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The ecological genetics of growth in *Drosophila*

1. BODY SIZE AND DEVELOPMENTAL TIME ON DIFFERENT DIETS

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1. INTRODUCTION

The interrelations between environment and the phenotypic expression of genetic differences have not received the attention they merit. Laboratory studies in the quantitative inheritance of *Drosophila* have so far shed little light on the problem, due either to choice of character or experimental conditions. Such favourite characters as the numbers of sternital or sternopleural bristles are comparatively unaffected by gross environmental variation and are unsuitable material for studying gene-environment interaction. On the other hand, growth, measured in terms of body size and duration of the developmental period, is very sensitive to environmental variation and offers therefore ideal material. But in earlier studies on the inheritance of body size in Drosophila (Robertson & Reeve, 1952; Robertson, 1955, 1957), nutrition and temperature were kept as uniformly favourable as possible to simplify the analysis. Nevertheless, the evidence and concepts from this earlier work provide a basis for the next stage in which genetic behaviour is studied in different controlled environments.

As soon as we select for apparently the same 'character' in different conditions we meet the problem of how far qualitatively different physiological changes contribute to the response. For example, Falconer & Latyszewski (1952) showed that mice, selected for body size on high and low planes of nutrition, differ in carcase composition. Waddington (1957) has demonstrated how sub-threshold effects may be brought to light by appropriate stimuli and then selected for. Also (Waddington, 1959), when *Drosophila* larvae were grown for some twenty generations on media with high salt concentration, the size of the anal papillae—believed to function in osmotic regulation—was increased above the normal level even on the ordinary medium while the responsiveness to increasing salt concentration was also enhanced. I have shown, from a comparative survey of the cellular make-up of the *Drosophila* wing (Robertson, 1959*b*, *c*), that the way in which growth is apportioned between cell size and cell number differs according to the nature of the environmental variation and also the kind of genetic change which is imposed on a population. Qualitatively different causes may lead to outwardly similar variation in wing or

body size, although closer inspection often reveals characteristic contrasts in development. Clearly, therefore, changes in physiology and development must be regarded as essential information in a general study of gene-environment interaction.

Such information is likely to prove relevant to the rate of change and also how far the response to selection can be pushed in a given direction. It is evident from the various selection experiments reported over the last decade or so, that the statistical parameters derived from the correlations between relatives are of low predictive value in selected populations and the origin of the so-called 'plateau', which marks the end of selection response, remains obscure. A comparative study of the level at which selection response falls off in different controlled conditions, coupled with parallel records of physiological changes, could set these problems in a fresh light and suggest new ways of interpreting familiar data.

Thus, the statistical techniques and concepts which have been developed for the analysis of quantitative variation must be combined with physiological description and analysis in a more comprehensive approach. Statistical variation between individuals must ultimately be interpreted in biological terms, and this can hardly be avoided, without generating inscrutable confusion, when different environments are involved. At present, quantitative and physiological genetics are kept in separate compartments to their mutual disadvantage. I suggest that a deliberate breaking-down of barriers is long overdue to clear the way for a greater variety of experimental analysis which will lead to new data and fresh concepts.

In planning a systematic attack along these lines, the ecology of the animal provides an essential guide to the kind of environmental variation which first merits attention, since there are so many ways in which conditions can be altered. Any species or population is normally subject to an array of environmental conditions, which have a certain average consistency to which the population is adapted. By reference to ecology we can create, in the laboratory, environmental conditions which are normally encountered by many or few individuals in a population or which are perhaps only experienced from time to time by the population as a whole. A comparison of the level of phenotypic variation, selection response and its physiological attributes is likely to be instructive under suitable contrasted conditions. Also, by following in the laboratory genetic and physiological changes which accompany adaptation to different kinds of environment, we may be able to detect regularities which can be related to the differences between geographical races or species living under different conditions. Thus, the analysis can be progressively broadened to embrace in a wider synthesis data from field ecology, including breeding structure and effective population size.

Since the procedure advocated here does not fall neatly within the confines of what are currently termed quantitative, physiological or population genetics, but exploits the techniques and concepts of all of them to a common end, it is useful to have a descriptive label. Professor Waddington has suggested to me that 'ecological genetics' might do. This has the merit of emphasizing the animal's relation to its environment as the point of departure and I have therefore adopted it.

The present introductory paper is the first of a series in which this approach is devoted to the genetics of growth in *Drosophila melanogaster*. It deals generally with methods of comparing and interpreting differences in growth on different diets.

2. MATERIALS AND METHODS

For this—or any other species—environmental variation is often largely a question of variation in the quantity and chemical composition of the diet during the stages of growth and reproduction. Genetic differences is response to nutritional conditions might be anticipated, but the relative importance and properties of such variation, both within and between populations, is unknown. Hence comparisons of growth on different controlled diets provide the obvious starting point.

With *Drosophila* the study of gene-environment interaction entails the use of chemically defined, aseptic media in place of the heterogeneous complex of yeast and other micro-organisms on which the animal is normally cultured. Various workers have contributed to the perfection of such chemically defined media, especially Tatum (1939), Schultz, St Lawrence & Newmeyer (1946), Begg & Robertson (1950), Hinton, Noyes & Ellis (1951) and Sang (1956), who has determined the quantitative requirements of essential nutrients. Since shortage of any essential constituent, or an unfavourable balance, especially of amino-acids, will lengthen the larval period and may reduce final body size as well, there are many ways of preparing an unfavourable diet. Of chief interest is the sort of nutritional variation commonly encountered in nature. This is unknown, but circumstantial evidence suggests that the choice of suitable conditions for comparing genotypes need not be entirely a matter of guesswork. Thus Sang (1959) concluded, from various published estimates of the composition of yeast upon which *Drosophila* mainly feeds, that B vitamins, with the possible exception of folic acid, are unlikely to be limiting factors under natural conditions. Hence media deficient in protein and/or yeast nucleic acid, probably offer the most realistic choice of sub-optimal conditions to begin with. As information accumulates, it should be ultimately possible to state more precisely the principal attributes of environmental variation in nature, population cage or culture bottle.

The media upon which larvae were grown aseptically comprise either Medium C of Sang (1956) or various modifications of it. The complete medium contains vitamin-free casein, fructose, yeast nucleic acid (RNA), cholesterol, lecithin, salts and seven B vitamins in an agar gel. The modified diets involve (*a*) omission of the fructose, (*b*) reduction of the RNA level from 0·36% to 0·09%, (*c*) reduction of the casein concentration from 5% to 2%.

After the medium had been autoclaved, eggs, which had been dechorionated and freed of micro-organisms by repeated washing in various antiseptic fluids and sterile water, were introduced, forty at a time, into each culture tube. Four or five replicates per genotype and treatment were set up in this way. Body size has been recorded generally on eight females per culture, by measuring thorax length on the live flies in the manner described elsewhere (Robertson & Reeve, 1952). For live yeast cultures the usual maize-meal molasses medium has been used, fortified with

dried yeast and seeded with live baker's yeast. All experiments have been carried out at 25°C.

Development time is based on the morning and evening count of the adults hatching in individual cultures. The duration of the pupal period is comparatively independent of genetic differences in body size as well as nutritional variation during the larval period and is about 4·3 days at 25°C., as Sang (1956) has noted. Hence this value has been subtracted from the total period of development to estimate the larval period more precisely. In comparisons between strains reared on live media, the duration of the larval stage is, of course, appreciably shorter than on sub-optimal media, and so, in most cases, newly emerged larvae have been set up in about fifteen small tubes—10 larvae per tube—to provide greater accuracy and more replicates without undertaking the labour of recording pupation time on individual larvae. The average development time in the egg is added to the estimates of larval life to make them comparable with the other data.

The basic records have been transformed to a log scale. Body size is expressed as three times the natural logarithm of thorax length, in hundredths of a millimetre. Unpublished tests have shown that a unit change on this scale corresponds closely with a unit change in the log weight of newly emerged flies. As we shall see, there is good internal evidence that the log scale is the most appropriate for biological interpretation of the data, while there is the additional advantage that differences between means on the log scale can be converted roughly into percentage differences by multiplying by 100, and this helps the reader to keep the relative magnitude of such differences in perspective. Development time, or, more exactly, duration of larval life, is expressed as log days. Since development time was recorded for all the flies hatching from the cultures, whereas body size was recorded on a random sample of them, the degrees of freedom for mean development time is almost always considerably higher than for body size; only the data for females have been analysed.

The wild population used in these tests is known as *Pacific*; it was derived from a large number of wild flies and had been run for about a year in a population cage before the tests began. To provide material for comparison, a large and small strain have been created by selection on the ordinary live yeast medium. The procedure was essentially the same as that used in earlier long-term experiments (Robertson, 1955). The thorax length of twenty pairs of flies from each of five replicate cultures was recorded and the extreme four pairs combined to give a total of twenty pairs selected from 100 pairs examined in each generation. The experiment was stopped after seven generations. Flies of the unselected, wild population were also reared each generation.

3. RESULTS

(*a*) *The effects of selection*

Figure 1 shows the effects of selection, expressed as deviation from the mean of the unselected flies. The points refer to the tests carried out after 2, 3, 4 and 6 rounds of selection on live yeast medium and the flies to which the points refer were the grandchildren of selected flies. Comparing their deviations with the F_1 of

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selected flies showed that a generation of mass mating did not lead to appreciable change and so the points shown in the figure accurately represent the course of selection.

Selection, as usual, quickly separated out two distinct populations which, by generation 6, differed by some 30% in body size. Judged by the deviations from

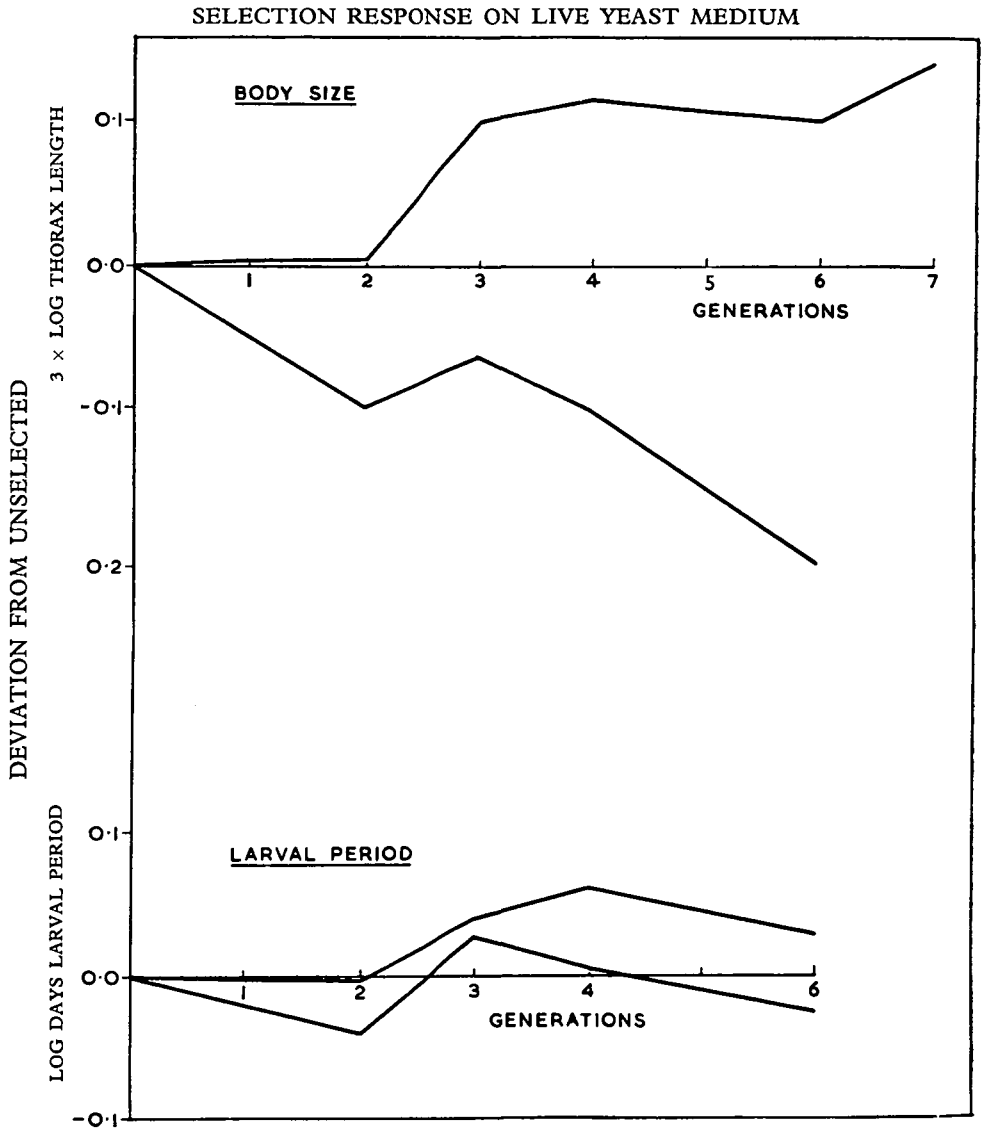

Fig. 1.

unselected, selection for large body size was apparently ineffective until generation 3; an alternative explanation will be given later. The strains also came to differ in development time—the larger strain taking longer to develop. This difference is

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chiefly due to change on the part of the large strain, and the small strain does not differ consistently from the unselected flies. This difference in duration of larval period is quickly established and shows little evidence of progressive increase as body size diverges; that is indicated in Table 1.

Table 1. *Divergence between selected strains in body size and development time; log units*

Generation	Body size	Larval period
2	0·11	0·04
3	0·17	0·01
4	0·22	0·05
6	0·32	0·06

It is quite clear that the positive relation between body size and development time which may be inferred from the variation between wild flies or the first generation or so of selection would be quite misleading as a guide to later changes due to selection; this point will be taken up again later.

(*b*) *Reaction of the unselected strain to different diets*

Before considering the effects of rearing different strains on alternative media, it is helpful to get an idea of the order of effect of these media on growth and for this purpose the behaviour of the unselected flies can be taken as a guide. These were grown on the live yeast medium and on the three aseptic media—without fructose, with low RNA, and with low protein concentration—as part of the tests carried out after 2, 3, 4 and 6 generations of selection. There is no reason to anticipate that genetic differences will contribute to differences between tests and so comparison of performance at different times will provide a measure of ability to reproduce a particular environment as well as indicating the order of effect of the different treatments. Table 2 sets out the variance analysis for these four tests; the following points must be noted.

(i) The media lacking or deficient in fructose, RNA and protein cause approximately 10%, 20% and 30% reduction in body size, and some 30%, 50% and 70% lengthening in the duration of larval life.

(ii) Variance between replicated cultures within tests is quite unimportant for body size, except on the low-protein medium. For larval period, the culture effects are just over the 0·05 level of significance for the fructose- and RNA-deficient media, but are clearly significant for low protein.

(iii) Significant heterogeneity between tests was generally present, and since genetic differences are unlikely, this calls for comment. Heterogeneity between tests on the ordinary live yeast medium is not surprising. It is worth noting in the case of body size, that the heterogeneity, although significant, is not very great, whereas it is very marked in the duration of larval period. This is a good illustration of the tendency for sub-optimal conditions, provided they are not too severe, to be accompanied by a lengthening of development with little or no reduction of body size, i.e.

there is a well-marked capacity for regulating body size by prolonging the larval period (Sang, 1956; Robertson, 1959*a*). Further evidence on this phenomenon will be presented in the next paper. Such heterogeneity in the larval period also shows how variable the live yeast medium may be, even though we try to make it uniformly favourable.

On the aseptic media, such differences in repeat tests are at first sight unexpected. Temperature was held constant, and variation due to temperature differences can be ruled out anyway on internal evidence based on the lack of correlation between body size on contrasted media in successive tests. Other observations suggest that such differences in performance are probably due to variation in autoclaving tem-

Table 2. *Comparison of repeated tests with the unselected population on alternative media*

	Mean of all tests—log units			
	Live yeast	No fructose	Low RNA	Low protein
Body size	14·08	13·98	13·85	13·75
Larval period	1·43	1·79	1·94	2·12

	Variance analysis—mean squares × 10^2							
	d.f.	Mean square	d.f.	Mean square	d.f.	Mean square	d.f.	Mean square
				Body size				
Between tests	3	2·39*	3	3·39*	3	1·34	3	16·75**
Between cultures	40	0·46	13	0·47	13	0·24	13	2·26**
Within cultures	132	0·39	110	0·52	111	0·55	108	0·77
				Larval period				
Between tests	3	148·0**	3	0·1	3	18·7**	3	389·0**
Between cultures	64	0·5	14	1·4*	13	1·0*	13	2·1**
Within cultures	253	0·6	250	0·6	189	0·4	243	0·6

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

perature, since sub-optimal media are generally rendered less adequate by prolonging the time of autoclaving, and such an effect will be relatively more marked the more sub-optimal the diet; hence the greatest evidence of heterogeneity in the low-protein medium. Evans & Butts (1949) have shown that certain amino-acids are inactivated when autoclaved in the presence of sugar and it is possible that some such reaction is involved here. With the equipment available it is practically impossible to ensure that the total heat exposure of the medium will be identical in successive tests, although the autoclaving time is carefully standardized. Also, heterogeneity between cultures could arise from uneven heating due to temperature gradients within the autoclave. However, such heterogeneity between tests is really quite minor compared with the differences between treatments and, as we shall shortly discover, can in fact be turned to advantage to bring to light an unsuspected phenomenon, which would have been missed if such heterogeneity had not occurred.

(iv) With respect to the within-culture variance of body size, representing the combined effects of genetic segregation and irreducible environmental variation, the lowest value is found on the live yeast medium (0·0039), it rises on the fructose deficient and low-RNA media to respectively 0·0052 and 0·0055, and to the still higher value of 0·0077 on the least favourable, protein-deficient diet. According to Bartlett's test (1937), such differences in variance are highly significant ($\chi^2 = 330$ for 3 degrees of freedom, $p < 0{\cdot}001$). In view of the chemical and physical homogeneity of the synthetic medium, there is little doubt that the greater within-culture variance represents an increase in the phenotypic expression of gene differences. This could derive from a kind of scalar transformation such that a unit difference under optimal conditions is represented by more than a unit difference as the mean falls or, on the other hand—and much more likely—from the segregation of genetic differences which make little or no contribution to the variance under favourable conditions but do so when the diet is sub-optimal, to the extent of doubling the variance on the low-protein diet. In development time there is virtually no difference between treatments in the within-culture variance. This may partly reflect the much lower precision with which development time is recorded, compared with body size, rather than a real difference in the behaviour of these two measures of growth.

(c) *Reaction of selected strains to different diets*

We can now turn to the problem of whether or not the genetically different strains behave in the same way when exposed to different conditions, using body size as the criterion of performance. Since the data are expressed in logarithms, absence of gene-environment interaction will be reflected in the same difference in mean—within sampling limits—when the strains are compared on alternative media. Table 3 sets out the mean deviations for body size on live yeast and on the alternative deficient media for unselected flies, for the large and small strains and also for the cross between them. With regard to the latter, reciprocal matings were made and eggs set up separately, but since maternal effects were apparently absent or very slight, the data from the progeny of reciprocal crosses have been combined. The F-values quoted below the sets of differences refer to tests of heterogenity of the differences between means for flies grown on the live and on the appropriate sub-optimal diet. The error variance for each set is twice the variance of a mean based on the pooled variance for live yeast and whatever other treatment is involved. This procedure is open to some objection due to the heterogeneity of the within-culture variances, but this is not so great as to make the tests seriously misleading. Generally where interaction exists, it is obvious from mere inspection of the data.

In the comparisons at generation 2, 3 and 4, only on the low-RNA medium at generation 2 is there unequivocal evidence of interaction, although the F-values are consistently high for the differences between the live yeast and the fructose-deficient medium, and almost certainly indicate genuine differences in reaction. But by generation 6 there are really striking contrasts in response to the alternative diets. Thus strain differences in gene-environment interaction are clearly established after only a few generations of mass selection.

There is no very obvious regularity about the table of differences with respect to treatment and direction of selection, except perhaps for the 10% greater reduction of the large strain on low protein at generation 6, compared with controls or small

Table 3. *Differences between strains in average body size (log units) of flies reared on live yeast medium and alternative sub-optimal diets*

Reduction in body size below that on live yeast medium

Genotype	No fructose	Low RNA	Low casein
	Generation 2		
Unselected	0·08	0·23	0·29
Large	0·03	0·16	0·26
Small	0·04	0·20	0·26
Cross	0·05	0·15	0·27
F	1·8	6·8**	< 1
	Generation 3		
Unselected	0·11	0·21	0·27
Large	0·07	0·22	0·26
Small	0·09	0·25	0·23
Cross	0·12	0·25	0·21
F	1·9	1·0	1·2
	Generation 4		
Unselected	0·12	0·19	0·44
Large	0·12	0·20	0·44
Small	0·11	0·19	0·45
Cross	0·05	0·19	0·47
F	1·8	< 1	< 1
	Generation 6		
Unselected	0·07	0·28	0·24
Large	0·07	0·23	0·34
Small	0·00	0·28	0·24
Cross	0·08	0·28	0·30
F	4·7**	1·5	5·5**

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

strain. This recalls the similar reaction of other large strains after five generations of selection (Robertson, 1959*a*).

However, the properties of these gene-environment differences cannot be understood merely from records of body size; development time must also be taken into account. In searching for a possible relationship between body size and duration of the larval period, the data from the live yeast cultures are of little use, since they fall on the horizontal part of the curve where great variation in larval period leaves body size comparatively unchanged. The low-protein diet is apparently much more affected by autoclaving than the other sub-optimal media and this may involve an additional complication. Hence the media which either lack fructose or have a low

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RNA content have been used for comparing body size with development time; it will be recalled that these media result in some 10% and 20% reduction of body size along with about 40% and 50% increase in larval period.

Since there is heterogeneity between tests, the differences in body size and larval period between flies grown on the fructose-deficient and the low-RNA media in successive tests vary. What is the relationship between the two measures of growth

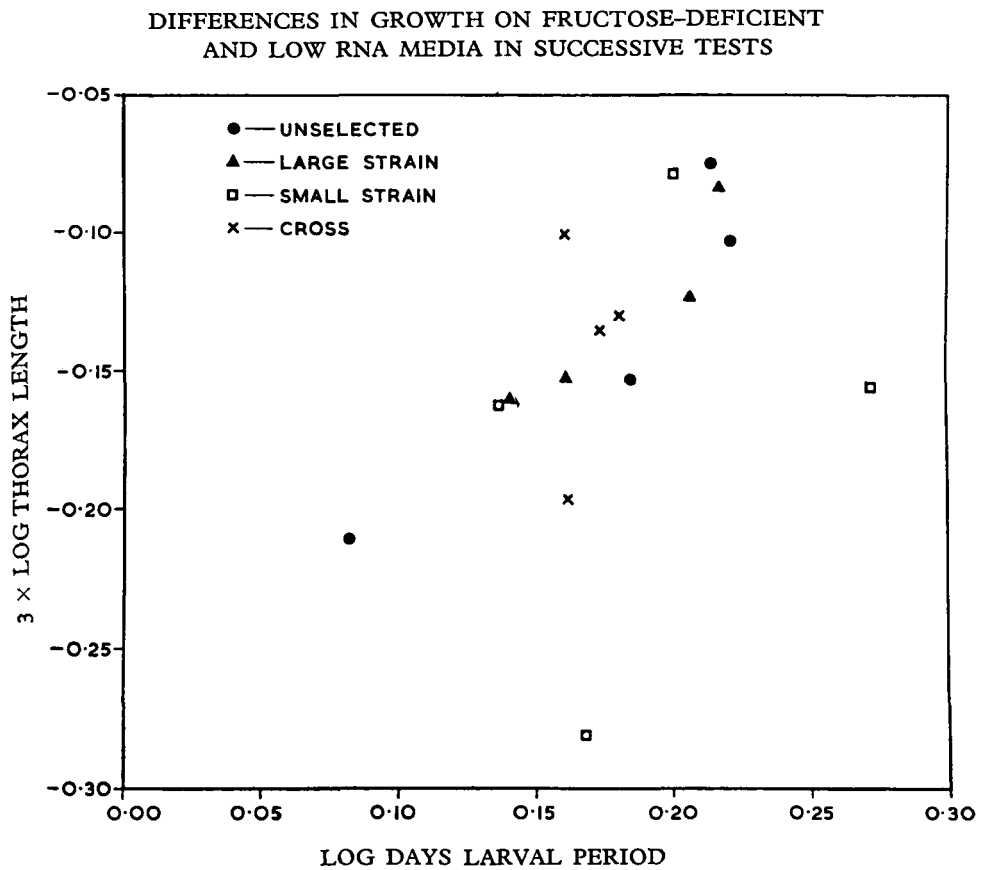

Fig. 2. Body size and larval period are expressed as deviations from performance on the fructose-deficient medium.

for such variation in the difference between the two media? The answer turns out to be both unequivocal and a little unexpected. In Fig. 2 the differences in body size, expressed as a deviation from the larger flies grown on fructose-deficient medium, are plotted against the corresponding differences for development time for the four tests. There is evidently a strong correlation; the smaller the differences in larval period, the greater the difference in body size between flies grown on the two media. Only two points stand apart from the rest; these refer to the small strain at generations 3 and 6. Excluding all four values relating to the small strain, to avoid bias, the regression of differences in body size upon differences in larval period works out

at 0·75 ± 0·26—a value not far off unity. Thus the observed value is just the opposite of what might be expected from the comparison of differences between means, averaged for all tests, in Table 2. Along with the reaction to gross nutritional differences, there is this evidence of an inverse relationship with respect to minor changes. The range of differences in body size, compatible with this positive regression, is about 0·15 log units, which is equivalent to about twice the within-culture standard deviation on these media.

The basis for this relationship is clarified in Fig. 3, which shows mean body size and development time of the unselected flies grown on the pair of alternative media

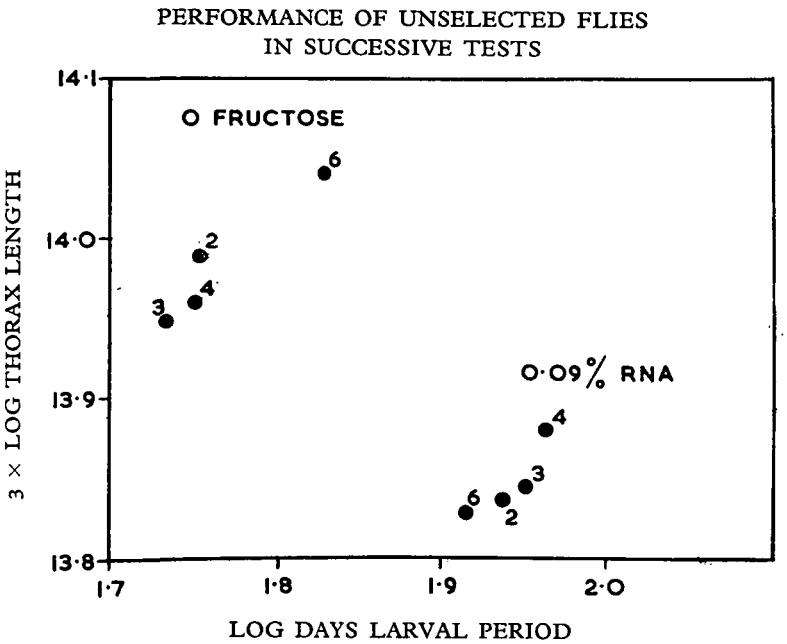

Fig. 3. The unselected flies drawn from the cage population served as controls in comparisons with selected strains after 2, 3, 4 and 6 generations of selection. In each test the cultures of synthetic medium with no fructose or with low RNA content were all autoclaved together.

in the four tests. For the differences between successive tests on the same medium, the regression of body size on development time is 0·92 ± 0·19, and 0·98 ± 0·37 for the fructose-deficient and low-RNA diets respectively. Hence the minor differences in final composition of each type of medium, previously attributed to uncontrolled variation in heat exposure during autoclaving, are associated with a positive regression of body size on larval period, such that the ratio of body size to duration of larval period remains constant. This relationship, characteristic of minor differences in the composition of the diet, is in sharp contrast with the negative regression of body size on development time when major differences exist; the average regression in the latter case is close to −1 on the log scale.

It is worth noting that the ranking of mean body size in tests at different generations is 6, 2, 4 and 3 on the fructose-deficient medium, compared with 4, 3, 2 and 6

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for the low-RNA series; i.e. the order is more or less reversed. This may mean that temperature-labile effects on nutritional imbalance, which favour relatively larger size and longer development time on the fructose-deficient medium, have the reverse effect when the level of RNA is reduced.

The kind of direct comparisons carried out on the unselected flies in the four tests cannot be applied to the selected strains, since their average size is steadily changing with selection; hence the need to deal in differences within tests, as in Fig. 2. Since all the latter comparisons, except two tests with the small strain, fall into line with the controls, this apparent plasticity of response to minor nutritional variation seems well established. Its distinguishing feature is the constancy of the ratio of body size to development time. When the variation in diet becomes too great or alters in some specific way, this ratio declines since the lengthening of development time is accompanied by smaller, not larger, body size. Thus the relations between the two aspects of growth can be used to distinguish and study different kinds of gene-environment interaction. If only body size were recorded, the existence of general gene-environment interaction could be recognized, but could not be analysed further. It is likely that the expression of this kind of response, which involves a constant 'growth rate', varies genetically. Hence individuals may react to an identical difference in nutrition by taking relatively longer or shorter time to develop and by growing to correspondingly larger or smaller size. Behaviour of this sort probably contributes to the gene-environment interaction which has been detected in comparisons between strains grown on different media. Superimposed on such variation in response are the effects of differences in the level of adaptation to sub-optimal diets which will be reflected in a sharp decline in 'growth rate'. When we compare different strains on different types of diet these two kinds of reaction are intermixed and it will require further experiments to evaluate their relative importance.

Such considerations immediately raise the question whether responses which lead to a positive correlation between body size and larval period commonly occur in live yeast cultures, and in particular, whether this is relevant to the genetic correlation of these two measures which exists among individuals of wild populations (Reeve, 1954; Robertson, 1957), and for which we have evidence in the present selection experiment. This seems likely from the following argument. It was shown in Fig. 1 that two generations of selection for large size were apparently without effect when selection response was measured as deviation from unselected on the live yeast medium. But is it quite clear that selection had in fact led to a significant shift of genotype, since body size is significantly and consistently greater on the three sub-optimal media. This is shown in Table 4.

It also looks as if the deviation of the small strain, on live yeast medium, is overestimated as much as the large strain deviation is underestimated. If the unselected flies reacted to prevailing conditions in the live yeast cultures by growing relatively bigger and taking longer to develop than either of the selected strains, the observed discrepancy can be accounted for.

Hence the initial selection for large and small size favours genotypes which respond

to nutritional conditions in opposite ways, and therefore fluctuation in conditions from generation to generation will lead to variation in the estimates of how much progress selection has effected. How far body size can be increased or diminished by picking out genotypes which are responsible for characteristically different or opposite reactions to the diet will depend in part at least on how far nutritional

Table 4. *Deviation in body size from unselected after two generations of selection; log units*

Strains	Media			
	Live yeast	No fructose	Low RNA	Low protein
Large	0·01	0·05	0·08	0·04
Small	−0·10	−0·06	−0·07	−0·07
Total difference between large and small strains	0·11	0·11	0·15	0·11

conditions can be reproduced in successive generations. In the present test, selection for this kind of change appears to have been comparatively ineffective and the major changes of body size due to selection are more or less independent of the duration of the larval period.

DISCUSSION

When the diet is sub-optimal the larval period is prolonged but final body size is not necessarily reduced below the maximum at a given temperature. There is therefore a well-marked ability to regulate body size in the presence of such adverse conditions, but when the diet becomes too deficient for one or more essential nutrients, body size is reduced as well. Also, when larvae are grown on increasingly poorer diets there is a distinct tendency for a given percentage reduction in final body size to be associated with an equivalent proportional lengthening of the larval period. It is to be expected therefore that genetic differences in reaction to the larval diet will be revealed not only in the degree of reduction in body size or lengthening of larval period but also in the variable capacity to regulate body size when the larval diet is sub-optimal. Evidence on this point will be presented in later papers of this series.

This basic pattern of response suggests a systematic approach to genetic differences in reaction to similar conditions. Earlier work (Robertson, 1959*a*) has shown that a few generations of selection for either large or small body size generally results in greater proportional reduction of adult body size on deficient diets than is true for unselected individuals. A similar situation developed after six generations of selection for large body size in the present selection experiment. How far such genetic differences in response vary according to the particular kind of nutritional deficiency or imbalance is an interesting topic for future study. It is quite clear, however, that genetic differences in reaction to nutritional variation are widespread in the population. Changes in body size due to selection are no doubt especially likely to involve different growth responses in relation to diet, although it remains to be seen whether selection for other characters reveals any regularity in this respect.

There do not appear to be any precise data about how much of the observed variation of body size in wild flies in nature can be attributed to nutritional variation, after allowing for temperature fluctuation. Sokoloff (1957) has suggested that food shortage is unimportant because the body size of wild flies does not vary greatly. However, as we have seen, the record of body size alone may be a poor guide to the adequacy or otherwise of the larval food supply, since regulation may lead to longer development time without much effect on body size. Also it might be expected that species will differ considerably in this capacity for maintaining a constant body size. But whatever the situation in nature, there is no doubt about the importance of nutritional variation in the laboratory cultures of *Drosophila* either in bottle or population cage.

The reaction of the growing larva to its diet is not confined solely to maintaining a genetically predetermined size or suffering a characteristic decline in the size at which pupation occurs. It appears that the duration of the growing period may also vary so that a longer growing period leads to larger body size and the converse. The clearest evidence for this reaction is provided by the successive tests on the media deficient in fructose and RNA. Judging from the behaviour of the wild stock, differences between repeat tests were such that larger body size was regularly associated with proportionately longer larval period. This was quite unexpected, since crude variation of the diet, as between say media deficient in fructose and RNA, leads to a negative correlation between these measures of growth—the longer the development time, the smaller the fly.

The essential causes of the differences in repeat tests are unknown, but possible origins may be suggested in diminishing order of likelihood. They may arise from uncontrollable differences in the heat exposure of the synthetic media during autoclaving, so that although the successive batches of a given medium start the same, they end up slightly different, due to heat-labile reactions. The tendency for the order of ranking of body size in the four tests to be reversed on the different media suggests that variation in heat exposure leads to inverse changes in the concentration or availability of one or more critical nutrients, according to the initial composition of the medium. Alternatively, the differences may be due to a maternal effect, whereby the diet of the mother can influence the growth of the progeny. Or conceivably, differences in the timing of critical stages of growth in relation to diurnal rhythm may play a part.

But, whatever the final explanation, there is evidently a plasticity of response in relation to environmental variation which is expressed by parallel changes in body size and development time. Such a plasticity of response may in turn reflect a buffering system which favours a characteristic ratio between final size and duration of the growth period. If it can be shown that genotypes, which are atypical with respect to the normal gene arrays in a population, are relatively less successful in maintaining such a characteristic ratio, this reaction could provide an empirical measure of physiological homeostasis. This problem is being examined further.

This type of growth response is probably relevant to the positive correlation between body size and development time among wild flies grown on the live yeast

medium. Genetic differences which determine whether individuals react to prevailing conditions by growing for relatively longer or shorter periods to become correspondingly bigger or smaller may be expected to contribute to selection response. Also the extent to which, say, larger body size can be attained by this kind of change depends on the repeatability of the appropriate conditions in successive generations. If these vary, consistent selection for this kind of change will be comparatively ineffective and the selection response will depend on changes which alter growth rate but not duration of the growth period.

Gene-environment interaction was invoked to account for the discrepancy between the estimates of selection response on the live yeast medium, on one hand, and various synthetic media, on the other. It was suggested that the unselected individuals had reacted via relatively longer development time and larger body size on the live yeast medium, compared with either of the selected strains, whereas on the synthetic media the three populations were more alike in this respect. Both from these comparisons and from the repeat tests on the media deficient in fructose and RNA, it appears that the range of body size associated with this kind of reaction is equivalent at least to between one and two times the within-culture standard deviation.

A general comment is relevant here. Had the data in these experiments been confined to the 'character' body size alone, this interesting phenomenon would not have been detected, since it would have been impossible to distinguish differences in growth response under different conditions. The heterogeneity between repeat tests would have been classified as unavoidable error variance, whereas, by taking development time into account, such variation is deprived of some of its apparently irreducible confusion and fresh possibilities of analysis and interpretation appear. In particular, the extent to which body size may be increased either by altering the growth rate or by increasing the duration of the growing period, the genetic behaviour and reaction to environment of strains differentiated in this way, together with the physiological interrelations between such alternative kinds of change, raise problems of great interest which indicate some of the next steps in this study.

Finally, when wild flies are grown on media deficient in essential nutrients, the within-culture variance between individuals increases. For example, on the low-protein diet it was approximately twice as great as on live yeast medium. The greater variance under adverse conditions may be attributed to the segregation of genetic differences which are indistinguishable when the diet is improved. This raises the question of how far parallel selection for, say, large body size on such different diets, leads to qualitatively different physiological changes and characteristically different reactions to controlled changes in the environment. Experiments bearing on this problem will be described in succeeding papers.

SUMMARY

1. The interrelations between environment and the phenotypic expression of genetic differences have not received the attention they merit. Laboratory studies in quantitative inheritance, either by choice of character or experimental conditions,

have not shed much light on this problem. Selection for the same character in different environments is likely to involve qualitative differences in physiology and development. Comparative study of such changes will throw light on the genetics of development generally, which in turn is relevant to how far the selection response can be pushed in a given direction. Since statistical variation between individuals must ultimately be interpreted in biological terms, the unnatural barriers between quantitative and physiological genetics must be broken down to clear the way for a greater variety of experimental analysis and a more widely based approach to the interpretation of individual differences in populations. The ecology of the animal provides the point of departure and guide to the kind of environmental variation which should be studied first. Since the suggested approach cuts across the conventional limits of quantitative, physiological and population genetics and exploits the concepts and methods of these alternative approaches to a common end, it is convenient to have a descriptive label. The term 'ecological genetics' has been adopted.

2. This introductory paper is the first of a series dealing with experiments orientated along these lines. Since environmental variation largely consists of variation in the quantity and composition of the diet, the growth of individuals from a cage population of *Drosophila melanogaster* and also other strains has been studied on a variety of aseptic, synthetic diets. Body size and duration of the larval period are taken as measures of growth. There is a well-marked ability to regulate body size, by extending the duration of development, provided the diet is not too deficient. When the diet is further reduced development time is further lengthened and body size is reduced as well.

3. To test for genetic differences in reaction to the diet, strains have been created by selecting for large or small body size, and their performance, together with that of the cross between them, has been compared with the performance of unselected individuals on alternative diets for the first few generations of mass selection. There is evidence of gene-environment interaction quite early in selection, and after six generations striking differences were detected. It is concluded that genetic differences in reaction to different sub-optimal diets are widespread in the population.

4. The within-culture variance is increased by growing larvae on progressively more deficient diets and is approximately twice as great on a low-protein diet as on the usual live yeast medium. This increase is attributed to the segregation of genetic differences which are unimportant and contribute little to the variance under more favourable conditions.

5. Comparison of body size and development time in repeated tests with two diets lacking fructose or deficient in ribonucleic acid revealed evidence of a plasticity of response to minor nutritional variation which is characterized by a positive association between body size and the duration of the growth period. This relationship is the reverse of that associated with crude variation in the diet which leads to a negative association between development time and body size. This plasticity of response probably represents an aspect of physiological homeostasis. Genetic differences in the magnitude and direction of this response probably contribute to

gene-environment interaction generally, and this probably accounts for apparent discrepancies in alternative estimates of the response to selection for large and small body size when these are based on deviations from the unselected. This suggests the need for determining how far body size may be increased either by altering the growth rate or by extending the growth period, and also how far strains differentiated in such respects differ in their reaction to controlled differences in nutrition.

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