THE BALANCE OF POLYGENIC COMBINATIONS

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1. Linkage and variation

It is axiomatic that selection can produce an effect on the genetical composition of a population only if there exists a certain amount of genetical heterogeneity. This heterogeneity will commonly depend on the segregation and recombination of genes controlling the character on which selection is being exercised. Indeed, unless segregation and recombination are both occurring the reaction to selection will cease after a very few generations. Segregation itself, without recombination, can produce only a short-lived advance with selection. The reaction to a selective force must be largely dependent on the recombination of genes where a number of these affect the character in question, as is the case with polygenic characters.

The number of genotypes possible with n segregating genes, each of two allelomorphs, is 3^n in a diploid organism, and it is clear that if n is at all large, say 10 or more, it would be necessary to raise a great number of individuals in order that each genotype should be represented in a progeny or population. Even, however, if the possible genotypes are not all represented in any one generation some of those present will enjoy a selective advantage over the others and advances with selection will occur. The selected individuals will in turn give rise to fresh progenies containing segregant types and further selection will thus be possible in the next generation. This process will be repeated for a number of generations depending on the population size, the rigour of selection and the organization of the polygenes affecting the character. In general, all the valuable genotypes can be produced with a relatively small number of individuals in each generation provided that a sufficient number

of generations are raised, during each of which selection is exercised. The advance in each generation is small, but when continued over many generations will be in total a large one.

The speed of this reaction is conditioned by the freedom of recombination of the genes involved, as Goodale (1938) has pointed out. If all genes recombined freely the rate of advance would depend on the number of genes segregating, the size and breeding system of the population, the rigour of selection and the magnitude of the damping effect exerted by environmental influences on the manifestation of the character. Where, on the other hand, many of the genes are linked into a number of groups, depending on the number of chromosomes of the organism, the rate of advance with selection must also be influenced by the organization of the combinations of these polygenes within the chromosomes. If genes acting in opposite directions, conveniently described as plus and minus, are intermingled along the chromosomes many cross-overs will be necessary before the combination most favoured by selection is produced. Selection will be slow in action. If, however, the plus genes tend to occur in one or more chromosomes and the minus genes in others, the number of effective recombinations necessary for the full advance with selection will be small—smaller indeed than where all the genes are unlinked. Selection will then be very rapid. In this way the rate of advance in a given stock may be either decreased or increased by linkage. So in order to understand the action of selection on populations it is necessary first to know something of the organization of the polygenic combinations in the chromosomes.

Now the effect of linkage on the rate of advance will vanish when a large number of experimental crosses or a heterogeneous and previously unselected population is considered; for in such a case all types of combination in a chromosome will be involved, and for every combination which slows down advance there will be another which has an accelerating action. This can be seen from a simple example. Consider the effect of selfing doubly heterozygous individuals **AaBb**. When the loci of **A**, **a** and **B**, **b** recombine freely the various types of progeny are expected with the relative frequencies

	AA	Aa	aa
BB	1	2	1
Bb	2	4	2
bb	1	2	1

If we then suppose that the degree of expression of some polygenic character affected by both genes is equal to the number of capital letters in the genotype, the phenotypes will be

	AA	Aa	aa
BB	4	3	2
Bb	3	2	1
bb	2	1	0

and the mean expression in the family is 2·0.

Next let these individuals be selfed and selection exercised so that the relative numbers of progeny of each plant surviving to the stage at which the character is measured in the next generation is the same as the phenotypic expression, i.e. that **AABB** leaves on the average 4 offspring, **AaBB** and **AABb** 3 each, **aaBB**, **AaBb** and **AAbb** 2 each, etc. The next generation will then consist of the various genotypes in the proportions

	AA	Aa	aa
BB	30	16	18
Bb	16	8	8
bb	18	8	6

These will show the polygenic character to the same degree as the corresponding genotypes in the previous generation, and the mean expression of the population will be 2·5.

If the genes are linked in the original double heterozygote, the recombination frequency being 0·25, on both male and female sides, selfing will give the ten genotypes in the proportions

	AA	Aa	aa
BB	q^2	$2pq$	p^2
Bb	$2pq$	$2p^2/2q^2$ (*R*) (*C*)	$2pq$
bb	p^2	$2pq$	q^2

where $p+q=1$ and $p=0{\cdot}25$ when the parent was in the coupling phase **AB/ab** and $q=0{\cdot}25$ for the repulsion phase **Ab/aB**. Note that the class **AaBb** is divisible into coupling (*C*) and repulsion (*R*) double heterozygotes. The mean expression of the polygenic character is, as before, 2·0. This it may be noted is true no matter what values p and q may take.

These individuals are then selfed and selection is exercised as in the example of two unlinked genes already considered. The various types of progeny are found in the next generation with the relative frequencies

	Coupling ($p=0{\cdot}25$)				Repulsion ($q=0{\cdot}25$)		
	AA	Aa	aa		AA	Aa	aa
BB	401	102	73	BB	145	102	201
Bb	102	10 / 90 (*R*) (*C*)	54	Bb	102	90 / 10 (*R*) (*C*)	54
bb	73	54	65	bb	201	54	65

Taking the same phenotypic values for each genotype as were used in the previous generation the mean expression of the coupling progeny is 2·75 and of the repulsion progeny in 2·25. Thus when $p=0{\cdot}25$, i.e. in the

coupling phase, the advance in the mean expression is $2{\cdot}75 - 2{\cdot}00 = 0{\cdot}75$; when $p = 0{\cdot}5$, i.e. free segregation, the advance is 0·5; and when $p = 0{\cdot}75$, i.e. repulsion, the advance is 0·25. If equal numbers of coupling and repulsion linked doubly heterozygotes were used the mean advance would be $\frac{1}{2}(0{\cdot}75 + 0{\cdot}25)$, i.e. 0·5, just as when the genes recombined freely.

With three linked genes the maximum rate of advance is given by the combination **ABC**/abc and the minimum by **Ab**C/a**B**c, with **AB**c/ab**C** and **A**bc/a**BC** in intermediate positions. Still more grades are possible with four genes, but the same general principle holds. In each case freely recombining genes give rates of advance intermediate between those of coupled and repulsed linked genes. So in each case the advance typical of freely recombining genes can and will be simulated by that of mixtures of the various linkage types, provided that nothing prevents the occurrence of such mixtures in the appropriate proportions. Selection can, however, affect the rate of advance under its own action by changing the proportions of the various linkage types.

Reverting to our simple example of two genes the original individuals were all doubly heterozygous and so displayed the same potential variability. The difference in reaction to the same selective force was due to different rates of exposure of this heterozygosity-variability to the action of selection. The remaining variability remains hidden, in the sense that it exists as heterogeneity in the population of a type on which selection is not operative. Thus **AA**bb and aa**BB** are different genotypes having, in our example, the same phenotype. Their difference represents genetic variability which is not exposed to selective action. **AA**bb and **aa**bb also differ in genotype, but here the phenotypes are different and the genetic variability is open to the action of selection. The variability is free, not potential as in the case of the difference between **AA**bb and aa**BB**.

Potential variation can be freed by suitable methods. If **AA**bb and aa**BB** are intercrossed, their doubly heterozygous progeny will on selfing produce a range of genotypes with phenotypic differences on which selection will act. The variability is no longer wholly potential; some of it has been freed.

With breeding systems other than continued selfing variability can be stored in other ways. Heterozygous types will be produced which are not subject to the full rigour of selection but which can be made to produce progeny in which the variation is free. The method of freeing variability is dependent on the way in which it is stored and hence on the breeding system of the population.

It has been shown (Mather, 1941) that the relative amounts of potential and free variation will be in equilibrium in wild populations, the precise level of balance depending on the rate of change of the condition to which adaptation is advantageous. In the majority of cases, where conditions are changing but slowly from generation to generation, the balance will be such that there is much more potential than free variation. The free variation will tend to be diminished by the action of selection in each generation but will be continually replaced from the hidden store. In this way the opportunity for selective response will be maintained, the major part of the variation not being exposed to the risk of depletion from this cause. This can be expressed in another way by saying that the population will be capable of showing variation from which future adaptation to changed conditions may flow, but will remain sufficiently stable not to lose adaptation to present conditions. Present fitness and the possibility of future change will be balanced against one another.

With potential variation maintained at a higher level than free variation there will exist in the population more linked combinations of the types which give slow response to selection than of the types which allow of rapid change. Both types will arise by random mutation and recombination of the genes, but the internally balanced types will be favoured by selective action.

Evidence of the existence of these types of linked and balanced polygenic combinations has been obtained from a selection experiment in *Drosophila melanogaster* (Mather, 1941), the polygenic character used being abdominal hair number. This experiment eventually yielded a

Table 1. *The mean abdominal hair number of the two parental, two selected, and tester stocks*

Stock		Mean number of hairs on segments 4 and 5	
		Males	Females
Parental	Or+	39·88	44·59
	BB	36·06	43·48
Selected	H-BB	44·35	52·25
	L-+	27·63	34·45
Cy/Pm; H/Sb*		25·20	36·70

* This stock contains the gene H, which reduces the abdominal hair number.

stable high selection stock, denoted as H-BB, and a stable low selection stock, denoted by L-+, whose means differed by about 17 hairs. The two parental lines, Or+ and BB, each had a mean of approximately 40 hairs (see Table 1). The advance with selection was thus considerable in both directions. It then seemed desirable to analyse in more detail the

organization of the linked polygenic combinations on which selection had acted in order to determine (*a*) how the ordering of the polygenes in the chromosomes has been affected by selection, and (*b*) how much of the potential variation had been used up by the large response to selection obtained during the experiment.

This type of analysis can only be undertaken using marker genes to enable particular chromosomes and segments of chromosomes to be recognized and followed. The gene **BB** was actually segregating in the material on which selection was exercised, and its presence permitted the demonstration of a balance between whole chromosomes in the two parental stocks; but in general the experiment can be better made using special tester stocks to analyse the parental and derived lines after selection has ceased, rather than by maintaining marker genes segregating in the material during the course of selection. The details of the method of making the necessary comparisons will be described in the account of the experimental results.

2. The effects of selection on the whole chromosomes

The first series of tests was designed to ascertain which of the major chromosomes had been affected by selection during the production of the selected lines from the cross **Or +** × **BB**. Males from the four stocks, two parental and two derived by selection, were crossed with females of a tester having the constitution **Cy/Pm**; **H/Sb sr In** (3R) **Mo**. The F_1 generation gave **Cy Sb**, **Cy H**, **Pm Sb**, and **Pm H** flies of each sex. In the crosses which involved the **BB** parental stock and the **H-BB** selected stock the female progeny were also **BB/+**, though the males, of course, had wild-type eyes. This enabled the sex chromosome to be partially followed in these tests; but as **Or +** and **L- +** did not contain **BB** or any other similar marker, the effect of the sex chromosome could not be determined in these two cases.

Pm Sb males from each of the four F_1's were backcrossed to the tested stocks, **Or +**, **BB**, **H-BB** and **L- +**, and F_2's were also raised from each of the four crosses. **Pm** and **Sb** were used because **H** has an effect in reducing the number of abdominal hairs while **Cy** makes the flies awkward to handle during the counting process.

Various types of progeny were produced. In each backcross **Pm Sb**, **Pm**, **Sb** and **+** flies were found, as expected, in equal numbers in both sexes. Since, however, the **BB** and **H-BB** stock females to which the F_1 males were backcrossed gave only **BB** sons, **Pm** was classified only in the **BB/+** daughters of these backcrosses. **Pm** is not always classi-

fiable with certainty in **BB** males. In the F_2 all combinations of **Pm** and **Sb** were classified in the **Or +** and **L- +** test crosses, while in the **BB** and **H-BB** crosses all combinations of **Pm**, **Sb** and **BB** were found. **Pm** was not classified in **BB** males for the same reason as in the case of the backcrosses.

After classification for these marker genes the abdominal hairs of the various types of progeny were counted. In each backcross 10 females of the four types **Pm Sb**, **Pm**, **Sb**, and **+** were counted, these being further subdivided into 5 **BB** and 5 not Bar wherever that character was segregating. In the males from the crosses involving **Or +** and **L- +** 10 each of the **Pm Sb**, **Pm**, **Sb**, and **+** classes were also counted. In the **BB** and **H-BB** crosses 20 **Sb BB** and 20 **BB** males were counted from the backcrosses (**Pm** being unclassified as noted above) and 7 **Pm Sb**, 7 **Pm**, 7 **Sb**, 7 **+**, 6 **Sb BB** and 6 **BB** (**Pm** being unclassified in the last two classes) from the F_2's. The mean numbers of hairs of these various types of progeny are given in Table 2.

Let us first consider the further analysis of the **Or +** and **L- +** tests. In each case the **Pm Sb**, **Pm**, **Sb**, and **+** flies were counted in equal numbers in both sexes. Now the **Pm Sb** and **Sb** flies differ in that the latter class receives both of its II chromosomes from the stock under test, i.e. **Or +** or **L- +**, while the former class receives one chromosome II from the tested and one from the tester stocks. **Pm** and **+** flies differ in exactly the same way. So as equal number of flies were counted in all classes we may take the difference between the hair number means of the **Pm** and not **Pm** flies, irrespective of whether they are **Sb** or not, as a measure of the difference in polygenic content of chromosome II in the tested and tester stocks. It may be noted that only dominant or partially dominant polygenes in the **Pm** chromosome will have an effect on this difference. The fully dominant genes of the tested chromosome are not detectable by the crosses.

Similarly the differences in polygene content of chromosome III in the tester and tested stocks are measured by the differences between the means of all the **Sb** flies and all the not **Sb** flies, irrespective of their being **Pm** or not. The sexes are kept separate throughout and so the experiment is in effect duplicated, the two sets of data serving as mutual checks.

Exactly the same analytical principle was adopted for the results of the **BB** and **H-BB** tests. Here there may be up to eight recognizable classes of progeny in one sex. In determining the effect of chromosome II **Pm** flies were compared with not **Pm** flies, the classifications for **BB** and

Sb being neglected, and so on for the other two major chromosomes. It will be observed that this type of analysis is dependent on the **Pm** and not **Pm** classes including equal numbers of **Sb BB**, **Sb**, **BB** and **+** individuals. The effects of differences in chromosomes X and III are thus evened out. Similarly, when analysing chromosome III the effects of the other chromosomes are removed by neglecting **Pm** and **BB** classification. The experiment and analysis are of the type termed 'factorial' by agronomists.

The mean differences are given in the right-hand three columns of Table 2. Their numerical derivation is quite simple. Thus the chromosome II difference in the females of the **Or** + F_2 is representable symbolically as $\frac{1}{2}$ (+ +**Sb** − **Pm** − **PmSb**), i.e. numerically as

$$\tfrac{1}{2}(43{\cdot}2+43{\cdot}8-39{\cdot}6-43{\cdot}4)=\tfrac{1}{2}(87{\cdot}0-83{\cdot}0)=2{\cdot}0.$$

In most cases all the differences attributable to each chromosome have the same precision, but in some cases where classification for **Pm** was prevented, or partially prevented, by **BB** the precisions are not all alike because some flies were of necessity omitted when determining certain of the differences. In every case the value of the tester chromosome from the **Cy**/**Pm**; **H**/**Sb** stock was subtracted from that of the tested chromosome from **Or** +, **BB**, **H-BB** or **L**- + as the case may be.

We are now in a position to compare the homologous chromosomes from the four tested stocks. Thus chromosome II of the **H-BB** selected line contains genes which give in F_2 females 3·3 more hairs on the average than those in chromosome II of the tester. In **L**- + F_2 females the genes of the tested chromosome II give an average of 1·5 hairs less than the tester chromosome II. Hence the chromosome II from **H-BB** has genes giving 3·3 + 1·5, i.e. 4·8, more hairs than the corresponding chromosome from **L**- +. There are, however, three different estimates of this difference available from the whole experiment, viz. those from F_2 females, from F_2 males and from backcross females respectively. Their differences provide an estimate of the standard error to which the difference itself is subject.

To obtain this standard error we must go back a stage in the analysis to the difference between the tested and tester chromosomes. Each of these differences is estimated from various portions of the data. Thus the difference between chromosome II in **Or** + and the tester stock is estimated from four sources, F_2 males and females and backcross males and females. A sum of squares of deviations from the mean of the four is obtained. The four values are 2·00, −2·40, 0·90 and 3·20, with a mean of

Table 2. *Mean numbers of abdominal hairs in the various classes of flies in the different tests, together with the single differences and mean differences derived from them as described in the text*

Test			Marker genes. Pm Sb	Pm Sb B	Pm	Pm B	Sb	Sb B	+	B	Differences. X-chromosome		II chromosome		III chromosome	
Or+	F_2	♀	43·40	—	39·60	—	43·80	—	43·20	—	—		2·00	0·9250	−2·20	−0·6750
		♂	36·80	—	36·00	—	32·90	—	35·10	—	—		−2·40		0·70	
Or+	backcross	♀	42·40	—	41·80	—	43·90	—	42·10	—	—		0·90		−1·20	
		♂	35·60	—	34·90	—	38·10	—	38·80	—	—		3·20		0·00	
BB	F_2	♀	40·60	43·80	40·20	40·60	41·40	42·40	38·60	40·40	1·60	0·8900	−0·60	0·2300	−2·10	−0·9625
		♂	34·28	—	33·71	—	34·14	35·00*	34·14	34·17*	0·18		0·14		−0·65	
BB	backcross	♀	—	42·40	—	40·90	—	42·50	—	43·10	—		1·15		−0·45	
		♂	—	—	—	—	—	34·80*	—	34·15*	—		—		−0·65	
H-BB	F_2	♀	41·20	45·20	43·20	43·40	45·40	49·00	42·20	49·60	3·80	3·1000	3·30	3·0900	−0·60	0·4750
		♂	35·57	—	35·71	—	38·57	39·17*	37·86	39·50*	2·40		2·57		−0·10	
H-BB	backcross	♀	—	43·30	—	43·70	—	46·60	—	47·20	—		3·40		0·50	
		♂	—	—	—	—	—	38·45*	—	40·85*	—		—		2·10	
L-+	F_2	♀	40·90	—	35·60	—	37·30	—	36·20	—	—		−1·50	−1·0500	−3·20	−2·6500
		♂	30·40	—	29·30	—	31·50	—	28·50	—	—		−0·15		−1·85	
L-+	backcross	♀	37·90	—	35·50	—	37·00	—	34·20	—	—		−1·10		−2·60	
		♂	30·00	—	27·10	—	28·60	—	25·60	—	—		−1·45		−2·95	

* Not classified for **Pm**.

0·925. The sum of squares of deviations from the mean is then 17·3875. Similar sums of squares are obtained from the tests of this chromosome in each of the other stocks, and also from the various tests of the other two major chromosomes. The sums of squares so found are independent of one another. Where different families are used this independence is obvious; but where the groups added are from the same progenies, though involving different chromosomes, the data are independent only by virtue of the factorial design of the experiment. The grand total sum of squares is 34·3666 and it corresponds to 24 degrees of freedom as shown by the analysis of Table 3. The mean square, or variance of a single difference is thus 34·3666/24, i.e. 1·4319.

Table 3. *The portions of the error sum of squares*

	Item	Sum of squares	Degrees of freedom
Or+	chromosome II	17·3875	3
	chromosome III	4·9475	3
BB	chromosome X	1·0082	1
	chromosome II	1·5434	2
	chromosome III	1·7519	3
H-BB	chromosome X	0·9800	1
	chromosome II	0·4106	2
	chromosome III	4·1275	3
L-+	chromosome II	1·1750	3
	chromosome III	1·0350	3
Total		34·3666	24

This method of estimating the variance is to be preferred to the alternative of estimating it from differences between individuals of the same kind in the same culture as the latter takes no account of interculture differences. These may be very large when compared with intra-culture variation.

Table 2 also shows the mean differences between given chromosomes of the tester and of the four tested stocks. These may be the averages of 4, 3 or 2 single differences. Where 4 values are averaged the variance of the mean so obtained is 1·4319/4, i.e. 0·3580 and the standard error is $\sqrt{0{\cdot}3580}$ or 0·5983. The variances and standard errors of means based on 3 and 2 single differences are found in the same way but with 3 and 2 respectively substituted for 4 in the denominator.

The final analysis is given in Table 4, which contains the mean differences between the homologous chromosomes from the various tested stocks, the tester chromosomes having been eliminated by subtraction as described above. The standard errors of the comparisons are also given in this table. Only one such difference is available for the *X*-chromosome as this could be analysed only in the **BB** and **H-BB**

stocks. Not all the differences of Table 4 are significant, but many are and the rest are consistent. So the whole story is reasonably clear.

The two parental lines differ only slightly in the hair producing strength of the chromosomes II and III. In each of these **Or +** is slightly stronger than **BB**. It will be recalled that this was already inferred from the behaviour of the *X*-chromosome in the account of the origin of the selected lines (Mather, 1941), where it was found that of the two stocks **Or +** was slightly more hairy than **BB** though the *X*-chromosome of **BB** was stronger than that of **Or +**. Thus the earlier analysis depending on the use of the **BB** marker segregating in the material during the process of selection has been fully confirmed by a somewhat different test.

Table 4. *Difference between homologous whole chromosomes of the tested stocks*

	Chromosome		
Difference	*X*	II	III
Or+ −BB	—	0·6950 ± 0·9139	0·2875 ± 0·8461
Or+ −H-BB	—	−2·1650 ± 0·9139	−1·1500 ± 0·8461
Or+ −L-+	—	1·9750 ± 0·8461	1·9750 ± 0·8461
BB −H-BB	−2·2100 ± 1·1966	−2·8600 ± 0·9770	−1·4375 ± 0·8461
BB −L-+	—	1·2800 ± 0·9139	1·6875 ± 0·8461
H-BB −L-+	—	4·1400 ± 0·9139	3·1250 ± 0·8461

The two selected lines differ markedly and significantly from each other in both of their large autosomes, and it is clear from the comparison of each of them with their parents that effective recombinations of polygenes has occurred in both these chromosomes during the course of selection. Thus, for example, chromosome II in the **BB** parental stock produces 2·860 less hairs than chromosome II of **H-BB**. The **Or +** parental stock similarly has a chromosome II producing 2·165 less hairs than chromosome II of **H-BB**. So the **H-BB** chromosome must have received + polygenes from both parental II chromosomes.

It would appear that chromosome II has played a larger part than has chromosome III in the selection of the high line. The two autosomes have on the other hand been of roughly equal importance in the development of the low line.

The difference in the *X*-chromosome of the **BB** and **H-BB** lines though in the direction expected is not significant. So there is no good evidence of selection having acted on recombinant sex chromosomes. This is fully borne out by evidence presented in a later section.

3. Selection and chromosome III

So far we have considered changes in the total effects of the different chromosomes, but an extension of the same analytical technique can be used to obtain information as to the action of different parts of the same chromosome. Two chromosomes have been analysed, chromosomes *X* and III. Chromosome II was omitted as a suitable tester stock was not immediately available.

Chromosome III has been shown in the last section to have undergone internal changes during the production of both selected lines. Of the two parental stocks, **Or +** displayed a slightly greater hair producing strength in its third chromosome than did **BB**. In the high selection line, **H-BB**, the third chromosome is stronger than either parent and in the low selection line, **L-+**, it is correspondingly weaker. Effective recombination has occurred and been selected in both cases.

The nature of these internal arrangements has been analysed using **h**, **th**, e^s and **ca** as the marker genes. The tester stock was actually **Mé ca/ru h th st cu sr** e^s **ca**; ey^2, and each of the four stocks, two parental and two selected, was crossed to flies of this constitution. In the F_1 generation not-**Mé** flies were chosen and crossed back to **ru h th st cu sr** e^s **ca**; ey^2 males. The offspring of these crosses were classified for the marker genes and their numbers of abdominal hairs counted. Not all the markers were followed. The fourth chromosome was neglected and as **ru** proved difficult to classify on flies showing various combinations of ey^2 and **BB** it too was omitted from the account. The flies were then classified for all the remaining genes, but as recombination in the regions **th–st**, **st–cu**, **cu–sr** and **sr–**e^s was rather rare these regions were grouped and treated as one. Thus effectively four marker genes were followed as detailed above. They are reasonably well distributed along the length of the genetical chromosome.

Double and higher recombinant types were neglected since they are too rare to be useful in this type of analysis. So eight classes of offspring were counted, two non-recombinant classes, two showing recombination in the region **h–th**, two showing recombination between **th** and e^s and finally two showing recombination between e^s and **ca**. The mean numbers of hairs on the fourth and fifth abdominal segments and numbers of individuals counted are shown in Table 5. It will be observed that males and females are presented separately as are flies from the two cultures of each kind. In some cases the numbers of flies are very small, indeed the whole experiment was rendered difficult by the rather marked infertility

Table 5. *Data for the analysis of chromosome III polygenes*

Each dual entry in the table consists of (i) the mean number of hairs in the class concerned, and (ii) the number of individuals on which the mean is based (in italics)

Test	Culture	Sex	Class 1: +	Class 2: h th e^s ca	Class 3: th e^s ca	Class 4: h	Class 5: e^s ca	Class 6: h th	Class 7: ca	Class 8: h th e^s
Or+	1	♂	38·50 *4*	39·33 *3*	40·00 *2*	42·00 *1*	41·00 *2*	— *0*	39·00 *3*	39·00 *1*
		♀	43·80 *5*	49·50 *2*	54·00 *1*	48·00 *1*	44·00 *2*	— *0*	46·00 *1*	48·33 *3*
	2	♂	39·70 *10*	39·38 *8*	37·67 *3*	40·50 *6*	40·33 *6*	42·00 *1*	37·67 *3*	39·33 *3*
		♀	47·10 *10*	51·40 *10*	47·67 *3*	45·75 *4*	47·25 *4*	48·20 *5*	45·88 *8*	47·75 *8*
BB	1	♂	37·40 *10*	35·00 *7*	35·50 *4*	37·00 *3*	38·80 *5*	— *0*	39·13 *8*	37·25 *4*
		♀	43·00 *10*	44·20 *5*	— *0*	46·33 *3*	44·40 *5*	44·00 *2*	40·50 *2*	46·67 *3*
	2	♂	37·00 *9*	36·50 *4*	38·00 *2*	38·00 *1*	36·67 *3*	— *0*	37·78 *9*	31·50 *2*
		♀	42·70 *10*	46·67 *6*	43·00 *2*	45·17 *6*	43·60 *5*	50·00 *1*	42·60 *5*	40·50 *2*
H-BB	1	♂	38·00 *9*	41·33 *3*	37·00 *1*	42·00 *3*	37·50 *2*	— *0*	41·10 *10*	37·50 *2*
		♀	47·40 *10*	46·00 *1*	— *0*	51·00 *1*	48·57 *7*	— *0*	48·33 *9*	— *0*
	2	♂	41·30 *10*	38·00 *6*	38·75 *4*	43·00 *4*	39·60 *5*	38·67 *3*	40·43 *7*	40·80 *5*
		♀	47·20 *10*	44·67 *3*	46·67 *3*	50·80 *5*	46·00 *4*	47·50 *2*	48·60 *10*	46·50 *10*
L-+	1	♂	31·70 *10*	34·80 *10*	36·67 *3*	36·67 *3*	30·00 *1*	35·67 *3*	32·10 *10*	35·20 *5*
		♀	38·70 *10*	44·60 *10*	42·71 *7*	42·71 *7*	40·00 *7*	40·75 *4*	39·70 *10*	42·33 *9*
	2	♂	31·90 *10*	36·00 *7*	36·38 *8*	33·50 *4*	31·50 *2*	36·00 *9*	33·10 *10*	37·25 *4*
		♀	40·20 *10*	45·33 *10*	42·75 *4*	41·67 *6*	39·25 *4*	46·00 *5*	40·60 *10*	42·57 *7*

of the **ru h th st cu sr** e^s **ca**; ey^2 parents. Care was taken to provide a means of estimating the sampling error and so the paucity of numbers gives rise to little difficulty of interpretation. It may be added that the difference between the duplicate results, from which the variance is estimated, was so much higher than the variance in hair number of similar flies from the same culture that increasing the numbers would have availed little in reducing sampling error. Some method of ensuring more comparable results from different classes would have been of more value in giving the experiment greater precision.

Before discussing the further analysis of these data it is necessary to digress a little in order to consider the nature of the information which is obtained. Each of the marker genes may have associated with it a number of polygenes, detectable only by this association. These associated genes may or may not affect the abdominal hair number. If an effective gene rarely or never recombines with the marker its full effect on hair number will be expressed by the mean difference in hair number existing between the two classes separable by the marker. If, however, a certain degree of recombination is found between the polygene and the marker the full effect of the polygene is not detectable in this way. The effect as measured by the difference between two classes separated on the basis of the marker must be less than the full effect. Thus a polygene, **A**-**a**, showing 33·3 % recombination with the marker **B**-**b** will give four classes in the backcross in the proportions 2 **AB**:1 **Ab**:1 **aB**:2 **ab**. These are separated into **B** and **b** types, the former containing **A** and **a** allelomorphs in the ratio of 2:1 and the latter having the same allelomorphs on the ratio 1:2. So calling the difference between **A** and **a** unity the mean difference between the **B** and **b** classes will be $\frac{1}{3}[(2\mathbf{A}+1\mathbf{a})-(1\mathbf{A}+2\mathbf{a})]=\frac{1}{3}(\mathbf{A}-\mathbf{a})=\frac{1}{3}$. In other words the use of the marker gene allows only one-third of the effect of **A**-**a** to be detected. It gives a measure of what may be termed the 'linkage moment' of the polygene about the marker. (Unlike the familiar moment of statics this linkage moment decreases as the distance from the marker increases.) There may be several polygenes associated with each marker and so the total difference observed is the sum of the moments of the individual genes. This question has been discussed in more detail, though in a different connexion, by Bartlett & Haldane (1935).

The summed linkage moments of the genes associated with any marker are measured as the difference in the mean hair number between flies of the two classes separable by means of the marker. Thus the effect of the polygenes associated with the **th** locus may be found by taking the

difference in hair number of any two classes which differ solely in that one has **th** and the other the corresponding **+** allelomorph. In our crosses two estimates of this difference are obtainable. The not **th** class which shows recombination in the region **th**–e^s, viz. e^s **ca**, differs from the **th** class showing recombination between **h** and **th**, viz. **th** e^s **ca**, in this way. Similarly the classes **h th** and **h** also provide an estimate of the effects of the genes associated with **th**. The two differences in hair number provided by these comparisons should be the same, apart from the effect of sampling error, unless there is some non-additive relation between those polygenes associated with **th** and those in other parts of the chromosome. This possibility cannot be adequately tested from the present data, but if the estimate of sampling variance is based on the discrepancies between such pairs of differences it will make due allowance for any distortion caused by interactions in effect of sets of polygenes.

The analysis of the data was carried out by differencing in the way outlined above. Eight classes of progeny were observed, viz.

1.	+	non-recombination.
2.	**h th** e^s **ca**	
3.	**th** e^s **ca**	recombination in region 1.
4.	**h**	
5.	e^s **ca**	recombination in region 2.
6.	**h th**	
7.	**ca**	recombination in region 3.
8.	**h th** e^s	

If we call the groups of polygenes associated with the loci **h**, **th**, e^s and **ca**, **A**-**a**, **B**-**b**, **C**-**c**, and **D**-**d** respectively, the capital letter denoting the group from the tested stock and the small letter the group from the **h th** e^s **ca** tester, we can find

A-**a**	as	1–4	or	3–2
B-**b**	as	5–3	or	4–6
C-**c**	as	7–5	or	6–8
D-**d**	as	1–7	or	8–2

the numerals denoting the mean hair number of the classes numbered correspondingly in the preceding list. It may be noted here that the use of this differencing technique minimizes disturbances due to sex variations and to environmental variations between culture bottles.

Each culture provides a duplicate estimate of the effect of each marked association of polygenes. The sexes were kept separate and two

cultures of each kind were raised, so that in addition to the internal duplication, sex and cultures give a further quadruplication. The four sets of data were combined into one estimate of the hair number difference, only the internal duplication being allowed to remain in order to provide an estimate of error. The means and hence the differences of the quadruplicates are based on different numbers of observations and so are of unequal precision. They must be weighted during the process of combination.

The weighting system used was based on the numbers of individual observations involved in each of the means. Suppose each individual observation is subject to a variance V. Then the mean of n_1 observations has a variance V/n_1, and the mean of n_2 observations a variance of V/n_2. The variance of their difference is thus $V\left(\frac{1}{n_1}+\frac{1}{n_2}\right)$, and since V may be taken as constant over a series of observations the variances of the differences will be in the proportions $\left(\frac{1}{n_1}+\frac{1}{n_2}\right)$, $\left(\frac{1}{n_3}+\frac{1}{n_4}\right)$, etc. The precision of an observation is conveniently measured by the reciprocal of its variance and so the weights used in combining the differences are of the type $\frac{1}{\frac{1}{n_1}+\frac{1}{n_2}}$, i.e. $\frac{n_1 n_2}{n_1+n_2}$. The use of these weights may be illustrated by considering the combination of the four estimates of the difference 1–4 in the tests of **L-+**. These four estimates, derived from the males and females of the two like cultures, were found to be $-4{\cdot}97$, $-4{\cdot}01$, $-1{\cdot}60$ and $-1{\cdot}47$. The first was found as the difference of two means one based on 10 observations and the other on 3 observations. So its weight is $\frac{10\times 3}{10+3}=2{\cdot}307$. The weights of the others are similarly 4·117 (10 and 7 observations), 2·857 (10 and 4 observations) and 3·749 (10 and 6 observations). The weighted mean of the four is then calculated as

$$-\frac{1}{2{\cdot}307+4{\cdot}117+2{\cdot}857+3{\cdot}749}[(4{\cdot}97\times 2{\cdot}307)+(4{\cdot}01\times 4{\cdot}117)+(1{\cdot}60\times 2{\cdot}857)+(1{\cdot}47\times 3{\cdot}749)]$$

$$=-\frac{38{\cdot}0527}{13{\cdot}050}=-2{\cdot}920.$$

The other entries in Table 6 are calculated in the same way.

At this stage of the analysis we are left with eight mean differences, forming four duplicate pairs, in each of the tests of **Or+**, **BB**, **H-BB**

and L-+. The final differences, denoted above as A-a, B-b, C-c and D-d are then obtained as the unweighted means of the corresponding pairs of differences shown in Table 6. Thus for example in the Or+ test, 1–4 has a value of −0·660 and its homologue, 3–2, a value of −0·930. Each measures the effect of the polygenes associated with the h locus. The average effect of these polygenes is thus $-\frac{1}{2}(0{\cdot}660+0{\cdot}930)=-0{\cdot}795$.

The variance of these final mean differences is found from a consideration of the discrepancies between the two estimates of each difference. In the example of the previous paragraph this discrepancy is 0·930 − 0·660 = 0·270. There are 16 such values to be found, four from each of the four tests; but not all of these may be used as they are not all independent. Thus A-a is estimated by the two differences 1–4 and 3–2,

Table 6. *Dual and mean estimates of the polygenic differences between the tested and tester stocks of chromosome III*

	(h) A–a			(th) B–b		
Test	First estimate	Second estimate	Mean	First estimate	Second estimate	Mean
Or+	−0·660	−0·930	−0·795	−0·567	−2·183	−1·375
BB	−1·452	−0·415	−0·934	1·359	−0·673	0·343
H-BB	−2·037	0·333	−1·302	0·233	3·862	2·048
L-+	−2·920	−1·467	−2·194	−2·903	−1·282	−2·093
	(e[a]) C–c			(ca) D–d		
Test	First estimate	Second estimate	Mean	First estimate	Second estimate	Mean
Or+	−1·540	−0·162	−0·851	0·798	−2·096	−1·298
BB	−0·492	1·678	0·593	−0·480	−0·770	−0·625
H-BB	1·310	−0·656	0·327	−1·211	1·164	−0·024
L-+	0·754	0·295	0·520	−0·750	−1·023	−0·887

while the calculation of B-b involves 5–3 and 4–6. Two of the observations, 4 and 3, are common to the two pairs of estimates, which are in consequence correlated. We may, however, use two sets of data from each test, either the A-a and C-c figures, or alternatively B-b and D-d. Each such pair involves every observation once. We then find the discrepancies between the two estimates of A-a and of C-c in each of the four tests. These are squared and the squares summed and divided by 2, in accordance with the usual practice of estimating sums of squares. Eight such values are summed and so the sum of squares corresponds to eight degrees of freedom. The mean-square, or variance, of each estimate of A-a, B-b, etc. is then found by dividing eight into the sum of squares. If the A-a and C-c discrepancies are used to supply material for this calculation the variance is 1·5377 but if B-b and D-d are employed it is estimated

as 2·2899. The latter value will be used as a precaution against over-estimation of significance.

This is the variance of a single estimate of any difference. The final differences obtained were, however, found as the means of a pair of individual differences. The variance of each of these final mean differences is thus $\frac{1}{2}(2{\cdot}2899) = 1{\cdot}14495$.

The last stage of the calculation is the comparison of the tested stocks with each other. The results so far have consisted of differences between the individual tested stocks and the common tester. A positive value shows that the tested stock has stronger hair producing genes than does the tester, and a negative that the tester stock genes are stronger than those in the tested line. We now find the differences between the gene associations of the tested stocks themselves by subtraction. **Or+** gives a value of −0·795 for A-a when compared with the tester and **BB** gives a value of −0·934 for the same association. Then the difference between **Or+** and **BB** in this region is 0·934 − 0·795, i.e. 0·139. Both are weaker here than the tester but **Or+** is less so than **BB**. The other differences of this kind are given in Table 7. Each is the difference of two mean values and so will have twice the variance of a single mean. This will be

Table 7. *Polygenic differences between the tested stocks in various regions of chromosome III*

Difference	A (h)	B (th)	C (e^s)	D (ca)
Or+ −BB	0·139	−1·718	−1·444	−0·024
Or+ −H-BB	0·507	−3·423*	−1·178	−0·625
Or+ −L-+	1·399	0·718	−1·371	0·238
BB −H-BB	0·368	−1·705	0·266	−0·601
BB −L-+	1·260	2·436	0·073	0·262
H-BB −L-+	0·892	4·141*	−0·193	0·863

All differences have a standard error of 1·5132.
* Probability of less than 0·05.

$2 \times 1{\cdot}14995$ or 2·2899. The standard error of the entries in Table 7 is thus $\sqrt{2{\cdot}2899}$, i.e. 1·5132. Any difference between two tested stock means which exceeds 3 may be considered to be significant.

It appears that only two entries in Table 7 are significant on this test, viz. the difference between **H-BB** and **L-+** in the genes associated with **th**, i.e. **B-b**, and the difference between **H-BB** and **Or+** in this same region. **H-BB** has polygenes making for more hairs than those of **L-+** and **Or+**. It may, however, reasonably be supposed that, as the results are consistent, **H-BB** is also stronger than **BB** and **L-+** weaker than either **Or+** or **BB** in this region of chromosome III. Effective recombination of polygenes has occurred near the **th** locus and the

recombinants have been selected in both high and low lines. The rest of the chromosome, with the possible exception of the h region in the L- + line, has not transgressed the parental limits during the selection of either derived line. All the release of stored variability has occurred in the section near to th.

4. SELECTION AND THE *X*-CHROMOSOME

The experiment described in § 2 provided no evidence that a change had occurred in the total hair producing strength of the *X*-chromosome during selection of the H-BB line. This has been fully confirmed by the analysis of the distribution of polygenes along the length of the chromosome. It also appears from this later experiment that the same conclusion holds good for the L- + line.

In outline the experiment was like that conducted for the analysis of chromosome III. Males of each of the four stocks, two parental and two derived, were mated with sc ec cv ct^6 v g^2 f females, and daughters of these four crosses were mated to their brothers who contained, of course, an unchanged sc ec cv ct^6 v g^2 f chromosome. It would perhaps have been better to use stock males carrying this chromosome because segregation of the autosomes no doubt increased the error variability of the results. This advantage was offset by the increased vigour of the outbred males, and since sterility constituted a very real problem in these experiments it was decided to use the brothers.

In each case two females were bred separately, each being allowed to lay in two bottles successively. Thus four cultures of each test cross were obtained. In addition males and females from each culture were recorded and their hairs counted separately, just as in the analysis of chromosome III.

The analysis of the *X*-chromosome yielded results differing in two ways from those of the previous section. In the first place one of the marker genes used in the tester *X*-chromosome, viz. sc, itself reduced the number of abdominal hairs to a very marked degree. So sc flies could not be employed in determining the differences attributable to the polygenes associated with the various loci.[1] Hence no analysis of the polygenes associated with sc was possible and, what is even more troublesome, a single culture provided in each sex only one estimate of the effect of the polygenes associated with each of the other markers. The duplicate estimates necessary for the estimation of error variance must be obtained

[1] The gene h in chromosome III does not appear to affect abdominal hair number, as it might perhaps have been expected to do.

in a way differing from that used in chromosome III where internal duplication was obtained.

Secondly, there is no reason to suppose that the two sexes will give similar results in this analysis; for the males will show the effects of all the sex-linked polygenes, except those whose action is suppressed by the *Y*-chromosome, while the females will show only the effects of those genes from the tested stocks which are not recessive to their allelomorphs in the tester chromosome. In the case of chromosome III, males and females were alike in the evidence which they supplied.

One consequence of this possible sex difference is that males and females cannot be used to supply duplicate results for the estimate of the error variance. The duplicate cultures must be used instead. It has already been noted that in each test cross two heterozygous females were used each being allowed to lay in two bottles. The two bottles from each female may be combined and the discrepancies between the results from the two individual females used to provide the estimate of error.

The mean hair numbers of the different classes distinguishable by means of the markers are given in Table 8, together with the numbers of individuals on which they are based. There are six classes, the gene **ec** not being followed as it is difficult to classify on **BB** flies. These classes are

1.	+	non-recombination.
2.	cv ct^8 v g^2 f	recombination between **sc** and **cv**.
3.	ct^6 v g^2 f	recombination between **cv** and ct^6.
4.	v g^2 f	recombination between ct^6 and **v**.
5.	g^2 f	recombination between **v** and g^2.
6.	f	recombination between g^2 and **f**.

It will be seen that the **sc** classes are omitted from account.

The following differences are then found:

3–2	giving an estimate of	A-a
4–3	,,	B-b
5–4	,,	C-c
6–5	,,	D-d
1–6	,,	E-e

where **A-a** measures the difference in effect of the polygenes associated with the **cv** locus in the tested and tester stocks, **B-b** that associated with ct^6, **C-c** that associated with **v**, **D-d** that associated with g^2 and **E-e** that associated with **f**. The two mean differences from the bottles in which a single female has laid are then combined, using weights based on

Table 8. Data for the analysis of X-chromosome polygenes

Each dual entry in the table consists of (i) the mean number of hairs in the class concerned, and (ii) the number of individuals on which the mean is based (in italics)

Test	Culture	Class 1 +	2 cv ct^6 v g^2 f	3 ct^6 v g^2 f	4 v g^2 f	5 g^2 f	6 f
				Males			
Or+	1–1	35·50 *10*	33·50 *10*	34·20 *5*	33·70 *10*	34·00 *3*	35·00 *7*
	1–2	36·30 *10*	34·00 *4*	35·00 *2*	34·13 *8*	32·17 *6*	35·71 *7*
	2–1	35·30 *9*	30·00 *1*	35·00 *2*	35·33 *3*	38·50 *2*	37·00 *2*
	2–2	34·23 *9*	31·86 *7*	34·17 *6*	34·71 *7*	34·67 *3*	36·25 *4*
BB	1–1	34·10 *10*	32·25 *4*	— *0*	30·30 *2*	33·50 *2*	30·00 *3*
	1–2	32·20 *10*	29·00 *1*	32·50 *2*	31·00 *3*	32·00 *1*	32·80 *5*
	2–1	33·70 *10*	31·50 *2*	30·33 *4*	32·56 *9*	32·71 *7*	32·75 *4*
	2–2	34·20 *10*	29·33 *3*	29·80 *5*	29·67 *3*	30·00 *6*	30·00 *2*
H-BB	1–1	37·33 *10*	35·83 *6*	35·40 *5*	36·29 *8*	37·17 *6*	37·17 *6*
	1–2	38·90 *10*	36·80 *5*	34·67 *3*	36·20 *10*	36·29 *7*	35·67 *6*
	2–1	36·60 *10*	35·29 *7*	32·25 *4*	33·67 *9*	35·50 *6*	33·14 *7*
	2–2	37·80 *10*	36·00 *1*	33·33 *6*	32·75 *4*	32·83 *6*	36·14 *7*
L-+	1–1	29·20 *10*	27·80 *5*	26·67 *3*	27·40 *10*	28·20 *5*	26·83 *6*
	1–2	30·90 *10*	28·75 *4*	28·00 *3*	28·00 *5*	29·00 *4*	27·14 *7*
	2–1	32·40 *10*	33·00 *1*	— *0*	31·00 *6*	33·00 *2*	29·00 *2*
	2–2	30·60 *10*	29·40 *10*	30·33 *3*	30·20 *10*	30·50 *2*	31·00 *1*
				Females			
Or+	1–1	38·50 *10*	40·00 *1*	42·40 *5*	37·75 *8*	37·50 *4*	39·60 *10*
	1–2	39·30 *10*	41·50 *2*	36·00 *5*	38·88 *8*	38·00 *1*	37·33 *6*
	2–1	40·25 *4*	39·00 *1*	— *0*	39·00 *1*	42·50 *2*	39·33 *3*
	2–2	40·40 *10*	39·14 *7*	38·67 *3*	41·80 *10*	39·67 *3*	43·00 *2*
BB	1–1	38·20 *10*	38·00 *2*	37·20 *5*	37·20 *5*	36·60 *5*	36·50 *2*
	1–2	38·10 *10*	38·50 *2*	35·67 *3*	35·67 *9*	36·50 *2*	36·60 *5*
	2–1	39·10 *10*	38·00 *1*	36·33 *3*	36·71 *7*	37·33 *9*	35·80 *10*
	2–2	38·10 *10*	35·40 *5*	33·67 *3*	35·40 *5*	37·75 *4*	35·88 *8*
H-BB	1–1	43·20 *10*	42·80 *10*	47·00 *1*	42·40 *10*	41·67 *9*	41·00 *5*
	1–2	43·10 *10*	43·37 *8*	36·00 *1*	41·60 *5*	42·75 *4*	41·50 *2*
	2–1	42·70 *10*	40·50 *6*	37·14 *7*	39·20 *10*	40·75 *4*	39·50 *4*
	2–2	41·90 *10*	— *0*	38·50 *2*	39·40 *10*	38·00 *4*	40·33 *6*
L-+	1–1	35·70 *10*	36·00 *4*	30·33 *3*	35·75 *8*	31·50 *2*	33·67 *3*
	1–2	34·20 *10*	36·50 *4*	32·00 *2*	32·20 *5*	32·57 *7*	34·80 *5*
	2–1	37·90 *10*	— *0*	— *0*	38·00 *1*	36·00 *3*	36·50 *2*
	2–2	38·10 *10*	36·80 *5*	37·33 *3*	36·22 *9*	34·14 *7*	38·20 *5*

Table 9. *Dual and mean estimates of the polygenic differences, as shown in the two sexes, between the tested and tester stocks in chromosome X*

Test	A–a (cv)			B–b (ct^6)			C–c (v)			D–d (g^2)			E–e (f)		
	First estimate	Second estimate	Mean	First estimate	Second estimate	Mean	First estimate	Second estimate	Mean	First estimate	Second estimate	Mean	First estimate	Second estimate	Mean
							Males								
Or+	0·786	2·770	1·778	−0·620	0·483	0·069	−1·051	1·127	0·038	1·933	0·445	1·189	0·545	−1·840	−0·648
BB	3·500	−0·211	1·645	−1·500	1·277	−0·112	2·143	0·211	1·177	−1·738	0·025	−0·744	0·967	2·148	1·558
H-BB	−0·715	−3·013	−1·864	0·736	0·491	0·614	0·995	1·130	1·063	−0·321	0·475	0·077	1·695	2·560	2·128
L-+	−0·949	0·930	−0·010	0·403	−0·130	0·137	0·880	1·105	0·993	−1·607	−2·199	−1·903	3·098	2·059	2·579
							Females								
Or+	−2·483	−0·470	−1·477	−0·885	3·130	1·123	−0·408	−0·873	−0·641	1·461	0·080	0·771	0·216	−1·279	−0·532
BB	−2·270	1·713	−0·279	0·000	1·017	0·509	−0·034	1·244	0·605	0·500	−1·652	−0·576	1·507	2·792	2·180
H-BB	−1·521	−3·360	−2·441	0·277	1·726	1·002	−0·130	0·091	−0·020	−0·840	0·703	−0·069	2·000	2·314	2·157
L-+	−4·721	0·530	−2·095	3·354	−1·110	1·122	−1·267	−2·067	−1·667	2·213	3·022	2·618	0·476	0·400	0·438

the numbers of individuals of each class involved, exactly as in the case of chromosome III. Male and female results were kept separate.

The remainder of the analysis is the same as that of the previous section. The means of the two estimates of **A-a**, **B-b**, etc. in each test are found and the discrepancies between the two estimates of effect of each set of polygenes are used as the basis of the estimate of error. In order to avoid difficulties arising from the non-independence of some of the discrepancies only those from **A-a**, **C-c** and **E-e** are used, male and female results being pooled. In all there are 24 such differences, as 3 are taken from the data concerning each sex in four different tests. The sum of squares is 98·270864 which for 24 degrees of freedom gives a mean square of 4·0946. This is the variance of each mean difference between the tested and tester stocks.

Table 9 gives all these differences between tested and tester stocks while Table 10 gives the difference between the tested stocks themselves as derived from Table 9 by subtraction. These correspond to Tables 6 and 7 respectively for the chromosome III data, but unlike the earlier

Table 10. *Polygenic differences between the tested stocks in various regions of chromosome X*

	Males				
Difference	A (cv)	B (ct^6)	C (v)	D (g^2)	E (f)
Or+ −BB	0·133	0·181	−1·139	1·933	−2·206
Or+ −H-BB	3·632*	−0·545	−1·025	1·112	−2·776
Or+ −L-+	1·778	−0·068	−0·955	3·092	−3·217
BB −H-BB	3·509*	−0·726	0·114	−0·821	−0·570
BB −L-+	1·655	−0·249	0·184	1·159	−1·021
H-BB −L-+	−1·854	0·477	0·070	1·980	−0·451
	Females				
Difference	A (cv)	B (ct^6)	C (v)	D (g^2)	E (f)
Or+ −BB	−1·198	0·614	−1·246	0·195	−2·712
Or+ −H-BB	0·964	0·121	−0·621	0·702	−2·689
Or+ −L-+	0·618	0·001	3·026	−1·841	−0·970
BB −H-BB	2·162	−0·493	0·825	0·645	0·013
BB −L-+	1·812	−0·613	2·272	−2·042	1·742
H-BB −L-+	−0·346	−0·120	1·647	−2·687	1·719

All differences have a standard error of 2·0235.

* Probability between 0·10 and 0·05

ones they contain two sets of entries since the sexes are kept separate in this analysis. Each of the entries in Table 10 has a variance equal to that of the single items of Table 9, viz. 4·0946. The standard error is $\sqrt{4{\cdot}0946}$, i.e. 2·0235. Any entry in Table 10 which is less than 4 in value cannot be considered as fully significant. On this basis no item in that table may

be considered highly significant and there is thus no fully demonstrable change in the hair number polygenes of the *X*-chromosome in either sex.

Though not quite significant, two of the differences of Table 10 are very suggestive. In the males, where maximum effects are expected, the polygenes associated with cv, denoted as **A**, show little difference between the **Or +** and **BB** parental stocks, yet the selected **H-BB** line is markedly lower than either parent in this region. It gives values of 3·632 and 3·509 when subtracted from the parental values. The probability of differences of this magnitude is less than 10 %. Such a change is clearly against the action of selection but it appears to be compensated, at least to some extent, by smaller changes in the opposite direction in other parts of the chromosome. In this way the total effect of the chromosome would be unaltered.

Such a redistribution of the + and − genes along the chromosome could occur as a result of recombination, and indeed would be expected to occur on occasions if unlike balanced polygenic combinations were allowed to recombine with each other. In this way the stored variability which the balanced combinations contain can change without any great quantity being released and exposed to the action of selection. The storage of polygenic variability is not a static process. It is essentially dynamic in that recombination must always be bringing about redistributions of the genes within the combinations. The extent to which this process can go on without causing a corresponding release and loss of variability must depend on the precise way in which the + and − genes of the combinations are intermingled along the chromosomes.

5. Polygenic combinations in populations

Three results emerge from these analyses. In the first place selection affects all chromosomes. Balanced polygenic combinations are not the monopoly of any one chromosome. They may occur and be acted on by selection in any member of the complement. Secondly, a large phenotypic change may be brought about by recombination of polygenes in a small portion of a single chromosome. Finally the polygenes in combination in a chromosome may recombine with those of another combination to give new types with different arrangements of the + and − genes without causing any marked release of variation in the form of changes in the effect of the chromosome as a whole.

The first two results when taken together emphasise the very large amount of heritable quantitative variation that can be stored in the form of balanced combinations. In the origin of the lines tested above

selection gave rise to a difference of about 17 hairs in eight generations, i.e. a change equal to about 40 % of the hair numbers of the parental lines (Table 1). The selective changes were about equally divided between two large autosomes as shown by the analysis of § 2. Yet the detectable change in one of these chromosomes was shown in § 3 to be confined to a relatively small portion of that chromosome. This section of chromosome III is not peculiar in containing polygenic differences, as experiment has shown the other two major chromosomes to have effects.

It would be unwise to attempt, on the basis of these data, to arrive at any estimate of the total change possible if all portions of all chromosomes recombine in such a way as to release all their stored variation. It is, however, clear that when so much can depend on one section of a chromosome very large total selective advances are possible. Quite possibly greater effects were not obtained in the production of the **H-BB** and **L-+** lines as the small number of parents, never greater than 6, used in each generation allowed homozygosity to develop before the full selective potentialities had been realized. This conclusion as to the great selective changes possible is fully verified by Wigan (1941) who showed that on selecting wild *Drosophila melanogaster* the number of sternopleural chaetae could be doubled in five generations even though a very small number of parents was used in each generation.

There is thus every reason to believe that the organization of polygenes into balanced combinations provides storage for an amount of variability amply sufficient to give rise to changes of the magnitude which must occur during the separation of species in the wild. During this process which takes many generations and is concerned with larger populations than are possible in the laboratory, a greater release can be achieved than would be possible in experiment, even though the same combinations be used. Not all the variability will be so readily available as that released and selected during the course of the experiments in which **H-BB** and **L-+** were built up. Some sections of chromosome may have combinations of genes so tightly linked as to prevent recombination except as a rarity. In an experiment such combinations would seldom, if ever, be broken down, yet in nature tight linkage of this kind would serve merely to prolong the processes of release and reaction to selection, as homozygosity would be expected to supervene.

The third result of the present analysis, viz. the redistribution of genes along the chromosome without much change in total effect brings out the dynamic nature of polygenic organization. The number of combinations possible with a few score genes is enormous and each will have

its characteristic properties both of phenotypic expression, homozygous and in combination with other genes, and of variation release by recombination with other associations. Under any given set of conditions some combinations will be advantageous in giving a well adapted phenotype and others will be disadvantageous owing to maladaptation. The former will tend to increase in relative frequency as a result of natural selection while the latter will tend to become rarer. There will, of course, be every gradation of fitness between the extremes observable in the population and many combinations may show roughly the same effect.

Those combinations which tend to increase in frequency will, however, show continual recombination and hence will throw others which may either be equally well adapted to conditions, or, more likely, less well adapted. Similarly the poor combinations will recombine to give a certain proportion of better types. In general the balance of change must be towards the loss of fitness by recombination except possibly in populations subject to a steadily changing environment. The possible increase in frequency of any good type as a result of natural selection is limited and must be counterbalanced by some equal process causing a diminution in frequency. In the same way the steady loss from the population of relatively disadvantageous types by selection must in general be replaced by recombination; otherwise such types would vanish and the variability of the population would decrease to the vanishing point, with the consequence that further evolutionary change would become impossible and ultimate extinction certain.

One further agent of change in polygenic combinations remains to be mentioned, viz. mutation. Though this will clearly not be so important as recombination in causing redistribution of genes, and consequent alteration in adaptation, it plays a fundamental part in that it is the ultimate source of all heritable variation. The gradual disappearance of extremely disadvantageous combinations tends to reduce the reservoir of variability but this reduction is counteracted by mutation steadily adding to the store of variation. In this way mutation makes possible the storage of variability and the balancing of gene combinations. Its role in changing one combination into another is secondary, such changes being more easily brought about by recombination.

Thus we can form some picture of the behaviour of polygenes in a wild population. The polygenic combinations which give extreme phenotypes are at a disadvantage as compared with those that give better adapted individuals. The former decrease and the latter increase in frequency under the action of selection. The variability lost by the

selective elimination of poor types is continually replaced by mutation of the polygenes. Heterozygosity and linkage provide the means of storage, while recombination is the distributing agency. It is continually reshuffling the genes in the chromsomes, continually giving new combinations which may be better, but are usually worse, adapted than their progenitors. Recombination thus lowers present fitness but maintains the possibility of future change and its frequency will be subject to the action of selection by virtue of these properties. The opposing advantages of stability and variability will be balanced against each other by adaptation of the recombination frequency.

The phenotypic characteristic of a population may be much more stable than the genotypes. Some polygenes are always becoming hómozygous as a result of both random and selective loss, and others are mutating at the same time. The proportion of heterozygous and homozygous loci may, indeed probably will, be fairly constant but the actual loci which are heterogeneous are changing all the time. Similarly the polygenic combinations are continually changing both as a result of mutation and, more especially, from the redistribution of already heterogeneous loci by means of recombination. In spite of this the superficial stability of the phenotypic expression is maintained. The numerous polygenes, each having a small effect indistinguishable from that of any other, allow of a continual state of genotypic flux under cover of a statistically constant phenotypic array, in much the same way as the numerous molecules of a gas each act in an unpredictable way, yet taken together behave according to certain regular laws. The picture given by a study of qualitative genetical variation in the wild is completely changed when the behaviour of polygenes is considered.

6. SUMMARY

Linkage of polygenes may either increase or decrease the rate of advance under the action of selection, according to whether the genes are coupled or repulsed. Recombination is, in fact, the means by which the rate of release of potential, i.e. hidden, genetical variation is made free to the action of selection. In this way both the recombination frequency and the phase of linkage are adaptive and hence are themselves subject to selective control. In general a slow rate of release of variation will be favoured.

Experimental analyses of certain stocks of *Drosophila melanogaster* selected for their number of abdominal hairs, and of the parental stocks from which they were derived, show that

(*a*) balancing of polygenic combinations may occur in any chromosome, .

(*b*) a large change can occur under the action of selection as a result of unbalancing the polygenes in a small segment of chromosome, and

(*c*) reordering of the genes may occur in a chromosome in such a way that the effects of individual segments are changed while the effect of the chromosome as a whole remains nearly constant.

From these results it is seen that balanced polygenic combinations provide ample storage for the variation necessary to give selective changes of the magnitude required by species formation. Mutation initially provides the reservoir of variation and is continually maintaining its level, while at the same time recombination maintains the gene combinations in a state of flux and slowly exposes the variation to the action of selection. Stable, or nearly stable, phenotypic characteristics of a population hide an ever changing genotypic constitution.

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