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The ecological genetics of growth in Drosophila

6. THE GENETIC CORRELATION BETWEEN THE DURATION OF THE LARVAL PERIOD AND BODY SIZE IN RELATION TO LARVAL DIET.

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1. INTRODUCTION

The magnitude and properties of the genetic correlation between adult size and the duration of the period of growth in an animal like *Drosophila* are relevant to the influence of natural selection on mean body-size since the duration of the larval period is probably an important component of fitness. Such information is also relevant to the developmental origin of phenotypic differences in a quantitative character like body-size and how far different kinds of developmental change can be shown to contribute to apparently continuous differences. Such analysis must take account of environmental variation, especially nutritional variation. Earlier work (Robertson, 1960*a*, *b*, *c*) has demonstrated abundant genetic variation in populations of *Drosophila melanogaster* which can influence growth on certain diets, so we have some guide to the kind of situation which may be of most interest. The present paper, and the succeeding one in this series, are concerned with an association between the presence or absence of correlated change, when either body-size or development time is selected for, according to the nature of the diet which is provided during the selection.

Earlier experiments have provided estimates of the genetic variance for both body-size and duration of the larval period (Robertson, 1957). These were derived from comparisons between the phenotypic variance of individuals from a laboratory population and from the genetically uniform cross between inbred lines derived from it. These tests, carried out on the unrestricted live-yeast medium, suggested that more than half the total variance in either character is genetic, and later experience has suggested that this figure is about right for other populations as well. But in spite of the statistical similarity of the variance estimates the genetic behaviour of the two characters is, of course, quite different. For body-size selection is immediately effective in either direction (Robertson, 1955), whereas for the larval period all who have worked for this character are agreed (Sang & Clayton, 1957; Clarke, Maynard Smith & Sondhi, 1961) that selection for shorter time under the usual conditions of culture is ineffective, although Sang (1962) has recently reported successful selection for shorter periods in a synthetic population derived from crosses between inbred lines on certain sub-optimal diets. Selection for longer time is generally effective.

With respect to correlated behaviour between the two characters we encounter all possible relations in different environmental situations. Thus, many tests have shown (Robertson, 1959, 1960*a*) that there is a well-marked tendency for body-size to be maintained at a fairly constant level on sub-optimal diets which may cause a great extension of the larval period. The correlation here is nil. But, if the quantity and quality of the diet becomes too inadequate body-size is reduced as well, so that progressive worsening of the diet leads to a negative correlation between size and development time which approaches unity on a log scale. It has also been shown (Robertson, 1960*a*) that certain kinds of nutritional variation can cause extension of the larval period and roughly proportional increase in body-size, i.e. a high positive correlation. The origin of this relationship has been further examined and will be considered later.

Since particular changes in the composition of the diet can determine the presence or absence and sign of the correlated changes, it may be inferred that suitable genetic changes are potentially capable of doing likewise. But the requirements of adaptation are likely to have imposed restrictions on the kinds of change which can be caused by the freely segregating variation in the population and this will determine the degree and sign of the correlation when selection is applied to either character in different environmental conditions. Controlled variation of the composition of the larval diet provides an essential tool for examining the properties of these restrictions and their significance in adaptation generally.

The published evidence on genetic correlation between body-size and larval period in *Drosophila* is insufficient to draw any useful conclusions. General experience has suggested the tendency for development time to be a little longer in strains selected for larger size and this is in line with evidence of positive correlation from progeny tests (Reeve, 1954) and from estimates of genetic and environmental variance and co-variance (Robertson, 1957).

2. MATERIALS AND METHODS

The flies used in most of these experiments were derived from the cage population known as *Pacific*. Some data are also included from experiments carried out on the *Crianlarich* population which has been kept as a large population in bottles. Presence of absence of correlation has been followed with repeated selection for larger or smaller body-size or faster or slower development time on several diets, which include either the unrestricted live-yeast medium or different, generally sub-optimal aseptic, synthetic diets made up by modifying Sang's Medium C (1956). Most commonly the limiting factor has been either protein or RNA, since these two types of nutrient are probably the commonest deficiencies in nature or laboratory. The general procedure for aseptic culture is such experiments has been described elsewhere (Robertson, 1960*a*).

After at least five generations of selection the strains and the unselected population have been grown on various media to see how far the phenotypic expression of differences, caused by selection, are influenced by particular nutritional changes.

The evidence on this point will be given in the succeeding paper, while the present one will be restricted to the main features of the selection responses.

As in earlier papers, body-size refers to the measurement of thorax length and the values are transformed to three times the natural logarithm of thorax length in $\frac{1}{100}$ mm. Methods of scoring live flies are the same as those described by Robertson & Reeve (1952). Larval development time is estimated by subtracting the average duration of the pupal period (4·3 days) from the total duration of development and is expressed as log days. In the experiments with the *Crianlarich* stock, the larval period was measured directly by noting the time of eversion of the anterior spiracles. Multiplying by 100 roughly converts differences between means to percentages. Variances have been multiplied by 10^4 to make comparisons easier. For any given treatment generally three to four replicated cultures have been set up for each genotype and eight to ten females per culture were measured. Records of development time included all the females which hatched. Sometimes there were fewer flies than ten per culture, due to the hazards of sampling and to a lower viability with certain diets, but in most cases the numbers are quite adequate for reliable comparisons. The occasional infected culture was discarded from tests with the synthetic medium. Tests of significance are based on estimates of error variance which are derived from estimates of within- and between-culture effects, pooled over the appropriate series. All experiments have been carried out at 25° C. The live-yeast medium refers generally to the usual yeast fortified cornmeal molasses medium, but three selection tests were carried out on a medium recommended by Sang in which a thick suspension of yeast is autoclaved along with salts and agar.

For either character mass selection has been used. For body-size, generally fifteen pairs of parents were selected from fifty pairs each generation, representing ten pairs from each of five tubes. For development time, the same number of parents were selected from all the flies which hatched—100–125 pairs.

During the selection experiments on the low RNA medium great difficulty was encountered for a time in preparing viable cultures or, at least, cultures with the usual rate of growth. This meant that selection had to be relaxed for a generation on several occasions. This unexpected difficulty was eventually traced to a change in the solubility of the casein in the medium which was in turn due to a change in the place of manufacture to a district with a more acid water supply. By raising slightly the pH of the medium this difficulty was eventually overcome.

3. EXPERIMENTAL RESULTS

(i) *The Crianlarich population*

Evidence on the relations between body-size and development time in the Crianlich population is available from three different experiments, two of which have been already described, but which acquire added significance when compared with the third additional test. In all these experiments larvae have been grown individually in small tubes. In the first experiment, summarized by Reeve (1954), parents and progeny were scored for both characters and the faster and slower

group of parents were retained to provide a number of pair-matings. The original data were given in terms of total development time and the linear dimension of thorax length. Expressing the values in terms of larval period and the cubic measure of size, the two groups of parents differed on average by about 20% in larval development time and by about 6% in body-size. The two groups of progeny differed by 4–5% in development time and about 3% in size, from which it might be inferred that the heritability of development time was of the order of 30%, and that the regression of size on development time, due to predominantly additive genetic effects, was rather high.

In the second set of data (Robertson, 1957), estimates of total variance and co-variance due to genetic segregation were derived by subtracting the variance between individuals of the population from the genetically uniform F_1 of crosses between inbred lines. At least 50% of the variance of either character was genetic, while the regression of size on development time, due to purely genetic effects,was about 0·4. This refers to total genetic variance, while the progeny test measure primarily additive effects. Thus, the two rather different kinds of experiment agree in suggesting that there is an appreciable genetic correlation between size and larval period, which might, on a simple view, be taken to imply that selection for either would produce some change in the other.

Table 1. *Deviations from unselected of Crianlarich strains*

Genotype	Body-size (3 × log thorax length)	Larval period (log days)	*N*
Large line	0·14**	0·06	69
Small line	−0·14**	0·01	58
Cross—reciprocals combined	0·03	0·03	75

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

To test this inference mass selection for large and also small body-size was carried out, and after five generations the selected lines were compared with the unselected controls. Table 1 shows that large and small strains each deviate some 14% from the original population in body-size. In development time the average for the unselected and small strains almost coincide, while, although the mean of the large line exceeds the unselected by 6%, this difference is statistically quite insignificant. There is, however, some doubt about the validity of this comparison since the variance in development time among individuals of the large strain is several times greater than that of the unselected or small strain (Table 2). This increased variance also appears in one of the crosses in which the female parent is from the large strain. For unknown reasons, eggs laid by females of this strain appear to give rise to a proportion of individuals with unexpectedly longer development time. There is no evidence of such a presumably maternal effect on body-size, either in the mean or the variance. This heterogeneity of variance detracts from the genetic significance of the 6% difference in development time between the means. So, in spite of strong

Table 2. *Variance of body-size and larval period* ($\times 10^4$) *of Crianlarich strains*

Genotype	Body-size	Larval period	d.f.
Unselected	42	10	82
Large iine	4i	63	68
Small line	35	22	57
Cross:			
S × L	25	73	25
L × S	20	35	48

indications of genetic correlation in the tests on the unselected population, selection for size in either direction fails to demonstrate a comparable correlated change in development time. It may be significant, in view of what follows, that in the tests on the unselected population, larvae were grown individually in small tubes, whereas in the selection experiments they were grown together in ordinary vials. The larval nutrition may have been rather different in the two situations.

Table 3. *The phenotypic relations between body-size and development time in the Crianlarich strains and crosses*

Genotype	Correlation coefficient	Regression coefficient S on D	d.f.
Unselected	0·13	0·26	82
Large line	0·17	0·14	68
Small line	−0·54**	−0·68	57
Cross—reciprocals combined	0·41**	0·27	73

S and D refer respectively to body-size and duration of the larval period.
**indicates significance at the 0·01 level of probability.

The phenotypic correlation between the two characters is of interest; the values are shown in Table 3 along with the coefficients of regression of size on development time. In the unselected and large strains, the correlations, although both positive, are not statistically significant. In the small strain there is a highly significant negative correlation, i.e. smaller individuals tend to take longer to develop, while in the reciprocal crosses the correlation is positive and highly significant. The regression coefficients are of the same order for the unselected population, the large strain and the crosses. The striking discrepancy on the part of the small strain may arise because selection for small size not only favours genotypes which behave more or less additively with respect to both genetic background and diet, but will also include gene-gene and gene-environment interactions which are sufficiently adverse to diminish size and slow down larval growth and thereby contribute to a negative correlation between the two characters. Such effects generally behave as recessive or hypostatic in crosses to unselected populations (Robertson, 1955, 1962*a*) and if

they make an important contribution to the total variance, this could generate a negative correlation between the two characters in the small strain. In the cross to the large strain we might anticipate, and indeed find, a return to positive correlation. The appreciably higher positive correlation in the F_1 compared with the unselected population, implies some change in the composition of the variance with respect to effects which influence body-size alone or the duration of the larval period as well.

4. THE PACIFIC POPULATION

(i) *Selection for body-size*

It is convenient to describe in two groups the experiments in which selection for body-size on different diets led to corresponding differences in the evidence for genetic correlation between size and the duration of the larval period. In the first group the diet was either the usual unrestricted cornmeal molasses medium or the yeast-enriched gel referred to earlier. Data are also included from earlier experiments on the usual medium (Robertson, 1960*a*), or on low protein or diluted aseptic media (Robertson, 1960*b*, *c*); together they provide a series of repeated tests on the same population. In the second group of experiments, selection was carried out on media deficient in RNA, an essential nutrient for maximum growth rate.

Table 4. *The effects of selecting for large body-size in the Pacific population on live-yeast media or on media with relatively high RNA/protein content*

	Deviation from unselected	
Selection	Body-size (3 × log thorax length)	Larval period (log days)
	Live yeast	
Large 1	0·12**	0·02
Large 2	0·14**	0·06*
Large 3	0·07**	0·00
Large 4	0·11**	−0·07*
Large 5	0·09**	−0·01
Small	−0·17**	0·01
	High RNA/low protein	
Large 1	0·18**	0·00
Large 2	0·22**	0·02

Dealing with the first group, in which RNA is unlikely to be an important limiting factor in growth, we can look for evidence of genetic correlation between the two characters among this array of lines. The comparisons of deviations from unselected were carried out after five to seven generations of selection. Table 4 sets out the relevant comparisons. Of the five large strains selected on the live-yeast medium, one shows a significant positive and another a significant negative deviation for development time, and the other three hardly differ from the average of

the unselected. This is also true of a small line in which a 17% reduction in size involves no change in development time. This conclusion is also supported by the effects of selecting on the deficient synthetic media, in which either protein is reduced (line 1), or all nutrients are reduced to one-third the standard concentration (line 2). A 20% deviation from the unselected in body-size leaves development time unchanged. So, on this fairly extensive body of evidence, we would conclude that genetic variation in either character is due to independent effects and that occasional instances of positive or negative correlation could be referred to sampling or linkage effects rather than pleiotropy.

(ii) *Selection for development time*

Here we have several experiments carried out at different times on the *Pacific* population. In one test selection for slow development time was carried out on the usual live-yeast medium and in the others selection for either faster or slower development time was carried out on an aseptic medium in which fructose was omitted. Such a diet is equivalent to a mildly protein-deficient one, since addition of fructose has a sparing effect on protein. The results are summarized in Table 5. Selection for faster development time is ineffective as expected, and so the lack of change in body-size requires no comment. Selection for slower larval period is effective on both media and leaves average body-size unchanged. Thus, these observations agree with the other evidence from the lines selected for body-size, in failing to demonstrate genetic correlation between the two characters.

Table 5. *The effects of selecting in the Pacific population for slower or faster larval period on the live-yeast medium or on a medium with high RNA content*

	Deviations from unselected	
Selection	Body-size	Larval period
	Live yeast	
Slow	0·01	0·04**
	High RNA/No fructose	
Fast	0·03	−0·01
Slow	0·00	0·10**

(iii) *Selection on low RNA media*

Since deficiency of protein, including amino-acid imbalance, and RNA are probably the commonest limiting factors in the usual diet, and since genetic reaction to protein deficiency has already been studied to some extent, the next logical step is to see whether the deficiency of RNA resembles or differs from that of protein with respect to genetic behaviour of the two characters. Selection for larger size has been carried out in two replicated lines on each of two levels of RNA deficiency, one in

which the medium is mildly deficient (0·3% RNA), the other severely deficient (0·075%), compared with the level of 0·35–0·40% required for maximum growth rate. In addition, selection for faster development time was carried out in one line on each of the two media. The lines are labelled in the following way: HF and LF refer to the lines selected for faster development time on the higher (HF) or lower (LF) RNA level while HA, HB refer to the lines selected for larger size on the higher RNA level and LA, LB stand for the lines selected on the medium with the lower level. The effects of selection for body-size will be considered first.

Table 6. *The response to selection for large body-size in the Pacific population on media deficient in RNA*

	Deviations from unselected							
	0·30% RNA				0·075% RNA			
	HA		HB		LA		LB	
Generations averaged	S	D	S	D	S	D	S	D
1–5	0·07	0·07	0·08	0·04	0·14	0·04	0·08	0·03
6–10	0·11	0·11	0·14	0·09	0·18	0·13	0·10	0·02
11–13	0·17	0·10	0·17	0·10	0·17	0·13	0·14	0·06
Regression coefficients S on D	1·28		1·58		1·11		2·10	

S and D refer to body-size and duration of the larval period.
HA and HB refer to lines selected for large size on 0·30% RNA.
LA and LB refer to lines selected on 0·075% RNA.

The results are set out in Table 6 for each of the four lines, as average values for generations 1–5, 6–10, 11–13.

The results are in striking contrast to those set out in Table 4, since there is a well-defined increase in development time in all lines, and in three of them (lines HA, HB and LA) the proportional increase in development time is of the same order as that in body-size. For these three lines the regression of size on development time, derived from the three average values, including, of course, the zero deviation corresponding to the unselected population, works out at respectively 1·28, 1·58 and 1·11. There is little evidence that the level of RNA deficiency has much effect on the nature of the changes. It is instructive to compare these values in Table 6 with those in Table 4, which include the effects of selection for large size also on synthetic media since approximately 20% increase in body-size was unaccompanied by change in development time. The principal difference in the two series of experiments is the ratio of RNA to protein in the diet, and this apparently determines whether or not body-size and development time are correlated in the response to selection for larger size.

(iv) *Selection for faster development time*

The results of selection for faster development time on the two media with different levels of RNA are summarized in Table 7 and Fig. 1, which shows the effects on body-size which was not scored after generation 7. The figure shows that selection for faster development time led in both cases to an immediate decline in body-size. Most of the effects of selection were achieved by generations 3–4 by which time the reduction in body size was equivalent to 1–2 standard deviations, calculated from the within-culture variance of body-size on favourable diets. But

Table 7. *The response to selection for faster development time in the Pacific population on media deficient in RNA*

	Deviations for unselected			
	0·30% RNA		0·075% RNA	
Generations averaged	S	D	S	D
1–5	−0·07**	−0·02	−0·07**	0·00
6–10	—	0·03	—	−0·08*
11–13	—	0·00	—	−0·07*

S and D refer respectively to body-size and duration of the larval period.
* and ** indicate significance at the 0·05 and 0·01 level of probability.

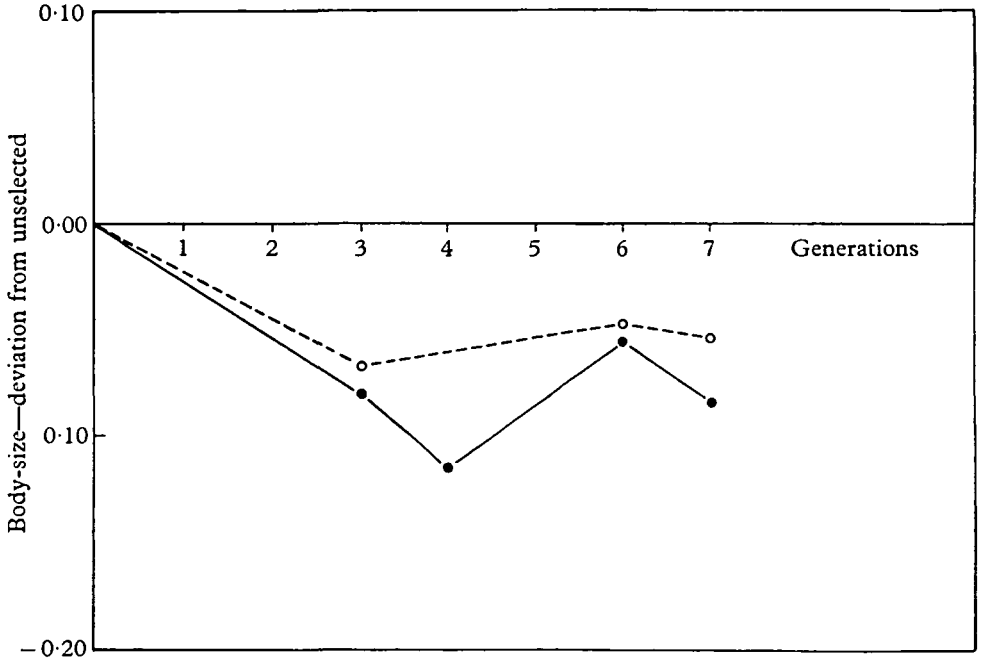

Fig. 1. The effects on body-size of selecting for shorter larval period on media with mild (unbroken line) or more extreme deficiency of RNA (broken line).

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comparison of Table 7 and Fig. 1 reveals an apparent anomaly in that there is comparatively little or no average change in development time in HF and only in later generations of LF are the average deviations statistically significant. Lines selected for faster development time appear to be especially susceptible to minor uncontrollable environmental variance which is reflected in inconsistency of performance. Data will be given in a later paper showing that when these lines are reared on a variety of different diets there is no doubt that, on average, both lines develop faster than the controls although there are striking differences in reaction to different diets. The relative insolubility of the casein, noted earlier, may have affected the expression of differences in development time but not body-size. A further possible explanation for the apparent anomaly will be given in the discussion.

PHYSIOLOGICAL EVIDENCE

(i) *General*

Apparently, according to the nature of the larval diet, selection may either alter the larval growth rate or change the duration of the growing period. If this is so, genetic variation among the individuals of a population is compounded of these two kinds of physiologically different effects; and environmental, especially nutritional, differences may alter their relative contribution to the total variance in such a way that similar selection leads predominantly to one or other type of change if the diets differ appropriately. If both kinds of effects contribute to the variance on the ordinary live-yeast medium, this could account for the low but recurring evidence of positive correlation. Since selection on this medium provides so little evidence of correlation, this suggests that such a medium is effectively more like one with a relatively high RNA content. But, before we can usefully discuss the implications of the contrasts in selection response, we need to know whether there is any direct physiological evidence to support the distinction between differences in growth rate and differences in growing period.

(ii) *Stages of larval growth*

It has long been known (Beadle, Tatum & Clancy, 1938) that larvae must be allowed to feed for a certain period before they acquire the capacity to pupate and differentiate. We also know that there is a general tendency for adult body-size to be held constant on sub-optimal diets which lead to a considerable extension of the larval period. These two observations suggest the questions: (1) At what stage in larval development is this capacity to maintain a normal size lost?—and (2) What relation does this bear to the duration of the larval period? The following simple experiments provide an answer.

Larvae of the *Pacific* population were grown under restricted, i.e. crowded conditions which reduced body-size and greatly extended the larval period. At daily intervals larvae were removed, weighed and transferred to favourable conditions in which each larva was grown individually on well-yeasted medium. One group of

larvae was kept on this medium all the time, while another group was allowed to reach the adult state on the restricted media. Adult flies were scored for size and development time and compared with the flies kept throughout larval life under one or other set of conditions. Such tests have been repeated several times with essentially the same results. A typical set of data is summarized in Figs. 2 and 3. Figure 4 illustrates a similar test in which unfavourable conditions were provided by growing larvae on a highly sub-optimal synthetic diet.

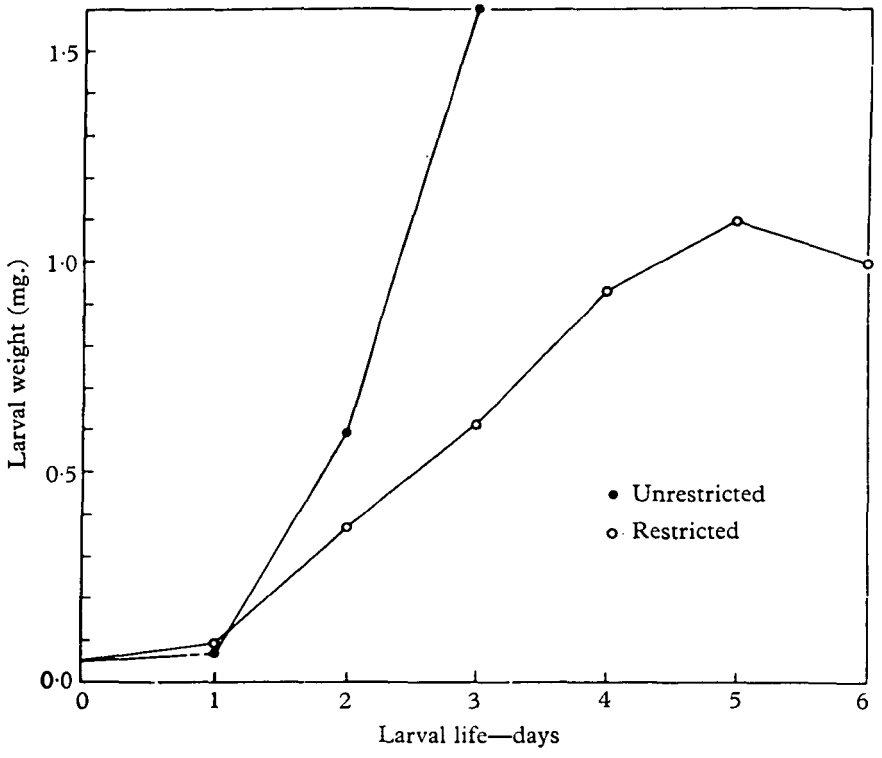

Fig. 2. Larval growth rate on restricted, i.e. crowded, and unrestricted live-yeast media.

Figure 2 shows the average weight of female larvae grown on the unrestricted yeast diet and on the crowded media, while Fig. 4 shows the corresponding comparisons for the low protein series. From Fig. 3 we can see that up to day 4, larvae still retain their ability to achieve more or less normal size, although the total development time is progressively longer. After this time the capacity is lost, and the longer they are kept on this crowded medium the smaller their final size although the development time is not further increased. Flies transferred at days 4, 5, 6 or not transferred at all have the same average development time although they differ greatly in body-size. A couple of qualifications can be noted. The fact that flies from larvae transferred after two and three days on the crowded medium hatch at

the same time can be attributed to heterogeneity between cultures which is difficult to avoid under crowded conditions, i.e. the three-day group happen to come from more adversely affected cultures than the two-day-old group. Secondly, flies from the larvae transferred on days 2, 3 and 4 do not grow quite so big as the controls, but are some 10% smaller; this will be considered shortly. Figure 4 shows a similar result when larvae are transferred at intervals from the highly sub-optimal synthetic

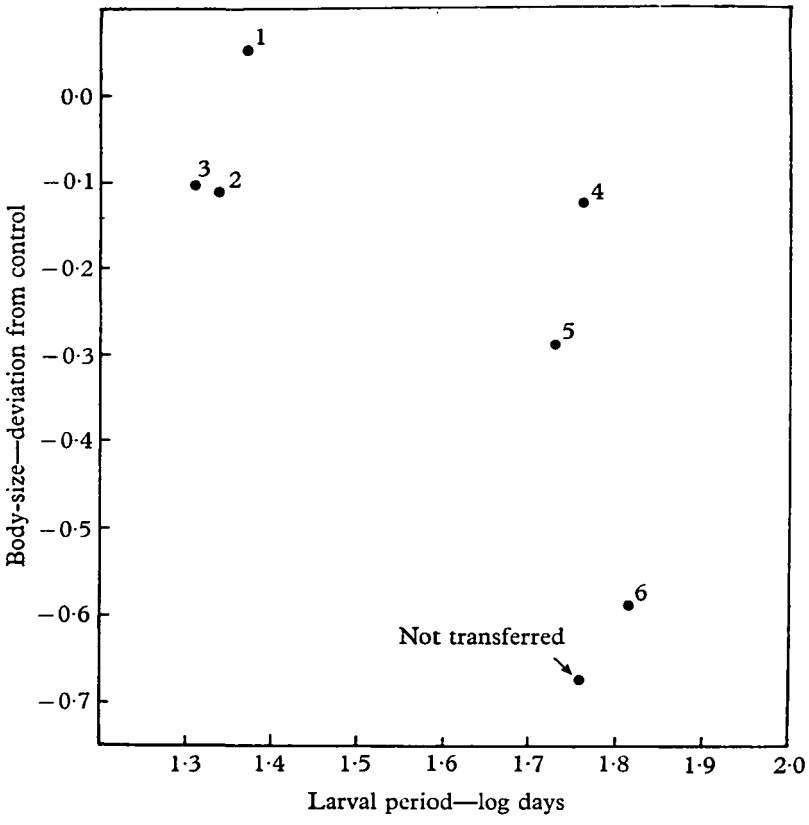

Fig. 3. The effects on adult body-size of transferring larvae at successive times from restricted to unrestricted live-yeast media, compared with the size of flies reared on the latter throughout larval life. The numbers refer to the age of the larvae in days when they were transferred.

diet, and demonstrates how greatly the larval period may be extended without appreciable reduction in adult size, which is close to the control level in this test.

These relations may be interpreted as follows. Larvae have to grow to a minimum size which corresponds to a critical stage in development early in instar 3 before they can enter on the next stage of larval growth. After it is reached the remaining time to pupation is more or less constant and so far it has not been possible to alter it by modifying larval diet. At the critical stage, larvae have grown to less than half their final maximum weight and so adult size is chiefly determined by the genetically

determined growth rate and the available food supply in this latter stage of larval life. If this is adequate the larva will attain its maximum size however adverse the early development, with the qualification noted above. Generally, when the diet is such as to prevent maximum growth in mass in the last larval stage it will be also

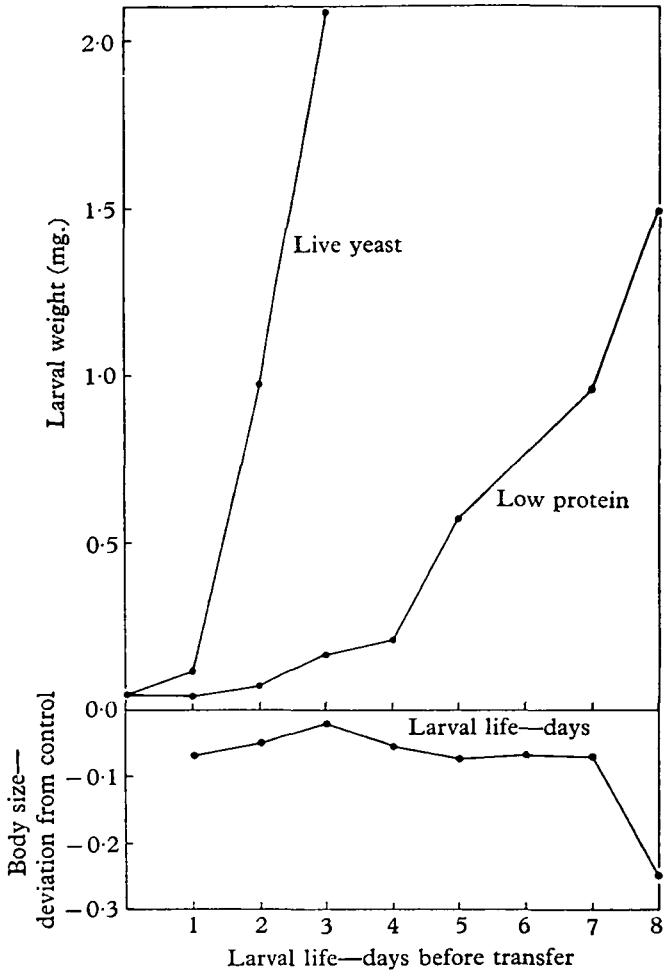

Fig. 4. Growth rate on a sub-optimal synthetic diet and the live-yeast medium (upper figure), together with the effects on body-size of transferring larvae from the sub-optimal to live-yeast medium on successive days.

insufficient to allow fastest growth to the critical period, so there will be a correlation between the extension of the larval period and the relative reduction of adult size. In the course of these tests Bakker (1959) published a valuable study of growth in *Drosophila* which agrees with these conclusions, although somewhat different methods were used.

We have further evidence in favour of these distinctions. Attainment or non-attainment of the critical stage is closely correlated with the ability of the larvae to

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pupate when they are removed from food to containers in which food is entirely absent. We can, therefore, test whether the large lines with the longer development time grow to a larger size before they can pupate. Several tests have been carried out on the relations between larval size, time and ability to pupate of the unselected population and on two of the large lines (HB and LA) selected on the low RNA diets. Figure 5 shows a representative set of data. Five sets of larvae from each strain

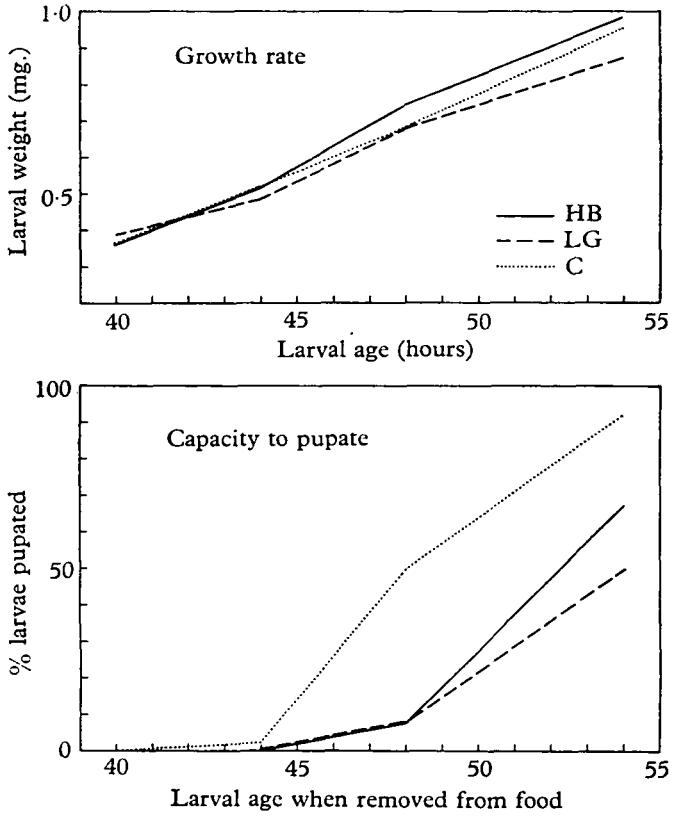

Fig. 5. Growth rate and attainment of the capacity to pupate, when removed from food, in larvae of the unselected population and large lines selected on low RNA media.

were weighed at respectively 40, 44, 54 and 58 hours of growth under favourable, unrestricted conditions. The larvae were then deprived of food and the number of pupae scored. Two points must be noted. Firstly, over the period which covers more than half the total larval period under favourable conditions, the rate of growth for the different strains is the same. The minor differences in the position of the points shown in the Figure are not statistically significant. Secondly, the critical stage is reached a good deal earlier in the unselected population than in either of the large strains which behave rather similarly. Thus, by 48 hours, 50% of the unselected larvae were able to pupate compared with about 8% for either of the

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large strains. By 54 hours over 90% of the unselected larvae could pupate compared with 68%, and 50% for the HB and LA lines respectively.

Comparison of the growth rate with the curve relating capacity to pupate and larval age (Fig. 5) shows that the weight is 30–35% greater for the selected than for the unselected individuals at the time when half the larvae can pupate.

As a working hypothesis we can infer that larval growth is divisible into two more or less distinct phases—growth to a critical period and growth from then until pupation—and that these two phases may be influenced independently by genetic changes. Hence body size may be altered either by increasing or decreasing the critical size, thereby effectively increasing or decreasing the period of growth or by changing the growth rate in the final stage of growth. Naturally when selection is

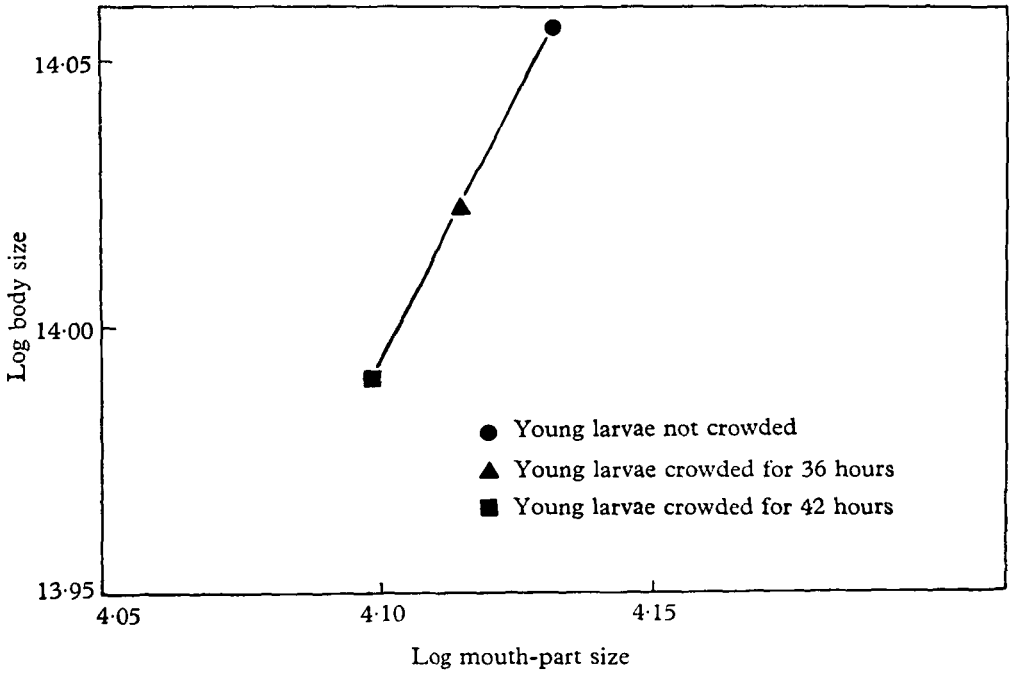

Figure 6. The correlation between adult body-size and the size of 3rd instar mouth-parts in larvae subjected to different degrees of starvation in 1st and 2nd instar.

for body-size alone both kinds of change may be selected for, but the contrasts in correlated response according to the nature of the diet suggest that selection has favoured different genes with distinct effects on development, and that environmental, especially nutritional, conditions influence the relative contribution to the variance of genes with such different effects and may have other effects as well which determine the degree of correlation. This hypothesis provides a basis for further analysis designed to evaluate the apparent genetic independence of the two kinds of change and their general genetic behaviour, and also the relevance of this distinction to the correlation of other characters with body-size.

Finally, we can deal with the minor exceptions to the rule that transference from a low to a high-plane diet before the critical period allows growth to maximum size. Repetition of tests like the ones shown in Fig. 3 produces flies which do not quite reach normal size, but are a few per cent smaller. This may have a simple explanation. As part of a separate study of early larval growth, the size of the 3rd instar mouth-parts have been recorded on larvae drawn at successive times from crowded cultures and transferred to unrestricted conditions. The mouth-parts were dissected from the puparium and measurements were taken from the tip to the posterior extremity of the plough-shaped part. Larvae were removed at 36 and 42 hours from the crowded media and their mouth-parts compared with those from an uncrowded control group. At 36 and 42 hours all larvae were still in the 2nd instar, and had reached 0.2–0.3 mg.

The results, shown in Fig. 5, show that early feeding conditions clearly influence both the mouth-part size and the final body-size. Also, within each group of larvae, there is a distinct tendency for the positive regression of adult size on larval mouth-part size to increase as the total exposure to adverse conditions increases, so that after 42 hours crowding the regression rises to the significant value of 0·64. These relations are shown in Table 8. So there is no doubt that early conditions of nutrition

Table 8. *The regression of log body-size on log larval mouth-part size in larvae exposed to different periods of crowding in early life*

	Uncrowded	Crowded 36 hours	Crowded 42 hours
Regression coefficient	0·11 + 0·19	0·24 + 0·25	0·64 + 0·22
Degrees of freedom	20	33	34

in the first, and especially the 2nd instars, can influence final size to a limited extent. Since food supply in the 2nd instar can influence the size of the mouth-parts of the 3rd instar, which is so important in determining final adult size, and, assuming that the rate of food intake is influenced by the size of mouth-parts for mechanical reasons, then there is no great difficulty in accounting for the observed differences between adults from the control and the transferred larvae in the early experiments. Such effects are secondary, however, and cannot conceal the more important relations between body-size and duration of the larval period.

6. DISCUSSION

These experiments have shown that body-size in *Drosophila* can be altered by changing either the growth rate or the duration of the period during which growth is taking place, judged by the lack or presence of correlation between size and development time. Both kinds of change may be influenced by genes whose segregation contributes to the variance of body-size and, by appropriate adjustment of the environment, it is possible to effect equivalent changes in size by different kinds of developmental change.

That body-size can be so readily increased by acceleration of the growth rate is at first sight rather paradoxical. In an adapted population we might expect growth rate to be at a maximum. However, the paradox is resolved by distinguishing between the stages at which this accelerated growth rate occurs. There is no evidence that the rate of growth up to the critical period has been altered in these experiments. The unselected and the large strains grow at the same rate while selection for faster growth rate, on media other than those deficient in RNA, has proved quite ineffective. We might infer therefore that the maximization of growth rate applies to the first stage of larval growth up to the critical period and that the effects of inbreeding or other genetic changes which upset the original equilibrium or balance with respect to the environment will particularly apply to this stage. The duration of the post-critical phase is virtually unaffected by drastic nutritional changes which can greatly influence body-size. So it is understandable that growth rate up to the critical stage is of major importance with respect to fitness and will be difficult or impossible to increase by selection in an environment to which the population is adapted.

The critical stage is reached when the larvae have grown only to some 40% or less of their potential size. Hence, genetic and environmental variation which influence growth in the post-critical phase are especially important with respect to the determination of final size. This accounts for the predominance of genetic variation which is uncorrelated with changes in the duration of the larval period in the unselected population. The stabilization of body-size about an intermediate optimum therefore refers especially to growth in the latter part of the 3rd instar.

The rate of increase in mass is considerably higher in the first than the second stage of larval growth and on general grounds the minimum nutritional requirements probably differ in the two stages. Probably early growth is more exacting and sensitive to nutritional variation and such a difference may be relevant to the ability to arrive at more or less normal adult size on diets which are very unfavourable for early growth. If successful adaptation ensures that growth to the critical stage is as fast as can be attained whereas post-critical growth is stabilized at a more or less intermediate level, the differences in tempo of growth may alter the effects of segregation at heterozygous loci sufficiently to generate different genetic properties at the earlier and later stages of development. We might expect the predominance of non-additive and additive genetic effects in the earlier and later stages respectively. The same genetic differences may influence development time and final body-size and this raises the intriguing possibility of analysing the relations between the two 'characters' in terms of the relative effect and behaviour of particular genetic differences at different stages of development. Experiments to test this possibility are in progress.

Since the total duration of the larval stage is probably important for fitness under competitive conditions, it follows that not only is maximal early growth rate favoured on the array of diets commonly encountered by the species, but the critical stage will be stabilized at the smallest larval size compatible with the other requirements of adaptation. Especially significant is the great sensitivity to minor

nutritional variation on the part of the lines selected for faster development time on the low RNA media. Consistent reduction of the larval period, either by speeding up growth to the critical stage or, as is probably the case here, by reducing the larval size at which this stage is reached, probably requires rather special conditions not commonly met with in the usual habitat.

It will be recalled that selection for shorter development time on low RNA media immediately reduced body-size but had little apparent effect on average development time itself. The anomaly may have been due to the relative casein insolubility which altered the environment sufficiently to obscure the changes in potential development time which were taking place. But there is also the possibility that reduction in the critical size below the usual level is negatively correlated with growth rate up to this stage so that two opposite tendencies cancel each other. This possibility merits further study since it could shed light on why natural selection for shorter total development time does not lead to a smaller critical size and also on the relation between general metabolism and the origin of the special threshold situation which marks the attainment of the critical size.

The organization of larval growth evidently provides a flexible system for adjustment to different ecological conditions. The same or different body-sizes can be achieved by adjusting the proportion of total growth completed in the pre- and post-critical stages of larval growth. Such changes will involve characteristically different reactions to variation in diet, and, on the evidence of the experiments described in this paper, particular kinds of nutritional conditions are likely to influence the way in which such adjustments are made under the pressure of natural selection.

7. SUMMARY

1. The low but regular positive correlation between body-size and the duration of the larval period in populations of *D. melanogaster* has been studied by selecting for large size or shorter development time on aseptic defined diets deficient in RNA and comparing the results with parallel selection on unrestricted yeast diets or on media in which RNA is not a limiting factor.

2. There is a striking contrast according to the nature of the diet during selection. On unrestricted diets and where RNA is adequate there is little or no evidence of correlation between the two characters, but on low RNA media there is a striking correlation whether selection is for large body size or shorter development time.

3. This contrast is accounted for in terms of particular changes in larval growth which can be divided into a first stage of growth to a critical size in the early 3rd instar and a second stage thereafter. The duration of the first stage can be greatly prolonged by inadequate diet but the duration of the later stage appears to be virtually unaffected by such variation although the amount of growth and hence final body-size, may be drastically reduced. The different diets which lead to presence or absence of correlation have enabled selection either to extend the growing period, so that the critical stage is reached later at a larger larval size, or to accelerate the growth rate in the later stage.

4. Variation in the final stage of growth predominates on unrestricted diets and is responsible for the greater part of the variation in body size in unselected populations. Stabilization of body-size about an intermediate optimum refers especially to growth in this later stage.

5. Lines selected for fast development on low RNA media are especially sensitive to minor nutritional variation. Probably only under rather special conditions is it possible to shorten the duration of the larval period and this is compatible with the importance of development time in fitness generally.

6. There is evidence that the restriction of early growth, in the 2nd instar, reduces the size of the 3rd instar mouth-parts. Such reduction is correlated with changes in adult size probably because smaller mouth-parts restrict food intake.

7. The pattern of larval growth suggests a flexible system which can be adjusted to different ecological conditions since the same body-size can be attained by adjusting the amount of growth effected before or after the critical stage. Differences in this respect will involve characteristic differences in reaction to environmental variation and particular nutritional conditions are likely to influence the way in which adaptive changes are realized.

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