Studies in Quantitative Inheritance.

by

Forbes W. Robertson.

CONTENTS

From JOURNAL OF GENETICS, Vol. 50, No. 3,
pp. 414–448, February, 1952.]
 PRINTED IN GREAT BRITAIN

STUDIES IN QUANTITATIVE INHERITANCE

I. THE EFFECTS OF SELECTION OF WING AND THORAX LENGTH IN *DROSOPHILA MELANOGASTER*

By FORBES W. ROBERTSON and ERIC REEVE

Genetics Laboratory, Animal Breeding and Genetics Research Organization, Edinburgh

(With Fourteen Text-figures)

CONTENTS

1. Introduction

Developments in the field of quantitative inheritance have lagged behind other branches of genetics, but animal-breeding research is making ever greater use of biometrical methods, which have to be based on assumptions about the nature of gene action. In order to provide a broader foundation for such assumptions, there is a need for detailed investigation of the inheritance of typical quantitative characters in a variety of laboratory animals. With these considerations in mind we began to study the inheritance of body size in *Drosophila melanogaster* four years ago. Limited facilities at the start of the work made it necessary to use *Drosophila*, which has obvious advantages for extensive selection and breeding tests, and we chose body size for study as it is one of the classical quantitative characters. The genetic control of this character is being investigated by selecting a number of lines from wild stocks for large or small size, observing different aspects of their behaviour under continued selection, reversed selection, etc., and using intercrosses, progeny tests and chromosome assays to analyse the changes brought about by selection.

Work of this kind is not easily broken up into natural sections for presentation, since new results are continually coming forward which force us to modify our working hypotheses, and critical tests are hard to find. In fact, it has become abundantly clear to us that the genetic mechanisms under study are extremely complex, and no simple formal theory of polygenic action is adequate to describe them, let alone to predict their behaviour under experimental treatment.

The present paper is intended to provide a background for the more detailed analyses of particular selection lines which will follow. It describes the technical procedure, gives a general account of the behaviour of our different selection lines, and examines the theoretical background against which we are viewing the problems of quantitative inheritance.

2. TECHNICAL PROCEDURE

(*a*) *The measurement of body size*

The first technical problem was to find a method of measuring the body size of live flies rapidly and accurately. This was solved by constructing a small movable platform (Fig. 1) which is attached to the moving stage of an ordinary microscope, and can be

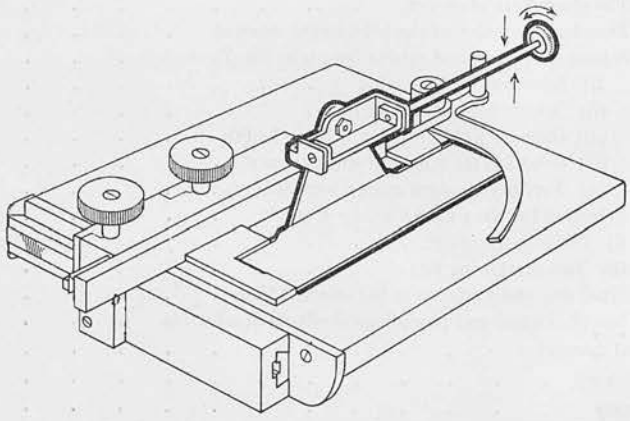


Fig. 1. Instrument for measuring flies, shown attached to moving stage of microscope. The arrows indicate the vertical and rotational movements of the lever by which the position of the fly on the platform is adjusted. The platform has a layer of thin cloth glued on its surface to prevent the fly sliding off when the platform is tilted.

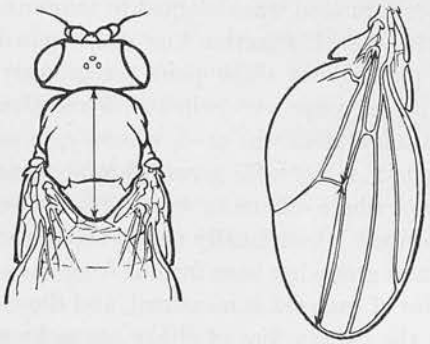
rotated about either of two horizontal axes at right angles to each other by twisting or moving vertically an attached lever. The platform is covered with thin cloth to which the etherized fly readily adheres; the fly is placed on the platform in approximately the correct position, and the dimension required can be brought into the exact position and alinement for measuring with a calibrated ocular micrometer by use of the moving stage and platform adjustments, and by rotating the axis of the micrometer. By this method it is possible for a skilled operator to measure 150–200 flies in a day before fatigue ruins her temper and the accuracy of measurement.

Provided that the two ends of the dimension to be measured are brought into focus simultaneously, accuracy is limited in practice only by the degree of definition of the two end-points. For our purpose wing length and thorax length were chosen as closely correlated measures of body size. Thorax length is measured from the tip of the scutellum

to the anterior edge of the thorax when the supra-alar bristles are approximately in focus at the same time as the tip of the scutellum (Fig. 2*a*). This dimension was about 0·87 mm. long in males and 1·0 mm. in females in our unselected stocks reared at 25° C. Wing length was measured along longitudinal vein IV in two roughly equal segments which add up to almost exactly twice the thorax length in our unselected stocks at 25° C. (Fig. 2*b*). The inner angle of the second basal cell is not always clearly defined, but in all stocks used it was found that inaccuracy of measurement of wing length due to ill-definition of points was negligible in comparison with phenotypic variation.

(*b*) *Culture methods and the control of environmental variation*

Overcrowding and temperature fluctuations are well known to affect body size in *Drosophila* cultures, and methods of reducing environmental influences to a minimum are therefore essential in studying the genetics of size. A standard sterile medium technique is available (Begg & Robertson, 1950), but was found impracticable for large-scale selection work. Preliminary tests also showed, to our surprise, that the phenotypic variance



a *b*

Fig. 2. The dimensions measured. The arrows indicate thorax length (above) and the two segments of wing length (below).

of size was not reduced by rearing on a sterile medium. We had therefore to rely on an agar medium of maize meal and molasses, fortified with dried baker's yeast. A generous allowance of live baker's yeast, made into a pasty consistency, is smeared over the surface of the medium before use. For selection work and size tests flies are reared in vials containing about $\frac{1}{2}$ in. depth of the medium, and overcrowding is prevented by introducing a maximum of seventy eggs into each culture vial, instead of allowing the parent flies to lay eggs freely in it. A very convenient way of collecting eggs from a small number of parents (1–6 pairs) is to confine the flies in an inverted vial, stoppered with a cork carrying a round disc of agar, about $\frac{1}{2}$ cm. deep. The disks are cut from a cylinder prepared by allowing agar to cool in a vial of slightly smaller diameter than the oviposition vial, and are attached to the cork by wrapping a piece of absorbent paper round both cork and disk before fitting them into the vial, which also contains a square of absorbent paper to take up excess moisture. A core of constant diameter is removed from the agar disk with a cork borer and the cavity filled with live yeast. Food and oviposition surfaces are replaced every 24 hr. This method permits standardization of food supply and oviposition area, the former being an important factor in fecundity measurements (Robertson & Sang, 1944*a*,*b*) carried out in the course of the selection experiments. These culture

methods keep down environmental variation by ensuring that flies are always reared in presence of excess food supply, and that all the flies in a single vial emerge within a fairly short period of time. All the work to be described was carried out at 25° C. ± 0·5 unless otherwise stated.

Variability of environmental origin is still troublesome, in spite of all our precautions, and significant differences are often found in the mean size of flies from different vials put up at the same time and given identical treatment, while considerable differences may occur in the mean size of flies put up on different days. Labour was not available for handling regular controls before generation 20 in the Nettlebed selection experiments, but since then flies from an unselected mass-mating stock of the same origin as the selection lines have always been reared concurrently with each selection generation, so that the response to selection can be measured in terms of deviation from the unselected size, and different generations and experiments can be directly compared.

(c) *Selection procedure*

The type of mating procedure used was designed to minimize the inbreeding inevitable in a small closed population. Each selection line was begun by measuring a sample of flies—generation 0—and picking out three pairs (or groups) of extremes of the kind required. The offspring of these groups were called *A*, *B* and *C* respectively of generation 1. Thereafter, a factorial mating system was used, whereby, in each generation, $A♂$ mated to $B♀$ gives offspring denoted *A*, $B♂ \times C♀$ gives offspring denoted *B* and $C♂ \times A♀$ gives offspring denoted *C*. Except where otherwise stated, this system has been used in all the selection experiments described. Occasionally one of these matings has failed to produce any offspring, and then a new group has been formed from one or both of the other groups. A maximum of twenty flies of each sex is measured, and the most extreme three selected from each mating. When the twenty flies of either sex to be measured cannot be drawn from a single vial, ten from each of two vials are measured (or as many as are available) and the flies are selected on the basis of their deviations from the vial mean rather than by their absolute size. At the beginning of the *Nb* selection experiment, several individual pair matings of *A*, *B* and *C* were cultured each generation, and the most fecund pair from each was chosen to continue the line, so that only six parents were used per generation; but individual females often gave insufficient offspring for our requirements, and after twelve generations the method was changed so that the most extreme three females and males in each group were mated together to give each new *A*, *B* and *C* generation.

When flies begin to hatch in the cultures, they are removed morning and evening to ensure that the majority are virgin. After measurement and selection the mated flies are kept overnight in a culture vial (with yeasted medium) before being transferred to the oviposition vials. Thus there is little danger that mating of females before they are isolated will affect the selection.

3. EXPERIMENTAL RESULTS

(a) *The characters observed*

The following characters have been studied in each selected line.

(i) *The mean lengths of wing and thorax*

These are best measured as deviations from unselected controls reared concurrently; but usually only limited numbers of controls (20–30 of each sex) could be measured in

each generation, and at times their size fluctuated out of step with the lines selected in both directions, due perhaps to some variation in treatment which escaped notice. Graphs of the total deviation between the long and short lines selected at the same time are sometimes smoother than those of deviations from controls, and we cannot assume that irregular movements in the deviations of individual lines always reflect periods of alternating stability and advance.

(ii) *The phenotypic variance*

This is calculated as the coefficient of variation, in order to eliminate most of the effect of change of mean size on variance. The use of this index is justified by the fact that males and females of the same stock are found to have roughly equal coefficients of variation though they differ by about 15% in mean size. The effect of the (often significant) size differences between vials has been eliminated since an early stage in the experiments by using the variance within vials.

Trends in the phenotypic variance during selection have been neglected in most previous work, which is surprising, since they may bring to light important changes in the genetic situation not obvious from the progress of the selection line. Short-term fluctuations must, of course, be interpreted with great caution, since the variance not only has a high sampling error but appears to be rather sensitive to uncontrollable environmental variations from one generation or experiment to another. Moreover, it is likely that different genotypes vary in their sensitivity to the normal range of conditions in our culture vials. Nevertheless, broad changes in variance, extending over many generations, make themselves perfectly clear in some of our selection lines, and provide an important clue to the interpretation of their behaviour.

(iii) *The effect of relaxing or reversing selection*

These tests throw a valuable light on the genetic changes resulting from selection, but limited assistance enabled us to carry them out only at long intervals, and critical stages in the progress of our selection lines have doubtless been missed in consequence.

The relaxed selection lines to be described were maintained by allowing about twenty pairs of flies, picked at random each generation, to mate and lay eggs together. These were then cultured under the usual (surplus food) conditions, seventy per vial. The lines thus avoided the severe competition found in ordinary bottle cultures. The two reversed selection experiments were treated in the same way as the forward selection lines, except that more intense inbreeding was practised in the first (started from generation 23 of the *Nb* wing selection lines). In this case the three extreme males and females from each of the *A*, *B* and *C* sublines were mated together to form three separate reversed selection lines, and each was continued by mating together the three extreme males and females.

(iv) *Fertility changes*

These depend on rate of egg production and the proportion of eggs which yield adults (emergence percentage). Both components are known to be sensitive to environmental variations, and changes in them must be interpreted cautiously unless elaborately controlled tests can be made. Records have been kept from an early stage of the number of eggs cultured and the number of adult flies emerging in each vial of the selection lines, so that

we can plot changes in the percentage emergence and can also tell when the fecundity of the females fell very low.

(*b*) *The characteristics of the wild stocks selected*

Selection experiments have so far been confined to two wild-type stocks, each descended from a single impregnated wild female, whose progeny had been kept in mass cultures under ordinary laboratory conditions for about ten generations before the experiments began. The stocks are called *Nettlebed* (*Nb*) and *Edinburgh* (*E*) according to their districts of origin.

These stocks differ slightly in relative wing and thorax lengths (Table 4) and in the phenotypic variance of wing length (Table 1). Brother-sister mating without selection for thirty generations leads to a significant and marked decline in the coefficient of variation of each (Table 1), equivalent to a loss of variance of nearly one-half for *Nb* and one-third for *E*. This indicates that both stocks contain a large amount of free genetic variance.

Table 1. *Coefficients of variation in unselected and inbred stocks*

	Stock	
Culture	*Nb*	*E*
Unselected	1·80	1·55
Inbred 30 generations	1·35	1·26

Inbreeding produced an average decline of 3·5 % in size in the three *Nb* lines tested, but no change in size was observed in the single *E* inbred line. We can therefore assume that progressive inbreeding in the selection lines will not of itself produce much change in size.

(*c*) *Selection of wing and thorax length in the Nb stock*

Parallel selection lines for long and short wing, long and short thorax, have now been continued for fifty generations. Started with inadequate facilities in London during the autumn of 1946, these lines were affected to such an extent by the severe winter that year (in particular by the power cuts it caused), that selection had to be abandoned for generations 6–8. Conditions rapidly improved after generation 12, when we moved to Edinburgh, and technical assistance enabled us to maintain unselected controls regularly after generation 19. The lines were kept at about 23·5° C. in a constant temperature from generations 13–26, and afterwards under rather better temperature control in an incubator at 25° C.

Progress is shown in terms of mean size in Figs. 3 and 7, and after generation 19 as deviations from control size in Figs. 5 and 8. Controls for wing length were measured for generations 11, 12, 16 and 17 as shown in Fig. 3. The values for the two sexes are averaged in these graphs, since they show the same general trends. Fig. 4 shows the rates of divergence from each other of the two wing selection lines, and Fig. 9 shows their variance.

(i) *Selection for long wing*

The rate of divergence between the long- and short-wing lines during generations 1–5 is believed to be exaggerated in Figs. 3 and 4, owing to the differential effects of the early temperature variations on the two lines, since these could not always be cultured simultaneously because of varying rates of egg production. But it seems clear that the two lines

responded immediately selection began, and that much of their early progress was lost during the enforced random mating of generations 6–8. The long-wing line must have declined right back to the unselected level during these three generations, since it was still there at generation 11. It seems to have advanced again at least from generations 11–17, and then remained almost stationary at some 9 units above controls till about generation 30, since when there has been slight progress; but the line became stationary again soon after generation 40.

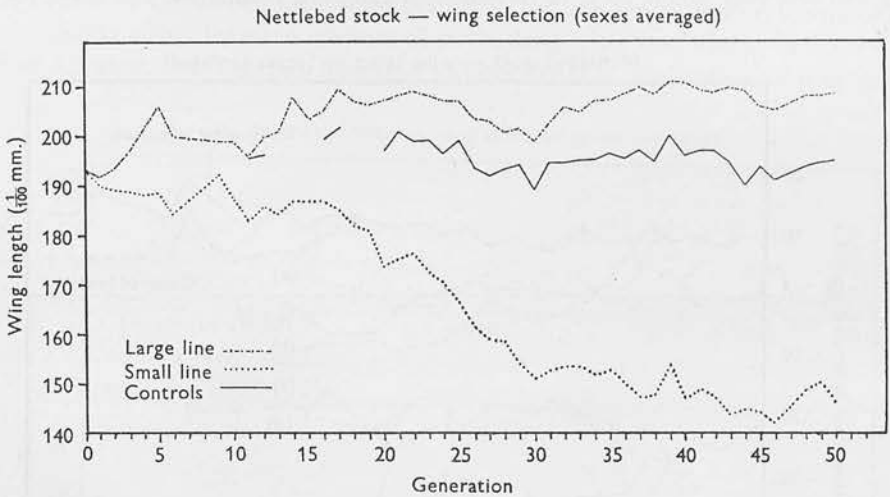


Fig. 3. Mean wing length in the *Nb* wing selection lines, during the first 50 generations. The marked fluctuations of the controls, measured regularly from generation 20, indicate the disturbing effects on size of uncontrollable variations in temperature, nutritional conditions, etc. (cf. Fig. 7).

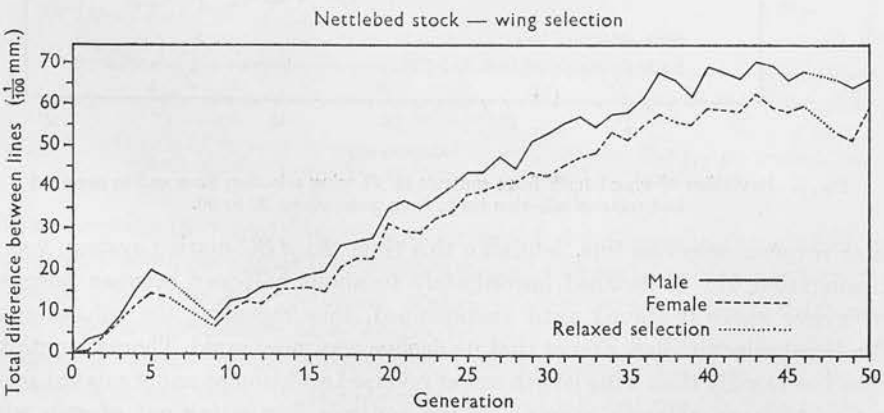


Fig. 4. Divergence of *Nb* long and short wing-selection lines from each other.

Three-reversed selection lines, with increased inbreeding intensity (as already noted), were taken off at generation 23 (Fig. 5). One failed, but the others (*a* and *b* in the graph) returned within three generations to unselected size, where (*a*) remained while (*b*) declined about 9 units more and then also remained stationary. Eventually a third back-selection line was made from the F_2 of the cross between these two lines ($a \times b$ in Fig. 5), but remained intermediate between them.

Lines in which selection was relaxed were taken off the main selected line at generations 27 and 37. The first had returned completely to unselected size when measured six generations later, while the second returned about half-way, mainly in the first two generations, and then remained fairly steady. It is interesting that thorax length declined relatively less than wing length in both these lines. Thus in the first case it remained significantly above the control level, while in the second test the average values for generations 8, 9 and 13 of random mating (intermediate stages were not measured) show that thorax length had lost only about 20% of its selection progress, compared with 50% for wing length.

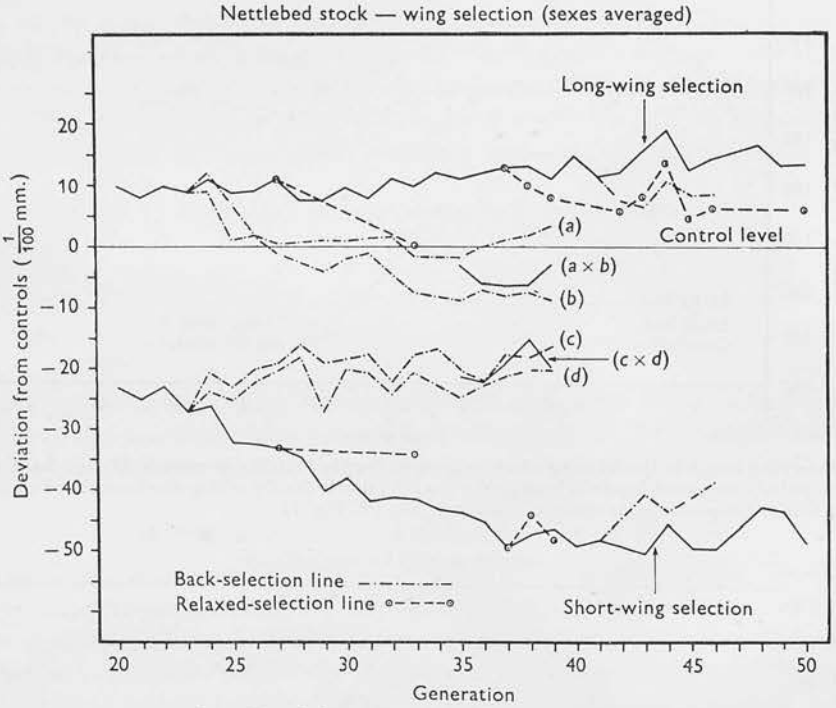


Fig. 5. Deviation of wing length from controls in *Nb* wing selection lines and in reversed and relaxed selection tests, from generations 20 to 50.

A later reversed-selection line, retaining this time the *ABC* mating system, was taken off at generation 41. It declined immediately to about half-way between selected and control levels, where it stayed until discontinued, thus repeating the behaviour of the second relaxed selection-line, except that its decline was more rapid. Thorax length tended to decline less rapidly than wing length under reversed selection as under relaxed selection. Generation 44 is an extreme example of the controls fluctuating out of step with the forward, back and relaxed selection lines. The peak in all three for this generation (Fig. 5) is obviously artificial.

The trends in the coefficient of variation of these lines are of great interest, and are shown in Fig. 6. Here the values of the two sexes are averaged, and only the average level of the controls is shown, since their variance did not show any marked time trend. Bearing in mind the uncertainty of the estimates for the first ten generations, when between-vial effects were not eliminated, we can say that the variance of the long-wing line perhaps

rose early in its history, and certainly remained well above control level from the early twenties. Reversal of selection on both occasions pulled the variance down well below unselected level, the decline being particularly sharp on the second occasion, and the second relaxed-selection test also showed a marked decline in variability. No decline was actually observed in the first relaxed-selection line (not shown), but this was only tested once and the result is not conclusive. Several inbred lines, without selection, were taken off the long-wing line at generation 30 and showed a decline in both size and variability similar to that of the second back-selection line. They are not shown on the graphs.

These results taken together indicate that the long-wing line, during or after a short period of advance, developed an increased genetic variance, and entered a long period of

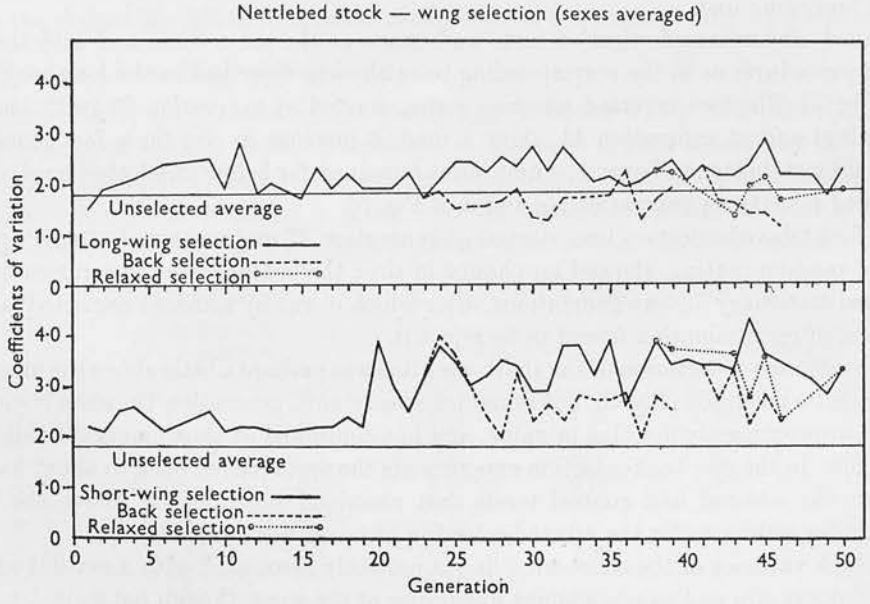


Fig. 6. Coefficient of variation of wing length in *Nb* wing-selection lines and in reversed- and relaxed-selection tests. Average level of unselected controls is shown.

equilibrium between the force of our wing selection and some opposing force, which limited further advance and forced down both mean size and variance as soon as selection pressure was relaxed. This state of equilibrium probably arose before generation 20 and still continues, but genetic changes have evidently taken place in spite of it, since average wing length has increased since generation 30, and the second relaxed- and back-selection lines contrast with the first in remaining well above unselected size. It follows that some genes which increase size must have become fixed in the line since generation 27. We can also infer that the barrier to further advance is not simply infertility, since the selected females were still laying plenty of eggs at generation 50, and Fig. 9 shows that the percentage emergence had only declined slightly between generations 15 and 40. Clearly some complex genetic mechanism must be involved in the equilibrium, and this is now under investigation by special tests. These will be described in the next paper.

(ii) *Selection for short wing*

Here there is a marked contrast to the previous case. Progress before generation 16 is uncertain, for the reasons already given, and the trends shown in Fig. 3 are misleading. Assuming that the long-wing line was approximately the same size as the controls during generations 9–11, we can deduce that the short-wing line declined at least 7 units during the first six generations of selection, and showed little or no regression during the three generations of relaxed selection. Slow progress was made from generations 9–16, when the total advance was about 12 units. From that point there was a rapid and steady advance for about twenty generations, since when there has been little change. The total change in wing length is about 50 units (23 %)—more than three times as great as the change made by the long-wing line.

Relaxed- and reversed-selection tests were made at the same times and with the same mating procedures as in the corresponding tests already described in the long-wing selection (Fig. 5). The two reversed-selection series, started at generation 23 (with increased inbreeding) and at generation 41, show a modest increase in size for a few generations followed by stability, and even the first series remained far below unselected level, though continued for sixteen generations (*c* and *d* of Fig. 5).

The first relaxed-selection line, started at generation 27 and measured after six generations of random mating, showed no change in size; the second, from generation 37, also remained stationary for two generations, after which it was by accident neglected, and the suspicion of contamination forced us to reject it.

The coefficient of variation of the short-wing line was perhaps a little above the unselected level from its inception (Fig. 6), but remained steady until generation 19, when it suddenly became approximately doubled in value, and has continued at this remarkably high level ever since. In the two back-selection experiments the variance fell back to about half-way between the selected and control levels, but remained substantially above the latter. Satisfactory estimates for the relaxed-selection lines are not available.

The high variance of the short-wing line is probably associated with a sex-linked effect which reduces size and causes a slight upcurving of the wing, though not enough to make measurement difficult. This syndrome of effects behaves in outcrosses like a sex-linked recessive gene with incomplete exhibition. Flies showing slight wing curvature began to be noted about generation 16, and were rejected as parents, but the line soon became homozygous for this effect. An effect later identified as an allele of *vestigial* also began to appear in the line at about the same time. This usually caused slight and variable notching of the wings when heterozygous, and complete sterility when homozygous in females (Beatty, 1949), and it probably reduced wing length in the heterozygote even when no notching occurred, as we had some difficulty in eliminating it from the selection line.

It would be easy to explain the increase of variance for a few generations after generation 19 as due to segregation of one or both of these factors, but since one became fixed and the other eliminated long before generation 30, we are left with no reason why the high variance should continue for another twenty generations. Another hypothesis is that the increased variance was caused by high sensitivity of the wing curvature factor (in its effect on length but not curvature of the wing) to environmental variation. But this theory is also unsatisfactory, for we can infer that a major fraction of the increased variance is of genetic origin (i.e. due to segregation), since the response to selection was rapid during

the first eighteen generations of increased variance. An environmental effect which doubled the coefficient of variation (i.e. multiplied the phenotypic variance by 4) would inevitably result in a very low heritability and therefore in a very slow rate of advance. This conclusion is also supported by the decline in variance when selection is reversed. It is therefore all the more surprising that the short-wing line still retains its high variance long after response to selection has apparently ceased.

It might be possible to explain the high variance as due to metric bias, if it had increased gradually, but this theory is difficult to reconcile with its sudden rise and approximate constancy afterwards. We are forced to conclude that the high variance is due to more than one agency, perhaps acting in succession, and further tests are necessary before we can say more. Tests on the curved-wing factor are in progress, and genetic tests will be carried out on the short-wing line as soon as the completion of work on the long-wing line permits.

(iii) *Changes in heritability of wing length*

The heritability (or fraction of the phenotypic variance which is additively genetic) can be estimated for any period of selection as the ratio of rate of advance to selection differential. The latter is simply the average deviation of parents from the mean size of their generation (each deviation is taken from the mean for the vial containing the fly), and the rate of advance per generation has been estimated as the linear regression coefficient of mean deviation from controls on generation number. Values have been calculated for both five and ten-generation intervals. In the former, the rate of advance has been calculated as the regression for the sets of six points given by generations 20–25 inclusive, 25–30, etc.; and in the latter for the sets of 11 points given by generations 20–30 inclusive, 30–40, etc. The results are shown in Table 2, for both long- and short-wing lines, during generations 20–50.

Table 2. *Heritability of wing length in Nb wing-selection lines*

	Selection differential ($\frac{1}{100}$ mm.)		Advance per generation ($\frac{1}{100}$ mm.)		Percentage heritability	
Generation	Long wing	Short wing	Long wing	Short wing	Long wing	Short wing
20–25	5·1	7·6	−0·14	1·53	−2·7	20·1
25–30	5·4	6·4	0·38	1·68	7·0	24·7
30–35	5·7	6·1	0·36	0·95	6·3	15·7
35–40	5·5	6·8	0·48	0·75	8·7	11·1
40–45	5·6	7·1	0·30	−0·15	5·4	−2·1
45–50	5·3	5·4	0·11	−0·77	2·1	−14·1
20–30	5·2	7·0	−0·08	1·83	−1·5	26·1
30–40	5·6	6·4	0·46	1·00	8·1	15·6
40–50	5·5	6·2	0·12	−0·40	2·1	−6·4

The estimates taken over five and ten generations are in reasonable agreement, although sampling errors in the former may be rather high. In the long-wing line heritability appears to have been zero for generations 20–25 or 30, then to have risen to about 8% until around generation 40, when it fell again to about 2%. In the short-wing line the heritability was about 25% for generations 20–30, declined during the next ten generations and then became negative, so that there seems to have been a tendency on the part of this line to increase in size after generation 40, in spite of continued selection. These results bring out quite clearly the point already made, that a large fraction of the increased variance of the short-wing line must be due to segregation, at least for the first twenty generations after its appearance.

(iv) *Selection for long and short thorax*

The graphs showing changes in thorax length are on twice the scale of those for wing length, to allow for the fact that the thorax is half as long as the wing. Equal proportional changes will therefore have the same magnitude in the graphs for the two characters. Selection for long and short thorax was relatively ineffective during the first twenty generations (Fig. 7), and there was hardly any regression during the early period of random mating. The long-thorax line appears to have advanced steadily during generations 20–40 and then to have become stable (Fig. 8), so that its total progress has been greater than that of the long-wing line in thorax length, though less in wing length (Table 4).

Soon after generation 20, a light-eyed mutant, later identified as a recessive allele of *lt*, was noticed in the long-thorax stock, and has continued to segregate in it ever since. This suggests the probability that we have been selecting it in heterozygous form because of

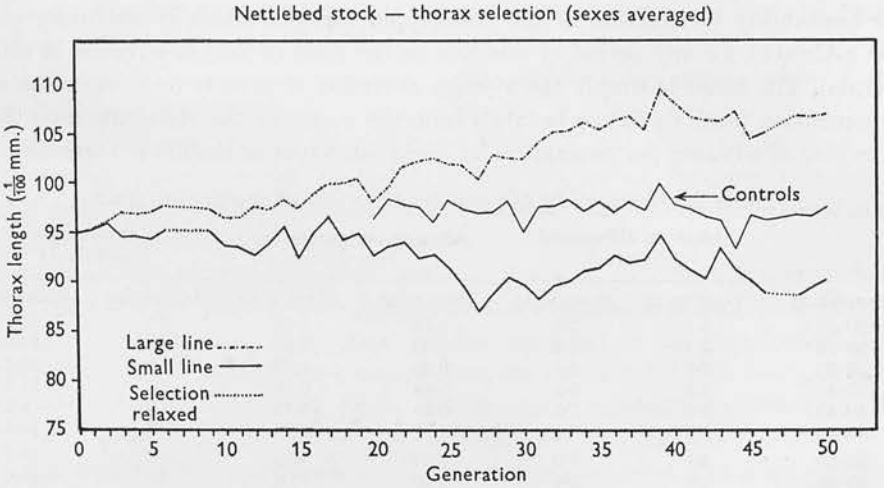


Fig. 7. Mean thorax length of *Nb* thorax selection lines during the first fifty generations.

an effect on size, since otherwise it should have been fixed or eliminated in less than thirty generations. The homozygote appears to have no size advantage, but we have yet to test the effect on size of the heterozygote.

Two relaxed selection experiments have been taken off the long-thorax line. The first was a subculture from generation 25 homozygous for the *lt* allele, and was measured after fifteen generations of normal laboratory culture. No change in wing or thorax length was detected. The second was a normal relaxed selection experiment started at generation 37 and measured regularly for a few generations. This line lost about 15% of its deviations from the controls and then remained stationary. These tests are not shown on the graphs.

The coefficients of variation for wing and thorax length in the long-thorax line are almost identical with those for the unselected stock, so that selection does not appear to have altered the variance. There has thus been no sign in this line of the unstable equilibrium which characterizes the long-wing line, nor of the increase in variance found in all the other lines, and it seems probable that there has been simply fixation of most of the available genetic variability.

The short-thorax line acquired rather low fertility early in its history, so that selection intensity has not been very high, and it never made great progress. After reaching its lowest point at generation 27 (Fig. 8), it appears to have regressed for the next seventeen generations and then to have declined rapidly to its previous lowest level. The upward movement during generations 27–44 is almost parallel to that of the long-thorax line, so that the total deviation between the two lines remains almost stationary during this time, and it is possible that these trends are due to both lines remaining steady while the controls declined in size. If so, the controls seem to have regained their original size by a rapid rise during generations 44–48, and it is more likely that there was a real regression

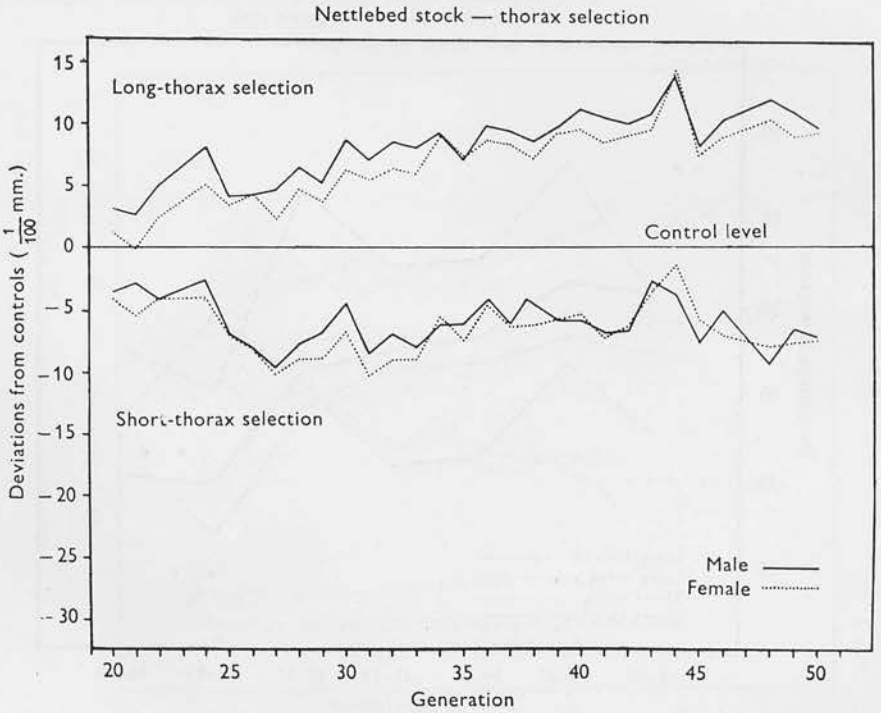


Fig. 8. Deviation of thorax length from controls in *Nb* thorax selection lines from generations 20 to 50. The two sexes are shown separately.

in size in the short-thorax line, due to low selection intensity caused by the low fertility. It has not been possible to make additional tests on this line, so that there is no further evidence on this point. However, it is interesting to find that the coefficients of variation were 30% above the control level for wing length, and nearly 50% up for thorax length, taking average values for generations 35–50. This, coupled with the slow progress and the probability that size regressed when selection pressure was less stringent, suggests that the line may have developed some genetic mechanism limiting further advance comparable to that in the long-wing line. If so, it is interesting that such a mechanism should develop in the long-wing and short-thorax lines but not in the short-wing and long-thorax lines. Evidently chance plays an important part in the response to selection, and we cannot assume that different lines taken from a single stock will react in the same way.

In the course of selection for short thorax a mutation appeared and was isolated; it is characterized by change in shape of the eggs, which have a semi-globular form. Exhibition is variable and viability appears to be unaffected. This mutant has been called 'hen-egg' (*hg*).

(v) *Fertility changes during selection*

Throughout the experiments we have recorded the percentage of adults emerging from each generation of eggs cultured, and the values for the four *Nb* lines, averaged over five generation periods, are shown for generations 14–50 in Fig. 9. This measure depends

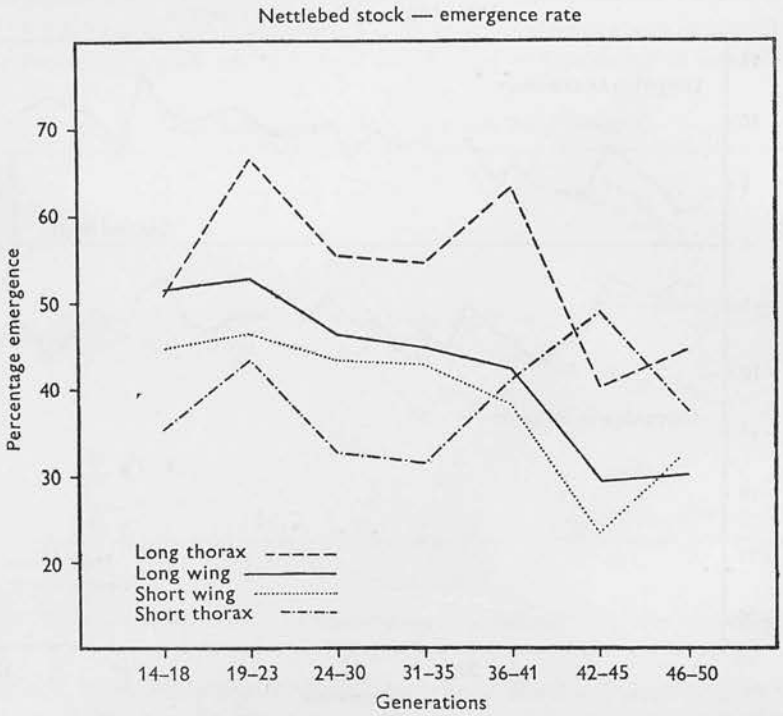


Fig. 9. Percentage of eggs yielding flies in *Nb* selection experiments, generations 14–50. Each value is an average of four generations, but the interval is not constant, since figures are not available for some generations.

on the viability of the sperm and unfertilized ova, as well as on that of the zygote, but experiments suggest that viability of the zygote is by far the most important factor.

It will be noticed in Fig. 9 that the two thorax lines showed a remarkably similar trend up to generation 40, the changes from one period to the next being absolutely parallel, while the two wing lines also follow a single (although quite different) trend very closely. This result is quite inexplicable at present, since all four lines were run together each generation, and as far as we are aware were treated identically. Since their genetical behaviour was quite different in other respects, it can only be supposed that some unnoticed similarity of treatment between the two wing lines and between the two thorax lines caused this striking result. It cannot be due merely to the fact that we selected for thorax length in one case, for wing length in the other.

(d) Selection for wing length in the E stock

It was possible to measure controls from the start of this experiment (owing to an error they were omitted from generations 1 and 3), so that we have a much better picture of the effects of selection in the early generations. Parallel selection for long and short wings has been continued for twenty-nine generations, and the progressive deviations of the two lines from the controls and from each other are shown in Figs. 10 and 11. Since the start of the experiment there has been a steady response in both directions, with little evidence of alternating periods of change and stability, but the total rate of divergence between the two lines (Fig. 11) appears to have been faster over the first eight generations than afterwards, and has again declined since generation 22.

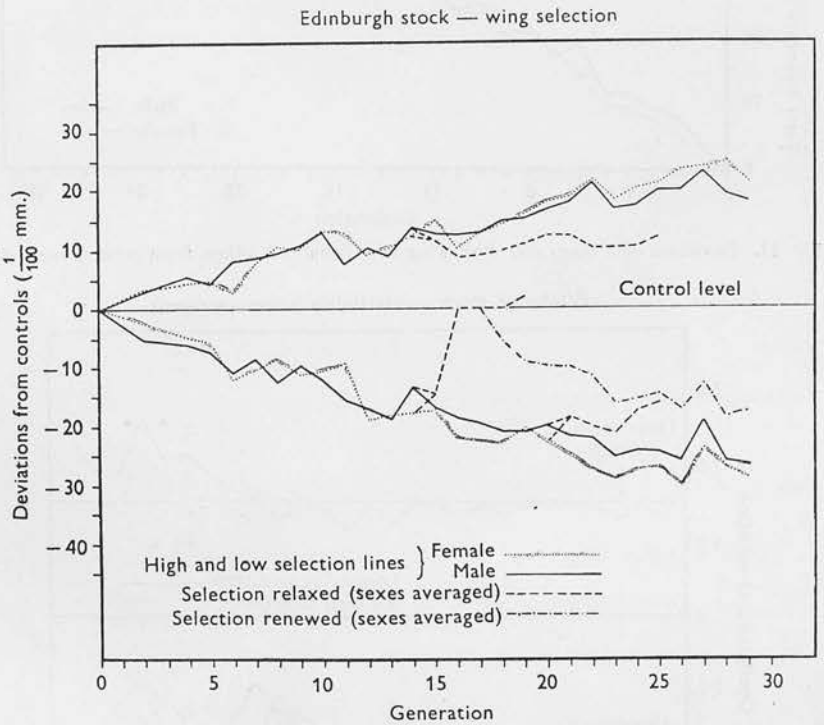


Fig. 10. Deviation of wing length from controls in *E* wing-selection lines and relaxed-selection tests, from generations 0–29. The two sexes are shown separately.

(i) *The long-wing line*

A single relaxed-selection line was taken off at generation 14 (Fig. 10) and remained almost constant in size. The coefficient of variation remained very steady and rather below the unselected level during the first nineteen generations, in spite of the steady advance throughout that period (Fig. 12). It then rose and remained well above the unselected level. The variance of the relaxed-selection line also seems to have risen after a time, but this is probably an environmental effect, since the variance returned to the unselected level. The percentage emergence has remained very high throughout the experiment (Fig. 13), and the rate of egg production has also remained high, so that selection has not affected fertility.

Heritability estimates, similar to those described above, are given in Table 3. The value for the long-wing line is rather variable, but it seems doubtful whether the difference

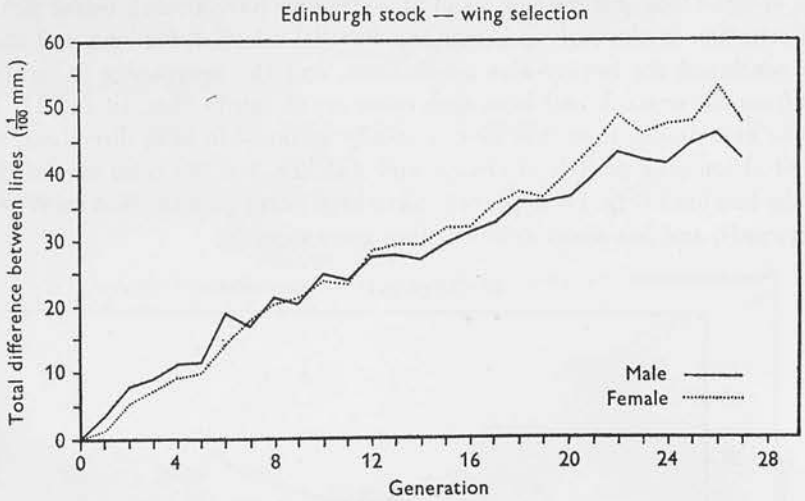


Fig. 11. Deviation of *E* long- and short-wing lines from each other, from generations 0–27.

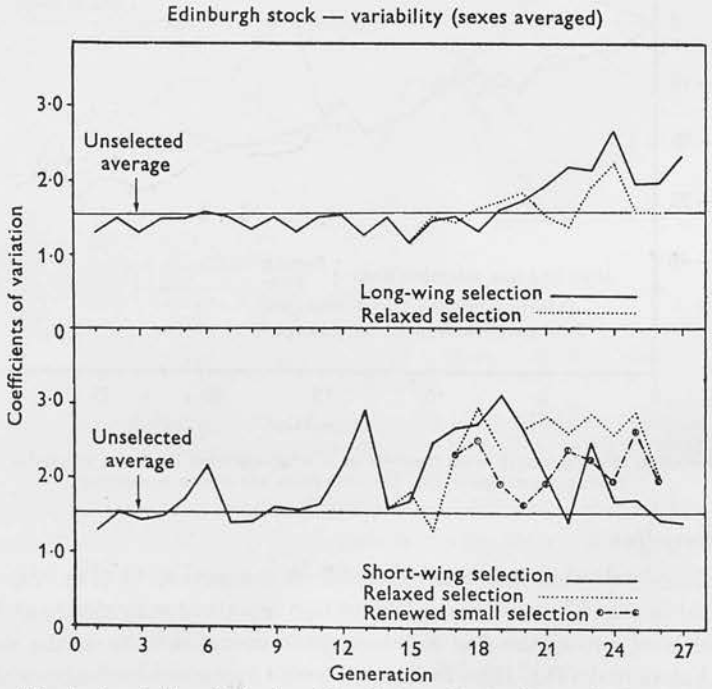


Fig. 12. Coefficients of variation of wing length in *E* wing-selection lines and relaxed-selection tests. The average value of the unselected controls is shown.

between the first two five-generation periods is significant, while the high value of 25·1 % for the fourth period is probably an artefact, due to some temporary trend in the controls. This is indicated by the drop for the same period in the value for the short-wing line.

It seems probable that the heritability in the long-wing line was about 30% for the first ten generations and then maintained a level of 6–8% for the rest of the experiment.

The behaviour of this line forms an interesting comparison with the large lines in the *Nb* experiment. A much longer and steadier advance was obtained than in the *Nb* long-wing line, and there was no sign of regression when selection pressure was relaxed. But

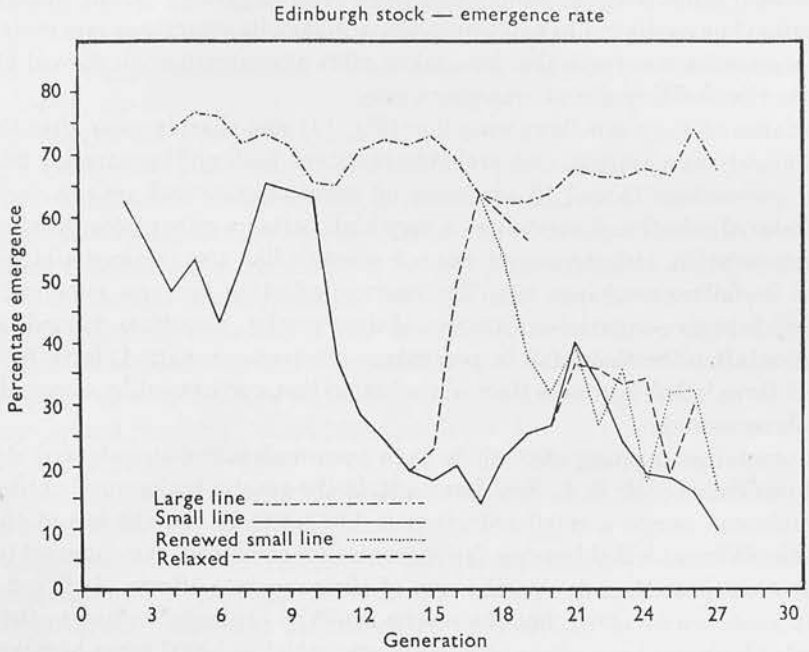


Fig. 13. Percentage of eggs yielding flies in *E* wing-selection lines, and in relaxed- and renewed-selection tests on short-wing line.

Table 3. *Heritability of wing length in Edinburgh selection lines*

	Selection differential		Advance per generation		Percentage heritability	
Generation	Long wing	Short wing	Long wing	Short wing	Long wing	Short wing
0–5	4·0	3·9	0·78	0·94	19·3	24·2
5–10	4·0	3·8	1·96	0·95	48·5	24·7
10–15	3·5	4·6	0·23	1·00	6·7	21·6
15–20	3·7	4·5	0·92	0·53	25·1	11·6
20–25	5·9	3·6	0·40	0·91	6·7	25·6
25–30	5·7	2·3	0·34	0·29	5·9	12·7
0–10	4·0	3·9	1·20	1·16	29·9	30·0
10–20	3·6	4·6	0·52	0·94	14·7	20·5
20–30	5·8	2·9	0·49	0·45	8·3	15·2

the decline in the rate of progress, and particularly the recent rise in the variance, point to underlying changes which may perhaps reflect the development of a kind of balance similar to that of the *Nb* long-wing line.

(ii) *The short-wing line*

Although the general rate of progress was very similar to that of the long-wing line (Fig. 10), this line shows striking differences in its behaviour, and interpretations based purely on the rate of its response to selection would be highly misleading. The percentage

of eggs yielding adults fell sharply to under 20% after generation 12 (Fig. 13), so that it was difficult to maintain the selected line. A relaxed-selection line taken off at generation 14 showed a rapid rise in both size and percentage emergence, so that it had returned to unselected size in two generations and to unselected emergence rate in three. Renewed selection, after three generations of random mating, reduced both the size and the emergence rate again sharply, though not quite to their previous level. Meanwhile, the main selection line continued to make progress, although its emergence rate remained very low, and a second relaxed-selection line, taken off it at generation 20, showed little or no tendency to rise in either size or emergence rate.

The variance of the main short-wing line (Fig. 12) rose sharply soon after the decline in rate of emergence occurred, and probably remained high until generation 24. The low values for generations 14 and 15 are based on small samples and are not very reliable. The first relaxed-selection line retained a very high variance either from the start or after its second generation, and the second relaxed-selection line also retained a high variance, in spite of its failure to change size. The renewed-selection line also remained variable.

It is possible to give a partial explanation of these results. Genetic tests made at generation 15, soon after the sharp fall in percentage emergence occurred, have revealed the presence of three lethal factors in the main selection line, one carried by a second and two by third chromosomes.

The chromosomes carrying the lethals have been maintained in balanced stocks, and tests made on them by Mr B. K. Sen show that, in the genetic background of these stocks, each chromosome causes a small reduction in body size in the absence of the others; but when the different lethal-bearing chromosomes are combined, they interact to produce a size reduction more than double the sum of their separate effects. It is not yet clear how many genes are involved, but the results could be explained as due to the presence in each lethal-bearing chromosome of a single gene which is lethal when homozygous and reduces size when heterozygous, these genes interacting with one another to cause a disproportionate decrease in size when they are together. An alternative possibility is that genes causing lethality and reduced size are linked together on each chromosome.

Selection of parents carrying these factors would explain the decline in percentage emergence and the rise in the variance of size in the short-wing line, and also the rapid rise in size and percentage emergence when selection was relaxed at generation 14. Since size and viability declined sharply when the first relaxed-selection line was again selected for small size, it is evident that the lethal effects had not been eliminated by three generations of mass mating. When the main selection line was relaxed a second time at generation 20, its size did not increase and the viability remained low, suggesting that the small size and lethal effects has become fixed in the line by this time. Some reduction in the severity of the lethal effects, either by selection of modifiers or by recombination eliminating genes responsible for part of the lethality, must have occurred between generations 14 and 20.

Various morphological abnormalities appeared sporadically in the short-wing selection line from generation 10 onwards, becoming fairly common by generation 15. They included blistered wings, plexus-like venation, deformed tarsi, absence of halteres, hemi-thorax, small eyes, and disarranged abdominal tergites. Selection for increased frequency of exhibition of these malformations in subcultures from the selection line has had little success except in the case of plexus venation, whose exhibition could be greatly increased, though much phenotypic variability always remained. Most of the abnormalities continue

to appear sporadically in both the main short-wing line and the line in which selection was renewed after three generations of random mating. It seems probable that they are pleiotropic effects of the lethal factors, or of interactions between them.

It will be noticed that our short-wing line shows many similarities of behaviour to the first high bristle number selection line described by Mather (1949). Thus both lines returned very rapidly to unselected level when selection was relaxed shortly after a period of low fertility had set in, and renewed selection produced a response much more rapid than occurred in the original selection line. When selection was relaxed a second time, a few generations later, no change in size or bristle number occurred. The two experiments differed, however, in the behaviour of viability when the first relaxed selection line was reselected. Viability declined in our experiment but remained high in Mather's.

Mather has explained the results of his selection experiment as due to the recombination of polygenes affecting bristle number, so that blocks of plus polygenes in coupling are built up in each chromosome; these blocks include genes adversely affecting fertility, so that they cannot become homozygous and fertility falls very low as selection continues. Under relaxed selection, sufficient of the low-fertility genes have apparently become separated from these blocks by recombination to enable reselection to make the blocks homozygous, and so to establish a stable high line. The very rapid change of size during the mass mating and reselection of the high line shows that each block behaves as a tightly linked unit, and is difficult to break down once it has been built up; and tests based on crosses between high, medium and low bristle number lines suggest that there is only a single such block in each chromosome.

Mather's explanation may be criticized on general grounds, for reasons we shall discuss later, and his data need to be reconsidered in view of their similarities to our own results. The behaviour of the short-wing line cannot be explained in terms of a number of elementary polygenes, some affecting wing length and some fertility, since there is marked interaction between the different chromosomes carrying the lethal effects. The results are best interpreted in terms of a few genes with rather large effects and subject to some degree of modification by selection of the genetic background. A similar hypothesis would explain the results obtained by Mather, in his high bristle number line, and is supported by the fact that tests based on crosses between high, medium and low bristle number lines suggested that there was only a single effective unit (of small genetic length) affecting bristle number of each major chromosome.

Changes in the heritability of wing length, calculated as before, are shown in Table 3. Heritability over the first ten generations was the same in both directions of selection (30%). Later it declined in the short-wing line, but more slowly than in the long-wing line, averaging 20% in the second and 15% in the third period of ten generations. Allowing for the artificially high value for the five-generation period 20–25, which has already been noted, the heritability seems to have been remarkably steady in view of the complex genetic changes which appear to have occurred in the short-wing line.

(*e*) *Final size and variance in the selected lines*

The relative changes produced by selection in the various lines are summarized in Table 4. It will be seen that the long-wing (*E*) line is already the largest in both wing and thorax length, while the short-wing (*Nb*) line is much smaller in wing length and slightly smaller in thorax length than the other small lines (Table 4(*a*)).

The relative changes in each dimension are shown in Table 4(*b*). From the values averaged for the two sexes, we find that wing and thorax length have made about equal progress under selection for long wing or short thorax; but the thorax has advanced twice as much as wing length in the long-thorax line (10·8% compared with 5·4%), while the wing has advanced twice as far as the thorax in the short-wing (*Nb*) line (23·5% compared with 11·4%).

Clearly change in one dimension generally produces a similar change in the other, presumably through its effect on general size, but there is also some degree of genetic independence between the two dimensions, since in one line wing length has made twice the

Table 4. *Dimensions of the selected lines at* 25° C.

(*a*) Mean size ($\frac{1}{100}$ mm.)

Line	Wing length		Thorax length	
	Males	Females	Males	Females
Long wing (*Nb*)	194·4	221·5	97·5	109·9
Long wing (*E*)	199·1	229·1	102·1	116·0
Long thorax (*Nb*)	191·1	218·0	100·1	112·7
Unselected (*Nb*)	179·9	208·4	89·0	103·4
Unselected (*E*)	178·8	205·2	92·0	105·6
Short wing (*Nb*)	139·7	157·3	80·0	91·3
Short wing (*E*)	152·9	177·0	82·3	94·0
Short thorax (*Nb*)	164·8	190·3	82·4	95·7

(*b*) Percentage deviation from unselected size

Line	Wing length			Thorax length		
	Males	Females	Average	Males	Females	Average
Long wing (*Nb*)	+8·0	+6·3	+7·2	+8·3	+6·3	+7·3
Long wing (*E*)	+11·3	+11·6	+11·5	+11·0	+9·9	+10·5
Long thorax (*Nb*)	+6·2	+4·6	+5·4	+12·5	+9·1	+10·8
Short wing (*Nb*)	−22·3	−24·6	−23·5	−11·1	−11·7	−11·4
Short wing (*E*)	−14·5	−13·8	−14·2	−10·5	−11·0	−10·8
Short thorax (*Nb*)	−8·4	−9·5	−9·0	−7·5	−8·8	−8·2

(*c*) Ratio of wing to thorax length (*d*) Percentage of female over male

	(*c*)		(*d*)	
	Male	Female	Wing length	Thorax length
Nb lines				
Long wing	1·99	2·02	13·9	12·7
Long thorax	1·91	1·94	14·1	12·6
Unselected	2·00	2·02	15·8	16·2
Short wing	1·75	1·72	12·6	14·1
Short thorax	2·00	1·99	15·5	16·1
E lines				
Long wing	1·95	1·97	15·1	13·6
Unselected	1·95	1·94	14·8	14·8
Short wing	1·86	1·88	15·8	14·2

Note. These values are based on the averages for generations 48–50 of the *Nb* lines and for generations 28–30 of the *E* lines.

advance, in another line half the advance, of thorax length. Moreover, the two short-wing lines have changed about equally in thorax length, but the *Nb* line has made nearly twice the progress of the *E* line in wing length (23·5% compared with 14·2%).

The ratio of wing to thorax length (Table 4(*c*)) shows very similar changes in each sex. In the *E* lines selection has tended to raise the ratio in the large line and lower it in the small line, whereas in the *Nb* lines the ratio has declined in the selection for long thorax and short wing, as we should expect, but has not risen in the other two lines.

The relation between wing and thorax length in flies of different body sizes raises problems of absolute-size allometry (Reeve & Huxley, 1945). The allometric relationship will not necessarily be the same in different strains and under different conditions, but

may vary with the factors causing variation in size. As we have seen, genetic changes induced by selection have different effects on proportions, according to the character selected and the direction of selection. Various kinds of environmental change may also have different effects on proportions. Thus preliminary experiments suggest that the ratio of wing to thorax length is decreased when body size is reduced by rearing flies at a higher temperature, while the ratio tends to be increased when size is reduced by restricting the food supply. Qualitative changes in nutrition might also affect this ratio. Finally, it is worth noting that certain environmental changes will alter one dimension without affecting the other. Thus unpublished experiments made by Mr L. Ragab under our direction showed that when temperature is decreased after pupation has begun, the thorax length remains unaltered but wing length may be considerably increased.

The changes made by males and females are compared in Table 4(*b*) and (*d*). Selection in either direction has tended to reduce the size difference between the sexes, with the exception of wing length in the two *E* lines. As a result, the sex which, on the scale of absolute size, is behind in the direction of selection has tended to catch up with the one in advance. This is most apparent in the *Nb* long and short-wing and long-thorax lines. One possible explanation of this effect is that there is a metric bias acting as a kind of law of diminishing returns, i.e. the net effect on size of a given gene substitution declines in proportion as the flies in which the substitution is made differ from unselected size in either direction. But this seems unlikely, since the line with the greatest plus deviation (the *E* long-wing line) shows no such effect, and in the other lines, the effect appears to have occurred early and not to be progressive.

Dobzhansky (1929), using crosses between normal and triploid flies, found a striking positive correlation between cell size and volume of chromosome material, excluding the *Y*-chromosome. The latter, though relatively large in volume, had a negligible effect on size, but the small fourth chromosome had an effect much more than proportional to its volume. This indicates that only part of the sex difference in size could be due to the difference in the volume of chromosome material present, and individual size genes must play an important part. It is thus clear that the changes we have observed in the size differences between the sexes are due at least in part to changes in the balance of the size genes on the *X*-chromosomes and autosomes, which must have reduced the difference between the effects on size of a single and a double dose of the genes carried by the *X*-chromosomes. The analogy between this situation and the interactions between autosomes and *X*-chromosomes which underlie sex determination in *Drosophila* is worth noting.

Table 5 gives the coefficients of variation of wing and thorax length of the four *Nb* lines averaged over generations 35–50, and for the *E* lines averaged over generations 25–30. The ratio of the variance of selected lines to controls is also shown in the last two columns. The variance is calculated as the squared coefficient of variation, in order to correct for differences between the lines in average size.

It is clear that the coefficients of variation of both dimensions always show the same trend, although there is a tendency for the selected dimension to become a little more variable than the other in most of the lines. Evidently the genetic changes which are responsible for increasing the variance under selection have affected both dimensions.

The relative variance of the selected dimension has remained practically unchanged in the *Nb* long-thorax line, but it has risen 50–70 % in the two long-wing lines, over 100 %

in the short-thorax line, and has been nearly quadrupled in the *Nb* short-wing line. In the case of the *E* short-wing line, it will be remembered that the variance rose very sharply after generation 11, but returned to the unselected level after generation 23 (Fig. 12), so that the figures quoted in Table 5 do not bring out these changes.

(*f*) *Morphological and physiological effects of selection*

We hope shortly to make a detailed study both of the correlated changes made by characters not selected in our lines and of general changes in cell size, growth rate, etc., which may throw light on the physiological effects of selection. Only preliminary studies of this kind, made some time ago, are at present available.

Dobzhansky (1929) has demonstrated that there is a one-to-one relationship between the cells and hairs of the wing surface, so that it is possible to estimate cell size in the wing by counting the number of hairs in a region of known area. But cell size varies in a rather complex manner throughout the wing surface (Henke, 1947), so that cell size in a given wing region may not bear an absolutely constant relation to average cell size for the whole

Table 5. *Average variability of the Nb selection lines* (*generations* 35–50)

	Coefficients of variation		Ratio of variance to controls*	
Line	Wing length	Thorax length	Wing length	Thorax length
Nb lines:				
Long wing	2·2	2·2	1·5	1·3
Long thorax	1·7	1·9	0·9	1·0
Unselected	1·8	1·9	1·0	1·0
Short wing	3·5	3·3	3·8	3·0
Short thorax	2·4	2·8	1·8	2·2
E lines:				
Long wing	2·0	2·2	1·7	1·6
Unselected	1·5	1·7	1·0	1·0
Short wing	1·5	2·0	1·0	1·3

* Ratio of squares of coefficients of variation in selected lines and unselected stock.

wing, in different stocks and environments. Alpatov (1930) compared cell size and wing size in stocks of *D. melanogaster* reared at 28° C. and on normal and low nutrition conditions at 18° C. Using estimates of cell size in an area of 0·1 sq.mm., near the distal end of the second posterior wing cell, he concluded that increase in wing area, whether caused by increased food or decreased temperature, was accompanied by an increase in number of cells.

We estimated wing-cell size from three central regions of the wing similar to those used by Dobzhansky (1929), and measured the total wing area by camera obscura drawings. These estimates were made on the 18th generation of the *Nb* long- and short-wing lines reared at approximately 23·5 and 28° C., and the results are given in Fig. 14. The mean values of males and females of both lines at each temperature are shown separately, and the line drawn on the graph represents the proportional rate of increase in cell size and wing area, i.e. the line of constant cell number.

All the points lie close to the line of proportional increase except the point for small males at 28° C., and it therefore seems probable that the changes of wing length caused by selection—at least for the first eighteen generations—are due entirely to change in cell size. The deviation of the point for small males indicates that these have fewer cells at the high temperature, and this may be an example of the effect found by Alpatov. However,

our other series do not show the same tendency, and it is possible that the choice of different wing regions for estimating cell size may account for the divergence in results. It remains to be seen whether change in thorax size in our lines is also due to change in cell size, though this seems probable. Our lines have made considerable progress since this test was made, and we have still to test whether cell number has remained constant throughout the period of selection.

Preliminary observations made at generation 25 revealed no differences between the *Nb* long- and short-wing lines in either egg size or developmental rate within the egg, and general observations suggest that there are no striking differences between the various lines in developmental time between hatching of the egg and emergence of the adult. Thus the main differences in size must be the result of differing larval growth rates.

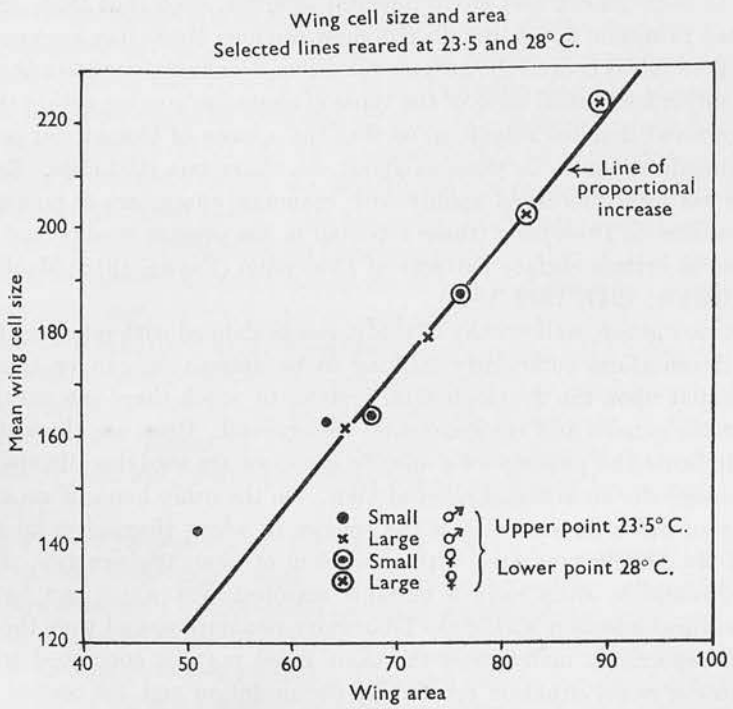


Fig. 14. Wing cell size and wing area in *Nb* wing-selection lines after eighteen generations of selection.

4. General discussion

Mendelian and biometric genetics in a sense represent opposite points of attack on the same general problems of heredity, since they start at the two ends of the developmental chain and take respectively the gene and the character as the unit. But the two approaches have generally been concerned with different kinds of variation, and the distinction between 'biometric' and 'Mendelian' characters has been used to infer a difference between the kinds of gene 'polygene' and 'oligogene' which control them (Mather, 1943). We must first ask whether there is any biological basis for this inference.

A survey of the Mendelian characters studied in any organism suggests at once that they form a highly heterogeneous group biologically, united solely by their quality of showing segregation under suitable conditions and thus acting as markers for particular genes.

The kind of character studied varies with the type of organism and the laboratory techniques which it requires. Thus in *Drosophila* we have a miscellaneous group ranging from lethal and sterility effects, through gross physical defects to minor changes in normally constant features of surface pattern or colour, and but few internal or biochemical effects. In micro-organisms, by contrast, work is largely confined to biochemical changes, since there are often few constant morphological features for study. If we can imagine the full range of genetic characters theoretically available for study in any organism, it becomes clear that those studied by Mendelian methods form in no sense a biologically distinct subclass, from which we can infer special properties of the genes controlling them.

It is equally instructive to examine the ill-defined class of quantitative characters from its biological aspect. Biometrical characters are apparently all those which show variations attributable to both genetic and environmental agencies, such that these variations can only be defined in metric terms and do not show obvious Mendelian segregation, though it is inferred that this is behind the genetic variation. Confining our attention to animals, we find that only a few special cases of the types of character coming within this definition have so far received detailed attention, so that the science of biometrical genetics is too young for generalizations to be possible about the characters it studies. Existing work with animals has been concerned mainly with economic characters in domestic animals, metrical characters in *Drosophila* (those reported in the present study), and variation of bristle number in certain surface patterns of *Drosophila* (Payne, 1918; MacDowell, 1915, 1917, 1920; Mather, 1941, 1942, 1943).

An entirely new group, well worthy of study, can be defined with reference to Mendelian characters. A mutation, sufficiently striking to be detectable, can be considered as a specific strain put upon the developmental system, to which there is a varying reaction, depending on the genetic and environmental background. If we use the visible evidence of strain to indicate the presence of a specific gene, we are studying Mendelian genetics, but this is biologically an artificial point of view. On the other hand, if we are interested in the ability of different members of the species to adapt themselves to the strain in question, we are usually presented with a problem of biometric genetics. For example, mutants in *Drosophila* often have a variable manifestation which can be changed by artificial or natural selection within the laboratory population, and they thereby present a biometrical aspect. In many cases the same genes may be concerned with both the adaptation to the novel situation created by the mutation and the control of its manifestation, although this will not always be so. It would be wrong to dismiss this aspect as merely concerned with modifiers, since it remains to be shown that the ability of these modifiers to react with one or other of a heterogeneous assembly of identified mutations defines a special class of genes. In fact, the range of easily measured quantitative characters in normal individuals is very limited in the case of small laboratory animals such as *Drosophila*, and variable exhibition of mutants enables us to extend the range and variety of such characters.

The economic characters usually measure the total productive ability of the animal, concentrated by selection into a particular channel, e.g. milk or egg production. They are particularly complex characters which have been pulled right out of their normal place in the adapted organism by artificial selection and man-controlled environment. Artificial selection for these characters has already been going on for many generations, and this must be borne in mind when interpreting their genetic behaviour with reference to adapted

characters in wild populations—the entire adaptive background will be different. It is also obvious that the genetic control of such characters must be very complex, and cannot depend on a simple system such as the balanced series of elementary polygenes envisaged by Mather. They will in fact be influenced by genetic and environmental agencies acting at many different levels and on many different organs. Moreover, quite major variations in internal organs and conditions, sufficient to be easily detected if their effects had been externally visible, could be responsible for an important fraction of the variance in economic characters without their Mendelian behaviour being detectable.

Biometrical characters are in general very susceptible to environmental variations such as change in temperature or in quantity or quality of food. But the bristle variations studied by Mather appear to be of a different kind. The number of abdominal chaetae is almost as variable in highly inbred lines as in unselected stocks, and yet it is extremely resistant to changes of environment which are great enough to have profound effects on other quantitative characters such as body size, fecundity and the expression of many mutant genes. Thus flies raised on fresh and on very stale culture medium have almost the same mean chaeta number (41·03 and 42·00 respectively), while the average number only declines by about one chaeta during the first 10 days of emergence of flies from normal cultures (Mather & Harrison, 1949). Also change of temperature and partial starvation appear to have remarkably little effect on average chaeta number. This suggests that much of the variability is independent of external conditions and must be due to 'intangible' accidents of development. Such a case is reminiscent of the variation of coat colour pattern found by Wright (1920) in highly inbred lines of guinea-pigs. Variation of this kind must be clearly distinguished from what is usually described as 'environmental variation', i.e. phenotypic variation which can be attributed to differences in the environment experienced by different individuals. The importance of this class of variation has recently been emphasized by Gordon (1950). Thus from this point of view bristle number falls into a special category.

The general pattern of chaetae on the body is sufficiently constant throughout the Drosophilidae to form a taxonomic character of the family, while rectangular abdominal tergites carrying a number of small bristles are characteristic of the many species of diverse habitat forming the genus *Drosophila* (Sturtevant, 1921). The bristles studied by Mather are, therefore, of ancient phylogenetic origin. They also seem to vary in a haphazard manner between populations of the same species, and between different species, which often overlap in this respect. Mather appears to believe that their genetic control is due to elementary polygenes, concerned purely with bristle number, and subject to natural selection which nicely adapts bristle number in each population to its external conditions. But it is difficult to believe that moderate variations in abdominal bristle number can have any adaptive significance (in contrast to variations in, say, body size), and this conclusion is borne out by the fact that bristle number is subject to a considerable amount of 'chance' non-genetic variability, apparently unconnected with environmental variations. In view of the comparative stability of the pattern throughout the genus, it is surprising to find that artificial selection can produce such enormous changes in bristle number.

The key to this problem is suggested by the work of Stern (1938), who found that every thoracic bristle in *Drosophila* was fed by a single bipolar nerve cell of the peripheral network, and the one-to-one relation between nerve cell and bristle was retained in mutants with changed numbers of bristles. The same relation probably occurs with the abdominal

bristles, and it follows that bristle number bears a definite morphogenetic relation to the underlying peripheral nervous system. One system presumably induces the formation of the other, and the number of abdominal bristles must depend on the developmental balance between different morphogenetic fields concerned with the formation of the peripheral nervous system.

We know little of the forces which control the boundaries between different patterns, but they must act by controlling the relative strength of the morphogenetic fields, and this implies control of rate of production, diffusion, etc., of different substances. It may be suggested that control of abdominal bristle number depends on a developmental balance of this sort. The relative strengths of these morphogenetic fields are doubtless under adaptive control, but the adaptive value will depend on some more important result than the exact number of bristles produced. It is thus probable that genetic variation in abdominal bristle number is due to genes having other primary tasks, and there are good reasons for believing that such genes will usually have pleiotropic effects, contrary to the view expressed by Mather. The 'intangible' environmental variations in bristle number, mentioned above, are perhaps the result of a degree of indeterminacy in the details of the developmental pattern.

Variations in bristle number thus appear to be secondary effects of genes whose primary job lies elsewhere. In this sense they seem analogous to the genes responsible for variation of exhibition of mutants, since these also will have other normal effects, and only under special conditions become important for their modifying effect on the character in question. It is likely that the segregation of genes acting as modifiers may often be more easily detected than that of genes influencing adaptive quantitative characters, since selection towards an intermediate optimum can only be important in the latter, where the phenotypic effects of individual segregation will be reduced to a minimum by the influence of natural selection upon the genotype generally.

We have seen that no general inferences can be drawn from features of Mendelian and biometric characters to the type of gene controlling each, but Mather (1949) has made far-reaching deductions about the nature of the genes responsible for Mendelian segregation and quantitative variation respectively, and these require further examination. He considers that major genes, or oligogenes, are responsible for the grand plan of the organism. They are highly specific and their effects cannot in general be duplicated by other genes, so that when mutation occurs the mutant form 'is drastically different from normal, even to the extent of being completely inviable'. Such genes are 'the backbone of the genotype and being essential like a backbone, their normal allelomorph has an unconditional advantage over its mutant alternative'. In contrast, the minor genes or polygenes are thought of as interchangeable in effect, individually undetectable, more or less additive in function, and generally without pleiotropic effects; and they combine together to adapt the grand plan nicely to the environmental conditions and are chiefly responsible for the continuous variation of quantitative characters. Finally, Mather has concluded that oligogenes are carried by the euchromatin and polygenes by the heterochromatin, though not confined to heterochromatic areas.

These assertions seem to conflict with a great deal of widely accepted genetic knowledge, in particular, the following facts:

(*a*) Major genes do not always have large effects. Study of Bridges & Brehme (1944) shows that nearly one-quarter of the gene loci identified in *D. melanogaster* carry at least

one mutant allele which overlaps wild type, and this list doubtless excludes many so-called 'bad' alleles discarded by geneticists because their exhibition was too variable for linkage tests. Moreover, the vast majority of mutants discovered by inbreeding from wild populations by Gordon, Spurway & Street (1939) were so variable in exhibition that they were difficult to isolate.

(*b*) The effects of mutant genes are frequently susceptible to genetic and environmental changes, and their exhibition often completely disappears in laboratory cultures, until outcrossing reveals the mutant effect again. It is frequently possible for different genes to do each other's jobs.

(*c*) Where technical difficulties permit, careful study of loci usually reveals the presence of multiple alleles. These are often graded in effect from alleles hardly distinguishable from wild type to those with striking effects. Recent highly suggestive work by Stern (1938) and Stern & Schaeffer (1943) has shown differences between what had been assumed to be identical wild-type alleles from different stocks. Stern refers to these as iso-alleles, and points out that the term wild-type allele is misleading, since it is likely that many distinct iso-alleles exist in wild populations. It should be noted that to describe two genes as iso-alleles implies only that the differences between them do not affect the very limited range of characters which the geneticist can easily observe. Spencer (1944) discovered numerous alleles of *bobbed* in a population of *D. hydei*; these could be classified according to their bristle effects and their pleiotropic effects on general morphology, vigour, etc., and included iso-alleles whose effects could not be distinguished when homozygous. Also, severity of bristle effects was only partially correlated with severity of other effects. Goldschmidt (1944) has reported a similar case in *D. melanogaster*, and others are referred to by Stern & Schaeffer (1943).

(*d*) It is a familiar experience with *Drosophila* that newly arisen visible mutants exert pleiotropic effects on such typical biometric characters as size and fertility. These effects can often be greatly modified in laboratory cultures without parallel changes in the exhibition of the gene's visible effect.

The theory that polygenes are located in heterochromatin was suggested by the discovery that *Y*-chromosomes drawn from different wild stocks of *D. melanogaster* differed in chaeta-producing power when placed against a common genetic background and showed recombination with the *X* (Mather, 1944). Some plants carry a variable number of heterochromatic chromosomes, which are associated with slight effects on vigour and fertility, and are thought to have survival value, although their significance is still very obscure. It was suggested that such chromosomes carried polygenes, and this idea was linked with the assumption that members of a polygenic system 'have small, similar and supplementary effects because...they have less differentiated products and less elaborate action' (Darlington & Mather, 1949). Since the supernumerary chromosomes are not constant in number and heterochromatic chromosomes do not always divide cleanly at mitosis, it was held that 'duplication and deficiency for a small number of polygenes is not likely to be unconditionally deleterious' (Mather, 1949).

Examination of the bristle-producing power of different parts of the *X*-chromosome showed that the highest activity is found in the distal (euchromatic) region, and not in the heterochromatic zone (Wigan, 1948), so that the only direct evidence yet available does not support the heterochromatin theory. The word 'heterochromatin' is open to criticism, since it implies the existence of a special chromosome material with characteristic

properties other than its staining reaction (Baker & Callan, 1950). A similar criticism applies to the word 'polygene' with its implication that there is a special class of gene responsible for biometrical characters under polygenetic (i.e. multigenic) control. As we have seen, biometrical characters do not in any biological sense represent a distinct class of character, and can hardly be supposed to be under the control of a distinct class of gene. A further logical fallacy arises in the argument that, because 'polygenes' have small effects on biometrical characters, therefore the alleles responsible for these effects are themselves correspondingly unimportant in the gene complex, and produce simpler substances than the 'oligogenes'. Inferences cannot be drawn about the importance of a given locus from knowledge of the magnitude of the phenotypic variations caused by differences between its various alleles. In fact, as Waddington (1943) has pointed out, a pleiotropic gene may have striking effects on some characters and very slight effects on others. Hence the very same gene could fit Mather's definition of both 'oligogene' and 'polygene'. It follows from these points that there are strong grounds against making any general distinction between the 'polygenes' supposed responsible for the multigenic variation of biometrical characters and other genes.

As a starting point for the interpretation of future selection results, it seems necessary to conclude that the genes affecting quantitative characters have no special qualities distinguishing them from other genes, and must often represent pleiotropic effects of genes having other, perhaps more important, effects. Much of the variation may well be due to differences between numerous alleles which differ little from each other in their primary effects, and it is possible that a single locus can carry a number of different alleles, each of which is most frequent in a particular locality. The differences between the effects of these alleles will be small either because they are very similar chemically or because natural selection has modified the genetic background so as to diminish the effect of the substitution of one for the other. The gene effects which have been studied seem generally to be very plastic in the sense that their action can be modified considerably by selection of the genetic background. Natural selection must inevitably act on this plasticity by reducing to a minimum the effects of segregation on biometric characters which vary about an intermediate optimum.

Since natural selection must reduce the differences between the effects of unfixed alleles and thus makes segregation difficult to detect in quantitative characters, it seems likely that strong artificial selection for an extreme value of the character will cause the opposite result of magnifying the effects of individual alleles affecting the character. Any gene whose fixation is delayed by linkage (to harmful genes) or adverse pleiotropic effects when homozygous, may have its heterozygous effect on the selected character so magnified by fixation of genes which interact with it that segregation may become detectable where none was before. It is, of course, impossible to predict the importance of this magnification effect in a given case, but judging from the remarkable variation in exhibition of so many *Drosophila* mutants when the genetic background is varied, it may play an important part both in our selection for body size in *Drosophila* and in selection of economic characters in domestic animals. It is not difficult to envisage changes in development involving alteration of thresholds and competitive and co-operative interactions which would greatly increase the differences between alleles that previously seemed very similar.

Among the consequences of this magnification of the effects of allelic differences under artificial selection, we may expect that marked non-additive gene interactions may some-

times be created. This can easily be imagined in a case where two genes at separate loci both have modifiable effects on the selected character, and neither can be fixed. Fixation of modifiers of both these genes might result in interactions between them, so that when both were present the total effect was much greater than the sum of the effects produced by each alone. Such interactions, and the magnified effects of each gene separately, would be likely to disappear in outcrosses, as a result of the alteration of the genetic background, so that such crosses would lead one to underestimate the degree both of interaction and of dominance present. As a final consequence, we may expect that the phenotypic variance will tend to increase whenever the effects of unfixed genes are magnified in this way by artificial selection.

Granted the likelihood of this effect, the existence of different iso-alleles at a given locus suggests interesting evolutionary possibilities. As we have seen, differences between these alleles can only be detected by special genetic methods, which involve changing the genetic background and the environmental conditions. It therefore seems likely that natural selection will hardly be able to discriminate between iso-alleles under normal conditions. They will segregate more or less freely, and the frequency of each will be determined mainly by their reciprocal mutation rates, the chances of random sampling in small populations, and the more immediate history of the population in terms of migration. But artificial selection or a striking change of environment may so alter the genetic background as to magnify the differential effects of some of these alleles until they become large enough to have selective value. In a sense, selection may create internal conditions which make possible a further response. Thus iso-alleles seem to provide a possible source of genetic variation in adapted populations.

These lines of thought can be followed a little further. If change of genetic background can magnify the differences between alleles, we might expect suitable changes of environment to have the same effect, and this is consistent with what we know about the effect of changes of environment on gene exhibition. Such a possibility is perhaps worth bearing in mind when designing animal breeding or artificial selection experiments. It is generally assumed that selection is most efficient when the environmental contribution to the phenotypic variance is kept as small as possible. But it may be possible to go further and choose those environmental conditions which give maximum expression to the genetic differences affecting the character under study. These conditions are not necessarily those which favour maximum growth rate as Hammond (1947) apparently believes, but would have to be determined empirically. They might differ in different strains and at different stages of selection.

It has been suggested above that natural selection may be unable to discriminate between different iso-alleles. In discussions of the role of natural selection it is usually assumed that in any population there is an intermediate optimum phenotype, and that deviations about it are more or less normally distributed. The adaptive level of the individual falls off in proportion as its phenotype deviates from the optimum.

This is perhaps too simple a model of the action of natural selection, and gives us an exaggerated idea of its power to discriminate between small genetic differences. Thus in the developing *Drosophila* culture, which has been studied in detail (Gordon & Sang, 1941; Robertson & Sang, 1944*a*, *b*; Sang, 1949 *a*, *b*), we find a complex series of interactions between the changing microflora and the different stages of the insect. Competition between the microflora causes progressive changes in the composition of the medium, and larvae

hatching early and late will be exposed to widely different types and intensities of selection. The general effect of this variety of conditions must be to reduce the discriminatory power of natural selection, so that a considerable degree of genetic variability will be able to survive in the normal laboratory culture. Conditions in nature will be even more variable, and we can expect natural selection to be a very variable agency.

This brings us to the final problem in the evolution of quantitative characters which needs discussion here: the question of how stability of the phenotype can be reconciled with the presence of considerable genetic variability, available for further evolution and adaptation to changing conditions. Artificial selection can reveal this variability by carrying the mean of a quantitative character very far beyond the limits of variation in the original population.

Wright has pointed out that variability may be stored by the division of a species into many partially isolated local populations. Thus 'each local population may be expected to approach fixation of a particular balanced combination of the many genes that act on each character. But as there may be a very large number of different balanced genotypes with the same or nearly the same phenotype each local population may be expected to centre about a different genotype. There will then be little apparent variability either within local populations or within the species as a whole, yet an enormous field of potential variability, available by mere increase in the amount of migration between populations' (Wright, 1945). He suggests the likelihood that each species carries many slightly different alleles at each locus, giving rise to an astronomically great number of possible gene combinations, and the crux of evolutionary change lies in the way one harmonious combination of alleles can be replaced by another in the face of the conservative agency of selection.

Mather (1949) assumes that genetic variability is stored within individuals by the evolution of a definite system of polygenic balance for each biometric character. Given a large number of polygenes with small and interchangeable plus or minus effects, he deduces that natural selection for an intermediate optimum will favour plus and minus genes linked in repulsion, since these will give the fewest extreme individuals as a result of segregation. Continued selection of this kind will therefore lead to their chromosomes carrying series of linked polygenes with plus and minus genes tending to alternate with each other. It is also suggested that in an outbreeding organism a 'relational' balance will be developed between the different homologous chromosomes which may be brought together. In other words, they will all tend to have the same numbers of plus and minus genes, although these may be arranged differently in each, so that any combination of chromosomes will tend to give the same phenotype. Artificial selection for an extreme value of the character will only gradually be able to assemble all the plus polygenes together, since this will require a large number of chiasmata.

Wright (1945) has criticized this ingenious theory on the grounds that segregation of the different polygenes would in the long run be independent of each other unless linkage between them was almost complete. Thus the development of elaborate systems of balanced polygenes, in which polygenes affecting different characters are intermingled, seems unlikely. Moreover, linkages tight enough to survive over a long period would only rarely be broken during artificial selection, and would hardly be sufficient to explain the selection results obtained by Mather and ourselves. Mather's theory also seems artificial in its reliance on a set of hypothetical elementary polygenes without pleiotropic effects, as we have pointed out earlier.

Genetic variability may be present in small populations for a variety of reasons. These include linkage of genes affecting the character with one another or with deleterious recessives, selection in favour of heterozygotes, and the occurrence of iso-alleles capable of developing increased effects in a changed genetic or environmental background. The partial isolation mechanism discussed by Wright will increase the number of distinct iso-alleles which can be maintained in the species, though we may wonder whether individual iso-alleles will necessarily become fixed in each local population. It seems certain that Mather has overrated the importance of linkage, although it doubtless plays a more important part in a species with very few chromosomes such as *Drosophila*, than in others.

Mather (1949) has also pointed out that the multiple recombinations caused by artificial selection towards an extreme will cause correlated effects by reassorting linked genes affecting other characters. He believes that pleiotropic effects of the 'polygenes' are not to be expected, and suggests that it is profitless to look for them. This view is difficult to accept, since genes with pleiotropic effects are of widespread occurrence. There are, in fact, morphogenetic grounds, as we have seen earlier, for believing that the genes selected in his bristle selection experiments will in general have pleiotropic effects, and that their effect on bristle number will be secondary from an adaptive point of view.

It should be noted that in developmental studies of pleiotropic effects it has been found that apparently unrelated end-effects can be caused by the same primary action of the gene (Grüneberg, 1938). Thus we cannot assume that the occurrence of apparently unrelated effects in selection experiments must be due to linkage, though this will often be the case. The distinction between a pleiotropic and a linkage effect may often be unimportant in practice, since it may be easier to modify the effect by selection of the genetic background than to eliminate it by breaking a tight linkage.

The relative importance of mutation in maintaining the variation of quantitative characters is quite unknown. There are obviously great difficulties in obtaining even a very rough measure of the frequency of mutations affecting such characters, and it would be unwise to assume that the mutation rates are of the same order as, say, sex-linked lethals. Selection of highly inbred lines for large and small size during ten generations resulted in no increase of size in the high line but a slight decrease in the low line. This fact, coupled with the long periods of stability occurring in our other selection lines and other preliminary tests, suggests that mutation is of minor importance in contributing to the continued response to selection.

Mutation, migration between populations, selection of heterozygotes—in short, all the agencies which interact to determine gene frequencies in natural populations—must endow populations which can successfully adapt themselves to changing environmental conditions with a great wealth of largely concealed genetic variation. There seems no reason, even on general grounds, why we need look further for special mechanisms to conserve variation. It need cause no surprise if, in an immensely complex character such as body size, the origins of variation in particular instances which appear alike turn out to be quite different, although it is quite possible that detailed experimental study of a variety of cases may reveal unsuspected similarities which could not be anticipated. Finally, we are still far from penetrating to the really fundamental similarities in terms of the genetic control of the developmental system which creates the end products we study.

5. CONCLUSIONS

We shall not attempt a detailed interpretation of the behaviour of each selection line at this stage, since genetic tests on each still remain to be done, and many aspects of their behaviour need further study. But certain features of our results can now be reviewed in the light of the ideas examined in the previous section.

The behaviour of the phenotypic variance under continued selection is of particular interest. In five of the six lines, it has shown a marked increase, though of varying magnitude and duration. Usually there has been a fairly steady response for ten to twenty generations before any obvious rise in the variance occurs, and afterwards the response may still continue at much the same rate, or it may fall almost to zero.

In the two short-wing lines the increase of variance was accompanied by the appearance of visible or lethal effects at least partly attributable to individual genes, and it seems probable that part of the effect of selection has been to pick out certain alleles which reduce size but are difficult to fix, and to magnify their effects by selection of the genetic background. A mechanism of this kind may explain some of the visible and lethal effects which have appeared in various lines at different times.

At least part of the rise in variance is probably due to the magnification of the effects of alleles which are not easily fixed in the stock. But in the short-wing *Nb* line other factors must be present. Here the variance rose very sharply at generation 19, soon after two visible wing effects began to appear in the line. But the variance remained high, and response to selection continued long after these two effects had become fixed in and eliminated from the line respectively. Finally, the variance maintained its high level long after response to selection had stopped. These results are difficult to explain on any single theory, and it seems probable that both the magnification of gene effects and sensitivity of one or more selected genes to environmental fluctuations affected the variance.

An equally interesting case is provided by the long-wing *Nb* line. Here a period of unstable equilibrium between wing selection and an opposing force—either overdominance or some form of natural selection—has been maintained for a very long period. The high phenotypic variability results from the enforced genetic variability maintained by this equilibrium, and it declines sharply when wing selection is relaxed or reversed. It is not yet clear how this equilibrium is maintained, since fertility as measured by percentage emergence of adults did not decline sharply when it first developed. Tests are now in progress to determine whether overdominance or other agencies are mainly responsible for the barrier to further advance, and the results will be described in the next paper.

A close linkage between fertility and size has developed in the short-wing *E* line, where three lethal genes or groups of linked genes have appeared which cause a reduction in size either directly or through close linkage with other genes. But here, in contrast to the last case, these severe effects have not set up a complete barrier to further advance, since size continued to decrease long after their appearance, and they now seem to have been fixed in the line after modification of their lethal effects. Finally, we have a case in the long-thorax line where the variance has remained normal and the advance seems to have come to an end as a result of the exhaustion of most of the available genetic variability for size.

6. Summary

1. This paper, the first of a series dealing with the inheritance of body size in *Drosophila melanogaster*, describes the effects of selecting for large and small size in lines taken from two wild stocks, *Nb* and *E*. Size, variance, viability and the effects of relaxing and reversing selection have been studied in each line. Four *Nb* lines have been selected for long- and short-wing and thorax, respectively, during fifty generations, and two *E* lines have been selected for long- and short-wing during thirty generations. A cyclical mating system was used to minimize the rate of inbreeding in a small population.

2. The *Nb* long-wing line, after increasing 7% in size, entered a long period of unstable equilibrium, characterized by failure to respond to selection, high phenotypic variance, and a sharp decline in size and variance whenever selection was relaxed. The *Nb* short-wing line declined steadily in size until about generation 37, and remained constant. Its variance became very large after generation 19, and remained high when response to selection had ceased. Size did not increase when selection was relaxed.

3. The *Nb* long-thorax became stable after increasing 11%, and its variance remained unchanged. The *Nb* short-thorax line developed low fertility early in its history, and only declined by 8%. It developed a high variance.

4. The *E* long-wing line has advanced steadily during thirty generations, to 11·5% above control size. Variance increased after generation 19 and has remained high. Relaxed-selection lines did not lose size, in contrast to the *Nb* long-wing line. Fertility has remained very high.

5. The *E* short-wing line has declined about 14%. Viability fell sharply after generation 12, but both size and viability rose immediately to control level when selection was relaxed, and fell again when the relaxed-selection line was reselected. When the main selection line was relaxed later, neither size nor viability increased. Variance was very high during the first twelve generations of low viability. One second chromosome and two third chromosome lethals were detected at generation 15. Each of these reduces size slightly when heterozygous, but they interact to reduce size greatly when together.

6. Selection of either dimension changes both wing and thorax length in the same direction but to a different extent. This is discussed with reference to size allometry.

7. Current theories about the inheritance of quantitative characters are examined, and it is concluded that there are no valid biological grounds for distinguishing between genes responsible for the variation of quantitative characters and other genes. Thus no special qualities can be attributed to the former as a class.

8. The possible effects of continued selection on a quantitative character are discussed. Progressive alteration of the genotype may create conditions in which alleles previously indistinguishable from each other can be selected, and appropriate changes in the environment may sometimes have the same effect. Selected genes which cannot be fixed may have their effect on the character magnified by selection of modifying genes. This provides one possible explanation for a rise in variance of a character after it has been selected for many generations.

We particularly wish to thank Miss Brenda Green and Mrs C. H. Wearmouth who have looked after the stocks and made most of the measurements. Without their devoted assist-

ance in this laborious work we should have made very little progress. We are also grateful to our colleagues in the Animal Breeding and Genetics Research Organization for their continued interest and for many valuable suggestions made in discussions.

REFERENCES

ALPATOV, W. W. (1930). Phenotypical variation in body and cell size of *Drosophila melanogaster*. *Biol. Bull. Woods Hole*, **58**, 85.

BAKER, J. R. & CALLAN, H. G. (1950). *Nature, Lond.*, **166**, 227.

BEATTY, R. A. (1949). Studies on reproduction in wild-type and female-sterile mutants of *Drosophila melanogaster* (Meig.). *Proc. Roy. Soc. Edinb.* **63**, 249.

BEGG, C. M. & ROBERTSON, F. W. (1950). The nutritional requirements of *Drosophila melanogaster*. *J. Exp. Biol.* **26**, 380.

BRIDGES, C. B. & BREHME, K. S. (1944). The mutants of *Drosophila melanogaster*. *Publ. Carneg. Instn*, no. 552.

DARLINGTON, C. D. & MATHER, K. (1949). *The Elements of Genetics*. London.

DOBZHANSKY, T. (1929). The influence of the quantity and quality of chromosomal material on the size of the cells in *Drosophila melanogaster*. *Roux Arch. Entw. Mech. Organ.* **115**, 363.

GOLDSCHMIDT, R. B. (1944). Cryptic bobbed alleles in *Drosophila melanogaster*. *Amer. Nat.* **78**, 564.

GORDON, C. (1950). Unpublished.

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

GORDON, C., SPURWAY, H. & STREET, P. A. R. (1939). An analysis of three wild populations of *Drosophila subobscura*. *J. Genet.* **38**, 37.

GRÜNEBERG, H. (1938). An analysis of the 'pleiotropic' effects of a new lethal mutation in the rat (*Mus norvegicus*). *Proc. Roy. Soc.* B, **125**, 123.

HAMMOND, J. (1947). Animal breeding in relation to nutrition and environmental conditions. *Biol. Rev.* **22**, 195.

HENKE (1947). Einfache Grundvorgänge in der tierischen Entwicklung. *Naturwissenschaften*, Heft 5 and 6, 1.

MACDOWELL, E. C. (1915). Bristle inheritance in *Drosophila*. I. Extra bristles. *J. Exp. Biol.* **19**, 61.

MACDOWELL, E. C. (1917). Bristle inheritance in *Drosophila*. II. Selection. *J. Exp. Biol.* **23**, 109.

MACDOWELL, E. C. (1920). Bristle inheritance in *Drosophila*. III. Correlation. *J. Exp. Biol.* **30**, 419.

MATHER, K. (1941). Variation and selection of polygenic characters. *J. Genet.* **41**, 159.

MATHER, K. (1942). The balance of polygenic combinations. *J. Genet.* **43**, 309.

MATHER, K. (1943). Polygenic inheritance and natural selection. *Biol. Rev.* **18**, 32.

MATHER, K. (1944). The genetical activity of heterochromatin. *Proc. Roy. Soc.* B, **132**, 308.

MATHER, K. (1949). *Biometrical Genetics*. London.

MATHER, K. & HARRISON, B. J. (1949). The manifold effect of selection. Part I. *Heredity*, **3**, 1. Part II. *Heredity*, **3**, 131.

PAYNE, F. (1918). An experiment to test the nature of the variations on which selection acts. *Ind. Univ. Stud.* **5**, no. 36.

REEVE, E. C. R. & HUXLEY, J. S. (1945). Some problems in the study of allometric growth. In *Essays on Growth and Form*. Oxford.

ROBERTSON, F. W. & SANG, J. H. (1944*a*). The ecological determinants of population growth in a *Drosophila* culture. I. Fecundity of adult flies. *Proc. Roy. Soc.* B, **132**, 258.

ROBERTSON, F. W. & SANG, J. H. (1944*b*). The ecological determinants of population growth in a *Drosophila* culture. II. Circumstances affecting egg viability. *Proc. Roy. Soc.* B, **132**, 277.

SANG, J. H. (1949*a*). The ecological determinants of population growth in a *Drosophila* culture. III. Larval and pupal survival. *Physiol. Zoöl.* **22**, 183.

SANG, J. H. (1949*b*). The ecological determinants of population growth in a *Drosophila* culture. IV. The significance of successive batches of larvae. *Physiol. Zoöl.* **22**, 202.

SANG, J. H. (1949*c*). The ecological determinants of population growth in a *Drosophila* culture. V. The adult population count. *Physiol. Zoöl.* **22**, 210.

SPENCER, W. P. (1944). Iso-alleles at the bobbed locus in *Drosophila hydei* populations. *Genetics*, **29**, 520.

STERN, C. (1938). The innervation of setae in *Drosophila*. *Genetics*, **23**, 172.

STERN, C. & SCHAEFFER, E. W. (1943). On wild-type iso-alleles in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci., Wash.*, **29**, 361.

STURTEVANT, A. H. (1921). The North American species of *Drosophila*. *Publ. Carneg. Instn*, no. 301.

WADDINGTON, C. H. (1943). Polygenes and oligogenes. *Nature, Lond.*, **151**, 394.

WIGAN, L. G. (1948). Distribution of polygenic activity in the *X*-chromosome of *Drosophila melanogaster*. *Heredity*, **3**, 53.

WRIGHT, S. (1920). The relative importance of heredity and environment in determining the piebald pattern of guinea-pigs. *Proc. Nat. Acad. Sci., Wash.*, **6**, 320.

WRIGHT, S. (1945). Tempo and mode in evolution: a critical review. *Ecology*, **26**, 415.

Interactions Between Chromosomes from Large and Small Strains of *Drosophila melanogaster*

by FORBES W. ROBERTSON*

Institute of Animal Genetics, Edinburgh University

SELECTION experiments provide one of the most effective methods of studying the inheritance of a quantitative character, and they may be coupled with progeny tests, inbreeding and crossing experiments, and sometimes with lethal tests, to give a great deal of indirect information about the genetic control of the character and how it responds to different kinds of experimental treatment. But even the most detailed investigations of this kind generally leave us unable to go far in determining the nature of the gene effects involved, except in the particularly favourable case where one is studying the genetic variability, presumed due to a small number of genes, which long-continued selection has failed to fix or eliminate (Reeve, 1952). But these genes are only a residue of those affected by selection, and it is important to find means of analysing the permanent effects of selection. A number of strains differing in body-size have been obtained by selection for large and small size in two wild stocks of *Drosophila melanogaster* (Robertson and Reeve, 1952) and these provide valuable material for further analysis. Crosses between the large strains and the unselected parent stocks gave progeny which were generally larger than the parental mean, and one may ask whether this heterosis is due simply to partial dominance of the genes selected for large size, as might well be expected, or to some other effect, such as metric bias (use of the wrong scale of measurement), or gene interaction.

Such questions are generally very difficult to answer, but *Drosophila melanogaster* provides unique opportunities for their analysis, since the small number of chromosomes, the absence of crossing-over in males, and the existence of special stocks carrying dominant markers and cross-over suppressors for the major chromosomes, make it possible to create genotypes carrying a specified chromosome complement, and thus to examine the differences between the chromosomes from the various selected strains. Theoretically, one should be able to build up all the possible combinations of the three major chromosomes from two different strains, giving 18 genotypes in males and 27 in females, but this requires much laborious work and four or five generations of crosses, during which errors may arise due to the incomplete suppression of crossing-over in females. An attempt is being made to obtain complete comparisons between some of our strains by this technique, but the work is still in progress.

A much more rapid, though incomplete, analysis of differences at the chromosome level can be made by an extension of the chromosome assay technique used by Mather and Harrison (1949), and others who have carried out selection experiments on *Drosophila*. Females of an experimental strain are mated to males carrying the genes Plum and Hairless, dominant markers of the second and third chromosomes, which are lethal

*This work was financed by the Agricultural Research Council.

when homozygous. F_1 males carrying both marked chromosomes (for which the symbols *P* and *H* will be used), are backcrossed either to the same or to a different strain, and the segregation of *P* and *H* is used to distinguish four genotypes among the progeny. There is no danger that crossing-over will affect the differences between these genotypes, since the only heterozygous parents used are males. Four such backcrosses can be made in comparing two strains, say *T* and *N*, since each strain can be used in either the first or second stage, or both, giving crosses which may be indicated by the symbols *TxT*, *TxN*, *NxT*, and *NxN*, where the order of the letters indicates the order in which the strains were used. These crosses give sixteen identifiable genotypes of each sex, of which the females are shown in Table 1. The corresponding males are obtained by substituting a *Y* for the lower *X* chromosome in each type. Two genotypes in females and two pairs of genotypes in males are identical for the three major chromosomes, but differ in the origin of their fourth chromosomes, and also, in the case of the females, in the origin of their cytoplasm. Comparisons of the size of such identical pairs thus provides a method of testing whether differences in the fourth chromosome have a significant effect on size.

TABLE 1.—GENOTYPES SEGREGATING IN CROSSES INVOLVING TWO STRAINS

Cross	*Genotypes of female progeny*			
T × T ..	$\frac{T}{T}\ \frac{T}{T}\ \frac{T}{T}$	$\frac{T}{T}\ \frac{T}{P}\ \frac{T}{T}$	$\frac{T}{T}\ \frac{T}{T}\ \frac{T}{H}$	$\frac{T}{T}\ \frac{T}{P}\ \frac{T}{H}$
T × N ..	$\frac{N}{T}\ \frac{N}{T}\ \frac{N}{T}$	$\frac{N}{T}\ \frac{N}{P}\ \frac{N}{T}$	$\frac{N}{T}\ \frac{N}{T}\ \frac{N}{H}$	$\frac{N}{T}\ \frac{N}{P}\ \frac{N}{H}$
N × T ..	$\frac{T}{N}\ \frac{T}{N}\ \frac{T}{N}$	$\frac{T}{N}\ \frac{T}{P}\ \frac{T}{N}$	$\frac{T}{N}\ \frac{T}{N}\ \frac{T}{H}$	$\frac{T}{N}\ \frac{T}{P}\ \frac{T}{H}$
N × N ..	$\frac{N}{N}\ \frac{N}{N}\ \frac{N}{N}$	$\frac{N}{N}\ \frac{N}{P}\ \frac{N}{N}$	$\frac{N}{N}\ \frac{N}{N}\ \frac{N}{H}$	$\frac{N}{N}\ \frac{N}{P}\ \frac{N}{H}$

From Table 1 it will be seen that a number of different kinds of comparison can be made between the various genotypes. Thus differences between items from a single row enable us to estimate the effect of substituting a *P* or *H* chromosome for one from either of the tested strains, in a variety of different genetic backgrounds. By taking differences between items from a single column, we can estimate the effect of substituting one or more chromosomes of one strain for the corresponding chromosomes from the other, in various genetic backgrounds, most of which, it will be noted, contain at least one of the marked chromosomes. Such estimates of the effect of individual chromosome substitutions may be added together and compared with direct, but independent, estimates of their joint effects. Analysis along these lines can obviously provide a great deal of information about the average effects and combining characteristics of each major chromosome from the different selected strains, although many of the comparisons we should like to make are not included in the scheme.

This method has been used to test the strains listed in Table 2, which are

products of the Nettlebed and Edinburgh selection experiments (Robertson and Reeve, 1952). Strains *T* and *W* were obtained by selection for long thorax and long wings, respectively, from a cross between the two Nettlebed large lines, after these had ceased to respond to selection. *L* is the Edinburgh long-wing strain and *D* is the Nettlebed short-wing strain. The Edinburgh short-wing strain has not been included, since it was not sufficiently stable under mass-mating. The standard strains *N* and *E* are highly inbred, unselected lines from the Nettlebed and Edinburgh parent stocks.

TABLE 2.—WING LENGTH OF STRAINS TESTED

Nettlebed strains	*Deviation from standard strain* (1/100mm.)	
	Males	*Females*
T (Long thorax)	25·3	26·2
W (Long wing)	22·6	25·4
D (Short wing)	−31·2	−36·3
Edinburgh strain		
L (Long wing)	26·4	30·5
Size of standard strains		
N (Nettlebed)	176·6	203·6
E (Edinburgh)	176·3	201·6

Crosses of the kind indicated in Table 1 were made between each selected strain and both standard strains, and also between the pairs of strains *T* and *W*, *L* and *D*. One cross, $D \times E$, failed and has not yet been repeated. Samples of flies of both sexes, from the four genotypes segregating in each cross, were measured; and all crosses were replicated so that adequate standard errors could be obtained for the different kinds of comparison contemplated. Wing and thorax length, body-weight and egg-to-adult mortality have been examined, but only the more interesting results relating to wing length will be discussed here, and a more detailed analysis of the complete data, together with details of the technical methods, is given elsewhere (Robertson and Reeve, in the press).

Altogether 27 successful crosses were made, giving 108 genotypes of each sex, and so many comparisons can be made between such a large array of types that it is necessary to proceed systematically, in order to avoid the danger of confusion. We shall, therefore, consider first the simplest possible hypothesis, namely that the chromosomes combine together additively, for which a general test is available; and if this hypothesis breaks down, it must be modified by stages to test different hypotheses about the causes of the non-additive effects found. We shall consider metric bias, dominance or partial dominance, simple epistatic effects and more complex interactions between groups of genes in turn, in so far as ways can be found of discriminating between them.

As a preliminary, we must ask whether cytoplasmic and fourth-chromosome effects can be ignored in our analysis. There is ample evidence from reciprocal crosses between strains of different size that cytoplasmic and maternal effects have little or no influence on body size, and we shall not test these further.

The effect of the fourth chromosome can be tested by comparing the *PH* males of any pair of crosses for which the same strain was used in the second stage, e.g. $T \times N$ and $N \times N$. These males receive identical sets of major chromosomes and cytoplasm, and will differ only in their fourth chromosome complements. In fact, if a, t and n indicate the fourth chromosomes from the original marker stock and the T and N stocks, respectively, the fourth chromosome complements will be $\frac{1}{2}\left(\frac{a}{n} + \frac{t}{n}\right)$ and $\frac{1}{2}\left(\frac{a}{n} + \frac{n}{n}\right)$ for $T \times N$ and $N \times N$ It follows that the difference between *PH* males from the two crosses measures $\frac{1}{2}\left(\frac{t}{n} - \frac{n}{n}\right)$ i.e. half the effect of substituting a T for an N fourth chromosome in the presence of an N chromosome. Similarly, $T \times T - N \times T$, for *PH* males, measures $\frac{1}{2}\left(\frac{t}{t} - \frac{n}{t}\right)$, so that, if there is a significant effect, comparison of the two differences gives an indication of any dominance of t over n. It will be sufficient to apply this test to the most extreme cross, involving L and D. We obtain:

$$L \times L - D \times L = -0{\cdot}7 \pm 1{\cdot}3$$
$$L \times D - D \times D = -0{\cdot}3 \pm 1{\cdot}3$$

Evidently selection for large and small size leading to a total difference of nearly 60 units has caused no significant fourth-chromosome effects, and we may therefore assume that the size-differences between the various genotypes are the effect of differences in their major chromosomes.

The fact noted earlier, that the F_1 of crosses between the large and the unselected strains generally exceeds the mid-parent size, is a good indication that we are not dealing with simple additive effects among the major chromosomes, but this may be tested more generally for each group of four crosses involving two strains. For this purpose, we take as unknowns the size of the standard wild-type (N), and the effects of substituting the three major T chromosomes (T_1, T_2, and T_3) and the two marked chromosomes (P and H) for the corresponding N chromosomes. The expected size of any of the sixteen types occurring in the set of four crosses can be expressed as a linear function of one or more of these unknowns, e.g. the full T wild-type female is $N + 2T_1 + 2T_2 + 2T_3$. The six unknowns can then be estimated by the method of least squares, and the significance of the variance due to differences between the expected and observed sizes of the 16 types can be tested against a suitable error variance. This test shows that there are strongly significant deviations from additive combinations of the chromosomes in both sexes, for all sets of crosses with the exception of $L \times N$ males and $L \times E$ females.

Evidently a simple additive scheme will not do, as we had anticipated, and we must try out more complex hypotheses One could examine the deviations between the observed sizes and their least-squares estimates, in the hope of discovering where the greatest deviations from additive effect occur, but this is of little use, since the least squares method distributes the deviations as evenly as possible over the different items, and so does not enable us to single out particular chromosome combinations. Such general methods of analysis must give way to methods based on inspection of individual chromosome combinations and the differences between them. But before considering theories based

on genetic effects, we must examine the possibility that the deviations from an additive scheme are simply due to the use of the wrong scale of measurement, so that the effect of a given gene substitution on size becomes progressively changed as it is made at different points of the size range. Such a scale distortion would occur if the genes responsible for the strain differences had effects proportional to the size of the individual in which they occurred, so that they would have additive effects when measured on a multiplicative, or logarithmic, scale.

Perhaps the simplest test of metric bias is to examine the effects of the *P* and *H* substitutions in the different crosses. The *P* substitution has a substantial effect on size in most of the genetic backgrounds tested, and we may therefore measure the effect of substituting an *H* for the corresponding wild-type chromosome at two different size levels (i.e. in the presence and absence of the *P* chromosome) within a single cross. The difference between the two estimates of *H* is simply the interaction between *P* and *H*, and its significance can be tested by the usual standard statistical procedure. If there is much metric bias, we should expect significant interactions, since the effect of the *H* substitution at the two different size levels caused by the presence and absence of *P* would differ. Table 3 shows the average effect of substituting each of the marked chromosomes for their corresponding wild-type chromosomes, and the interaction between them, in crosses involving the largest and the smallest strains.

TABLE 3.—EFFECT OF *P* AND *H* SUBSTITUTIONS ON FEMALE WING LENGTH

Cross	*Average effect of*		*Interaction between P and H*
	(+) − *P*	(+) − *H*	
L × L ..	11·6	4·4	1·0
T × T ..	13·8	3·5	0·0
D × L ..	10·6	4·4	−0·8
D × D ..	3·0	−8·0	1·5

Standard deviation of all estimates = 0·85

In spite of the large effect of the *P* substitution in most of these crosses, the interaction is in each case small and not significant. Of the 27 crosses tested in this way, only a few showed significant interaction between the *P* and *H* effects, and these could be attributed to specific interactions involving the selected and marked chromosomes, so that there is no evidence of a general scale distortion. More detailed study of the non-additive effects which occur also makes it clear that they could not be eliminated by a change of scale, as will be apparent from the following analysis.

Since metric bias does not appear to be the cause of the deviations from an additive system, we must look for an explanation in genetic terms. The simplest hypothesis is that they are due to dominance; and one might reasonably expect the genes which have been selected for their effects on size to show some degree of dominance to their alleles in the standard strains, since selection would obviously tend to pick out first genes dominant in the direction selected.

Dominance at a particular locus cannot be tested, since our units are whole chromosomes, but tests can be made which give a good indication of whether dominance is present in the aggregate. If there is a tendency for the size genes in a particular T chromosome to be dominant to their N alleles, then the size difference $T/N - N/N$ will be greater than $T/T - T/N$. Such differences can only be estimated in different genetic backgrounds, but this will not destroy the validity of the comparisons unless complex interactions between non-homologous chromosomes occur, and even then one may accept the absence of dominance as shown by this test at its face value.

The dominance tests which can be made are shown in Table 4, where the two heterozygotes and the two homozygotes are each grouped together. Both groups carry the same chromosome complement, but one chromosome pair is heterozygous in the first group and homozygous in the second group. The deviation of the mean of the homozygotes from the mean of the heterozygotes gives a measure of the non-additive, or dominance, deviation, which may be tested for significance against an appropriate standard error. Table 4 shows that chromosomes 2 and 3 may be tested both separately and together in males, while the first chromosome and the three chromosomes together may be tested

TABLE 4.—COMPARISONS USED IN TESTS FOR AGGREGATE DOMINANCE

Chromosome Tested	*Heterozygotes*	*Homozygotes*
Males		
2	$\frac{T}{Y}\ \frac{T}{N}\ \frac{T}{H} + \frac{N}{Y}\ \frac{T}{N}\ \frac{N}{H}$	$\frac{T}{Y}\ \frac{T}{T}\ \frac{T}{H} + \frac{N}{Y}\ \frac{N}{N}\ \frac{N}{H}$
3	$\frac{T}{Y}\ \frac{T}{P}\ \frac{T}{N} + \frac{N}{Y}\ \frac{N}{P}\ \frac{T}{N}$	$\frac{T}{Y}\ \frac{T}{P}\ \frac{T}{T} + \frac{N}{Y}\ \frac{N}{P}\ \frac{N}{N}$
2 + 3	$\frac{T}{Y}\ \frac{T}{N}\ \frac{T}{N} + \frac{N}{Y}\ \frac{T}{N}\ \frac{T}{N}$	$\frac{T}{Y}\ \frac{T}{T}\ \frac{T}{T} + \frac{N}{Y}\ \frac{N}{N}\ \frac{N}{N}$
Females		
1	$\frac{T}{N}\ \frac{T}{P}\ \frac{T}{H} + \frac{T}{N}\ \frac{N}{P}\ \frac{N}{H}$	$\frac{T}{T}\ \frac{T}{P}\ \frac{T}{H} + \frac{N}{N}\ \frac{N}{P}\ \frac{N}{H}$
1 + 2 + 3	$\frac{T}{N}\ \frac{T}{N}\ \frac{T}{N} + \frac{N}{T}\ \frac{N}{T}\ \frac{N}{T}$	$\frac{T}{T}\ \frac{T}{T}\ \frac{T}{T} + \frac{N}{N}\ \frac{N}{N}\ \frac{N}{N}$

in females. If there are only dominance deviations present, then the separate deviations for each chromosome should add up to the corresponding combined estimate.

Table 5 gives the dominance deviations on testing each selected strain against the standard strains, and on testing the two standard strains against each other. The first three rows show the deviations for each chromosome separately, which are summed in the fourth row, and the last two rows give the combined estimates for chromosomes 2 + 3 and 1 + 2 + 3.

Among the individual estimates there are only a few deviations which are clearly significant; the W third chromosome against both standard strains, and the N first chromosome against the small D strain, while there are additional cases of significance at the 5 per cent level. But the combined estimates

TABLE 5.—TEST FOR AGGREGATE DOMINANCE. WING LENGTH (1/100 mm.)

Chromosome and Sex	*Mean of heterozygotes minus mean of homozygotes*							
	$T-N$	$T-E$	$W-N$	$W-E$	$L-E$	$L-N$	$N-D$	$N-E$
1 Female	1·6	−0·3	1·8*	0·4	2·1*	−1·4	7·3**	−0·3
2 Males	0·4	0·6	0·1	0·5	0·2	−0·5	2·4*	1·6
3 Males	0·3	0·9	4·5**	3·0**	2·4*	−1·2	1·1	0·3
Sum	2·3	1·2	6·4	3·9	4·7	−3·1	10·8	1·6
2 + 3 Males	0·0	3·9**	2·6*	2·6*	1·6	−1·6	4·2**	4·0**
1 + 2 + 3 Females	6·8**	9·8**	3·7**	5·6**	3·4**	−0·9	14·0**	7·4**

*Values significant at 5% level
**Values significant at 1% level

for the three chromosomes show a large deviation in all cases except the $L - N$ test, and in the $T - N$, $T - E$ and $N - E$ series the combined estimate far exceeds the sum of the three individual estimates. It is true that we are combining estimates from both sexes in these comparisons, but this should not introduce any major error, since selection has generally caused approximately the same progress in both sexes. Also, no complications from dosage-compensation of the X chromosome have been introduced, since its effect is always estimated in females. Finally, it will be seen that the second and third chromosomes, whose effects are estimated separately and in combination in males, show a similar discrepancy in the $T - E$ and $N - E$ tests.

Perhaps the most striking case is the cross between the two standard strains N and E, both of which have been intensely inbred and are of approximately the same size. The triple heterozygote female shows marked heterosis, being considerably larger than either parent. In the absence of further data, one would naturally explain this by one of the classically assumed causes of heterosis, i.e. the fixation of different dominant size genes in the two strains, or the occurrence of direct overdominance for size at certain loci. But neither of these theories is acceptable, since each requires that the total heterosis must be the sum of heterosis within each chromosome pair, while in fact the summed effects of the individual chromosome pairs add to a mere 1·6 units, compared with their combined effect of 7·4 units. It appears that interaction between non-homologous chromosomes must be the main cause of the heterosis in this cross, and the same conclusion applies to the crosses of the T strain to the two standard strains.

A further point of interest in these comparisons is that the dominance deviations, when they occur, are always in the direction of large size, even in the cross to the small line, D, so that selection for small size has shown no tendency to pick out genes dominant for small size, but has built up mainly recessive effects. Nevertheless, only a small part of the total advance of the large lines can be due to dominant genes.

It is possible to carry the analysis a little further by setting out all the estimates which can be obtained of the effect of substituting each chromosome

of a selected strain for its homologue from a standard strain, and then combining these estimates for comparison with independent estimates of the joint effect of substituting groups of chromosomes of one strain for those from the other. Such an approach may give us further insight into the kinds of non-additive effect present in the different strains. All the estimates which can be obtained of the effect of substituting a *T* for an *N* chromosome are shown in Table 6 with a suitable notation to indicate the two genotypes which are compared to give each estimate.

TABLE 6.—ESTIMATES OF THE EFFECT OF AN INDIVIDUAL CHROMOSOME SUBSTITUTION

Chromosome	*MALES*			*FEMALES*			
	Background	*Estimate*			*Background*	*Estimate*		
1	—	$\frac{T-N}{Y}$	$\frac{T}{N}$	$\frac{T}{N}$	Selected	$\frac{T-N}{T}$	$\frac{T}{P}$	$\frac{T}{H}$
					N	$\frac{T-N}{N}$	$\frac{N}{P}$	$\frac{N}{H}$
					E	$\frac{T-N}{E}$	$\frac{E}{P}$	$\frac{E}{H}$
2	Selected	$\frac{T}{Y}$	$\frac{T-N}{T}$	$\frac{T}{H}$	—	$\frac{T}{N}$	$\frac{T-N}{P}$	$\frac{T}{N}$
	N	$\frac{N}{Y}$	$\frac{T-N}{N}$	$\frac{N}{H}$				
	E	$\frac{E}{Y}$	$\frac{T-N}{E}$	$\frac{E}{H}$				
3	Selected	$\frac{T}{Y}$	$\frac{T}{P}$	$\frac{T-N}{T}$	—	$\frac{T}{N}$	$\frac{T}{N}$	$\frac{T-N}{H}$
	N	$\frac{N}{Y}$	$\frac{N}{P}$	$\frac{T-N}{N}$				
	E	$\frac{E}{Y}$	$\frac{E}{P}$	$\frac{T-N}{E}$				

NOTE.—$\frac{T-N}{Y}\ \frac{T}{N}\ \frac{T}{N} = \frac{T}{Y}\ \frac{T}{N}\ \frac{T}{N} - \frac{N}{Y}\ \frac{T}{N}\ \frac{T}{N}$, etc.

In males there are three estimates for each of the second and third chromosomes, in *T*, *N* and *E* backgrounds, respectively, and one estimate for the *X* chromosome. Females provide three estimates for the first chromosome and one each for the two autosomes. It will be noticed that one or both of the marked chromosomes are used in all the estimates except that for the first chromosome in males, and in the estimates for autosomes in females the homologous marked chromosome is opposite the chromosome whose effect is being estimated. Where there are estimates of the same effect in both a selected and an *N* background, the difference between them provides the dominance deviation of Table 4, so that we can see how the dominance deviation compared with the effect of a single dose of the chromosome.

A large number of such estimates of substitution effects can be obtained from the different crosses, and as there is no reason to suppose that the various strains will show the same kind of effect, we will only examine here the two related strains, *T* and *W*. The estimates obtained on crossing each to both standard strains are given in Table 7.

TABLE 7.—THE EFFECT ON WING LENGTH OF SUBSTITUTING A SELECTED FOR A STANDARD CHROMOSOME

Chromosome substitution

Chromosome substituted	*Background*	T – N	T – E	W – N	W – E
			MALES		
1	—	5·7	1·6	3·5	2·8
2	Selected	5·0	2·6	2·1	−0·3
	N	5·8	2·5	1·4	(0·4)
	E	3·8	3·7	(2·7)	0·7
3	Selected	4·2	6·8	−1·6	1·3
	N	4·7	8·0	7·3	(7·8)
	E	4·8	8·7	(3·2)	7·2
			FEMALES		
1	Selected	−1·6	2·4	−1·9	0·0
	N	1·6	2·5	1·8	(0·6)
	E	1·6	1·8	(0·5)	0·8
2	—	4·6	4·9	4·6	1·5
3	—	7·0	8·7	7·1	5·4

The standard errors of these estimates are about 1·2 in males and 1·3 in females, except for the values in brackets, which were based on comparisons between flies measured at different times, and are therefore somewhat less reliable. There is a remarkable lack of order about these figures, which makes it difficult to fit them into any simple theory. Thus in males the distribution of the size effects of the *T* chromosomes is very different when compared against the two standard strains. Averaging the estimates in the selected and unselected backgrounds, we should conclude that the three *T* chromosomes contribute 5·7, 5·4, and 4·5 units to size, respectively, judged against the *N* strain, compared with 1·6, 3·1 and 7·8 units when judged against the *E* strain. The marked difference between these sets of figures is surprising, since the two standard strains are both of the same size, and dominance does not appear to affect the results. Perhaps it is more remarkable that the estimates for each chromosome effect show a considerable difference in the two sexes in the *T*−*N* comparisons, although they agree fairly well in the *T*−*E* comparisons. The fact that the substitution of a *T* for an *N* first chromosome produces so much more effect in males than in females (5·7 compared with 1·6 units) might be attributed to dosage compensation, were it not that the order of effects is reversed for the *T*−*E* comparisons (1·6 compared with 2·5 units).

Turning now to the *W* strain, we find much better agreement between the *W*−*N* and *W*−*E* estimates in males than in females, in contrast to the situation

with the T estimates. It is also interesting to find that the $W-N$ and $T-N$ estimates for females agree almost exactly with each other, although there are differences between the other sets of estimates. The greatest difference between the two strains occurs in the third chromosome, since W_3 shows complete dominance over both N_3 and E_3, its effect being 7·3 and 7·2 units, respectively, in the N and E backgrounds, compared with $-1{\cdot}6$ and 1·3 units in the selected background. It is difficult to decide from these comparisons whether the distribution of size effects between the three chromosomes is much the same or very different in the two selected strains, since our conclusion depends on which set of estimates we compare.

Before attempting to interpret these results it is instructive to compare the sums of individual substitution effects with independent estimates of their combined effects, thus using a test analogous to the previous test for dominance. To obtain the combined estimates genotypes must be chosen which are not used for the individual estimates, and this almost limits us to the homozygotes of each strain and the heterozygotes between them, including the male heterozygote carrying a selected X chromosome. The observed differences between these genotypes and the corresponding standard strains are shown in Table 8, together with the differences between the observed size and that estimated by adding the chromosome substitutions of Table 7 to the same standard strain. In the case of the homozygotes, we have two estimates, indicated by N

TABLE 8.—The comparison of observed and expected sizes

T strain				*W strain*		
Genotype	*Observed size*	*Observed −Estimated size*	*Genotype*	*Observed size*	*Observed −Estimated size*
HOMOZYGOTES					
$\frac{T}{T}\ \frac{T}{T}\ \frac{T}{T}$ (N)	27·8	1·4	$\frac{W}{W}\ \frac{W}{W}\ \frac{W}{W}$ (N)	25·2	−1·8
$\frac{T}{T}\ \frac{T}{T}\ \frac{T}{T}$ (E)	30·5	−0·3	$\frac{W}{W}\ \frac{W}{W}\ \frac{W}{W}$ (E)	26·5	11·1*
$\frac{T}{Y}\ \frac{T}{T}\ \frac{T}{T}$ (N)	27·1	0·4	$\frac{W}{Y}\ \frac{W}{W}\ \frac{W}{W}$ (N)	21·9	1·0
$\frac{T}{Y}\ \frac{T}{T}\ \frac{T}{T}$ (E)	26·7	0·3	$\frac{W}{Y}\ \frac{W}{W}\ \frac{W}{W}$ (E)	22·0	3·4
HETEROZYGOTES					
$\frac{T}{N}\ \frac{T}{N}\ \frac{T}{N}$	19·7	6·5*	$\frac{W}{N}\ \frac{W}{N}\ \frac{W}{N}$	16·3	2·8
$\frac{T}{E}\ \frac{T}{E}\ \frac{T}{E}$	25·1	9·7**	$\frac{W}{E}\ \frac{W}{E}\ \frac{W}{E}$	18·9	11·2**
$\frac{N}{Y}\ \frac{T}{N}\ \frac{T}{N}$	10·7	0·2	$\frac{W}{Y}\ \frac{W}{N}\ \frac{W}{N}$	11·8	3·1
$\frac{E}{Y}\ \frac{T}{E}\ \frac{T}{E}$	16·5	4·1*	$\frac{W}{Y}\ \frac{W}{E}\ \frac{W}{E}$	14·8	6·9*

and *E* respectively. The first is obtained by starting from the size of the *N* wild-type and adding the required *T*−*N* estimates, and the second by adding *T*−*E* estimates to the size of the *E* wild-type. The observed size will, of course, differ in the two cases, since the same selected strain wild-type is measured from two different starting points.

Several estimates may be obtained of expected size, since we have alternative estimates of some of the substitutions in Table 7, from which to choose. The values given are based on the estimates made in the appropriate standard strain background, so that the estimate for the first genotype given in Table 8 is $2(1{\cdot}6 + 4{\cdot}6 + 7{\cdot}0)$, while that for the third item is $5{\cdot}7 + 2\ (5{\cdot}8 + 4{\cdot}7)$. This method has the advantage of including the full possible effect of each selected chromosome, on the assumption that there is no dominance, and dominance should, therefore, be indicated by a tendency for expected size to exceed observed size.

In the *T* strain there is a quite remarkable accuracy in predicting the size of the homozygotes of both sexes, whether we use the (*T*−*E*) or (*T*−*N*) estimates, and this accuracy is all the more surprising, in view of the marked differences which we noted earlier between the individual *T* chromosome effects, when estimated in the two sexes and against the two standard strains. It is difficult to believe that such accurate prediction is merely the result of coincidence, and does not imply the existence of an underlying additive system. But in the case of the heterozygotes, three of the four estimates are far too low, so that the homozygotes and heterozygotes do not appear to fit into the same scheme. One could, perhaps, explain this result by the theory that the underlying additive scheme is disturbed in the triple heterozygote by the occurrence of interactions leading to heterosis; but there is then the difficulty of understanding why the substitution effects, some of which are estimated in a heterozygous background, are not influenced by the same interactions.

The *W* strain shows a rather different pattern. All estimates based on *W*—*N* substitutions are in fair or good agreement with observation, while the W—*E* estimates generally fall very short in the case of both the homozygotes and the heterozygotes. Here, in fact, the greater measure of agreement between the four sets of substitution effects of the *W* chromosomes, shown by Table 7, does not lead to a generally accurate prediction. A further point of interest is that the marked dominance of the third *W* chromosome over both *N* and *E* chromosomes, suggested by Table 7, is not supported by the analysis of Table 8, since the estimates for the male homozygotes, which include twice the full effect of W_3 in a standard background, fall slightly short of the observed size, instead of exceeding it. It is not possible to resolve this contradiction without further tests, but the apparent dominance could, of course, be due to a specific interaction between the *W* and marked chromosomes.

It is too early to generalise on the basis of these results, but they are certainly suggestive, and some tentative conclusions may be drawn. It seems clear that neither a simple additive scheme, nor one modified by dominance of some of the selected genes over their alleles in the standard strains, is adequate to explain the differences between the genotypes which we have been able to test. One is left, therefore, with the conclusion that some kind of interaction between genes on different chromosomes makes an important contribution to these differences. Contradictions occur in some of the tests, particularly in the case of the *W* third chromosome, which is shown as dominant in the first test and

as lacking dominance in the second test; and this must cast some doubt on the meaning of the various estimates of the individual and joint substitution effects which we have examined. The two marked chromosomes, which are a common feature of all the crosses, may be responsible for these contradictions, although there is no sign of the specific interactions we should expect if this is the case.

The most striking non-additive effect is the heterosis shown by the cross between the two standard inbred strains, which cannot be explained in terms of either the fixation of different dominant genes in the two strains, or of the occurrence of overdominance at certain loci. It appears, rather, to depend on the fact that more than one pair of chromosomes is heterozygous. The heterosis in the crosses between the large (T) strain and both standard strains seems to be of the same kind, and cannot be attributed simply to the selection of genes dominant for large size. These facts suggest that gene interaction may play a more important part in heterosis than is generally assumed, but a greater range of comparisons is necessary before we can determine the part played by the different chromosomes in these interactions, and experiments are now in progress which should provide the basis for a more complete analysis.

REFERENCES

MATHER, K., and HARRISON, B. J. 1949. "The manifold effects of selection." Heredity: *3*, (1): pp. 1-52.

ROBERTSON, F. W., and REEVE, E. C. R. 1952. "Studies in quantitative inheritance. I. The effects of selection of wing and thorax length in *Drosophila melanogaster*." J. Genet. *50* (3): pp. 414-448.

ROBERTSON, F. W., and REEVE, E. C. R. "Studies in quantitative inheritance. IV. The effects of substituting chromosomes from selected strains in different genetic backgrounds." J. Genet. (in the press).

REEVE, E. C. R. 1952. "Genetical analysis of a strain of *Drosophila melanogaster* selected for long wings." This volume, p. 113.

10110. Wt. P3019. 5197. 25. 12/52. S.P. & S. G9-8-8.

(*Reprinted from Nature, Vol.* 170, *p.* 296, *August* 16, 1952)

Heterozygosity, Environmental Variation and Heterosis

When a wild strain of *Drosophila melanogaster* is inbred under optimum conditions, the phenotypic variance of body-size is reduced by not much more than 50 per cent[1]. This suggests that nearly half the variance of the original strain was due to the effects of uncontrollable environmental variations within the culture. But highly inbred lines tend to have a much higher variance than crosses between them, so that the environmental variance in such quantitative characters is not constant for all genotypes and tends to be smaller in heterozygotes than homozygotes. This is illustrated in Table 1 for six highly inbred lines and the crosses between them. The lines came from two unrelated wild stocks, Nettlebed and Edinburgh, and include two lines selected for long wings (*LN* and *LE*), two selected for short wings (*SN* and *SE*) and two unselected lines (*UN* and *UE*). Wing-length is closely correlated with body-size, and variance is expressed as squared coefficient of variation, since this measure eliminates most of the effect of differences in absolute size.

Table 1. Phenotypic Variance of Inbred Lines and Crosses. Variance of wing-length in squared coefficients of variation

Strains crossed	Parental average	F_1	
LN × *SN*	1·79	1·37	females only
LE × *SE*	1·44	0·52	males only
UN × *UE*	2·27	1·11	sexes combined
SN × *UN*	2·85	1·88	,,
SE × *UE*	1·78	0·55	,,
SN × *SE*	4·00	2·00	,,
Average	2·35	1·24	

The inbred lines have, on the average, almost double the phenotypic variance of the crosses between them, and the same tendency is shown by crosses involving large, small and unselected lines. There are probably also differences in variance between the different inbred lines; but part of the differences between the parental averages for the six comparisons must be attributed to environmental differences between the experiments in which they were tested.

The relation between heterozygosity and environmental variance has been further analysed by preparing genotypes containing specific combinations of the three major chromosomes from inbred lines *SE* and *UE*, using methods which will be described elsewhere. If these genotypes are grouped according to

the numbers of heterozygous pairs of chromosomes they carry, we find that the average variance of wing-length declines progressively with increase of heterozygosity (Table 2), so that the environmental variance of a particular genotype appears to be intimately related to the degree of heterozygosity.

A partial analysis of the *SN* × *UN* cross by the same technique shows the same general trend for

Table 2. VARIANCE AND HETEROZYGOSITY IN *SE/UE* GENOTYPES

No. of major chromosome pairs heterozygous	No. of genotypes tested	Average variance of wing-length
Females		
0	5	2·54
1	9	2·23
2	5	1·44
3	1	1·20
Males		
0	5	1·64
1	7	1·31
2	2	1·19

size, and in view of the differences in variance between inbred lines and their crosses, it is likely to be a general phenomenon. The same general relationship also appears to hold for rate of egg production, both in comparisons between inbred lines and their crosses, and in comparisons between the specific genotypes of differing heterozygosity referred to above. But problems of scaling make interpretation of these figures difficult, since the heterozygotes tend to have a much greater output than homozygotes. It seems probable that many quantitative characters in different animals and plants will show the same tendency for environmental variability, under given conditions, to decrease as heterozygosity increases. It is not to be expected, of course, that the same rule would hold good when one is dealing with individual genes with large effects.

Size in *Drosophila* shows the same phenomena of decline under inbreeding and heterosis in crosses between inbred lines as other quantitative characters. This suggests that heterosis, or increased size, vigour, etc., and reduced susceptibility to environmental variations are both manifestations of the same phenomenon of heterozygosity. There may be a general explanation for this relationship: the more heterozygous individuals will carry a greater diversity of alleles, and these are likely to endow them with a greater biochemical versatility in development. This will lead to heterosis, because of the more efficient use of the materials available in the environment, and also to a reduced sensitivity to environmental variations, since there will be more ways of

overcoming the obstacles which such variations put in the way of normal development.

FORBES W. ROBERTSON
E. C. R. REEVE

Institute of Animal Genetics,
King's Buildings,
West Mains Road,
Edinburgh 9.
March 11.

[1] Reeve, E. C. R., and Robertson, Forbes W., *J. Genet.* (in the press).

Printed in Great Britain by Fisher, Knight & Co., Ltd., St. Albans.

[From JOURNAL OF GENETICS, Vol. 51, No. 3,
pp. 586–610, July 1953.]

 PRINTED IN GREAT BRITAIN

STUDIES IN QUANTITATIVE INHERITANCE

IV. THE EFFECTS OF SUBSTITUTING CHROMOSOMES FROM SELECTED STRAINS IN DIFFERENT GENETIC BACKGROUNDS IN *DROSOPHILA MELANOGASTER*

By FORBES W. ROBERTSON and E. C. R. REEVE

STUDIES IN QUANTITATIVE INHERITANCE

IV. THE EFFECTS OF SUBSTITUTING CHROMOSOMES FROM SELECTED STRAINS IN DIFFERENT GENETIC BACKGROUNDS IN *DROSOPHILA MELANOGASTER*

By FORBES W. ROBERTSON* and E. C. R. REEVE*
Institute of Animal Genetics, University of Edinburgh

(*Received* 26 *May* 1952)

CONTENTS

1. Introduction

A number of large and small strains of *Drosophila melanogaster* have been obtained by selection of wing or thorax length (Robertson & Reeve, 1952*a*), and two additional large strains have been created more recently by selecting from a cross between two of these lines. This paper describes an attempt to analyse, at the chromosome level, the nature of the gene effects responsible for the permanent differences in size between some of these strains and standard inbred strains originating from the same wild stocks. Not all the effects of selection were permanent, and one case where size declined sharply whenever selection was relaxed has received detailed study (Reeve & Robertson, 1952).

D. melanogaster provides unique opportunities for analysing the effects of selection on each chromosome, in view of its small number of chromosomes, the absence of crossing-over in males, and the existence of dominant markers and cross-over suppressors for each of the major chromosomes. Various of these properties were used by Payne (1918, 1920), Sturtevant (1918) and others to study the location of genes affecting a quantitative character, but their methods may be said to have lacked statistical precision. More recently, improved methods of chromosome assay have been developed by Mather and his associates for investigating the distribution of effects between different chromosomes (Mather & Harrison, 1949) and within the *X*-chromosome (Wigan, 1949).

* Members of the Agricultural Research Council Scientific Staff.

Mather used a 2^3 factorial design, in which the three 'factors' were the effects of substituting marked first, second and third chromosomes of a tester stock for those of the strain being examined, and he expressed the results in terms of 'main effects' and 'interactions'. These tests gave an interesting picture of the relative changes in each major chromosome caused by selection for high and low abdominal bristle number in a series of lines, and also provided some rather indirect information about the average amount of dominance of each line to the tester stock. But this method, while an improvement on those of previous workers, is still open to certain objections. In the first place, it yields a very limited range of genotypes for study, so that one cannot test different hypotheses about the nature of the observed effects; secondly, there was some loss of efficiency due to crossing-over, which was estimated as an average of 10%, but would appear to have been much larger in some of the tests (cf. Mather & Harrison, 1949, Table 8); finally, it is questionable whether the statistical concepts of main effect and interaction, derived from field-plot experiments, are adequate for analysing genetic effects, where the amount of dominance and epistasis present, and the stability of a given effect when the genetic background is changed, are problems of primary importance.

For the most complete analysis of the difference between two strains, in which chromosomes are treated as units, we should need to create all possible genotypes consisting of major chromosomes from one or both strains (18 in males, 27 in females) in sufficient numbers to enable accurate measurements of each to be obtained. We have recently developed a method of creating the full range of genotypes with little chance of error, and this is being used to analyse the differences between various selected and unselected strains. Meanwhile a much more rapid survey has been carried out, using a method which avoids crossing-over and provides sufficient genotypes, from two generations of crosses, to enable a number of tests to be made, of the extent and nature of non-additive effects arising in genotypes containing chromosomes from different sources. Some of the results of this analysis have already been described (Robertson, 1952), but the present paper contains both additional data and some additional tests.

2. THE DESIGN OF THE EXPERIMENTS

(*a*) *The system of crosses*

In order to compare two strains, say T and N, virgin females of each strain are mated to males of a marker stock, carrying the genes Plum and Hairless, dominants on the second and third chromosomes. F_1 males carrying both marked chromosomes (for which the symbols P and H will be used) from each cross, are backcrossed to both the T and N strains, giving four backcrosses, which may be distinguished by the symbols $T \times T$, $T \times N$, $N \times T$ and $N \times N$, according to the type of female used in the first and second stages of the cross. The segregation of the P and H chromosomes among the progeny enables us to identify four genotypes in each cross, or sixteen in the set of crosses, containing different combinations of the T, N and marked chromosomes. These genotypes are listed in Table 1. There is obviously no danger of crossing-over between chromosomes of different strains, since only pure T and N females are used as parents. The Y-chromosome always comes from the marker stock, and so does not affect comparisons between the different genotypes, while the fourth chromosomes differ in the different crosses in a way which allows us to test the magnitude of their effects.

some tests being repeated each time so that different experiments could be compared if necessary. The crosses made in each experiment are shown in Table 3, but Exp. 3 has not been used in the present analysis, except to give additional estimates of the error variances.

Table 2. *Size of strains tested*

	Deviation from standard strain (1/100 mm.)			
	Wing length		Thorax length	
	Males	Females	Males	Females
Nettlebed strains:				
T (Long thorax)	25·3	26·2	15·5	14·6
W (Long wing)	22·6	25·4	8·6	8·6
D (Short wing)	−31·2	−36·3	−7·1	−11·0
Edinburgh strain:				
L (Long wing)	26·4	32·3	9·8	13·1
	Size of standard strains			
N (Nettlebed)	176·6	203·6	91·1	105·1
E (Edinburgh)	176·3	201·6	92·4	103·1

Table 3. *Crosses made in each experiment*

Exp. 1				Exp. 2				Exp. 3				Exp. 4				
	T	*N*	*E*		*W*	*N*	*E*		*T*	*W*	*N*		*L*	*N*	*E*	*D*
T	×	×	×	*W*	×	×	×	*T*	×	×	×	*L*	×	×	×	×
N	×	×	×	*N*	×	×		*W*	×	×	×	*N*	×	×		×
E	×	×	×	*E*	×		×	*N*			×	*E*	×		×	×
												D	×	×	(×)	×
7 replicates				6 replicates				6 replicates				6 replicates				

Note. Each × indicates that the cross was made in which the first and second strain used in the crossing scheme are indicated by letters at left end of row and top of column, respectively, containing the cross; e.g. in Exp. 4, second × in top row indicates that cross $L \times N$ was made. The cross $D \times E$ in Exp. 4 failed.

(*c*) *Culture methods and measurements*

The cultures were reared in an incubator at 25° C., and great care was taken to keep the experimental conditions as constant as possible, by avoiding overcrowding and randomizing the arrangement of the cultures within the incubator, so as to avoid any systematic effect from temperature gradients. Cultures were made in $\frac{1}{4}$-pint milk bottles, containing about 3 cm. depth of the usual cornmeal-molasses-agar medium, fortified with additional dried baker's yeast and well yeasted with live yeast. 300 eggs were introduced into each culture bottle, and two cultures of each mating were made on three successive days, except for Exp. 1, where 2, 2, 1 and 2 cultures per mating were made on four successive days.

After emerging, the flies in each culture were classified into the four genotypes and counted, and five males and five females of each type were measured. The males were also weighed in groups of five on a torsion balance. The measurements of wing and thorax were the dimensions described by Robertson & Reeve (1952*a*). Altogether, about 67,000 eggs were cultured and their progeny counted, and 9000 flies were measured.

3. STATISTICAL ANALYSIS OF THE DATA

Each cross provides four distinct types of fly (+, *P*, *H* and *PH* phenotypes) which segregate within a single culture. Five flies of each type were measured per culture, and the cultures were usually replicated six times (two cultures put up on three successive

days). Within a single culture we have three degrees of freedom for differences between types, which may be separated into the average effects of substituting (+) chromosomes for P and H, respectively, and the interaction between them, i.e.

$$p = \tfrac{1}{2}[(+) - P + H - PH],$$
$$h = \tfrac{1}{2}[(+) + P - H - PH],$$
$$i = \tfrac{1}{2}[(+) - P - H + PH].$$

Environmental conditions must inevitably vary from culture to culture, and any genotype-environment interaction would tend to make the variance of p, h and i between cultures greater than would be expected from the variance within types and cultures. Differences between types from the same cross may thus be affected by two sources of error, the variance within types and cultures (σ^2) and the interaction between types and replicates (TR). The latter could be split up into components within and between days, but this turns out to be unnecessary. It is estimated from the mean square for interaction between types and replicates, which is simply the mean of the variances of p, h and i within crosses.

Two additional sources of error affect comparisons between type means from different crosses. These are differences between replicates due to effects of culture differences on the bottle means (R), and interaction between days and crosses, due to differential responses of the types in different crosses to the environmental differences between days (DC). The measurements were divided equally between two observers, each taking one culture per day of each cross, and using microscopes giving slightly different magnifications. (R) must therefore be calculated from the differences between replicates put up on the same day after eliminating the differences between observers. The relation between the various mean squares and components of error are shown in Table 4 for a typical experiment, consisting of n crosses each replicated twice over three days.

Table 4. *Error variances affecting type means*

Mean square	Degrees of freedom	Expected value
(1) Within types and cultures	$96n$	σ^2
(2) Types × replicates within crosses	$15n$	$\sigma^2 + 5(TR)$
(3) Between replicates	$3n - 1$	$\sigma^2 + 5(TR) + 20(R)$
(4) Days × crosses	$2(n - 1)$	$\sigma^2 + 5(TR) + 20(R) + 40(DC)$

In Exp. 1 there were 2, 2, 1 and 2 cultures per cross on four successive days, of which one observer measured 1, 2, 0, 1, both cultures of day 2 being measured by the same observer owing to a misunderstanding. This will affect the degrees of freedom and the component of (DC). A few missing samples or samples of less than five also reduce the number of degrees of freedom. Adjustments were made for these gaps in the usual way.

Table 5 shows the four error mean squares for wing length in each sex, expressed on the basis of an individual fly. The four experiments, which include 9, 7, 7 and 13 crosses, are shown separately and also averaged. Significance tests have only been applied to the average variances, on the assumption that there is no heterogeneity between experiments, since the different experiments were fairly consistent.

The variance within types and cultures (1) is much the same in the four experiments, and there are, on the average, significant interactions between types and replicates (2) and a significant variance due to differences between cultures reared together (3) in each sex.

The interaction variance between types and replicates was at first thought to be a real genotype-environment interaction effect, but further analysis showed that its cause was quite different. This is clear from Table 6, where the variance within types and cultures (1) and the interaction variance (2) are calculated separately for the data measured by each observer.

Table 5. *Error variances in each experiment* (1/100 *mm.*)^2

Variance	Degrees of freedom				Mean square				
	1	2	3	4	1	2	3	4	Average
			Males						
(1) σ^2	1008	636	656	1248	10·6	12·3	13·3	11·7	11·8
(2) $\sigma^2+5(TR)$	159	99	102	195	15·4	19·2	21·8	13·9	16·7**
(3) $\sigma^2+5(TR)+20(R)$	25	18	17	38	17·4	32·0	12·2	55·4	33·9**
(4) $\sigma^2+5(TR)+20(R)+40(DC)$	24	12	12	24	31·6	49·6	18·2	56·9	40·8
			Females						
(1) σ^2	1008	649	634	1248	14·6	15·0	16·0	13·3	14·5
(2) $\sigma^2+5(TR)$	158	99	90	195	24·2	19·5	19·2	14·4	19·0**
(3) $\sigma^2+5(TR)+20(R)$	25	18	15	38	53·4	45·5	31·5	42·9	44·3**
(4) $\sigma^2+5(TR)+20(R)+40(DC)$	24	12	10	24	36·8	40·1	58·0	28·5	37·5

** Significant at 1% level.

Table 6. *Error variances of wing length for each observer*

Exp. ...	1		2		3		4	
	B.M.G.	C.H.W.	B.M.G.	C.H.W.	B.M.G.	C.H.W.	B.M.G.	C.H.W.
Males:								
(1) σ^2	9·9	11·3	11·7	12·8	11·5	15·1	10·9	12·5
(2) $\sigma^2+5(TR)$	10·5	20·6	12·2	22·6	13·7	23·5	8·4	20·8
Females:								
(1) σ^2	13·2	16·1	16·4	13·7	14·3	17·8	12·2	14·4
(2) $\sigma^2+5(TR)$	17·0	34·1	13·8	27·4	17·1	21·8	12·9	19·3

The variance within types is about the same for the two observers, though B.M.G. appears to be a little more accurate in her measurements. But while variances (1) and (2) are almost identical for the measurements of B.M.G., (2) is very much larger than (1) for those of C.H.W., so that the significance of the interaction variances is entirely attributable to one observer. The cause was evidently a tendency of this observer to misclassify the phenotypes of samples when measuring them. Several obvious misclassifications, all made by the same observer, were corrected at the time of the analysis, and other discrepancies in the sample sums were noticed, where the cause of the discrepancy could not be determined with certainty. Probably there has been an occasional mixing of samples between phenotypes of the same culture, but no samples have been excluded because of discrepancies, since these were not frequent enough to affect the means seriously. In view of the large mass of material which had to be sorted out, weighed and measured, under rather cramped conditions, it is perhaps more surprising that one observer maintained such a high standard of accuracy than that some mistakes occurred.

In order to obtain standard errors for the various comparisons, it has been assumed that (DC) is zero, so that variances (3) and (4) can be combined, and the variances of the four experiments have been pooled, since the differences between them were not in general very great, and this leads to a great simplification in computing standard errors. The components of variance affecting wing length of an individual of each sex are then as shown in Table 7.

Table 7

Components	Males	Females
σ^2	11·8	14·5
(TR)	0·98	0·90
(R)	1·00	1·12

The variance of a type mean based on n replicates is

$$V_1=\frac{1}{5n}[\sigma^2+5(TR)] \quad \text{or} \quad V_2=V_1+\frac{1}{n}(R),$$

depending on whether we wish to compare two means from the same or different crosses, so that $nV_1=3{\cdot}34$ and $3{\cdot}80$ for males and females, and $nV_2=4{\cdot}34$ and $4{\cdot}92$ for the two sexes.

We shall require certain additional error variances, which can be deduced from those already given: These are:

(1) The error variance for a type mean chosen at random from the set of sixteen means given by four crosses such as $T\times T$, $T\times N$, $N\times T$, $N\times N$, which is required for the least squares analysis of § 5. These means consist of four sets of four, each four coming from a single set of replicates, as indicated in Table 1. Thus, if we choose two such means at random from the set of sixteen, there are twenty-four ways of choosing two from the same set of replicates and ninety-six ways of choosing two from different sets. The variance will thus consist of a weighted mean of V_1 and V_2 in the ratio of 1 : 4, i.e. $V_3=\frac{1}{5n}[\sigma^2+5(TR)+4(R)]$. The same result may be obtained by considering the analysis of the set of sixteen means, on the assumption that there are no differences between types or between crosses. The analysis would then be as shown in Table 8.

Table 8

Source of variance	Degrees of freedom	Mean square
Between type means within crosses	12	$\frac{1}{5n}[\sigma^2+5(TR)]$
Between crosses	3	$\frac{1}{5n}[\sigma^2+5(TR)+20(R)]$
Between type means	15	$\frac{1}{5n}[\sigma^2+5(TR)+4(R)]$

The last row gives the variance (V_3) of a type mean chosen at random from the sixteen of the set. The actual degrees of freedom of this error variance will lie somewhere between those on which (TR) and (R) were based (Cochran, 1951), and we can give them a minimum value of 50.

(2) In § 7 (*c*) standard errors will be required of various linear combinations of type means, of which some come from the same cross and some from different crosses. Any linear combination may be written

$$X=\sum_r (a_r A_r+b_r B_r+c_r C_r+d_r D_r),$$

where A_r, B_r, C_r and D_r are the four type means from the rth cross and the small letters

are the coefficients by which these means are multiplied. Summation is made over the crosses. Then the error variance of X is

$$V(X) = V_1 \sum_r (a_r^2 + b_r^2 + c_r^2 + d_r^2) + (V_2 - V_1) \sum_r (a_r + b_r + c_r + d_r)^2.$$

V_1 and V_2 are the error variances of type means from the same and different crosses, as defined earlier. This formula arises directly from the fact that the between-culture component of variance $\left(V_2 - V_1 = \frac{1}{n}(R)\right)$ affects only the sums of type means from the same set of replicates, while the component V_1 acts independently on all the means included.

The error variances for weight will be discussed in the section analysing the relation of weight to thorax length in males.

4. FOURTH-CHROMOSOME EFFECTS

Reciprocal crosses between selected strains of different size indicate that cytoplasmic and maternal effects have little or no influence on body size; but nothing is known about effects of genes carried by the fourth chromosome, apart from the fact that change in the number of fourth chromosomes changes cell size much more than in proportion to the change in volume of chromosome material (Dobzhansky, 1929).

The present series of tests enables us to compare males which differ only in their fourth-chromosome complement, and thus provides a direct measure of the importance of fourth-chromosome size effects. *PH* males from any two crosses for which the same strain was used in the second stage (e.g. $T \times N$ and $N \times N$) receive identical sets of major chromosomes and cytoplasm, and differ only in their fourth chromosomes. If a, t and n are the fourth chromosomes of the original marker stock, the T and N stocks, respectively, then the chromosome complements of $(T \times N)$ and $(N \times N)$ will be $\frac{1}{2}\left(\frac{a}{n} + \frac{t}{n}\right)$ and $\frac{1}{2}\left(\frac{a}{n} + \frac{n}{n}\right)$. Hence the difference $(T \times N) - (N \times N)$ measures $\frac{1}{2}\left(\frac{t}{n} - \frac{n}{n}\right)$. Similarly, the difference $(T \times T) - (N \times T)$ measures $\frac{1}{2}\left(\frac{t}{t} - \frac{n}{t}\right)$, and comparison of the two differences, which may be symbolized as $(T-N)/N$ and $(T-N)/T$, gives a measure of the extent of dominance of t over n. Table 9 gives all the comparisons which can be made between the different strains.

It will be seen that most of the differences do not differ significantly from zero, but there are interesting exceptions in the comparisons involving W. In fact, the W fourth chromosome, when homozygous, appears to reduce size compared with any of the heterozygotes W/N, W/E and W/T, with which it can be compared. Thus

$$(W-N)/W = -3{\cdot}6,$$
$$(W-E)/W = -1{\cdot}6,$$
$$(W-T)/W = -2{\cdot}6,$$

and the first and third of these differences are significant at the 1 and 5% levels, respectively. Since the genotypes compared differ only in their fourth chromosomes, it appears that the W-chromosome carries a recessive gene or genes which reduce size when homozygous. It is surprising to find such an effect in a line selected for large size, but

a chance fixation of such a gene might have been caused by the gradual inbreeding which accompanied selection of the *W* strain.

The average significance of the remaining eighteen differences can be tested approximately by χ^2, since their standard errors are based on large numbers of degrees of freedom. Summing the squares of the ratios of each difference to its standard error, we obtain $\chi^2 = 25$, with 18 degrees of freedom. This value is not significant ($P = 0{\cdot}10$), so that there is no evidence of fourth-chromosome effects on size, apart from the one already noted. Selection has evidently had little or no effect on the fourth chromosome.

Table 9. *Effects of fourth-chromosome substitutions on male wing length. Differences based on mean size of P, H males* (1/100 *mm.*)

Comparison	Wing lengths	Comparison	Wing lengths
$(T-N)/T$	0·0	$(T-E)/T$	1·0
$(T-N)/N$	0·1	$(T-E)/E$	2·2*
$(W-N)/W$	−3·6**	$(W-E)/W$	−1·6
$(W-N)/N$	0·8	$(W-E)/E$	0·0
$(T-W)/T$	−1·6	—	—
$(W-T)/W$	−2·6*	—	—
$(L-N)/L$	2·0	$(L-E)/L$	1·0
$(L-N)/N$	−2·7*	$(L-E)/E$	−0·3
$(N-D)/N$	2·2	—	—
$(N-D)/D$	−0·3	$(E-D)/D$	−1·5
$(L-D)/L$	−0·7	$(N-E)/N$	0·2
$(L-D)/D$	−0·3	$(N-E)/E$	1·7

The standard errors of these estimates are 1·11 for the first two rows, and 1·20 for the remainder. One star and two stars indicate significance at 5 and 1% levels.

5. Test of the hypothesis that the chromosomes combine additively

The simplest hypothesis about the effects of the major chromosomes on size is that they combine additively. The four crosses involving two strains (e.g. $T \times T$, $T \times N$, $N \times T$ and $N \times N$, as in Table 1) yield sixteen genotypes, of which two are identical in males and two pairs identical in females, for the major chromosomes; and, on the hypothesis that they combine additively, each of these genotypes may be expressed as a linear combination of the six constants N, T_1, T_2, T_3, P and H, where N is the size of the strain N, and the other constants are the effects of substituting a T for an N first, second and third chromosome, and P and H for the corresponding N chromosomes. By equating the observed size of each genotype with the linear expression in terms of the constants we obtain sixteen equations, e.g.

$$\frac{NNN}{NNN} = N,$$

$$\frac{TPH}{TTT} = N + 2T_1 + T_2 + T_3 + P \div H, \text{ etc.},$$

from which the values of the constants may be estimated by the method of least squares. This solution ignores the effects of fourth-chromosome differences, which would require two extra constants to represent the effects of substituting a T and an N fourth chromosome for the corresponding chromosome of the original marker stock. But whether or not these effects are included, we have only seven independent equations for estimating the constants, since nine of the sixteen original equations can be eliminated by means of

equations of the form $\frac{TTT}{TTT}-\frac{TPT}{TTT}=\frac{TTT}{NNN}\times\frac{TPT}{NNN}$. It is, therefore, impossible to solve for the eight constants which included the fourth-chromosome effects, and we can only solve for the six constants measuring the average effects of the major chromosomes.

It is obvious, from the preceding section, that fourth-chromosome effects can be ignored in most of the crosses, and they are only likely to have a noticeable effect in crosses involving the *W* strain.

Table 10 sets out the solution of the equations for each sex. The small letters *a*, *b*, *c*, etc., in § 10(*a*) are the observed means of the sixteen genotypes obtained from a set of four crosses such as $T\times T$, $T\times N$, $N\times T$ and $N\times N$, and R_{1-4} and S_{1-4} are the sums of rows and columns respectively, S being the sum of all sixteen items. The six normal equations are not shown, but (*b*) gives the constants A, B, ..., F on the right-hand side of the normal equations, in terms of *a*, *b*, *c*, ..., etc., and (*c*) gives the values of the unknowns T_1, T_2, etc. in terms of A, B, ..., F (i.e. the inverse matrix). Thus in males

$$T=\tfrac{1}{24}[22A-8B-8C-\ldots+5F].$$

Table 10. *Least squares estimates of chromosome substitution effects*

(*a*) Type means

Cross	Genotype +	P	H	PH	Sum
$T\times T$	*a*	*b*	*c*	*d*	R_1
$T\times N$	*e*	*f*	*g*	*h*	R_2
$N\times T$	*i*	*k*	*l*	*m*	R_3
$N\times N$	*n*	*o*	*p*	*q*	R_4
Sum	S_1	S_2	S_3	S_4	S

(*b*) Constants in normal equations

Males	Females
$A=R_1+R_3$	$A=2R_1+R_2+R_3$
$B=A+a+c+e+g$	$B=A-(b+d+f+h)$
$C=A+a+b+e+f$	$C=A-(c+d+g+h)$
$D=S_2+S_4$	$D=S_2+S_4$
$E=S_3+S_4$	$E=S_3+S_4$
$F=S$	$F=S$

(*c*) Effect of chromosome substitutions

	Males						Females					
	A	*B*	*C*	*D*	*E*	*F*	*A*	*B*	*C*	*D*	*E*	*F*
$24T_1=$	+22	− 8	− 8	− 4	− 4	+ 5						
$24T_2=$	− 8	+16	− 8	+ 8	− 4	− 4						
$24T_3=$	− 8	− 8	+16	− 4	+ 8	− 4						
$24P=$	− 4	+ 8	− 4	+10	− 2	− 5						
$24H=$	− 4	− 4	+ 8	− 2	+10	− 5						
$24N=$	+ 5	− 4	− 4	− 5	− 5	+10						
$16T_1=$							+11	− 6	− 6	− 3	− 3	+1
$16T_2=$							− 6	+12	− 4	+6	− 2	− 2
$16T_3=$							− 6	− 4	+12	− 2	+6	− 2
$16P=$							− 3	+ 6	− 2	+7	− 1	− 3
$16H=$							− 3	− 2	+ 6	− 1	+7	− 3
$16N=$							+ 1	− 2	− 2	− 3	− 3	+6

The estimates of the six unknowns, T_1, T_2, ..., N, enable us to estimate the sixteen genotype means, *a*, *b*, ..., *q*, on the basis of our hypothesis. The variance due to differences between the observed and estimated values of these sixteen means as 10 degrees of freedom, and will be an estimate of the error variance of a type mean if our hypothesis that the chromosomes combine additively is correct, and fourth-chromosome effects are ignored. The significance of deviations from an additive system may, therefore, be tested by comparing the variance for each set of crosses with the error variance, V_3, of § 3. Table 11 shows the variances of both sexes for both characters in each set of crosses.

Only five of the variances are not significantly greater than the error variance (items in heavy type), two are significant at the 5% level (starred items), and the remainder are significant at the 1% level. Evidently the chromosomes do not combine in a completely additive manner in most of the crosses, and the deviations from an additive system are often substantial and much too large to be attributed to fourth-chromosome effects. The variance is also generally much greater in females than in males, suggesting that the chromosomes show greater non-additive effects in the former.

Table 11. *Test of goodness of fit of least squares estimates. Variance due to difference between observed and expected genotype means*

	Wing length		Thorax length	
Strains crossed	Males	Females	Males	Females
(1) *E* and *N*	2·6	8·4	**0·05**	**0·13**
(2) *T* and *N*	1·9	6·0	0·39*	1·33
(3) *T* and *E*	3·0	20·1	0·65	2·54
(4) *W* and *N*	4·3	4·6	1·06	1·43
(5) *W* and *E*	5·3	8·0	1·40	1·53
(6) *L* and *E*	1·6*	3·7	**0·24**	0·95
(7) *L* and *N*	2·2	**1·1**	**0·44**	0·95
(8) *D* and *N*	3·2	54·3	0·98	2·29
(9) *D* and *L*	3·7	50·6	0·75	2·36
	Error variances			
Rows 1–3	0·59	0·66	0·19	0·21
Rows 4–9	0·68	0·77	0·23	0·24

Values given in heavy type are not significant. * indicates significance at 5% level. All other values are significant at 1% level.

Several hypotheses could be put forward to explain the deviations from an additive system, and they will be considered in turn. The following non-additive effects can be distinguished:

(1) Metric bias, due to use of an unsuitable scale of measurement.

(2) Interaction between homologous chromosomes, due to either dominance of individual genes or interactions between genes carried on the same chromosome pair.

(3) Interaction between genes carried by different pairs of chromosomes.

Since we are dealing with the segregation of whole chromosomes, it is not possible to distinguish between the two effects under item (2), and these will be included under the term 'aggregate dominance'. The term 'potence' has been introduced by Wigan (1944) to describe non-additive effects between groups of polygenes, but this term appears to include effects of types (2) and (3), so that it is avoided here.

6. Test for metric bias

As has often been pointed out, there are no *a priori* reasons for believing that the scale of measurement is necessarily the most appropriate for genetical analysis (Mather, 1949; Wright, 1952), and the question arises how far distortion of additive gene effects, due to the wrong choice of scale, could have contributed to the non-additive effects of Table 11. A glance at the table shows that metric bias cannot be the main cause of these effects, since crosses of any large strain to the two standard strains, which are of about the same size, would then be expected to give variances of much the same amount. But the variances are very much greater when *T* or *W* is crossed with *E* than when they

are crossed with N, so that marked dominance or interaction effects must be present. Metric bias might nevertheless be a contributing factor, and some test of its importance is required.

Such a test may be based on the average effects of the substitutions of (+) for P and H chromosomes, and the interaction between them, in the different crosses. As noted earlier, we define these effects in the usual way:

$$p=\tfrac{1}{2}[(+)-P+H-PH],$$
$$h=\tfrac{1}{2}[(+)+P-H-PH],$$
$$i=\tfrac{1}{2}[(+)-P-H+PH].$$

i actually measures the difference between the effects of the H substitution at two different levels of size (i.e. in the presence and absence of P), and it is obvious that any metric bias will tend to increase this interaction, since the same substitution effect will be measured on different scales at different levels of size. Moreover, the extent of the bias will vary with the magnitude of the substitution effect and the difference in size levels at which it is estimated. Metric bias will, therefore, introduce a correlation between i and the joint magnitude of the P and H substitutions, which may be measured by their geometric mean $\sqrt{(ph)}$, where p and h are both taken with the positive sign. In the case of wing length, p varies from 12·6 to 1·9 units in males and from 16·9 to 3·0 units in females, while h varies from 5·3 to −8·3 units and from 6·4 to −8·6 units in the two sexes. The corresponding variations in the geometric mean of the absolute values of p and h are 7·7 to 1·0 and 9·5 to 3·0 units respectively, so that there is a good range of values for testing metric bias. All p values are positive, and when h is negative the sign of i is changed, so that a positive value of i always indicates that the substitution effect is reduced when the level of size is reduced.

One can distinguish between what might be called *monotonic* and *divergent* metric bias. The former consists of a progressive trend from one end of the scale to the other, as when an arithmetic scale is transferred to logarithms. 'Divergent' bias occurs if the point of zero distortion is at the mean size, or size of the unselected stocks, and distortion of scale increases as we move away from this level in either direction. Bias of this kind would exist if there were some kind of resistance to movements away from the optimum (unselected size), such that given gene substitutions produced progressively less effect on size as the size level deviated more from the optimum in either direction. Monotonic bias would lead to a correlation between i and $\sqrt{(ph)}$ over the whole series of crosses, thirty-six in all; divergent bias would lead to a correlation if we reverse the sign of i when considering deviations below the size of the standard lines, and omit crosses which include genotypes both above and below this level. Actually, for this test we shall also omit the single small strain (D), and take only the twenty-one crosses in which N and E occur not more than once and not together.

Table 12 gives an analysis of the interaction variances for wing length in each sex. The mean squares are shown for the deviation of the average interaction from zero, the regression of i on $\sqrt{(ph)}$, the residual variance, and the error variance. The last item is simply the variance V_1 of § 3, adjusted for the fact that some estimates are based on seven and some on six replicates.

In all cases the variance due to the regression of i on $\sqrt{(ph)}$ is not significant, whether judged against the remainder or error variance, so that there is no evidence of metric

bias in this test. This cannot be taken as proof that we are using the best possible scale, but it suggests that no great improvement would be gained by transforming to another scale, and will be taken as sufficient justification for retaining the arithmetic scale. Table 12 enables us to draw some additional conclusions. The average interaction is significant in both sexes, as shown by the first row, the mean over all crosses being $+0{\cdot}61 \pm 0{\cdot}14$ in males and $+0{\cdot}58 \pm 0{\cdot}17$ in females, so that the means of the two sexes are almost identical. Thus the presence of the *P*-chromosome, which always decreases

Table 12. *Test for metric bias: analysis of interaction variance*

	Test for monotonic bias			Test for divergent bias		
		Mean squares			Mean squares	
Variance of i	Degrees of freedom	Males	Females	Degrees of freedom	Males	Females
Deviation from 0	1	13·3	12·3	1	4·9	5·4
Regression on $\sqrt{(ph)}$	1	1·09	0·29	1	0·08	0·11
Remainder	34	0·72	1·03	19	0·70	1·18
Error	—	0·54	0·61	—	0·54	0·61

size, tends also to decrease the effect of the *H* substitution on size, whether this increases or decreases size. This must be a genuine epistatic effect, since it cannot be attributed to metric bias. The variance of the interaction about its mean, after eliminating any regression on $\sqrt{(ph)}$ (row labelled 'Remainder'), is a little greater than the error variance in males and significantly so in females, so that there is probably some heterogeneity between the different crosses in the amount of interaction between the *P* and *H* substitutions, though it is less than one might expect.

An excellent discussion of the problems raised by metric bias has recently been given by Wright (1952), who describes tests for the presence of bias and transformations suitable for removing different types of bias, including examples of what we have classified as monotonic and divergent bias. Scaling problems have also been discussed by Mather (1949). In the present case tests based on comparisons of the variances were not tried, since not all genotypes were constant, in view of the genetic variability remaining in some of the strains tested. Moreover, there is evidence that genotypes which are homozygous tend to have a higher phenotypic variance than heterozygous genotypes (Robertson & Reeve, 1952*b*), so that comparisons of variance might prove misleading.

7. Tests for dominance and interaction

(*a*) *Methods of analysis*

Having eliminated metric bias as the cause of the non-additive variations, it remains to see how far we can distinguish between the effects due to aggregate dominance and interaction between non-homologous chromosomes. Neglecting fourth-chromosome effects, a set of crosses such as $T \times T$, $T \times N$, $N \times T$ and $N \times N$ (Table 1) gives fourteen different genotypes in males and fifteen in females. These are only a small proportion of the fifty genotypes in males and seventy-five in females which can be built out of *T*, *N*, *P* and *H* chromosomes (*P* and *H* are lethal when homozygous), so that general theories about the nature of the genetic differences between the strains obviously cannot be tested.

We can choose thirteen independent differences between pairs of the fourteen genotypes in males, and fourteen such differences in females, and these will contain all the information provided by the set of crosses. There are various ways of choosing these comparisons, depending on whether we are interested in the effects of P and H in different backgrounds, as in the test for metric bias, or in the effects of substituting one or more T chromosomes for their N homologues. The latter point of view enables us to compare the effects of selection on the different chromosomes of each strain. If we consider only differences between pairs of genotypes which carry the same complement of chromosomes from the P, H stock (i.e. differences within the columns of Table 1), there are ten independent differences for males and eleven for females, of which five in males and four in females give estimates of the effects of substituting individual T for N chromosomes. The remainder provide estimates of the effects of substituting two or more chromosomes together and these substitutions may be chosen in various ways. In addition, of course, there are the differences between columns, which consist of the average effects of P and H and their interaction. These do not concern us for the time being.

The comparisons which provide estimates of substitutions of individual T for N chromosomes are set out in Table 13, where a simple notation is used to indicate the difference chosen. Thus $\frac{T-N}{Y}\frac{T}{N}\frac{T}{N}$ means the difference $\frac{T}{Y}\frac{T}{N}\frac{T}{N} \times \frac{N}{Y}\frac{T}{N}\frac{T}{N}$, and measures the effect of substituting a T for N first chromosome when II and III are both heterozygous for T and N.

Table 13. *Estimates of the effects of individual chromosome substitutions* $(T-N)$

	Males		Females	
Chromosome substituted	Genetic background	Estimate	Genetic background	Estimate
I	Heterozygous for II and III	$\frac{T-N}{Y}\frac{T}{N}\frac{T}{N}$	(*a*) N	$\frac{T-N}{N}\frac{N}{P}\frac{N}{H}$
			(*b*) T	$\frac{T-N}{T}\frac{T}{P}\frac{T}{H}$
II	(*a*) N	$\frac{N}{Y}\frac{T-N}{N}\frac{N}{H}$	Heterozygous for I and III	$\frac{T}{N}\frac{T-N}{P}\frac{T}{N}$
	(*b*) T	$\frac{T}{Y}\frac{T-N}{T}\frac{T}{H}$		
III	(*a*) N	$\frac{N}{Y}\frac{N}{P}\frac{T-N}{N}$	Heterozygous for I and II	$\frac{T}{N}\frac{T}{N}\frac{T-N}{H}$
	(*b*) T	$\frac{T}{Y}\frac{T}{P}\frac{T-N}{T}$		

For the X-chromosome in females and both autosomes in males, we have two estimates of the substitution effect: $(a)=\frac{T-N}{N}$, and $(b)=\frac{T-N}{T}$. The (a) and (b) estimates also differ in their genetic background, since (a) is homozygous for N and (b) heterozygous for T/N, apart from the presence of P or H. If there is no interaction due to the differences of background, then the difference between (a) and (b) measures the amount of aggregate dominance for the chromosome pair in question. We can also obtain both (a) and (b) estimates of the joint substitutions (II + III) in males, (I + II), (I + III) and

(I+II+III) in females, as shown in Table 14. If the non-additive effects are due entirely to dominance, then the sum of the individual substitution effects of I and II will equal the effect of the group substitution (I+II), when either (*a*) or (*b*) estimates are considered separately, and the same will be true for the other group substitutions. We thus have a direct test for interaction between genes on non-homologous chromosomes from the different strains.

Table 14. *Estimates of the effects of group substitutions of* $(T-N)$

Substitution	Estimate (*a*)	Estimate (*b*)
II+III in males	$\frac{Y(T_2T_3-N_2N_3)}{NNN}$	$\frac{Y(T_2T_3-N_2N_3)}{TTT}$
I+II in females	$\frac{(T_1T_2-N_1N_2)\,H}{NNN}$	$\frac{(T_1T_2-N_1N_2)\,H}{TTT}$
I+III in females	$\frac{(T_1T_3-N_1N_3)\,P}{NNN}$	$\frac{(T_1T_3-N_1N_3)\,P}{TTT}$
I+II+III in females	$\frac{(TTT-NNN)}{NNN}$	$\frac{TTT-NNN}{TTT}$

The (*a*) and (*b*) individual estimates are made in the presence of P and H for I, of H for II, and of P for III. In addition, we have an estimate of I in males, made in a (+) background heterozygous for T/N, and one estimate each of II and III in females, made opposite the P and H chromosomes, respectively, in a T/N background. Apart from the possibility of sex differences, the estimates for the autosomes made in the two sexes may differ because of the different dominance relations of the T, N and P or H chromosomes. H decreases size when substituted in a large strain and increases it when substituted in a small strain, so that it is probably fairly similar to a standard third chromosome, but P always reduces size.

We shall examine first the individual chromosome substitutions and the consistency of the individual and group estimates.

(*b*) *Individual substitution effects on wing length*

Table 15 gives the effects on wing length of the individual chromosome substitutions, as set out in Table 13, and their standard errors, for each pair of strains compared.*

The two standard strains N and E are of almost identical size, but their chromosomes differ in their effects on size, as is shown by the $(N–E)$ substitutions in males. These substitutions reduce size when made for chromosomes I and III, but increase size for II. Their effects differ in males and females for I and III, presumably due to differences in genetic background. The chromosomes of the other strains show differences, when substituted for N and E chromosomes, which roughly correspond with the direct $(N–E)$ differences, apart from a few discrepancies. The most striking of these is $L\,\mathrm{III}\,a$, which appears to have too small an effect when substituted for N. Thus $(L–N)\,\mathrm{III}\,a=2{\cdot}3$, which is too small compared with $(L–E)-(N–E)=5{\cdot}8$, or with $(L–D)-(N–D)=4{\cdot}0$. Possibly a specific interaction is involved here.

* Three of these values differ slightly from figures quoted in a previous discussion (Robertson, 1952), due to subsequent corrections in the analysis.

There is a general tendency for partial dominance to occur in the direction of large size, since the (*a*) estimates usually exceed the (*b*) estimates, but the large strains differ markedly in their dominance relations, when tested against the standard strains. Thus *T* shows only a slight tendency towards dominance of II and III to *N* and *E*, which is not significant; while the related strain *W* shows little dominance of II, but complete dominance of III to *N* and *E*. *L* shows complete dominance of I, measured in females, and partial dominance of III, to the chromosomes of the related strain *E*, but the pattern is strikingly reversed in the (*L*–*N*) comparisons. Here the (*b*) estimates always exceed the (*a*) estimates, so that there appears to be partial dominance in the direction of small size for all three chromosomes.

Table 15. *Effects of individual chromosome substitution on wing length* (1/100 *mm.*)

Chromosome substituted	Strains compared								
	N–*E*	*T*–*N*	*T*–*E*	*W*–*N*	*W*–*E*	*L*–*N*	*L*–*E*	*L*–*D*	*N*–*D*
				Males					
I	−2·1	5·7	1·6	3·5	2·8	7·0	4·8	26·8	19·6
II (*a*)	−0·1	6·3	3·7	2·4	0·7	1·1	1·5	5·5	4·1
II (*b*)	−2·5	5·0	2·6	2·1	−0·3	3·3	1·2	1·4	0·5
III (*a*)	3·9	5·5	8·7	7·3	7·2	2·3	9·7	11·3	7·3
III (*b*)	2·5	4·2	6·8	−1·6	1·3	5·4	4·8	8·1	4·3
S.E.	0·88	0·88	0·88	1·02	1·02	1·02	1·02	1·02	1·02
				Females					
I (*a*)	0·2	1·6	1·8	1·8	0·8	−0·5	4·4	22·5	21·6
I (*b*)	0·9	−1·6	2·4	−1·9	0·0	2·2	0·2	5·7	7·0
II	−2·9	4·6	4·9	4·6	1·5	5·0	4·9	3·8	−2·0
III	−1·7	7·0	8·7	7·1	5·4	11·7	13·0	16·6	3·2
S.E.	0·99	0·99	0·99	1·16	1·16	1·16	1·16	1·16	1·16

The chromosomes of the *L* and *N* strains show only partial dominance to those of the small *D* strain, so that selection in the latter has not picked out only recessive genes. *D* could not be compared with strain *E*, since one of the crosses required for this test failed.

The distribution of size effects between the three major chromosomes is much the same in the three large strains, and shows that selection has caused most change in III, and rather small effects in I and II. The only discrepancy occurs with the (*T*–*N*) estimates in males, which show roughly equal effects on all three chromosomes. Selection for small size in strain *D* has evidently had most effect on the *X*-chromosome, and least on II.

A single substitution of I in males often exceeds in effect the corresponding double substitution (*a*+*b*) in females, e.g. in the case of (*T*–*N*), (*W*–*N*), (*W*–*E*) and (*L*–*N*). These discrepancies seem too great to be attributable to dosage compensation of the *X*-chromosome, and must be due to the fact that the estimates of I are made in a (+) background in males and in a *PH* background in females. This appears to be an example of interactions between genes on non-homologous chromosomes.

(*c*) *Consistency of individual and group substitution effects*

The test for consistency between the individual and group substitutions is given in Table 16, which shows the differences between the observed group substitution effects and the corresponding sums of the individual effects, as set out in Tables 13 and 14. In

the case of (II+III) both group and individual estimates are made in males, and the differences of Table 16 are of the following form, taking $(T\text{–}N)$ as an example:

$$(a)\quad \frac{N}{Y}\frac{(TT\text{–}NN)}{NN} - \frac{N}{Y}\frac{(T\text{–}N)}{N}\frac{N}{H} - \frac{N}{Y}\frac{N}{P}\frac{(T\text{–}N)}{N},$$

$$(b)\quad \frac{T}{Y}\frac{(TT\text{–}NN)}{TT} - \frac{T}{Y}\frac{(T\text{–}N)}{T}\frac{T}{H} - \frac{T}{Y}\frac{T}{P}\frac{(T\text{–}N)}{T},$$

where the expressions in brackets indicate the substitutions made. A significant difference for either estimate must obviously indicate the presence of interactions between genes on non-homologous chromosomes; but such interactions might be of several kinds, e.g. between T_2 and T_3, between N_2 and N_3 or between T or N genes and genes in the P and H chromosomes. It is not possible to distinguish between these possibilities without further comparisons which are not available in the present data.

The other differences are of essentially the same kind, except that estimates made in the two sexes have to be combined. Thus the $(T\text{–}N)$ differences are as follows:

$$\begin{aligned}
\text{I+II} &= (TT\text{–}NN)\,H - (T\text{–}N)\,PH - Y(T\text{–}N)\,H,\\
\text{I+III} &= (TPT\text{–}NPN) - (T\text{–}N)\,PH - YP(T\text{–}N),\\
\text{I+II+III} &= (TTT\text{–}NNN) - (T\text{–}N)\,PH - Y(T\text{–}N)\,H - YP(T\text{–}N),
\end{aligned}$$

where the genetic background of all genotypes consists of NNN for (a) estimates and TTT for (b) estimates. The group estimates and the estimates of I are made in females, while the individual estimates for the autosomes are made in males, since this alone enables us to compare estimates always of the same form: $\frac{(T\text{–}N)}{N}$ or $\frac{(T\text{–}N)}{T}$.

Significant differences in these comparisons might be due to three kinds of effect:

(1) An unequal distribution of size effects between the three chromosomes in the two sexes—no evidence on this point is yet available, but it seems unlikely that the two autosomes would have differential effects in males and females.

(2) The autosomes might have slightly greater effects in females than in males, because of the difference in absolute size of the two sexes and the fact that the deviations caused by selection tend to be proportionately greater in females (Table 2). The ratio of female to male size in the standard strains and of the standard errors in the two sexes is about 1·15, and the substitution estimates from males have been multiplied by this factor, before use in Table 16. This should eliminate any effects due to size differences between the sexes.

(3) Interactions affecting size may occur between genes on different chromosomes. It can be assumed that most of the significant effects of Table 16 are of this kind.

Table 16 shows immediately that dominance cannot account for all the non-additive effects present in the different crosses, since no less than thirteen of the seventy-two interaction differences are significant at the 1% level and twenty-three at the 5% level. Substantial interactions must occur between genes on non-homologous chromosomes in a number of genotypes, and there is some tendency for these to be larger in crosses involving unrelated strains (columns 3, 5 and 6) than in those involving related strains (columns 2, 4 and 7), so that interactions appear to be greatest when chromosomes from unrelated strains are brought together. Selection for small size also appears to have involved epistatic effects as well as the accumulation of recessive and partly recessive

genes, since strain *D* shows interactions with both *L* and *N*. Significant differences are shown almost equally by the (*a*) and (*b*) estimates, so that one cannot interpret the interactions as simply a modification of the degree of dominance of genes which increase size, caused by the changes in genetic background.

Table 16. *Comparison of group and individual substitution effects on wing length. Observed minus estimated group substitution effect* (1/100 *mm.*)

Group substitution	Level	1 *N–E*	2 *T–N*	3 *T–E*	4 *W–N*	5 *W–E*	6 *L–N*	7 *L–E*	8 *L–D*	9 *N–D*	Standard errors Columns 1–3	Standard errors Columns 4–9
II+III in males	(*a*)	1·0	−1·1	4·1*	2·1	6·9**	5·9**	1·9	2·7	−1·4	1·78	1·92
	(*b*)	−3·1	1·5	−0·8	6·1**	3·4	1·9	3·9*	2·3	−3·2		
I+II in females	(*a*)	1·8	−0·8	3·8*	1·7	2·6	3·7	−0·9	3·6	1·8	1·89	2·04
	(*b*)	−0·1	−1·3	−7·9**	2·6	1·9	−2·0	−2·2	−1·8	−4·6*		
I+III in females	(*a*)	−0·3	0·6	−1·2	−0·1	0·2	5·8**	−4·2*	1·7	−1·4	1·89	2·04
	(*b*)	−3·3	1·2	−6·2**	5·3*	2·0	2·6	3·9	2·3	−4·5*		
I+II+III in females	(*a*)	3·1	4·4	7·9**	3·3	9·0**	10·5**	2·1	5·4*	0·1	2·41	2·62
	(*b*)	−6·9**	−0·8	−7·7**	10·2**	6·4*	3·8	5·4*	5·1	−8·5**		

Note. One and two stars indicate significance at 5 and 1% levels.

In the crosses involving a large and a standard strain, most of the significant differences occur in the estimates of (II+III) and (I+II+III), and with one exception they are then positive. For these differences group substitution effects of (II+III) and (I+II+III) in a (+) background are compared with the corresponding individual estimates in a background containing *P*, *H* or both chromosomes, and a significant excess of the group substitution effect is equally frequent whether a set of chromosomes from the large strain is present or not (*b* and *a* estimates). Two hypotheses could explain these results: either the chromosomes of a selected strain interact together to increase size by more than the sum of their separate effects, or the presence of a *P* or *H* chromosome in the individual substitution estimates reduces their effect. The latter hypothesis seems unlikely, since it would require the *P* or *H* chromosome to show specific inhibiting effects on chromosomes of three large strains. It is probable, therefore, that interactions occur between genes on different chromosomes of each selected strain which increase the size effect of these chromosomes when they are present together. Theoretical reasons for believing that selection will build up interactions of this kind have been put forward (Robertson & Reeve, 1952), and some practical evidence has also been found (Reeve & Robertson, 1952).

The possibility should also be borne in mind that inbreeding of the standard strains has fixed genes on different chromosomes which interact with each other to reduce size more, when two or more chromosomes are homozygous, than one would expect from the reduction when each chromosome is made heterozygous separately. This would cause positive differences in the (*a*) but not the (*b*) estimates of Table 16 for (II+III) and (I+II+III) and may account for some of these effects. Further work is being done to test these hypotheses.

The (*W–N*) differences might be explained by another hypothesis. In the (*W–N* column of Table 16, significant effects are only found in those (*b*) substitutions which involve chromosome III, and the average of these differences is 7·2, which is remarkably close to the (*a*) estimate of (*W–N*) III in Table 15. In fact, there would be no significant

differences if we used the (*a*) instead of the (*b*) estimate of (*W*–*N*)III, i.e. if we assumed that the dominance of *W*III to *N*III shown by Table 15 disappears when *W*III is substituted jointly with other *W* chromosomes (I, II or both). We could thus explain most of the non-additive effects in the (*W*–*N*) series by the hypothesis that *W*III is dominant to *N*III for size effects in certain genetic backgrounds only. It should be noted, however, that the same hypothesis will not account for the non-additive effects of the (*W*–*E*) series.

The (*L*–*N*) series only show significant—and positive—differences for the (*a*) substitutions involving chromosome III, and these could be eliminated by taking a larger value for (*L*–*N*)III*a* in Table 15. It will be remembered that (*L*–*N*)III*a* seemed too small judged by other substitutions in Table 15, so that here again it may be a specific chromosome substitution which is responsible for an apparent array of non-additive effects, although dominance is not involved in this case, since the (*a*) and not the (*b*) estimates show the disagreement.

An idea of the relative magnitude of some of the interactions shown by Table 16 may be obtained by comparing them with the total difference in size between the pairs of strains in question (Table 2). The largest individual difference is 10·5, for (I + II + III)*a* in the (*L*–*N*) comparisons, which is no less than 35 % of the total deviation between the wild-type females of *L* and *N*; and a number of the interaction deviations of Table 16 are more than 20 % of the difference between the two strains compared. This brings out clearly that substantial interactions occur in a number of cases, apart from the general prevalence of some degree of dominance in the direction of large size.

8. Differences in the behaviour of wing and thorax length

In unselected strains the thorax is half as long as the wing dimension, so that proportional changes in the two characters would be in the ratio of 2 : 1, but the genetic correlation between wing and thorax length is less than unity (Reeve & Robertson, 1952), so that selection of wing length tends to cause less than proportionate changes in thorax length, and vice versa. This is well illustrated by comparing the effects of the individual chromosome substitutions between related strains on each character (Table 17). Each selected strain is compared with the standard strain of the same origin, and the figures for males only are given.

Table 17. *The effects of individual chromosome substitutions on wing and thorax length (males; units of* 1/100 *mm.)*

Chromosome substituted	*T*–*N*			*W*–*N*			*L*–*E*			*N*–*D*		
	Wing	Thorax	Ratio	Wing	Thorax	Ratio	Wing	Thorax	Ratio	Wing	Thorax	Ratio
I	5·7	3·6	1·6	3·5	1·3	2·7	4·8	1·5	3·2	19·6	1·0	0·05
II (*a*)	6·3	3·1	2·0	2·4	2·0	1·2	1·5	1·3	1·2	4·1	1·6	2·6
II (*b*)	5·0	3·1	1·6	2·1	1·2	1·8	1·2	2·8	0·4	0·5	−0·6	—
III (*a*)	5·5	3·3	1·7	7·3	2·1	3·5	9·7	3·2	3·0	7·3	4·0	1·8
III (*b*)	4·2	2·7	1·6	−1·6	−1·8	0·9	4·8	1·5	3·2	4·3	2·1	2·0
S.E.	0·88	0·45		1·02	0·49		1·02	0·49		1·02	0·49	

Strain *T* was selected for long thorax, and we find that the individual substitution effects on thorax length are generally a little more than half those on wing length, the two series running closely parallel. *W* and *L* were both selected for long wings, and the

values for wing length are rather more than twice those for thorax length, except in the case of chromosome II, which shows relatively large deviations for thorax length in both strains. The large strains thus show much what we should expect from the selection of two dimensions, each of which is closely related to body size. *D* was selected for short wings, and shows the expected ratios of wing to thorax length for chromosomes II and III, but there is a marked discrepancy for I. The *X*-chromosome of *D*, when substituted for *N* in males, causes a barely significant decline in thorax length (1·0 unit), although wing length is reduced by 19·6 units. The substitution of I in females gives essentially the same picture, since the (*a*) and (*b*) estimates are 3·1 and 1·4 for thorax length, compared with 21·6 and 7·0 for wing length. Evidently selection in strain *D*, which has caused most effect on the *X*-chromosome, has done so mainly by accumulating genes in this chromosome which affect wing length only. Further work is in progress to study the nature of these effects.

Apart from the one major discrepancy in strain *D*, analysis of the individual and group substitution effects on thorax length gives results closely parallel to those for wing length, and gives the same indications of the existence of substantial interactions between genes on non-homologous chromosomes, so that these interactions are not specific wing effects, but mainly affect general body size.

9. THE RELATION OF WEIGHT AND THORAX LENGTH

Experiments have shown that weight changes significantly during adult life. Females may increase by as much as 40% when the ovaries are maturing during the first few days after emergence, while males increase by about 5%; and later both sexes decline in weight. Thus weight is difficult to measure accurately, compared with the linear dimensions, which can be taken as constant during adult life. For these reasons males only were weighed—the flies of each sample being weighed in a batch—and all cultures were weighed at approximately the same age in terms of date of culturing the eggs.

We shall confine ourselves to a general analysis of the relation of weight and thorax length, working with measurements transformed to logarithms so as to eliminate dimensional complications. It is of interest first to examine the error variances of log weight in the different experiments. The error components are the same as in Table 4, except that we cannot calculate a within-sample variance (1), and the $T \times R$ variance (2) of Table 4 has been separated into two mean squares:

(2*a*) Types × replicates within days and crosses.

(2*b*) Types × days within crosses.

Table 18 gives the four mean squares and their interpretation. The logarithms to base 10 were multiplied by 1000, and the variances then calculated on the basis of a type mean. The degrees of freedom differ from those in Table 5 because there was no difference between observers to eliminate and only four crosses in Exp. 3 were weighed.

The different experiments were reasonably consistent in their error variances and generally show substantial components for differences between replicates cultured on the same day (*R*), and for interaction between days and crosses (*DC*). Evidently adult weight is rather susceptible to uncontrollable variations in culture conditions, while the large $D \times C$ interactions may reflect variations in the rate of change of adult weight, due to variations in the time of transferring samples from different cultures to fresh vials, before weighing.

Table 18. *Error variances for $\log_{10}$ weights of males. The unit is [1000 $\log_{10}$ (sample mean)]/no. of replicates*

	Source of variance	Degrees of freedom 1	2	3	4	Mean square 1	2	3	4	Components of variance
2 (*a*)	$T \times R$ within D and C	81	54	36	117	34	48	29	46	(TR)
2 (*b*)	$T \times D$ within C	81	36	24	78	20	65	44	70	$(TR)+2(TD)$
3	R within D and C	27	18	12	39	277	230	48	113	$(TR)+4(R)$
4	$D \times C$	24	10	6	24	483	300	170	393	$(TR)+2(TD)+4(R)+8(DC)$*

* Except for Exp. 1, where one of the 4 days had only one replicate.

If form remains constant, the coefficients of variation of weight should be about three times those for thorax length, and this enables us to compare the relative magnitude of errors affecting the two characters. Any variance (V) in Table 18 may be converted into a coefficient of variation (C), using the formula

$$C = 0{\cdot}23(5rV)^{\frac{1}{2}}\ \%,$$

where r is the number of replicates. Taking the average variances of $T \times R$ and $D \times C$, we obtain the following comparison, C for thorax length being multiplied by 3:

	Thorax length ($3C$)	Weight (C)
$T \times R$	7·6	8
$D \times C$	10	26

The error variances based on the $T \times R$ interaction are in close agreement for the two characters, but interactions between Days and Crosses introduce disproportionately large errors affecting weight. Thus comparisons between different crosses are likely to be rather inaccurate in the case of weight.

In spite of this fact, there are high correlations between log weight and log length for the type means, the correlation coefficients being 0·91, 0·65, 0·93 and 0·91 for the four experiments. These correlations are analysed in Table 19 for Exps. 1, 2 and 4. The first four rows give the regression of log weight on log thorax length when all the types means for a given experiment are pooled. Linear regression accounts for about four-fifths of the total variance in Exps. 1 and 4 and nearly half in 2, but the residual variance is still significantly greater than the error variance in each case, so that weight appears to show some variation independent of thorax length.

The error term in the fourth row requires explanation. It is calculated as

$$3n[T \times D]/(4n-1) \text{ plus } (n-1)\,[D \times C]/(4n-1),$$

where $[T \times D]$ and $[D \times C]$ are the mean squares of Table 18, and n is the number of crosses contained in the experiment. The justification for this estimate may be found by reference to Table 8 $[T \times D]$ and $[D \times C]$ being taken as the error variances within and between crosses. The degrees of freedom are calculated approximately following Cochran (1951).

The variance of log weight within crosses has also been analysed in the second part of Table 19. Here the average regression accounts for two-thirds of the variance within crosses in each experiment, and the remaining variance is divided into differences in slopes of the regression lines fitted to the four types of each cross separately and the

residual variance about these regression lines. The error variance in this case is the error within crosses, i.e. item $T \times D$ of Table 18, but for Exp. 1 we have pooled the $T \times R$ and $T \times D$ error variances.

There are clearly no significant differences between the slopes of the regression lines within the different crosses, but the residual variance is still a little greater than the error, in all experiments, so that the marked chromosomes probably have differential effects on the two characters in some of the crosses.

Table 19. *Regression of log weight on log thorax length (type means)*

Experiment	1		2		4	
Source of variance	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.
			Total variance			
Total	35	1,320	27	455	51	2,600
Linear regression	1	38,000	1	5,300	1	109,000
Remainder	34	240*	26	284*	50	468**
Error	34	131	27	117	54	146
			Variance within crosses			
Total	27	254	21	296	39	410
Average regression	1	4,900	1	4,300	1	11,600
Slope differences	8	55	6	101	12	91
Remainder	18	86**	24	93	26	126*
Error	162	27	36	65	78	70
			Slope of regression line			
Average within crosses		1·29±0·17		1·28±0·18		1·58±0·16
Means of crosses		1·64		0·67		2·12

Stars indicate significance of remainder variances at 5 and 1% levels.

The regression slopes within crosses and between the cross means are given at the foot of Table 19. Within crosses the three experiments do not differ significantly in slope, the average regression being about $1{\cdot}4 \pm 0{\cdot}1$. If the P and H substitutions leave form unchanged we should expect to obtain a value of a little less than 3, the difference from 3 being due to the fact that both characters are subject to errors. A functional relationship of the kind discussed by Kermack & Haldane (1951), e.g. the ratio of the standard deviations of log weight and log thorax length, would have an expected value of about 3, but the average value of this ratio over the three experiments is only 1·64, so that weight obviously changes at a rate much less than proportional to the cube of thorax length, when the P and H substitutions are made. This is a surprising result, which must mean that these substitutions alter either form or specific gravity, and it requires further study.

The regression slope for the cross means is higher in Exps. 1 and 4, but lower in 2, so that differences in regression within and between crosses account for part of the residual variances given in row 3 of Table 19. Part can also be attributed to the fact that strain E was consistently about 13% heavier than N, though it was only about $1\frac{1}{2}$% longer in the thorax. In Exp. 2, E was actually heavier than W, and this doubtless explains the low regression coefficient for the cross means.

In view of the rather large sampling errors of the mean weights, it did not seem worth while to carry the analysis further by comparing the ratio of log weight to log thorax length for particular genotypes.

10. Conclusions

Tests have shown that, when chromosomes from pairs of selected and standard strains were put together in the arrangements which can be obtained by a simple crossing scheme, there were in most cases significant deviations from additiveness in the effects of the different chromosomes on size. It was possible to prove that metric bias was not a major cause of these non-additive effects and that they could not be attributed entirely to dominance, although there was a variable amount of dominance in the direction of large size, when large strains were crossed with the standard unselected strains or the latter were crossed with the short-wing strain. There were obviously substantial interactions between genes on non-homologous chromosomes in several cases, and these tended to be greatest when chromosomes from unrelated strains were combined.

Among the most significant interaction effects was a general tendency for the joint substitution of II+III or I+II+III of a large strain in a (+) background to increase size by more than the sum of the individual substitution effects of the same chromosomes in a background containing one or both of the marked chromosomes, *P* and *H*. It seems unlikely that either of these chromosomes would have a specific inhibiting effect on the chromosomes of three large strains, so these differences probably indicate that interactions occur between genes on different chromosomes of each large strain which tend to increase size when they are together. This result, expressed in a different way (Robertson, 1952), has been used to support a suggestion that gene interactions may contribute more than is generally supposed to heterosis effects, since neither of the accepted theories of heterosis—dominance and overdominance—could cause the interactions between genes on different chromosomes brought to light by the present study. The interactions seemed to be most marked when chromosomes of unrelated strains were brought together, and it may well be that epistasis contributes substantially to the heterosis often found in crosses between unrelated strains. It is hoped to test these conclusions by a technique which provides a greater range of genotypes and does not require the presence of special marked chromosomes, whose peculiar properties may complicate the picture.

Comparison of changes in wing and thorax length show that they vary closely in parallel in the different genotypes, with one striking exception. This is strain *D*, selected for short wings, which differs from the standard strain *N* mainly in a very large *X*-chromosome effect which causes practically no reduction in thorax length. On this chromosome evidently genes were selected with a specific effect on wing length and not on body size, but in the other cases the relative effects of the various chromosome substitutions on the two characters were much what one would expect from the fact that there is a high but not perfect genetic correlation between them (Reeve & Robertson, 1952).

The relation of weight to thorax length, studied in males, shows that these two characters generally varied together in the different genotypes, but weight tended to change at a rate much less than the cube of thorax length. This was particularly the case when comparing the effects of substituting the *P* and *H* chromosomes on the two characters. The relative rate of change of weight and thorax length was much the same in all crosses, the substitutions causing weight to change in proportion to about the $1\frac{1}{2}$ power of thorax length. It would appear that these substitutions cause a change in form with a relatively great effect on thorax length, or also a change in specific gravity of the flies, and further work is necessary on this problem.

It should, perhaps, be emphasized that the substitution effects we have been examining were estimated by comparing genotypes most of which contained major chromosomes from two or even three different strains; and one obviously cannot draw inferences about the effects of genes segregating in an unselected population from study of whole chromosomes under these somewhat artificial conditions. The interactions discovered are average interactions between the groups of genes on non-homologous chromosomes, and individual epistatic effects may well be more important than one would judge from study of these group effects, since plus and minus interactions would cancel each other out. In the same way, dominance and epistasis occurring within a given chromosome pair may sometimes be of different sign, so that the measures of aggregate dominance we have used probably underestimate the relative importance of non-additive gene effects.

11. SUMMARY

1. A simple crossing technique was used to produce cultures of *Drosophila melanogaster* segregating for chromosomes marked by the genes *Plum* and *Hairless*, in which the genotypes were otherwise either homozygous for chromosomes of a given strain or heterozygous for chromosomes of two strains; and samples of flies of the four segregating genotypes were measured.

2. This method was used to compare three strains selected for large size and one selected for small size with two standard inbred strains, and enabled tests to be made of the importance of fourth-chromosome effects on size, of the extent to which the chromosomes combine additively, and of various hypotheses as to the nature of the non-additive effects.

3. In nearly all cases there were substantial non-additive effects on size and little if any of these could be attributed to metric bias.

4. There was generally some 'aggregate dominance'—i.e. summed dominance effects over a whole chromosome—in the direction of large size, in crosses of both the large lines and the small line to the standard strains, which varied from complete dominance to a slight deviation from additiveness in different chromosome pairs. But substantial interactions were demonstrated between genes on non-homologous chromosomes, apart from the dominance effects. Thus both dominance and gene interaction must contribute to the non-additive effects previously noted. The interaction effects tend to be greatest when chromosomes from unrelated strains are combined.

5. Selection for large size has generally produced most effect in the third chromosome, while selection for small size in the one strain tested produced effects mainly in the *X*-chromosome. Changes in this chromosome reduced wing length but had very little effect on thorax length. In the other cases wing and thorax length showed more or less proportional changes, so that selection of either character has mainly affected body size.

6. Variations in weight in males follow closely those in thorax length. But substitution of the Plum and Hairless chromosomes, which have substantial effects on size, caused changes in weight proportional to less than twice the power of thorax length, so that either the form or the specific gravity changes. Comparable data for females are not available.

7. There was no evidence that selection for large or small size had produced changes in the fourth chromosomes acting in the direction of selection.

We are most grateful for the assistance of Miss B. M. Green and Mrs C. H. Wearmouth, who were responsible for the handling, sorting and measurement of the cultures, and to Mr C. M. Marr, who took the weights and bore the brunt of the computational work. We also wish to thank Prof. Sewall Wright for some valuable suggestions made in discussion.

REFERENCES

Cochran, W. G. (1951). Testing a linear relation among variances. *Biometrics*, **7**, 17.

Dobzhansky, T. (1929). The influence of the quantity and quality of chromosome material on the size of the cells in *Drosophila melanogaster*. *Roux. Arch. EntwMech. Organ.* **115**, 363.

Kermack, K. A. & Haldane, J. B. S. (1951). Organic correlation and allometry. *Biometrika*, **37**, 30.

Mather, K. (1949). *Biometrical Genetics*. Methuen, London.

Mather, K. & Harrison, B. J. (1949). The manifold effect of selection. *Heredity*, **3**, 1.

Payne, F. (1918). An experiment to test the nature of the variations on which selection acts. *Indiana Univ. Stud.* Study no. 36.

Payne, F. (1920). Selection for high and low bristle number in the mutant strain 'Reduced'. *Genetics*, **5**, 501.

Reeve, E. C. R. & Robertson, F. W. (1952). Studies in quantitative inheritance. II. Analysis of a strain of *Drosophila melanogaster* selected for long wings. *J. Genet.* **51**, 276.

Robertson, F. W. (1952). Interactions between chromosomes from large and small strains of *Drosophila melanogaster*. In *Quantitative Inheritance*. London: H.M.S.O.

Robertson, F. W. & Reeve, E. C. R. (1952*a*). Studies in quantitative inheritance. I. The effects of selection of wing and thorax length in *Drosophila melanogaster*. *J. Genet.* **50**, 414.

Robertson, F. W. & Reeve, E. C. R. (1952*b*). Heterozygosity, environmental variation and heterosis. *Nature*, **170**, 296.

Sturtevant, A. H. (1918). An analysis of the effect of selection. *Publ. Carneg. Instn.*, no. 264.

Wigan, L. G. (1944). Balance and potence in wild populations. *J. Genet.* **46**, 150.

Wigan, L. G. (1949). The distribution of polygenic activity on the *X*-chromosome of *Drosophila melanogaster*. *Heredity*, **3**, 1.

Wright, Sewall (1952). The genetics of quantitative variability. In *Quantitative Inheritance*. London: H.M.S.O.

STUDIES IN QUANTITATIVE INHERITANCE

V. CHROMOSOME ANALYSES OF CROSSES BETWEEN SELECTED AND UNSELECTED LINES OF DIFFERENT BODY SIZE IN *DROSOPHILA MELANOGASTER*.

Forbes W. Robertson[+]

Institute of Animal Genetics, Edinburgh.

[+] Member of the Agricultural Research Council Scientific Staff.

Contents

1. Introduction.

Practical information about the properties of genes which control the hereditary variation of quantitative characters is almost non-existent. For any such character we have little idea how far it is legitimate to think in terms of dominance and recessiveness, additive or epistatic effects in relation to the observed genetic variation. The general concepts of gene behaviour are derived principally from comparatively simple genetic situations, in which it is possible to follow a few substitutions by more or less striking phenotypic effects. An earlier discussion (Robertson and Reeve, 1952a), based on general considerations, stressed that an allele substitution which affects a quantitative character is likely to be greatly influenced by both genetic background and environmental conditions. At first sight it might appear fruitless to attempt study of such allele differences and safer to rely instead on the average effects of statistical analysis. For obvious reasons, this is unavoidable in most animals, particularly in livestock. There are, however, considerable dangers in this, since the constant emphasis on average or so-called additive effects of allele differences, encourages students to think in terms of truly additive effects of allele substitutions, whereas the genetic situation may be really far removed from an additive one. It is usually extremely difficult, even in such a convenient animal as Drosophila, to discriminate between alternative genetic interpretations, as in

the instance discussed by Reeve and Robertson (1953a). And yet if we really knew more of the properties of the genetic variation, it might greatly alter our interpretation of the effects of selection and shed light on such problems as the stability of adaptive characters, heterosis and the adverse effects of inbreeding. It might also suggest profitable lines of inquiry which would be unlikely to find favour when the preoccupation with average effects is too great. Drosophila therefore presents something of a challenge to take genetic analysis as far as possible, since it alone is sufficiently well known genetically to offer some prospect of discriminating between alternative interpretations.

Although, in quantitative inheritance, it is generally impossible to follow the behaviour of individual gene substitutions, in Drosophila at any rate, it is possible to follow the behaviour of particular chromosomes in different genetic situations. This might not appear particularly encouraging, since a chromosome carries many loci. However, we may reasonably assume considerable genetic similarity between a selected strain and the unselected stock from which it was derived. Hence chromosomes of the selected strain differ chiefly from their unselected homologues in the loci affected by selection. It is at present unknown approximately what fraction of the total this is likely to be, but the method represents a step in the breakdown of the total genetic differences into more manageable

proportions. Also such a method of analysis, by encountering novel genetic situations and by providing tests for various hypotheses, is quite a valuable tool for deepening our insight into the properties of genetic variation.

In a previously published study of this kind (Robertson and Reeve, 1953), the effects of substituting, in different genetic backgrounds, chromosomes from large and small strains were studied with the aid of autosomal markers, using a system of crossing which provided a sample of the theoretically possible combinations. These experiments revealed substantial non-additive effects, arising in part at least from interactions between non-homologous chromosomes. They also suggested that study of a complete set of all possible combinations of chromosomes from pairs of contrasted strains might resolve some of the difficulties which are unavoidable with incomplete sets of combinations. Accordingly the present paper deals with such a complete chromosome analysis of an unselected and a small selected line from each of two unrelated wild stocks. A similar analysis of unselected and large lines is in progress and will be reported in a later paper of this series.

I should like to thank Dr. E. C. R. Reeve for much fruitful discussion during the analysis of the data and the preparation of this paper, which presents a contribution to our joint studies on quantitative inheritance.

2. The Experimental Method.

The two small lines used in these experiments are derived by inbreeding from the strains selected for short wing length which were descended from the Nettlebed and Edinburgh stocks; they are referred to as the D and S lines respectively. The origin and selection of these small strains have already been described (Robertson and Reeve, 1952a).

The line D was taken off the selected strain after the latter had ceased to respond to selection either way and was inbred by brother-sister mating for more than 40 generations before the start of the present experiments. The small Edinburgh line, S, was founded by making an isogenic line from the selected stock after it had made a considerable response to selection, but when it still retained considerable genetic variability. The S line has also been inbred for many generations (40+) before being used. The unselected lines were derived by more than 100 generations of brother-sister mating from the Nettlebed and Edinburgh stocks and are referred to as N and E respectively.

When a mass mating stock is intensively inbred, wing and thorax length decline in size, and therefore the unselected lines used in the present experiments are a little smaller than the outbred stocks from which they were derived. The substantial differences in thorax and wing length between the contrasted lines D/N and S/E (Table 1) reflect corresponding differences in general body size, with which these dimensions are highly correlated.

Table I

Wing and thorax length of the contrasted lines.

	Male		Female	
Line	Wing	Thorax	Wing	Thorax
N	177·4	89·8	201·1	101·9
D	135·9	80·0	154·8	91·8
Percentage reduction	23·4	9·2	23·0	9·9
E	171·2	91·0	196·3	101·5
S	156·8	80·5	182·8	92·2
Percentage reduction	8·0	11·5	6·9	9·2

Average Standard Errors of mean wing length are: .56 (male), .62 (female); and of thorax length: .29 (male), .32 (female).

A partial chromosome analysis of several other inbred lines is also described; discussion of their origin and attributes will be deferred until later.

3. Design of the Experiments

(a) The chromosome combinations

If the Y and IVth chromosomes are ignored for the moment and only the combinations of the three pairs of major chromosomes are considered, then for each pair of chromosomes there are, in females, 2 alternative homozygous and 1 heterozygous combinations, i.e. 3 x 3 x 3 or a total of 27 possible combinations. In males, since there are only two alternatives for I, there are only 18 combinations. To simplify reference to so many different genotypes, a notation is used in which any genotype can be specified by 3 letters whose order corresponds to chromosome pairs I, II and III. Thus the pure lines of Nettlebed origin are designated as DDD and NNN, while other combinations can be referred to by such formulae as XXX, DNX, NDN, etc.; the X of course refers to the heterozygous combination. It must be remembered that in males, the first letter of any formula represents a single chromosome I. Numerical subscripts as in N_1, D_2, etc., indicate particular chromosomes of the origin specified by the letter.

Provided all possible combinations of pairs of homozygous chromosomes are available, then all the types with one or more heterozygous pairs of chromosomes can be created by appropriate

crosses between these 8 basic types. In the D/N comparisons for example, the basic types are: DDD, NDD, DND, DDN, DNN, NDN, NND, NNN. The genotypes with one or more heterozygous pairs of chromosomes can be created by more than one type of cross. This is particularly true of males, since there are only 2 alternatives for the X chromosome. Thus, as a specific instance, males of the type DXX can be prepared from such crosses as NNN x DDD, DNN x DDD or NDD x DNN; the male parent is always quoted first. Similarly, females of the constitution XXX can be derived from no less than 5 different crosses. This is of considerable value, since it provides an exacting test of the genetic constitution of the basic types. If the latter have the constitution attributed to them, then the mean size of theoretically identical types, produced by different crosses, should agree within the limits of sampling. Although all possible combinations of major chromosomes have been produced in these experiments, not all of the possible crosses have been carried out; however, there are enough crosses yielding the same type to provide a check on the method, and these crosses are indicated in Fig. 1.

Figure 1.

The production of Different genotypes.

Females \ Males	DDD	NDD	DND	DDN	DNN	NDN	NND	NNN
DDD	DDD	XDD D-	DXD	DDX		XDX D-	XXD D-	XXX D-
NDD		NDD	XXD N-	XDX N-	XXX N-			
DND			DND					
DDN			DXX	DDN	DXN		XXX D-	
DNN	DXX		DNX		DNN			
NDN		NDX		XDN N-		NDN		
NND		NXD	XND N-			NXX	NND	
NNN	XXX N-	NXX	XNX N-	XXN N-	XNN N-	NXN	NNX	NNN

In crosses which produce females heterozygous for the X-chromosome, the origin of the latter in males is shown by a single letter below the row which indicates the constitution of the females.

(b) The Preparation of the Basic Types

The preparation of the 6 basic types with one pair of homologous chromosomes from one strain in the presence of homozygous pairs from the other strain, presented something of a problem, since there must be no reasonable doubt as to their genetic constitution. Preliminary experiments suggested that the usual method of replacing a pair of homologous chromosomes of one strain by chromosomes from another were insufficiently rigorous for the present purpose, since it relies on the suppression of recombination in I, II and III by large inversions marked by dominants, and encounters two technical difficulties. The most suitable inversions are marked by dominant eye effects, e.g. B in ClB and M-5 (I); L^4 in Cy L^4 (II) and Me′ in the case of III. Since both B and L^4 reduce eye size and, in combination, often lead to the appearance of flies with very small eyes, it is often difficult to detect the rather subtle appearance of Me′. By doing the experiments on a large scale and testing all doubtful flies, these difficulties could be overcome, although the labour would be considerable. More serious is the fact that when several major inversions co-exist in the same female, there is a general, if sporadic, tendency for the efficiency of individual inversions in reducing crossing-over to be lowered, sometimes dramatically so. For example, in females heterozygous for Me′ Sb, recombination between Me′ and Sb occurs to the extent of about 4-5%, but when Cy is also present, the frequency rises on the average to about

15%, and it was also noted that M-5, normally such an excellent suppressor, was similarly affected by the presence of other inversions. This general phenomenon, which is familiar to all who have worked with multiple inversion stocks of Drosophila melanogaster, has been studied by Steinberg (1936) and commented on by Gowen et. al. (1946).

These difficulties have been satisfactorily by-passed by means of the crossing procedure described in Fig.2. Only autosomal inversions are used and these include two distinguishable inversion complexes for each major autosomal chromosome. Thus, for II, the inverted chromosome with inversions in left and right arms is marked by the dominant Curly wing (Cy), with or without the dominant eye effect Lobe (L^4). Similarly for III we have an inversion complex marked by Moire (Me´), with or without the dominant Stubble (Sb). The differently marked chromosomes are referred to in Fig. 2 and hereafter as Cy, CyL, Me´ and MeSb. Even in the presence of either Me´ or MeSb and Cy and CyL inversions are excellent cross-over suppressors, although recombination does occasionally occur. Me´ alone or in combination with the Cy inversions is also good, although there appears to be about 3% recombination at the extreme tip in the region of ru. MeSb is the least satisfactory for the reasons noted earlier, but its shortcomings can be largely overcome by testing flies of doubtful constitution; there remains a small proportion of double cross-overs which escape detection.

Fig. 2. The preparation of basic homozygous types from inbred lines B and C.

Back-crosses

1. $\frac{B}{Y_B}\ \frac{B}{B}\ \frac{B}{B} \times \frac{+}{+}\ \frac{Cy}{+}\ \frac{Me}{+}$

2. $\frac{+}{Y_B}\ \frac{Cy}{B}\ \frac{Me}{B} \times \frac{B}{B}\ \frac{B}{B}\ \frac{B}{B}$

3. $\frac{B}{Y_B}\ \frac{Cy}{B}\ \frac{Me}{B} \times \frac{B}{B}\ \frac{B}{B}\ \frac{B}{B}$

Similarly for Cy MéSb, CyL Mé, CyL MéSb.

I II III

Chromosome replacement

4. $\frac{B}{Y}\ \frac{Cy}{B}\ \frac{MéSb}{B} \times \frac{C}{C}\ \frac{CyL}{C}\ \frac{Mé}{C}$ $\qquad$ $\frac{C}{Y}\ \frac{CyL}{C}\ \frac{MéSb}{C} \times \frac{B}{B}\ \frac{Cy}{B}\ \frac{Mé}{B}$

5. $\frac{C}{Y}\ \frac{CyL}{B}\ \frac{Mé}{B} \times \frac{C}{C}\ \frac{Cy}{C}\ \frac{MéSb}{C}$ $\qquad$ $\frac{B}{Y}\ \frac{Cy}{C}\ \frac{MéSb}{B} \times \frac{B}{B}\ \frac{CyL}{B}\ \frac{B}{B}$ $\qquad$ $\frac{B}{Y}\ \frac{CyL}{B}\ \frac{Mé}{C} \times \frac{B}{B}\ \frac{B}{B}\ \frac{MéSb}{B}$

6. $\frac{C}{Y}\ \frac{Cy}{B}\ \frac{MeSb}{B} \times \frac{C}{C}\ \frac{Cy}{B}\ \frac{MéSb}{B}$ $\qquad$ $\frac{B}{Y}\ \frac{CyL}{C}\ \frac{B}{B} \times \frac{B}{B}\ \frac{CyL}{C}\ \frac{B}{B}$ $\qquad$ $\frac{B}{Y}\ \frac{B}{B}\ \frac{MéSb}{C} \times \frac{B}{B}\ \frac{B}{B}\ \frac{MéSb}{C}$

Pair matings; parents tested

C B B

B C B

pair matings; parents tested

B B C

As shown in Fig. 2 the four combinations of inversions marking II and III are introduced into a background of each of the lines used in these experiments, by repeated backcrossing. The first cross is made to males of the experimental lines, in order to bring in the Y chromosome from the latter. Thereafter only males carrying both markers are used to backcross to the females from the various lines. In this way it is possible to build up large numbers of suitably marked flies - an important consideration when fertility is low as in some of the lines used in the experiments.

The further steps are set out in the diagram, but one or two points are worth noting. In the replacement of I, there is a risk that recombination in Mé´Sb females will lead to confusion as to the genotype of males which are phenotypically Cy Mé´Sb, since some may be Mé´/Sb due to recombination. However, Mé´/Sb males can be identified from the segregation of their offspring in Cross 6, since Mé´ and Sb will occur separately, and, as a further check, each male is also mated separately to + females. Only progeny from the right matings are retained. In the replacement of II and III, only a single marker is used in crosses 5 and 6, a considerable advantage, while in III recombination between Mé´ and Sb is detected as already noted.

There is no evidence that the presence of the autosomal inversions used here causes an increase in the frequency of non-disjunction beyond the normal rate.

(c) The Y and IVth Chromosomes

The main assumptions likely to affect the validity of the experimental methods are that cytoplasmic or maternal effects are absent and that the effects of the Y and IVth chromosomes can be ignored. So far there is no evidence of cytoplasmic or extra-chromosomal differences between strains of different body size. This is supported by a great variety of reciprocal crosses between lines of different size and also by the earlier chromosome combination experiments which have been already noted. The possibility of Y-borne differences was tested directly. With the aid of the dominant markers Cy, Me´ and Ci^D for II, III, IV respectively, the Y chromosome of the large line was replaced by the Y from the small line in the following way:

$$\frac{D}{Y_D}\ \frac{D}{D}\ \frac{D}{D}\ \frac{D}{D} \times \frac{+}{+}\ \frac{Cy}{+}\ \frac{Me´}{+}\ \frac{Ci^D}{+}$$

$$\downarrow$$

$$\frac{+}{Y_D}\ \frac{Cy}{D}\ \frac{Me´}{D}\ \frac{Ci^D}{D} \times \frac{N}{N}\ \frac{N}{N}\ \frac{N}{N}\ \frac{N}{N}$$

$$\swarrow$$

$$\frac{N}{Y_D}\ \frac{Cy}{N}\ \frac{Me´}{N}\ \frac{Ci^D}{N} \times \frac{N}{N}\ \frac{N}{N}\ \frac{N}{N}\ \frac{N}{N}$$

$$\frac{N}{Y_D}\ \frac{N}{N}\ \frac{N}{N}\ \frac{N}{N}$$

The procedure for the S/D comparison was identical. Males carrying the Y from the small line, but otherwise genetically identical with the larger line, were compared with males of the latter. The means quoted in Table 2, as in the rest of the paper,

Table 2.

Test of Y chromosome differences.

Origin of Y	Background	Wing	Thorax
D	N	190.43 ± .59	94.95 ± .26
N		190.25 ± .59	94.98 ± .26
S	E	179.25 ± .98	93.60 ± .40
E		180.29 ± .98	93.08 ± .40

The means are in 1/100th mm.

are expressed in 1/100th mm. and are based on the measurement of 5 males from each of 5 cultures set up simultaneously in the manner described below. There is no evidence that the Y chromosome from either of the small lines differs from the Y of the corresponding larger line. It had been intended to compare also males carrying the Y from the large line in a background of chromosomes from the small line, but the tests failed and were not repeated. It has been assumed that the Y chromosome may be safely disregarded in the subsequent analysis.

The IVth chromosome has also been ignored. It had been intended to test different IV chromosomes directly, but the analysis of the combinations suggested that any such differences must be quite trivial and therefore it is unlikely that any appreciable error of interpretation is introduced by disregarding IV as well.

(d) The Accuracy of the Method

The general validity of this method of preparing the basic types can be tested in a variety of ways. Thus independent replicates of the various basic types, reared at the same time, should agree within the limits of sampling and this is found to be the case. Using as error variance the variation between repeated cultures of the same series, we find satisfactory consistency between replicated types for wing and thorax length. Table 3 shows a representative series of comparisons based on the wing length of females; the thorax lengths show equally close agreement.

Table 3.

Comparison of mean wing length and coefficient of variation of replicates of the basic types (♂♂)

DNN		NDN		NND		NDD		DND		DDN	
180.7	1.1	207.1	1.0	200.0	1.1	191.7	1.9	175.0	1.3	175.4	1.2
180.9	1.2	207.2	1.2	197.1	1.6	193.1	1.5	175.5	2.0	173.4	0.8
179.8	1.8	207.9	0.6	201.2	0.9	194.0	1.4	174.5	1.5	173.2	1.2
180.3	1.4	205.9	1.1	199.5	0.9			172.7	1.4	175.4	1.1
				199.3	1.2			172.9			

The Standard Error of the difference between Means based on pooled variation within replicates of the same type is .99.

The figures on the right in each double column refer to the coefficient of variation.

An additional check on the method is provided by the coefficient of variation of flies reared together within the same culture. This is a sensitive test, since even the occurrence of occasional flies which differ substantially from the mean, although they will have little effect on the latter, will greatly increase the variance. Since the basic types should be completely homozygous, except for the IVth chromosome, the coefficient of variation should be of the same order as that observed in the untreated pure lines. Any appreciable increase in this coefficient suggests that otherwise undetected recombination has occurred. There is actually a very striking homogeneity of the variance in the types listed in Table 3; indeed, in all the experiments described here, only one case of very high phenotypic variance has been encountered, and this was obviously due to the segregation of flies which differed considerably in size. This type and all the crosses in which it was involved, was discarded; it will be referred to again later. Since only IV should be segregating in the basic types and since the phenotypic variance of the basic types does not exceed on the average the variance of the pure lines themselves, this can be taken as evidence for the identity in effect of IVth chromosomes derived from the contrasted lines.

Finally we may compare the size of the theoretically identical heterozygous types produced by crosses between different basic types. Males have been chiefly used in this test, since the

same type is produced by relatively more different matings than in females.

The comparisons are set out for males of the D/N and S/E series in Table 4 and reveal good agreement. The few blanks in the set of S/E comparisons are due to the rejection of the types ESS and its crosses, since this is the defective type just referred to. There are a few instances in which the replicates differ significantly and presumably this represents evidence of some recombination in the course of preparing the basic types. But in general, there is excellent agreement and it appears that the genetic constitution of the various basic types used in these experiments can be relied on with some confidence.

(e) The methods of Culture

After the basic types were prepared, they were expanded quickly. Plenty of virgin females of each type were collected and mated appropriately according to the scheme outlined in Fig.1. The mated flies were well fed for several days and then transferred to oviposition bottles for the collection of eggs. Wherever possible 10 vials containing 50 eggs each were set up for each type, except when replicated in different crosses in which case 5 or 6 cultures were used. In each test the cultures were all set up on a single day or on two successive days, so that error due to environmental differences affecting cultures started on different days either did not occur or could be allowed for. The cultures were randomised within an incubator at 25±.5°C. Both wing and thorax

Table 4.

The deviation between the wing lengths of theoretically identical types produced by different matings (Male).

D/N Comparison				S/E Comparison			
Parent Mating ♂	Parent Mating ♀	Type	Deviation	Parent Mating ♂	Parent Mating ♀	Type	Deviation
NND	NND	NND	0.7 ± .84	EES	EES	EES	1.1 ± .77
DND	NND			SES	EES		
NDN	NDN	NDN	1.1 ± 1.30	ESE	ESE	ESE	1.5 ± .81
DDN	NDN			SSE	ESE		
NNN	NNN	NNN	0.5 ± .86	EEE	EEE	EEE	3.0 ± .82 xx
DNN	NNN			SEE	EEE		
NDD	NND	NXD	1.4 ± 1.25				
NND	DDD						
NND	NNN	NNX	1.0 ± 1.10	EES	EEE	EEX	0.8 ± .75
DND	NNN			SES	EEE		
DDN	NNN	NXN	0.1 ± 1.10	SSE	EEE	EXE	1.4 ± .79
NDN	NNN			ESE	EEE		
DDN	DDD	DDX	1.7 ± 1.15	SSE	SSS	SSX	0.8 ± 1.08
NDN	DDD			ESE	SSS		
DND	DDD	DXD	1.8 ± 1.10	SES	SSS	SXS	0.6 ± 1.03
NND	DDD			EES	SSS		
NDD	NDN	NDX	1.6 ± 1.13				
DDN	NDD						
DDD	DNN	DXX	S	SSS	SEE	SXX	N.S
DDN	NND			SSE	EES		
NNN	DDD			EEE	SSS		
DDD	NNN	NXX	N.S	SSS	EEE	EXX	0.4 ± .91
DNN	NDD			ESE	EES		
NDN	NND						
NDD	NNN						

Differences among the standard errors are due to variations in sample size. Where 3 or more crosses are compared, S and N.S. indicate significance and non-significance at 5% level.
xx indicates significance at 1% level.

length of 5 males and 5 females from each culture were measured directly. The general methods of handling, the culture media and the method of measurement have been already fully described (Robertson and Reeve, 1952a).

4. The statistical analysis of the data.

5 flies from each of 10 cultures were measured to provide the mean for each genotype, except that occasionally fewer flies were available, due to low fertility. The variance of the mean thus includes within-culture effects (σ^2) and between-culture effects (σ^2_c). The within-culture variance is not constant for all genotypes, since the phenotypic variability of body-size appears to decrease with increasing heterozygosity of the genotype (Robertson and Reeve, 1952b, Reeve and Robertson, 1953b), so that a standard error based on the pooled variances would be too low for the homozygotes and too high for the most heterozygous genotypes. To obtain more accurate standard errors, an average value of σ^2_c has been calculated from all the data, while σ^2 has been averaged separately for groups of genotypes with 0, 1, 2 and 3 pairs of chromosomes heterozygous. The variance of a genotype mean is then $V_m = \frac{1}{N}(\sigma^2 + n\sigma^2_c)$, where N is the total number of flies and n the number of flies per culture, and σ^2 is chosen according to the number of chromosome pairs heterozygous. Standard errors of various linear combinations of the means are then easily obtained (cf. Robertson and Reeve, 1953c).

A few minor adjustments of the raw data must be noted. In

one or two of the crosses between the basic types in the D/N analysis, a few flies appeared, which differed greatly from the average size of the rest of the flies in the crosses. Such aberrant individuals were within the same size range as the type represented by the female parents and were probably due to an occasional fly not being virgin; tests of speed of first mating show that females may occasionally mate within a few hours of emergence. These aberrant flies, which were excluded from the data, occurred in the following crosses:- NNN x DDD (1♂, 1♀), NND x DDD (1♂), NDN x DDD (1♀).

The chromosome analysis which was described in an earlier paper (Robertson and Reeve, 1953c) provided tests of how far metric bias of one sort or another might obscure the interpretation of purely genetic effects. There is no evidence that metric bias is of any importance in this respect, at any rate within the range of size studied in the present experiments. A theoretical case could be made for the transformation of the data into logarithms, but over the range studied here it would make very little difference to the interpretation and would not justify the extra labour. Accordingly all means are based on the actual measurements and are expressed in 1/100 mm.

The following account of the chromosome combinations is designed to analyse the difference between the contrasted strains and discover how far and in what way the situation departs from an additive one, especially in relation to the direction of selection in the small strains.

5. The analysis of the D and N Lines.

(a) General. These experiments represent a continuation of the analysis described in an earlier paper (Robertson and Reeve, 1953c), which dealt with only some of the possible combinations of chromosomes from contrasted strains of different size. A complete chromosome analysis should provide more critical information about some of the problems which were encountered. But the almost embarrassing array of different genotypes in the present analyses poses rather a problem of description and interpretation, since the data can be considered from various angles, according to the aspect to which we wish to draw particular attention. We are obliged, therefore, to proceed empirically. Widening experience and the exploration of a greater variety of genotypes should bring to light regularities which are at present unsuspected or but dimly perceived.

Following the procedure in the earlier paper, we can look for answers to a few clear-cut questions of general interest relating to (a) the importance of additive gene effects, (b) regularity in the direction of dominance and (c) the existence of interaction between non-homologous chromosomes.

(b) Non-additive effects. Mere inspection of the effect of crossing the D and N lines shows that we are dealing with a highly non-additive situation. This is evident from Table 5 which lists the observed size of all types in the D/N analysis, expressed as deviations from the size of NNN. Thus, in both sexes, flies of

Table 5.

Observed sizes of types in the D/N analysis (1/100 mm.)

Type	Female		Male	
	Wing	Thorax	Wing	Thorax
NXN	3.2	2.0	1.2	1.6
NNX	0.6	-0.1	-1.9	-1.2
NXX	0.0	-0.3	-1.5	-0.6
XNN	-3.7	-0.9	-	-
XXN	-3.7	0.6	-	-
XNX	-4.1	-1.6	-	-
XXX	-5.1	-1.6	-	-
DNN	-24.2	-3.1	-22.9	-3.0
DXN	-25.8	-1.5	-24.9	-2.4
DNX	-27.7	-3.8	-26.4	-4.9
DXX	-24.8	-2.6	-27.0	-3.4
NDN	-1.5	-0.2	-2.3	0.5
NDX	-4.1	-0.2	-6.3	-0.7
NND	-10.2	-5.1	-9.8	-4.2
NXD	-9.9	-4.3	-11.1	-3.9
NDD	-15.3	-5.2	-16.9	-5.3
XDN	-8.3	-1.5	-	-
XDX	-10.1	-1.3	-	-
XND	-14.0	-6.7	-	-
XXD	-15.1	-6.2	-	-
XDD	-22.3	-7.3	-	-
DDN	-33.4	-3.2	-29.1	-3.0
DDX	-27.3	-1.9	-26.0	-1.8
DND	-33.8	-8.2	-31.3	-8.1.
DXD	-26.9	-4.4	-26.3	-4.0
DDD	-46.3	-10.1	-41.7	-9.8

The observed sizes of wing and thorax length are expressed as deviation from NNN.

the type NXX are as large as NNN, suggesting the presence of dominance in the direction of larger size. The triple heterozygote, XXX, is 5.2 units shorter in wing length and 1.6 units shorter in thorax length than the NNN type; hence it appears that, unlike the N autosomes, the N first chromosome is not completely dominant to the D homologue.

(c) Aggregate dominance of chromosomes. The apparent dominance shown by the crosses may depend on summation of the effects of true dominance between alleles, or upon interaction between non-allelic genes, or both may occur. Obviously we cannot test for the presence of true dominance, but we can find out whether this effect is primarily due to the dominant behaviour of individual chromosomes. The term "aggregate dominance" has been used to refer to the dominance properties of whole chromosomes (Robertson and Reeve, 1953c), to draw attention to the probably complex origin of this phenomenon. We can test for aggregate dominance by comparing the effect of making single or double substitutions of D- chromosomes for their N homologues. Such comparisons, carried out in a background of N chromosomes, are set out in Table 6. There is a considerable tendency for the substitution of a single D chromosome to have little effect on size, compared with the double substitution. Hence it appears that the aggregate dominance of N chromosomes is primarily responsible for the observed size of the crosses between the D and N lines. It is worth noting that the dominance appears incomplete in I, as has

Table 6.

Comparison of the effects on wing length of single and double substitutes of a D chromosome for its N homologue in an N background.

Chromosome	Male		Female	
	Single	Double	Single	Double
I	-22.9	-	-3.7	-24.2
II	1.2	-2.3	3.2	-1.5
III	-1.9	-9.8	0.6	-10.2

The effects are expressed in 1/100 mm. as deviations from NNN.

been suggested previously, and that the substitution of a single D_2 chromosome actually increases size, suggesting that phenomena other than aggregate dominance may also be involved.

(d) Interactions between chromosomes. Having demonstrated substantial aggregate dominance in the direction of larger size, we must now look for the existence of interactions between non-homologous chromosomes. In particular we wish to know whether they are randomly distributed and quite unpredictable, or whether they occur primarily between chromosomes from the small, selected line and, if so, whether there is any sign of regularity. The most fruitful approach seems to be to estimate the effects of single and double substitutions of each chromosome, and the expected value of each genotype, by Least Squares, on the assumption that there is no interaction between non-homologous chromosomes. Examinations of the deviations between expected and observed values of each genotype should throw light on the pattern of interactions between chromosomes. This is more appropriate than calculating first and second order interactions between chromosomes I, II and III, by the usual factorial method, since these interactions would be the average of the individual interactions in all possible genetic backgrounds, and it is the individual interactions, rather than the averages which are likely to be of interest.

Assuming no interaction between chromosomes, the 27 genotypes in females can all be expressed as linear combinations of one or

more of 7 constants (see Robertson and Reeve, 1953c), which may be taken as:

n = size of N inbred line,

a = effect of substituting single 1st chromosome of D for N ($N_1 - X_1$),

A = effect of substituting two 1st chromosomes of D for N ($N_1 - D_1$),

b, B, c & C = single and double substitutions of II and III.

Thus we have NNN = n

NXN = n - b

DDN = n - A - B, etc.

Solving by Least Squares, giving equal weights to all genotypes, we find that the constants a, A etc. representing the substitution effects, are the means of the 9 differences representing each substitution effect, calculated in all possible genetic backgrounds. In other words

$A = \frac{1}{9}$[Sum of the 9 genotypes with homozygous N first chromosome minus the sum of the 9 genotypes with homozygous D first chromosome]

$a = \frac{1}{9}$[Sum of the 9 genotypes with homozygous N first chromosomes minus sum of the 9 genotypes with heterozygous first chromosome] etc.

Finally, n is estimated as

$$n = \frac{1}{27}[\Sigma + 9a + 9A + 9b + 9B + 9c + 9C]$$

where Σ is the sum of the observed values of all genotypes.

In males we have only 18 genotypes, and the equations are modified

as follows:

b, B, c and C are now averages of 6 differences

e.g. $b = \frac{1}{6}$[Sum of 6 genotypes with homozygous second chromosome minus sum of 6 genotypes with heterozygous second chromosome]

Also, $A = \frac{1}{9}$[Sum of 9 genotypes with N first chromosome minus sum of 9 genotypes with D first chromosome]

and $n = \frac{1}{18}[\Sigma + 9A + 6b + 6B + 6c + 6C]$

From the constants n, A, a etc. we can calculate the expected values of the various genotypes and compare these with their observed values. The differences observed - expected size are shown for wing length of both sexes in Table 7.

It is convenient to deal first with the wing length of females. As might be expected, the mean square of the deviation of all types shows a highly significant degree of interaction; this is shown in the lower section of Table 8. But when we compare the deviations between the observed and expected size of individual types in column 1 of Table 7, in which the types are arranged roughly in order of increasing number of D chromosomes, we find comparatively small deviations in the majority of types. Most of the variance of the deviations is due to major deviations of a few types, especially DDN, DDX, DXD and DDD, i.e. types with a preponderance of chromosomes from the D line.

Now if the situation were truly one in which non-interaction is the rule, then inclusion of the aberrant types in the least

Table 7.

Least Squares analysis of the D/N series: Wing Length (1/100mm.)

Deviations: Observed - Expected Size

Type	Female		Male	
	1	2	1	2
1 NNN	-1.2	-1.9^{x}	-0.2	-1.4
2 NXN	1.1	1.0	0.7	0.3
3 NNX	-0.1	0.1	-0.1	-0.5
4 NXX	-1.6	-0.8	-0.1	0.4
5 XNN	0.6	-0.2		
6 XXN	-0.3	-0.4		
7 XNX	0.7	0.8		
8 XXX	-1.4	-0.4		
9 DNN	0.5	0.9	0.1	0.8
10 DXN	-2.0^{x}	-1.0	-2.4^{xx}	-0.8
11 DNX	-2.5^{x}	-1.2	-1.6^{x}	0.1
12 DXX	-0.7	1.4	-2.6^{xx}	-0.1
13 NDN	3.1^{xx}	1.5	2.7^{xx}	1.2
14 NDX	1.0	0.3	0.5	0.0
15 NND	-0.7	0.0	0.0	1.0
16 NXD	-1.3	0.0	-1.7^{x}	0.2
17 NDD	-0.1	-0.2	-2.1^{x}	-1.2
18 XDN	1.7^{x}	0.2		
19 XDX	0.4	-0.2		
20 XND	0.9	1.6		
21 XXD	-1.1	0.3		
22 XDD	-1.6	-1.7^{x}		
23 DDN	-2.9^{xx}	-3.4^{xx}	-1.1	-0.6
24 DDX	3.7^{xx}	4.1^{xx}	3.8^{xx}	5.4^{xx}
25 DND	1.6	3.4^{xx}	1.5	4.6^{xx}
26 DXD	7.7^{xx}	10.0^{xx}	6.1^{xx}	10.0^{xx}
27 DDD	-5.2^{xx}	-4.2^{xx}	-3.9^{xx}	-1.0

Columns 1 and 2 refer to estimations based on the least squares analyses of respectively all types and types 1-22.

x Significant deviation from zero at P = 0.05:

xx Significant deviation from zero at P = 0.01.

Table 8.

Alternative least squares estimates of chromosome substitutions and their relation to the mean square of deviations in the D/N analysis.

Constants	Double Substitutions	Female				Male			
		Wing		Thorax		Wing		Thorax	
		1	2	1	2	1	2	1	2
A	I	-25.89	-26.99	-2.82	-3.25	-23.00	-25.05	-2.97	-3.53
B	II	-5.77	-4.97	-0.40	-.41	-5.00	-4.88	0.20	-0.02
C	III	-10.67	-12.11	-5.28	-5.53	-9.85	-12.22	-4.83	-5.16
	Single Substitutions								
a	I	-5.47	-5.47	-1.46	-1.46	-	-	-	-
b	II	0.91	0.26	0.75	0.38	0.45	-0.46	1.43	0.90
c	III	-0.49	-1.41	-0.13	-0.31	-1.85	-2.84	-1.03	-1.48
Mean square of deviations									
All types		7.53	8.85	1.21	1.32	8.37	13.25	1.57	1.93
Excluding types 23-27		2.64	1.30	0.78	0.63	4.35	1.09	0.55	0.20
Average error variance		0.70		0.32		0.60		0.30	

Columns 1 and 2 are based on the Least Squares analyses of respectively all types and types 1-22 only. In males the estimates of a single substitution of I is listed in the same row as the estimates of double substitutions of I in females.

squares analysis will obviously inflate the average deviation between observed and expected values in the types which really combine additively; the least squares estimates of their size will be biased, because interacting types have been included in the estimations. Accordingly the 7 constants have been re-calculated, excluding the symmetrical group of 5 types numbered 23-27 inclusive in Table 7. With this alternative least squares analysis, the average deviation between the observed and expected values in types 1-22 is substantially reduced. Table 8 shows that the Mean Square of the deviations of this group is reduced from 2.63 with the first estimations to 1.30 with the second. Column 2 of Table 7 illustrates the same point in terms of the individual deviations. Naturally the total variance of deviations is greater in the second analysis, because evaluation of the constants is not based on all the types. The reduction in average deviation of types 1-22 with the second least squares estimates supports the view that interactions are generally absent or very small. The greater deviations of types 23-27 shown in the second analysis probably gives a clearer impression of their magnitude than those shown in column 1 of Table 7.

DDN and DDD involve interactions which reduce wing length below the expected value, while, on the other hand, DDX, DXD and DND increase wing length quite strikingly. There is further evidence of atypical behaviour among these types, since DXD and DDX exceed DND and DDN respectively in wing length (Table 9),

Table 9.

Wing and thorax length of types showing major interactions in the D/N analyses (1/100 mm.).

Type	Male		Female	
	Wing	Thorax	Wing	Thorax
DND	146.3	81.7	167.3	93.7
DXD	151.3	85.8	174.2	97.5
DDN	148.5	86.8	167.7	98.7
DDX	151.6	88.0	173.8	100.0

i.e. instead of the more usual dominance of the N autosome there appears to be over-dominance. This problem will be discussed in more detail later.

Dealing now with the wing lengths of males, we find a very similar situation. The deviations based on estimates derived from the full series of types show that major interactions in the same direction generally occur in the same types as in females. Recalculation of the constants, excluding the last five types, leads to a striking reduction in the Mean Variance of deviations of the other 13 types from 4.32 to 1.07 (Table 8). A contrast with the situation in females appears in the type DDN, which, in males, shows only a minor deviation and also in the type DDD which appears to involve a very much smaller deviation than in females. It will be remembered from Table 5 that the absolute deviation of wing length of the DDD type is 4.8 units greater in females than males and it is possible that this difference may be partly or completely due to this sex-limited interaction.

The analysis of thorax length may be carried out in the same fashion. In both sexes the degree of interaction with respect to this dimension is relatively less than in wing length (Table 8), but comparison of Tables 7 and 10 shows a largely parallel behaviour between wing and thorax length in the direction of their deviations. The corresponding group of five types is also responsible for most of the variance due to deviations and, when

Table 10

Least Squares analyses of the D/N series: Thorax length (1/110mm.)

Deviations: Observed - Expected Size

Type	Female		Male	
	1	2	1	2
1 NNN	-0.20	-0.47	0.11	-0.41
2 NXN	1.05	1.15^{x}	0.28	0.29
3 NNX	-0.17	-0.26	0.04	-0.03
4 NXX	-1.12	-0.84	-0.89	-0.43
5 XNN	0.36	0.09		
6 XXN	1.99^{xx}	1.90^{xx}		
7 XNX	-1.09	-0.99		
8 XXX	-1.23^{x}	-0.68		
9 DNN	-0.48	-0.32	0.08	0.12
10 DXN	0.37	0.90	-0.75	-0.18
11 DNX	-1.05^{x}	-0.71	-0.79	-0.31
12 DXX	-0.60	0.11	-0.72	0.30
13 NDN	0.00	0.26	0.41	0.11
14 NDX	0.13	0.05	0.24	0.39
15 NND	-0.02	-0.04	0.74	0.55
16 NXD	0.03	0.38	-0.39	0.05
17 NDD	0.28	0.27	-0.56	-0.53
18 XDN	0.16	-0.10		
19 XDX	0.49	0.41		
20 XND	-0.16	-0.18		
21 XXD	-0.41	-0.06		
22 XDD	-0.36	-0.37		
23 DDN	-0.18	-0.01	-0.12	0.14
24 DDX	1.25^{x}	1.60^{x}	2.11^{xx}	2.82^{xx}
25 DND	-0.30	0.11	-0.19	0.18
26 DXD	2.75^{xx}	3.53^{xx}	2.48^{xx}	3.38^{xx}
27 DDD	-1.80^{x}	-1.38^{x}	-2.09^{xx}	-1.50^{x}

Columns 1 and 2 refer to estimations based on the least squares analyses of respectively all types and types 1-22.

x Significant deviation from zero at P = 0.05

xx " " " " " P = 0.01

they are excluded from the least squares estimates, Table 8 shows there is an appreciable reduction in the variance of deviations of the other types.

(e) Comparison of the effects of the substitutions.

Table 8 summarises the least squares estimates of the constants A, B, C, a, b and c, i.e. estimates of the effect of replacing single or pairs of N chromosomes by their D homologues. The estimates based on the analysis of all types and numbers 1-22 only are listed in columns headed 1 and 2 respectively. For the reasons just discussed, estimates of the substitutions from the latter analysis probably provide a better basis for discussing the effects of the substitutions. There is excellent agreement between the sexes in the estimates of the effect of corresponding substitutions on both wing and thorax length. In wing length, the X-chromosome is chiefly responsible for the total difference between the D and N lines. D_3 ranks next in effect, a double substitution reducing wing length by appreciably 12 units, while a double substitution of D_2 causes a reduction of 5 units. Comparison of the estimates of single substitutions with those of the corresponding double substitutions, demonstrates the striking tendency to aggregate dominance of the N-chromosomes. One interesting feature is that the single substitution of a D X-chromosome in males is about as fully effective in reducing wing length as the corresponding double substitution in females, suggesting a high degree of dosage compensation. However, as noted in Table 5,

the absolute reduction of wing length of the D below that of the N line is 4.7 units greater in females than males. The deviations between observed and expected values in Table 7 have suggested the existence in females of the type DDD of an interaction which reduces wing length by 4.2 units, while in males the corresponding deviation is insignificant; and this may account for the difference between the sexes in their absolute reduction of wing length.

The relative changes in wing and thorax, produced by selection in the different chromosomes, have a bearing on the genetic correlation between the two dimensions. If size is changed while body proportions remain constant, wing length changes at about twice the rate of thorax length. Actually, the difference in length between the N and D lines is about 4 times as great for wing as for thorax, so that selection for short wings has caused a relatively greater reduction in wing length than in thorax length of the D strain. This is what we should expect from the fact that the two dimensions have a genetic correlation less than unity (Reeve and Robertson, 1953a), but we may carry the analysis further by comparing the relative changes caused by selection in the different chromosomes, as judged by the estimates of their effects given in Table 8. The ratio of wing to thorax length is about 2:1 for chromosome III, so that here selection must have picked out genes mainly affecting general body size. Genes affecting wing length only seem to have been selected in chromosome

II, and genes mainly effecting wing length in chromosome I. It will be noted that the greatest contribution to thorax length comes in chromosome III, although the main wing length difference is due to sex-linked effects.

(f) Comparison with earlier chromosome analysis.

The selected short wing strain from which the inbred D line was descended, and a different inbred line (taken from the same Nettlebed stock), were used in an earlier chromosome analysis of a different sort (Robertson and Reeve, 1953a), the earlier experiments may be compared with the present ones to see how far they show similar features. The procedure in the earlier experiments was as follows: a crossing system was used to produce cultures segregating for chromosomes marked by the dominants Pm and H, in which the genotypes otherwise consisted of chromosomes from one or other strain alone or were heterozygous for chromosomes of the two strains. By finding the difference in size between appropriate types, which were identical but for a single substitution, it was possible to estimate the effect of substituting an N chromosome in place of its D homologue for each pair of chromosomes. But, except in the X chromosome substitution in males, either the foreign Pm or H chromosomes or both were present. Hence the individual effects were estimated against a background with one or two pairs of heterozygous chromosomes. The variety of comparisons which could be made are set out in column A in Table 11 while column B shows the corresponding estimates in the present experiments, using comparisons which are as similar as possible to the others,

Table 11

Comparison of substitution effect on wing length in different chromosome analysis experiments. (1/100 mm.)

	Male						Female					
	A				B		A			B		
I	$\frac{N\text{-}D}{Y}$ $\frac{N}{D}$ $\frac{N}{D}$	19.6		$\frac{N\text{-}D}{Y}$ $\frac{N}{D}$ $\frac{N}{D}$	25.5		(a) $\frac{N\text{-}D}{D}$ $\frac{D}{P}$ $\frac{D}{H}$	21.6		$\frac{N\text{-}D}{D}$ $\frac{D}{N}$ $\frac{D}{N}$	19.7	
							(b) $\frac{N\text{-}D}{N}$ $\frac{N}{P}$ $\frac{N}{H}$	7.0		$\frac{N\text{-}D}{N}$ $\frac{D}{N}$ $\frac{D}{N}$	5.1	
II (a)	$\frac{D}{Y}$ $\frac{N\text{-}D}{D}$ $\frac{D}{H}$	4.1		$\frac{D}{Y}$ $\frac{N\text{-}D}{D}$ $\frac{D}{N}$	-1.0		$\frac{N}{D}$ $\frac{N\text{-}D}{P}$ $\frac{N}{D}$	-2.0		$\frac{N}{D}$ $\frac{N\text{-}D}{N}$ $\frac{N}{D}$	1.0	
(b)	$\frac{N}{Y}$ $\frac{N\text{-}D}{N}$ $\frac{N}{H}$	0.5		$\frac{N}{Y}$ $\frac{N\text{-}D}{N}$ $\frac{D}{N}$	-0.4							
III.(a)	$\frac{D}{Y}$ $\frac{D}{P}$ $\frac{N\text{-}D}{D}$	7.3		$\frac{D}{Y}$ $\frac{D}{N}$ $\frac{N\text{-}D}{D}$	-3.1		$\frac{N}{P}$ $\frac{N}{P}$ $\frac{N\text{-}D}{H}$	3.2		$\frac{N}{D}$ $\frac{N}{P}$ $\frac{N\text{-}D}{N}$	1.6	
(b)	$\frac{N}{Y}$ $\frac{N}{P}$ $\frac{N\text{-}D}{N}$	4.3		$\frac{N}{Y}$ $\frac{D}{N}$ $\frac{N\text{-}D}{N}$	2.7							

A and B refer to the earlier and the present experiments respectively.
a and b indicate substitutions in the presence of a D or N homologous chromoseme.

in terms of the presence of heterozygous pairs of chromosomes. It will be noted, in the earlier experiments that two estimates are available for I in females and II and III in males, according to whether the genetic background consists only of D or N chromosomes apart from the presence of one or other of the foreign chromosomes. Dominance is indicated by the excess of the (a) estimate over the (b), if no interaction between non-homologous chromosomes is present.

Allowing for the probable existence of genetic differences between the two inbred lines and also between the selected strain (which was not so highly inbred) and the inbred line derived from it, there is nevertheless considerable agreement between corresponding estimates, as shown in Table 11. Thus in the wing length of females, the effects of substituting a single N_1 in the presence of either a D_1 or N_1 chromosome agree quite well with the estimates from the later experiments, including the evidence for incomplete dominance of N_1. The parallel estimates for N_2 and N_3 substitutions also show fair agreement in the two experiments. In males the position is a little different. Thus the estimate of the N_1 substitution is less in the earlier experiment, and this may be attributable to the genetic differences already referred to. In the case of the second chromosome substitution, the difference between the (a) and (b) estimates is consistent with the assumption that the types which carry the H chromosome do not involve interactions, since the (a) estimate is

close to the estimate shown in Table 11 while the (b) estimate does not differ significantly from zero and indicates complete dominance of N_2. In the substitution of N_3, the (a) and (b) estimates do not differ greatly in the earlier experiments and dominance appears to be incomplete. Thus, as far as the analysis of the Nettlebed short wing strain is concerned, the earlier analysis, which involved only a fraction of the available genotypes, nevertheless provides a fairly satisfactory picture of the distribution of major effects, dominance and the presence of unpredictable interactions.

6. The Analysis of the S and E Lines.

The small S and the unselected E lines, which are derived from the Edinburgh stock, differ in thorax length by 10.5 and 9.3 units in males and females respectively (Table 1). This is quite close to the differences in thorax length between the N and D lines but the corresponding differences for wing length are 14.4 and 13.5 units. Although it is not surprising that the S and E lines should differ less in wing length than the D and N lines, it is interesting that they depart from the 2:1 ratio in the opposite direction.

It has been noted earlier that the basic type ESS had a very high variance and was obviously heterozygous; hence this type and the three crosses in which it was involved have been rejected from the data. This accounts for the gaps in Table 12, which lists the observed size of the type, since ESS, ESX, EXS and XSS are not

available for comparison.

The first striking resemblance to the D/N comparisons is evident in the apparent dominance in the direction of larger size. Table 12 shows that both wing and thorax length of EXX in both sexes and of XXX in females are almost identical with the size of EEE. There is no evidence here of any difference in behaviour between the X chromosome and the autosomes. The departure from an additive system of gene combination could hardly be more complete.

Using the least squares procedure, adjusted for the absence of the types ESS, ESX, EXS and XSS, we here find little or no evidence of interactions between non-homologous chromosomes. The mean square of the deviation between observed and expected values does not significantly exceed the error mean square (Table 13), for either dimension in either sex.

The least squares estimates of the effect of making double and single substitutions of S chromosomes are also set out in Table 13. In both sexes the X chromosome of the S and E lines appear to be indistinguishable. There is a different distribution of the effect of double substitutions of the S autosomes in the sexes. Although in males and females, S_3 produces a greater reduction in size than S_2, the effect is greater in females, while in the double substitution of S_2, wing length is only slightly though significantly reduced and thorax length not at all in females, while in males, on the other hand, there is a striking

Table 12

Observed size of types with S/E analysis (1/100 mm.)

	Female		Male	
	Wing	Thorax	Wing	Thorax
EXE	0.9	1.8	0.2	0.5
EEX	-2.1	-2.0	0.0	-1.4
EXX	1.1	0.8	-1.5	-0.6
XEE	1.1	0.8	-	-
XEX	-0.5	-1.1	-	-
XXE	2.2	2.0	-	-
XXX	0.3	0.6	-	-
SEE	1.1	0.6	2.4	-0.7
SXE	1.7	2.2	-0.4	0.3
SEX	-1.1	-1.2	0.8	-1.5
SXX	1.7	1.2	-2.0	-1.2
ESE	-0.4	0.6	-5.3	-2.1
EES	-12.7	-9.6	-7.9	-8.4
XSE	-0.3	0.9	-	-
XSX	-2.6	-0.9	-	-
XES	-12.8	-9.2	-	-
XXS	-13.8	-7.4	-	-
SSE	-3.4	-0.5	-6.0	-2.5
SSX	-4.3	-1.6	-7.0	-3.4
SES	-11.8	-9.0	-9.4	-7.8
SXS	-9.8	-7.2	-11.6	-8.4
SSS	-13.5	-9.3	-14.4	-10.5

The observed size of wing and thorax is expressed as a deviation from the type EEE.

Table 13

Least squares estimates of the effect of substituting S chromosomes.

Substitution	Wing		Thorax	
	Male	Female	Male	Female
Double				
I	-0.08	0.27	0.28	0.40
II	-6.74	-1.94	-2.02	0.01
III	-10.01	-12.76	-8.09	-9.75
Single				
I	-	0.37	-	0.54
II	-1.96	1.57	0.45	1.96
III	-1.18	-1.51	-1.14	-1.58
Mean square deviations	0.87	0.10	1.03	0.16
Error variance	0.60	0.30	0.70	0.32

reduction in wing and a significant reduction of thorax length. A further minor difference between the sexes is that the substitution of a single S chromosome, slightly reduces wing length in males and increases it in females, i.e. the same chromosome shows incomplete dominance in one sex and over-dominance in the other. Thus, although within each sex interactions between chromosomes are absent, there is nevertheless evidence of chromosome interaction controlled by the different chromosome constitution of males and females. Phenomena of this sort have been encountered in the earlier chromosome analysis (Robertson and Reeve, 1953♦), while the apparent wing reducing interaction in females of the pure D type provides a further example of sex differences in chromosome action. Effects of this kind raise interesting problems related to the genetic control of the sex difference in size, and merit further attention.

Finally, instead of the 2:1 ratio in the effects of the substitutions on wing and thorax length, expected if body proportions remain constant, the substitutions show a relatively much greater effect on thorax than on wing length, even though wing length was the dimension selected.

7. General features of the D/N and S/E analysis.

The generally recessive behaviour of chromosomes from the small lines is the most striking feature revealed by the foregoing analysis. Since the Nettlebed and Edinburgh stocks, from which the two small lines are descended, are quite unrelated, the

parallel phenomena shown by the D/N and S/E analyses suggest that the apparent dominance relations between chromosomes from the unselected and small lines illustrate a general feature rather than a coincidence. It is unlikely that the more or less recessive behaviour of chromosomes from a small line is a peculiar feature attributable to the use of an inbred, unselected line in the comparisons. The small S line has also been crossed to the mass mating Edinburgh stock and the mean of the F_1 was very close to that of the unselected.

It is interesting to consider the implications of these analyses for a general understanding of the inheritance of size in Drosophila. The usually recessive behaviour of chromosomes from the small lines, together with the comparative scarcity of interactions between non-homologous chromosomes, might suggest that we are dealing largely with dominant and recessive alleles which tend to combine additively. The genetic variation in the original population, inbreeding decline and the heterosis which usually occurs when inbred lines are crossed, could be formally accounted for in such terms. However, earlier experiments (Robertson and Reeve, 1953a), have suggested that the heterosis in crosses between inbred lines cannot be explained as simply due to summation of the effects of dominance, and this throws doubt on the general validity of the first simple deduction from the D/N and S/E analysis. In order to secure more information on this point, chromosomes from a number of inbred lines have been combined in various ways, and these experiments will now be described.

8. Chromosome Analysis of Unrelated Inbred Lines.

(a) The effects of single and joint substitutions.

The lines used have been called A, B and C and were derived by more than 100 generations of brother-sister mating from the following wild stocks respectively: Nettlebed, Edinburgh and Oregon K. Chromosomes were combined from the pairs A/B and B/C and only females were studied since the chief interest lay in the effect of different combinations on egg production. Altogether 21 out of the possible 27 types were studied; these comprise the 8 basic homozygous combinations, all possible types with one or two heterozygous pairs accompanied by chromosomes from one or other of the lines, together with the fully heterozygous types. The experiments met the usual tests for homogeneity and the general procedure was identical with that used in the other comparisons. The different combinations were prepared by Mr. B. K. Sen, as part of a general study of the inheritance of egg production - in preparation for publication - and the flies were also available for the measurement of wing and thorax by our assistants.

The A/B and B/C tests were carried out at different times, but the observed sizes of the pure B type, common to both tests, are almost identical. Hence temperature conditions and the environment generally must have been very similar in the two tests. The lines B and C have about the same wing and thorax length, while A has a larger body size since it exceeds them in both

dimensions, especially in wing length.

The F.1 produced by crossing the inbred lines shows substantial heterosis, and is larger than either parent (Table 14). Following the same sort of approach as in the earlier analyses, we can see whether this heterosis can be interpreted as the sum of the separate effects of making each pair of chromosomes heterozygous. However, we do not have to look far to find difficulties in the way of such a simple interpretation.

We can find the effect of making each chromosome pair heterozygous in two different homozygous backgrounds. For example in a B background, we can find the differences between BBB and respectively XBB, BXB and BBX; similarly in an A background we compare AAA with XAA, AXA and AAX. By adding the appropriate separate effects to AAA or BBB, according to the type of substitution, we should get values for double and single heterozygotes of the sort XXA, XBX or XXX, which closely correspond with the observed values. Table 15, however, shows that this is far from being so. Whatever the background in which the original substitutions are made, there are striking deviations between the observed size and the sum of the separate effects. Thus in the A/B analysis, the sum of the separate effects in a B background exceeds the observed size of XXX by 30.5 units and the corresponding deviation in the B/C analysis is about as great. On the other hand, it is particularly interesting that the sum of the separate effects falls short when the latter are derived from substitutions

Table 14

Heterosis in crosses between unselected inbred lines.

Dimensions in 1/100 mm.

Line	Wing	Thorax
A	208.6	104.5
B	196.0	101.4
C	192.0	102.2

Cross	F.1 - Mid-parent	
A x B	10.3 ± .85	4.1 ± .51
B x C	12.0 ± 1.45	5.5 ± .64

Table 15

Deviation between the sum of separate substitution effects on wing length and joint effects.

	A/B Comparisons	
Joint Effects	A Background	B Background
I + II	-8.1	-25.2
I + III	-5.7	-17.5
II + III	-3.5	-12.8
I + II + III	-8.9	-30.5

	B/C Comparisons	
	C Background	B Background
I + II	10.9	-22.7
I + III	7.6	-21.2
II + III	13.0	-11.1
I + II + III	14.0	-27.2

The values are obtained by subtracting the sum of the appropriate separate effects from the observed size of double or triple heterozygotes. All the deviations are highly significant.

in the pure CCC type. Thus in A and B background substitutions the sum of separate effects exceeds the double and triple heterozygotes, while in the C background substitutions the reverse is true.

Further light can be thrown on the sort of interactions which occur here by comparing the size of the fully heterozygous type with types in which only one or two pairs of chromosomes are heterozygous. For example, in the A/B series we can compare XAA or BXB with XXX by finding the differences: (XXX-XAA) or (XXX-BXB). These differences are set out in Table 16. A negative sign before the figures means that the single or double heterozygote is larger than the fully heterozygous type. This table reveals a very remarkable fact, namely that, in A or B backgrounds, almost any individual substitution increases size up to the level of the fully heterozygous type, and in some cases, e.g. XBB in the B/C series, actually exceeds it appreciably. In other words, the presence of a single heterozygous pair of chromosomes overcomes the decline in size due to inbreeding in the pure type and restores size to about the normal outbred level. A double substitution, i.e. the presence of two heterozygous pairs, may lead to no further increase or may actually decrease size below the level of the single heterozygote. Substitutions in a background of C chromosomes behave differently. The individual substitutions, except in II, produce little or no increase in size but increase in the number of heterozygous pairs leads to an increase which is

Table 16

Deviation between the full heterozygote and the single and double heterozygotes (1/100 mm.)

	A/B		B/C	
Heterozygotes	A Background	B Background	B Background	C Background
I	-0.9	1.6	-6.0	13.8
II	0.4	-0.1	1.0	7.1
III	0.4	1.2	-2.8	11.8
I + II	3.6	10.1	8.0	3.1
I + III	0.4	3.7	2.7	6.4
II + III	-0.5	-0.7	-0.4	1.0

The values quoted are obtained by subtracting from the size of the XXX type, the size of single or double heterozygotes, e.g. XXX-AXA. A negative sign indicates that the latter is greater than the full heterozygote.

greater than the sum of the separate effects. Thus the substitutions in the A and B backgrounds, on the one hand, and the C background, on the other, behave in opposite ways.

Although the inter-chromosome interactions are very striking, it is worth seeing whether there is any trace of regularity in the effect of the different chromosomes, as suggested in the report by Straus (1942), who found evidence of a correlation between the length of the different chromosomes and their individual effect on rate of egg production. Thus we can find the difference between heterozygous and homozygous combinations in fully heterozygous and fully homozygous backgrounds, i.e. we can compare differences of the sort (XAA-AAA) and (XXX-AXX). The average value for all such differences from the A/B and B/C analysis are set out in Table 17 which reveals quite a contrast in the two backgrounds. Thus in heterozygous backgrounds there is no reduction in size when I is made homozygous, whereas size is definitely reduced when II and specially III is made homozygous. In homozygous background, however, the average effect turns out to be the same for all chromosome pairs. In the heterozygous backgrounds, therefore, there is a suggestion of a relationship between probable total genetic activity of a chromosome and its effect, but none in the homozygous backgrounds; however further comparisons are needed before we can be certain of these points.

(b) The substitution of homozygous pairs of chromosomes.

Although the foregoing discussion has referred to interactions

associated with heterozygotes, interaction is also frequent among different homozygous combinations. The substitution of the same pair of homozygous chromosomes in different backgrounds produces different results, and Table 18 shows that the same substitution may sometimes increase and sometimes decrease size. Thus in the case of I and III, the effect of substituting a pair of A chromosomes for a pair of B homologues may be strongly positive or negative according to the background, while in the case of II there may be an increase in size or no effect at all. Similarly in the B/C substitutions the genetic background greatly influences the effect of a substitution and this is particularly striking in the substitution of II. It is also worth noting that some of the combinations of homozygous pairs, e.g. CBB and BBC are as large as the cross between the two parent lines.

Table 17

The relative effects of different chromosomes (female wing length 1/100 mm.).

Chromosome Pair	Background	
	Homozygous	Heterozygous
I	11.9	-0.3
II	11.9	-4.6
III	11.5	-5.6

The left-hand column shows the average difference between single heterozygotes and fully homozygous types, while the right-hand column shows the average reduction in wing length caused by making I, II or III homozygous in a fully heterozygous background.

Table 18

The effect of substituting homozygous pairs of chromosomes in different homozygous backgrounds (1/100 mm.).

A/B Comparisons

Chromosome Pair	Substitution	Wing	Thorax
I	(A-B)BB	11.0	5.7
	(A-B)AB	-6.9	-3.9
	BAB-BBB	10.2	4.4
II	BAA-BBA	-0.5	-0.8
	AAA-ABA	4.0	2.7
III	BB(A-B)	12.9	4.4
	AB(A-B)	-2.4	-4.8
	Standard error	.98	.60

B/C Comparisons

I	(B-C)CC	4.7	0.6
	(B-C)BC	6.3	2.8
	CBC-CCC	6.0	0.2
II	CBB-CCB	25.9	8.2
	BBB-BCB	-1.2	-2.6
III	CC(B-C)	-7.9	-3.5
	BC(B-C)	0.8	1.7
	Standard error	1.34	.72

9. Discussion

The analysis of the D/N and S/E lines, on the one hand, and the different, unselected inbred lines, on the other, provide a number of contrasts. Thus in the former the chromosomes of the N and E lines show a high level of dominance over their D or S homologues. There is widespread additive combination of the effects of non-homologous chromosomes, although interactions do occur, especially when most of the chromosomes come from the D line. Thus the size of the F_1 of the cross between the small and unselected lines could be largely interpreted in terms of chromosome dominance in the direction of larger size.

But in the analysis of the crosses between the unselected lines, which show heterosis, interactions are very striking and there is little evidence of additive combination. The substitution of a single chromosome from another line in the otherwise homozygous background, may increase size up to the level of the cross between the lines, or, in another case, the sum of the effects of such individual substitutions may fall short of the observed size of the cross. These contrasts appear to suggest rather different interpretations of the genetic control of body size, and our understanding of the inheritance of size would be a good deal further advanced if such divergences could be reconciled. It is possible, of course, that the contrasts may not be so important as they first seem, since the behaviour of the combinations of chromosomes from inbred lines rests on

comparatively few comparisons, and fortuitous choice of the lines may give an exaggerated impression of the differences in behaviour between the two groups. Experiments are in progress to test this. Perhaps more important is the regularity with which the F_1 of all crosses departs strikingly from the mid-parent level in the direction of more normal size. In the crosses between small and unselected lines, the F_1 closely resembles the latter, while in the other crosses it exceeds either parent.

Dealing first with the unselected lines, the interactions suggest that the heterosis shown by the crosses between the lines, cannot be accounted for merely as a summation of the independent dominance or over-dominance effects of particular chromosomes. This agrees with the conclusion derived from the earlier chromosome analysis (Robertson and Reeve, 1953c). Particular substitutions may have no effect or they may increase or decrease size according to the genetic background and some of these naturally resemble the effects of dominance or over-dominance. Since whole chromosomes can behave in this way, presumably the effects of individual genes may also be indistinguishable from those of their alleles, or there may be dominance or over-dominance or some degree of intermediate expression. This raises the question as to how far it is useful to think in terms of the classic antithesis between dominance or over-dominance as the cause of heterosis in crosses between inbred lines,

since to do so is to assume that the dominance or over-dominance relations between alleles is stable or sufficiently stable over the range of genotypes which are involved in the comparisons. The less this is so, the greater the interest attaching to the genotype as a whole rather than the role of independent genes. The present experiments clearly do not support an interpretation based on independent effects.

Inbreeding wild stocks leads to a variable decline in size, and when such lines are crossed the F_1 tends to fall within the normal range of size of non-inbred strains. In so far as the heterosis shown by crosses between inbred lines is to be interpreted in terms of gene interaction, it seems likely that the decline due to inbreeding must rely on a similar interpretation.

It appears that striking heterosis may be associated with less than the maximum degree of heterozygosity. Thus the presence of a single heterozygous pair of chromosomes in the background of the unselected line B, increases size up to or beyond the level of the fully heterozygous type. On the other hand, substitutions in a C background show maximum heterosis with maximum heterozygosity. Also homozygous combinations of chromosomes from two lines may lead to as great a size as that of the cross between them. Doubtless a great variety of gene arrays can lead to the same result. Whether or not a particular set of chromosome combinations show interaction, may depend, to some extent, on the more or less chance distribution of genes on

different chromosomes, i.e. whether the interactions are between linked or unlinked genes. It might be thought that such interactions are a peculiar feature of combinations of chromosomes from unrelated lines. This seems unlikely however, since other experiments, in which the parent lines are from the same stock, show the same sort of phenomena.

The most striking feature of the D/N and S/E analysis is the dominance of chromosomes from the unselected lines. This is probably a general feature since crosses between different unselected and small strains also show dominance in the direction of the larger parent; these will be described elsewhere. This situation is particularly interesting since it demonstrates regularity in the changes produced by parallel selection in different wild populations. At first sight it might appear that selection for small size involves the selection of recessive genes, which combine in a largely additive fashion. This may be in part true, but it seems unlikely that this is the whole story. The clear cut interactions which appear in the D/N combinations, the extensive interaction in the unselected line analysis, the earlier experiments (Robertson and Reeve, 1953c), together with the probability that the study of whole chromosomes underestimates the importance of gene interactions, cast doubt on any explanation which relies entirely on purely independent effects. It seems likely therefore that gene interaction has played a part in the selection for small size.

Table 19

Type	Deviation from DDD (Wing length)
DNN	22.1
DXX	21.5
DXD	19.4
DDX	19.0

A possible clue is provided by comparing the sizes of the types, responsible for major interactions i.e. DXD, DDX and DXX, with that of DNN. Table 19 shows that all four types, though genetically different, have approximately the same wing length. Heterozygosity of either pair of autosomes in an otherwise D background is almost as effective as when all autosomes are replaced by N chromosomes. This situation recalls the effects of single substitutions in a B background, which increase size up to the level of the fully heterozygous type, and formally resembles the effects of dominance not confined to single loci but extending over a number.

Inbreeding reduces body size, hence selection for small size is likely to create a bias in favour of homozygous combinations, especially those which particularly reduce size. Unpublished experiments in which several different stocks were mass selected for small size, have demonstrated a steady response to selection which ceased when the selected strains became apparently homozygous with respect to size, so there is good evidence that selection for small size involves a progressive trend to homozygosity. Possibly as selection proceeds, remaining heterozygous combinations which increase size, are thrown into greater relief thereby making their elimination easier. Much of the variability, revealed by selection for small size, was probably concealed in the wild stocks, either by dominance or epistasis, possibly of the type indicated in Table 19, while the positive deviation from mid-parent value

for the F_1 of all crosses implies at least a partial return to the original conditions.

The wild stocks, from which the different lines are derived by selection or inbreeding, are highly heterozygous and appear to be phenotypically stable with respect to size. Selection in either direction leads to an immediate response, while progeny tests yield high estimates of heritability (40-50%), suggesting considerable consistency in the expression of gene differences. Selection and inbreeding alter the genetic situation and expose an underlying assymetry in the genetic control of size, which is probably least evident in the normal wild stock. Mather (1943) has drawn particular attention to the adaptive stability of wild populations in the presence of a high level of genetic variability and has proposed a solution in terms of more or less elementary, largely additive, polygenes - a view which has been criticised elsewhere (Robertson and Reeve, 1952a). Further progress in this field would appear to hinge on greater understanding of the properties of genes and gene complexes which influence the development of different characters. Genetic analysis of the effects of selection may bring to light regularities, as in the behaviour of the small lines in the present experiments. As further experimental data becomes available it may be possible to discuss the situation prevailing in wild populations in more realistic terms than at present.

10. Summary.

1) A crossing method is described for creating all possible combinations of major chromosomes from pairs of inbred lines of Drosophila melanogaster. The twenty-seven different genotypes in females, eighteen in males, provide the basis for different tests which throw light on the genetic control of body size.

2) Complete chromosome analyses have been carried out on two pairs of contrasted lines of different size, descended from the Nettlebed and Edinburgh wild stocks. Each such pair comprises a small line, descended from a strain selected for small body size, and an approximately normal-sized line, inbred without selection from the same stock. Three unrelated lines inbred without selection have been studied in a similar way, except that twenty-one out of the twenty-seven possible combinations for each pair have been studied in females only.

3) The accuracy of the method of combining chromosomes was demonstrated by the agreement between preparations of the same genotype by different means, and also by the level of the within-culture variance, which was generally of the same order as that for untreated inbred lines.

4) The within-culture variance is not constant for all genotypes, but tends to decline with an increase in the number of heterozygous pairs of chromosomes.

5) When the unselected and small lines are crossed, a highly

non-additive situation is revealed by the size of the F_1 which may be as great, or nearly as great, as the size of the unselected parent line. This effect is primarily due to the aggregate dominance of chromosomes from the larger line.

6) In the analysis of the unselected and small Edinburgh lines the size of the different types could be accounted for by aggregate dominance of the chromosomes of the larger line.

7) In the Nettlebed combinations, aggregate dominance and additive combination of non-homologous chromosomes account for the size of the majority of the types. But there are also a number of striking interactions which increase or decrease size, leading to different effects of particular substitutions and different dominance relations in different genetic backgrounds. Most of the larger interactions occur in genotypes carrying several chromosomes from the small line. The behaviour of the X-chromosome of the small line is exceptional in being incompletely recessive in all backgrounds.

8) In the combination of chromosomes from the unrelated, unselected, inbred lines, interactions between non-homologous chromosomes are much more frequent and striking. The substitution of a single chromosome or of a homozygous pair may increase or decrease size, according to the genetic background.

9) Inter-crossing these unrelated inbred lines always leads to heterosis in the F_1, which exceeds both parent lines in size. This heterosis cannot be accounted for merely in terms of the

summation of the effects of dominance or over-dominance on different chromosomes, but must be considered in terms of gene interaction. The effects of making each pair of chromosomes heterozygous in otherwise homozygous backgrounds may be compared with the joint effects of making two or more pairs heterozygous. In several cases, the presence of a single pair of heterozygous chromosomes may lead to a body size quite as large as in the fully heterozygous type, and actually exceeding the size of types with two heterozygous pairs. But, in one series, on the other hand, increase in the number of heterozygous pairs of chromosomes increases size more than the sum of the individual effects.

10) The results are discussed in relation to the mechanism of heterosis, inbreeding decline and possible ways in which selection has changed the genotype to produce small size.

Acknowledgments.

I wish to thank Miss Brenda M. Green and Miss Phyllis Cameron for valuable technical assistance and also Mr. C. M. Marr for carrying out most of the basic computation.

References.

Gowen, J. W., Stadler, J. & Johnson, L. E. (1946). On the mechanism of heterosis - the chromosomal or cytoplasmic basis for heterosis in Drosophila melanogaster. Amer. Nat. 80: 506.

Mather, K. (1943). Polygenic inheritance and natural selection. Biol. Rev. 18, 32.

Robertson, F. W. & Reeve, E. C. R. (1952a). Studies in quantitative inheritance. I. The effects of selection of wing and thorax length in Drosophila melanogaster. J. Genet. 50: 414.

_____ (1952b). Heterozygosity, environmental variation and heterosis. Nature 170: 296.

Reeve, E. C. R. & Robertson, F. W. (1953a). Studies in quantitative inheritance. II. The analysis of a strain of Drosophila melanogaster selected for long wings. J. Genet. 51L 276.

_____ (1953b). The analysis of environmental variability in quantitative inheritance. Nature, 171: 874.

Robertson, F. W. & Reeve, E. C. R. (1953c). Studies in quantitative inheritance. IV. The effects of substituting chromosomes from selected strains in different genetic backgrounds. J. Genet. 51: 586.

Steinberg, A. G. (1936). The effect of autosomal inversions on crossing-over in Drosophila melanogaster. Genetics, 21: 615.

Straus, F. S. (1942). The genetic mechanism of heterosis as demonstrated by egg production in Drosophila melanogaster. Ph.D. Thesis, Iowa State College Library, Ames, Iowa.

Studies in Quantitative Inheritance

VII. Crosses between strains of different body size in *Drosophila melanogaster*.

by

Forbes W. Robertson[x] and E. C. R. Reeve[x]

---

[x] Members of the Agricultural Research Council Scientific Staff.

Contents.

1. Introduction

In the course of a general study of the inheritance of body size in Drosophila melanogaster, by means of selection and chromosome analysis, many crosses have been made between strains and inbred lines of similar and also widely differing body size. In some cases these crosses were more or less incidental to other aspects of the work and this limits the variety of available comparisons between different sorts of cross. However, it is of some interest to bring these data together, since the size of the F.1 in relation to the size and degree of inbreeding of the parents, amplifies the evidence on the genetic control of body size, derived from more analytical experiments, provides further information on inbreeding and heterosis, and generally directs attention to the interpretation of apparent dominance in crosses.

2. Material

The flies used in these experiments are derived from one or other of five wild stocks, known as Nettlebed, Edinburgh, Crianlarich, Renfrew and Ischia, each of which was descended from a wild impregnated female and maintained in the laboratory in large populations. The types of cross which have been carried out fall into three groups, as follows:

1) Between strains selected for long or short thorax length, before the response to selection had ceased and also, when forward selection no longer led to any progress. At this later stage, reversal of selection in the large strains demonstrated the presence

of a great deal of genetic variability, but the small strains behaved as if they were homozygous with respect to size, since reversal of selection was ineffective.

2) Between unselected inbred lines.

3) Between inbred lines derived from selected strains after selection progress had ceased.

Crosses noted under (1) were compared with strains derived from the Crianlarich, Ischia and Renfrew stocks in mass-selection experiments which will be described in detail elsewhere. Selection was carried out by mating together the 20 extremes from 100 flies of each sex, every generation, a large and small strain being established in this way from each stock. Progress under selection continued for 12-15 generations upwards and for 16-20 generations downwards, after which reverse selection tests suggested that the large strains retained considerable genetic variability and the small strains little or none. Lines inbred from some of these selected strains together with inbred lines from the Nettlebed and Edinburgh selection experiments (Robertson and Reeve, 1952a) provided the material for the third group of crosses. For the second group the lines were obtained by long inbreeding from the different stocks.

Since wing and thorax length are generally highly correlated with body size, discussion is mostly confined to thorax length. Mean size and variance of the parent strains and crosses are based on a sample of 30-50 flies drawn equally from 6-10 replicated

cultures. Methods of culture, measurement, etc. have been fully described elsewhere, (Robertson and Reeve, 1952a). Only females have been studied, to avoid having to deal with sex linkage, which is irrelevant to the problems considered here. In almost all cases reciprocal crosses have been carried out, and the progeny of the reciprocal matings have been combined. All measurements are expressed in 1/100 mm. A logarithmic or multiplicative scale seems most appropriate for analysing size variations; but the size range is small compared with average size, and the use of a logarithmic scale would not have any noticeable effect on the results of the analysis (Robertson and Reeve, 1953b).

3. Description of the crosses

(a) Crosses between non-inbred strains.

The crosses between the selected strains will be described first and the results will then be compared with the crosses between inbred lines. While all strains were still responding to selection, all possible crosses have been carried out between the large, small and unselected strains of different origin, both within and between strains. Since there were many crosses and the measurement of so many flies is no small labour, the crosses were distributed between three experiments made in successive generations, each of which dealt with a symmetrical group of crosses, in which strains of different origin were equally represented. The parents were derived from generations 8, 9 and 10

in the Renfrew and Ischia, 10, 11 and 12 in the Crianlarich series. All the selected and unselected strains were reared along with the crosses in each experiment, and it is therefore possible to compare the size of the former in terms of deviation from the unselected stocks, a procedure which minimises the importance of slight environmental, especially temperature, differences between separate experiments. Although the Crianlarich strains were a generation ahead of the others, the three large strains and the three small strains were very similar in thorax length in each experiment, while the three unselected stocks were almost identical in thorax length. Hence the average deviation of the selected from the unselected strains provides a good indication of the changes produced by selection. Table I shows that the small strains deviate from the level of the unselected more than the large, a comparatively frequent occurrence in selection experiments. Also, the mean deviation is progressively greater in consecutive experiments, due to the parents being drawn from successive generations of selection.

Strains of similar size also resemble one another in variability. The average within-culture variability, expressed in squared coefficients of variation, is set out in Table 2. Flies descended from selected parents of successive generations of selection show a progressive increase in variability and there is little doubt that this is related to corresponding changes in mean size due to selection. The error variance for

Table 1

Average deviation of thorax length of the selected strains from unselected stocks (1/100 mm.)

	Average deviation	
Experiment	Large strains	Small strains
1	4.3	-9.5
2	5.6	-10.5
3	7.0	-12.1

Mean thorax length of unselected strains = 111.03 $\pm$ .41

Table 2

Average variability of the selected and unselected strains (squared coefficients of variation).

Experiment	Unselected	Large	Small
1	3.45	2.55	4.77
2	3.59	2.86	5.17
3	3.81	4.84	9.16
Average	3.62	3.42	6.70

each group of experiments is based on the separate calculation of average components of variation due to variation within and between cultures. This gives a standard error which is a little too low for small strains and a little too high for the others, but the results are clear enough to make minor adjustments to the standard error unimportant.

For ease of reference, the unselected, large and small strains are referred to as U, L and S. The types of cross carried out in the three successive experiments were as follows.

Experiment 1. Between strains of approximately equal size, i.e. L x L, U x U and S x S (Table 3).

Experiment 2. Between large and small strains, L x S, of related and unrelated origin (Table 4).

Experiment 3. Between unselected strains and selected strains of the same or different origin: U x L, U x S (Table 5).

Finally experiment 1 was repeated when the selected strains had ceased to make further progress under selection (Table 6).

(b) Results.

The chief interest of these crosses is in showing the position of F1 size in relation to mid-parent size and tables 3-6 give the deviations (F1 - M.P.) and their statistical significance. The tables bring out the following points.

(1) Table 3 shows the results of crossing strains of similar size. When either unselected stocks or large strains of different origin

Table 3.

Deviation of F1 from mid-parent level in crosses between unrelated strains of similar size.

Origin of parent strains	Crosses		
	U x U	L x L	S x S
C x R	1.0	0.3	2.55^{xx}
C x I	0.7	1.15	3.6^{xx}
R x I	-0.1	1.25	3.35^{xx}

C, R, and I refer respectively to the Crianlarich, Renfrew and Ischia stocks.

Table 4.

Crosses between large and small strains.
F_1 deviation from mid-parent level.

Small parent	Large parent C	R	I	Average
C	0.00	0.35	0.75	0.37
R	0.25	0.05	0.55	0.28
I	2.40^{xx}	2.00^{xx}	2.30^{xx}	2.23

The crosses between large and small strains of the same origin are shown along the diagonal.

are intercrossed, the F1 does not differ significantly from the mid-parent level, although in crosses between large strains there is a tendency to a positive deviation. But when the small strains are inter-crossed, the F1 significantly exceeds the mid-parent value, and these crosses provide a clear contrast with the others.

(2) In crosses between large and small strains, summarised in Table 4, the F1 deviation is positive whether the strains are related or not. But the Ischia small strain differs from the other small strains in giving a consistently larger F1 deviation. Such characteristic behaviour is not attributable to this strain being smaller than the others, since the three strains are very similar in size.

(3) When the small strains are crossed to the unselected stocks (Table 5a), the same characteristic difference in their behaviour is seen. With one exception (R x R), the F1 signficantly exceeds the mid-parent size, but the deviation is greatest in crosses which involve the Ischia small strain. In the crosses between unselected and large strains (Table 5b), there is a general tendency for the deviation to be positive although only in the crosses, R x R, and R x I, is the deviation significant. Comparing tables 4 and 5 we note that the deviation F1 - M.P. is generally greater when the small strains are crossed to the unselected stocks (average 2.08) than when they are crossed to the large strains (average 0.96). The difference between these two averages

Table 5.

Cross of unselected with large and small strains.

Unselected parent	(a) Small parent			(b) Large parent		
	C	R	I	C	R	I
C	2.55^{XX}	1.25^{X}	3.35^{XX}	-1.00	0.55	0.60
R	2.05^{XX}	-0.10	3.30^{XX}	0.75	2.00^{X}	1.80^{X}
I	2.70^{XX}	1.30^{X}	2.30^{XX}	0.00	1.90^{XX}	-1.00
Average	2.43	0.82	2.98	-0.08	1.15	0.47

seems too great to be explained by the fact that one generation of selection intervened between the two sets of crosses.

Further information is provided by crosses between similarly selected strains after response to selection had ceased, (Table 6). In the large strains these crosses were carried out after 19 generations of selection in the Crianlarich and after 17 in the Renfrew and Ischia strains. Since the selection for small size was effective over a longer period, the crosses between small strains occurred after 27 generations in the Crianlarich and 25 in the other strains. As noted earlier, at the time of these crosses, the large strains retained considerable genetic variability, unlike the small strains which behaved as if they were homozygous with respect to size. The F1 is larger than the mid-parent size in all cases, but the absolute deviation is considerably greater in the crosses between small strains, as shown in Table 6. We again find that the crosses involving the small Ischia strain show the greatest deviation. It happens that the average deviation of the F1 from the mid-parent level, expressed as a percentage of the deviation of the parent strains from the unselected size, is roughly the same, i.e. 30 and 37% in the crosses between the large and between the small strains; but it is not clear whether there is any special significance in this fact, particularly in view of the contrast in the amounts of genetic variability remaining in the large strains compared with the apparent homogeneity of the small strains.

Table 6.

Crosses between strains of similar size at the end of the period of selection.

Origin of parent strains	F1 deviation from mid-parent level	
	L x L	S x S
C x R	1.15^{x}	4.5^{xx}
C x I	3.45^{xx}	7.45^{xx}
R x I	2.70^{xx}	7.70^{xx}

Average deviation of parent strains from unselected stocks:- Large - 8.2
Small - 17.9

The most consistent feature of these crosses is the evidence for assymetry in the direction of larger size. Whenever the F1 deviates significantly from the mid-parent level it does so in a positive direction, while less significant deviations show the same trend. The greatest deviation occurs when small strains are used as one or, especially, both parents. Crosses between large and unselected strains give an intermediate F1, close to the mid-parent value while crosses between unselected or between large strains, of different origin, show no significant deviation, although there is some suggestion of a positive deviation in the crosses between the large strains. Whether the parent strains are related or not appears to make no difference to the F1 relative to the size of the parents. Repetition of the cross between similarly selected strains at the end of the experiment leads to positive deviations in all cases, the deviations are significant for the crosses between large strains, and of greater magnitude than the earlier crosses in the case of the small strains. In general, later crosses between unrelated strains demonstrate more clearly tendencies which were apparent at earlier stages of selection. Finally the crosses between large and small strains, whether related or not, show comparatively little departure from strict intermediacy, except where the small Ischia strain is used as one of the parents. Although the differences in size, between the parent unselected and small strains, is less than that between large and small, the F1 deviation is less in the second type of cross.

4. Crosses between unselected inbred lines

When sustained inbreeding is carried out in wild stocks, there is generally a decline in size, the degree of which, however, appears to be rather variable. The inbred lines were all taken off the various wild stocks shortly after they were established in the laboratory, and we cannot be certain that the wild stocks have not changed in size since that time. This makes it difficult to obtain satisfactory estimates for the effects of inbreeding on size, since an objective standard is lacking. Nevertheless different wild stocks, kept in the laboratory, are very similar in size when reared under similar, optimal conditions, and, with the above qualification in mind, the best we can do is to base estimates of inbreeding decline on the average deviation between such stocks and different inbred lines. Many records are available, over several years, of the size of various wild stocks as well as of lines descended from them. Since these estimates were made at different times, often as part of other experiments, it is impossible to reduce them all to a common basis in terms of deviation from a control stock. However a general impression of the effects of inbreeding can be gained from the list of mean sizes quoted in Table 7. Since many of these lines were reared separately, minor differences of temperature etc. will contribute a little to the differences between them, but they can be assumed to give a fair indication of the extent of the variation in size to be expected among inbred lines.

Table 7.

Average wing and thorax length of inbred lines (1/1000 mm.)

Origin of line	Number	Wing	Thorax	Wing/Thorax Ratio Inbred lines	Wild stocks
Nettlebed	1	193.9	96.0	2.02	
	2	207.0	103.9	1.99	2.02
	3	213.0	105.7	2.02	
Edinburgh	1	201.9	109.1	1.85	
	2	194.2	101.8	1.91	1.96
	3	204.6	105.9	1.93	
	4	201.6	104.2	1.93	
Crianlarich	2	205.2	110.1	1.86	
	6	200.6	106.6	1.88	
	9	205.4	107.5	1.91	1.96
	10	202.5	105.6	1.92	
Renfrew	1	204.8	106.9	1.92	
	2	200.9	101.0	1.99	1.94
Average size of inbred lines		202.7	104.8		
" " of wild stocks		213.1	107.6		
" " of crosses		208.6	109.4		

The estimate of the average size of crosses between inbred lines is based on 14 such crosses between lines of related and unrelated origin.

Table 7 also shows the average wing and thorax length for all the inbred lines, for a number of their crosses together with the average of 10 estimates of the size of each of the wild stocks from which they are derived. Bearing in mind the possibility that the wild stocks may have changed in size during the period of laboratory culture, inbreeding appears to have led to an average reduction of at least 5% in wing length and 2.5% in thorax length, while the F1 returns approximately to the wild stock level in the case of thorax length and remains below it for wing length. There are slight differences in the wing/thorax ratio in different wild stocks, and since the genetic correlation between the two dimensions, although high, is not complete, (Reeve and Robertson, 1953), slight differences in the ratio are to be expected between inbred lines. Comparison of the ratio in wild stocks, with the related inbred lines, suggests that a reduction of the ratio has accompanied inbreeding in the Edinburgh and Crianlarich series, but the available comparisons are too few to draw any conclusions in the others.

When inbred lines from unselected stocks are crossed, the F1 always exceeds the mid-parent level, generally significantly so. Crosses between lines derived from the same and different stocks, summarised in Table 8, behave in a very similar manner and may be treated as a homogeneous group. There appears to be a distinct tendency for the F1 deviation to increase, as the difference between the parent lines becomes greater; the regression of the deviation

Table 8.

Crosses between unselected, inbred lines - thorax length.

Cross	P_1-P_2	F_1-M.P.	Cross	P_1-P_2	F_1-M.P.
$C_6 \times C_9$	0.9	2.1	$E_3 \times C_{10}$	0.3	1.7
$C_2 \times C_9$	2.6	1.5	$E_3 \times C_6$	1.4	2.6
$C_2 \times C_6$	3.5	1.6	$N_2 \times R_2$	2.1	2.5
$E_2 \times E_3$	4.1	2.1	$R_1 \times C_{10}$	3.3	2.9
$R_1 \times R_2$	5.9	4.3	$E_2 \times C_{10}$	3.8	2.3
$N_1 \times N_2$	7.9	5.0	$E_2 \times C_6$	5.6	3.5

P_1, P_2 and M.P. refer respectively to the larger and smaller parent and the mid-parent size.

on parental difference is 0.396 ± .092 which is nearly 0.5, or in other words, the difference between F1 and P1 - the larger parent is approximately constant. The deviations between F1 and P1 are tabulated opposite the appropriate value of P1 in Table 9; the comparisons are divided into 3 groups according to the size of P1. There is no evidence of a trend and the average value of the deviations in the three groups are very similar. Thus, in these crosses, the size of the larger parent appears to be the chief determinant of F1 size, and the size of the smaller parent seems unimportant.

5. Crosses involving lines from selected strains.

We may now consider crosses between lines which differ to greater extent than in the cases just considered. Inbred lines have been taken off most of our selected strains, generally when response to selection had ceased, and these have been crossed together, or crossed to inbred unselected lines, in various ways. Table 10 summarises the results of the crosses between inbred selected and unselected lines of different size (i.e. large x unselected, large x small and unselected x small lines). With all these crosses there is a considerable difference in size between the parents and it is therefore of particular interest to examine the position of the F1 with regard to the parent and mid-parent sizes. Table 9 shows the size difference between the parents (P1 - P2 where P1 is the larger parent), the absolute deviation (F1 - M.P.) and the relative deviation and dominance

Table 9

The relation between the size of the larger parent and the F1 in crosses between unselected, inbred lines.

P_1	$F_1 - P_1$	P_1	$F_1 - P_1$	P_1	$F_1 - P_1$
110.1	0.2	106.9	1.4	105.7	1.5
110.1	-0.1	106.7	0.7	105.6	0.4
107.5	1.5	106.1	1.2	105.6	1.8
107.3	1.9	105.9	0.0	103.9	1.1
Average	0.9		0.8		1.2

Table 19.

Crosses between inbred lines of different size.

Cross	P_1-P_2	F_1-M.P.	Dominance ratio
	Unselected x Large lines		
C_2	4.7	1.9	0.81
$C_9 \times L_C$	7.3	0.2	0.05
C_6	7.9	1.8	0.43
R_1	10.4	5.0	0.96
$R_2 \times L_R$	16.3	3.8	0.47
	Unselected x Small lines		
C_2	15.7	2.8	0.43
$C_9 \times S_C$	13.1	4.0	0.64
C_6	12.5	0.2	0.05
$R_1 \times S_R$	15.4	5.4	0.70
R_2	9.5	5.1	1.07
$N_2 \times S_N$	10.1	3.5	0.72
$E_2 \times S_E$	9.3	5.3	1.14
	Large x Small lines		
$L_C \times S_C$	20.4	2.5	0.25
$L_R \times R_R$	25.8	1.9	0.15
	Large x Large lines		
$L_{R1} \times L_{R5}$	1.7	3.9	1
$L_{I3} \times L_{I4}$	1.5	4.8	1
	Small x Small lines		
$S_C \times S_R$	4.4	4.5	1
$S_C \times S_I$	3.8	9.7	1
$S_R \times S_I$	0.6	7.5	1

The dominance deviation is calculated as $\frac{F_1\text{-M.P.}}{\frac{1}{2}(P_1-P_2)}$. Where several unselected lines are crossed to a single large or small line the former are arranged in order of decreasing size.

ratio: $\frac{F1 - M.P.}{\frac{1}{2}(P1 - P2)}$. The ratio will, of course, be 1.0 for complete dominance and 0 for strict intermediacy. In the table, the symbols (L, W and S) indicate whether the line originated from a large, ~~unselected~~ or small strain, while the second letter refers to the wild strain involved. Subscript numerals indicate different inbred lines of the same origin and the unselected inbred lines of each group are arranged in order of decreasing size.

In both the Crianlarich and Renfrew series, we can compare the effect of crossing two or three unselected inbred lines, generally differing in size, to a single large or small, selected line. Considering first the crosses to the large lines, Table 10 suggests that large, rather than smaller, unselected lines, when crossed to a single large line, favour greater F1 deviations and a higher degree of dominance. The indication that the greater the difference between the parents the less the departure from intermediacy in crosses involving large lines as one parent, is supported by the cross between the large and small lines of either Crianlarich or Renfrew origin, in which the parental difference is respectively 20.4 and 25.8 units and the departure from intermediacy is very slight. The crosses between the unselected and small lines imply that large F1 deviations, and a high level of dominance is most likely to occur when unselected parents are drawn from a relatively small line. This is particularly clear in crosses which involve R_2, N_2 and E_2 - all comparatively small lines, compared with the larger lines: C_2, C_6, C_9 and R_1.

Support for the existence of regularity is provided by evidence from the crosses between the mass selected strains. There it was found that the crosses between strains which differed most in size, i.e. large x small, produced an intermediate F1, - reminiscent of the parallel crosses between the present inbred lines, while significant F1 deviations occurred when small strains were crossed with the unselected stocks. The general implications of these findings will be discussed later, after we have considered variability in the different lines and crosses, since there is an advantage in considering all the data together. The table also quotes the results of crosses between different inbred lines descended from the Renfrew and Ischia strains selected for large size, i.e. $L_{R1} \times L_{R5}$; $L_{I1} \times L_{I2}$. These are appreciably larger than the unselected stocks but, in terms of deviation from the latter, are smaller than the selected strains from which they were derived, after selection had failed to produce further advance. The F1 in both cases considerably exceeds the size of either parent, and is approximately similar in size to that of the original selected strain. Also shown in the table are the crosses between the different strains selected for small size, after they had attained an apparently homozygous condition. It is instructive to bring the results of the different types of cross together in one diagram. (Figure 1).

The various crosses provide consistent evidence of a uni-directional departure of the F1 from strict intermediacy in favour

CROSSES BETWEEN LINES OF DIFFERENT SIZE

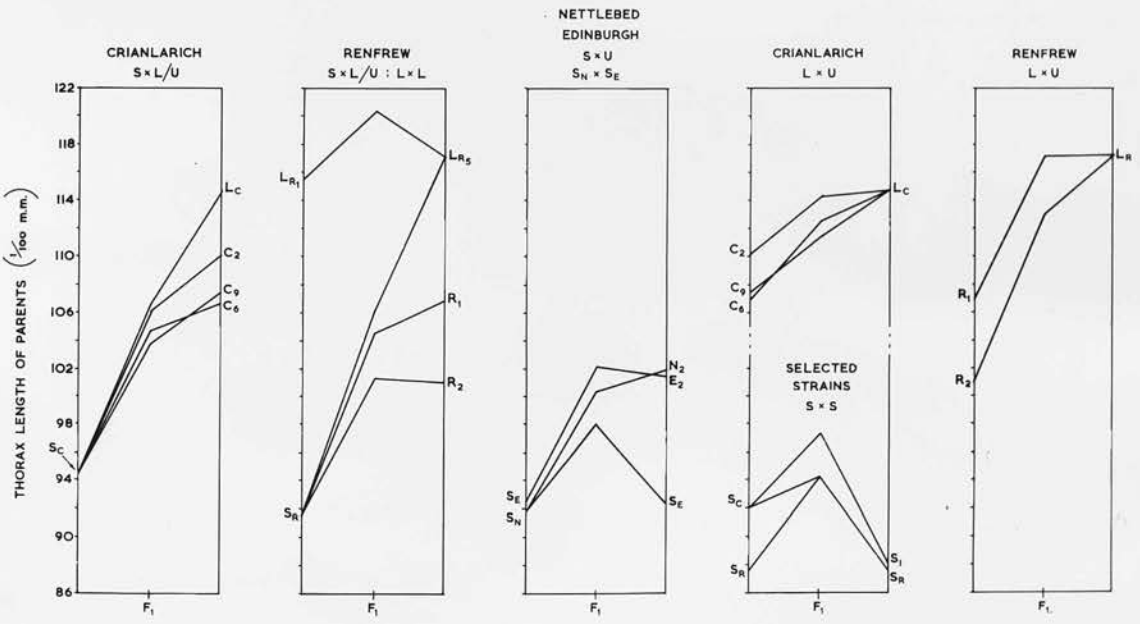


Figure 1

of the larger parent. The F1 is never less than the mid-parent value, and, in crosses between inbred lines, generally exceeds it significantly, especially in crosses between small lines. Inbreeding causes a variable decline below the level of outbred stocks and crosses between such lines generally take the F1 to within the normal range of variation encountered in wild stocks. On the simplest view, these observations might suggest dominance in the direction of larger size, and the F1 deviation could be attributed to summation of the independent effects of dominance at a number of loci. Although, as noted in the Introduction, the present series of crosses are only partly analytical in design, they nevertheless present serious difficulties for this interpretation. For example, if the uni-directional bias is due to dominance, then selection should lead to an accumulation of dominant alleles in the large strains and a reduction of their frequency in the small strains. Hence we might expect a greater F1 deviation in crosses between large and small strains than in crosses between the unselected and small strains; but, we have seen that the reverse is true (Tables 4 and 5a).

An even greater difficulty is presented by the crosses between the unselected inbred lines and respectively the large and small selected inbred lines, as well as the crosses between the latter. Using as a convenient measure of the "dominance ratio" $\frac{2x(F1-MP)}{P1-P2}$, we can compare the deviations in the different crosses. This ratio is plotted against the parental difference

in figure 2. If size were dependent mainly on dominant genes, we should expect to find a positive correlation between the dominance ratio and the difference between the parents, with the greatest ratio occurring in the crosses between the large and small lines. But the graph shows a striking negative correlation, so that the nearest approach to intermediacy occurs in crosses between the large and small lines of Crianlarich and Renfrew origin, which differ respectively by 20.4 and 25.8 units. Crosses between lines of about the same size, whether large, unselected or small, generally have a positive deviation (F1-MP), so that they would give very large dominance ratios with small values of (P1-P2). If these crosses were added to figure 2, the relationship between the parents would appear to be roughly hyperbolic, of the form $xy = K$, where $K = 2(F1-MP)$. A glance at Table 10 shows that the F1 deviation (F1-MP) is not constant for the different crosses, but that its variation is small compared with that of (P1-P2) and that it shows little correlation with the latter. Figure 2 apparently reflects these facts.

Evidently the simple hypothesis of independent effects of more or less dominant alleles cannot fit our results, and the same difficulties would obviously apply to the hypothesis of over-dominance.

6. Variability

Only the variation within cultures is considered here since it constitutes a much greater portion of the total variation than

DOMINANCE RATIO IN CROSSES, IN RELATION TO THE DIFFERENCES IN SIZE BETWEEN PARENTS.

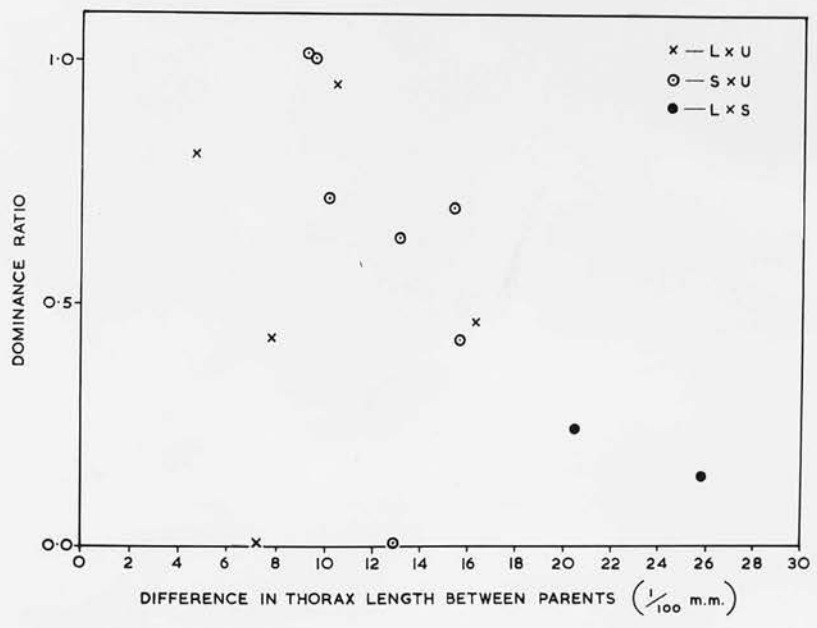


Figure 2.

Table 11

Within - culture variability of parents and progeny in crosses between selected and unselected strains(squared coefficients of variability).

Cross	Average variability	
	Parents	F1
Parent variability similar		
U x U	3.35	2.53
L x L	2.55	3.04
S x S	4.77	3.74
U x L	3.81 (U), 4.84(L)	3.61
Parent variability dissimilar		
U x S	3.81(U) 9.16 (S)	4.75
L x S	2.86(L) 6.17 (S)	2.86.

The discrepancy between the variabilities quoted for the same strain is due to the three stages in which the various crosses were carried out.

Table 12

Average variability in large and small strains and crosses at the end of the selection experiments.

Unselected	2.56	Unselected	2.96
Large	4.50	Small	2.97
Large x Large	4.18	Small x Small	2.96

The comparisons between the unselected stocks and the crosses between respectively the different large and small strains were carried out at different times, hence the two quotations of the variability of the unselected stocks.

that due to variation between cultures. Variability is expressed in squared coefficients of variation. Dealing first with the selected strains, comparison of Table 11 with Table 2 indicates that unselected and large strains are about equally variable in successive experiments, with, perhaps, a general tendency for the latter to increase in variability. The small strains are more variable than the others and show a rise in successive experiments i.e. in successive generations of selection. When the strains are crossed (Table 10) the F1 generally resembles the parents when the latter are approximately similar in variability, as in crosses of the type: U x U, U x L, L x L and S x S. But when the more variable small strains are crossed to large or unselected parents, the F1 is less variable than the average of the parents, and more closely resembles the larger strain.

The variability in the final crosses between similarly selected strains is shown in Table 12. Comparison of this table with Table 2, suggests that the unselected stocks are less variable in the later experiments, but this difference may be of environmental origin and is probably unimportant. More significant contrasts appear in the variability of large and small strains, for in these later tests, the variability of the large strains exceeds that of both the unselected and small strains, while the latter are now as variable as the unselected stocks. The increased variability of the large strains is doubtless connected with the ineffectiveness of continued selection in the presence of a high level of genetic variability, suggesting the selection of

heterozygous combinations; this will be discussed in more detail elsewhere. Since there is good evidence that the small strains lack genetic variability for size, some decline in their phenotypic variability is to be expected. But they are actually as variable as the highly heterozygous wild stocks, thus providing further examples of the increased sensitivity to environmental conditions, which has been reported for other small lines and inbred lines generally (Robertson and Reeve, 1952b).

Further evidence of this phenomenon appears in the variability of inbred lines and their crosses, set out in Table 13; the F1 is consistently less variable than the parent lines. The reduction in variability is least in crosses between large lines, but since only two such crosses are available, it is uncertain how far this reflects a genuine trend in relation to the size of the parent lines.

7. Discussion

The attributes of an individual, in relation to the attributes of its parents, is the initial fact in any study of inheritance. It is customary to describe a situation in which the progeny more closely resembles one parent as exhibiting dominance. The concept of dominance is derived from the behaviour of alleles at a locus, in genetic situations where the salient differences between parents and offspring can be attributed to variation at a single locus and where other genetic differences are unimportant. In the study of mutant genes which affect morphology, pattern, colour,

Table 13

The variability of inbred lines and crosses (squared coefficients of variation).

Type of cross	Number	Average variability		Percent reduction
		Parent line	F1	
U X U Related	6	3.06	2.30	25
U x U Unrelated	8	2.60	1.69	35
L x U	5	3.59	1.90	45
S x U	5	3.73	2.44	35
L x S	5	2.63	2.08	22
L x L	2	3.51	3.12	11

etc. there is usually not much risk of confusion in understanding the origin of the observed differences. But when we deal with continuously varying characters like size, in which genetic variation is due to the segregation of alleles at many loci, the occurrence of deviations from intermediacy raises considerable problems of interpretation. If we are satisfied that a given deviation is not an artefact of scale and reflects a biologically valid effect, then various interpretations are available.

As a first approach it is reasonable to carry over the concepts derived from the known behaviour of alleles at single loci, and attribute such deviations to summation of independent effects at a number of loci. This, of course, is the basis for the most widely held theory of heterosis. When inbreeding leads to a decline in size, vigour or fertility and outcrossing restores the normal level, there is evidently a uni-directional tendency which has been correlated with the occurrence and direction of dominance at many loci. On the other hand, there is the alternative view that such heterosis rests primarily on over-dominance, such that the presence of unlike alleles at corresponding loci confers an advantage compared with either of the homozygous combinations and that the observed deviations in crosses may be attributed to summation of such effects of over-dominance. The extent to which it is useful to consider heterosis in terms of dominance or over-dominance depends on how far the relations between alleles are constant over the range of genotypes which

are being studied. The less this is true, or, in other words the more sensitive is the effect of any substitution to variation in the genetic background, the less useful are these concepts, which may indeed prove mis-leading in so far as they involve a hidden assumption about gene behaviour derived from much simpler situations.

Thirdly inbreeding decline and heterosis in crosses may be attributed respectively to disruption and restoration of the genetic balance which characterises the normal outbred population. The general idea of genetic balance or a harmonious gene complex has been a commonplace of genetic thought for many years but we have few data relating to the way inbreeding or selection may affect the essential nature of the genetic system with respect to particular characters. Mather (1943) has recognised this problem, but has dealt with it in terms of the linkage relations of a special kind of gene - polygenes - with hypothetical attributes which are hard to reconcile with what is known of gene behaviour generally (Robertson and Reeve, 1952a). Experimental information is so sparse in this field, that it appears advisable to proceed empirically with as few pre-conceived notions as possible. The problem is further complicated by the likelihood that in studying genetic variation in different characters, which are similar in being amenable to quantitative measurement, we may be dealing with variation of widely different significance in the economy of the organism. The present data, derived from the effect of crossing lines and strains of different size, may now be considered to see

what light they throw on these problems and alternative interpretations.

As we have seen the simple hypothesis of the independent effects of dominance or over-dominance is of little value in helping us to understand the results of the different types of cross. This conclusion is supported by evidence of a different type derived from the effects of interchanging chromosomes between lines which differ in size or show heterosis when crossed (Robertson, in the press; Robertson and Reeve, in the press). The experiments referred to have revealed numerous examples of genetic interaction between non-homologous chromosomes, for which the properties of homozygous combinations are largely responsible, especially homozygous combinations which favour smaller size. In the face of such widespread interaction it is obviously impossible to account for the observed heterosis or inbreeding decline in terms of the summation of independent effects. Since it is unlikely that any biologically reasonable scalar transformation can be of much help, we have to adopt a different approach and see how far the apparent regularities in the genetic control of size suggest corresponding properties in the underlying behaviour of gene combinations.

If we consider first, the variation of the relative position of the F1 in relation to the difference in size between the parents, it is obvious that intermediacy is conditional on the gene complex and is not to be interpreted in terms of the inherent properties of genes which control size. It is assumed that genes

responsible for variation in body size are not operating on a single developmental system, such that genes at different loci can be regarded as interchangeable. Underlying the continuous variation is a complex system in which it is theoretically possible to distinguish different sorts of gene action, whose relative importance will influence the properties of the genetic variation of the character under study. If the relative position of the F1 is conditional, then the occurrence of intermediacy, in which the F1 shares the attributes of its parents, implies the existence of certain inter-relations among the gene-controlled processes of development. Any regularities in the result of crossing parents of different size provides empirical evidence which may help us to understand the inter-relations in due course.

We may first enquire what are the implications of intermediacy in a cross. With respect to genetic variation at a single locus, intermediacy is usually taken to imply that the alternative contrasted alleles intervene in the same developmental process, variation in the rate or intensity of which is reflected in the eventual phenotype. It has been suggested by Wright (1945) that the occurrence of dominance between alleles may imply a low ratio of substrate to the immediate gene products which react with it, since there is likely to be competition between alleles. A high ratio favours more independent effects. In the study of dominance at particular loci, we can usually disregard variation in quantity and quality of substrates which are dependent on the

rest of the gene complex. But where variation at many loci is involved, the gene controlled inter-relations which determine the availability and quality of substrates is really the important problem. Gene complexes which favour dominance or intermediacy, might be taken to imply intensification or reduction of allelic or, more generally, gene competition. Although detailed speculation as to how such differences may be brought about is not very helpful, when we have only variation in phenotypic size as a measure of effect, any regularities in departures from intermediacy are of empirical value. The present data are of some interest in this connection.

In general intermediacy does not occur in crosses between inbred lines. When unselected inbred lines are crossed, the F1 apparently exceeds the size of the larger parent by a fairly constant amount, as if the effect of the haploid complement of the smaller parent merely contributes to the heterosis which is nevertheless limited in its extent by the nature of the complement from the larger line. The controlling influence of the contribution from the larger parent becomes even more evident in crosses between the unselected and small inbred lines, in which the F1 is very close to the size of the former. Within these crosses, there appears to be a trend in relation to the difference in size between the parents. Thus when small lines are crossed to the smaller unselected lines, the F1 is almost identical with the latter, but as the parental difference increases, i.e. as the size of the unselected line increases, the F1 tends to fall short of the

larger parent to increasing degree (Figure 2). When the parental difference is further increased by crossing the same small lines to large selected lines it appears that the same trend exists, since the F1 approximates to intermediacy in the extreme crosses. It is particularly interesting that crosses of small lines to unselected lines should produce an F1 close to the latter in size, provided the difference in parent size is not too great. It does not appear as if this trend is necessarily related to the peculiar properties of small selected lines, since the same tendencies appear in crosses between the large and unselected lines. Thus the more alike the size of inbred parents, the greater the likelihood of heterosis; the greater the difference the greater the tendency towards the occurrence of an intermediate F1. Heterosis in crosses between similar parents means that the same or very similar phenotypes may be associated with different genotypes. The general trend noted above refers to crosses between homozygous parents, so that the F1 is qualitatively different from the former in being to greater or lesser degree heterozygous. It appears as if the basis for intermediacy of the F1 is progressively favoured as such differences between the parents become greater.

These considerations raise the question as to how far the results of crossing homozygous lines may be validly compared with the effects of crossing heterozygous parents, as in the crosses between the large, small and unselected strains at the early stages

of selection. It is true that the corresponding crosses show some parallel tendencies, i.e. F1 is intermediate in crosses between large and small strains, shows a positive deviation when unselected and small strains are crossed, while crosses between small strains are accompanied by striking heterosis. In terms of the absolute differences in size between parents it appears that intermediacy of the F1 may occur when the parents differ much less than is the case in those crosses between homozygotes which produce an approximately intermediate F1. There is other evidence that intermediacy is comparatively wide-spread in wild stocks, in the high estimates of heritability (40 - 50%) found in the course of unpublished progeny tests. It is perhaps significant that the closest approach to intermediacy in crosses between homozygous lines occurs when the lines have been selected in opposite directions from the same wild stocks; it might be expected that such crosses involve a higher level of heterozygosity in the F1 than is true of the other crosses. Comparison of the effects of intercrossing inbred lines has suggested that intermediacy is in some way related to the occurrence of opposing tendencies in the F1. If the existence of such opposing tendencies is largely equivalent to the occurrence of heterozygosity, these may point to a way of linking the results of intercrossing inbred lines and genetically variable stocks. Analysis of the effects of exchanging chromosomes between inbred lines (Robertson and Reeve, in the press) has shown that interaction between non-homologous

chromosomes is detected much less frequently when chromosomes are substituted in heterozygous than in homozygous backgrounds. This is, of course, consistent with the high estimates of heritability observed in the highly heterozygous wild stocks. It appears as if the conditions which sustain the heterosis which exists in such stocks also generally reduce the incidence of gene competition. However, further consideration of this problem must await more detailed analysis of the phenomena associated with heterozygous gene combinations in relation to body size.

Summary

1. The paper describes the results of many crosses between inbred and non-inbred strains of Drosophila melanogaster, differing in body size and descended from several different wild stocks. The results of the different types of cross are compared with respect to the departure of the F1 from the mid-parent value. Body size here refers to thorax length.

2. The large and small strains were created by selection for thorax or wing length. When the strains no longer responded to continued selection they were inbred to establish the lines used in the present tests. A number of inbred lines have been created by inbreeding unselected stocks.

3. Large, small and unselected non-inbred strains descended from three different wild stocks were intercrossed in all possible ways after 6-8 generations of mass selection, which had led to substantial

differences. Intercrossing the different small strains led to striking heterosis in the F1; when these small strains were crossed to related or unselected wild stocks the F1 deviated significantly from the mid-parental value in the direction of the larger parent, but when crossed to the large strain, the F1 was found to be almost intermediate, except for the crosses involving a particular small strain. Crosses between different large strains and between large and unselected strains produced slight, but statistically insignificant departures from intermediacy; there was no heterosis in crosses between unrelated, unselected stocks which were very similar in body size.

4. When unselected stocks are inbred by brother sister mating, there is generally a variable decline in body size, equivalent, on the average, to about 2.5% reduction in thorax length. When such long inbred lines are intercrossed the F1 falls within the normal range of variation of outbred stocks. Although the size of the F1 exceeds that of the larger parent in such crosses, there is nevertheless a positive correlation between them; in the series of crosses between selected and unselected lines the deviation between F1 and larger parent appears to be comparatively constant.

5. When large, small and unselected lines are intercrossed, the F1 most clearly exceeds the larger parent when the differences between different small and also different large lines. There appears to be a tendency toward greater intermediacy of the F1 as the difference in size between the parents increases. Thus in crosses between small selected lines and the smaller of the

unselected lines the F1 is identical with or closely resembles the larger parent; but if the same small line is crossed to larger unselected lines the F1 falls short of the latter, while in crosses to the large selected lines the F1 is almost intermediate.

6. Phenotypic variability, as measured by the within culture variation, is consistently higher in the inbred lines than in the crosses between them, and presumably reflects the increased resistance of heterozygous combinations to variation in environmental conditions.

7. These results are considered in relation to the origin of heterosis and apparent dominance. Attention is drawn to the general inadequacy of explanations based on the summation of more or less independent genetic effects while the possible implications of some of the regular features of these crosses are discussed.

Acknowledgements

We wish to thank Miss Evelyn Davidson and Miss Alexa Hunter for technical assistance and Mr. Charles Marr for carrying out much of the computation.

References.

MATHER,K.(1943) Polygenic inheritance and natural selection. Biol. Rev. 18, 32.

REEVE, E.C.R. & ROBERTSON, F.W. (1953a) Studies in quantitative inheritance. 11 The analysis of a strain of Drosophila melanogaster selected for long wings. J.Genet. 51,276.

_____ (1953b). The analysis of environmental variability in quantitative inheritance. Nature, Lond., 171, 874.

ROBERTSON,F.W. & REEVE, E.C.R. (1952a) Studies in quantitative inheritance. 1 The effects of selection of wing and thorax length in Drosophila melanogaster. J.Genet. 50,41+

_____ (1952b). Heterozygosity, environmental variation and heterosis. Nature, Lond. 170, 296.

_____ (1953) Studies in quantitative inheritance. 1V The effects of substituting chromosomes from selected strains in different genetic backgrounds, J.Genet. 51, 586.

_____ (in the press) Studies in quantitative inheritance. v111 Further analysis of heterosis in crosses between inbred lines of Drosophila melanogaster.

ROBERTSON, F.W. (J in the press). Studies in quantitative inheritance. V Chromosome analyses of crosses between selected and unselected lines of different body size in Drosophila melanogaster J. Genet.

WRIGHT,S. (1945). Physiological aspects of genetics. Ann. Rev. Physiol. V11, 75.

Studies in Quantitative Inheritance

VIII. Further analysis of heterosis in crosses between inbred lines of *Drosophila melanogaster*.

By

Forbes W. Robertson[X] and E. C. R. Reeve[X]
Institute of Animal Genetics, Edinburgh.

[X]Member of the Agricultural Research Council Scientific Staff.

CONTENTS

1. Introduction.

Discussion of the behaviour of genes and gene complexes which control the development of quantitative characters relies largely on hypothesis and convenient assumption, rather than empirical demonstration. Further understanding in this field calls for more detailed experimental analysis, although this encounters many practical difficulties and much depends on the use of appropriate material. Deductions based on the comparison of responses to artificial selection and the usual methods of population analysis are often limited by the difficulties found in discriminating between widely different interpretations of the same phenomena. One way of enlarging the scope of experimental analysis in Drosophila melanogaster, is to construct different combinations of chromosomes from pairs of lines which differ strikingly in some respect, due to selection for example, or which produce striking heterosis when crossed. Many genotypes can be created at will and the comparison of genetic constitution with the level of expression of the character under study makes it possible to test different hypotheses about gene and chromosome behaviour.

Such methods have already been used in a partial chromosome analysis of strains selected for large and small size (Robertson and Reeve 1953b), and in a complete analysis of all possible combinations of major chromosomes from inbred lines obtained without selection and after selection for small size (Robertson,

in the press). These experiments suggested that the positive F_1 deviation from the mid-parental level, in most crosses, could not be accounted for in terms of the addition of the effects of individual chromosomes acting in a more or less dominant manner, nor of the addition of over-dominance effects arising from heterozygous pairs. There was clear evidence of gene interaction which tended to be uni-directional, in the sense that gene combinations which favour smaller size tend to be hypostatic to those responsible for more normal or larger size. Also the phenotypic variability of the body size of inbred lines was found to exceed that of the crosses between them, while the comparison of types heterozygous for different numbers of chromosome pairs suggested that this reduction in variability is closely related to the level of heterozygosity (Robertson and Reeve, 1952b, Reeve and Robertson, 1953).

The present paper describes an extension of a partial chromosome analysis to a further series of crosses between inbred lines, and deals with the inheritance of egg production as well as body size. It is particularly instructive to compare, on the same material, the genetic control of quantitative characters which are so different in their reaction to environmental variability and response to inbreeding. Straus (1942) also studied egg production by similar methods and concluded that output is linearly related to level of heterozygosity.

2. Methods and Material.

(a) The chromosome combinations. An earlier publication (Robertson, in the press) has described methods of preparing all possible combinations of major chromosomes from pairs of inbred lines with the aid of marked, autosomal inversions and a system of backcrossing which minimises possible error due to recombination. The present experiments deal with only 15 of the possible 27 combinations of major chromosomes in females. These comprise the parent lines, and the types characterised by individual and joint substitutions of single, non-homologous chromosomes of one line for those of another. The array of types is set out in Table 1, using a convenient notation in which such letters as A or B represent homozygous, and X indicates heterozygous pairs of homologues; the particular chromosome pair is indicated by the order of the letters.

The method of preparing the types is shown in Fig. 1. Curly (Cy) and Curly Lobe (Cy al^2 L^4 sp^2) - with the inversion in both arms - are used for balancing II, and Moiré (Mé) and Moiré Stubble (MéSb) for III. Cy L^4 and Mé, either alone or together, are particularly effective in suppressing recombination; they occur in females at only one stage of the procedure while the other inversions are carried in males, and so error due to crossing-over should be very slight. Males, carrying the markers Cy Mé Sb or Cy L^4 Mé, are repeatedly backcrossed to females of the two lines, say A and B. Crosses are then made between Cy Mé Sb

Table 1.

The genotypes creased by interchanging chromosomes between two inbred lines A and B.

Heterozygous Pairs	Genotypes	
	AAA	BBB
I	XAA	XBB
II	AXA	BXB
III	AAX	BBX
I + II	XXA	XXB
I + III	XAX	XBX
II + III	AXX	BXX
I + II + III	XXX	

Fig. 1.

The creation of different genotypes by combining chromosomes from lines A and B.

Backcrosses

$$\frac{+}{Y}\ \frac{Cy}{+}\ \frac{MéSb}{+} \times$$

AAA → $\frac{A}{Y}\ \frac{Cy}{A}\ \frac{MéSb}{A}$

BBB → $\frac{B}{Y}\ \frac{Cy}{B}\ \frac{MéSb}{B}$

$$\frac{+}{Y}\ \frac{CyL}{+}\ \frac{Mé}{+} \times$$

AAA → $\frac{A}{Y}\ \frac{CyL}{A}\ \frac{Mé}{A}$ x AAA → $\frac{A}{A}\ \frac{CyL}{A}\ \frac{Mé}{A}$

BBB → $\frac{B}{Y}\ \frac{CyL}{B}\ \frac{Mé}{B}$ x BBB → $\frac{B}{B}\ \frac{CyL}{B}\ \frac{Mé}{B}$

The crosses which produce the types studied

$\frac{A}{Y}\ \frac{Cy}{A}\ \frac{MéSb}{A}$ x $\frac{B}{B}\ \frac{CyL}{B}\ \frac{Mé}{B}$

$$\frac{B}{Y}\ \frac{Cy}{B}\ \frac{Mé}{A} \times$$

AAA → XXA

BBB → BBX

$$\frac{B}{Y}\ \frac{CyL}{A}\ \frac{Mé}{A} \times$$

AAA → XAA

BBB → BXX

$$\frac{B}{Y}\ \frac{CyL}{A}\ \frac{MéSb}{B} \times$$

AAA → XAX

BBB → BXB

$\frac{B}{Y}\ \frac{CyMe}{B}\ \frac{Sb}{B}$ x $\frac{A}{A}\ \frac{CyL}{A}\ \frac{Me}{A}$

$$\frac{A}{Y}\ \frac{Cy}{A}\ \frac{Me}{B} \times$$

BBB → XXB

AAA → AAX

$$\frac{A}{Y}\ \frac{CyL}{B}\ \frac{Me}{B} \times$$

BBB → XBB

AAA → AXX

$$\frac{A}{Y}\ \frac{CyL}{B}\ \frac{MéSb}{A} \times$$

BBB → XBX

AAA → AXA

males backcrossed to A or B and Cy L^4 Me females produced by backcrossing to respectively B or A. From the F_1 various types of male are chosen, and crossed to virgin females of the appropriate line, as shown in the diagram. In the next generation, wild type flies, of known constitution, are separated and measured or used for recording egg production. The pure lines and the reciprocal crosses are reared at the same time to complete the array of types. The 4th chromosome has been disregarded, since the earlier tests, with a greater variety of genotypes, suggested that differences due to variation in this chromosome are likely to be unimportant.

(b) The material and general procedure. Most of the lines used here are derived from different wild stocks of Drosophila melanogaster, by inbreeding with brother-sister mating. With the possible exception of the Oregon line, the early history of which is uncertain, inbreeding began shortly after the stocks were established in the laboratory. When crossed the F_1 shows heterosis for both size and egg production. The size of the parent lines and their crosses is set out in Table 2, along with an estimate of the average size of a number of different non-inbred, wild stocks, taken from another publication (Robertson and Reeve, in the press). The data relating to the chromosome analysis of the three lines described in an earlier paper (Robertson, in the press) are included here, and in the subsequent analysis, since there is an advantage in comparing as many

parallel genetic combinations as possible.

Egg production is based on the total eggs laid during a 4-day period corresponding to the phase of maximum daily yield, i.e. approximately the 4th to 8th day of adult life (Hanson and Ferris, 1929 ; Shapiro, 1933 ; Robertson and Sang, 1944; [illegible]), when the number of eggs laid on consecutive days by an individual fly is usually comparatively constant. Unpublished experiments have shown a high correlation between the 4-day total and life-time yield. The egg production of inbred lines and their crosses are also summarised in Table 2, and may be compared with the average production of the vigorous, mass mating, Crianlarich stock. Evidently inbreeding causes a striking decline in egg output and there is clear-cut heterosis when the lines are crossed. Comparison with the similar data relating to body size, suggest that egg production is more sensitive to inbreeding - a promising contrast for analysing the effects of chromosome substitutions on the two characters.

The mean values of wing and thorax length of the different chromosome types are based ideally on a series of 5 females from each of 5 replicated cultures set up on two successive days, i.e. 50 individuals in all. Egg production, in view of the labour involved, has been recorded only on flies drawn from the five cultures set up on a single day, hence the individual egg production as well as size is known for approximately half the total flies studied. Generally less than the ideal numbers are

Table 2.

Average thorax length and egg production of inbred lines and of the crosses between them.

Parent lines	Thorax length (1/100 mm.)		Egg production over 4 days	
	P_1	F_1	P_1	F_1
R1	107.3	111.3	108	292
C6	106.3		114	
R2	106.1	107.3	148	342
C10	102.8		124	
N1	96.0	105.0	190	305
N2	103.9		122	
N2	105.5	107.2	174	284
R2	103.6		181	
N3	104.2	107.6	-	-
O1	98.8		-	
O2	102.2	107.3	-	-
E4	101.9		-	
N3	104.5	107.0	-	-
E4	100.9		-	
Wild stocks (Average)		107.6		320

available, due to the hazards of segregation and low viability associated with a few genotypes, while, in the case of egg production, occasional death or escape of a fly further reduces the number. Error variances are based on within- and between-culture effects, suitably adjusted for variation in the number of flies per culture.

All experiments are conducted at 25 ± 0.5°C. Methods of culture, measurement and the recording and collection of eggs have been fully described elsewhere (Robertson and Reeve, 1952a). Measurements of wing and thorax are expressed in 1/100 mm.

(c) Scale. For a statistical test of the adequacy of any scale, we must change the mean by altering either the environment or the genotype, and study the effect on environmental variability. But it proves to be very difficult to change the mean by methods which leave no theoretical ground for believing that the variance will also be changed. Thus change by environmental means - level of nutrition or temperature - certainly alters the range of environmental conditions in the culture, and a transformation which left the variance constant for changes in these factors would obviously give a biased result. If we compare heterozygotes with homozygotes, the former generally have larger means, but again it is likely that the heterozygotes will be better able to cope with a given range of environmental conditions than the homozygotes, so that their variance will actually be less. It is possible that even comparison of inbred lines with different

means would introduce similar difficulties, since those with the larger means might have "better" genotypes and show less variability for a given range of environment - in other words, change of mean may indicate a change in ability to maintain constancy under given conditions. For these reasons a scale chosen for its statistical value in making the variance constant may actually obscure some interesting biological phenomena, and it seems better to rely on theoretical considerations

Theoretically, one would expect any cause of variation, genetic or environmental, to act in proportion to the mean, rather than independently of the mean - e.g. genetic or environmental factors affecting the rates or duration of processes, and it is in fact difficult to imagine a change which would add the same amount on to a large and a small organ. It seems natural, therefore, to use a logarithmic scale rather than the scale of measurement in which the data are recorded. For wing and thorax length, however, the range of variation is comparatively small and a log transformation makes little difference to the analysis and is hardly worth the additional labour of computation. The coefficient of variation provides a sufficiently valid measure of variability and earlier work has supported this view (Robertson and Reeve, 1953b). With egg production the range of variation is very much greater. Accordingly all 4-day totals have been transformed to natural logarithms and all computations are based on such transformed data.

3. Experimental Analysis.

(a) Genotype and mean performance.

(i) Body size. Since crosses between lines show heterosis we are evidently not dealing with a purely additive genetic system. The chromosome is here the lowest unit and we must first see how far interaction between non-homologous chromosomes appears in the array of types.

To test for departure from additive combination of non-homologous chromosomes, it is necessary to avoid dominance effects by considering separately each series of 8 genotypes consisting of an inbred line and the genotypes obtained by substituting one or more chromosomes of another line, in single dose - e.g. AAA, XAA, AXA, AAX, XXA, XAX, AXX, XXX. Representing the means of these genotypes by A, A + a, A + b, A + c, A + a + b, A + a + c, A + b + c and A + a + b + c, where A is the expected mean of AAA, and a, b and c are the average effects of substituting a first, second and third chromosome of another genotype B, we can solve for A, a, b and c by Least Squares and test whether the fitting of the four constants leaves any significant interaction. This form of analysis is, of course, well known from factorial field plot experiments. The solution may be written:

$$A = \tfrac{1}{8}\Sigma A - \tfrac{1}{4}[\Sigma a + \Sigma b + \Sigma c]$$

$$a = -\tfrac{1}{4}[\Sigma A - 2\Sigma a]$$

$$b = -\tfrac{1}{4}[\Sigma A - 2\Sigma b]$$

$$c = -\tfrac{1}{4}[\Sigma A - 2\Sigma c]$$

where, on the right hand side, ΣA = sum of all genotype means, Σa = sum of means of genotypes with heterozygous first chromosome, etc. The interaction variance is the mean of the interaction variances between chromosomes i.e. 1 x 2, 1 x 3, 2 x 3, 1 x 2 x 3, with four degrees of freedom, and may be calculated as quarter the sum of squares of differences between expected and observed values of the genotype means. It is tested against the error variance of a genotype mean. Each crossing experiment provides two series of 8 genotypes with a common type XXX (Table 1), for which the error variances are pooled. In one such series a genotype was missing, and a modified Least Squares analysis was made.

The tests of the presence of interaction in the various series are set out in Table 3. Evidently the majority of the sets of comparisons show significant departure from a system of additive combination. Particularly interesting are the cases in the E_4/O_2, and E_4/N_3 comparisons with respect to thorax length, where substitution of chromosomes from one line in the background of another is associated with interaction, while the reciprocal exchanges are not.

It is interesting to compare these results with the effects of interchanging chromosomes between small lines, descended from selected strains, and unselected lines derived from the same initial population (Robertson, in the press). The F_1 of such crosses closely resembled the larger parent in size, but in the Nettlebed series there was clear evidence of genetic interaction

Table 3.

Test of additive combination of chromosomes by the method of Least Squares.

Series	Wing		Thorax	
	Mean square of deviations	Error variance	Mean square of deviations	Error variance
R1	1.01	0.60	0.53^{xx}	0.12
C6	4.94^{xx}		1.43^{xx}	
R2	0.37	0.60	0.20	0.18
C10	1.86^{x}		0.32	
N1	9.16^{xx}	0.48	2.14^{xx}	0.15
N2	1.23^{x}		0.58^{xx}	
N2	0.52	0.21	0.20	0.11
R2	0.59		0.07	
N3	6.52^{xx}	0.60	0.33^{x}	0.12
O1	3.47^{xx}		1.35^{xx}	
O2	4.45^{xx}	0.78	0.60	0.35
E4	42.10^{xx}		8.58^{xx}	
N3	2.27^{xx}	0.69	0.51	0.21
E4	41.50^{xx}		5.26^{xx}	

"Series" refers to the homozygous background in which the substitutions are carried out.

between non-homologous chromosomes, but none in the Edinburgh series. The chromosome analysis of the selected lines presents the same sort of phenomena as are shown in the present combinations.

The analysis can be carried a stage further by using a modification of the Least Squares analysis which enables us to separate the interaction variance into three components, attributable respectively to inconsistencies among the 6 single and double heterozygotes, and to deviations of the homozygote and triple heterozygote from the values expected on the basis of linear combination of chromosomes in the single and double heterozygotes. The equations to be solved by Least Squares become:

$XAA = n + a$ $\quad AXX = n + b + c$ $\quad AAA = n - h_A$

$AXA = n + b$ $\quad XAX = n + c + a$ $\quad XXX = n + a + b + c - h_X$

$AAX = n + c$ $\quad AXA = n + a + b$

As before, a, b and c measure the average effects of making the first, second and third chromosomes heterozygous (when one other chromosome pair is already heterozygous), n is the expected size of AAA assuming linear combination of chromosomes, and h_A and h_X are the deviations of AAA and XXX below their expected values, assuming linear combination of chromosomes. After fitting the 5 constants there remain 2 degrees of freedom for deviations between actual and expected values, giving an interaction variance which measures inconsistencies in the substitution effects when one chromosome pair is heterozygous, (i.e. within the group of 6 partial

heterozygotes). This variance and h_A and h_X give us the three components of the original interaction variance, and enable us to detect non-linear chromosome combinations within the group of single and double heterozygotes and in the homozygotes and triple heterozygote, respectively.

The equations for obtaining the various estimates and their standard errors are set out in Table 4. Table 5 lists the components of interaction for the 13 series; these are tabulated in order of increasing difference between the fully heterozygous type (XXX) and the homozygous background in which the substitutions are carried out.

Of the three components of interaction in the case of thorax length (Table 5) I is evidently the least important, so that there is comparatively little interaction between chromosomes substituted in a partially heterozygous background. Several significantly negative values of h_X occur, suggesting that interactions in the triple heterozygote tend to make it larger than would be expected from the size of the partial heterozygotes, while the most important interaction effects are with h_A, which is usually positive when significant. This implies that the homozygote tends to be smaller than the sizes of the partial heterozygotes would lead us to expect, suggesting that interactions between the different homozygous pairs of chromosomes in the inbred lines are often partly responsible for their small size. Finally, it is of particular interest that significant interactions are most frequent with substitutions in the smallest lines, e.g. E_4, O_1 and N_1, and in cases where the greatest deviation XXX - AAA occurs.

The analysis of the interactions for wing length (Table 6)

Table 4.

Estimates of substitution effects and interactions.

$a = \frac{1}{2}[XAX + XXA - AXA - AAX]$

$b = \frac{1}{2}[XXA + AXX - AAX - XAA]$

$c = \frac{1}{2}[AXX + XAX - XAA - AXA]$

$n = \frac{1}{3}[2(XAA + AXA + AAX) - (AXX + XAX + XXA)]$

$h_A = n - AAA$

$h_X = n + a + b + c - XXX$ S.E. of h_A and $h_X = \sigma\sqrt{\frac{8}{3}}$

where σ^2 is variance of a genotype mean.

Interaction variance (I) = $\frac{1}{18}(x^2 + y^2 + xy)$, with 2 degrees of freedom;

where $x = 2(AXX + XAA) - (XAX + AXA + AAX + XXA)$

$y = 2(XAX + AXA) - (AAX + XXA + XAA + AXX)$

σ^2 is the error variance for testing I.

Table 5.

Effect of the presence of heterozygous pairs of chromosomes on the degree of additive combination - thorax length.

			Components of Interaction		
	AAA	XXX-AAA	h_A	h_X	I
N2	103.9	1.1	-2.0^{XX}	0.1	0.2
N2	105.7	1.5	1.0	-0.6	0.2
N3	104.5	2.5	2.1^{XX}	-0.3	0.3
N3	104.2	3.4	-1.5^{XX}	0.7	0.3
R2	103.6	3.6	-0.2	-0.6	0.0
R1	107.3	4.0	-0.1	-1.6^{XX}	0.4^{X}
C10	102.8	4.5	0.3	-1.7^{X}	0.1
C6	106.3	5.0	3.6^{XX}	-2.4^{XX}	0.3
O2	102.2	5.1	-2.1^{X}	0.8	0.2
E4	101.9	5.4	6.4^{XX}	-2.9^{XX}	2.8^{XX}
E4	100.9	6.1	9.1^{XX}	-4.3^{XX}	1.6^{XX}
O1	98.8	8.8	1.8^{XX}	1.0	1.2^{XX}
N1	96.0	9.0	4.0^{XX}	0.0	0.3

Single and double asterisks indicate significance at the .05 and .01 levels of probability.

Table 6.

Effect of the presence of heterozygous pairs of chromosomes on the degree of additive combination - wing length.

Series	(A)	(X) - (A)	Components of Interaction h_A	h_X	I
N2	207.0	2.1	-1.9	-1.1	0.7
N2	212.7	0.9	2.0^{XX}	-0.6	0.2
N3	208.6	4.0	5.8^{XX}	-2.6	2.6^{XX}
N3	208.4	-1.6	-6.6^{XX}	5.2^{XX}	4.0^{XX}
R2	203.9	9.7	-1.7	2.3	0.1
R1	204.2	7.8	-1.8	-0.3	1.1
C10	201.4	5.3	-1.0	-3.2^{X}	0.1
C6	204.7	7.3	4.7^{X}	-3.4^{X}	5.5^{XX}
02	192.0	14.0	-4.3^{X}	3.9	0.3
E4	196.3	9.7	18.3^{XX}	-9.5^{XX}	19.9^{XX}
E4	196.0	16.6	19.2^{XX}	-7.8^{XX}	14.6^{XX}
01	184.8	22.0	1.9	2.1	3.8^{XX}
N1	193.9	15.2	8.6^{XX}	-1.4	2.4^{XX}

Single and double asterisks indicate significance of the .05 and .01 ~~lines~~ levels of probability.

tells much the same story, except that there is a large interaction I for both tests with E_4. Evidently the estimates of each chromosome substitution effect are very inconsistent for chromosomes of E_4, even when made in a partially heterozygous background. There is also more evidence of interactions in the partial heterozygotes in other series for wing length than for thorax length.

The two E_4 series show particularly striking inconsistencies in the substitution effects for both characters. Thus taking the first E4 series for wing length and subtracting 196.3 units from all dimensions we can compare the observed and expected sizes of AAA and XXX as follows:

	Observed size	Expected size.
XXX	9.7	$9.7 + h_X = 0.2$
AAA	0	$0 + h_A = 18.3$
XXX - AAA	9.7	-18.1

Although the triple heterozygote is larger than the homozygote, substitutions of the form (X-A) in a partially heterozygous background reduce size, so that the expected value of XXX is much less than that of AAA. Similar results are obtained with both wing and thorax length for the two E_4 series. In these cases all three components of interaction are substantial, and the attempt to locate the genotypes which are the main sources of interaction fails. It is of particular interest that the degree of interaction may be very different when chromosomes from

different lines are substituted on the same line. Thus N_2, N_3 and E_4 have each been used in two such tests. Only in the case of substitutions in E_4, do the estimates of h_A, h_X and I run parallel. In the others, the estimates of h_A are opposite in sign and generally significantly different. On theoretical grounds, we might expect that such effects are more typical of substitutions from lines drawn at random, than the consistency of the effects of substitutions in E_4, but this problem requires more detailed study.

Summing up, groups of genotypes of a series AAA→XXX tend to show interaction between non-homologous chromosomes particularly when the homozygote AAA is small or the difference XXX - AAA is large, and in these cases the major source of interaction is

usually a reduction of size in the homozygote, with some tendency for the triple heterozygote also to be longer than we should expect from substitution estimates made in a partly heterozygous background.

(ii) Egg production. Fewer experiments are available for study of the effects of chromosome substitution on egg production than for size. The analysis is carried out in exactly the same way; except that the data have been transformed to natural logarithms for the reasons discussed above. Applying the general Least Squares test, we find that half the comparisons show significant departure from an additive scheme, (Table 7). Egg production and size agree, therefore, in providing examples of both additive and non-additive combination of non-homologous chromosomes. It will be noted that the error variance is particularly large in the experiment involving the N_1/N_2 substitutions. High phenotypic variability is often encountered in the study of egg production and presumably depends largely on uncontrollable variations in the nutrition of the larvae.

The results of the more discriminating least squares analysis are set out in Table 8, following exactly the same scheme as for size. The most striking feature of the Table is that h_A is consistently positive, even in series where the interactions are not statistically significant. Hence, there is good evidence that the performance of homozygotes is lower than might be expected from the partial heterozygotes with even greater

Table 6.7

Least squares test of additive combination of chromosome effects on egg production.

Units of 10 $\log_e$ (eggs per day)

Series	Mean square of deviations	Error variance
R1	9.17^{xx}	0.69
C6	3.23^{xx}	
R2	2.97^{xx}	0.64
C10	0.85	
N1	1.80	1.96
N2	7.10^{xx}	
N2	0.42	1.06
R2	0.53	

The comparisons are expressed in units of 10 $\log_e$ (egg output) for ease of reference. There are 4 degrees of freedom in all series other than that relating to substitutions of C10 chromosomes in R2 background, where there are only 3.

consistency than in body size. The interaction variance is insignificant in all except one case, while h_X, although not statistically significant, is generally of smaller absolute magnitude than h_A, and tends to be negative. Thus it appears that the attributes of the homozygous combinations are chiefly responsible for the occurrence of interaction, and residual interaction, though present, is relatively less important. There are indications of the same trend as that reported for size, namely h_A tends to be correlated with the difference between the homozygous and fully heterozygous type (XXX - AAA), and lines with the lowest egg output tend to be associated with higher values of h_A. Hence homozygous genotypes which are responsible for low egg production are particularly associated with the interactions observed in chromosome exchange and the presence of at least one heterozygous pair of chromosomes tends to establish conditions which are more favourable for additive combination of chromosomes. It will be noted where the line N_2 occurs in two tests, that h_A is insignificant in one, highly significant in the other, and that the performance of the homozygote is considerably lower in the second test. It is unlikely, however, that the significant value of h_A is due to low value of the homozygous type alone, since the general level of egg production of different genotypes often shows parallel fluctuation in successive tests, due presumably to differences in the constitution of different batches of medium and the growth of the yeast with which it is seeded. Hence these contrasts may probably be taken

Table 8.

Effect of the presence of heterozygous pairs of chromosomes on degree of additive combination - egg production. (Units of 10 $\log_e$ daily output)

			Components of Interaction		
Series	AAA'	XXX - AAA	h_A	h_X	I
N2	38.2	4.35	1.20	-0.84	0.07
$R2^{xx}$	38.0	4.56	2.06	-1.35	0.26
N1	38.3	4.88	3.09	-1.47	1.80_{xx}
$C6^{xx}$	33.8	8.86	2.31	0.51	4.77^{xx}
$R1^{xx}$	32.4	10.29	8.45^{xx}	-0.41	1.29
$N2^{xx}$	32.8	10.45	7.74^{xx}	-1.93	2.06
C10	33.6	10.50	1.18	1.20	0.59

' It may be noted that on this scale an addition of 4 units represents a 50% increase in egg output.

as a further illustration of the different degrees of interaction often encountered when chromosomes from different lines are substituted in the same background.

(b) Variability.

(i) Body size. The phenotypic variation between individuals of a single genotype reflects their sensitivity to the variation of external conditions. Inbred lines are consistently more variable than the crosses between them and earlier chromosome analysis demonstrated an inverse relation between the variability and the level of heterozygosity, as measured by the number of heterozygous pairs of chromosomes (Robertson and Reeve, 1953b).

The present experiments take the analysis of this problem a stage further.

The analysis of the variability is based purely on the variation within cultures. The variance of wing and thorax length has been expressed in squared coefficients of variation, which is equivalent to using a logarithmic transformation, but for egg production the data were transformed to natural logarithms. The variability of corresponding types has been averaged over all experiments and tabulated in order of increasing heterozygosity in Tables 9 and 10. The analysis for wing and thorax length, given in Table 9, shows the familiar reduction of variability in crosses between inbred lines, compared with the parent lines - a reduction on the average of 40% for thorax length and 27% for wing length. Comparing the averages for genotypes with particular chromosomes heterozygous, it will be seen that the presence of a single pair of heterozygous chromosomes always causes a marked decline in variability below the level of the homozygote, but there is little sign of any further reduction when a second chromosome pair is made heterozygous. The variance is, however, further reduced in the triple heterozygotes. The exact relationship between variance and level of heterozygosity cannot be judged from these figures, since a small part of the variance in the partial heterozygotes may be genetic, due to segregation of the uncontrolled fourth chromosomes.

Turning now to egg production (Table 10), we find a striking

Table 9.

Variability of wing and thorax length in relation to the number of heterozygous pairs of chromosomes.

Chromosome pairs heterozygous	Number of types tested	Squared % coefficient of variation: Average variability		Percentage reduction in variability	
		Wing	Thorax	Wing	Thorax
0	14	2.30	2.79		
I	13	1.87	2.13		
II	14	1.92	2.02		
III	14	1.54	2.39		
Average		1.78	2.18	23	22
I + II	14	1.94	2.43		
I + III	14	1.87	2.15		
II + III	14	1.76	1.86		
Average		1.85	2.15	20	23
I + II + III	7	1.68	1.67	27	40

Table 10.

Average variance and mean of log egg production for different levels of heterozygosity.

Chromosome pairs heterozygous	Units of 10 $\log_e$ (daily egg production) Variance		Mean
0		26.7	35.7
I	6.8		38.8
II	4.4		41.0
III	3.2		42.4
Average		4.77	
I + II	4.4		39.9
I + III	3.0		42.3
II + III	3.7		43.1
Average		3.70	
I + II + III		2.47	43.7

inverse relation between variability and number of chromosome pairs heterozygous. The variance of the homozygote is reduced by about 80% when a single pair of chromosomes is made heterozygous, and a further steady (though much less rapid) decline in variance occurs as the number of heterozygous pairs of chromosomes increases from 1 to 3. There is even a tendency among the single heterozygotes for the variance to decrease in the order I, II, III, i.e. as length of chromosome heterozygous increases, although such a tendency is not visible in the variance of body-size. It should be noted that the variance of fecundity in Table 10 (units of 10 $\log_e$ daily output) must be multiplied by 100 to bring it on to the same relative scale as the variance of size in Table 11 (units of $(100\sigma/m)^2$), so that the relative variance of fecundity is about 200 times as great as that of body-size.

It is clear from these results that the mean, variance and level of heterozygosity are all inter-related in the case of each character, in that the mean generally increases and the variance falls as heterozygosity increases; and it is therefore of interest to attempt to separate the effects on variance of variations in mean and in level of heterozygosity, by a partial regression analysis. For this purpose, we note that the 14 of the 15 genotypes provided by each experiment (as listed in Table 1) can be grouped in pairs such as (AAA, BBB), (XAA, XBB), (XXA, XXB), etc., such that the two genotypes of a pair are identical

for their heterozygous chromosomes and carry respectively A and B homozygotes for the rest. This gives seven pairs and the unpaired triple heterozygote XXX, making eight groups in all. If y is a suitable measure of the variance of a genotype, and x of its mean, we can calculate the regression of y on x both within pairs of genotypes of equal heterozygosity and between pair-means (XXX being considered as a pair mean).

The within-pair regression is a partial regression of variance on mean, with level of heterozygosity held constant, and may be compared with the regression on the pair averages, for which both mean and heterozygosity vary. Further, the level of heterozygosity (H) may be roughly measured in terms of length of chromosome heterozygous, and the partial regression of variance on mean, with H constant, calculated within and between pairs, can be compared. This comparison, together with the between-pair regression of variance on H, should give some idea of the independent effects of mean and heterozygosity on variance.

This analysis has been confined to fecundity and thorax length, since wing length adds little to the latter. Both variables are measured in logarithmic units, for reasons given earlier, and the variance has been measured as $y = \log_e s$, where s is the standard deviation for any genotype. Weighted mean squares of y are calculated, the weights being the numbers of degrees of freedom of s, so that the mean squares have the expected value of 0.5. The use of $\log_e s$ reduces the effect of the rather

striking non-linear trend of variance of fecundity with increasing heterozygosity, shown in Table 10. H has been taken as proportional to the metaphase chromosome length heterozygous (after Gowen, 1952), I, II and III being rounded off as 3.1, 4.4 and 5.6 units. No significant differences between experiments were found, and the mean squares etc. were pooled within experiments.

The analysis is summarised in Table 11, and gives the regression and residual mean squares, with their degrees of freedom (D.F.) and some regression coefficients (b). Rows are numbered for easy reference. (A) shows the analysis within pairs of genotypes of equal heterozygosity, and (B) and (C) give two ways of looking at the regression of variance on mean and H for pair averages.

(A) shows that the regression within pairs is negative, but not significantly so, for both characters; hence the environmental variance has at most a slight tendency to decrease as the mean increases without changing the heterozygous chromosomes. Comparing (2) with (3), there is some heterogeneity in the residual variance, due either to genetic effects on variance but not on mean, or possibly to the segregation of the uncontrolled fourth chromosome.

The analysis of pair averages differing in heterozygosity (B and C) shows that the variance has a strong negative correlation with both H and mean (items 4 and 7); but if we keep H constant there remains a significant negative correlation between variance and mean (5), while keeping the mean constant leaves no significant

Table 11.

Regression of log σ^2 on mean and level of heterozygosity. (All variances pooled within experiments).

Source of variance	Thorax Length			Daily egg output		
	D.F.	Mean square	b	D.F.	Mean square	b
(A) Within Pairs of Equal Heterozygosity						
(1) Regression on mean	1	2.54	-0.028	1	1.00	-0.37
(2) Remainder	47	0.91^{xx}		25	1.07^{xx}	
(3) Theoretical error	-	0.50		-	0.50	
(B) Between Pairs Averages						
(4) Linear regression on H	1	10.6^{xx}	-0.017	1	24.0^{xx}	-0.05
(5) Partial regression on mean	1	4.0^{x}	-0.048	1	31.2^{xx}	-1.45
(6) Remainder	46	1.33		24	1.25	
(C) Between Pair Averages						
(7) Linear regression on mean	1	14.5^{xx}	-0.054	1	52.0^{xx}	-1.07
(8) Partial regression on H	1	0.1	-0.003	1	3.2	+0.03

x Statistically significant at 1 in 20 level
xx " " " 1 in 100 level.

partial correlation between variance and H (8). The analysis between pairs thus shows a stronger trend for variance to decline with increase of mean when H is constant, than is suggested by the analysis within pairs, particularly for egg production. This does not appear to be caused by H being an inadequate measure of chromosome length, since it turns out that no other choice of lengths will noticeably increase the linear regression of variance on H. The residual variances between and within pairs (items 6 and 2) are of about the same magnitude, and thus suggest the same level of heterogeneity.

In interpreting these results, it must be noted that mean and H are highly correlated ($r = +0.8$ for both characters), so that their independent effects are hard to separate. In view of the origin of the genotypes, much of the variations in mean must be the result of variations in heterozygosity, and it is natural to take heterozygosity as the primary variate. It then appears that variations in heterozygosity have little or no effect on variance apart from their effects on the mean, while increase of mean without changing the level of heterozygosity tends to reduce the variance. This would lead us to suppose that large inbred lines would have a lower variance than small inbred lines, while homozygotes and heterozygotes of the same mean would not differ in variance. But such a conclusion must be accepted with reserve, in view of the rather contradictory results of analyses (A) and (B). Thus for fecundity, the within-pair regression coefficient of

variance on mean (-0.37 ± 0.38) is significantly less than the partial regression coefficient between pairs (-1.45 ± 0.29), at P = 0.05. Fortunately this tentative conclusion is open to further test.

It is interesting that there is evidence of similar relations between the mean and variability of egg production, when different levels of the former are due to environmental rather than genetic causes. The data refer to several experiments in which samples of the wild Crianlarich stock were reared in a series of replicates and the flies were measured and their egg production recorded. The flies in any experiment represent random samples from the same population, and differences between cultures may be attributed primarily to environmental causes. The regression of variability on the mean, calculated in the same terms as above is negative and clearly significant (b:=-1.7 ± .16, D.F. = 70) for egg production, but quite insignificant for thorax length (b:= -.03 ± .04). It will be noted that the regression for egg production does not differ significantly from that quoted in Table 11, but it is uncertain how this resemblance should be interpreted. The contrast in the behaviour of size and egg production is doubtless related to the greater general sensitivity of the latter to environmental variation.

(c) Relative effects of different chromosomes.

As we have seen already, there is a high correlation between the degree of heterozygosity, measured in terms of metaphase

Table 12.

Relative chromosome substitution effects.

	Effects on Mean			Effects on Variance			
Chromosome	Wing length	Thorax length	Egg output	Wing length	Thorax length	Egg output	Metaphase length (Gowen)
I	20	18	23	30	27	53	56
II	59	51	65	61	163	59	79
III	100	100	100	100	100	100	100

chromosome length, and both mean and variance. It is not possible to reduce significantly the residual variance in the partial regression of variability on heterozygosity, holding the mean constant, by choice of alternative estimates of chromosome length. It is therefore of interest to bring together all the available estimates of the relative activity of the different chromosomes for the different characters. Simple least squares estimates of chromosome length, using all genotypes of a series, have been taken, e.g.

$$I = 1/4[XAA + XAX + XXA + XXX - AAA - AAX - AXA - AXX]$$

and the averages are expressed as percentages of the average effect of III in Table 12. These least squares estimates seem to be most appropriate, in spite of the occurrence of interaction, since they include estimates in the greatest variety of genetic background. The estimates based on the means are consistent for the three characters, and only one of the doubtless less reliable estimates based on variances, shows much disagreement with them. There is therefore little evidence of difference in the distribution in the chromosomes of genes which affect size and egg production, which rather suggests that the number of gene differences are large. Genetic differences in II and I appear to be about 60% and 20% as effective as genetic differences in III, in contrast to their relative metaphase length of 79% and 56%, so that the X chromosome appears to have only half as much activity per unit length as the autosomes. This may well be connected with the presence of single and double dose of the

X chromosome in males and females, and the possible advantages of a restriction of sex-linked effects.

The present analysis may be compared with the data of Straus (1942) which have been summarised by Gowen (1952), and which consist of a study of the effects on egg production of all combinations of major chromosomes from a pair of inbred lines, which showed heterosis when crossed. No interactions between non-homologous chromosomes were detected; the greatest effect was associated with the substitution of II and this was regarded as consistent with estimates of chromosome length based on per cent visible loci and cross-over units. However the present experiments, which have demonstrated both interaction and its absence in different tests, suggest caution in generalising from the effects of combining chromosomes from a single pair of lines, both with respect to presence or absence of interaction and also relative chromosome activity. Chance must obviously play a large part in deciding the distribution along the chromosomes of gene differences in any two inbred lines and the relation between chromosome length heterozygous and mean character expression could not be expected to have much significance when calculated for substitutions between two lines only. It is worth noting, too, that the error variance in Straus' experiments, due presumably to uncontrolled environmental variation, was much greater than in the present tests, and this would naturally lessen the chance of detecting statistically significant interactions.

(d) The correlation between size and egg production.

Since inbreeding lowers both size and egg production and since

the creation of different levels of heterozygosity, by substitution in homozygotes, presents so many parallels, we might expect a positive correlation between the two characters in the different experiments. In practice we find a consistently positive and generally significant correlation between mean thorax length and egg output for the 15 different types in each experiment, (Table 13). This, of course, raises the problem of how far such a correlation is truly genetic in that heterozygosity at the same loci and the same gene combinations produce parallel effects on both characters, or whether such correlation may be attributed merely to linkage between independently acting genes which resemble one another in the effects of their heterozygous combinations. We can test whether there is any evidence of close genetic correlation by grouping together parallel pairs of types in the same heterozygote categories as those used in the analysis of mean and variance and estimating the correlation from the variation within these categories, ~~due to differences in the level of heterozygosity~~. The four correlations for the different experiments are rather revealing (Table 13); in two there is no correlation, while the other pair show respectively a significant positive and negative correlation. On physiological and developmental grounds, it would be hard to reconcile such violent differences, including reversal of sign, with a close correlation between the effects of genetic changes in both characters. But, on the other hand, since we are dealing with the substitution of whole chromosomes and the possible replacement of many loci at a time, it would be unwise to place too much emphasis on the

Table 13.

The correlation between thorax length and egg production for all types and within specific heterozygote groups.

Experiment	All types	Within heterozygote groups
R1/C6	.757^{x}	-.074
R2/C10	.711^{x}	.774^{x}
N1/N2	.286	-.788^{x}
N2/R2	.548^{x}	.055

inconsistency of the 4 correlations as evidence for general lack of genetic correlation between size and egg production.

Discussion.

Body size and egg production represent two "characters" in the sense that variation in each can be separately evaluated in quantitative terms. Since they constitute different aspects of the integrated, adaptive responses of the animal, we might expect that variation in either character will have a somewhat different significance in relation to adaptation generally. Thus ability to produce more rather than fewer eggs, even under adverse conditions, would appear to be an adaptive asset; variation in body size is related to fitness more indirectly (and it is by no means obvious whether natural selection tends to favour a maximum or intermediate size). Such contrasts in the economy of the animal may be reflected in the qualities of both genetic and environmental variability, especially variation in larval nutrition, even under our culture conditions which are generally favourable for rapid growth, and it is of interest to look for contrasts in genetic behaviour as well.

Body size and egg production both decline with inbreeding, and when inbred lines are crossed the F_1 generally falls within the range of variation encountered among the individuals of outbred stocks but there is little doubt that size is relatively less affected than egg production. Thus, judged by thorax length,

body weight may decline by anything between 0 and 30%, while egg production is usually reduced by more than 50% when newly established wild stocks are inbred. Random fixation of genes which affect egg production is unlikely, since homozygous combinations which reduce egg production too much will be selected against during inbreeding; it is possible that comparable selection is less important in relation to body size. Evidently then, egg production may be regarded as far more sensitive than body size to both environmental and genetic changes.

In view of the widespread evidence of interaction between non-homologous chromosomes, revealed in the course of chromosome exchange between lines, it is clear that the heterosis which appears in crosses cannot be generally accounted for in terms of the summation of independent effects of dominance or overdominance; this supports the conclusions derived from a somewhat different method of chromosome analysis, already described (Robertson and Reeve 1953). Such analyses can, of course, only detect interaction between unlinked genes; but there is little doubt that the same sort of interaction occurs between linked genes as has been shown for genes located on non-homologous chromosomes. It appears that gene interactions play an important part in the genetic control of typical quantitative characters so that the effect of any gene substitution is likely to be far from constant when the genetic background is changed.

If we measure the heterosis in a cross by the deviation of

the F_1 from the mid-parental level, there appears, in the case of body size, to be an association between the extent of such heterosis and the likelihood of detecting genetic interaction in the course of exchanging chromosomes between the lines. Such interactions are detected most clearly when both lines are particularly small, or when chromosomes from a larger line are substituted in the background of a smaller one. In one set of reciprocal exchanges, i.e. between the lines R_1 and C_6, where the parents are comparatively normal in size, significant interactions are encountered; in this case the F_1 apparently exceeds the average size of wild stocks.

Analysis of the effects of chromosome exchanges on egg production provides parallel indications. There is a consistent tendency for the full homozygotes to have a lower egg production than we should expect from estimates based on chromosome substitutions made in a partially heterozygous background (h_A is always positive). Moreover the homozygotes with the lowest output generally show the most marked interaction, since they deviate most from expectation. It must be remembered that study of the effects of whole chromosomes greatly underestimates the variety and extent of genetic interaction generally, but, since the properties of whole chromosomes are assumed to reflect the properties of the genes they carry, the degree of interaction between chromosomes may be taken as a rough, but reasonably reliable guide, to the occurrence of interaction between genes.

Evidence for gene interaction, in studies of genetic variation,

generally implies that the effect of a given gene substitution is not constant, but may be enhanced, reduced or inhibited according to the genetic background. The genetic interactions revealed in these experiments do not appear to be of a haphazard nature. The second least squares test suggests that a good deal of, but by no means all, the responsibility for such interaction may be attributed to the properties of the homozygous combinations. The substitution of even a single chromosome from another line tends, on the average, to create conditions which favour a more additive combination of chromosome effects.

In view of the one way effect of inbreeding on size and egg production, it is hardly surprising to find an average relation between the increase towards the normal level of outbred stocks and the level of heterozygosity, as measured by length of metaphase chromosome. But such an average relation conceals a variety of individual differences in relative effect of corresponding substitutions in different series. Thus, in some cases, the presence of a single heterozygous pair of chromosomes, in an otherwise homozygous background, raises the level of one or other character to that of the cross between the two lines from which the chromosomes are drawn. The general tendency for single substitutions to return most of the way to the level of the fully heterozygous type is most evident for egg production and rather less so for size, in which the highest level is sometimes associated with the fully heterozygous combination. When chromosomes from

two different lines are substituted in the same homozygous line, the degree of interaction may be very different, although there is one example of consistency with respect to body size.

A further line of evidence is provided by the inverse relations between variability and both mean and level of heterozygosity. Within the limits studied, variability increases as size or egg production falls. The high correlation between mean and level of heterozygosity lowers our ability to test how far differences in heterozygosity may influence variability independently of the mean, but there are indications that it is the particular heterozygous combination of genes, rather than mere level of heterozygosity as measured by the metaphase chromosome length, which determines the level of both mean and variability. Thus there is no significant regression of variability on heterozygosity after removing the variance due to regression on the mean. Within equally heterozygous types, the regression of variability on the mean is negative, for both size and egg production, although not significantly so. It may also be noted that a highly significant residual variance is left after taking out the regression on the mean, suggesting that particular gene combinations may influence variability, to some extent, independently of their effects on the mean.

For egg production there is also evidence of an inverse relation between mean output and variability, when the latter is primarily of environmental origin. There is no sign of this in

body size, but it must be remembered that the same range of environmental variation has less effect on size than egg production. The association of effects - more normal size or egg yield, decline in variability and diminished evidence of genetic interaction - evidently all stem from the replacement of homozygous by heterozygous combinations, and we must consider the wider implications of these apparent inter-relations.

Inbreeding highly heterozygous wild stocks produces a genotype not likely to arise as a result of the normal processes of segregation, and leads to less favourable conditions for normal development which results in diminished size and egg production and increased sensitivity to environmental variation. It could be maintained that inbreeding destroys the original "genetic balance" while outcrossing restores it. But to use such a term as "genetic balance" begs the main question and throws no light on the basis for regularities associated with inbreeding, outcrossing and chromosome exchange. These are, however, raw material for any attempt to understand the genetic attributes of adapted wild stocks.

These experiments have focussed attention on the effects of heterozygous, as opposed to homozygous, combinations. Presumably size and egg production may be influenced by loci distributed over all chromosomes; hence increase in the number of heterozygous pairs of chromosomes is equivalent to making numerous gene substitutions. It does not follow that normal size or egg production can only be attained with heterozygous gene combinations.

Thus, as noted earlier, the average reduction in size by inbreeding is small, while, by selection, followed by inbreeding, it has been possible to establish a number of homozygous lines which exceed the average size of the parent stocks (Robertson and Reeve, in the press). It is likely that characters differ with respect to the variety of homozygous combinations which are consistent with a normal level of performance and this will be reflected in the average response to inbreeding as in the contrasted behaviour of size and egg production. It is significant that the latter appears to be relatively more sensitive than body size to environmental variation. A high level of egg production provides a stringent test of the animals' efficiency in the rapid conversion of nutrients and makes exacting demands on environmental conditions; we might expect such a character to be relatively more sensitive to genetic changes than body size. It is a reasonable inference that it would be relatively more difficult to find homozygous combinations which are consistent with normal egg production than is the case for body size.

These considerations are not intended to underrate the possible importance of heterozygous gene combinations, but rather to underline the qualifications which must be borne in mind, when interpreting the results of the chromosome analysis. Since heterozygous combinations involve greater genetic diversity, it could be argued that they are therefore more likely to approximate to the genetic conditions for normal growth which appear to be consistent with a great variety of gene combinations. The

relative importance of specific heterozygous combination is likely to vary with the genetic background and the more unfavourable the genetic background for normal development, the greater the superiority, on the average, of heterozygous combinations. This is consistent with the evidence that the most striking interactions are associated with the substitution of chromosomes in lines of smaller size and lower egg production.

In general, as the genotype changes in the direction of smaller size or lower egg production, the greater the evidence for genetic interaction. Gene combinations which favour normal performance and which reduce the importance of interaction will be responsible for a reduction in the phenotypic variability of many loci are heterozygous, and this has an obvious bearing on the interpretation of genetic variability of quantitative characters in wild stocks. Inbreeding, leading to more or less random fixation, is likely to cause changes which make segregation of genes at unfixed loci more evident than is normally the case, especially if the genetic changes are generally unfavourable. Thus the interpretation of phenotypic variation in populations undergoing inbreeding is likely to be particularly difficult; fixation tends to reduce variation due to segregation, but is also likely to be accompanied by an increase in the phenotypic effects of segregation, and, also, an increase in the sensitivity to environmental conditions.

The genetic control of differences in sensitivity to external conditions merits more detailed study, since interpretations will

vary somewhat according to the nature of the environmental variation and the way in which it influences the phenotypic level of size or egg production. Such variation may be effective at all stages of development, but some stages are likely to be more influenced than others by the sort of variation encountered in a given series of experiments. The increase in sensitivity to environmental conditions with genotypes which lead to lower levels of performance, and which are also associated with increased evidence of genetic interaction, suggests that developmental processes are subject to limitations arising possibly from the slower rate of basic reactions and/or increased competition for nutrients. Changes, either of genetic or external origin, are likely to lead to a disproportionately large effects in such a situation. In general, the relative importance of environmental variation on the outbred population is likely to provide a reasonable guide to the resistance to inbreeding of different characters.

Although body size and egg production represent different aspects of adaptation, the present experiments indicate similarity in the general properties of the genetic variation which determines their levels of expression. The phenomena encountered in the chromosome analyses of size appear in more exaggerated form in egg production, which is more sensitive to external conditions and suffers a greater decline with inbreeding, while individual substitutions in homozygous backgrounds return relatively further

towards the normal level. It is possible that the level of egg production may provide a better measure of the rate and efficiency of growth than body size, and this naturally raises the question as to how far the genetic conditions which favour the normal level of both characters are the same or different. However, further discussion must await the outcome of experiments designed to throw further light on this problem.

SUMMARY

1. By a special chromosome assay technique, it is possible to prepare genotypes consisting of various combinations of chromosomes from two inbred lines, and thus to study the effect of individual chromosome substitutions in different genetic backgrounds.

2. This method produces 15 genotypes for any pair of inbred lines, A and B, consisting of the parent lines and their F_1, together with the 12 genotypes carrying one or two chromosome pairs heterozygous for A and B and the remaining chromosomes either all A or all B. It has been used in an analysis of the heterosis for size and egg production in a number of crosses between unselected inbred lines.

3. All pairs of lines show heterosis for each character on crossing. A least squares test shows that in many cases the separate effects of making the three chromosomes heterozygous do not combine additively in the triple heterozygote, so that interactions between genes on non-homologous chromosomes often have

marked effects on all characters. Similar interactions between linked genes doubtless also occur, and it is likely that epistatic gene effects are generally of importance in quantitative characters.

4. By studying the set of genotypes consisting of a pure line A and all genotypes obtained by making one or more of the major chromosome pairs heterozygous for line B, we can divide the interaction into three portions consisting of (a) interaction between chromosomes in a partially heterozygous background, (b) deviation of the triple heterozygote A/B, and (c) deviation of the homozygote A from their expected values as estimated from substitution effects in a partially heterozygous background. This test has been applied to each set of genotypes for all three characters.

5. With a few exceptions, interactions among partial heterozygotes were slight, while the triple heterozygote tended to be a little greater than expectation. But the main source of interaction was located in the inbred line homozygotes, which were rather consistently lower in mean than their expected values. Generally, the lines with the lowest means showed the most interaction of this kind, so that the highest level of interaction in the homozygote tended to occur where the inbreeding decline was greatest.

6. Difficulties of interpretation arise from the fact that the amount of interaction shown by a given inbred line could be very different when it was tested against chromosomes of two different lines, and it is not yet clear how consistent such estimates are

likely to be.

7. In the series examined, the environmental variance declines with increasing level of heterozygosity for all three characters, though not necessarily in a linear manner. An attempt was made to separate the effects of the mean and the level of heterozygosity on the environmental variance, using a partial regression analysis. The mean and the level of heterozygosity (length of metaphase chromosome heterozygous) are closely correlated in these series, and both are closely correlated with the variance, so that a complete separation of their effects was impossible. But the analysis suggests that it is the mean and the particular gene combination responsible for it, rather than level of heterozygosity per se, which is the main factor in determining the variance.

8. Estimates of chromosome substitution effects averaged for a number of inbred lines should give a measure of the relative activity of genes on the different chromosomes, activity depending on the number of genes on a chromosome affecting a given character and their mean effect. Estimates of relative activity, averaged over all lines, were obtained for changes in mean and variance of wing and thorax length and egg output. They were remarkably consistent in showing II to have about 60% and I only about 20% the activity of III. The three characters showed essentially the same distribution of effects, which suggests that many genes are involved per chromosome. The activities of II and III are roughly in proportion to their metaphase chromosome lengths, but

that of ♀ much less in proportion. This is thought to be a dosage compensation effect, arising from the fact that I is hemizygous in males.

9. The general implications of these results are discussed and it is suggested:

(a) Interpretation of the phenotypic variation in populations undergoing inbreeding has to take account of the increased effects of segregation at unpaired loci as well as the greater sensitivity to environmental variation.

(b) The relative importance of environmental variation in different characters in out-bred populations may provide a reasonable guide to their resistance to inbreeding.

(c) The increased sensitivity to external variation and the greater importance of interaction, which accompanies changes in the genetic constitution which lowers the level of performance, opens possibilities of investigating the nature of the environmental variation and the stages of development at which it is most effective. This is likely to throw further light on the attributes of the gene-controlled changes which lower performance as well as the effects of gene combinations, heterozygous or otherwise, which are involved in striking interactions.

Bibliography

Hanson, F. B. & Ferris, F. R. (1929) A quantitative study of fecundity in Drosophila melanogaster. J. Exp. Zool., 54, 485-506.

Robertson, F. W. & Reeve, E. C. R. (1952a). Studies in quantitative inheritance. I The effects of selection of wing and thorax lengths in Drosophila melanogaster. J. Genet. 50, 414.

_____ (1952b) Heterozygosity, environmental variation and heterosis. Nature, 170, 296.

_____ (1953) Studies in quantitative inheritance. IV The effects of substituting chromosomes from selected strains in different genetic backgrounds. J. Genet. 51, 586.

_____ (1) Studies in quantitative inheritance. V. Chromosome analysis of crosses between selected and unselected lines of different body size in Drosophila melanogaster. J. Genet. (in the press).

_____ Studies in quantitative inheritance. VII. Crosses between strains of different body size in Drosophila melanogaster. (In the press).

Robertson, F. W. & Sang, J. H. (1944). The ecological determinents of adult flies. I. Fecundity of adult flies. Proc. Roy. Soc. B, 132, 259.

Reeve, E. C. R. & Robertson, F. W. (1953). The analysis of environmental variability in quantitative inheritance. Nature, Lond., 171, 874.

Shapiro, H.(1933). The rate of oviparation in the fruit fly, Drosophila. Biol. Bull. Woods Hole, 63, 456.

Acknowledgements.

We wish to thank Miss Evelyn Davidson and Miss Alexa Hunter for technical assistance and Mr. Charles Marr for assistance in computation.