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The ecological genetics of growth in *Drosophila*

V. GENE-ENVIRONMENT INTERACTION AND INBREEDING

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1. INTRODUCTION

It is well known that inbreeding in a normally outbred species increases the sensitivity to environmental variation during growth and that this often leads to greater variability in performance. But there is comparatively little information about how arrays of inbred lines derived from the same population differ in their response to controlled differences in the environment. We need to know what particular environmental conditions promote the greatest heterogeneity of response, since this may prove a useful guide to the experimental study of genetic and environmental conditions which influence the stability of development in the outbred population.

The present paper deals with some comparisons between the growth of various inbred lines and the non-inbred parent population in *Drosophila melanogaster* as well as a number of crosses between lines derived from the same population. Controlled differences in environment have been provided by growing larvae, at one or more temperatures, on chemically defined, aseptic media which differ in nutrient composition.

2. MATERIAL AND METHODS

Body size and development time have been recorded on females only and both are expressed on a log scale. Three times the natural logarithm of thorax length is taken as the measure of body size, while development time refers to the duration of the larval period unless otherwise stated. When multiplied by 100, differences on the log scale are roughly equivalent to percentage differences. For ease of reference all differences shown in the tables, relating to both body size and development time, have been multiplied by this factor. The sub-optimal diets have been provided by modifying the more or less complete medium of Sang (1956) by reducing the concentration of such essential nutrients as protein, RNA or fructose. The general experimental procedure and the reasons for choosing these particular diets have been discussed elsewhere (Robertson, 1960).

The lines, inbred for more than fifty generations by brother-sister mating, are derived from the Pacific cage population, and five such lines have been studied.

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Eight to ten females from four to five replicated cultures were generally scored for each genotype or treatment. Unless otherwise stated, heterogeneity between lines in their reaction to different treatments is tested against the error variance of a mean, derived from the pooled within- and between-culture effects; the latter are generally unimportant. The numbers on which the means are based are not quite the same in all cases since occasionally cultures of synthetic medium were infected and had to be discarded. The average number of flies per line per treatment has been used in computing the error variance of a mean.

3. RESULTS

The five inbred lines studied here comprise lines numbered 3, 4, 6, 7, 10. They have been grown at 25° C. and 18° C. on the usual live yeast medium and also two sub-optimal diets: (1) with the protein level reduced from the usual 5% to 2%, and (2) with the concentration of all nutrient halved. In addition, these lines, together with various crosses, have also been grown at 25° C. on media deficient in RNA or lacking fructose. The comparisons between the control population and the five inbreds will be considered first.

Table 1. *Comparisons of body size of Pacific inbred lines and population under different conditions. Deviations from size on live yeast medium*

	25° C.				18° C.	
Genotypes	No Fructose	Low RNA	Diluted	Low protein	Diluted	Low Protein
Line No. 3	−1	0	−17	−29	−25	−33
4	−7	−21	−33	−27	−40	−54
6	−4	−16	−30	−20	−47	−31
7	−7	−32	−27	−49	−47	−75
10	−1	−27	−28	−28	−37	−58
Average of inbreds	−5	−19	−27	−31	−39	−50
Pacific population	−15	−31	−29	−27	−27	−36

Table 1 shows the deviation from the body size on the live yeast medium when flies are grown under alternative conditions. In all sets of comparisons there is significant heterogeneity of differences which may be very great. Thus, on the fructose-free medium, the wild stock suffers the greatest decline—some 15% compared with an average of 5% for the inbreds. Two of the latter, numbers 3 and 10, are quite unaffected by this change in diet. When grown on low-RNA diets, the wild stock is again more affected than the inbred lines, of which No. 3 is unaltered by this treatment which reduces the controls by 30%. On the diluted medium, at 25° C. the inbreds and controls are comparatively alike in their reaction. The inbreds deviate, on the average, by 27—on the $\times 100$ log scale—from their size on the live yeast medium, compared with 29 for the controls.

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For the low-protein medium, there is also fair consistency, except for line 7 which suffers a very drastic decline in size compared with the others. At 18° C. the relative performance of the inbreds is clearly inferior to that of the wild stock.

Table 2. *Deviations in body size of Pacfic inbred lines from wild population at* 25° *and* 18° C.

Line	18° C.	25° C.
3	−7*	−18**
4	−9*	−24**
6	3	−21**
7	3	−23**
10	−7*	−24**
Average	−3	−20

* and ** indicate significance at the 0·05 and 0·01 levels of probability.

Evidently the particular chemical composition of the diet determines the magnitude of the heterogeneity of response. Thus low RNA and the diluted medium lead to approximately equal reduction in the body size of the controls but an entirely different array of responses on the part of the inbred lines. On the diluted medium they behave like the controls while on the low-RNA medium they behave quite differently. The general tendency in these comparisons for culture on both diluted and low-protein media to result in greater proportional decline at 18° C. than at 25° C. shows how the effect of a given change in diet is influenced by the temperature during growth. This is apparent even under favourable conditions of culture at 25° C. and 18° C., although the order of effect is reversed. Table 2 lists the deviations from the size of the controls at the two temperatures on the live yeast medium. At the lower temperature the inbred lines are, on the average, only slightly smaller than the controls, whereas at 25° C. they are some 20% smaller. It may be noted that Parsons (1959) has reported gene-environment interaction with respect to survival at different temperatures.

We can now consider the behaviour of the crosses. Lines 3, 4, 7 and 10 and all possible crosses between them have been grown on the live yeast medium and also the fructose-deficient and low-RNA media. In addition, the F_1 of the cross between lines 6 and 10 was grown on all three media while the F_1's between line 6 and lines 3 and 4 were grown on the live yeast alone. So in the latter medium we have altogether nine crosses and seven on each of the other diets. Reciprocal crosses between lines 3 and 4, 3 and 10, and 4 and 10 on the live yeast medium failed to demonstrate any differences in development time, according to the direction of the cross, while for body size only 3×10 showed a difference, just significant at the 0·05 level; the reciprocal crosses have been averaged.

The data are plotted graphically in Figure 1. The main points to note are as follows:

(i) The crosses closely resemble the wild stock in both body size and development time for the three alternative diets. The average deviation from the con-

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trols for either body size or development time does not exceed 3% for any of the three diets.

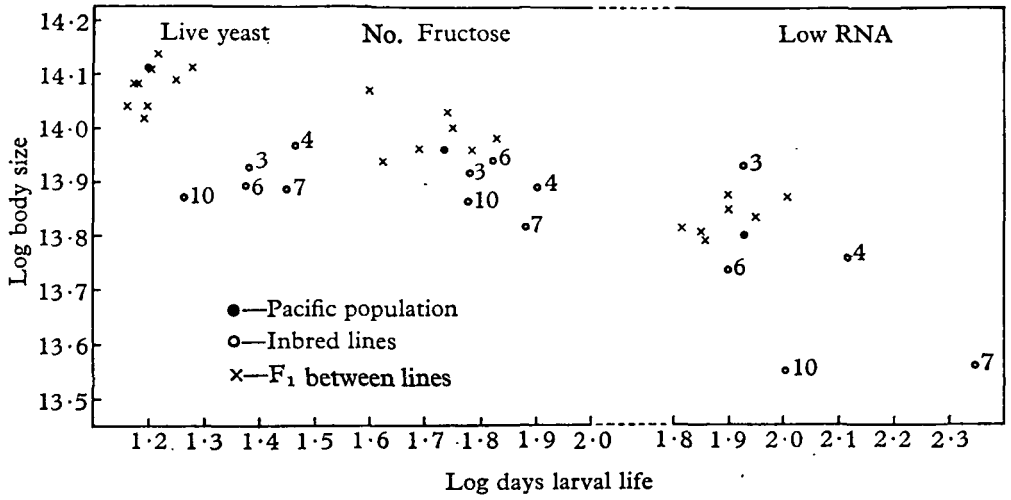

Fig. 1. Mean body size and development time of Pacific inbred lines, crosses and the foundation population, on the favourable live yeast and on two sub-optimal synthetic diets which either lack fructose or are deficient in RNA content.

(ii) It is obvious from Fig. 1 that the crosses, as a class, show much less between-genotype variance than the inbreds. The contrast is particularly striking on the low-RNA diet. A statistical measure of the behaviour of the inbreds and crosses is provided by the estimate of genotype-treatment interaction based on the mean body size of the inbred lines and the six crosses which are represented in all treatments. The results of this analysis are given in Table 3. The gene-environ-

Table 3. *Comparison of gene-environment interaction among inbred lines and crosses*

	Interaction variance		
	d.f.	Mean square	Ratio to error variance
Inbreds	8	51	12·8**
Crosses (*a*)	12	12	3·0*
Crosses (*b*)	10	5	1·2

Crosses (*a*) and (*b*) refer to the analyses for either all crosses or with one excluded.
* and ** indicate significance at the 0·05 and 0·01 levels of probability.

ment interaction is very great among the inbred lines but much lower, although statistically significant, among the crosses. However, inspection of the data shown in Fig. 1 shows that one cross (6 × 10) is unaffected by the change from live yeast to the medium without fructose. This genotype is responsible for a characteristically different response. When the data are recalculated, excluding this cross, the

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gene-environment interaction among the crosses is no longer significant, so, unlike the homozygous parents, the heterozygous F_1's, apart from one exception, respond in the same way to the alternative treatments.

(iii) Such contrasts in response on the part of parents and offspring can be looked at from a different angle by estimating the regression of mean F_1 size on the average value of the parents in the different environments. The regressions for the crosses which are represented on all treatments are summarized in Table 4, which also shows the residual variance between means tested against the error variance. For the live yeast medium there is a highly significant regression of progeny mean on mid-parent size of $1{\cdot}60 \pm 0{\cdot}33$, a value of at least unity. Also the residual variance is not statistically significant. Thus, on live yeast, the inbreeding decline which is dissipated by outcrossing is roughly the same for the five lines, and this apparently acts more or less independently of the genetic differences which are responsible for the correlation between F_1 and mid-parent. On the medium lacking fructose the regression, although it works out at 1·0, is statistically quite insignificant; while the heterogeneity of response among the inbreds is so great on the low-RNA diet that all trace of correlation between the size of parents and the size of F_1 vanishes.

Table 4. *Regression of F_1 body size on average of parents*

		Residual variance	
Treatments	Regression	d.f.	Mean square
Live yeast	$1{\cdot}60 \pm 0{\cdot}33$	5	3·7
No fructose	$1{\cdot}00 \pm 0{\cdot}72$	5	18·4**
Low RNA	$0{\cdot}02 \pm 0{\cdot}17$	5	12·4**

The residual variance is tested against the error variance of a mean.
** indicates significance at the 0·01 level of probability.

(iv) A glance at Fig. 1 shows, for any treatment, a distinct negative correlation between size and development time when inbreds are compared with the control wild stock and the crosses. It has been inferred from earlier work (Robertson, 1960) that a decline in body size accompanied by a more or less proportional increase in development time is the best measure of increasingly unfavourable reaction to the diet. The negative relationship in the present data suggests that the effects of inbreeding in reducing body size and lengthening development time are largely due to less successful utilization of nutrients. It is not surprising that the phenotypic effect of inbreeding should be extremely sensitive to changes in the composition of the diet.

DISCUSSION

Although inbred lines are generally more variable in their response to changes in diet than either the crosses between them or the parent population, it does not follow that conditions which lead to smaller adult size or longer period of larval

development necessarily lead to greater proportional changes on the part of the inbreds. This was clearly shown in the comparisons between the Pacific inbred lines and crosses on the media which were deficient in RNA or which lacked fructose. Both media caused much less reduction below the live yeast level on the part of the inbreds than either crosses or wild population. Contrasts of this nature may be related to the inherently faster growth of the non-inbred individuals. This may create a relatively greater requirement for a high concentration of certain essential nutrients. It is worth noting here that lower protein concentration, which leads to roughly similar proportional reduction in body size on RNA-deficient media, was associated with a good deal less heterogeneity of response among the inbreds and the parent population than on the medium with lower RNA. Hence RNA-deficient media may provide especially favourable conditions for the study of gene-environment interaction in relation to diet.

Estimates of the average effects of inbreeding are highly dependent on the diet and temperature during growth. Thus, on the live yeast medium, the Pacific inbreds averaged some 20 % smaller than the parent population at 25° C., but only about 3 % smaller at 18° C. The parent population and the inbred lines are normally kept at 25° C. and it might be thought that the lines would be better adapted to 25° C. than 18° C. and that the inbreeding decline would be relatively less; clearly this is not so.

There appears to be a high degree of unpredictability in the reaction of inbred lines to changes in diet and temperature. It would be valuable to have comparisons of performance, for a series of different environments, of arrays of inbred lines drawn from a number of different populations. This might afford some evidence of regularity which could never be detected on only a few comparisons.

The crosses show an impressive level of homeostasis in their consistency of response under nutritional conditions which are responsible for wide heterogeneity among the inbred parents. This suggests that the contribution of gene-environment interaction to the phenotypic variance of the foundation population is of a low order. But, on the other hand, Robertson (1960) showed that selection for larger body size on qualitatively different diets quickly leads to striking differences in response to alterations in the composition of the diet. However, the origin of the gene-environment interaction is rather different in the two situations. In the latter case, it originates in selection of genetic differences with greater effect under certain restricted conditions, whereas with the inbred lines we are dealing with numerous unpredictable departures from normal metabolism.

SUMMARY

1. The growth of a number of inbred lines from the Pacific cage population have been compared under different conditions of temperature and nutrition. Body size and duration of the larval period were taken as measures of performance. Sub-optimal diets were provided by growing larvae on chemically defined synthetic media.

2. Gene-environment interaction is widespread and often very great. The phenotypic effects of inbreeding on body size, even on a live yeast medium, may be greatly influenced by temperature. In one set of comparisons, inbred lines averaged 20% smaller at 25° C. but only 3% smaller at 18° C.

3. Sub-optimal diets of different chemical composition, which lead to about the same average decline in body size, may differ greatly in the level of heterogeneity of response among the same set of inbred lines. Thus much greater heterogeneity was found on diets deficient in RNA than on diets with low protein levels. Such information is a useful guide to further study of gene-environment interaction in the outbred population.

4. Diets which lead to a decline in body size of flies of the foundation population do not necessarily cause greater proportional decline on the part of inbred lines. Individual lines have been encountered in which body size is quite unaffected by changes in diet which reduce the size of the outbred flies by 25% or more.

5. A series of crosses between lines from the same foundation population showed a striking level of homeostasis. The average body size and development time of the F_1's was close to that of the population of flies on the favourable and two alternative sub-optimal diets. Also, compared with the parent lines, there was little evidence of gene-environment interaction among the crosses.

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