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Selection at the Acid Phosphatase Locus in *Drosophila melanogaster*

R. Nassar

Department of Statistics, Kansas State University,
Manhattan, Kansas 66506, U.S.A.

Abstract

Results of fitness estimates at the acid phosphatase locus in *D. melanogaster* revealed that the observed gene frequency equilibrium at the locus was maintained by frequency-dependent selection. Selection was due mostly to larva–adult viability. Gene-frequency response on media with substrate (α-naphthyl acid phosphate) added may have been caused in part by selection at the acid phosphatase locus, although the possibility of a correlated response cannot be excluded.

Introduction

Of importance in the study of evolution in natural populations is finding mechanisms that maintain genetic variability. Frequency-dependent selection has been recognized by many as an important mechanism in maintaining genetic polymorphisms. Kojima and Yarborough (1967), Kojima and Tobari (1969), and Nassar (1979) have presented experimental evidence for frequency-dependent selection at the esterase 6, the alcohol dehydrogenase and the *Lap* loci in *D. melanogaster*. The mode of selection for Lap and esterase 6 was such that all genotypes had equal fitnesses at equilibrium. Frequency-dependent selection has also been demonstrated for mating success (Petit 1958; Ehrman 1966, 1968) and for inversion polymorphism (Tobari and Kojima 1967; Nassar *et al.* 1974). Yamazaki (1971) failed to find evidence for frequency-dependent selection at the esterase 5 locus in *D. pseudoobscura*. Kojima (1971*a*, 1971*b*) argued for the generality of frequency-dependent selection as a mechanism maintaining genetic variability in natural populations. More experimental evidence is needed to adequately assess the generality and the importance of frequency-dependent selection.

We report in this study on selection experiments regarding the acid phosphatase locus in *D. melanogaster*. Evidence is presented that frequency-dependent selection acts to maintain a stable genetic polymorphism at the locus.

Materials and Methods

The base population for this experiment was founded with 15 inseminated females collected in Manhattan, Kansas. The females were trapped at two locations and over a period of 1 week, so it is likely that close to 30 wild-type individuals were represented in the initial sample. Ten lines homozygous for a fast-moving band (F) at the acid phosphatase locus (*Acph-1*^{A}; III; 101·4) and 10 lines homozygous for a slow-moving band (S) were isolated from the base population through single-pair matings. Each line could be traced to one-pair mating in the base population. Inseminated

females representing each of the 10 *FF* lines, were brought together in a bottle to form an *FF* subpopulation. An *SS* subpopulation was initiated in a similar manner from the 10 *SS* lines. The possibility was excluded that inversions (like the Payne inversion) were present on the right arm of the third chromosome of the wild-type population. This was based on the finding that there was a normal crossover rate between ebony (III; 70·7) and taxy (III; 91) when they were balanced against chromosomes from the wild-type population (++/*e tx*). Fifty ++/*e tx* virgin females were individually crossed to *e tx*/*e tx* males and 1500 (30 per female) progeny were scored for recombinants. The percentage recombination between ebony and taxy was 0·213 (319 recombinants). As one reviewer pointed out, it is possible that the test would not have detected small inversions adjacent to the *Acph* locus.

The first experiment was started 10 generations after the *FF* and *SS* lines were established. Four types of crosses were made (*FF* × *FF*, *FF* × *SS*, *SS* × *FF* and *SS* × *SS*) and after 2 days of mating, males and females were separated and 100 mated females from the four type crosses were brought together in a half-pint bottle containing 45–50 c.c. of cornmeal–Karo medium and allowed to lay eggs for 3 days. Different proportions of females from each of the four type crosses were chosen so that the genotypes laying eggs were in Hardy–Weinberg proportions in which the frequencies of the *Acph F* allele were 0·2, 0·5 and 0·8. There were three replicates for each frequency.

In the next generation the frequencies of the three genotypes were determined using starch-gel electrophoresis. The same experiment was repeated over a year later (30 generations) with six replications per frequency. These two experiments will be designated I and II.

Estimates were taken on the average number of eggs laid and eggs hatched per *FF* and *SS* female. *FF* or *SS* females were crossed in a bottle to *FF* and *SS* males. After 2 days the females were transferred to individual vials and a count was made of the number of eggs laid and eggs hatched. The technique was the same as described by Nassar (1979).

A second experiment with six replications was started from the same *FF* and *SS* lines 50 generations after their initiation. In all, 100 inseminated females initiated each replication. For each replication four of the females came from cross *FF* × *FF*, 16 from *SS* × *FF*, 16 from *FF* × *SS* and 64 from *SS* × *SS*. Three of the replications were on media containing 0·5% α-naphthyl acid phosphate (a substrate specific for the Acph enzyme) and the remaining three on normal media (no substrate present). A concentration of 0·5% α-naphthyl acid phosphate in the media did not cause death among adult flies, but was close to the threshold level at which death occurred. These experiments are designated M-α (substrate present in the medium) and M-N (substrate not present in the medium). Replicates were kept separate and the electrophoretic determination for each generation was run on adults after emergence and the genotypic frequencies of *FF*, *FS* and *SS* were determined. Daily random sampling commenced (in all replicates simultaneously) on the first day of adult emergence and ended 4 days later. On the seventh and eighth day after adult emergence started, all flies were transferred to a new bottle to start a new generation. Parents were discarded after 3 days of egg-laying. The number of adult flies transferred per replication was over 600.

Throughout this work flies were maintained at room temperature under discrete generations. In the analysis of results, data were pooled over replicates. This was justified as there were no significant differences in genotypic frequencies among replicates and replicates had nearly equal sample sizes. Horizontal starch-gel electrophoresis was used to assay for *FF*, *FS* and *SS* genotypes. The gel buffer consisted of 0·045 M Tris, 0·925 M boric acid and 0·001 M EDTA. The electrode buffer consisted of 0·054 M Tris, 0·03 M boric acid and 0·012 M EDTA. A 0·05 M sodium acetate was used for the stain buffer. The substrate was α-naphthyl acid phosphate and the stain Fast Garnet GBC salt. Stain and substrate were used at the rate of 1·2 mg per millilitre of stain buffer.

Statistical Analysis

In experiments I and II, the interest was in estimating the fitness over one generation of the *FF*, *FS* and *SS* offspring genotypes. This was done by taking the ratio of the observed offspring genotype frequency to its expected frequency. The latter was the Hardy–Weinberg frequency assuming no fitness differences among the genotypes. Fitness in this experiment was due to eggs hatched per *FF* and *SS* female parents and larva to adult viability of the offspring genotypes (*FF*, *FS*, *SS*). Hatchability and larva to adult viability are confounded. Fitness estimates for I and II (Table 1) are maximum likelihood estimates of

$$f_1\mu_1/w, (f_2+f_1)\mu_2/w, f_2\mu_3/w, \quad (1)$$

where f_1 and f_2 are the fitnesses due to eggs hatched per *FF* and *SS* female: μ_1, μ_2 and μ_3 are the fitnesses due to larva to adult viabilities of *FF*, *FS* and *SS* genotypes respectively. For more details on the methodology and rationale for this experiment see Nassar (1979).

In the two experiments M-α and M-N the enumeration of the genotype was at the adult stage. Hence, a generation was from adult to adult [adult–zygote–adult] and not, as commonly assumed, from zygote to zygote (Prout 1965; Crow and Kimura 1970). Fitness from adult to zygote may include survival to time of mating, differential mating and fecundity. Fitness from zygote to adult includes mainly viability.

The data in the following tabulation represent the observed adult frequencies over a number of generations for M-α and M-N experiments. Our interest is to find a selection model that best fits the data. This was done by estimating for a given model the fitness parameters and then testing by means of a χ^2 statistic for the goodness of fit of the model to the data. The fitness parameters of a specified model were estimated using minimum χ^2 values. (For details on methodology and rationale for this analysis see Nassar 1979.)

For this analysis we have considered two types of models—a constant-fitness model (model 1) and frequency-dependent models (models 2, 3, etc.):

Model	*FF*	*FS*	*SS*
1. Overdominance	$1-\theta_1$	1	$1-\theta_2$
2. Kojima (1971*a*)	$1+\theta_1(\hat{p}_1-p_1)$	1	$1+\theta_2(p_1-\hat{p}_1)$
3. cf. O'Donald (1969)	$1+\theta_1(E_1-p_1^2)$	$1+\theta_2(E_2-2p_1p_2)$	$1+\theta_3(E_3-p_2^2)$

For the Kojima model, $\hat{p}_1$ is the gene frequency equilibrium for the *F* allele. This was estimated from the data for M-N and M-α (Table 2). For M-N, $\hat{p}_1$ was estimated at generations 30 and 42 to be 0·656 and 0·635 with a pooled estimate of 0·646. This frequency was similar to the frequency in the base population which after more than 100 generations was estimated from a sample of 270 adults to be $0{\cdot}652\pm0{\cdot}029$. The estimate for generations 15 and 22 in M-α were 0·761 and 0·779 with a pooled estimate of 0·771. From the stability of gene frequencies over these generations it seemed safe to assume that the M-N and M-α populations had in fact reached an equilibrium.

Model 3 is similar to a model of frequency-dependent selection by O'Donald (1969). E_1, E_2 and E_3 in this model represent the frequencies of the three genotypes at equilibrium. They were estimated (as for $\hat{p}_1$, Table 2) to be, respectively, 0·424, 0·445 and 0·131 for M-N and 0·607, 0·328 and 0·065 for M-α.

Different combinations of the above models were used in specifying the adult–zygote and zygote–adult fitnesses. In addition, equal fitness values for the three genotypes at the adult–zygote stage was used. In such a case selection was assumed to act only in the zygote–adult stage. For each model, estimates of θ's were obtained and the fit of the expected genotype frequencies (obtained from the estimates of the fitness values for a certain model) to the observed genotype frequencies was tested by χ^2. The degrees of freedom for the χ^2 analysis were $2n-p$, where n is the number of generations and p the number of fitness parameters that were estimated. Our interest was not in estimating parameters as such, but rather in determining the type of selection operating in the population. For this reason the emphasis (Table 3) is on the χ^2 statistic for goodness of fit of different selection models to the data.

Results and Discussion

Fitness estimates in Table 1 are consistent for experiments I and II. When the *F* allele frequency was 0·2 and 0·5, the *FF* genotypic fitness was larger than the *FS* or *SS* fitnesses. On the other hand when the *F* allele frequency was 0·8 the *SS* genotypic fitness was larger than the *FF* and *FS* fitnesses. The equilibrium frequency for the *F* allele was estimated to be 0·646. These results suggest that selection was against the *F* allele when its frequency exceeded the equilibrium frequency of 0·646. Selection, however, was for the *F* allele when its frequency was less than 0·646. This demonstrates that selection for the *F* allele is frequency-dependent and of the kind that can maintain stable gene frequency equilibrium. The data also show that fitness differences among genotypes are reduced as the equilibrium gene frequency is

approached. This is in agreement with results on the *Lap* (Nassar 1979) and esterase 6 (Kojima and Yarborough 1967) loci and points out that frequency-dependent selection can be a powerful way of maintaining a high level of genetic polymorphism without an ensuing segregation load. This is of significance, since it has been shown that there is a limit on the amount of genetic variation that can be maintained under the classic overdominance model. For a discussion of this type of experiment in relation to the confounding of gene frequency with fitness, see Nassar (1979).

Table 1. Fitness estimates for the three genotypes (*FF*, *FS* and *SS*) as a function of their frequencies in experiments I and II

Expt No.	Freq. of *F* allele	Sample size	*FF*	*FS*	*SS*
I	0·2	220	3·86±0·610	1·38±0·104	0·632±0·052
II	0·2	536	2·33±0·314	1·17±0·065	0·831±0·034
I	0·5	345	1·345±0·101	0·980±0·054	0·695±0·082
II	0·5	458	1·205±0·086	0·917±0·046	0·943±0·079
I	0·8	320	0·908±0·043	1·11±0·083	1·56±0·338
II	0·8	656	1·02±0·028	0·876±0·055	1·61±0·239

Fitness in (1) includes viability of the offspring genotype confounded with hatchability of the *FF* and *SS* mother genotypes. It is possible to correct the fitness estimates for hatchability by obtaining estimates for the latter. Estimates of eggs laid and eggs hatched per *FF* and *SS* females were as follows:

Genotype	*FF*	*SS*
Eggs laid	37·25±2·31	35·56±2·34
Eggs hatched	34·90±2·27	32·89±2·11
No. of females	51	57

From these estimates one can conclude that no hatchability differences exist between *FF* and *SS* parent genotypes and that the fitness estimates from (1) represent mainly larva to adult viabilities.

Observed frequencies of *FF*, *FS* and *SS* are presented for M-α and M-N experiments in Table 2. It is seen from this data that the *F* allele reached a frequency of 0·761±0·027 at generation 15 in the M-α experiment. The frequency of the *F* allele at generation 18 in the M-N experiment was 0·646±0·028. This turned out also to be the equilibrium frequency. The equilibrium frequency of the *F* allele in the M-α population was estimated to be 0·771±0·025. It is clear that the rate of change of gene frequency of the *F* allele and its frequency at equilibrium were significantly higher for M-α than M-N.

The fitness of the *F* allele seemed to have increased relative to that of the *S* allele when substrate was present in the media. It is significant to note that between generations 22 and 33 the substrate was not added to the media. At generation 33 the observed *FF*, *FS* and *SS* frequencies in a sample of size 240 were 0·371, 0·471, and 0·158 respectively. Thus, the frequency of the *F* allele decreased from near 0·779±0·031 at generation 22 to 0·607±0·032 at generation 33. After generation 33 we resumed the substrate in the medium. This was continued until generation 44. At generation 44 the observed *FF*, *FS* and *SS* frequencies in a sample of size 243 were 0·6, 0·354 and 0·045 with a frequency of 0·777±0·027 for the *F* allele.

It is clear from the M-α experiment that viability differences among genotypes at the *Acph* locus were influenced by the presence of the enzyme substrate in the media. Similar results have been reported with regard to the Adh (Gibson 1970; Vigue and Johnson 1973; Kamping and van Delden 1978), *G-6-pdh*, *6-pgdh* (Bijlzma 1978) loci in *D. melanogaster* and to the *Odh* locus in *D. pseudoobscura* (Wills and Nichols 1971, 1972). For the *Adh*, *G-6-pd* and *6-pgd* loci, addition of specific enzyme substrate to the media affected the level of enzymatic activity indicating that selection was for the locus itself. This may have been the case for the *Acph* locus, but one cannot know without biochemical evidence on enzymatic activity. The change in the frequency of the *F* allele might be due to other genes tightly linked to the Acph locus.

Table 2. Observed frequencies of *FF*, *FS* and *SS* genotypes at the Acph locus for experiments M-N and M-α

Generation	*FF*		*FS*		*SS*		Sample size	
	M-α	M-N	M-α	M-N	M-α	M-N	M-α	M-N
1	0·108	0·091	0·547	0·522	0·344	0·386	276	132
2	0·258	0·168	0·529	0·517	0·211	0·314	236	172
3	0·256	0·217	0·512	0·526	0·231	0·256	242	230
4	0·308	0·279	0·529	0·483	0·162	0·237	240	240
5	0·384	0·250	0·504	0·496	0·111	0·254	216	240
6	0·416	0·208	0·466	0·528	0·116	0·264	240	250
7	0·403	0·254	0·461	0·529	0·136	0·216	243	240
8	0·440	0·256	0·436	0·524	0·113	0·219	238	242
9	0·475	0·308	0·422	0·510	0·102	0·181	225	237
10	0·524	0·314	0·372	0·467	0·091	0·219	242	242
11	0·508	0·357	0·421	0·489	0·070	0·153	242	241
12	0·520	0·333	0·387	0·465	0·091	0·201	240	243
13	0·473	0·362	0·439	0·436	0·087	0·202	241	188
14	0·539	0·355	0·419	0·498	0·041	0·146	241	239
15	0·598	0·360	0·326	0·484	0·075	0·155	239	161
16		0·417		0·429		0·149		242
17		0·400		0·465		0·135		230
18		0·431		0·431		0·145		234
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22	0·614		0·330		0·056		321	
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30		0·421		0·470		0·108		323
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42		0·420		0·429		0·150		240

Table 3 presents only the genetic models that gave the best fit to the data of Table 2 analysed over the first 15 (M-α) and 18 (M-N) generations. The goodness of fit of a model to the data was judged by its χ^2 value. For both the M-N and M-α data, it is seen that the best models were the zygote–adult frequency-dependent models (model 2 for M-α, M-N; model 1 for M-α). Model 4 (with frequency-dependent fitnesses at both the adult–zygote and zygote–adult stages) also gave good fit to the

Table 3. Estimates of fitness parameters for four models of selection and the χ^2 goodness of fit of each model to the data in Table 2

Model	Fitness			Expt	Estimates						χ^2	d.f.
	FF	*FS*	*SS*	No.	θ_1	θ_2	θ_3	θ_4	θ_5	θ_6		
1. Adult–zygote	1	1	1	M-N	0·30	1·10					54·90 ($0{\cdot}01 < P < 0{\cdot}025$)	34
Zygote–adult	$1+\theta_1(\hat{p}_1-p_1)$	1	$1+\theta_2(p_1-\hat{p}_1)$	M-α	0·5	1·2					29·62 ($0{\cdot}25 < P < 0{\cdot}5$)	28
2. Adult–zygote	1	1	1	M-N	−0·05	1·45	1·25				31·62 ($0{\cdot}5 < P < 0{\cdot}75$)	33
Zygote–adult	$1+\theta_1(E_1-p_1^2)$	$1+\theta_2(E_2-2p_1p_2)$	$1+\theta_3(E_3-p_2^2)$	M-α	0·5	0·05	1·25				26·76 ($0{\cdot}25 < P < 0{\cdot}5$)	27
3. Adult-zygote	$1+\theta_1$	1	$1+\theta_2$	M-N	−0·25	0·45	1·8	−0·5	1·2		81·37 ($P < 0{\cdot}001$)	31
Zygote–adult[A]	$1+\theta_3(E_1-q_1^2)$	$1+\theta_4(E_2-2q_1q_2)$	$1+\theta_5(E_3-q_2^2)$	M-α	−0·25	−0·20	1·30	−0·7	1·2		48·72 ($0{\cdot}001 < P < 0{\cdot}005$)	25
4. Adult–zygote[B]	$1+\theta_1(E_1-P_{11})$	$1+\theta_2(E_2-P_{12})$	$1+\theta_3(E_3-P_{22})$	M-N	−0·7	0·7	0·1	−0·5	−0·05	1·35	35·03 ($0{\cdot}1 < P < 0{\cdot}25$)	30
Zygote–adult*	$1+\theta_4(E_1-q_1^2)$	$1+\theta_5(E_2-2q_1q_2)$	$1+\theta_6(E_3-q_2^2)$	M-α	0·05	−0·8	0·05	1·0	−0·75	1·10	31·57 ($0{\cdot}1 < P < 0{\cdot}25$)	24

[A] q_1 = gene frequency at zygote stage after adult–zygote selection.

[B] P_{11}, P_{12}, P_{22} = adult genotype frequencies.

M-N and M-α data ($0{\cdot}1 < P < 0{\cdot}25$). It is significant that model 3 (with overdominance fitness at the adult–zygote stage and frequency-dependent fitness at the zygote–adult stage) gave poor fit to the data. The poorest fit to the data was for models with only overdominance fitnesses. These models were not presented in Table 3. As an example, the overdominance fitness model with selection at both adult–zygote and zygote–adult stages gave χ^2 goodness of fit of 190·65 for M-N and 341·05 for M-α. Also, for the overdominance fitness model with selection at the zygote–adult stage only the χ^2 values were 259·3 for M-N and 240·5 for M-α. Results of the analysis of the data in Table 2 agree with the results of the one generation data (Table 1) in showing that selection was frequency-dependent and due in large part to larva–adult viability.

Results presented in this paper were based on a sample size of about 30 wild-type individuals. The size of this sample, although not large, cannot be considered too small to affect the generality of these results. Frequency-dependent selection was demonstrated also for Lap and for an inversion polymorphism in similar size experiments (Nassar *et al.* 1974; Nassar 1979). The populations used in those experiments were not the same and were also different from the one used in the present study. These findings plus similar findings by others argue for the generality of frequency-dependent selection as a way of maintaining genetic variability.

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