

The ecological genetics of growth in *Drosophila*

4. THE INFLUENCE OF LARVAL NUTRITION ON THE MANIFESTATION OF DOMINANCE

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1. INTRODUCTION

Earlier work has demonstrated an association between genetic behaviour and development in the variation of the body size of *Drosophila* (Robertson, 1959 *a, b*). Using the cellular make-up of the wing as index, it appears that statistically additive changes are associated with changes in cell number whereas non-additive genetic behaviour, such as that encountered with inbreeding, heterosis and the effects of exchanging chromosomes between lines, is generally accompanied by correlated changes in body and cell size when flies are grown on the live yeast medium. When a small strain, which had been selected for small cell size, was crossed to the foundation unselected population the F_1 was the same size as the latter and the genetic differences accumulated by selection behaved as recessive. Such behaviour presents a sharp contrast with the more or less additive behaviour of comparable differences, due to selection for body size, which, at least in the early stages of selection, leaves average cell size unchanged. But, at the same time, it recalls the comparatively recessive behaviour on the part of small lines which have been long selected for small body size (Robertson, 1954). To clarify the origin of such differences, further experiments have been carried out and they are dealt with in this paper.

Other tests have shown that the composition of the larval diet may greatly affect the expression of genetic differences which influence body size, especially when selection and/or inbreeding has been practised (Robertson, 1960; Prabhu & Robertson, in press) and so comparisons under different nutritional conditions are essential for a fuller understanding.

Two strains have been selected for small cell size in a population which is unrelated to those which were used in the earlier selection for cell size. Repeated crosses between the unselected population and the selected lines have been carried out both on the usual live yeast medium and also on various chemically defined, aseptic media which were made sub-optimal by reducing the level of some essential nutrient. To test for genetic interaction, chromosomes from the unselected population have been substituted in the genetic background of the selected lines and the results of these tests afford useful comparisons with earlier studies of the effects of chromosome exchange.

2. MATERIALS AND METHODS

The flies used in these experiments are all derived from a cage population of *Drosophila melanogaster*, known as Pacific; only females have been scored. Estimates of cell size refer to the average surface area of cells on one side of the wing; details of counting procedure and various tests of reliability have been given in an earlier publication (Robertson, 1959 *a*). Cell area is measured in squared hundredths of a millimetre, transformed to natural logarithms.

Body size is generally recorded as three times log thorax length, in $\frac{1}{100}$ mm.; differences on the log scale can be converted approximately into percentage differences by multiplying by 100. The method of measuring live flies and general cultural procedure have been described before (Robertson & Reeve, 1952). All tests have been conducted at 25° C. All variances have been multiplied by 10⁴ to make comparisons easier.

Aseptic culture on chemically defined media has been used to control the larval diet, as described in an earlier publication (Robertson, 1960). Medium C of Sang (1956) has been taken as reasonably equivalent to a complete medium and sub-optimal diets have been prepared by reducing the level of either casein, which is the protein source, ribonucleic acid or choline. Generally only one kind of nutrient was reduced at a time. For both live and aseptic media, four to five replicated cultures have been set up and eight to ten flies from each culture have been measured, so the mean sizes are each based on data from about forty flies.

Selection for small cell size has been carried out in two lines started at the same time from the Pacific population and treated throughout in the same way; they are referred to as lines A and B. Each generation, fifty pairs of flies, drawn equally from five replicated cultures, were scored for cell size, by removing and mounting one wing from each fly. The two pairs in each culture with the smallest cell size were combined to give ten pairs of parents per generation. Controls from the unselected population were set up at the same time and selection response is expressed as deviations from unselected. Thorax length was also recorded, either on all the females scored or on a representative sample to see how body size changed as selection proceeded.

For the many crosses, numerous progeny have been reared from the selected parents of a given generation to provide the parent flies of the cross. F₁ and parent strains were always set up at the same time under similar conditions. A single generation of relaxed selection apparently makes no difference to mean body size in the tests dealt with here. Various other experiments have been carried out but it is more convenient to deal later with the procedure in their case. We shall deal first with the selection response.

3. EXPERIMENTAL RESULTS

(a) Selection for small cell size

Figure 1 shows the response to selection as deviations from the unselected. Controls were not reared every generation. The upper graphs represent the changes

in cell size while the lower ones show what happened to body size. Not all generations are equally represented by deviations for both characters in the two lines. Thus, at generation 5, there was no record of cell size in Line A because the hatch was poor and selection was relaxed in this generation although the thorax length was measured. Also, at generation 7, although selection was carried out as usual in both lines, the controls were scored for body size only, due to pressure of work.

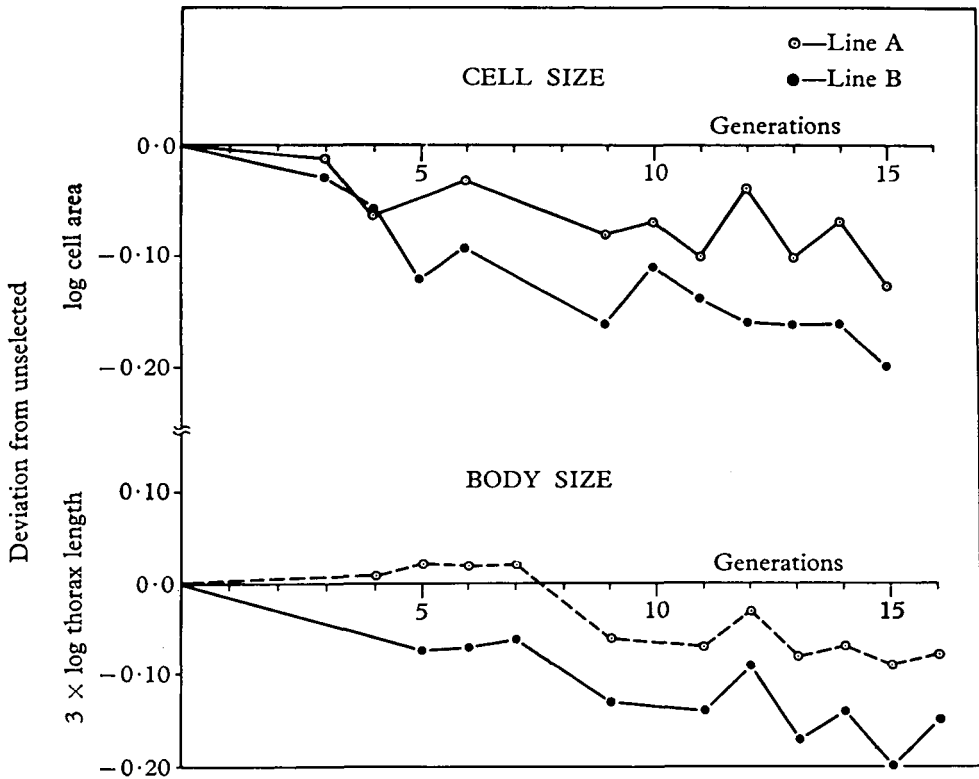


Fig. 1. The response to selection for small cell size. The upper curves show the changes in cell size; the lower ones show the changes in body size.

Selection led to an immediate decline in cell size which continued until about generation 10 and resulted in 10–12% reduction of cell area in Line A and 17–20% reduction in Line B. The decline in cell size was accompanied by a reduction in body size. This is shown in Fig. 2 in which the points for the two lines represent the average for the last few generations of selection. It should be noted in Fig. 1 that the initial response to selection for smaller cell size in Line A was unaccompanied by changes in body size which, however, declined later. In Line B there was an immediate decline in size. Lack of correlation between body and cell size can be attributed to variation in the inverse relations between cell size and number in the wing which can be responsible for considerable differences in

cellular make-up without corresponding changes in wing or thorax size (Robertson, 1959 *a*). Evidently selection for this kind of change has been more effective in Line A than Line B.

In Fig. 2 body size is expressed in square measure— $2 \times \log$ thorax length. If changes in wing cell size and number which are correlated with changes in body size, are accompanied by parallel changes in the rest of the exoskeleton, the graph

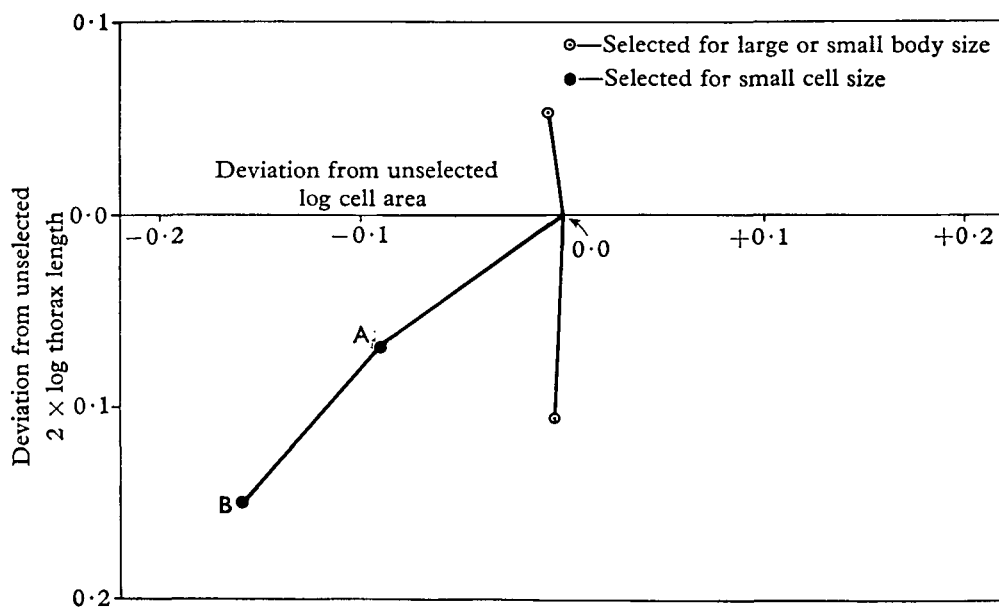


Fig. 2. The changes in body size which accompany selection for smaller wing cells are compared with the average effects on cell size of selecting for either large or small body size in several different populations.

suggests that the final reduction in body size is roughly proportional to the decline in wing cell size, and that changes in cell number are unimportant. Figure 2 also shows the average effects of selecting for large or small body size in several different populations; the data are taken from an earlier publication (Robertson, 1959 *a*). Selection for change in body size does not alter average cell size, at least in the early stages of selection, whereas selection for small cell size certainly reduces body size.

(b) Crosses on the live yeast medium

A number of crosses between the selected lines and the unselected population have been carried out at generation 12 or subsequently, i.e. when there was little evidence of continuing response to selection. There is some additional evidence of the stability of average body size during this period since a subline was taken off both A and B lines at generation 15 and allowed to mate at random in bottles while selection was continued as usual. Controls were not scored for these additional generations of selection so they are not shown in Fig. 1. After four generations

of such relaxed selection there was no evidence of any change in the direction of more normal size (Table 1). The relaxed line from A was actually slightly smaller than the selected line. The controls were always drawn from the large cage population and it is assumed that their performance remained effectively constant over the period of these comparisons.

Table 1. *The effects of relaxing selection for four generations*

	Deviation from unselected— body size	
	Line A	Line B
Continued selection	-10.4	-18.7
Relaxed selection	-14.2	-18.2
Difference	-3.8*	0.5

* Indicates significance at the 0.05 level of probability.

Table 2 presents the results of the five crosses between Line A and the unselected population and the six crosses in which Line B was used. They are tabulated in order of increasing body size of the selected line, of which the smallest average size is reckoned as zero and the others are expressed as deviations from this figure. The reason for doing this will be considered later. In some of the Line B crosses the same parent flies provided eggs for separate lots of cultures which were set up several days apart. Hence the double entries for generations 13 and 17. Variation between the means for each parent type or cross is heterogeneous ($p < 0.01$), so the nutritional conditions on the live yeast medium varied in successive tests.

Table 2. *The dominance deviation in successive crosses on the live yeast medium*

Body size of selected line—coded from smallest mean	Generation number	Differences		
		Unselected— selected	F ₁ —Mid- parent	Unselected—F ₁
Line A				
0.0	19	13.8	-1.8	8.7**
2.7	17	12.9	-0.3	6.1*
4.6	15	15.9	1.1	6.8**
9.7	18	12.8	2.1	4.3*
9.9	14	9.3	4.5*	0.1
Line B				
0.0	15	19.2	5.6*	4.0*
3.1	13	16.8	3.4*	5.0*
3.4	17	15.4	7.2*	1.4
3.5	19	13.5	-0.1	6.6**
4.4	17	21.3	7.7*	3.0*
8.8	13	10.6	5.3*	0.0

* and ** indicate significance at the 0.05 and 0.01 levels of probability.

The third and fourth columns in Table 2 show respectively the deviations of the F_1 from the mid-parent value and the unselected parent. There is evidently great heterogeneity in the size of the F_1 relative to that of the parents. In the A crosses the average size of the F_1 coincides with that of the unselected parent in one test, while in the others it fluctuates about an intermediate level. In the B series, the F_1 is intermediate only in one cross. In all the others it either does not differ appreciably from the size of the unselected parent or significantly exceeds the mid-parent value.

Thus the F_1 is either the same size as or nearly as big as the unselected parent often enough to justify the earlier emphasis on the apparently recessive behaviour of the differences created by selection for small cell size. The new feature is the variable manifestation of the genetic behaviour. There is no evidence of correlation between the relative size of the F_1 and the chronological order of the crosses, and since temperature was kept constant there is little doubt that such differences are due to variation in the composition of the larval diet.

As noted above, there is statistically significant heterogeneity between mean size in successive tests for both lines and also the unselected population. How far the selected lines and the unselected population respond in the same way to this environmental variation can be inferred from the regression of the former on the latter. For Line A and the F_1 of the cross to unselected, the regressions work out at 0.98 ± 0.40 and 1.34 ± 0.50 respectively, while the corresponding regressions for Line B and the F_1 are 0.06 ± 0.47 and 1.19 ± 0.37 . Thus only Line B shows possible departure from parallel fluctuation, although the difference in slope between it and the others does not exceed the 0.05 level of significance. Table 2 suggests that the positive departure from the mid-parent value in the crosses between Line A and unselected tends to increase under more favourable conditions which increase body size generally, so that the F_1 is intermediate, at one end, and the same size as the unselected, at the other end, of the 10% range in size. In the B series, the F_1 coincides with the unselected parent in the test in which the selected line attains largest size, but otherwise there is no evidence of a trend.

(c) Crosses on sub-optimal diets

To provide a further check on the influence of larval diet on the manifestation of apparent dominance, crosses have been carried out on aseptic synthetic media which comprise the so-called complete medium and several media in which the levels of either casein, RNA or choline have been reduced. The effects of these treatments are shown in Table 3. These tests were not all carried out at the same time. The relative order of the various treatments is indicated by how far they reduced the body size of the selected lines below the level attained on the complete medium.

It is quite clear that Line B suffers a relatively greater decline on all treatments than Line A. In the A crosses the F_1 does not differ significantly from the unselected population, either on the complete medium or on the medium with 3%

casein. But when the casein content is reduced to 2% or the RNA level is lowered to half (0.18%) or a quarter (0.09%) of the concentration in the complete medium, the F_1 does not differ significantly from the mid-parent value. In the B series, the F_1 is about the same size as the unselected on the complete medium and the media with 2% casein and 0.18% RNA, deviates significantly from the mid-parent value on the lowest concentration of RNA and actually exceeds the size of the unselected parents on the 3% casein diet. Finally on the low levels of choline the F_1 exceeds the size of the unselected parents in all four crosses, significantly so in three of them.

Table 3. *The dominance deviation in crosses on sub-optimal diets*

Medium	A Crosses			B Crosses		
	Deviation from complete medium	F_1 -mid-parent	Unselected- F_1	Deviation from complete medium	F_1 -mid-parent	Unselected- F_1
Complete	0.0	6.6**	-1.6	0.0	5.5**	-0.9
3% Casein	-13.1	4.3*	1.9	-10.0	-0.5	-4.1*
2% Casein	-22.8	0.7	7.3**	-28.5	10.3*	1.2
0.18% RNA	-5.0	-1.4	9.4**	-11.6	4.6*	1.6
0.09% RNA	-13.6	1.3	4.4*	-22.1	3.6*	5.1*
150 μ g. Choline	-18.6	11.2**	-6.1*	-28.2	15.0	-6.9**
100 μ g. Choline	-26.4	4.5	-2.0	-46.0	13.5	-7.5**

* and ** indicate significance at the 0.05 and 0.01 levels of probability.

These tests on different diets confirm that the chemical composition of the larval diet largely determines the relative size of the F_1 and hence the apparent genetic behaviour. But the distribution of differences is erratic and there is little evidence that conditions which lead to general decline in size, irrespective of the composition of the diet, are associated with a regular trend in apparent genetic behaviour. Thus in the A crosses, both the diets with 3% casein and 0.09% RNA lead to about 13% reduction in body size of line A but dominance was present in one case and absent in the other. Also, for both series, the effects of low levels of either casein or choline led to similar reduction in size but the relative position of the F_1 was quite different. Hence it is the specific, qualitative composition of the diet which matters rather than its quantitative effect on body size. The parallel behaviour, for both sets of crosses, on the low choline diet suggests that parallel selection for small cell size has led to similar alterations in metabolism in the two lines.

(d) Crosses to the Caribbean population

As a further test of genetic behaviour, both lines have been crossed to an unrelated cage population known as Caribbean. The crosses were made after fourteen generations of selection and the flies were grown on the live yeast medium and on two synthetic diets which either lacked fructose or had a low (0.09%) RNA content, but otherwise had the same composition as the complete medium.

Table 4. Comparisons of the body size of the Caribbean with the Pacific population and the selected lines

Genotype	Deviation from Caribbean		
	Live yeast	No Fructose	Low RNA
A	-6.4**	-7.7**	3.7*
B	-11.4**	-8.1**	-3.0*
Pacific population	5.7**	4.5**	13.1**

* and ** indicate significance at the 0.05 and 0.01 levels of probability.

On the more favourable diets provided by the live yeast medium and the fructose-deficient diet, which leads only to minor reduction of body size, the Caribbean is some 5% smaller than the Pacific population. But with the lower RNA concentration the difference is increased to 13%, indicating a relatively greater requirement for RNA on the part of the Caribbean population. These differences are shown in Table 4 while the results of the crosses are given in Table 5.

Table 5. Crosses of the selected lines to the unselected Caribbean population

Treatment	A × C		B × C	
	F ₁ -Mid-parent	C-F ₁	F ₁ -Mid-parent	C-F ₁
Live yeast	6.1**	-2.9*	5.7*	0.0
No fructose	0.1	3.1*	5.5*	-1.4
Low RNA	10.1**	-11.9**	6.1*	-4.4**

A, B and C refer to the selected lines and the Caribbean population.

* and ** indicate significance at the 0.05 and 0.01 levels of probability.

When Line A was crossed to the Caribbean population, the F₁ was significantly larger than the unselected parents on the live yeast medium but intermediate when fructose was omitted from the diet. With low RNA concentration, the cross significantly exceeded the larger parent. In the B crosses, on both live yeast and fructose-deficient media, the F₁ was the same average size as the Caribbean population but, on the low RNA diet, exceeded it. The parallel behaviour on the low-RNA diet in which the F₁ of both crosses exceeded the larger parent may be attributed to the genetic background common to both the lines derived from the same foundation population. These comparisons provide further evidence that the relative size of the cross is influenced by the nature of the larval diet.

(e) The chromosome substitutions

If we bear in mind the complex interrelations of metabolic pathways in growth, the observed differences in genetic behaviour suggest that selection has led to changes at a number of loci and that interaction between non-allelic genes may be important. These inferences have been tested directly by substituting chromosomes from the Pacific population in the genetic background of each of the selected lines, and the effects of their single and joint effects have been compared. This procedure tests only for interaction between genes on different chromosomes but it has been found useful in earlier analyses of heterosis in crosses between inbred lines (Robertson & Reeve, 1954). Details of procedure and analysis have been given in the earlier paper and only the minimum needed for interpreting the present experiments are given here.

With the aid of marked, autosomal inversions which suppress recombination and a system of back-crossing, we can create genotypes in which single doses of chromosomes I, II or III from the unselected population are substituted in the background of the selected line. The small fourth chromosome is ignored. If the

Table 6. *The effects of substituting chromosomes from the unselected population in the background of the selected lines*

Genetic background	Least-squares estimates of chromosome effect			Interaction variance
	I	II	III	
A	0.3	4.1	1.9	3.8*
B	2.1	0.4	4.6	7.3**

* and ** indicate significance at the 0.05 and 0.01 levels of probability.

genetic constitution of the selected line is represented as AAA, the three alternative single substitutions may be represented as XAA, AXA and AAX, to show that one pair of chromosomes consists of homologues derived from different sources. Double substitutions are represented as XXA, XAX and AXX, while the triple substitution—the F_1 of the cross between selected line and unselected population—is XXX. Including the selected line there are altogether eight genotypes for comparison. The stages which preceded the final test were carried out on a sufficiently large scale to ensure the substitution of a representative sample of chromosomes from the unselected population.

The estimates of the average effects of replacing particular chromosomes and the test for non-additive interaction is based on a least-squares analysis. If the size of the selected line is represented as A, then the effects of replacing one member of each chromosome pair in turn by chromosomes from the unselected population may be expressed as $A + a$, $A + b$ and $A + c$, while joint substitutions can be expressed as $A + a + b$, $A + a + c$, $A + b + c$ and $A + a + b + c$. The resulting equations can be solved to give the least-squares estimates of the average effect and a test

for the presence of interaction, which will take the form of discrepancy between the effects of joint substitutions and the sum of their estimated individual effects when substituted one at a time. The error variance is the error variance of a mean, derived from the within- and between-culture components of variance.

The results are shown in Table 6. The distribution of genetic differences, due to selection, differ in the two lines. In A, selection has especially altered II, while in Line B it is chromosome III which differs most. Also in Line B there is an appreciable sex-linked difference which has been confirmed in the males by reciprocal crosses between Line B and the unselected population. Both sets of comparisons show evidence of significant interaction between chromosomes, and the level of interaction is greater for the substitutions in the B than in the A background. No doubt this is related to the more consistent tendency to recessive behaviour in crosses between Line B and the unselected population.

Table 7. *Comparison of the effects on body size of single and joint substitutions of chromosomes from the unselected population in the genetic background on lines A and B*

Chromosomes substituted	Deviation from selected line, body size	
	A	B
I	3.6	7.4
II	3.7	6.5
III	2.8	6.6
Sum of single effects	10.1	20.5
I + II + III	6.8	14.0
Difference	3.3	6.5

The general implication of these results can be more easily appreciated by a simpler approach which is summarized in Table 7. This shows the effects of making single substitutions in the background of either line and also the comparisons between the sum of these individual effects with their joint effect in the F_1 of the cross of each line to the unselected population. Any substitution from the unselected population tends to restore normal body size, but there is a kind of law of diminishing returns so that the sum of the single effects exceeds the joint effect by some 50% in both series. Further implications of these experiments will be considered later.

(f) *Selection from the F_2 of the cross between the lines*

To throw further light on the genetic behaviour of the differences due to selection, the two selected lines were crossed reciprocally at generation 15, and taken to a large F_2 from which two lines were started. Selection for large body size was carried out by selecting ten out of fifty pairs.

When the selected lines were crossed, the F_1 was significantly larger than the larger A parent—by about 5%—but was still some 10% below the level of the unselected population. In the F_2 there was a sharp decline to a level about half-way between the selected lines. Selection resulted in an immediate increase for three to four generations but no further progress thereafter and both lines fluctuated at a level which, on average, was a little below that of the unselected population.

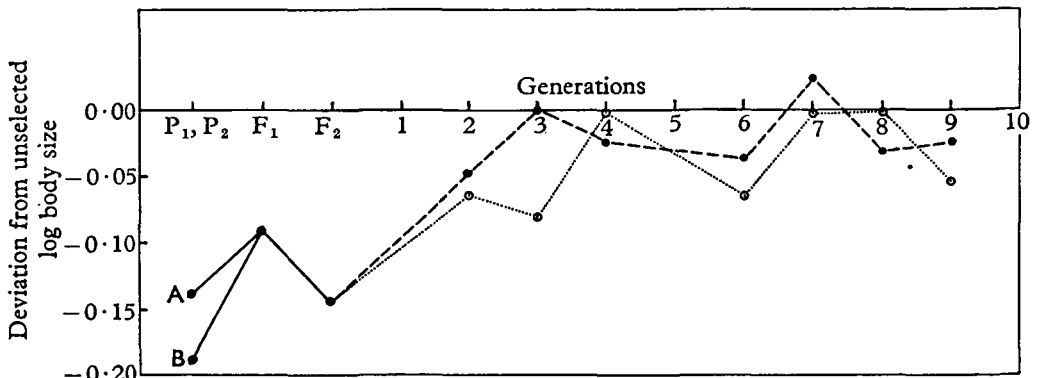


Fig. 3. The response to selection for large body size from the F_2 of a cross between the lines which had been selected for small cell size. Two selection lines were established from the F_2 .

DISCUSSION

The most significant feature of these experiments is the tendency for differences in body size, which have been produced by selection for small cell size, to behave as recessive in crosses to the unselected population. Such behaviour appears to be quite general. It was reported in a similar selection experiment with an unrelated population (Robertson, 1959 *a*). It also holds for crosses between the selected lines and the unrelated Caribbean population. The genetic behaviour of differences due to selection for small cell size differs from that shown by equally small or smaller lines produced by selection for small body size. When the latter are crossed to the foundation population the F_1 is intermediate or, where a positive deviation exists, it is relatively less than is generally true for lines selected for small cell size.

Selection for small cell size is not a particularly effective way to reduce body size. Thus, after some fifteen generations of selection, the two lines A and B were only about 10% and 15% smaller than the unselected population, whereas other experiments with the Pacific population, in which small thorax length has been selected for, have produced as great or greater deviations in five generations. Also, in other tests, in which small body size was selected for in several different populations, body size was reduced about 50% before the response ceased (Robertson, 1955). Part of such differences in response may be due to selection for change in the cell size and number relations in the wing, which can vary inversely within fairly wide limits without change of wing or body size (Robertson, 1959 *a*). This was also illustrated in the early stages of selection in Line A.

These facts, together with the tendency for wing cell size to remain unchanged when selection for either larger or smaller body size is carried out, suggest that the physiological changes which reduce body and cell size together differ qualitatively from the changes produced by selection for smaller body size, at least in the early stages of selection, and that such differences are closely related to the differences in genetic behaviour.

But prolonged selection for smaller body size has produced evidence of genetic behaviour which approximates more closely to that which distinguishes lines selected for small cell size, namely comparatively recessive behaviour (Robertson & Reeve, 1954). Selection will, of course, involve a general loss of heterozygosity (Lerner, 1954), while evidence derived from the exchange of chromosomes between lines suggests that selection for smaller body size is especially likely to accelerate the trend to homozygosity (Robertson, 1954). It appears that selection for smaller cell size enables us to make the kind of changes in development which contribute especially to the later stages of selection for smaller body size. Equivalent reduction in size, due either to selection in a highly heterozygous background or to inbreeding decline, will be generally distinguished by absence or presence of correlation between the size of the body and that of the wing cells (Robertson, 1959 *b*). So, for a given reduction in size, selection for smaller cells is likely to involve a greater loss of heterozygosity than selection for smaller body size. This could help to account for the comparative ineffectiveness of selection for smaller cell size in reducing body size.

This view is consistent with the results of substituting chromosomes from the unselected population in the background of the selected lines. The type of interaction revealed by this analysis recalls the effects of exchanging chromosomes between inbred lines (Robertson & Reeve, 1954). These earlier experiments suggested that the stability of body size rests on dominance and complementary interaction between genes at different loci. It is interesting that Breese & Mather (1960) have recently reported a similar genetic situation with respect to viability and so such behaviour is likely to be general for any character which is subject to inbreeding decline. In the present tests, as in the earlier ones, the sum of the effects of the single substitutions considerably exceeded their joint effect. This pattern of diminishing returns is a practical demonstration that genes at different loci overlap in function to ensure normal growth, although it does not follow that their primary effects are the same.

The genetic behaviour of the differences produced by selection is greatly influenced by the composition of the larval diet. When the same parent strains are crossed the F_1 may be either midway between them or the same average size as the unselected parent in successive tests on the live yeast medium or on slightly different sub-optimal synthetic media. The comparisons on the various diets did not show any correlation between genetic behaviour and general quantitative effect on body size, except, possibly, for the tendency for the deviation from intermediacy to be greater in these tests on the live medium which lead to all round increase in size, in the crosses between Line A and the unselected population. The

specific composition of the diet determines whether the F_1 is to follow the metabolic pathways characteristic of the unselected parent or whether the genetic differences produced by selection are to influence development as well.

If we consider the interrelations of metabolic processes, the polygenic nature of the differences due to selection and also the influence of nutrition on genetic behaviour, it may be inferred that the apparently recessive behaviour is progressively established during the course of selection and that it depends, to no small degree, on the replacement of heterozygous by homozygous combinations. The population may carry individual recessive genes whose effects are so insensitive to variation either of genetic background or environment that they behave as recessive in virtually any situation. But there is no evidence that such genes were important in the experiments described here. If the reduction of body size by selecting for smaller cells is a rather effective way of disturbing the normal homeostasis of growth, genes or chromosomes segments which behave as recessive in the altered gene complex are likely to be fixed and such fixation will provide a more stable genetic background for further changes of this nature.

It is uncertain how far parallel selection in the two lines led to similar changes. On the one hand, the early differences in selection response between the lines, the different distribution of effects on the major chromosomes, the heterosis when the selected lines were crossed and the greater tendency for crosses between Line B and the unselected population to resemble the latter than was the case in the crosses between Line A and unselected together imply that numerous genetic differences exist.

On the other hand, on the choline-deficient media, when either Line A or B was crossed to the unselected population, the F_1 exceeded the latter. This could mean that selection led to similar changes in metabolism, although the genes responsible for such changes may occur at different loci. Such considerations are relevant to the response to selection from the F_2 of the cross between the lines. This was not very great and ceased rather abruptly after three to four generations. Parallel selection in the two parent lines could have fixed the same genes at a number of loci so that the genetic variance is low, although, as noted above, there is evidence to the contrary. It might also mean that physiological changes common to both parent lines and the lines derived from the F_2 set a limit to the increase in body size, even though genetic variation remains and could be utilized in the absence of this limitation.

SUMMARY

(1) Two lines have been selected for small wing cell size from the cage Pacific population. Body size was reduced by about 10% and 15% in the two lines which did not regress when selection was relaxed.

(2) The effects of crossing each line to the unselected population has been determined in a number of repeated tests on the live yeast medium and also on various sub-optimal synthetic media.

(3) The size of the F_1 , relative to the size of the parents, is greatly influenced by the composition of the larval diet. The F_1 may coincide with the mid-parent value but generally significantly exceeds it and is often the same size as the unselected parent population.

(4) In crosses to an unselected population on alternative media the F_1 was either the same size as the unselected population or exceeded it.

(5) Crosses between the selected lines produced an F_1 which exceeded the larger parent but remained well below the level of the unselected population.

(6) To test for interaction between genes at different loci, chromosomes from the unselected population were substituted in the genetic background of each of the selected lines to provide an array of genotypes in which one, two or three pairs of major chromosomes had homologues derived from different strains. Least-squares analysis indicated differences between the lines in the distribution of effects among the chromosomes together with the presence of interaction between chromosomes and this was greater for the substitutions in the line which showed the greater consistency of recessive behaviour in crosses to the unselected population.

(7) At the end of the selection experiment two lines were selected for large body size from the F_2 of the cross between the two selected lines. Both responded to selection for three to four generations and then fluctuated at a level slightly below that of the unselected population.

(8) The physiological changes which involve correlated changes in body and cell size differ from those which result from selection for smaller body size, at least in the early stages of such selection, and are associated with differences in genetic behaviour. The apparently recessive property, which involves extensive non-allelic interaction, is progressively established during the course of selection. Apparently selection for smaller cell size is particularly effective in disturbing the normal homeostasis of growth and is accompanied by relatively greater loss of heterozygosity than is likely with equivalent reduction in size due to selection for smaller body as opposed to cell size.

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