STUDIES IN QUANTITATIVE INHERITANCE

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

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(With Two Text-figures)

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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 & Reeve, 1952*a*), based on general considera-

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tions, 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 & Robertson (1953*a*). 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 & 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. Experimental material

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 & Reeve, 1952*a*).

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 forty 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

Table 1. *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: 0·56 (male), 0·62 (female); and of thorax length: 0·29 (male), 0·32 (female).

corresponding differences in general body size, with which these dimensions are highly correlated. 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, two alternative homozygous and one heterozygous combinations, i.e. $3 \times 3 \times 3$ or a total of 27 possible combinations. In males, since there are only two alternatives for I, there are only eighteen combinations. To simplify reference to so many different genotypes, a notation is used in which any genotype can be specified by three 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 eight 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 two alternatives for the X-chromosome. Thus, as a specific instance, males of the type DXX can be prepared from such crosses as NNN × DDD, DNN × DDD or NDD × DNN; the male parent is always quoted first. Similarly, females of the constitution XXX can be derived from no less than five different crosses. This is of considerable value, since it provides an exacting

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

Fig. 1. The production of different genotypes. 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.

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 are indicated in Fig. 1.

(*b*) *The preparation of the basic types*

The preparation of the six 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 CyL^4 (II) and *Mé* 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 *Mé*. 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 *MéSb*, recombination between *Mé* 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 *D. melanogaster*, has been studied by Steinberg (1936) and commented on by Gowen, Stadler & Johnson (1946).

Backcrosses

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

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

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

Similarly for *Cy MeSb*, *CyL Me*, *CyL MeSb*.

	I	II	III
		Chromosome replacement	
4.	$\frac{B}{Y}\ \frac{Cy}{B}\ \frac{MéSb}{B} \times \frac{C}{C}\ \frac{CyL}{C}\ \frac{Mé}{C}$	$\frac{C}{Y}\ \frac{CyL}{C}\ \frac{MéSb}{C} \times \frac{B}{B}\ \frac{Cy}{B}\ \frac{Mé}{B}$	
5.	$\frac{C}{Y}\ \frac{CyL}{B}\ \frac{Mé}{B} \times \frac{C}{C}\ \frac{Cy}{C}\ \frac{MéSb}{C}$	$\frac{B}{Y}\ \frac{Cy}{C}\ \frac{MéSb}{B} \times \frac{B}{B}\ \frac{CyL}{B}\ \frac{B}{B}$	$\frac{B}{Y}\ \frac{CyL}{B}\ \frac{Mé}{C} \times \frac{B}{B}\ \frac{B}{B}\ \frac{MéSb}{B}$
6.	$\frac{C}{Y}\ \frac{Cy}{B}\ \frac{MéSb}{B} \times \frac{C}{C}\ \frac{Cy}{B}\ \frac{MéSb}{B}$	$\frac{B}{Y}\ \frac{CyL}{C}\ \frac{B}{B} \times \frac{B}{B}\ \frac{CyL}{C}\ \frac{B}{B}$	$\frac{B}{Y}\ \frac{B}{B}\ \frac{MéSb}{C} \times \frac{B}{B}\ \frac{B}{B}\ \frac{MéSb}{C}$
	Pair matings; parents tested		Pair matings; parents tested
	CBB	BCB	BBC

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

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 *Moiré* (*Mé*), with or without the dominant *Stubble* (*Sb*). The differently marked chromosomes are referred to in Fig. 2 and hereafter as *Cy*, *CyL*, *Mé* and *MéSb*. Even in the presence of either *Mé* or *MéSb* the *Cy* and *CyL* inversions are excellent cross-over suppressors, although recombination does occasionally occur. *Mé* alone or in combination with the *Cy* inversion is also good, although there appears to be about 3 % recombination at the extreme tip in the region of *ru*. *MéSb* 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.

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 *MéSb* females will lead to confusion as to the genotype of males which are phenotypically *Cy MéSb*, 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*, *Mé* 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:

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

$$\downarrow$$

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

$$\downarrow$$

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

$$\swarrow$$

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

The procedure for the S/E 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, are expressed in $\frac{1}{100}$ mm. and are based on the measurement of five males from each of five 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.

Table 2. *Test of Y-chromosome differences*

Origin of Y	Background	Wing	Thorax
D	N	190·43±0·59	94·95±0·26
N		190·25±0·59	94·98±0·26
S	E	179·25±0·98	93·60±0·40
E		180·29±0·98	93·08±0·40

The means are in $\frac{1}{100}$ mm.

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	2·3	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	2·1	—	

The standard error of the difference between means based on pooled variation within replicates of the same type is 0·99.
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.

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	♂	♀	Type	Deviation
NND	NND	NND	0·7±0·84	EES	EES	EES	1·1±0·77
DND	NND			SES	EES		
NDN	NDN	NDN	1·1±1·30	ESE	ESE	ESE	1·5±0·81
DDN	NDN			SSE	ESE		
NNN	NNN	NNN	0·5±0·86	EEE	EEE	EEE	3·0±0·82**
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±0·75
DND	NNN			SES	EEE		
DDN	NNN	NXN	0·1±1·10	SSE	EEE	EXE	1·4±0·79
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±0·91
DNN	NDD			ESE	EES		
NDN	NND						
NDD	NNN						

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

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 type 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 ten vials containing fifty eggs each were set up for each type, except when replicated in different crosses in which case five or six 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 randomized within an incubator at $25 \pm 0{\cdot}5°$ C. Both wing and thorax length of five males and five 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 & Reeve, 1952*a*).

4. The statistical analysis of the data

Five flies from each of ten 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_b^2). 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 & Reeve, 1952*b*; Reeve & Robertson, 1953*b*), 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_b^2 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 $V_m = \frac{1}{N}(\sigma^2 + n\sigma_b^2)$, 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 & Reeve, 1953).

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 × DDD (1♂, 1♀), NND × DDD (1♂), NDN × DDD (1♀).

The chromosome analysis which was described in an earlier paper (Robertson & Reeve, 1953) 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 $\frac{1}{100}$ 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 & Reeve, 1953), 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.

Table 5. *Observed sizes of types in the D/N analysis* ($\frac{1}{100}$ *mm.*)

	Female		Male	
Type	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.

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 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 & Reeve, 1953), 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

Table 6. *Comparison of the effects on wing length of single and double substitutions of a D chromosome for its N homologue in an N background*

	Male		Female	
Chromosome	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 $\frac{1}{100}$ mm. as deviations from NNN.

noting that the dominance appears incomplete in I, as has 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*) *Interaction 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 twenty-seven genotypes in females can all be expressed as linear combinations of one or more of seven constants (see Robertson & Reeve, 1953), 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 and C = single and double substitutions of II and III.

Thus we have

$$NNN = n,$$
$$NXN = n-b,$$
$$DDN = n-A-B, \text{ etc.}$$

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 nine differences representing each substitution effect, calculated in all possible genetic backgrounds. In other words

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

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

Finally, n is estimated as

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

where Σ is the sum of the observed values of all genotypes. In males we have only eighteen genotypes, and the equations are modified as follows:

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

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

Also, $A = \frac{1}{9}$[sum of nine genotypes with N first chromosome minus sum of nine 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 8, 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.

Table 7. *Least squares analysis of the D/N series: wing length* ($\frac{1}{100}$ *mm.*) *Deviations: Observed – expected size*

	Type	Female 1	Female 2	Male 1	Male 2
1	NNN	−1·2	−1·9*	−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
0	DXN	−2·0*	−1·0	−2·4**	−0·8
11	DNX	−2·5*	−1·2	−1·6*	0·1
12	DXX	−0·7	1·4	−2·6**	−0·1
13	NDN	3·1**	1·5	2·7**	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*	0·2
17	NDD	−0·1	−0·2	−2·1*	−1·2
18	XDN	1·7*	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*	—	—
23	DDN	−2·9**	−3·4**	−1·1	−0·6
24	DDX	3·7**	4·1**	3·8**	5·4**
25	DND	1·6	3·4**	1·5	4·6**
26	DXD	7·7**	10·0**	6·1**	10·0**
27	DDD	−5·2**	−4·2**	−3·9**	−1·0

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

* Significant deviation from zero at $P=0{\cdot}05$.

** Significant deviation from zero at $P=0{\cdot}01$.

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 Wing 1	Female Wing 2	Female Thorax 1	Female Thorax 2	Male Wing 1	Male Wing 2	Male Thorax 1	Male Thorax 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	−0·41	− 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 are listed in the same row as the estimates of double substitutions of I in females.

Now if the situation is truly one in which non-interaction is the rule, then inclusion of the aberrant types in the least 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 seven constants have been recalculated, excluding the symmetrical group of five 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 2·64 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

Table 9. *Wing and thorax length of types showing major interactions in the D/N analyses* ($\frac{1}{100}$ *mm.*)

	Male		Female	
Type	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

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), 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 thirteen types from 4·35 to 1·09 (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 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 summarizes 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

Table 10. *Least squares analyses of the D/N series. Thorax length* ($\frac{1}{100}$ *mm.*)
Deviations: Observed – expected size

		Female		Male	
Type		1	2	1	2
1	NNN	−0·20	−0·47	0·11	−0·41
2	NXN	1·05	1·15*	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**	1·90**	—	—
7	XNX	−1·09	−0·99	—	—
8	XXX	−1·23*	−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*	−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*	1·60*	2·11**	2·82**
25	DND	−0·30	0·11	−0·19	0·18
26	DXD	2·75**	3·53**	2·48**	3·38**
27	DDD	−1·80*	−1·38*	−2·09**	−1·50*

Columns 1 and 2 refer to estimations based on the least squares analyses of respectively all types and types 1–22.
* Significant deviation from zero at $P = 0{\cdot}05$.
** Significant deviation from zero at $P = 0{\cdot}01$.

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 & Robertson, 1953*a*), 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 & Reeve, 1953); 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, 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

Table 11. *Comparison of substitution effect on wing length in different chromosome analysis experiments* ($\frac{1}{100}$ *mm.*)

		Male					Female				
		A			B			A		B	
I		$\frac{N-D}{Y}\ \frac{N}{D}\ \frac{N}{D}$	19·6	$\frac{N-D}{Y}\ \frac{N}{D}\ \frac{N}{D}$	25·5	(*a*)	$\frac{N-D}{D}\ \frac{D}{P}\ \frac{D}{H}$	21·6	$\frac{N-D}{D}\ \frac{D}{N}\ \frac{D}{N}$	19·7
						(*b*)	$\frac{N-D}{N}\ \frac{N}{P}\ \frac{N}{H}$	7·0	$\frac{N-D}{N}\ \frac{D}{N}\ \frac{D}{N}$	5·1
II	(*a*)	$\frac{D}{Y}\ \frac{N-D}{D}\ \frac{D}{H}$	4·1	$\frac{D}{Y}\ \frac{N-D}{D}\ \frac{D}{N}$	−1·0		$\frac{N}{D}\ \frac{N-D}{P}\ \frac{N}{D}$	−2·0	$\frac{N}{D}\ \frac{N-D}{N}\ \frac{N}{D}$	1·0
	(*b*)	$\frac{N}{Y}\ \frac{N-D}{N}\ \frac{N}{H}$	0·5	$\frac{N}{Y}\ \frac{N-D}{N}\ \frac{D}{N}$	−0·4					
III	(*a*)	$\frac{D}{Y}\ \frac{D}{P}\ \frac{N-D}{D}$	7·3	$\frac{D}{Y}\ \frac{D}{N}\ \frac{N-D}{D}$	−3·1		$\frac{N}{P}\ \frac{N}{P}\ \frac{N-D}{H}$	3·2	$\frac{N}{D}\ \frac{N}{P}\ \frac{N-D}{N}$	1·6
	(*b*)	$\frac{N}{Y}\ \frac{N}{P}\ \frac{N-D}{N}$	4·3	$\frac{N}{Y}\ \frac{D}{N}\ \frac{N-D}{N}$	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 chromosome.

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 8 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 types, 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.

Table 12. *Observed size of types in the S/E analysis* ($\frac{1}{100}$ *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*

	Wing		Thorax	
Substitution	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 of deviations	0·87	0·10	1·03	0·16
Error variance	0·60	0·30	0·70	0·32

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 chromosomes 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 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 & 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 analyses

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 & Reeve, 1953), 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 twenty-one out of the possible twenty-seven types were studied; these comprise the eight 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.

Table 14. *Heterosis in crosses between unselected inbred lines*

Dimensions in $\frac{1}{100}$ 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 × B	10·3 ± 0·85	4·1 ± 0·51
B × C	12·0 ± 1·45	5·5 ± 0·64

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 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

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.

Table 16. *Deviation between the full heterozygote and the single and double heterozygotes* ($\frac{1}{100}$ *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.

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 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 values 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 especially III is made homozygous. In homozygous backgrounds, 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.

Table 17. *The relative effects of different chromosomes* (*female wing length* $\frac{1}{100}$ *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.

(*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.

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

Table 18. *The effect of substituting homozygous pairs of chromosomes in different homozygous backgrounds* ($\frac{1}{100}$ *mm.*)

Chromosome pair	Substitution	Wing	Thorax
	A/B comparisons		
I	(A – B) BB	11·0	5·7
	(A – B) AB	– 6·9	– 3·9
II	BAB – BBB	10·2	4·4
	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	0·98	0·60
	B/C comparisons		
I	(B – C) CC	4·7	0·6
	(B – C) BC	6·3	2·8
II	CBC – CCC	6·0	0·2
	CBB – CCB	25·0	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	0·72

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 & Reeve, 1953). 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 chromo-

Table 19

Type	Deviation from DDD (wing length)
DNN	22·1
DXX	21·5
DXD	19·4
DDX	19·0

somes 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 & Reeve, 1953), 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.

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 asymmetry 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 criticized elsewhere (Robertson & Reeve, 1952*a*). 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 become 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.

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.

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