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The ecological genetics of growth in *Drosophila*

7. THE ROLE OF CANALIZATION IN THE STABILITY OF GROWTH RELATIONS

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1. INTRODUCTION

The growth relations, which determine a character like body-size in *Drosophila*, are stable enough to confer a characteristic average phenotype on populations or species, in spite of a high level of individual variation of both genetic and environmental origin. A character such as body-size apparently differs from many pattern characters in which the stability and restriction of phenotypic variation are attributed to the special developmental relations which are generally associated with canalization (Waddington, 1957). With respect to characters such as body-size it is commonly assumed that decline in fitness with progressive deviation from the mean is the essential basis of the stability and no doubt this is in part true. But long-term familiarity with the genetic properties of this character leads us to suspect that canalization in one form or another must make a major contribution to the stability of species differences in body-size and growth generally, even though the apparently continuous nature of the phenotypic variation makes this seem unlikely at first sight.

Naturally this problem can only be tackled by determining how far apparently undifferentiated biometric variation produces similar effects by different developmental pathways. For this purpose, study of the physiological differences produced by selection in different environments provides a useful tool. Thus, in earlier papers of this series (Robertson, 1960, 1963) I showed that selection for large body size on different synthetic diets may lead to a similar increase in size by different routes. The clue to an interpretation was given by the presence or absence of correlated changes in body-size and duration of the larval period. On one diet there was no correlation while on the other there was almost proportional change in both characters.

Such contrasts led to a study of larval growth which is divisible into two stages, namely, potential exponential growth to a so-called 'critical size' in the early third instar when the larva attains the capacity to pupate, even if thereafter deprived of

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food, followed by the stage between then and pupation, a period whose duration is not noticably influenced by nutritional variation. It was suggested that the growth effected in either of these stages could be altered and that the different diets favoured the manifestation of genetic differences which influenced one or the other. Changes in size, correlated with the duration of the larval period, were attributed to a longer period of growth to a larger critical size, while uncorrelated changes were attributed to variation in the later phase of growth after the duration of the larval period had been determined.

Since that paper was published (Robertson, 1963) further support for the importance of this division of larval growth into two stages has come from comparisons of different species of *Drosophila* which differ in body-size (Royes & Robertson, in press). Thus *D. funebris* and *immigrans* are about the same size and about two and a half times as big as *melanogaster* and, in each, the critical size is much larger than in *melanogaster* but is appreciably different in the two species in spite of their similarity in adult size. Hence the same end result in body-size can be arrived at by different amounts of growth before and after the critical stage. Also, the comparative study showed that in all three species the latter occurs at the end of the exponential phase of growth while, thereafter, growth slows down and finally ceases when pupal differentiation sets in. Further, the two large species were shown to differ characteristically in their reaction to nutritional stress and these differences were attributed to differences in these growth relations.

This evidence raises the question of the relative contribution of alternative types of developmental change to the phenotypic variation of size, both genetic and environmental. We might expect equivalent changes in final size, by alternative routes, to differ with respect to fitness since in one case the duration of the larval period—a major component of fitness—is involved and in the other it is not. Published data suggest that the contribution is very unequal, since selection under favourable conditions on low protein diets leads to great changes in size, with little or no change in development time.

The present paper deals with comparisons between selected strains, which have arrived at similar deviations from the mean by different developmental pathways, to see how far they differ in gene-environment interaction. The evidence leads to a general interpretation of the stability of growth relations and body-size.

2. MATERIAL AND METHODS

Mass selection for large or small size or shorter development time has been carried out on synthetic diets obtained by modifying medium C of Sang (1956). The details of selection procedure are exactly as described in an earlier paper (Robertson, 1963), and all strains were derived from the same Pacific foundation population. We are not concerned here with the quantitative response to selection, but with the creation of sufficient differences to provide convenient material for experimental comparison. For the present purposes, flies have been drawn at different times from the selected lines and grown on the live yeast medium, uncrowded or crowded and on various

synthetic diets. Samples of the foundation population have always been cultured as controls. Approximately eight females from four to five or more replicated cultures per genotype and treatment were measured for size but all hatching females were scored for development time. Size is recorded as $3 \times \log_e$ thorax length 1/100 mm. and the larval period (development time minus pupal period) is expressed as the natural logarithm. Further details of methods have been given in earlier papers of this series, e.g. Robertson (1960*a*).

The media used for selection are deficient either in protein, 2% in place of the usual 5% level of casein, or ribonucleic acid in which the level is mildly, 0·3%, or drastically, 0·075%, reduced below the minimum level for optimal growth on this medium. It so happens that reduction of casein to 2% and reduction of RNA to the lower concentration produces flies of about the same average body-size although development is rather longer on the low protein diet.

Data from tests carried out at different times have been brought together for comparison and include experiments on the performance of lines which have been already described, as well as new lines, since the accumulation of fresh evidence sometimes sets earlier data in a slightly different light. The earlier lines include two selected on low protein or on medium diluted to one third normal concentration (Robertson, 1960*b*) and also two lines selected for large body-size and two for shorter development time on both the higher or lower levels of RNA. The low-protein and low-RNA lines, which were described earlier, were selected at different times.

They showed such well defined differences in correlated changes that it was decided to repeat the entire test running all lines together, to make sure that such differences were due to differences in diet rather than to conceivable, although unlikely, changes in the genetic composition of the foundation population or the protein supply, since a new batch of casein had been used in later tests. Selection for large, and small size and also shorter development time was started at the same time and duplicated on the two media. In addition, selection for large body-size was carried out on a diet in which both protein and ribonucleic acid were adequate but a different nutrient, namely choline, limited growth. Thus evidence from altogether thirteen different selection experiments and many other tests has been used to arrive at the general interpretation which is given later.

3. EXPERIMENTAL RESULTS

(i) *The later tests*

After four generations of selection, samples from the six lines and the control unselected population were compared on the medium on which they had been selected and also on the live yeast diet uncrowded, and also crowded. In the live yeast cultures, crowded or uncrowded, white-eyed control flies were grown in the same tubes and deviations from unselected represent the average of such within-tube differences. These controls had been established by repeated back-crossing of white-eye into the Pacific population and many tests have shown that the white-eye gene is neutral with respect to growth on a wide range of diets. The results are

shown in Table 1, in which the deviations from control for body-size and larval period are set out. We may note the following:

Table 1. *The performance of stocks selected on different diets when compared on alternative media; deviation from unselected controls*

Medium	Selected on low RNA		Selected on low protein	
	Body-size	Larval period	Body-size	Larval period
		LARGE LINES		
Original synthetic	0·11**	0·08**	0·09**	−0·06**
Yeast—uncrowded	−0·02	−0·02	0·00	0·04
Yeast—crowded	0·03	0·00	−0·21**	−0·04
		SMALL LINES		
Original synthetic	−0·07**	−0·01	−0·14**	−0·04
Yeast—uncrowded	−0·06**	−0·01	−0·13**	−0·05*
Yeast—crowded	−0·13**	−0·06*	0·00	0·09**
		FAST LINES		
Original synthetic	0·06**	−0·06*	−0·06*	−0·06*
Yeast—uncrowded	−0·01	−0·06*	−0·11**	−0·09**
Yeast—crowded	−0·05*	−0·08**	−0·05*	−0·13**

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

(i) Selection for large size on the RNA medium leads to parallel changes in body-size and larval period, whereas approximately equivalent increase in size on the low protein diet is accompanied by decrease in development time. This result is in excellent agreement with earlier data which showed that on low RNA selection for large size led to more or less proportional changes in larval period (Robertson, 1963), while, on low protein, larger size was associated with shorter development time in early generations although, later, the deviation from control (in development and time) was negligible (Robertson, 1960*b*, *c*).

With respect to small size we have no earlier evidence of selection on sterile media with which to compare the present data. We note, however, that on low RNA there is no change in the larval period but a 7% decline in size—just the kind of result which might be expected from previous experience with selection on live yeast cultures (Robertson, 1960*a*), but on low protein, during the same interval, the decline in size is twice as great and the larval period is some 4% shorter. Although the two media lead to different results, if we compare selection for larger or smaller body-size, there is no evidence that opposite changes in larval period are correlated with opposite changes in size on a particular diet. With respect to selection for shorter development time, both lines are reduced by 6% but, on low RNA, body-size is increased by 6%, while on low protein it is reduced by the same amount. There is a difference here with early experiments in which selection for shorter time on low RNA led to smaller size. Thus the presence or absence and the sign of correlated changes in the larval period imply that the array of genetic variation accessible to selection differs appreciably on the two media.

(ii) The effect of growing the various strains on the favourable live yeast medium can be considered next. In the large line, selected on low RNA, the deviation from the unselected line is eliminated, apart from a slight tendency for the flies to be smaller and to develop faster. In the large line selected on low casein, the difference in size is also obliterated although development time is slightly longer here. In the two small lines, the change in diet is without effect on the proportional differences in size and development time which are shown on the sterile media. So selection in opposite directions involves different reactions to the same nutritional change. Manifestation of the positive deviation from control in body-size requires special conditions provided by the synthetic medium. Comparable deviation in the opposite direction is more stable. With respect to the fast lines, in the low RNA line there is no change in development time but here the size difference is obliterated. In the other, low-protein, fast line the shorter development time and smaller size is enhanced.

(iii) Finally we have the comparisons on the crowded sub-optimal medium which reduces size generally to a considerable degree, including the controls. In the large lines there is a striking contrast. In the large RNA line there is no significant deviation from control, so that, in the transition from optimal to definitely sub-optimal conditions, selected and unselected behave alike. But the low protein line is quite different since it shows a drastic decline in body-size, some 20% below the level of the control. It might be thought that this represents the effect of selection on metabolism generally, so that growth would be adversely affected, but this simple explanation will not do since, in that case, we should expect the larval period to be correspondingly or relatively more extended in time, in view of abundant evidence from earlier data that sub-optimal diets or unfavourable gene-environment interaction reduce body-size and extend development time. It appears that the differential reduction is effected during the later stages of growth, after the duration of the larval period has been determined.

In the small lines there is a striking difference in the behaviour according to the medium upon which they are selected. Thus, in the low-RNA line, the larval period is shorter than the controls, compared with performance on the original synthetic and the live yeast media. The negative deviation for body-size is about doubled. In the low protein line, on the other hand, the difference in body-size, 13–14% on the original sterile and favourable media is eliminated, while the larval period, instead of being shorter, is now 10% longer than the controls.

With respect to the fast lines, the differences are not so great since in the low-RNA line body-size is a little further reduced, while, in the other line, the duration of larval life is relatively reduced while the deviation for size is less.

Perhaps the most striking features of these results are the general tendency for the larval period to be relatively less, rather than higher, in the competitive conditions to which we might expect the unselected population to be best adapted, the elimination of the deviation of the unselected in the low protein small line and also the disproportionate reduction of body-size, unaccompanied by corresponding changes in larval period, in the large low-protein line.

At first sight we have an apparently bewildering array of differences which might be attributed to the unpredictable effects of various changes in metabolism. But closer scrutiny of the relations between body-size and duration of the larval period shows that there is a considerable degree of regularity. In Fig. 1 we have plotted the deviations from the unselected controls, listed in Table 1, and they show clear

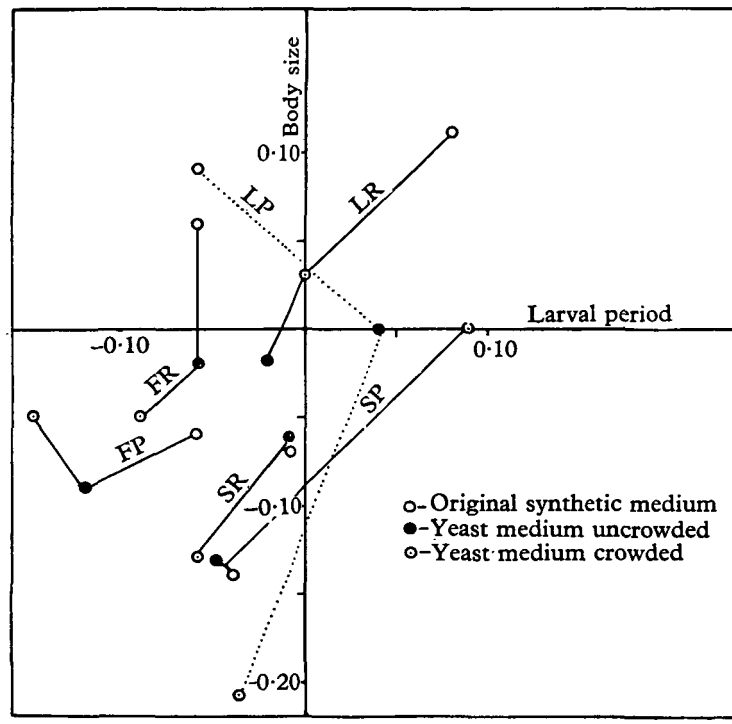

Fig. 1. The effects on body size and duration of the larval period of providing different larval diets for lines which have been selected for large or small body-size or shorter development time on axenic diets which are deficient either in protein or ribonucleic acid. The values are expressed as deviations from those of the unselected control population. For both body-size and larval period a logarithmic scale is used. The alternative diets comprise the original synthetic medium upon which the lines were selected, the favourable live yeast diet and also crowded, competitive conditions in live yeast medium. The first letter L, S or F refers to lines selected for large or small size or fast development respectively, while the second letter, P or R, refers to whether the line was selected on low protein or low RNA medium.

evidence of positive correlation between body-size and larval period. This is especially clear in both the small lines and also in the large line selected on low RNA. In the former, relative to controls, change to crowded conditions from either the sterile medium or live yeast, leads to opposite changes on the part of the low RNA and the low-protein line. In these three lines the regression of body-size on duration of the larval period is close to unity. In the fast lines, two of the points for each follow this relation while one is displaced. The outstanding exception is the large

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low-protein line in which, for the transition from sterile to the live yeast medium, the slope is negative, suggesting that selection on this medium has led to metabolic changes which affect both larval period and body-size, while on the crowded medium there is a drastic reduction in body-size which cannot be fitted into the general scheme.

This interpretation recalls parallel evidence described earlier (Robertson, 1960*a*), in which it was shown that both body-size and duration of the larval period varied between replicated tests on low RNA and fructose-deficient media, due probably to uncontrollable minor differences in the medium. But there was also a positive correlation between the two variables, with a regression close to unity, so that unspecified environmental differences were responsible for changes in growth which led to a positive correlation between body-size and larval period. Large and small strains selected on live media also showed the same relations. Such positive correlation presumably reflects relative extension and contraction of the first phase of growth and can be distinguished from the effects of variation of sub-optimal diets which leads to a negative correlation between body-size and larval period.

We can infer that the response to the kind of sub-optimal diets studied in the present test is compounded of two distinct reactions—the gross reduction in size and extension of larval life common to all the strains and a more subtle effect on the relative duration of the period of growth. It is likely that this represents variation in the growth to the critical stage, and is revealed in the deviation from the unselected population when gene-environment interaction, with respect to the time taken to reach this critical size and also the amount of growth effected thereafter, is the same. The general regularity focuses attention on the exceptions, especially the large low-protein line in which selection has altered metabolism so as to influence not only general growth rate—evident in the comparisons between performance on low protein and live yeast—but especially the later stages of growth on the crowded medium. Changes of the other kind may also be involved here, but they are obscured by these gross differences in reaction.

(ii) *Comparisons with earlier tests*

We can now compare these data with similar experiments carried out on selected large lines in the earlier separate tests. They are shown in Table 2. Considering first the lines selected on the low protein and the diluted medium, comparisons between their performance on the medium on which they were selected and on the live yeast medium shows that the deviation from control in body-size is greatly reduced although the negligible deviations from control in the larval life on the synthetic medium is unaffected. On crowded media the deviation from control is reduced further and development time is now significantly greater.

In the low-RNA lines there is marked contrast since the larger size and longer life is not obliterated by the change either to live yeast, low protein media or to conditions in crowded media and these lines show a remarkable stability in the expression of the genetic differences created by selection, compared with the other

lines selected on the low-protein or diluted media. The disproportionate positive deviation from controls on the crowded diet may have been due to some heterogeneity in conditions.

The contrasts between the two sets of comparisons, especially the presence or absence of stability of expression in later and earlier tests in low-RNA lines, can be accounted for. The data set out in Table 1 and Fig. 1 refer to the early stages of selection whereas in the tests referred to in Table 2, selection had been carried out for a longer time, especially in the line on low protein and diluted media. Presumably selection had led to more extensive changes in the genetic background so as to

Table 2. *The performance of large strains selected for longer periods on alternative diets on various media; deviation from unselected*

	Selected on low RNA		Selected on low protein or diluted media	
Medium	H	L	LP	D
		BODY SIZE		
2% protein	0·13	0·11	0·29	0·30
5% protein	0·14	0·15	0·19	0·24
Yeast—uncrowded	0·14	0·08	0·14	0·17
Yeast—crowded	0·26	0·19	0·05	0·07
		LARVAL PERIOD		
2% protein	0·10	0·12	−0·02	0·09
5% protein	0·09	0·08	0·04	0·01
Yeast—uncrowded	0·10	0·04	0·02	0·01
Yeast—crowded	0·11	0·10	0·07	0·12

H, L, refer to lines selected on two levels of RNA; LP and D refer to lines selected on low protein or diluted media.

stabilize the expression of the genetic differences more effectively, so that, in the large low-RNA lines, the phenotypic differences were comparatively unaffected by the change of conditions studied here. In the other lines, the effects of selection are partly expressed on the live yeast medium and not completely obliterated even on the crowded media. There is further evidence, in their longer larval life under such conditions, that long continued selection on low protein and diluted media had affected metabolism sufficiently to influence early larval growth rate as well.

So it appears that selection for large size on either type of diet alters development in different ways. At first these changes are only or best expressed under the particular conditions in which selection has been carried out. But as selection continues the phenotypic differences become increasingly stable with respect to changes in this diet, particularly in the lines in which the early exponential phase of growth has been extended.

(iii) *Selection on a low choline diet*

So far we have studied the effects of selection under optimal conditions and on axenic diets in which either protein or RNA are limiting. It would be useful to know the nature of the response to selection when the diet is made sub-optimal

for different nutrients, and so selection for large size has been carried out on a choline deficient medium. For this test, lecithin is omitted from the diet and choline is added at the rate of 30 mg./l. Choline is an essential nutrient for *Drosophila melanogaster* which has relatively high requirements compared with various other species of this genus (Royes & Robertson, in press). Also choline is unable to replace entirely the need for lecithin even at high concentrations, whereas in the other

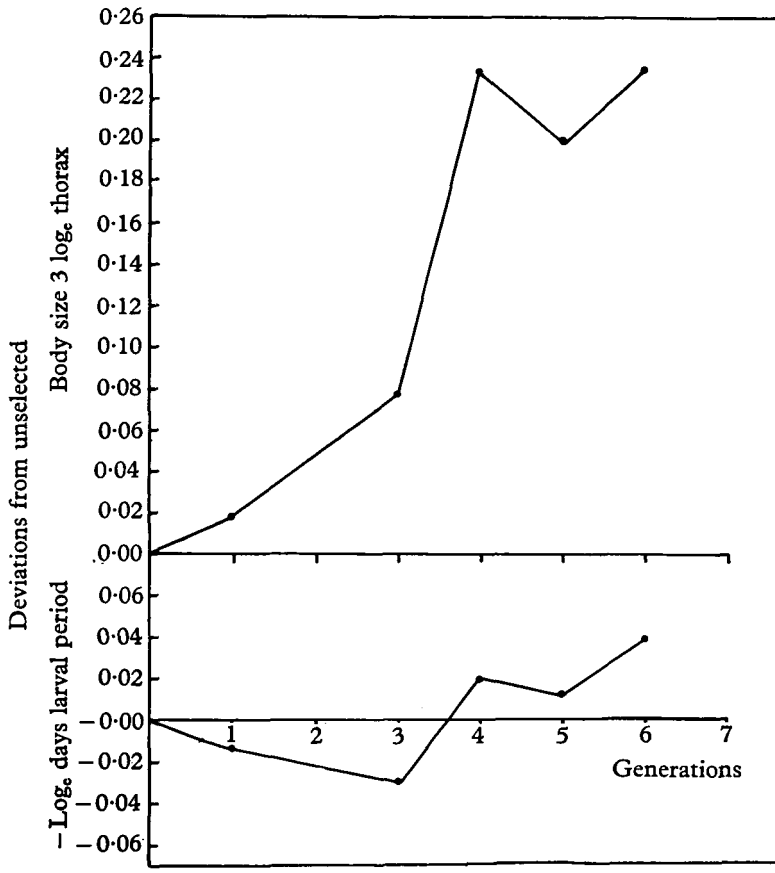

Fig. 2. The effects of selecting for large body-size on an axenic diet deficient in choline.

species which have been studied it can do so. The metabolic role of choline, provision of methyl groups, is very different from that of RNA and amino-acids generally.

The results of this test are shown in Fig. 2. Selection leads to a rapid and striking increase in body-size but average development time is virtually unaltered. Hence the response resembles that on the low protein and diluted diets and there is no evidence that the early exponential phase has been extended. The results from this test reinforce the conclusion that deficiency of RNA in the diet creates a unique situation for effecting changes in the duration of this phase.

(iv) *Correlated metabolic changes*

These observations raise the problem of the origin of the different physiological effects associated with selection on the different diets. With respect to changes uncorrelated with length of larval life, it appears that changes in growth rate after the larval period has been determined are responsible, and this implies considerable genetic independence between the two phases of growth. This may be related to the evidence that the first phase, under optimal conditions, follows an exponential path while in the later phase, growth is progressively slowed down.

(*a*) *Amino-acid requirements*

To shed further light on this problem, the unselected and also the two large and two fast lines from the earlier tests were grown on medium C, to which was added each of the amino-acids singly in an amount equivalent to that present in 5% casein.

This should indicate not only which, if any, amino-acids are deficient in casein, but should also demonstrate the relations between changes in body-size and larval period in instances where excess leads to an unbalanced, unfavourable concentration. At the same time, the experiment tests for differences between the various lines. Generally the L-form of amino-acid was added but, where not available, twice the corresponding amount of DL-amino-acid was used. The results are shown in Table 3. It was impossible to handle all the comparisons at the same time, but since the same unsupplemented medium was set up as control in each group of tests, all the data can be considered together as deviations from this control value.

For body-size, addition of different amino-acids is comparatively unimportant. There is evidence of minor heterogeneity between lines in the reaction, and when there is a significant deviation from control, addition of an amino-acid more often reduces than increases size. There is no obvious relation between the sporadic distribution of effects and the nature of selection.

But the duration of the larval period presents a rather different picture. Thirteen out of the nineteen amino-acids affect development time in at least one of the lines and some of the effects are very striking. When the increase in amino-acid has an effect it generally leads to a lengthening of development time. This is especially true of aspartic acid, cystine and glutamic acid and also, to a lesser degree, to methionine and histidine. That cystine and methionine have similar effects is not surprising in view of their metabolic relationships. For cystine, aspartic, and glutamic acid there is highly significant heterogeneity between genotypes but no obvious relation to the direction of selection.

Particularly striking in these data is how much the larval period may be extended without any effect on body-size. There is no consistent evidence of positive correlation between effects on body-size and larval period. The data therefore provide good evidence of metabolic differences between the two stages of growth which can apparently be influenced independently, and this is consistent with the existence of some degree of genetic independence as well, as the earlier data have suggested.

Further evidence was provided by an experiment in which the large and small low-protein lines, referred to in Table 1, and the unselected controls were grown on

Table 3. *The effects on the growth of large and fast lines selected on low RNA of supplementing the standard synthetic medium with individual amino-acids*

	Deviations from control medium									
	Body-size					Larval period				
		Large lines		Fast lines			Large lines		Fast lines	
Amino-acid added	Un-selected	H	L	H	L	Un-selected	H	L	H	L
Alanine	0·02	0·02	0·00	−0·03	0·02	−0·12*	−0·01	0·00	−0·14**	−0·18**
Arginine	0·03	0·00	−0·03	0·03	0·02	0·03	0·01	0·03	0·00	−0·05
Aspartic acid	0·01	−0·09*	−0·01	−0·02	−0·01	0·29**	0·25**	0·23**	0·30**	0·19**
Cystine	−0·05	−0·01	−0·02	−0·02	−0·03	0·20**	0·20**	0·18**	0·42**	0·31**
Glutamic acid	−0·01	−0·05	−0·05	−0·01	−0·05	0·30**	0·45**	0·17**	0·56**	0·16**
Glycine	0·03	−0·05	−0·04	−0·05	0·00	0·03	0·08*	−0·01	0·06	0·05
Histidine	0·01	−0·03	−0·04	−0·01	−0·01	0·04	0·13**	0·06	0·13*	0·06
Hydroxy-proline	0·12*	−0·04	0·05	−0·01	0·01	−0·02	−0·01	−0·06	0·03	−0·02
Isoleucine	−0·03	−0·08*	−0·02	0·01	−0·07*	−0·03	−0·08*	−0·02	0·07*	−0·07*
Leucine	−0·05	−0·01	−0·01	0·01	0·02	0·04	0·05	−0·01	0·03	0·00
Lysine	0·06*	0·02	0·00	−0·05	0·02	0·07*	0·05	0·09*	−0·01	0·09*
Methionine	0·00	0·02	0·01	0·04	−0·12*	0·11*	0·13**	0·16**	0·09*	0·08*
Proline	0·02	−0·03	−0·02	−0·01	−0·01	−0·11*	0·00	−0·04	0·04	0·02
Phenylalanine	0·01	−0·02	−0·08*	−0·06*	−0·03	−0·04	−0·01	0·04	−0·09*	−0·10*
Serine	0·04	0·05	0·01	0·03	0·07*	−0·11*	−0·02	−0·06	−0·01	−0·01
Threonine	−0·04	−0·04	−0·11*	−0·08*	−0·07*	−0·03	0·04	0·02	−0·03	−0·11*
Tyrosine	0·00	−0·01	−0·02	−0·01	−0·14**	−0·01	−0·07	−0·02	0·02	0·02
Tryptophan	0·06*	0·02	0·03	0·05	0·02	0·06	0·02	0·03	0·05	0·02
Valine	0·04	−0·01	−0·00	−0·05	−0·02	0·04	−0·01	0·00	−0·05	−0·02

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

H and L refer to mild and severe deficiency of ribonucleic acid in the media used for selection.

the 2% casein medium, which was supplemented with one or other of six amino-acids which might be expected to be particularly low on such a medium. The data are set out in Table 4. This test was carried out two generations after the one described in Table 1. Development time on the control medium is the same for all the genotypes, and separate addition of five of the amino-acids leads to a slight or well-marked shortening of the larval period in all but one case. But, in the unselected

Table 4. *The effects on the growth of unselected and large and small lines selected on low protein when individual amino-acids are added to the low protein diet*

	Deviation from unsupplemented diet					
	Body-size			Larval period		
Amino-acid added	Unselected	Large	Small	Unselected	Large	Small
Arginine	0·03	−0·07	0·04	−0·06*	−0·08*	−0·03
Cystine	0·18**	0·06	0·17**	−0·18**	−0·21**	−0·25**
Glycine	0·08*	0·03	0·03	−0·05	−0·09**	0·00
Histidine	−0·01	−0·01	−0·02	−0·03	−0·06*	−0·04*
Lysine	−0·06*	0·00	−0·07*	0·09**	0·12**	0·11**
Tryptophan	0·14**	0·02	0·12**	−0·13**	−0·14**	−0·10**

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

population and the small line, there are generally negatively correlated changes in body-size, suggesting that growth to the critical size and growth thereafter is improved by the supplement, but in the large line body-size is virtually unaffected. Hence the selection for large size on the low-protein diet has altered amino-acid metabolism with respect to the later stage of growth, since the supplements do not lead to larger body-size, but has had little apparent effect on the requirements for early growth during the exponential phase. In addition, increase in the lysine content of the diet extends the larval period in both selected lines and the unselected population, but whereas in the unselected population and the small line, body-size is reduced by 6–7%, in the large line body-size is unchanged. Hence selection for large size on a low-protein diet apparently influences amino-acid metabolism in the later stage of growth, which appears to differ in amino-acid metabolism generally from the earlier phase, which determines the duration of the larval period.

(*b*) *RNA requirements*

Since selection on low protein has involved changes in amino-acid metabolism we must determine whether selection on low RNA has affected the minimal requirement for this nutrient. The data here refer to large and fast lines selected earlier on the higher and lower sub-optimal RNA levels (Robertson, 1963). Each line and the unselected control were grown on different levels of RNA ranging from 0·1 to 0·4% on the normal 5% protein medium. If the selection for large size on low RNA has

affected the intrinsic ability to synthesize RNA, we expect this to be reflected in a different inflection point in the dose response curve. Figure 2 shows that there is little evidence of this. In the unselected population, the duration of the larval period continues to decline over the range tested up to 0·4% but there is a sharp inflection

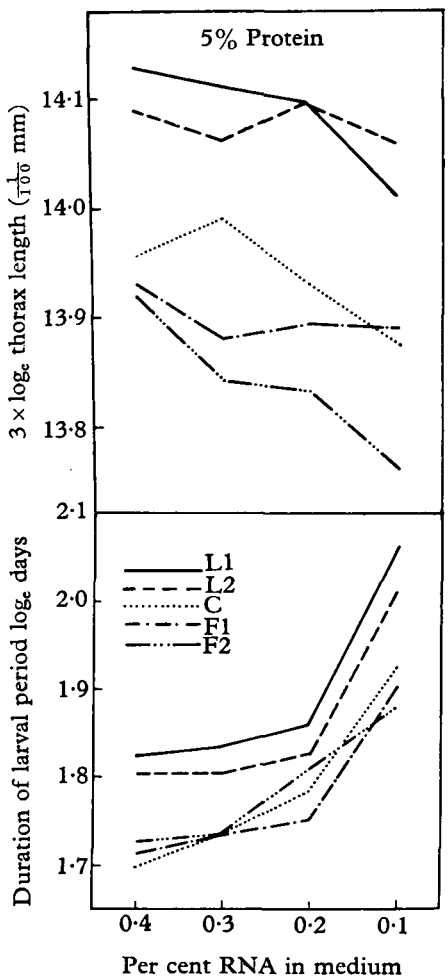

Fig. 3. The effects of varying the concentration of ribonucleic acid in the larval diet on the growth of lines selected for larger size or shorter development time on axenic diets in which the ribonucleic acid concentration is mildly inadequate (L_1,F_1), or very inadequate (L_2, F_2).

at 0·2% and the rate of change is less thereafter. In the two large lines the inflection point occurs at the same 0·2% level, and, although further increase tends to reduce development time, the effect of increasing the RNA beyond this point is relatively less in the two large lines. In the fast lines there is some evidence of difference in that one line shows little sign of an inflection point, but in the other it occurs at the same level as the rest.

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With respect to body-size, comparison between controls and one of the lines selected on low RNA (0·3%) level, there is some evidence of difference since the inflection point is at 0·2% in the selected line compared with 0·3% in the control, while in the line selected on low RNA reduction from 0·4 to 0·1% has hardly any effect on body-size. This might be taken as evidence for the specific effects of selection for a larger size on this medium, but it is improbable that this is the explanation since the fast line, selected on the same medium and which has become smaller in size, also shows a similar indifference to a comparable reduction in RNA. Perhaps in both lines there has been parallel selection for survival on these unfavourable conditions, and the independence of body-size reflects a pleiotropic effect of changes which influence survival. The other fast line is much more sensitive to RNA reduction.

Table 5. *A test of gene environment interaction for the unselected and large lines when grown on the media with five different levels of adenosine at each of four different levels of pyrimidine*

Concentration of pyrimidines (g./l.)	d.f.	Interaction mean square	p
		BODY-SIZE	
0·1	8	24	$< 0{\cdot}01 > 0{\cdot}001$
0·6	8	7	$> 0{\cdot}05$
0·8	8	6	$> 0{\cdot}05$
1·2	8	21	$< 0{\cdot}05 > 0{\cdot}01$
		LARVAL PERIOD	
0·1	8	40	$< 0{\cdot}01 > 0{\cdot}001$
0·6	8	8	$> 0{\cdot}05$
0·8	8	9	$> 0{\cdot}05$
1·2	8	26	$< 0{\cdot}01 > 0{\cdot}001$

As a further check, the variation in reaction to constituent nucleotides was studied since it is possible that differences in the reaction to puridine/pyrimidine ratio may have occurred. To test this the concentrations of both purines and pyrimidines were varied and the performances of the same large and fast lines were compared with that of the unselected population. The bases were supplied as nucleosides except in the case of cytidilic acid where allowance was made for the molecular weight differences between nucleotide and nucleoside. Purines were supplied in the form of adenosine, since guanylic was found to have only a small effect on size of the larval period and showed no interaction with the concentration of the rest of the nucleotides. Since Sang (1959) has shown that the normal requirement for RNA is essentially a requirement for adenylic acid, adenosine was supplied at the rate of 0·1, 0·5, 0·75, 1·0 and 1·5 g/l. of medium and pyrimidines at the rate of 0·1, 0·6, 0·8 and 1·2 g./l. So a much wider range of variation of purine/pyrimidine ratio was studied than is ever likely to be encountered in nature. This test was carried out

in two parts. The two large lines were compared with the unselected population separately from the test in which the two fast and unselected were compared. The results of these tests are set out in Tables 5 and 6 respectively. With respect to the comparisons between the large and unselected lines, at intermediate levels of pyrimidine there is no evidence of heterogeneity between the strains in their reaction to the variation in the purine from 0·1 to 0·5 g./l. for either body-size or larval period,

Table 6. *A test of gene environment interaction for the unselected and fast lines when grown on media with different levels of adenosine at each of three different levels of pyrimidine*

Concentration of pyrimidine (g./l.)	d.f.	Interaction mean square	p
		BODY-SIZE	
0·1	4	23	$< 0{\cdot}05 > 0{\cdot}01$
0·6	4	11	$< 0{\cdot}05 > 0{\cdot}01$
1·2	4	5	$> 0{\cdot}05$
		LARVAL PERIOD	
0·1	4	22	$< 0{\cdot}05 > 0{\cdot}01$
0·6	4	7	$> 0{\cdot}05$
1·2	4	20	$< 0{\cdot}05 > 0{\cdot}01$

thus confirming the early evidence that selection has not affected the intrinsic ability to synthesize adenylic acid. But at extreme levels of pyrimidine there is significant heterogeneity and this demonstrates how gene-environment interaction is likely to occur or be enhanced when the concentration of an important nutrient departs sufficiently from the optimum level, but, since the interaction is restricted to extreme ratios, it is not likely to be of importance naturally. A similar test on the fast lines, with fewer levels of adenosine, led to essentially similar results except that there was some evidence of interaction at the intermediate pyrimidine level as well.

4. DISCUSSION

The results of the various effects of selection on the different diets, combined with the other evidence on larval growth rates has led to a much better understanding of the processes which are responsible for both the variation of body-size and its stability. The dynamic environmentally caused variation in body-size, which is correlated with the larval period, probably represents variation in the potentially exponential phase of larval growth (Royes & Robertson, in press) which can also be extended by selection on low RNA diets without change in the early growth rate (Robertson, 1963). Presumably any individual of any genotype has the capacity to respond to particular differences in diet in this way, but the nature and magnitude of the response, whether nil, positive or negative is genetically determined. The best example of this was provided by the two small lines selected on either low RNA or low protein which responded in precisely opposite ways to the same change of environmental conditions. Since one became relatively smaller and had a

shorter larval period and the other became relatively larger and took longer, it might be expected that similar changes will be produced by selection, but this is only so under rather special conditions. On favourable diets a two-way selection for large or small size is associated with either none or, at most, a small tendency for larger size to be associated with longer larval period. On low protein or diluted media or media deficient in choline there is no trace of this kind of change even though selection leads to a great increase in body-size. The precise nature of natural sub-optimal conditions are unknown, but present evidence suggests that low protein is the chief limiting factor of the diet. Supplementing crowded live yeast media with either RNA or protein or both together showed that addition of RNA alone leads to a small improvement in growth rate and body-size but when protein is added, although not to a level sufficient to allow maximum size, then addition of RNA has no effect at all (Robertson, unpublished). We can suggest therefore that a diet in which RNA, especially adenylic acid, is the sole limiting factor represents a novel situation to which the species is not adapted. The inability of *D. melanogaster* to synthesize RNA at a sufficient rate for rapid growth and consequent need for an exogenous supply is further evidence that RNA shortage is not likely to be an important factor in the normal diet.

It follows from these observations that that genetic variation in body-size, which is correlated with the larval period, is relatively unimportant in the genetic variation of body-size generally. When exposed to different environmental conditions, which lead to increased or decreased size by extension or contraction of the first phase of growth, individuals of adapted populations are unlikely to differ much since the process of adaptation will confer similarity of response in this respect, i.e. gene-environment interaction will be unimportant. Earlier comparisons between unselected populations with large or small lines selected on the live yeast medium are consistent with this view (Robertson 1960*a*). Nevertheless, the gene pool of the population includes abundant variation which can increase or decrease the exponential phase of growth in a particular environment and can also influence the magnitude and direction of such changes when the environment is suitably altered.

Such restriction of the effects of genetic segregation in favour of phenotypic uniformity meets Waddington's (see Waddington 1957) definition of canalization. Thus, the classic quantitative character body-size—which at first sight appears to be completely uncanalized, as is indeed the case in certain attributes to be considered later, is highly canalized with respect to the growth effected in the exponential phase of larval growth whose end is marked by attainment of the ability to pupate when removed from food. In other words, the point in growth at which a decisive shift in the hormonal relations probably takes place, is canalized as far as the expression of genetic variation is concerned. Such canalization is dynamic since particular conditions can influence the absolute larval weight at which the shift occurs, leading to correlated variation between body-size and the duration of larval life—but individuals of an adapted population are likely to be alike in their response.

Such canalization is naturally effective for the conditions to which the species is

adapted and if these are sufficiently altered it is likely that the canalization will no longer be effective and normally undetectable segregation will now contribute to the variance and be accessible to selection. Some kinds of atypical environment will be more effective in this respect than others and, in the present instance, deficiency of RNA appears to be of such a kind.

Further support for this interpretation is provided by the conditional nature of such changes which are correlated with larval life, in the more recent selection on low RNA. Although body size and larval period were about 10% bigger and longer respectively on the synthetic medium, culture on live yeast diets, crowded or uncrowded, eliminated this difference entirely. But in the earlier tests, in which the comparisons were carried out at a relatively later stage of selection, this was not so and the changes produced by selection were remarkably stable to a wide range of diets. This suggests that continued selection in an environment, which favours gene expression for the character selected, will often raise the expression to a level at which the effect remains even when the particular stimulus, in this case, low RNA, is removed. This recalls the genetic assimilation of cross-vinless (Waddington, 1953), in which heat shock was the stimulus which exposed concealed genetic variation to selection. This phenomenon appears to be quite general and, as Robertson & Reeve (1952) pointed out, there is a wide range of potential developmental change latent in any variable population (see also Rendel, 1959). Particular developmental changes could be effected if only we knew sufficient about the environmental conditions and treatments most propitious for revealing the variation required. The relevance of this to applied problems is obvious.

The greater part of the genetic variation in body-size involves effects which are uncorrelated with development time and probably represent variation in growth in the post-critical phase after the duration of the larval period has been determined. There is evidence for considerable differences in metabolism between the earlier and later phases of growth since larval life can be greatly prolonged by some adverse diets, e.g. by adding excess of certain amino-acids, without any reduction in body-size, but with more drastic changes both characters vary together and this results in a negative correlation between them. Specification of the conditions in which such independence between later and earlier growth is permissible would be of great interest, since it would shed light on the physiological effects of the genetic differences which are responsible for the familiar variation at the later stage.

It appears likely that variation in amino-acid metabolism is of major importance since there was clear evidence of this in the reaction of the large line, selected on low casein, which is probably a particularly favourable diet for detecting genetic differences in amino-acid metabolism generally. In this case selection had altered the reaction to diets supplemented with different amino-acids with respect to body-size but not the duration of the larval period. Although the same amino-acids will be used in both stages of growth the metabolic relations are sufficiently different, perhaps because the hormonal situation also differs, to allow considerable independence in reaction to the same kind of nutritional change. It is not surprising, therefore, that we should find evidence of similar genetic independence.

What the metabolic relations are between changes which delay or hasten the hormonal shift and those which influence growth rate are at present obscure. It is clear that substantial extension can occur without any disturbance of the intrinsic growth rate, i.e. metabolism generally is unaffected. So far we cannot specify the conditions which will consistently increase or decrease body-size with correlated changes in the larval period although many unpublished experiments have been carried out with this in mind and continued efforts to do so are in progress. General experience suggests that the genetic changes which affect the duration of the exponential phase of growth and the point at which the change in hormonal balance occurs, to set the stage for eventual differentiation, are such as to regulate metabolism generally compared with the more restricted changes in particular metabolic pathways which alter body-size but have no effect on the duration of the larval period. It also appears that the nutritional conditions which favour phenotypic expression of the former at the same time diminish the expression of the latter since there is not much evidence that size can be increased by extending the growing period and increasing the later growth rate at the same time. Deficiency of RNA, especially adenylic acid, may exert a limiting effect on metabolism generally and, at the same time, may restrict the scope for developing alternative pathways in the amino-acid metabolism of the later stages of growth. Hence the expression of genetic variation in this latter respect, or of additive effects in particular, is diminished. This possibility, together with the canalization effect on relatively high RNA media, could account for the different developmental changes due to selection on the two media and for the contrasts between these lines in their gene-environment interaction.

It follows from this argument that, in *D. melanogaster*, the genetic variation in body-size, which accounts for about half the phenotypic variance under optimal conditions (Robertson, 1959), is composed of effects which are uncorrelated with the duration of the larval period, which have little or no effect on the exacting requirements of larval growth in the exponential phase and which chiefly reflect variation in amino-acid metabolism in the latter stages of growth. Underlying this variation is the canalized growth to the critical stage of larval life which determines the duration of the larval period. Stabilisation of the growth which must take place before the post-critical phase can be entered on lessens the need for relying exclusively on the inverse relations between deviation from the mean and fitness as the origin of long-term stability in body-size. Dynamic canalization of the exponential phase of larval growth sets a limit to the potential growth in the later stage whose duration is apparently independent of diet. Experience to date suggests that growth rate in this stage is equilibriated at a level which minimizes gene-environment interaction and that the usual arguments relating fitness and deviation from the mean are applicable. But the point at which the mean is equilibrated will be greatly influenced by the growth effected in the earlier canalized phase, which is of primary importance in determining the fraction of total growth which will be independent of the duration of larval life.

This general interpretation has the merit of relating a great variety of evidence

to a single coherent scheme which provides a basis for further tests designed to take the analysis to a deeper level. It is tempting to suggest that the evidence for partial genetic independence, between different phases of growth, reflects the duality inherent in holometabolous insect development, whereby the proliferation of the imaginal discs proceeds at an accelerated tempo in the later stages of larval life. But we are also led to enquire whether other animals, such as mammals, which have so much in common with *Drosophila* with respect to the genetic properties of variation in body-size, will reveal further similarity in parallel evidence for stability of their growth relations.

SUMMARY

1. Similar changes in the body-size of *Drosophila melanogaster* have been achieved by different developmental pathways, especially either by altering the duration of the early exponential phase of larval growth or by influencing the growth rate in the phase which is independent of time.

2. Such changes have been effected by selecting in the same population for larger or smaller size or shorter development time on chemically defined media, deficient in alternative nutrients. Selection for larger size on media deficient in protein or choline does not involve correlated changes in the larval period, whereas selection on media deficient in RNA does. The evidence suggests that shortage of this nutrient may be uniquely favourable for promoting a correlated change between body-size and duration of the larval period.

3. Strains which differ in presence or absence of such correlation are characteristically different with respect to gene-environment interaction. In the former, the differences due to selection are generally more fully or completely expressed when the diet is changed whereas in the latter this is not so, and different, especially competitive condition, leads to a drastic reduction of the difference.

4. How far the expression of the differences due to selection are affected, when the diet is altered, is also influenced by how long selection has been carried out. In early generations, the difference is only or best expressed in the special conditions provided during selection, but later on the changes due to selection are either fully expressed or partly so, as noted above.

5. Many of the differences in gene-environment interaction between selected strains can be accounted for in terms of variation in the duration of the exponential phase. Thus two lines selected for small body-size on low RNA or low protein diets responded in different ways to the same nutritional change—one became relatively larger and took porportionately longer to develop, the other became relatively smaller and developed in a shorter time.

6. There is clear evidence from various tests in which the amino-acid composition of the diet has been altered, that the nutritional requirements in the two stages of growth are not identical and this is consistent with the evidence for considerable genetic independence as well.

7. It is proposed that the first stage of larval growth, which principally determines

the duration of the larval period and may also influence body-size, is canalized. Genetic variation which can influence this stage is present in the population but contributes little to the phenotypic variation of adult size, except under special nutritional conditions as when ribonucleic acid is the sole limiting nutrient. But, at the same time, such canalization is dynamic in the sense that the absolute amount of growth which is completed in the first stage may vary with respect to diet and thereby lead to correlated variation in the duration of larval life and adult size. But individuals of an adapted population behave alike in this respect so that gene-environment interaction which leads to correlated variation in the two characters is of a very low order.

8. The canalized phase sets a limit to the potential growth in the later stage and thereby influences greatly the mean value about which such growth is equilibrated. This canalization plays a major role in the general stability of growth relations and body-size although this is normally concealed by the high level of phenotypic variation. This interpretation can account for a great variety of data and provides a rational guide to further analysis.

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