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The ecological genetics of growth in *Drosophila*

8. ADAPTATION TO A NEW DIET

By FORBES W. ROBERTSON*

Institute of Animal Genetics, Edinburgh

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1. INTRODUCTION

The developmental processes which determine the average body size of a given species of *Drosophila*, or any other animal, represent one solution to the problems of adaptation to a particular array of ecological conditions. There may be alternative solutions to the same or similar problems, but they can hardly be discussed with much profit until we know what particular ecological changes would be needed to cause a consistent change in body size, in any particular instance, as part of the adaptation to the new conditions. To elucidate this complex situation different kinds of evidence must be combined, including the genetic properties of variation in growth, the developmental and physiological aspects of such variation, the ecology of the species and also comparisons of the growth and ecology of different species.

One rather attractive approach is to create new conditions in the laboratory, alter the relative importance of components of fitness and follow what happens to body size. Evidence from such experiments will lead to more systematic manipulation of the environment to test alternative hypotheses as to the nature of the selective pressures which determine the average body-size characteristic of a population or species. The present paper deals with the adaptation of populations of *Drosophila melanogaster* to a new, initially unfavourable, environment, created by incorporating the chelating agent ethylenediaminetetra-acetic acid (EDTA) in the food medium. Steffensen (1957) showed that adding EDTA to the medium restricts growth and survival according to the concentration. Natural adaptation to such conditions has been studied with special reference to adult body size with or without minimization of natural selection for shorter development time. The results show that the kind of natural selection which acts during the process of adaptation influences the direction of changes in adult body size and also the way in which such changes are effected.

2. MATERIAL AND METHODS

(i) *General*

The flies used in these experiments were all derived from the Pacific cage population which has been maintained in the laboratory for a number of years. They

* Member of the Agricultural Research Council Unit of Animal Genetics.

were cultured on the usual cornmeal molasses medium, fortified with dried yeast. When EDTA was added it was present, unless otherwise stated, at a concentration equivalent to approximately 0·005 M with respect to the water content of the medium. This level is normally sufficient to cause an extended larval period and reduction both in body size and also in survival in the Pacific population. Hence the mean levels of these parameters provide a convenient index of adaptation. Comparisons of maximum body size, under favourable nutritional conditions, show whether or not there have been changes in the mean level of this character as a result of the adaptation. The severity of the effect produced by adding EDTA to the ordinary medium varies considerably between different experiments. In one test 0·01 M may be lethal to the Pacific population, while in another survival may be fair, although development time is always greatly prolonged and body size is reduced. The reason for such variation is uncertain, but it may be related to variability in the vigour of yeast growth in different experiments.

All tests were carried out at 25°C. Body size is expressed as three times the natural logarithm of thorax length in 1/100 mm. The larval period is expressed as the natural logarithm of days to pupation, measured as the total development time minus the average pupal period of 4·3 days.

(ii) *The experimental populations*

(1) One population was run in a cage; the only difference from normal procedure was that EDTA was incorporated in the food medium which was replaced at the usual intervals.

(2) Three populations were run in large flasks. Just before emergence of the flies, a fresh flask was attached to allow newly emerged flies to lay eggs on the fresh medium, and when the culture was well established the old flask was removed, and a fresh one added at the appropriate time. In two of the flasks the foundation flies carried the white-eye gene which had been incorporated into the Pacific background by repeated backcrossing.

(3) Two populations were kept in bottles with 100 pairs of parents per generation. Each generation the 100 pairs were randomly distributed between five bottles—twenty pairs to each—and in the following generation, twenty pairs were drawn from each of the five cultures, mixed and then distributed randomly between five bottles and so on each generation.

(4) In addition to these six populations in which there was full scope for natural selection in favour of adaptation to the EDTA medium, since the chelating agent was always added to the food, two other populations were established. In one, eggs were set up in successive generations and flies were selected for shorter development time. Generally fifteen pairs of flies were selected from 150 pairs each generation. This is referred to as the fast selected line. In the other population, selection for development time was minimal; all the flies in successive generations were allowed to hatch and the sexes were kept separate. When the last of the flies was out the entire set was kept for a few days further in the presence of abundant yeast

and then mass mated. Eggs were set up in a number of cultures containing EDTA in the medium so that individuals which laid most eggs would contribute disproportionately to the next generation, irrespective of the time taken to complete development.

3. RESULTS

(i) *The effects of EDTA*

Table 1 shows a typical result of adding different concentrations of EDTA to the usual medium. 0·0025 M was sufficient in this test to produce about 20% reduction in survival, 18% longer development time and about 8% smaller body

Table 1. *The effects of adding EDTA to the medium with or without zinc; deviations from performance on the usual medium; time and size deviation* $\times 100$

	Survival (%)	Larval period ($\log_e$)	Thorax length ($3 \log_e$)
Molarity of EDTA		Zinc not added	
0·0025 M	−19**	18**	−8**
0·0050 M	−21**	29**	−20**
0·0100 M	Nil	—	—
Molarity of zinc		Zinc added to medium with 0·005 M EDTA	
0·0050 M	−6	−3	1
0·0010 M	−22**	59**	−13**

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

size. Doubling the concentration increased the magnitude of the effects on development time and body size, while increase to 0·01 M prevented any flies from hatching. These adverse effects were completely abolished by adding equimolar zinc to the 0·005 M EDTA medium, but 0·001 M zinc was insufficient to remove the harmful effects of EDTA which, as Steffensen (1957) pointed out, evidently acts by binding zinc and/or other essential ions of lower stability constant.

(ii) *Evidence for adaptation*

After about twenty generations the populations were grown on various media which included the usual live yeast medium, without or with either 0·0025 M or 0·005 M EDTA. All the comparisons were carried out at 25°C. but performance on the medium with 0·005 M EDTA was also scored at 18° and 29°C. In addition, the growth of the strains was compared under crowded conditions on media with and without EDTA. About ten tubes of eggs were set up for each population and equal numbers of the adapted strain and the Pacific stock, with or without the white-eye gene, were introduced into each culture. The values shown in Table 2 represent

the average within-culture difference between the Pacific control and the EDTA-adapted strain.

Table 2. *Growth of EDTA-adapted strains on different media at different temperatures; 100 × deviations from performance of Pacific control population*

Population	Without EDTA 18°C.	25°C.	29°C.	Mean	With EDTA (25°C.) 0·0025 M	0·0050 M
			Thorax length [3 $\log_e$]			
Cage	−6**	−6**	−7**	−8	−5*	4*
Flask—1	−11**	−11**	−8**	−10	−3	9**
Flask—2	−7**	−4*	1	−3	−7**	7**
Flask—3	−4*	−10**	−9**	−8	−5*	1
Bottle—1	−4*	−4*	−10**	−6	−3	7**
Bottle—2	−3	−5**	−6**	−5	−3	3
Selected—fast	−7**	−9**	−10**	−9	−7**	5**
Selected—fecundity	9**	7**	5**	7	7**	14**
			Larval period [$\log_e$]			
Cage	−5*	−9*	−1	−5	−39**	−48**
Flask—1	−5*	−2	5*	−1	−4	−57**
Flask—2	4	8*	6*	6	−6*	−31**
Flask—3	−7*	−2	−5*	−5	−17**	−21**
Bottle—1	0	0	−3	−1	−15**	2
Bottle—2	−6*	−3	0	−3	−10**	−20**
Selected—fast	−12**	−8*	−18**	−13	−33**	−44**
Selected—fecundity	−6*	−3	−3	−4	−25**	−27**

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

On the standard EDTA-free medium body size was smaller than in the Pacific controls in all the EDTA-adapted lines; the average was about 7% less. There was also a tendency for development time to be shorter although this was not so well established. The differences in body size were maintained at both the higher and lower temperatures. In the line selected for fast development on the EDTA medium both body size and development time were reduced by 8–9%. The evidence for correlated changes of body size and development time may be compared with independent evidence of similar effects of selecting for shorter development time on different media (Robertson, 1963, 1964; Church & Robertson, 1966).

The significant exception to the otherwise regular reduction in body size was the population in which natural or artificial selection for differences in development time was minimized and in which selection favoured high egg production. In this strain body size was 7% greater than in the controls, on the EDTA-free medium, and about 16% greater than the average of the other lines which had been adapted to the presence of EDTA. The larval period was slightly shorter than in the Pacific controls.

When 0·0025 M EDTA was added to the medium, the difference in body size between the controls and the EDTA-adapted strains was not greatly altered, although the larval period of the Pacific controls was relatively increased by some

15%. In the lines selected for fast development and for fecundity the relative difference was even greater. On the medium with 0·005 M EDTA, body size was reduced in the Pacific controls, so that the adapted strains grew to a larger size than the controls, while the difference in the larval period was increased to about 30% for the adapted strains and the line selected for fecundity and even more for the line selected for fast development. Two features are of special significance:

(1) all the populations reared for successive generations on the EDTA medium developed substantial adaptation to the new environment;

(2) the line in which selection for development time was minimal and selection for fecundity was maximal differs from all the rest in reaching a substantially larger adult body size.

The comparison of growth under crowded conditions on media, with or without EDTA, provided additional evidence of differences in adaptation. Since the survival of the Pacific controls on 0·005 M EDTA was very low under crowded conditions, attention was confined to growth on medium with or without 0·0025 M EDTA. Crowding was held at a constant level sufficient to produce a considerable reduction of body size and a lengthened larval period. White-eyed Pacific controls and one or other of the adapted strains were cultured together in the same vials and distinguished by the difference in the eye colour.

Table 3. *Growth of EDTA-adapted strains under competitive conditions on media with and without EDTA;* 100× *deviations from Pacific control population*

Population	Without EDTA	With 0·0025 M EDTA
	Thorax length [3 $\log_e$]	
Cage	−7**	19**
Flask—1	—	—
Flask—2	−9**	10**
Flask—3	−14**	16**
Bottle—1	−17**	0
Bottle—2	−19**	10**
Selected—fast	−14**	8**
Selected—fecundity	−5**	−9**
	Larval period [$\log_e$]	
Cage	7*	−27**
Flask—1	—	—
Flask—2	12**	−28**
Flask—3	20**	−38**
Bottle—1	17**	−4
Bottle—2	2	−34**
Selected—fast	1	−27**
Selected—fecundity	7*	−13**

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

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Table 3 reveals a striking reversal of performance according to the presence or absence of EDTA in the medium. Thus, when EDTA was not added to the diet, the body size of the EDTA-adapted strains was relatively more reduced than that of the Pacific controls while development time was considerably longer, the sole exception being the line selected for fast development time. Successful adaptation to the EDTA medium evidently entailed lowered adaptation to the competitive conditions which the foundation population frequently encounters.

In the presence of 0·0025 M EDTA the situation was reversed so that the EDTA-adapted lines grew to a larger size, in a shorter time, than the Pacific controls. The only exception was the line selected for fecundity, in which potential maximum body size had been increased. In this case crowding on the EDTA medium led to a decrease in body size below the Pacific controls, and a 20% reduction below the other EDTA-adapted strains. The larval period, although less than the controls, exceeded that of most of the EDTA-adapted lines. This aspect will be considered later.

Table 4. *Average performance of EDTA-adapted strains compared with Pacific controls on medium with and without* 0·0025 M *EDTA*

EDTA	Survival, ratio to controls	3 $\log_e$ thorax length	Log_e larval period
		Uncrowded	
Nil	1·0	−7	−1
0·0025 M	1·0	−4	−15
		Crowded	
Nil	0·9	−14	12
0·0025 M	2·4	8	−26

The comparison of average performance of the adapted strains under the alternative conditions is summarized in Table 4 for growth on medium with and without 0·0025 M EDTA. The table also shows average survival of the EDTA-adapted strains expressed as a ratio to the survival of the controls. In this test, in uncrowded conditions, relative survival was unaffected by addition of 0·0025 M EDTA but, under competitive conditions, the survival of the EDTA-adapted strains was lower on the EDTA-free medium and relatively much greater in the presence of the chelating agent. The evidence from survival is therefore consistent with that relating to the other criteria of performance.

(iii) *Effects of varying EDTA and zinc*

Since the populations adapted to EDTA generally resembled one another in their reaction to different conditions, attention was confined, in subsequent experiments, to only a few of the adapted strains, especially the cage population. The effect on growth of varying the concentration of EDTA and/or zinc was compared in the cage population and the Pacific controls. When the EDTA concentration was increased to 0·01 M none of the control flies hatched whereas the survival of the adapted cage population was hardly reduced (Fig. 1).

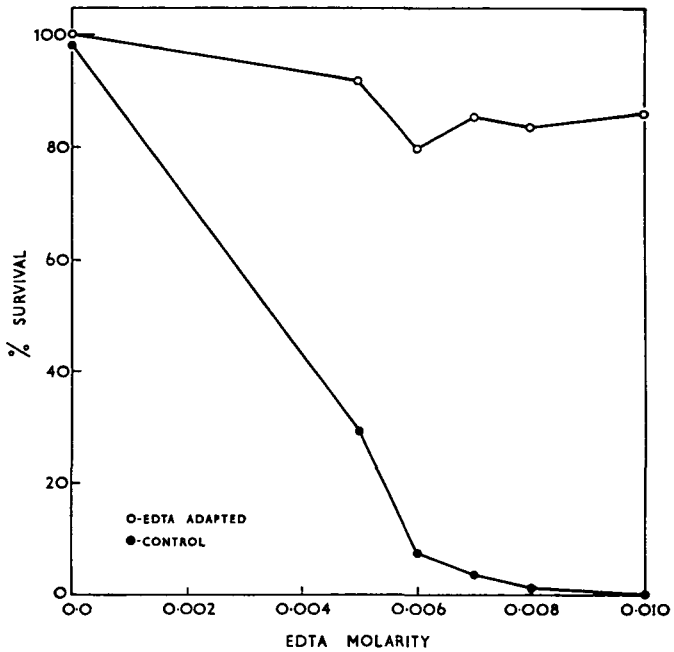

Fig. 1. Newly hatched larvae were set up on medium without, or with different concentration of, EDTA. The graphs show the proportion of larvae which reached the adult state in the EDTA-adapted cage population and the Pacific controls.

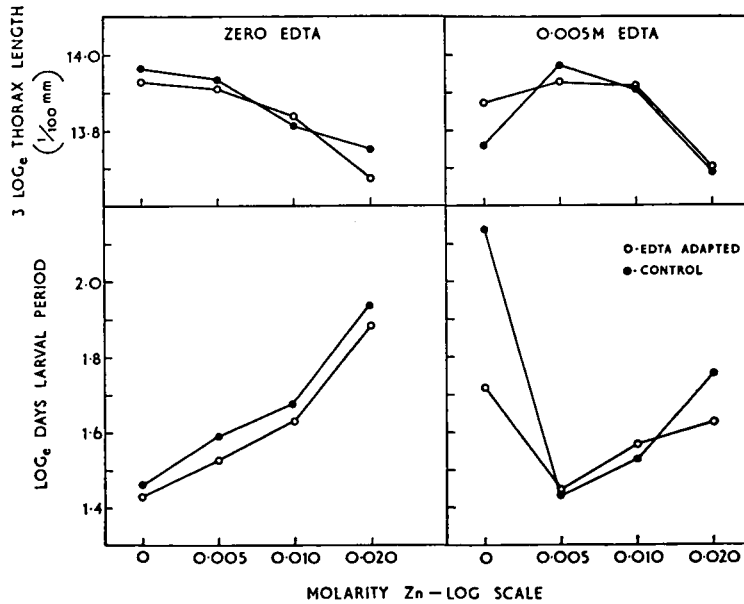

Fig. 2. The effects on body size and duration of the larval period of culturing larvae of the EDTA-adapted cage population and the Pacific controls on medium with different concentrations of zinc, in the presence of absence of EDTA.

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To provide further information about the EDTA–zinc relations, zinc was added as zinc sulphate to EDTA-free medium in increasing concentration, which led to progressive reduction of body size and lengthening of development time. Both the control and adapted lines behaved alike; the shorter development time of the EDTA-adapted strain was evident at all zinc concentrations (Fig. 2). When 0·005 M EDTA was present in the medium, equimolar zinc abolished its adverse affects but further increase of the zinc concentration to 0·01 M and beyond was unfavourable and led to small body size and longer development time. The two strains reacted in similar fashion. It appears, therefore, that the concentration of zinc in the medium may vary only within narrow limits to avoid adverse effects on growth.

(iv) *Stability of the adapted changes*

The EDTA-adapted population is less fit than the original one under competitive conditions on medium lacking EDTA. It was therefore of interest to discover whether or not it quickly recovered the original attributes when cultured in successive generations on the original medium. To test this a large sub-culture was taken off the EDTA-adapted cage population and run in bottles on EDTA-free medium under competitive conditions. After five generations the performance of this sub-population and of the parent population maintained on EDTA medium was compared with that of the foundation Pacific stock in crowded and uncrowded conditions on medium with and without appropriate concentrations of EDTA. The comparisons are summarized in Table 5. With respect to body size there was no evidence of any difference between the EDTA-adapted cage population and the relaxed sub-population derived from it. For development time there was evidence of a relatively longer larval period under all conditions in the relaxed sub-population especially in the absence of EDTA, which is the converse of what might have been anticipated. On the EDTA medium the slightly longer development time was not statistically significant. Thus there appears to be considerable stability of the genetic changes, which confer adaptation to EDTA, when the original conditions are restored.

Table 5. *The effects of five generations of culture of the EDTA–cage population on EDTA-free medium.* 100 × *Deviation from mean body size and larval period of the Pacific stock*

	3 $\log_e$ thorax length			Log_e larval period		
Medium	Unrelaxed	Relaxed	Difference	Unrelaxed	Relaxed	Difference
Zero EDTA						
(1) Uncrowded	1	4	3	3	14	11*
(2) Crowded	−10	−10	0	6	12	6*
0·0025 M EDTA	6	8	2	−4	0	4
0·005 M EDTA	10	9	−1	−30	−26	4

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

(v) *Physiological aspects*

In a number of the EDTA-adapted lines development time was shorter than in the controls. In the light of earlier evidence (Robertson, 1963, 1964; Church & Robertson, 1966) it is important to know whether this reduction reflects a general shortening of the growth period and for this purpose a convenient index is the time taken to reach the 'critical size'. This is the stage at which larvae can complete development even if no longer allowed to feed (Bakker, 1959; Robertson, 1963), and is believed to represent an important ontogenetic landmark. Newly emerged larvae of either the EDTA-adapted cage population or larvae of the strain selected for fecundity were set up along with equal numbers of Pacific control larvae, marked by the neutral white-eye gene. At about the time that the controls reach the critical size, larvae were removed to food-free vials and the surviving adults were scored for eye colour. If the two types reach their critical size at the same time we expect equal frequency of the two eye colours whereas if one reaches the stage earlier than the other that type will be in excess. Obviously the test required careful timing to remove larvae when about 50% of the controls had attained the capacity to pupate.

Table 6. *Comparison between Pacific controls and either the EDTA-adapted cage population or the large line in the rate of reaching the critical size*

	Cage population vs. controls	Large line vs. controls
Number of larvae per genotype	280	240
Percentage emergence of controls	56·4	31.0
Percentage emergence of EDTA-adapted line	72·4	56·6
Difference	16·0**	25·6**

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

The large line refers to the population in which there was maximum natural selection for higher rate of egg production.

Table 6 shows that both the cage population and the other selected line reached the critical size before the larvae of the foundation population when they were grown together on EDTA-free medium, and from this it may be inferred that the hormonal relations have been changed to reduce the period available for growth. This explanation may be applied also to the line selected for fast development and may be applicable to the other instances in which body size has been reduced. It should be noted that in the large strain, selected for fecundity, there has been a substantial increase in growth rate to allow growth to a larger size in a shorter larval period.

The origin of the larger body size in this strain is believed to be due to selection for higher egg production during the period of adaptation. It is well known that there is a high correlation between rate of egg production and body size, when variations in the latter is due to differences in larval diet. It has been shown (Robertson, 1957) that, under such conditions, there is a high correlation between

body size and the number of ovarioles. Hence, when the diet is suboptimal, the larger individual will be at an advantage so that, in the course of adaptation, selection will favour individuals which grow faster to a larger size. When adaptation has been established and an equilibrium reached, it does not follow that genetic changes which increase body size will necessarily lead to a further increase in egg production and it has been shown (Robertson, loc. cit.) that increase in body size by selection may occur without change in ovariole number. But in the course of establishing adaptation to an initially unfavourable environment the correlation between body size and egg production will be important.

To illustrate the proposed mechanism, flies of, respectively, the EDTA-adapted cage population, one of the bottle populations, the large line and the Pacific controls were reared on the EDTA medium, and egg production was recorded for flies allowed to feed on yeast growing on this medium. The test was arranged to provide conditions as similar as possible to those normally encountered. For comparison, flies were also grown and allowed to feed on the usual EDTA-free medium.

Table 7. *Egg production (loge.) of EDTA-adapted strain and Pacific population expressed as deviations* (× 100) *from the egg production of the large line*

	Larval and adult diet	
Genotype	With EDTA	Without EDTA
EDTA—cage population	−21**	13*
EDTA—flask 1	−22**	−12*
Pacific population	−82**	−9

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

The large line refers to the population in which there was maximum natural selection for higher rate of egg production.

Table 7 shows the difference in average daily egg production expressed as a deviation from the mean output of the large line. Egg production refers to the total eggs laid on the 4th-7th day of adult life inclusive. When grown as larvae and fed as adults on yeast growing on the EDTA medium, the large line is clearly superior in production to either of the other, smaller EDTA-adapted lines. As expected, the performance of the non-adapted control is particularly low. On the EDTA-free medium, however, there are only minor differences in egg production and the large line is not consistently superior to the others.

(vi) *Genetic evidence*

To ascertain whether or not adaptation to the EDTA medium has involved extensive changes in genetic composition, all possible combinations of homologous pairs of chromosomes from the EDTA-adapted cage population and the original Pacific population were constructed. Using essentially the same crossing scheme as that described elsewhere (Robertson & Reeve, 1955) dominant marked inversions

—effective as suppressors of recombination—were used to substitute pairs of chromosomes from one population for homologues of the other. Thus if we represent the control, Pacific foundation population as CCC and the EDTA-adapted population as EEE, then replacement of respectively the first, second or third pair of C chromosomes by E homologues would be represented as ECC, CEC, CCE, while the reciprocal substitutions would be CEE, ECE and EEC. Eight alternative genotypes are available for comparison. The experiment was carried out on a large enough scale to ensure that a large sample of pairs of first, second or third chromosome were incorporated in the substitutions to avoid any risk of homozygous combinations. The small fourth chromosome was ignored. Eggs of the two strains, CCC and EEE, and of the six synthetic types were set up in a series of replicated cultures on the medium without EDTA and also with 0·005 and 0·01 M EDTA. Body size and duration of the larval period were recorded.

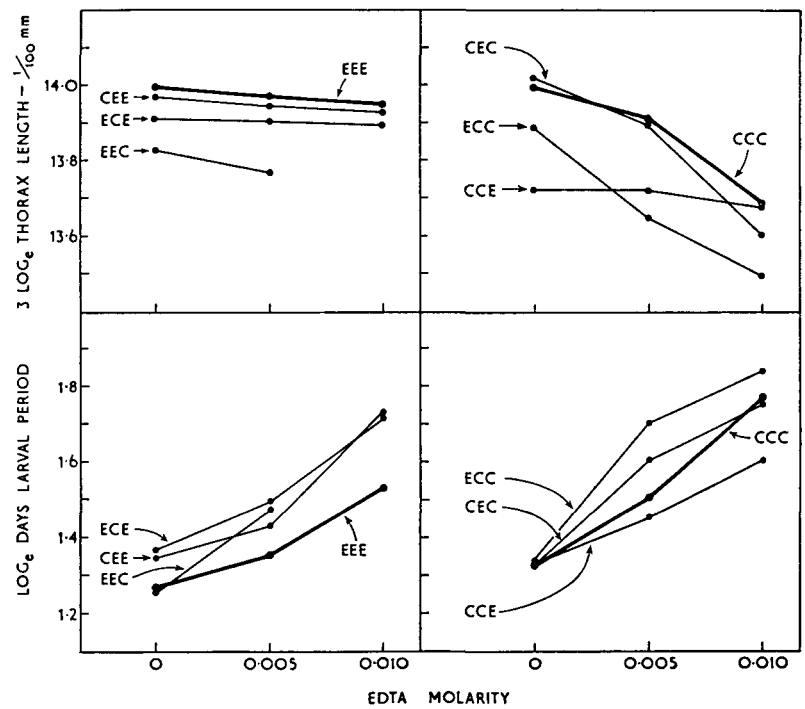

Fig. 3. The effects on body size and the duration of the larval period of substituting pairs of homologous chromosomes from the EDTA-adapted cage population in the background of the Pacific population and vice versa.

All three major chromosomes were shown to differ between the controls and the adapted population. There was highly significant between-chromosome interaction which was complex and not amenable to any simple interpretation. The pattern of interactions has been summarized diagrammatically in Fig. 3 which shows the effects of introducing chromosome pairs I, II or III from one strain into the genetic background of the other, in the different environments, and this evidence is sufficient to answer the question which prompted this experiment.

In the substitution of alternative chromosome pairs in the E background,

replacement of E by C chromosomes always reduced body size in the alternative environments and generally increased development time as well, and so such effects must be regarded as deleterious. With respect to body size on the optimal EDTA-free medium, the effect of substituting E by C chromosomes was least for the first and most for the third pair in which the substitution reduced body size by about 15%. Substitution of the second pair of chromosomes produced effects intermediate between those of the first and third pairs so the effects were roughly proportional to chromosome length. At the highest EDTA concentration, the type EEC reacted so unfavourably to the diet that the larvae were unable to complete their development. In addition, females of this combination were completely sterile irrespective of the kind of medium they were cultured in; although eggs were laid they failed to hatch.

In the reciprocal substitutions in the C background, body size was also reduced relative to the original type CCC. Replacement of the first pair of chromosomes of E homologues reduced body size by about 10% on the EDTA-free medium. Culture in 0·005 M EDTA medium reduced body size and lengthened development time but further increase of the EDTA concentration, although it lengthened development time, had little effect in further reducing body size. The substitution of the third pair of chromosomes (CCE) reduced body size by about 30% on the EDTA-free medium, although development time was unaffected, but there was no further change in the absolute body size in the presence of either concentration of EDTA although development time was increased. The second chromosome substitution (CEC) caused only minor deviation from CCC in body size or development time, apart from a tendency to relatively smaller body size at the highest EDTA concentration.

These effects show that adaptation to the new diet containing EDTA has involved changes in all chromosomes and also complementary interaction between non-homologous chromosomes. The differences between the EDTA-adapted and the original population have become so great that substitution of the third chromosome pair from the latter into the background of the EDTA-adapted strain causes complete sterility and environment-dependent lethality.

4. DISCUSSION

Adaptation to the new diet with EDTA involves selection of gene combinations which confer higher survival, faster development and growth to a body size which is nearer the maximum attainable under optimum conditions. The same gene combination is likely to improve fitness in several different ways so that the pressure of natural selection is reinforced through the life cycle. After survival, duration of the larval period is probably a major component of fitness in a competitive environment. Development time can be reduced either by improving the growth rate or by shortening the growth period or by both, and this will involve, respectively, either a negative or a positive correlation between body size and development time. Genetic variation which influences body size by altering the duration of the growth period requires special nutritional conditions for maximum expression (Robertson, 1963, 1964).

The EDTA-adapted strains show both kinds of change. Although shorter development time, due to better growth, is the major feature of adaptation, all strains, other than the one selected for fecundity, have declined in body size and, in several instances, development time has been reduced as well. Records of the time to reach the critical size in the cage population and in the strain selected for fecundity showed that both reached this stage more quickly than the controls, implying a reduction of the growth period. In the large strain the increase in body size, without increase in development time, recalls the extensive evidence of alteration of body size by changes in growth rate alone (Robertson, 1963, 1964; Church & Robertson, 1966). In view of the apparent independence between alternative pathways which influence final body size, both kinds of change may have contributed to the final size of the large strain, i.e. body size tends to be reduced by a shortening of the growth period and increased by acceleration of the growth rate. Evidence has been presented elsewhere (Church & Robertson, 1966), to suggest that presence or absence of genetic correlation between size and development time reflects changes in either the DNA content or the protein/DNA ratio which may respectively represent changes in either cell number or cell size. We may be justified therefore in relating changes in cellular composition to the kind of selection pressures which acted during the adaptation of this strain.

There is little doubt that the increased body size in the exceptional strain is due to the correlation between body size and egg production. It is significant that the increased size has been produced by a change in growth rate, not by extending the duration of the growth period. Earlier tests have shown that this is the route most commonly followed when body size is increased. Under favourable conditions there are only minor differences in egg production between the controls, the adapted cage population and the large strain, and this suggests that there is an upper limit to egg production and that, provided a certain body size is reached—probably that associated with normal ovariole number—further increase in body size is unimportant. Below that level the correlation of body size with ovariole number and hence with egg production will be of major importance.

There is a further aspect to the gene-environment interaction shown by the large strain which recalls earlier experience. It is commonly found in lines in which body size has been increased by changes in growth rate, as opposed to extension of the growth period, that body size is particularly susceptible to nutritional stress (Robertson, 1964). When grown under crowded conditions on the EDTA medium, body size in the large strain suffered greater decline than any of the other EDTA-adapted strains. Development time was not so disproportionately altered and this suggests that the crowded conditions particularly affected post-critical growth, the duration of which is not affected by diet.

The particular aspects of metabolism which are affected by the chelating action of EDTA are unknown. The genetic evidence that changes during adaptation have altered all major chromosomes and have involved extensive between-chromosome interaction suggests that many processes may be affected. The total sterility and conditional lethality caused by substituting the third chromosomes from the

control into the background of the EDTA cage population is the kind of chromosome incompatibility we might expect to find in comparisons between incipient species. In the present case, such differences are a by-product of the adaptation to the new environment and have arisen without selection for reproductive isolation. It is hardly surprising that the population shows phenotypic stability when natural selection for growth on the EDTA medium is relaxed.

Such chromosome incompatibility is very relevant to natural situations in which spatially isolated strains or biological races become adapted to a new diet, such as a different food plant, etc. In this context the use of a chelating agent in laboratory studies of adaptation may involve a closer parallel to commonly occurring natural conditions than, say, novel insecticides. Heed & Kircher (1965) have described recently an example of adaptation on the part of *Drosophila pachea* which breeds in the stem of the senita cactus—a habitat which is unfavourable for the growth of other species of *Drosophila* and which contains a particular sterol which is required by *D. pachea*.

The evidence in these experiments for systematic changes according to the relative importance of different components of fitness suggests there is considerable scope for manipulation of selection pressures to determine their effect on the mean score of quantitative characters such as body size. Experiments which are confined to populations which are already well adapted are handicapped by the unfavourable effects of virtually any major genetic change. The creation of new equilibria, by calculated intervention in the course of adaptation to a new environment, offers a technique for the study of the ecological origin of differences between species in body size and other characters.

SUMMARY

1. Populations of *Drosophila melanogaster* have been adapted to a new, initially unfavourable diet by adding to the food medium the chelating agent, EDTA, which lowers survival, lengthens development and reduces body size, according to the concentration.

2. Six populations were allowed to adapt to the new diet without intervention and compared with two additional populations in which there was either artificial selection for fast development time or in which the effects of variation in development time were minimized and higher egg production was favoured instead.

3. All populations adapted successfully and some were able to grow on medium with EDTA concentrations which were lethal for the original population.

4. Under uncrowded conditions on EDTA-free medium, in seven out of the eight populations, body size was reduced by about 7% below the level of the original population and the larval period was shorter in several instances. But in the population in which higher egg production was favoured, body size was 7% greater than in the original population and 16% greater than the average of the other EDTA-adapted populations. This contrast was attributed either to intense natural

selection for shorter development time or to selection for a higher rate of egg production, which is positively correlated with body size when larvae are grown on sub-optimal conditions.

5. Under crowded, competitive conditions, the fitness of the EDTA-adapted and the original populations was reversed according to the presence or absence of EDTA.

6. Genetic differences between one of the EDTA-adapted populations and the original population were studied by using marked inversions to interchange chromosome pairs. Larvae of the alternative genotypes were grown on different diets and adaptation was shown to have involved changes in all major chromosomes and also substantial, complementary interaction between non-homologous pairs. Substitution of the third pair of chromosomes from the original Pacific stock in the background of the adapted strain led to complete sterility of females, on all diets tested, and lethality of both sexes at higher levels of EDTA.

7. The creation of new equilibria, by manipulating the relative importance of components of fitness, in the course of adaptation to a new environment, offers a valuable technique for studying the selective forces which influence the mean value of quantitative characters generally.

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