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THE EFFECT OF CONDITIONING THE MEDIUM IN *DROSOPHILA*, IN RELATION TO FREQUENCY-DEPENDENT SELECTION

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SUMMARY

In the context of frequency-dependent selection, the effect of conditioning the medium, by the previous culture of larvae of a given strain, on the survival of subsequent larvae of the same or different strains was measured. Six strains were used, some inbred and some lines selected for low or high sternopleural bristles. The survival of larvae was not significantly less when the food had been conditioned by the same strain rather than by another strain, nor did conditioning by one strain have any special effect on the survival of another. The survival of lines on medium conditioned by the same strain was not less than on fresh medium, and in some cases was significantly greater.

1. INTRODUCTION

IN his series of experiments on frequency-dependent selection in *Drosophila melanogaster*, Kojima found that the relative survival of the three genotypes at the *esterase-6* (*Est-6*) locus depended on their initial frequency. The rarer the genotype the better its survival. He proposed frequency-dependent selection as " a possible general mechanism responsible for a large amount of the genic polymorphisms observed in natural populations ". Looking further into details, he and his colleagues investigated the effect on larval survival of " conditioning " the medium by allowing a group of larvae of a known genotype to develop to pupation before putting on the larvae whose survival was to be measured. Huang, Singh and Kojima (1971) and Kojima and Huang (1972) found that conditioning by larvae of a given genotype at the *Est-6* locus reduced preferentially the relative survival of larvae of the same genotype. They suggested that " individuals of a conditioning genotype either deplete nutrients or leave metabolic products harmful to its own genotype ".

A general failure to find frequency-dependent effects on larval viability associated with specific enzyme polymorphisms in a series of experiments which will be reported elsewhere led to this present work. We present the results of an evaluation of the effects of conditioning, using not genotypes at specific loci but rather highly selected or inbred lines.

In the first experiment, six lines were used, five deriving from the standard Kaduna population and one with a completely different origin. All lines were typed at the *Est-6* and *alcohol dehydrogenase* (*Adh*) loci, which are polymorphic in most wild populations and on which much experimental work

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has been done. The lines fall roughly into three pairs and information on them is summarised in table 1. The first pair consists of the lines selected from the Kaduna population for low (1) or high (2) sternopleural bristles respectively. This involved crossing between selected lines followed by further selection. The two are fixed for different alleles at both enzyme loci but they might also be expected to have different alleles fixed at all loci having a major effect on sternopleural bristles. The second pair (3 and 4) are lines from the Kaduna population, kept with 20 pairs of parents for over 400 generations. They proved to be segregating at both loci except that line 3 was fixed at *Est-6*. The final pair are two inbred lines, the first (5) produced by over 400 generations of brother sister mating from Kaduna and the

TABLE 1

Allele frequencies at the Adh *and* Est-6 *loci*

	Adh		*Est-6*	
Line	F	S	F	S
1	0·00	1·00	0·00	1·00
2	1·00	0·00	1·00	0·00
3	0·46	0·54	0·00	1·00
4	0·54	0·46	0·21	0·79
5	0·00	1·00	1·00	0·00
6	1·00	0·00	0'00	1·00

second (6) the white Oregon R line originally obtained from Schultz. Both were fixed at the enzyme loci and, purely by chance, the four lines fixed at both loci formed a balanced set of alternatives.

We decided to use inbred and selected lines to increase the chance of finding effects of conditioning since differences would be due to many loci. Inbred lines should differ at random over loci—lines selected for the same character in opposite directions should be fixed for different alleles at the loci affecting the character in addition to random differences due to genetic drift. Fortunately the lines proved to be fixed for different combinations of alleles at the *Est-6* and *Adh* loci, which have been much used by Kojima and his group.

2. RESULTS

Our intention was to examine the 36 combinations produced by measuring the viability of each of the six lines on all six kinds of conditioned media. The experiment was rather similar to that done on cornmeal molasses medium by Kojima and Huang and would correspond to their highest density for conditioning. In our case, 150 first instar larvae were transferred to 5 cc of cornmeal molasses medium in a vial. Vials were maintained at 25°C until almost all larvae had pupated. The pupae were removed from the vials which were subsequently frozen to kill any remaining larvae. When the vials had returned to room temperature, 300 first instar larvae were transferred to each. The vials were again maintained at 25°C and all emergent adults counted.

As one of the most important aspects of the experiment is the performance of a line in self-conditioned medium (*i.e.* medium which has been conditioned by the same line), more replicates were devoted to this treatment. Ten vials

were conditioned by each line, and on five of these the subsequent larvae were of the same line and one each of the remaining five contained larvae of each of the other lines.

Table 2(a) presents the number of adults of each emerging from 300 larvae cultured in conditioned medium. Each of the figures on the diagonal,

TABLE 2

Numbers of adults emerging from 300 first instar larvae in medium conditioned by 150 larvae. The numbers on the diagonal in (a) are the means of five replicates in self-conditioned medium given individually in (b)

(a)

Medium conditioned by:	Line 1	2	3	4	5	6	Average percentage survival for all lines
1	*114*	214	238	261	207	214	69
2	112	*205*	230	238	213	252	69
3	136	219	*237*	239	216	253	72
4	107	200	245	*259*	225	246	71
5	134	216	249	240	*234*	276	75
6	125	204	253	251	247	*237*	73
Average percentage survival on all media	40	70	81	83	75	82	

(b)

Line 1	2	3	4	5	6
128	198	238	252	236	209
124	207	241	262	231	254
99	204	230	253	223	247
110	197	229	254	228	236
109	218	247	275	250	241

TABLE 3

Analysis of variance in angles

Source	d.f.	M.S.	F	P
Lines as survivors	5	1038·07	124·77	$<0·001$
Lines as conditioners	5	17·22	2·07	$>0·05$
Self-conditioning				
Overall	1	0·28	$<1·0$	—
Between Lines	5	15·58	1·87	$>0·10$
Interactions between survivors and conditioners	19	9·80	1·37	$>0·10$
Between self-conditioned replicates	24	7·15	—	—
Pooled error	43	8·32	—	—

where the lines are self-conditioned, is the mean of five replicates and non-diagonal entries come from a single replicate. The five replicates in self-conditioned medium are given individually in table 2(b).

Frequency-dependent selection of multigenic origin would be shown in two aspects of the results. The first and most direct would be the effect of self-conditioning. The second would be the finding of an interaction between

a line as a survivor and another line as a conditioner. Table 3 presents the analysis of variance. As the survival percentages covered a wide range, the arcsin transformation was used. The analysis was carried out using least squares and the statistical model was as follows.

$$X_{ijk} = m + s_i d_{ij} + M_i + L_j + e_{ijk}$$

where X_{ijk} is the kth observation (with k up to 5 when $i = j$, $k = 1$ when $i \neq j$) of the jth line in the ith conditioned medium. s_i is the effect of self-conditioning (with $d_{ij} = 1$ when $i = j$ and $d_{ij} = 0$ when $i \neq j$), M is the effect of a line as a conditioner and L as a survivor. The variation between self-conditioned replicates is a basic error variance. As there was no indication of any interaction between a line as a survivor and another as a conditioner, this variance was combined with that between self-conditioned replicates to give an overall error for the rest of the table.

TABLE 4

Number surviving of 300 first instar larvae in fresh medium and medium conditioned by 150 larvae of the same line. In (a) in conditioned medium as presented in table 3. (b) Contemporaneous comparisons

	(a) Line				(b) Line	
	1	2	4	6	4	Kaduna
Mean survival in fresh medium	100	177	240	229	243	242
Mean survival in conditioned medium	114	205	259	260	260	252
t	1·41	3·39**	3·41***	0·97	2·78**	1·52
d.f.	7	7	9	9	10	10

** P 0·025. *** P 0·01.

There are clear differences between lines in survival, mostly due to a low value for the low bristle line. There are no significant differences between survival in medium conditioned by different lines. But we are here concerned mostly with the next three items in the analysis. We find no overall effect on survival due to conditioning by the same line (relative to survival in medium conditioned by other lines) nor do lines differ in this. Finally we find no indications of specific interactions due to survival of one line being particularly altered by conditioning by one other. Thus we have no evidence whatsoever of effects which would lead to frequency-dependent selection.

We therefore decided to look further at the direct effect of conditioning. Comparisons were made between fresh unconditioned and conditioned medium. For four of the six lines, the viability of 300 first instar larvae was measured in 5 cc of fresh medium to compare with the earlier results in self-conditioned medium. Line 4 and a sample from the outbred Kaduna cage population were used in a contemporaneous comparison when the same batch of medium was used both fresh and conditioned.

Table 4 presents the mean survival in fresh and self-conditioned medium for the six comparisons made. In all, viability is higher in the conditioned medium and in three cases enhancement is significant. The close agreement between the two sets of results for Line 4 indicates that there is little significant day to day variation in the medium. The comparisons made in table 4(a), although using medium produced at different times, therefore seem valid,

and together with the contemporaneous comparisons (table 4(b)) indicate that larval survival of these lines in conditioned medium is in fact higher than in fresh medium.

3. Discussion

Population density is obviously critical in these experiments, as it will influence the proportion of larvae surviving. All the work presented here and all that presented by Kojima and his colleagues was done in 5 cc of food. In the work by Huang, Singh and Kojima (1971), standard Kalmus medium was used with the addition of yeast. In that by Kojima and Huang (1972), the cornmeal-molasses medium used would have been similar to ours. Huang, Singh and Kojima used only a single combination of densities for conditioning and for the measurement of subsequent survival *i.e.* 100 larvae exposed to medium conditioned by 50. They give no indication of the average proportion of larvae surviving. In the second paper, six different treatments are used ranging from 100 to 200 larvae exposed after conditioning by 50, 100 or 150. The majority of viabilities fall between 80 per cent and 90 per cent. We deliberately chose a higher density of conditioning by 150, followed by measurement on 300 larvae, to try to reduce survival. Omitting one line with a survival of 40 per cent, our average value was 78 per cent.

Attention should be drawn to the discussion of significance in table 1 of Kojima and Huang where the mean viability and the standard errors are given. A superficial examination would suggest that none of the claimed significant differences are in fact so, when compared with their stated standard errors. Further examination showed, however, that each standard error had been calculated as though the observations were binomially distributed with the observed mean and the total number in each replicate. However each mean was based on five replicates. Significance tests had been based on the observed variation between replicates.

The increase in viability we found in conditioned medium above that in fresh medium has in fact been found earlier by other workers. Sang (1949) showed that "larval metabolic products do not impede but may even encourage larval development". Weisbrot (1966) found that conditioning, whether by the same or by different stocks, could result either in an improvement or in a reduction in viability. The experiments of Dawood and Strickberger (1969) provide the most direct comparisons for the present observations. They were not intended as an investigation of frequency dependent selection but, if such selection were operating through differential utilisation of the medium, it should have been apparent in their results. The density used was low, however, consisting of only 40 larvae per vial for conditioning and 70 for subsequent measurement. Viability of four genotypes was measured in medium conditioned by the genotypes themselves as well as in fresh medium. Medium conditioned by two of the genotypes increased survival for all four, in one case causing a three-fold increase above the control level. Conditioning by the other two genotypes produced both beneficial and harmful effects. However, the viability in self-conditioned medium was greater than that in fresh medium (comparable with our table 4) for all four genotypes, though for one the effect was not statistically significant.

In the papers of both Weisbrot and of Dawood and Strickberger, it is suggested that biotic residues are responsible for the alterations in viability. The conditioning process used by these authors was slightly different from the present procedure. A two or three day conditioning period was used after which the larvae were killed by freezing. In our experiments, the conditioning lasted from 6 to 7 days until the majority of the larvae had left the medium to pupate. Biotic residues, possibly different from those involved in the work of the other authors, may also have played a part in the present results. Another explanation for the increased viability due to conditioning may be an effect on the yeast population. In the conditioned medium, where the yeast had had 6 or 7 days to develop, the population is presumably larger than in fresh medium. As yeast is the primary food source of the larvae (Sang, 1949), an increased yeast growth in conditioned medium could result in higher larval survival rates.

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