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The ecological genetics of growth in *Drosophila*

3. GROWTH AND COMPETITIVE ABILITY OF STRAINS SELECTED ON DIFFERENT DIETS

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1. INTRODUCTION

The preceding paper in this series (Robertson, 1960*b*) dealt with characteristic differences in selection response for large body size when the larvae were supplied with different diets. Individual variation in adult body size represents—to a varying degree—the effects of genetic segregation and recombination on different processes of growth and metabolism when the conditions during larval growth differ. Hence selection for the same 'character' in different conditions leads to qualitatively different changes in the physiology of growth and this was advanced as the essential reason for the differing responses to selection on different diets. If this interpretation is valid it should be possible to provide further direct evidence by comparing the growth of such contrasted strains under different controlled conditions. Such comparisons should also throw light on genetic differences in the ability to maintain a characteristic body size as well as inter-relations between development time, body size and diet.

Accordingly the growth response has been determined for unselected flies and for three large strains created by selection either on the live yeast medium or on aseptic, synthetic diets, which were either deficient in protein or had the concentration of all nutrients reduced. These comparisons have been carried out after six to eight generations of mass selection on the live yeast medium and after six to twelve generations on the two deficient diets. Since the response to selection in the latter case ceased after seven or eight generations, and there is no reason to suppose that the performance of the lines on alternative diets changed thereafter, the comparisons can be taken to represent what happens after about six to seven generations of mass selection under such different conditions.

It has been noted, in the preceding paper, that the response to selection on the deficient diets involved better adaptation to these conditions. This was inferred from the decline in variance of adult size, the shortening of development time and also the increase in egg production—compared with unselected flies—which accompanied effective selection. Hence, these large strains can be regarded, to some extent, as adapted to different diets.

2. MATERIALS AND METHODS

The media upon which larvae have been grown aseptically comprise either synthetic Medium C of Sang (1956) or various modifications of it which include: (*a*) omission of the fructose, (*b*) reduction of the RNA level from 0·36 to 0·09%, (*c*) reduction of the casein concentration from 5% to either 4, 3, or 2%, (*d*) various combinations of these specific deficiencies and also (*e*) general dilution of Medium C to one-third normal concentration, except for the agar gel. Circumstantial evidence suggested that deficiencies of this kind might not be too far removed from the kind of adverse conditions commonly encountered by the population. In addition, the strains have been compared under competitive conditions on the live yeast medium, but the procedure in this case will be described later.

Body size is generally based on the average of eight females drawn from four or five replicated cultures. Since development time is scored on all females hatching from the cultures, there are more degrees of freedom for this measure of growth than for body size. The latter is based on the length of thorax, while development time is calculated from records of the morning and evening hatch of adults over successive days. The average duration of the pupal period is subtracted from the total duration of development to estimate the larval period. All data have been transformed to a log scale. Body size is expressed as three times the natural log of thorax length in 1/100 mm. while the larval period is reckoned in log days. Further details of procedure and general orientation are given in the introductory paper of this series (Robertson, 1960*a*). The population of *Drosophila melanogaster* used in these experiments is known as *Pacific* and had been run for about a year in a population cage before the start of the selection experiments.

3. RESULTS

The data are dealt with in three sections. Firstly, we have comparisons between the unselected population and the strain selected for large size in the low-protein medium; this includes the effect of backcrossing this strain to the unselected population. Secondly, we have comparisons of growth on synthetic diets, which differ in protein concentration, between the unselected population and the three large strains selected on different media. Finally, all the strains are compared under competitive conditions on the live yeast medium.

(i) *Tests on the low-protein strain*

(*a*) *Comparison with the unselected population*

After six generations of selection for large body size on the low (2%) protein diet, i.e. a generation before the response to selection ceased, eggs were collected from the selected strain and from the unselected population, and were set up on the live yeast medium and also on six different synthetic media. These were as follows: Medium C; (*a*) 'complete', (*b*) without fructose, (*c*) with low RNA, (*d*) with 2% casein, (*e*) with 2% casein and no fructose and (*f*) with 2% casein and low

RNA. The deviations from the performance of the unselected flies for the seven treatments are set out in Table 1.

In this and later Tables, which refer to the deviations of various strains from

Table 1. *Performance of the large strain selected on a low-protein diet, compared with unselected flies*

Medium	Deviations from unselected—logarithms	
	Body size	Larval period
Live yeast	0·12	0·01
5% casein	0·16	−0·01
5% casein, no fructose	0·21	0·03
5% casein, 0·09% RNA	0·15	−0·01
2% casein	0·17	−0·05**
2% casein, no fructuse	0·19	−0·01
2% casein, 0·09% RNA	0·21	−0·02

** indicates significance at the 0·01 level of probability.

their performance on the live yeast medium or from the performance of unselected flies on different diets, statistical significance of the differences are noted only for the larval period, since the deviations for body size are almost invariably highly significant; the few exceptions are noted in the text. In addition, the deviations from unselected flies on different diets, listed in columns in the Tables, have been shown to be highly heterogeneous for each set of comparisons. Hence gene-environment interaction is widespread and dramatic in magnitude. In dealing with such effects a statistical index of interaction—although useful as a first indication of a problem to be solved—is biologically meaningless and the origin of such differences has to be sought in terms of characteristic changes in growth.

It is obvious that the selected and unselected strains differ in how much body size is reduced on different diets; the striking heterogeneity of the differences has been confirmed by the usual statistical tests. Difference in body size between selected and unselected is least on the live yeast medium (12%) and greater on all the deficient diets, especially those deficient in fructose or RNA, in which the difference amounts to some 20%. It may be noted that the reduction of RNA from 0·36 to 0·09% in the presence of 5% casein leaves the difference between strains unchanged, while the same reduction of RNA, in the presence of a lower protein level, increases it—a good example of how the limiting effect of a given nutrient in short supply is influenced by the concentration of other constituents of the diet.

These differences in body size arise from differences in growth rate rather than alteration in the duration of the growing period. Thus, the selected strain, which may be some 20% greater than the unselected, either does not differ in average development time or tends to develop a little faster, especially on the 2% protein medium used for selection.

The interpretation of these results is clarified by reference to Fig. 1, in which

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body size is plotted against larval period on the log scale. Taking the performance on the live yeast medium as the reference point, Medium C, both with fructose (series 2) and without fructose (series 3) leads to a considerable reduction of body in size the unselected flies, whereas in the large strain there is no reduction below the maximum size. This capacity for maintaining body size constant is encountered when the diet is not too adverse. Development time may be prolonged to a variable degree so that, under such conditions, there is no environmental correlation between these two measures of growth. But when the diet becomes too inadequate body size declines and there is established an inverse relation between size and

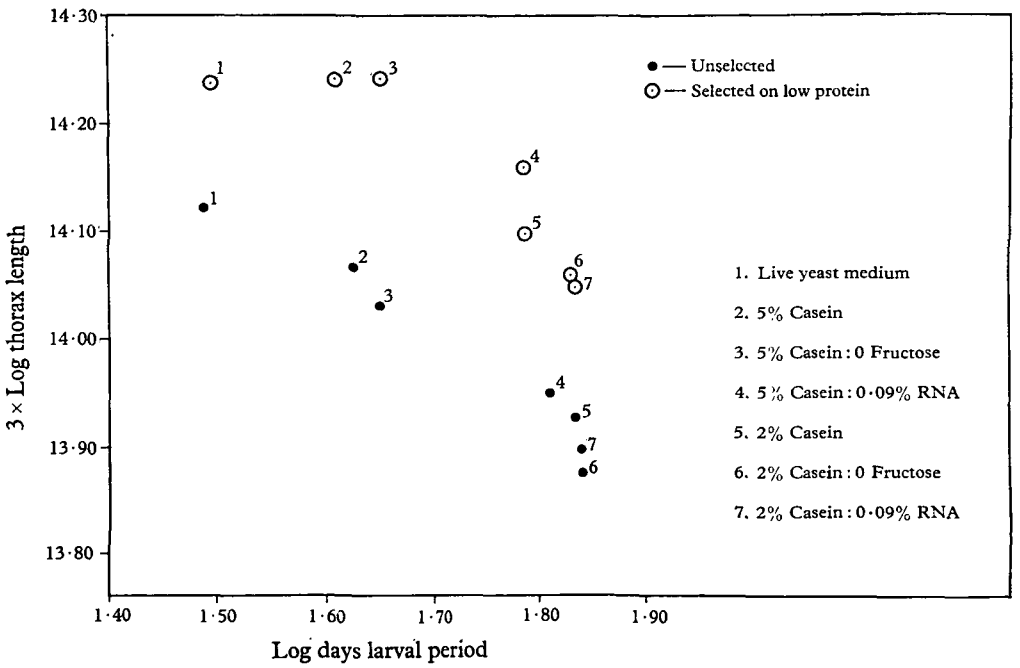

Fig. 1. Body size and development time of unselected population and the strain selected for large size on low protein, when grown on various media.

development time such that a given decline in size is accompanied by a more or less proportional increase in developmental time, as noted earlier (Robertson, 1959 and 1960*a*). In such situations the environmental correlation is very high. It is quite clear that the selected strain has much greater capacity to maintain a constant body size on diets which lead to about 10% reduction in the body size of the unselected flies. But when the diet is sufficiently sub-optimal to reduce body size of the selected individuals there is no evidence that they are better able to resist these more adverse conditions, than the unselected individuals, by relatively greater lengthening of the larval period.

Although sub-optimal diets reveal the inverse relation between decline in size and increase in development time, there is variation about the regression line due apparently to the specific composition of the diet. Thus, for the unselected flies,

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treatments numbered 5, 6 and 7 in Fig. 1 lead to appreciable differences in body size but to about the same average development time. It appears that under certain conditions, body size may be reduced without further delay in development. It is likely that such contrasts originate in characteristically different effects of such diets on particular stages of larval growth. This general problem is being studied in further experiments.

(*b*) *Performance of the backcross*

After seven generations of selection on the low-protein medium, selected flies were crossed to the unselected stock and the F_1 tested at the same time as the two parental strains on the live yeast medium and on Medium C: (i) without fructose, (ii) with 2% casein, (iii) with 2% casein and 0·09% RNA and (iv) Medium C diluted to one-third strength.

Table 2. *Selected, unselected and cross on different diets; deviations from body size on live yeast medium*

	Genotype			Difference in response	
Medium	Unselected (U)	Selected (S)	Cross (X)	U − S	U − X
No fructose	−0·04	0·03	0·00	−0·07**	−0·04*
2% casein	−0·24	−0·13	−0·15	−0·12**	−0·09**
2% casein, 0·09% RNA	−0·25	−0·11	−0·22	−0·14**	−0·03
Diluted	−0·23	−0·17	−0·21	−0·06**	−0·02

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

The results are shown in Table 2 and Fig. 2. Comparison of Fig. 2 with Fig. 1 shows that development time was considerably longer in the later test. This is almost certainly due to the greater exposure to autoclaving in this experiment. As noted in the introductory paper, differences in performance of the same strain set up on the medium made to the same formula are almost certainly due to variation in degree of heating, which cannot be controlled as precisely as would be desirable, with the equipment available.

This test shows that the body size of the selected strain is actually greater on the fructose-deficient medium than on live yeast (the difference is significant at the 0·05 level), compared with the unselected flies which are reduced on this medium. The cross is apparently intermediate and its body size does not differ on the two diets. Differences between strains in reaction to the deficient media are listed on the right of Table 2. The individual differences between body size on live yeast and other diets are tested against the sum of the four appropriate variances of a mean.

Figure 2 and Table 3, which summarizes the deviations from mid-parent value for both body size and larval period, show that the F_1 is generally intermediate. However, on the dilute medium, which causes about the same reduction of body size as 2% casein, but on which the larval period is considerably shorter, the F_1 is 3% smaller than the mid-parent value—a statistically significant difference.

With respect to larval period, none of the deviations from intermediacy are significant, but all are negative on the aseptic media, suggesting that the cross develops at a slightly faster rate than the parents.

The graph relating body size and development time shows the familiar features

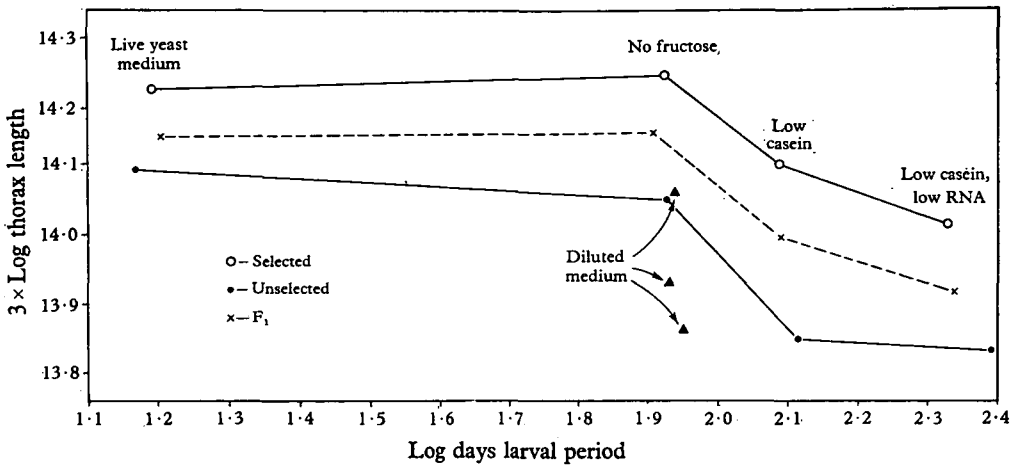

Fig. 2. Body size and development time of the unselected population, the large low-protein strain and the F_1 between them when grown on alternative media.

of lengthened development time and comparatively constant size on the least sub-optimal diet and the inverse relation with more extreme conditions. However, in this test, when the protein level is low, reduction of the RNA level does not further reduce the body size of unselected flies although it lengthens the larval period. In the selected strain, body size is reduced as well while the cross is more or less intermediate in this respect. The difference between the reaction of unselected

Table 3. *Deviation from mid-parent value in backcross*

Medium	Body size	Larval period
Live yeast	−0·02	0·01
No fructose	0·01	−0·01
2% casein	0·02	−0·02
2% casein, 0·09% RNA	−0·01	−0·02
Diluted	−0·03*	−0·01

* indicates significance at the 0·05 level of probability.

individuals to RNA reduction, in the presence of low protein, in this experiment and the one summarized in Fig. 1 is probably due to differences in exposure to autoclaving.

(ii) *Comparisons of large strains on protein-deficient media*

At the end of the various selection experiments, i.e. after 7, 12 and 9 rounds of selection on respectively live yeast, 2% casein and the diluted medium, eggs from

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Table 4. *Deviation from unselected of large strains on different protein-deficient diets—body size* (S) *and larval period* (L) *on log scale*

Medium of test	Medium used during selection of large strains: Low protein S	Low protein L	Diluted S	Diluted L	Live yeast S	Live yeast L
Live yeast	0·14	0·02	0·17	0·01	0·14	0·07**
Aseptic						
6% casein	0·23	0·04	0·25	0·06*	0·11	0·13*
5% casein	0·19	0·04*	0·24	0·01	0·12	0·14*
4% casein	0·25	−0·07	0·27	−0·01	0·06	0·14*
3% casein	0·27	−0·09	0·30	−0·10	0·13	0·09*
2% casein	0·29	−0·02	0·30	0·09	0·06	−0·01
Diluted	0·29	0·03	0·32	0·08*	0·02	0·23**

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

the unselected stock and from the large strains were set up on the live yeast medium, on the aseptic medium with either 6, 5, 4, 3 or 2% casein, and also on the diluted medium. At the same time eggs from these flies were allowed to hatch into larvae which were set up under competitive conditions on a modified live yeast medium. The response to varying protein concentration will be considered first.

The results are shown in Table 4 and Fig. 3.

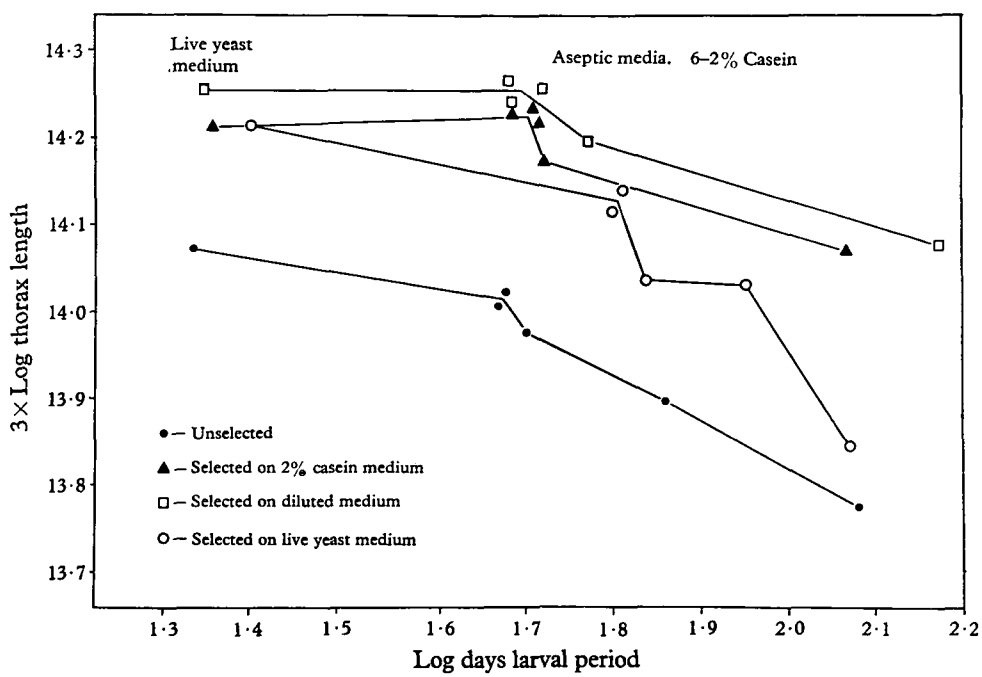

Fig. 3. Body size and development time of the unselected population and three large strains selected on different media, when grown on the live yeast medium and also on synthetic diets with different concentrations of protein.

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The main features are as follows:

(i) On the live yeast medium all three strains are about the same size—some 15% greater than unselected flies. This is a highly significant difference equivalent to some twelve to fifteen times the standard error of a mean. This equal deviation of body size is accompanied, however, by a difference in larval period, since the large strains selected on the sub-optimal diets do not differ from unselected flies, while the strain selected on live yeast takes appreciably longer to develop.

(ii) Both strains selected on the sub-optimal diets respond in much the same way to progressive protein deficiency and the deviation from unselected tends to increase as protein level declines up to a maximum difference of about 30%, which is really quite striking. In the strain selected on the live yeast medium, the deviation of 0·14 on the log scale is reduced to 0·06 on 2% casein and virtually to zero (0·02) on the diluted medium, while development time is greatly prolonged (Table 4).

(iii) These contrasts originate naturally in the differing extent to which body size is reduced by the alternative diets and it is instructive to look at the data from this angle. Accordingly, the deviations from body size on the live yeast medium are set out in Table 5. The two strains selected on sub-optimal diets show no decline in body size until the casein level falls to 3%, while the maximum decline at the lowest level used is about 15%. The strain selected on live yeast resembles the unselected in showing greater proportional decline at higher protein concentrations, but differs by being more adversely affected when the casein level falls to 2% or the medium is diluted; in the latter case while the unselected show a 30% decline, the large strain is 40% smaller.

Table 5. *Reduction in body size below the level on live yeast—log scale*

	Genotypes according to diet during selection			
Medium	Unselected	Low protein	Diluted	Live yeast
6% casein	−0·07	0·02	0·00	−0·10
5% casein	−0·05	0·00	0·01	−0·07
4% casein	−0·10	0·02	0·01	−0·17
3% casein	−0·18	−0·04	−0·05	−0·18
2% casein	−0·30	−0·14	−0·17	−0·37
Dilute	−0·29	−0·14	−0·15	−0·41

Thus, the earlier evidence, after six generations of selection, that the large strain suffered relatively greater proportional decline on a low-protein diet, is reinforced by this test carried out after a further generation of selection. Body size of the strains selected on sub-optimal diets is only slightly greater on 6, 5 and 4% casein than on live yeast, compared with the earlier test in which the difference was greater. Such differences may be due to variation in the composition of the live yeast medium in successive tests.

(iii) *The comparison of strains under competitive conditions*

Ideally we should like to relate the behaviour of the large strains selected on different diets to which they have become more or less adapted to the kind of sub-optimal conditions encountered in nature or population cage. One approach to this problem is to grow larvae at different levels of competition in the ordinary medium. This was done in the following way. The ordinary maize-meal molasses medium, unfortified with dried yeast, was poured into small vials (diameter 1·7 cm.), to a depth of approximately 1 cm.; only vials with apparently the same volume of medium were used. Different levels of food supply and competition were established by setting up different numbers of newly emerged larvae. To provide a check on the general homogeneity or otherwise of conditions, larvae carrying the dominant *Bar* were set up along with the unselected or one or other of the three large strains, so that each culture was started with an equal number of *Bar* and non-*Bar* larvae. The *Bar* strain had been created by backcrossing into the *Pacific* wild population for a couple of years, so we can regard the marked flies as genetically equivalent to the wild stock apart from, at most, a small region on the X chromosome in the immediate vicinity of *Bar*. The levels of crowding were 20, 40 and 50 total larvae per tube—divided equally between marked and unmarked flies respectively—and 10, 5 and 3 replicates for the three levels of crowding respectively, were set up for each of the four strains. Development time was recorded for all flies which hatched and all flies of both sexes were measured. Complete variance analysis has been carried out for the extensive data, but these are not quoted since their chief value lies in the provision of error variances for comparing averages.

The first question to be asked is whether the *Bar* flies can be regarded as entirely equivalent to unmarked wild individuals with respect to survival under these conditions. The answer is given in the left-hand column of survival values in Table 6, which shows differences in the percentage survival of *Bar* and non-*Bar* individuals. In competition with the unselected, unmarked flies, *Bar* individuals survive as well at all levels of crowding. The average difference in percentage survival is negligible. Also progressive increase of the level of crowding from twenty to fifty larvae per tube is virtually without effect on the survival of either marked or unmarked unselected flies, and the mean value is a little over 70%. Hence the survival of *Bar* flies, when competing with other strains, can be taken as a suitable measure of how the unmarked, unselected individuals would behave in these circumstances.

When the marked individuals compete with the selected strains, we find a considerably higher level of survival when competing with the strain selected on low protein, about the same value with the strain selected on dilute medium, and a considerably higher level in competition with the large live yeast strain, except where fifty larvae are present—a discrepancy which will be referred to later. The middle section of Table 6 shows the differences in percentage survival between the marked individuals and the various strains with which they are competing. Since, as far as survival is concerned, we have concluded that marked and unmarked flies

Table 6. *Survival at different levels of crowding*

Percentage survival of marked controls in presence of different strains

Larvae per culture	Large strains according to diet during selection			
	Unselected	Low protein	Diluted	Live yeast
20	74·2	99·0	72·0	80·0
40	70·5	79·0	77·0	82·0
50	71·3	78·5	68·0	60·6
	Difference from controls: percentage survival			
20	4·0	−29·0	1·0	−22·0
40	−1·0	−20·0	−14·0	−22·0
50	0·0	−25·2	−27·0	−0·7
	Average total flies per culture			
20	14	17	14	14
40	28	28	28	29
50	36	33	33	25

are equivalent, we may infer that all the selected large strains have lowered ability to survive under these conditions of limited food supply and competition, compared with the unselected individuals, but differ somewhat with respect to relative performance at different levels of crowding. Thus, the large strain, selected on the dilute medium, survives as well as unselected flies when the cultures are started with twenty larvae, but competes less effectively when the number is increased to forty and especially fifty. The apparent increase in survival of the large, live yeast, strain at fifty larvae per tube, is almost certainly an artifact; probably too few *Bar* larvae were set up in these cultures in error.

The lowest section of Table 6 lists the average total number of flies, irrespective of genotype, which hatch from the tubes at different levels of crowding. For any level, the total hatch appears to be independent of genetic composition, so that the more vigorous, marked, wild individuals must supplant their less efficient competitors; it would be hard to find a more elegant demonstration of competition which discriminates between alternative genotypes.

The next question is whether or not the *Bar* flies differ in body size according to the strain with which they are competing. Table 7 whows the mean body size of the marked females in the different situations. It turns out, for any level of

Table 7. *Mean body size of marked, unselected females cultured in the presence of other strains*—$3 \times$ *log thorax length*

Larvae per culture	Large strains according to diet during selection				
	Unselected	Low protein	Diluted	Live yeast	Average
20	13·76	13·69	13·76	13·72	13·73
40	13·59	13·55	13·57	13·57	13·57
50	13·46	13·50	13·49	13·41	13·47
Average	13·60	13·58	13·61	13·57	

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crowding, that the average body size of marked flies, does not differ according to which of the other strains is present; the variance analysis indicates that, for any row of values, the differences between means fall well within the range of error variances. The averages, quoted in the end column, show that an increase from twenty to fifty larvae per culture involves a decline of approximately 25% in body size. Since the average body size is comparatively constant for a given level of crowding, the performance of the various strains can be compared by reference to that of the marked flies whose behaviour can be regarded as typical of unselected flies. Table 8 lists the comparisons in terms of deviations of the values for body size and development time, at different crowding levels, from the values recorded for the unselected, while Table 9 sets out the comparisons as deviations from the performance on the uncrowded, live yeast medium.

Under the competitive conditions on the live yeast medium the large strains differ clearly in behaviour in spite of their outward resemblance when grown on the favourable live yeast medium. Thus, in the low-protein strain, crowding at twenty larvae per tube reduced body size below the level found on live yeast by some 50% compared with only 30% for the unselected individuals (Table 8). The relative difference was somewhat diminished at fifty larvae per tube since the large strain suffered about 12% greater decline than the unselected.

Table 8. *The effects of crowding on body size* (S) *and larval period* (L) *compared with uncrowded, favourable conditions; log scale*

	Large strains selected on different media							
	Unselected controls		Low protein		Diluted		Live yeast	
Larvae per culture	S	L	S	L	S	L	S	L
20	−0·28	0·29	−0·48	0·36	−0·31	0·39	−0·29	0·34
40	−0·43	0·45	−0·55	0·50	−0·63	0·57	−0·49	0·51
50	−0·53	0·57	−0·65	0·64	−0·59	0·63	−0·68	0·93

The strain selected on the dilute medium resembles the low-protein strain at higher levels of crowding but at twenty larvae per tube it does not differ appreciably from the unselected in proportional decline of body size. The other large strain, selected on the live yeast medium, shows the same proportional decline at twenty larvae per tube, somewhat greater decline at forty while at fifty larvae per tube this strain shows the greatest decline and also a greatly lengthened development time.

Table 9 sets out the data from the other point of view, i.e. in terms of the deviation from unselected under alternative conditions. Clearly the phenotypic difference between selected and unselected is greatest when conditions are favourable and substantially less under competitive conditions. For the two strains selected on aseptic media this situation offers a striking contrast to similar comparisons on different kinds of sub-optimal diet, i.e. protein deficient and dilute

Table 9. *Deviations from unselected of different large strains at various levels of crowding—body size* (S) *and larval period* (L) *on log scale*

	Large strains according to diet during selection					
	Low protein		Diluted		Live yeast	
Conditions	S	L	S	L	S	L
Optimum	0·14	0·02	0·17	0·01	0·14	0·07
Crowded twenty larvae	0·05	0·09	0·17	0·11	0·16	0·12
Crowded forty larvae	0·05	0·07	0·07	0·12	0·11	0·12
Crowded fifty larvae	0·06	0·09	0·06	0·05	0·03	0·42

media which lead to increased difference in size between selected and unselected as the diet becomes increasingly sub-optimal (Table 5).

The development time of the selected strains consistently exceeds that of the unselected flies when compared under competitive conditions. For any strain, the

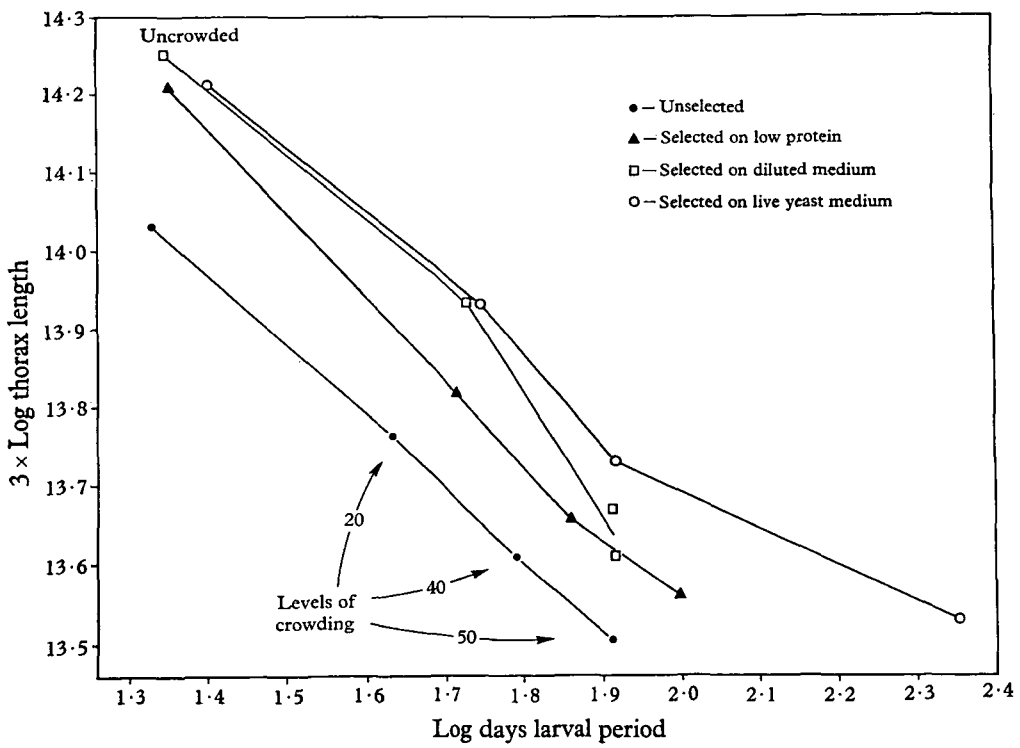

Fig. 4. Body size and development time of the unselected population and three large strains selected on different media, when grown in competition with genetically marked controls on the live yeast medium.

inverse relation between decline in body size and lengthening in development time is especially striking (Table 9 and Fig. 4). The only departure from the regular pattern occurs in the large strain selected on live yeast, where the larval period is disproportionately increased at fifty larvae per tube.

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Now it so happens that the reduction of body size of the unselected flies below the level on the optimum live yeast medium is about identical when larvae are set up either in the small tubes—twenty per tube—or when they are grown on the synthetic medium with 2% casein or on the medium diluted to one-third strength. Comparing the performance of the various strains under these particular alternative conditions, throws into sharp relief their characteristic differences in behaviour. Table 10 shows the deviation of body size and larval period from the values found on the favourable live yeast medium.

Table 10. *Growth under sub-optimal conditions, expressed as deviations from the performance on the live yeast medium*

	Body size				Development time			
Treatment	U.	L.P.	D.	L.Y.	U.	L.P.	D.	L.Y.
Crowded, twenty larvae per culture	−0·28	−0·48	−0·31	−0·29	0·29	0·36	0·29	0·34
Diluted medium	−0·29	−0·14	−0·15	−0·41	0·46	0·47	0·53	0·58
2% casein medium	−0·30	−0·14	−0·14	−0·37	0·75	0·71	0·83	0·67

U., L.P., D. and L.Y. refer respectively to the unselected strain, and the large strains selected on low protein, diluted medium and live yeast. Body size is measured as $3 \times \log$ thorax length and development time as log days larval life.

Although the large strain which has been selected for large body size on the live yeast medium, suffers much greater decline in body size on the sub-optimal synthetic media, it is relatively much less affected than the low-protein strain by crowding at the level of twenty larvae per culture. Under such crowded competitive conditions, the unselected flies and the strains selected either on diluted media or on live yeast suffer about the same reduction in body size although development time is relatively longer in the selected strains. On the sub-optimal aseptic media, the strain selected on live yeast shows the greatest reduction and the other large strains least reduction in body size with the unselected roughly intermediate. These contrasts provide an excellent illustration of how selection under different nutritional conditions has altered the pattern of growth responses when larvae are grown on different diets.

4. DISCUSSION

These tests have amply confirmed the earlier inference that, on different diets, the variance of body size represents the effects of genetic segregation on different processes of growth and metabolism, according to how much and in what way the diets differ. Hence selection for the same 'character' in different conditions leads to qualitatively different changes. These have been revealed by the striking differences in reaction to similar alterations of diet on the part of strains selected on different media. Perhaps the most dramatic demonstration is embodied in Table 10, showing that three treatments which lead to the same proportional

decline of body size in unselected individuals—just under 30%—result in reductions of body size in the other strains ranging from 14 to nearly 50%.

Such contrasts are obviously related to the conditions under which selection has been carried out. Thus, when tested on the deficient media, both the unselected population and the large strain selected on live yeast medium suffer a much greater decline in body size than either of the large strains selected on the synthetic diets. Under the competitive conditions tested, which probably approximate more closely to the conditions in nature, the strain selected on the low-protein diet is much more reduced in size, while the live yeast strain suffers about the same decline as unselected flies, at intermediate levels of crowding, but a greater decline when the level of competition is increased. As might be expected, there are many different ways in which a diet can be rendered sub-optimal, with respect not only to the deficiency of specific essential nutrients, but also to the relative concentration of nutrients which may affect nutritional imbalance. It looks, on present evidence, as if the competitive conditions on the live yeast medium may be more like those commonly met with than either of the synthetic diets, although the composition of the diluted medium may correspond more closely to natural sub-optimal conditions than is the case for the low-protein diet. By testing the reaction of suitable genotypes to graded levels of competition, supplementing the medium with specific nutrients, it should be possible to determine the more important limiting factors in growth under crowded conditions on the live yeast medium and arrive thereby at a more realistic, controlled reproduction of the natural sub-optimal conditions.

The relatively superior performance on the deficient synthetic diets, of the strains selected under these conditions, it is entirely consistent with the other indications given in the earlier paper, that the larger body size in these strains is partly due to better adaptation to these conditions. It was suggested in the earlier discussion, that adaptation to such diets probably lead to some loss of adaptation to the ordinary live yeast medium. This inference is consistent with the relatively greater decline in body size when larvae are crowded on the live yeast medium. There is also the tendency, most evident in the low-protein strain, for body size to be slightly smaller on the normally favourable live yeast medium, than on Medium C, even when fructose is omitted.

The ease with which a few generations of mass selection can improve performance on particular sub-optimal media, implies the existence of freely segregating differences which can provide the means of immediate adjustment to a wide variety of different nutritional conditions, such as are commonly encountered in natural or laboratory conditions (Gordon & Sang, 1941). Since the reaction to differences in the chemical composition of the diet is genetically controlled and since the composition of the diet in nature is subject to wide fluctuations, there will be corresponding variation in selection pressures. Although gene arrays will be favoured, which, on the average, confer greatest independence of such variation in environment, there will be a limit to the effectiveness of such adaptation at the level of the individual. In an outbreeding species, the high level of heterozygosity provides

the material for an immediate adjustment to either short or long term changes in the environment. The readiness with which we can select for better adaptation to the special diets chosen for these experiments is probably related to the variety of conditions normally encountered by the species. It would be interesting to see whether differences between species in this respect could be correlated with the ecological diversity of their habits.

The relations between the two measures of growth—adult body size and duration of the larval period—must be considered next. Provided the larval diet is not too deficient, the larval period may be considerably extended without any reduction of body size below that attained under the most favourable conditions for growth. But there is a limit to this capacity for maintaining a constant body size and when the diet becomes too inadequate body size declines and this reduction is accompanied by progressive and more or less proportional lengthening of the larval period. The strains differ in their capacity to achieve maximum size in relation to a given change of diet. Thus on media which drastically reduced the body size of both the unselected population and the large strain selected on the live yeast medium, the size of strains previously selected on sub-optimal aseptic media was not reduced, although development time was increased. It appears that differences in the level of adaptation to different kinds of diet are correlated with differences in ability to maintain a constant adult body size. Also, different gene arrays selected under different nutritional conditions, may lead to the same maximum body size, but the range of conditions which allow constancy of final size may differ sharply. The increase in the variance of body size when the diet is altered sufficiently to reduce average body size below the maximum level (Robertson, 1960*a*) can be attributed to the exposure of previously undetected differences in this capacity to maintain body size.

As noted above, more adverse conditions which reduce adult size show a distinct relation between smaller size and longer larval period. Although all the tests on alternative media show evidence of this kind of response, perhaps the most striking example occurs in the comparisons of performance with different levels of crowding. Here, the regression of body size on larval period was very close to -1, on the log scale. Sub-optimal diets may differ in how far they involve disproportionate lengthening of the larval period compared with the reduction of body size. Thus Table 10 showed that crowding at twenty larvae per tube and also aseptic culture on either low-protein or diluted media cause the same percentage reduction of body size of the unselected population but give widely different development times. With crowding the development time was increased by about 30%—just as much as body size was reduced—while on the diluted and low-protein media, development time was increased by about 50 and 70%.

Such differing relations may depend on the stage of larval growth and development primarily affected by the treatment in question. There is evidence from tests carried out by Bakker (1959) and supported by my observations, that, after a certain stage early in the third instar, the subsequent duration of the larval stage is determined. This was shown by removing larvae from the food medium at

different ages. It appears that after this stage is reached larvae can pupate without further food and that variation in food intake will lead to great variation in adult size but little or no differences in duration of development. Nutritional effects of this sort could account for differences in size which are associated with the same development time, as noted in Fig. 1, for example. What remains to be determined, however, is the relation between diet, early larval growth rate, the critical period and constancy or otherwise of the larval period after the critical stage is reached. There is little point in speculating about possibilities until current experiments have clarified the situation. However, a few general observations are relevant.

The relations between body size and duration of the larval period are probably controlled by antagonistic reactions between hormones which favour continued growth in the larval stage, on the one hand, and the onset of differentiation on the other. To quote from a recent review by Karlson (1956): 'The holometabolous form of development results from the special balance between hormones from the corpora allata and those from the prothoracic gland during the larval period followed by a marked change in the balance during pupation and imaginal differentiation.' If a certain critical ratio of concentration of antagonistic hormones or alternately some critical threshold relationship has to be established to set an end to growth, relative constancy of such a ratio or threshold will make for a constant body size, other things being equal. Even though growth is slowed down, the critical state will be approached more slowly and will be reached only when the total amount of growth characteristic of the strain has been completed. Possibly some such situation underlies the maintenance of a characteristic body size.

But, as we have seen, there is a limit to the effectiveness of this maintenance, since body size declines when the diet is too deficient. This sharp change-over in the pattern of response suggests a characteristic change in the hormonal relations of the critical threshold. If the antagonistic relations or critical threshold were independent of the nature of the diet, development time would be indefinitely prolonged until the essential conditions were fulfilled. On the other hand, a change in hormonal relations or threshold can ensure that the adult reproductive state will be reached more quickly. To put it rather crudely, it may often be better for the species to be a small fly today, rather than a bigger fly tomorrow or a fully grown one next week. Hence, if the diet is too deficient a kind of safety mechanism comes into operation which alters either hormonal relations or critical threshold in such a way as to allow the adult state to be reached more quickly than would otherwise be the case.

The relation between the nature of the diet and the readiness to sacrifice body size and potential fecundity for a quicker approach to adult-hood, probably varies from species to species. For some species, the capacity to produce the maximum number of eggs—only possible if body-size is not reduced—may be relatively more important than variation in the length of the larval period, and, in such cases, the capacity for maintaining body size will be more highly developed than in others for which shorter life cycle is relatively more important than maxi-

mum egg production. Exactly where the balance is struck, in relation to the composition and quantity of the diet, will differ according to ecological conditions and the nature and intensity of intra- and inter-specific competition. Differences of this kind may well influence the course of selection for apparently the same 'character' in different species. For example, if there is a highly developed ability to maintain a characteristic body size when the diet is poor, response to selection for, say, larger size, might be expected to be greater than in another species which responds to sub-optimal conditions by reducing body size more readily. Hence observed differences in response to parallel selection may be essentially due to characteristically different inter-relations between genotype and ecology, rather than, say, differences in linkage relations or chromosome number.

In general, therefore, the evidence from this and the earlier papers in this series, implies that the physiological nature and magnitude of the response to selection for body size—and doubtless other attributes too—can be fully understood only by relating the particular conditions in which selection is practised to those prevailing in the normal environment.

SUMMARY

1. The growth of strains of *Drosophila melanogaster* selected for large size under different nutritional conditions has been recorded on a variety of different media and compared with that of the unselected population. The experiments were designed to test the inference from earlier work that selection for the same 'character', body size, on different diets leads to more or less different changes in growth and metabolism. The inference has been amply confirmed.

2. When compared on a number of deficient synthetic diets, the strains which had been selected either on a low-protein diet or on one in which all the essential nutrients had been reduced, suffered a much smaller reduction in body size than either the unselected population or, especially, a large strain selected on the favourable live yeast medium. Some diets which drastically reduced the body size of the unselected population lead to no change in the size of strains selected on the synthetic media, although development time was prolonged. Hence selection had extended the capacity for maintaining a characteristic adult body size to diets which normally would lead to a decline. This is taken as evidence of improved adaptation to such conditions. There is also some evidence that selection on the synthetic diets had lowered the level of adaptation to the usual live yeast diet, since body size tended to be lower on this medium than on some of the normally sub-optimal diets.

3. To provide comparisons in adverse conditions which are probably more closely related to those commonly encountered by populations in nature or the laboratory, the performance of the strains has been compared in a graded series of competitive conditions on the live yeast medium. By using genetically marked flies of the foundation population, which were shown to react in the same way as unmarked flies—in terms of survival, body size and development time—the competitive ability of the different strains has been tested against that of unselected

individuals. The latter are generally superior to the selected strains, which differ among themselves, however, in a way which can be related to the conditions in which they were selected.

4. Under such competitive conditions, the strains selected on the synthetic diets suffer a much greater decline in body size than do the unselected individuals. For the strain selected on live yeast, the proportional reduction of body size is about the same for the unselected flies at lower levels of crowding, but is clearly greater under more severe conditions of competition.

5. The low-protein strain has been backcrossed to the unselected stock. When reared on a variety of synthetic diets, the performance of the F_1 was generally intermediate between that of the parents.

6. Nutritional variation may be responsible for either a high environmental correlation between the two measures of growth, body size and duration of larval period, or no apparent correlation. Provided the diet is not too unfavourable, body size remains constant although development time may be lengthened to a variable degree. With more adverse conditions, body size is reduced and development time is lengthened more or less proportionately. Such differences in reaction probably depend on the particular stage of larval growth and development primarily affected by the treatment; this problem is being examined further. The inverse relations between body size and development time may represent the operation of a kind of safety mechanism which ensures that the adult reproductive state is attained sooner than would be so if the capacity for maintaining a characteristic body size were more effective in relation to deficient diets. Populations and species adapted to different conditions are likely to differ as to where the balance is struck between effective maintenance of a characteristic adult size, with maximum potential egg production, and the alternative response, according to their ecology. This possibility must be borne in mind when the response to selection for, say, body size is compared in different species.

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REFERENCES

BAKKER, K. (1959). *Ent. exp. appl.* **2**, 171–186.

GORDON, C. & SANG, J. H. (1941). The relation between nutrition and exhibition of the gene antenneless (*Drosophila melanogaster*). *Proc. roy. Soc.* B, **130**, 151–184.

KARLSON, P. (1956). Biochemical studies on insect hormones. *Vitamins and Hormones*, **14**, 227–266. New York: Academic Press.

ROBERTSON, F. W. (1959). Gene-environment interactions in relation to the nutrition and growth of *Drosophila*. 'Biological Contributions.' *Univ. Tex. Publ.* No. 5914, 89–98.

ROBERTSON, F. W. (1960*a*). The ecological genetics of growth in *Drosophila*. 1. Body size and development time on different diets. *Genet. Res.* **1**, 288–304.

ROBERTSON, F. W. (1960*b*). The ecological genetics of growth in *Drosophila*. 2. Selection for large body size on different diets. *Genet. Res.* **1**, 305–318.

SANG, J. H. (1956). The quantitative nutritional requirements of *Drosophila melanogaster*. *J. exp. Biol.* **33**, 45–72.