computed using the error variance given in analysis of variance. They are adjusted for the ratios such as X_{ij}/X_{22} (= x_{ij}). Analysis of variance of the data showed a highly significant result for the genotypes × media interactions. The result of analysis of variance is given in Table 2. The significant comparisons of the medium effects and the genotype effects (viabilities) are also given in Table 2.

It is clear that there is no heterozygote superiority *per se* in the x values. Another clear indication is that the viability of a genotype is the lowest when grown in medium conditioned by the same genotype. This was consistent in 20 cases out of 24 such diagonal cases in eight replications when each replicate was con-

TABLE 1

Viability coefficients for various combinations of genotypes obtained by using conditioned media, and then expressed relative to X_{22}

Genotypes	Conditioning genotypes		
	<i>FF</i>	<i>FS</i>	<i>SS</i>
<i>FF</i>	0.923 ± 0.053	1.068 ± 0.056	1.130 ± 0.058
<i>FS</i>	1.090 ± 0.057	1.000 ± 0.055	1.087 ± 0.057
<i>SS</i>	1.146 ± 0.059	1.078 ± 0.057	0.928 ± 0.053

The common error variance was taken from the analysis of variance in Table 3.

TABLE 2

Analysis of variance for adult flies from conditioned media

Source	SS	df	MS	F	P
Replications	22057.28	7	3151.04		
Genotypes	40.58	2	20.29	0.35	
Media	3.08	2	1.54	0.03	
Genotypes × Media	4135.83	4	1033.96	17.64	<0.001
Error	3282.72	56	58.62		
Total	29519.50	71			

FF, FS, SS represent the conditioned media:
 Effect of three conditioned media on FF viability: FS > FF, P < 0.01; SS > FF, P < 0.001
 Effect of three conditioned media on FS viability: FS > FS, P < 0.05; SS > FS, P < 0.05
 Effect of three conditioned media on SS viability: FS > SS, P < 0.001; FS > SS, P < 0.01
 FF, FS, SS represent the three genotypes:
 Viability differences in three genotypes in FF conditioned media: FS > FF, P < 0.001;
 SS > FF, P < 0.001
 Viability differences in three genotypes in SS conditioned media: FF > SS, P < 0.001;
 FS > SS, P < 0.01

sidered separately. In the exceptional four cases, the values were not the lowest, but differed very little from the next lowest values.

More explicitly stated, for homozygous genotypes, the viability increased as the frequency of the allele involved decreased in the conditioner genotypes. The viability of FF was the lowest when the medium was conditioned by FF. For the SS genotype, its viability was the lowest when the medium was conditioned by SS, intermediate when conditioned by FS, and the highest when conditioned by FF. The viability of the heterozygote was the lowest when grown in media conditioned by FS, but significantly higher when conditioning was done by either homozygote. In the following diagram, the arrows indicate the direction of significant increase in the viability estimates of the genotypes shown in the left column, when grown in media conditioned by genotypes on the top row.

Genotypes		FF	FS	SS
		2	1	0
FF	2	—————→		
FS	1	←—————		
SS	0	←—————		
Marginal means		\bar{x}_{2k}	\bar{x}_{1k}	\bar{x}_{0k}

A lower fitness value for abundant genotypes, and higher fitness values for rare genotypes can be explained on the basis of the above findings. Since the survival value of a given genotype is expressed as in the following (1), the viability of a given genotype becomes an allele frequency-dependent value, which is a marginal average for a given genotype at a given allele frequency p_k .

$$\bar{x}_{ik} = p_k^2 x_{i2} + 2p_k(1-p_k) x_{i1} + (1-p_k)^2 x_{i0} \tag{1}$$

where p_k is the frequency of allele F.

TABLE 3

Relative fitness values estimated by using viability coefficients and estimated gene frequencies from Hardy-Weinberg ratios

$f(F)$	FF	FS	SS	$f(S)$
0.90	0.886	1.000	1.054	0.10
0.85	0.904	1.000	1.054	0.15
0.80	0.922	1.000	1.052	0.20
0.75	0.938	1.000	1.049	0.25
0.70	0.953	1.000	1.044	0.30
0.65	0.968	1.000	1.038	0.35
0.60	0.981	1.000	1.031	0.40
0.55	0.992	1.000	1.023	0.45
0.50	1.003	1.000	1.013	0.50
0.45	1.012	1.000	1.002	0.55
0.40	1.021	1.000	0.990	0.60
0.35 = \hat{p}	1.027	1.000	0.977	0.65
0.30	1.033	1.000	0.962	0.70
0.25	1.037	1.000	0.946	0.75
0.20	1.040	1.000	0.930	0.80
0.15	1.042	1.000	0.912	0.85
0.10	1.042	1.000	0.894	0.90

Using the estimates of the viability values, the fitnesses of the three genotypes were calculated according to equation (1). To find fitnesses at a given allele frequency, Table 3 was computed using the estimated x 's.

Considering Table 3, it is clear that with initially high F allele frequency, there are large differences among the fitness values; the SS genotype is superior to FS , which in turn is superior to FF in viability value. As the F frequency decreases, the differences in the viabilities decreased. At the initial point of equilibrium, $\hat{p} = 0.35$, genotypes are almost equal in viability. The pattern of viability changes strongly indicates that as equilibrium is reached, the three genotypes become equally fit. When the F frequency becomes smaller than the range of 0.45–0.35, genotype FF now becomes more viable than genotype SS . This reversal in viability is one of the most significant points in this paper.

According to the frequency-dependent selection model proposed by KOJIMA and YARBROUGH (1967), the abundant genotype is lower in fitness and the rare genotype has higher fitness in reference to the equilibrium frequency. Such a result is demonstrated in a quite striking manner in Table 3.

DISCUSSION

Frequency-dependent selection was not given sufficient attention until it was proposed by KOJIMA and YARBROUGH (1967), based on their laboratory data, as a possible mechanism responsible for large amounts of genetic polymorphisms observed in natural populations. However, there had been several authors who suspected its existence or proposed it as a theoretical possibility. While studying changes in the frequency of inversions in cage populations of *Drosophila pseudoobscura*, WRIGHT and DOBZHANSKY (1946) mentioned that there might be a tendency for a chromosome inversion to increase in frequency when rare. They suspected that such phenomena might be common. Some theoreticians such as TEISSIER (1954) and FISHER (1958) suggested such mechanisms. TOBARI and KOJIMA (1967) and KOJIMA and TOBARI (1969b) showed in second and third chromosome inversions of *Drosophila ananassae* that the minority karyotype always had the selective advantage over the other two inversion karyotypes. These inversions are polymorphic with about a 50:50 ratio in normal laboratory conditions. KOJIMA and TOBARI (1969a) found that in a small chromosomal segment (recombination fraction about 0.02 or less) marked by the alcohol dehydrogenase locus, when a homozygote of either *FF* or *SS* was present in a low frequency, its viability was enhanced; but when the frequency was high, the viability was reduced. This experiment was set up very similarly to that of KOJIMA and YARBROUGH (1967), but the detailed design was far more precise to measure egg-to-adult viability only. EHRMAN (1967) reported that in *Drosophila pseudoobscura*, minority males mate more frequently than the majority males. EHRMAN and PETIT (1968) observed a frequency dependence of the mating success in all three species, namely *Drosophila tropicalis*, *Drosophila willistoni*, and *Drosophila equinoxialis*. The mating component of fitness may have been operating principally in the population cages. However, after eliminating the mating component by using predated females in the desired proportions, the genotypes still showed dependence on frequency for their fitnesses (KOJIMA and TOBARI 1969a,b; KOJIMA and YARBROUGH 1967). From the results of the experiments of EHRMAN, PETIT, and KOJIMA and his associates, it appears that mating ability and egg-to-adult viability are probably the main components of fitness in frequency-dependent selection. In turn, these components are the major components determining *Drosophila* fitness.

The pattern of changes in allele frequency over a period of generations until the equilibrium is reached may be similar both in frequency-dependent and heterotic selection, unless one starts an experiment from an extremely low or high gene frequency. Therefore, one often cannot distinguish one mode of selection from the other in many studies. Heterotic selection generally assumes constant fitness, whereas fitnesses of the genotypes vary in frequency-dependent selection. The rare genotype has higher fitness and the abundant genotype lower fitness when their frequencies deviate on either side from an equilibrium frequency. At equilibrium, the three genotypes should be equally fit in some cases, as in this study (Table 3), but heterotic selection may be superimposed as in the

study by KOJIMA and TOBARI (1969b). Then to know whether the mode of selection is heterotic or frequency dependent, the fitnesses of the genotypes over a wide range of gene frequency must be investigated. The problem, therefore, reduces to obtaining reasonable estimates of fitness values from the observed changes in frequencies of genotypes, generation after generation. PROUT (1965, 1969), KIMURA, DEMPSTER, COMSTOCK, LERNER, and LEWONTIN (individual personal communications to KOJIMA) pointed out that the use of *zygotic* frequencies in a population determined by counting at the same stage of development in two successive generations might give incorrect fitness estimates. YARBROUGH and KOJIMA (1967) tried to estimate Wrightian fitness values by the minimum chi-square and least-square methods using the observed allele frequencies of the first 15 generations, and of 29 generations from eight population cages. Out of these eight, seven cages converged to the initial population cage equilibrium with fair to very rapid speeds. But the estimates fluctuated widely including zero and/or very high values. Had the estimation method not included a provision for avoiding negative values, they would have been included. *These estimates gave good agreement between observed and expected changes in allele frequencies.* Such changes can also be simulated using the estimates obtained experimentally with conditioned media. Thus, the curve-fitting methods to estimate fitness values may be considered unreasonable if the model is unknown. Negative and zero fitnesses without actual lethality have no biological meaning, but they are statistically possible values. PROUT (1969) using the method he proposed for estimating fitness values, obtained some negative and widely differing fitness estimates from the data of KOJIMA and TOBARI (1967). If one has previous knowledge of the mode of selection, he may use a model that will fit much better; and results are more meaningful.

Nine viability coefficients were determined experimentally from conditioned media, completely independent of YARBROUGH and KOJIMA's data. The only common factor was that the samples were taken from the same cage, but at two different times (approximately 80 generations apart).

One basis for frequency-dependent selection is indicated by the results from the conditioned medium experiment. In a high initial frequency of a given allele, individuals of the abundant genotype more *often* use the medium which is either being used or has been used by the same genotype. This lowers the fitness of the abundant genotype. On the other hand, a rare genotype *rarely* comes across the food used or being used by the same genotype. This would keep its viability higher, and therefore would force the frequency to increase. The control experiment, in a true sense, is not available and therefore it cannot be concluded whether a homozygote, in conditioning the media, renders a neutral or beneficial effect for the other genotypes. For this reason, the relative viability values were computed for each replication.

But the conditioning of the media by one genotype does make the media less unlivable for its own genotype. It is not known how this is accomplished. The medium may either be depleted or reduced in some required nutrients, or some byproducts may be left that could reduce the chance of survival of its own geno-

type. Many questions still remain unanswered. More elaborate studies involving the different proportions of conditioning genotypes and the biochemistry of media and byproducts are needed before one knows more fully all the mechanisms of frequency-dependent selection.

SUMMARY

Media were conditioned by growing 50 first-instar larvae of each genotype until the pupal stage. Then they were removed or killed. 150 first-instar larvae of each genotype were then grown in the conditioned media by the above method, until emergence of all adults occurred. Ratios of emerged flies to input larvae gave nine viability coefficients. Relative viability of a genotype was lowest when grown in media conditioned by the same genotype. A homozygote increased in viability as its allele frequency in the conditioning genotype decreased. It indicated that individuals of a conditioning genotype either deplete nutrients or leave metabolic products harmful to its own genotype.—Rare genotypes had higher fitness, and the abundant ones had lower fitness in comparison to the allele's equilibrium frequency. Fitnesses varied according to allele frequency, and were reversed when the high-frequency allele was reversed. Mode of selection was found to be frequency dependent. The agreement between the frequency changes using selective values obtained from the conditioned media and those of the original curves by YARBROUGH and KOJIMA (1967) was discussed. Furthermore, the new data provide support for KOJIMA and YARBROUGH's (1967) point, which was the near-neutrality of all genotypes at the point of equilibrium.

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