THE MANIFOLD EFFECT OF SELECTION

K. MATHER and B. J. HARRISON

John Innes Horticultural Institution, Bayfordbury, Hertford, Herts.

and

Department of Genetics, University of Birmingham

Received 21.x.48

CONTENTS

PART I

1. Variability and selection
2. Technical information
3. Chaeta number and fertility
 - (a) The low selection and low mass lines
 - (b) The high selection line and its derivatives
 - (c) The chromosome assays
 - (d) Crosses between the levels
 - (e) The relation between chaeta number and fertility
 - (f) Delayed responses to selection

PART II

4. Other correlated characters
 - (a) Spermathecae
 - (b) Discriminative mating
 - (c) Miscellaneous
5. Variability, correlated response and effective factors
6. Summary
7. References

PART I

I. VARIABILITY AND SELECTION

SELECTION, whether natural or artificial, distinguishes in its action only between different phenotypes. Its effects, however, can be permanent, in the sense of persisting after the generation which was subjected to the selection, only to the extent that different genotypes have been differentially favoured. The degree of correspondence between genotype and phenotype is therefore fundamental in determining the permanent response of a population to any selective pressure which it may experience. Genetic differences whose effects are completely expressed in the phenotype will be subject to the full rigour of selection, and will give the maximum immediate response. Genetic differences which are latent, in the sense that their full potential effect is not expressed in the phenotype, will be to that extent protected from selection, and the immediate response to selective pressure will be correspondingly diminished. Selection can in fact operate on this potential genetic variability only as it is released to express itself as free variation in the phenotype.

Thus where selection is of a given constant rigour, the nature of the response to it enables us to discover how the variability was distributed between the free and potential states in the initial population or group. Potential variability which fails to be released will, of course, remain undetected. The limit of advance under continued selection will be determined by the total amount of variability initially available, whether in the free state or in the potential state but capable of being freed. The rate of advance towards this limit will depend on the relative proportions of free and potential variability, and also on the rate at which the latter becomes freed. The greater the proportion of free variability, and the faster the potential is set free, the sooner will the limit of response be reached. The greater the available store of potential variability, and the slower its release, the longer will the advance under selection continue.

Even in the simplest of genetical situations, where the variation of phenotype upon which selection is acting depends on but one gene difference, the variability will be entirely free only where the mating system in force has led to every individual being homozygous for one or other allelomorph. Each allelomorph will then be exerting its full effect in the phenotype, and the variation associated with the gene will be at its greatest. Should there occur any cross-breeding, however, a proportion of the individuals will be heterozygous for the gene. Such a heterozygote may bear a close phenotypic resemblance to one or other of the two homozygotes, or it may lie between them, according to the dominance relations of the gene ; but it cannot closely resemble both homozygotes at once. Thus the effect of one allelomorph must be wholly, or the effect of each allelomorph partially, masked by association in the zygote with its alternative. The variability which is freely expressed as a phenotypic difference between homozygotes is hidden in the heterozygote. Such heterozygotic potential variability, the only type of potential variability possible with a single gene, must always be partly freed by segregation in the next generation, just as it is always produced by the fusion of two unlike gametes in the generation before.

Where the variability depends on two or more gene differences affecting the same character, a second type of potential variability will occur. The effect of one gene may be masked not only by its allelomorph but also by the balancing action of an allelomorph of a different gene. This additional means of storing variability must play an important part wherever there occurs continuous variation mediated by a polygenic system, whose member genes have effects similar to and supplementing one another, and individually small as compared with the non-heritable, or with the total, variation. With two such genes the extreme combinations will be *AABB* and *aabb*, where the particular allelomorphs which are associated have reinforcing effects. In the homozygotes *Aabb* and *aaBB* the genes will then have counter-balancing effects and the difference between phenotypes will

not fully represent the difference in genotype, in the sense that the genes in question can be reassociated to give phenotypic differences of greater magnitude. Potential variability is thus present in the difference between $AAbb$ and $aaBB$, but it is not the potential variability of heterozygotes. It is what has been called homozygotic potential variability, and it bears the same relation to heterozygotic potential as does free variability, for it is converted into heterozygotic potential by crossing and released from it by segregation. Thus the homozygotic potential variability must be released more slowly than heterozygotic potential, because it must go through this latter state as part of the process. A minimum of two generations is necessary as compared with a minimum of one (Mather, 1943).

The homozygotic potential state of variability is an alternative to the free state in that when variability passes from the heterozygotic state some of it is freed and the rest becomes homozygotic potential. Where the effective genes are segregating independently the distribution between these two states will be unaffected by the origin of the heterozygote. The actual proportion which passes into the homozygotic potential state, relative to that which becomes free, will of course increase as the number of genes increases: in fact with n genes of strictly additive and equal actions the proportion becoming free is $1/n$ of the variability which passes from the heterozygotic state. Where the genes are linked, however, the relative proportions taking the two courses are no longer independent of the origin of the heterozygote. If this arose by the fusion of gametes carrying the extreme combinations of genes ($AB \times ab$), a larger part of the variability passing out of the heterozygotic state will become free once more. Equally, of course, where balanced combinations went into the heterozygote ($Ab \times aB$) more of the variability which leaves the heterozygotic state will become homozygotic potential than would be the case in the absence of linkage.

When, as must generally be the case, there is an optimum degree of expression of a character in relation to the selective forces at work, balanced linked combinations of the genes affecting the character will be favoured at the expense of the less balanced (Mather, 1941, 1943). For any pair of additive genes these balanced combinations (Ab and aB) will exceed the unbalanced (AB and ab) by an amount depending on the ratio s/c where s is the selective disadvantage of the heterozygous unbalanced types (Ab/AB , Ab/ab , etc.) relative to the fully balanced types (Ab/ab , aB/aB , etc.), and c is the recombination frequency of the two genes (Wright, 1944). When $s = c$ the gametes balanced in respect of the two genes will be nearly $2\frac{1}{2}$ times as frequent as the unbalanced, with a corresponding excess of homozygotic potential variability over free. With $s = 2c$ the balanced types will be over 4 times as common as the unbalanced. Thus any tightening of the linkage will lower the proportion of free variability.

This reduction of free variability is, of course, itself the selective

advantage of the linkage. Now the loss of fitness due to the segregation of the unfavourable extreme phenotypes, so far as these are produced by any two genes, is proportional to something between the first and second powers of c , their recombination frequency. Two consequences then follow. On the one hand, any agency making for closer linkage must be favoured, and, on the other, the main balancing effect must be between closely linked genes. In fact the closer the linkage of the genes, the greater becomes their importance in storing variability by balancing one another's action when in combination. Such stored variability can be freed only by recombination, itself rare, in the appropriate heterozygote.

If only one such balanced linkage is broken the release of variability will be a sudden event ; but where, as might be expected with most polygenic systems, balancing is widespread, a number of different recombinations must be recovered, simultaneously or more likely successively, before the variability is freed. The release of variability, and with it advance under selection, will then be a slow, though not always steady, process as the unbalanced, or coupling, combinations are gradually built up from their balanced, or repulsion, forerunners. But once obtained, these coupling combinations will be as easy to keep in being as they were difficult to make, because they can be lost only by the same process of recombination. Response to selection will be as fast wherever it depends on these extreme combinations, as it was slow when the balanced ones were segregating.

The crossing-over by which the balanced combinations are broken down and the variability released, will recombine not merely the polygenic system affecting the character for which selection is practised, but also any other such system, affecting a further character, which might be distributed along the same chromosome. Thus selection for one character will, by picking out the unbalanced combinations of the one set of genes as they are produced by recombination, also pick out unbalanced combinations of other sets of genes mingled with them along the chromosome, and so cause, as a correlated response, change in characters for which no selection is practised. This correlated response could even cause change against selection for the character in question, provided that this selection were of a lower intensity than the transferred effect of that practised on the primary character (Wigan and Mather, 1942).

It is clear from these considerations that the nature of the response to selection will be determined to a great extent by the linkage relations of the polygenic systems concerned. Conversely, of course, suitable observations on behaviour under selection will enable us to infer these linkage relations. The effects of linkage will be expected in all higher organisms, but they should be in strongest evidence where the number of chromosomes is small and the average frequency of crossing-over within them is low. *Drosophila melanogaster* offers, therefore, particularly suitable material for the observation and

analysis of the ways in which advance under selection reflects linkage relations.

The delayed response to selection, due to the necessity for crossing-over to effect particular recombinations, has been seen in experiments on this fly by Mather (1941, 1942) and by Sismanidis (1942). In both cases it was possible to trace the chromosomes in which the recombination had occurred, and Sismanidis also showed that the effect of selection had all the determinacy to be expected from a dependence on linkage relations. He found that the same responses, due to change in the same chromosomes, and depending one must presume on recombinations of the same genes, occurred in lines of the same original constitution, even where these were maintained and selected quite independently of one another. The presence of the expected coupling linkages, which selection caused to replace the former balanced repulsion linkages, could also be inferred from the greater variance of the F_2 between Mather's (1941) high and low selected lines, as compared with that of the F_2 from which they were selected. No selection was, however, made from this second F_2 to show that the limits of selection were as easy to recover as they had initially been difficult to produce.

Correlated responses to selection have been described in *Drosophila* by Wigan and Mather (1942) and in *Lebistes* by Svårdsson (1944). In each case fertility fell, as a concomitant of selection for both higher and lower number of hairs in the flies and for number of fin-rays in the fish. In neither case, however, could the possibility be fully excluded that the relation between the primary character and fertility was simply physiological, due to the same genes affecting both characters, though Wigan and Mather's observations made this possibility the less likely one. A further limitation of these experiments requires notice. Neither of them shows both aspects of the correlated response relation, in the flies the effect of selection for fertility on hair number as well as the effect of selection for hair number on fertility.

Thus while these various experiments have given valuable evidence of the control of advance under selection by the linkage organisation, their results reflect their limitations in scope and time. The experiment with *Drosophila melanogaster*, which we are now to describe, is much more extensive in the number of lines kept, in the time over which the selection has been practised, and in the tests to which the lines have been subjected. Its results, by extending as well as confirming those of the earlier experiments, enable us to see the relation between linkage and selection with increased clarity, and to infer the system of variability with increased confidence.

2. TECHNICAL INFORMATION

The experiment was started on 4th December 1942 by Mr G. M. L. Haskell, who carried it to the 13th selected generation. Circumstances then forced him to give up the investigation, which was continued

thereafter by us. We are indebted to Mr Haskell for allowing us to use his data from the early generations in this publication.

The character chosen to supply the basis of selection was that used earlier by Mather (1941, 1942), *viz.* the number of chaetæ on the ventral surfaces of the 4th and 5th abdominal segments. Though counted separately, the numbers of chaetæ borne on the two segments have in every case been summed to give a single value for the fly. Twenty males and 20 females were counted wherever possible from each culture. Males have on the average some 5 or 6 chaetæ fewer than females (see tables 2 and 3), and so the mean of the two sex means has been used as the measure of the chaeta-producing capacity of the culture. Where, as in the great majority of cases, the full number of individuals could be counted, the mean of the sex means is also the simple mean of all 40 flies; but where the numbers of the two sexes differed this relation no longer holds. It is then essential to take the mean of the means, rather than the simple mean, in order to avoid distortion by the preponderance of counts from one or other sex.

The start of the experiment was a cross between the two wild-type stocks, Oregon (Or) and Samarkand (Sk). The former has been maintained in this laboratory for over nine years by brother-sister mating, apart from one generation's lapse into cousin mating in the early years. At the time of the cross in question, brother-sister mating had been practised for 74 generations since the lapse, which was itself preceded by 79 known generations of continuous brother-sister mating. The Or line has now reached over 200 generations of unbroken brother-sister mating since the lapse.

The Samarkand stock was being maintained solely by mass culture at the time of the initial cross, but since then an inbred line has been developed by brother-sister mating from it. This has been used side by side with the original mass stock, which is still maintained, for certain tests made later in the experiment. The inbred line and the mass stock gave no evidence in these tests of any difference which might affect the interpretation of the data. In fact the only noticeable difference between them was that flies in the inbred line mated rather less readily than those from the mass culture, and were perhaps less prolific.

An F_1 was raised from the cross between the two wild-type lines, and from it an F_2 . Selection was first practised at this stage by the choice of parents for the third generation, which thus became the first selected generation (S-1) of the experiment. Before leaving the F_1 and F_2 generations, it may be noted that the cross was remade and these generations repeated in 1946, for the purpose of observations on the spermathecae of the females as will be reported in a later section. At the same time Dr M. Westergaard, then visiting this Institution, kindly examined the salivary chromosomes of both F_1 and F_2 without finding evidence of any gross structural difference between the parental lines.

Selection was practised for increasing and decreasing numbers of chaetæ in two separate lines derived from the F_2 . In each of these selection lines, and indeed in all the various selection lines of the experiment, two cultures were set up in each generation. As already noted the chaeta numbers were determined for twenty flies of each sex from each culture, and the two most extreme (high in the high selection lines, and low in the low selection lines) were then selected from each twenty flies. The two males from the first of the two parallel cultures of a line were mated jointly to the two females from the second and *vice versa*, so giving two cultures, each with four parental flies, for the next generation. This method of mating was adopted as a means of minimising the risk of inbreeding. Where one of the two cultures of a selection line failed, twenty flies of each sex were taken for counting from the surviving culture in the usual way; but the four extreme flies of each sex were taken and mated two by two, to give parents for the two cultures of the next generation. Thus the failure of a culture meant a halving in the rigour of selection. Failure was, however, relatively uncommon during the experiment.

The four parents of a culture were mated in a vial for from one to three days, according to the circumstances of the moment. They were then transferred to a half-pint milk bottle and allowed to remain in it until just before the first of their offspring began to emerge. The cultures of all lines were placed in the incubator at 25° C. as soon as the parent flies were transferred to them from the vials, and remained at this temperature until finally discarded. With fully fertile flies this technique would ensure crowding in the bottles, but in some of the lines fertility, which was measured throughout the experiment after S-13 by the total output of flies from the bottle, was so low as to prevent the development of crowded conditions. The flies were shaken out of the bottles night and morning, and the sexes stored separately to preserve the virginity of females taken for mating.

As will be seen from later sections, a number of lines were developed and maintained during the experiment by mass mating. Only one culture was made of each of these lines per generation. The mean chaeta number was determined from counts of twenty flies of each sex as in the selection lines. The first ten flies of each sex to be counted were then taken, irrespective of their chaeta numbers, to be the parents of the culture in the next generation. Furthermore, these mass cultured lines were kept in step with the selection lines, so that, with the exception of two lines, *viz.* 2 and 8, which will be mentioned later, the representatives of all lines described as occurring in any particular generation of the experiment were in fact raised simultaneously in the incubator.

The flies were collected from each culture, whether of selection or mass line over at least the major part of the emergence period before the twenty of each sex were taken at random from among them for counting. The total emergence was used in estimating

fertility and for the observations on spermathecae which will be described later. Earlier experience had suggested that time of emergence and condition of food had only a small effect on the average number of chaetae, and certainly would not seriously distort observations on this character. Special tests were nevertheless made of their effects.

The test of the effect of culture conditions was made using 6 cultures each with 10 male and 10 female parents taken at random from a single culture of the mass line 3 (see section 3*b*). The flies were mated in vials for a day before transference to the culture bottles, 3 of which were two days old and 3 a week old. The two-day bottles were in good condition, but the others were stale, with dark yellow yeast growths and also some mould. A less vigorous stock would doubtless have died in them. The mean numbers (means of sex means) of chaetae per fly in the offspring obtained from these bottles are given in table 1. The means from the fresh culture bottles are

TABLE 1
The effect of initial culture condition on chaeta number

Condition of culture bottle	Mean chaeta number in culture			Grand mean
	A	B	C	
Fresh—2 days old . .	40.98	40.88	41.25	41.03
Stale—7 days old . .	42.38	42.10	41.53	42.00

all lower than those from the stale ones. The difference between them is significant, between the 0.05 and 0.02 probability levels, but it is just less than one chaeta. The initial state of the culture bottle cannot therefore have a seriously misleading effect on the interpretation of differences so large as those to be recorded in this experiment, particularly as it was not the practise to use culture bottles which had been standing for as much as a week. With rare exceptions, in fact, the flies were put into the bottles two or three days after these had been made up.

A second test concerned the effect of the time of emergence on chaeta number. Two single pair matings were made in the Or stock and cultured in the usual way. The emerging flies were shaken out each morning and evening over a period of ten days. The daily averages of the two cultures taken together are shown for males and females separately and also jointly in table 2. It appears that there is a fall, albeit an erratic fall, in mean chaeta number with time of emergence, and it seems to be more rapid in the early part of the emergence period than it is later. Indeed the grand average for days 8-10 is slightly higher than that for the period 5-7. The fall is, however,

nowhere great. The most extreme daily averages recorded for males are 40.67 and 39.00, and for females 46.94 and 45.32, a difference of less than 2 chaetæ in each case. As would be expected, the extreme values for the mean of the sexes, 43.76 and 42.37, differ by even less. Since the change in chaeta number with time of emergence should be over-estimated by the differences between the extreme values, the

TABLE 2
The effect of time of emergence on chaeta number

	Mean chaeta number on emergence day									
	1	2	3	4	5	6	7	8	9	10
Males . . .	...	40.57	40.67	40.11	40.30	39.00	39.42	40.58	39.16	39.54
Females . .	46.83	46.94	46.23	45.88	45.61	45.85	45.32	45.58	45.78	46.12
Mean of sexes	...	43.76	43.45	43.00	42.96	42.43	42.37	43.08	42.47	42.83
		43.40			42.58			42.78		

average fall in chaeta number over the main period of emergence seems unlikely to be much over one chaeta per fly. Like the similar difference caused by culture conditions in the previous test, this fall can cause little trouble in interpreting the results of selection. Even the slight effect it could have will have been minimised by the technique of collecting the flies over a large part of the emergence period before taking a sample for counting.

A further possible source of error remains to be mentioned, *viz.* personal differences in chaeta counting. It will be recalled that Mr Haskell made the counts until generations S-13, after which with a few exceptions they were made throughout the experiment by the junior author. On the exceptional occasions the senior author counted the chaetæ. Table 3 shows the average counts made by all three

TABLE 3
Personal differences in counting chaetæ in the Oregon inbred stock

Counter	Time of count	Mean number of chaetæ		
		Males	Females	Mean of sexes
K. Mather . . .	1939-40	39.6	44.4	42.0
G. M. L. Haskell . .	December 1942	32.0	40.1	36.0
B. J. Harrison . . .	February 1944	40.9	47.0	43.9
" " . . .	December 1946	40.8	45.5	43.2

people on the Or inbred stock. The two Harrison counts agree well with each other though separated by nearly three years. The Mather

count of some five years earlier differed by only $1\frac{1}{2}$ chaetæ from the average of the two Harrison counts. Such a difference could be due to a small change in the genetic constitution of the Or line; but it might also have been caused by the change in composition of the culture medium necessitated during the period 1939-46. It is in any case a difference of little moment, and in fact the occasional counts made by the senior author during the experiment agreed well with those of the junior author in all lines.

The situation is somewhat different when we turn to the Haskell counts. These average 6 chaetæ less than the Mather counts and 7.5 less than the Harrison average. The chaeta number of an inbred stock seems hardly likely from genetic causes to have fallen so much only to rise again. The discrepancy therefore suggests a genuine personal difference in counting, and warns us to be cautious in the detailed interpretation of the relations between the first thirteen generations and the rest of the experiment. Attention will later be drawn to certain specific effects of this change in counting.

3. CHAETA NUMBER AND FERTILITY

(a) *The low selection and low mass lines*

When selection was started in the F_2 of the cross between the Or and Sk stocks it was practised for both low and high chaeta numbers in separate lines. Much the greater part of the experiment is concerned with the high line and its derivatives, but the low line also has its interest. It will therefore be described before attention is turned to the results of selection for high chaeta number.

The progress in chaeta number of the low line under selection is shown for the first 61 generations in fig. 1, and for the next 61 in fig. 2. In these figures, as indeed in all the figures, solid lines are used to portray the chaeta numbers of lines upon which selection was being practised, and broken lines for the mass cultures kept without any artificial selection. Each point represents the mean of the chaeta numbers (each of which is, as already noted, itself the mean of the two sex means) of the two cultures in each generation of the selection lines, except where one of the two cultures failed, in which case the point on the graph is the chaeta number of the single surviving culture. In the mass lines each point is obtained from only one culture, as only one culture was kept in each generation. In referring to these graphs the generation numbers shown along the abscissa will be used, prefixed by an S to indicate that they start from the first generations of selection, *i.e.* F_3 and thus $F_2 = S-0$, and $F_3 = S-1$.

Progress under selection in the low line was fairly steadily downwards at first but the chaeta number receded sharply upwards in generations S-9 and S-10, only to fall again until S-15. At this

point the number had fallen by over 5 chaetæ from the F_2 mean value. Then followed a steady recession, amounting to some 2 chaetæ over the next 6 generations. This recession was most likely due to the change in counting consequent on the change in observers at S-13. Probably the second observer, B. J. Harrison, was influenced at first by his predecessor's counts and only gradually over several generations established his own level of counting. After S-22 the advance under selection was resumed and gradually increased in speed until by S-34 the lowest level was reached at 25 chaetæ, an advance of 11 (or quite likely really more in view of the change in counting) from the F_2 mean of 36. Sterility had also set in and only a few males were obtained in S-35 so that the line died out.

The low line thus behaved in the way expected from earlier experience. It progressed slowly, attaining its full advance only after 34 generations. This must indicate that the main progress depended on the release of variability by unbalance following recombination between balanced combinations within the chromosomes; for with only three major chromosomes progress due to the segregation and recombination of whole chromosomes should certainly have been over by S-5. At this point the advance had, in fact, only covered 3 chaetæ and there was a period of stability and even of recession which might well mark the period of waiting before intra-chromosomal recombination effectively began to release the variability upon which the main advance depended. This main advance was accompanied by the correlated response of loss of fertility, which eventually killed the line.

At S-20 a mass line (designated the low mass line or line 1) was started from the low selection line in parallel with one begun at that generation from the high selection line. The chaeta number of this mass line fluctuated somewhat during its history, to an extent perhaps a little greater than could fairly be ascribed to non-heritable effects. Such fluctuations would not be surprising in a mass line where selection is not acting to steady the random variation of gene frequencies. That no more deep seated cause of change was at work is attested by the remarkable overall stability of this mass line during the 95 generations that it was in existence. The line began with a mean of just over 32 chaetæ in S-21, and it had a mean of about 33 when it was terminated in S-115. Its peak value was 35, reached at S-44 and again at S-95, and its lowest value $30\frac{1}{2}$ at S-27, with a value of 31 attained or just passed six times.

A new low selection was taken off line 1 at S-31 and progressed to a value of 29 chaetæ in the 8 generations which elapsed before sterility killed it at S-39. A third low line begun at S-42 eventually died out after only 3 generations, during which progress had been of only 1 chaeta. The fourth low selection started from the mass at S-48, survived one generation longer, and progressed for nearly 3 chaetæ before it was extinguished by its sterility at S-52. The

next two low selections, at S-53 and S-54, were sterile from the first, inasmuch as no progeny were obtained from the parents originally selected from the mass line. The last low selection, attempted at S-56, survived one generation before sterility overtook it.

Selection from line 1 in the opposite direction, for high chaeta number, fared little better. Two such selections were attempted. One, begun at S-48, terminated through sterility after only two generations. Its chaeta number showed no advance; indeed it approximated so closely to that of the mass line that clarity demanded the omission of this selection line from fig. 1. The second high

FIG. 1.—Response to selection between S-0 and S-61 for abdominal chaetae in lines 1, 3, 6, 7 and 8 and certain other lines which were not continued. Selection lines are solid and mass lines broken. T indicates that the line was terminated deliberately, and D that it died out through sterility. The selection marked H was a high selection from line 1, the other selections from which were for low chaeta number. Note the low selections from lines 3 and 6 at S-48 and S-49 respectively. Ordinate is mean number of chaetae.

selection was a little more successful. Begun at S-56, it survived until S-61 before it was extinguished by sterility. It advanced little if at all, from the parent line in chaeta number, as can be seen from fig. 1 where it is distinguished from the various low selections, taken off the same mass, by the letter H.

Thus selection from the low mass line became progressively more difficult as the generations wore on, in the sense that both length of survival and progress made became lower. Some progress was made, however, in the second selection, and a little even in the fourth, before sterility supervened, so that the stability of the low mass itself can hardly have been due to homozygosity. Rather it seems that the low mass was building up a balanced sterility system within itself

and in the absence of any artificial intervention. The flies with the more extreme chaeta numbers seem to have been effectively sterile when the system was eventually fully developed—a sterility which might have been due either to their being more homozygous than their less extreme fellows, or to the combinations for which they were heterozygous being less well relationally balanced. The short periods of survival of some of the later selection lines could well have been due to flies with better balanced genotypes being selected among the 8 parents, with which each line commenced, owing to their extreme chaeta numbers being lower than usual in consequence of non-heritable effects. Such flies would keep the line going, though with little or no advance under selection, until among their descendants only genetically extreme, and hence sterile, flies were selected as the parents of a generation. Then the line would die out. The behaviour of all the selection lines after S-40 would accord in this way with the existence of a balanced sterility system in the mass line, a system which the second low selection suggests to be partially, though of course not fully, developed as early as S-31.

We shall meet several other cases of balanced, or partially balanced, sterility during the course of this report; but that in the low mass line was remarkable among them in two respects. First, it was of sharper action, in that deliberate selection from the mass became virtually impossible; and second, it seems to have grown in strength during the history of the mass line itself.

Three attempts were made to obtain more direct evidence of the sterility in this low mass line. In generation S-75, 27 flies of each sex were counted for chaeta number and then made up into 27 pair matings. For this purpose those of each sex were divided into three groups, each of 9 individuals, with the highest (H), the middle (M) and the lowest (L) chaeta numbers respectively. They were then mated to give 3 H×H matings, 3 H×M and so on, as shown in table 4 where the actual chaeta numbers are given. The mated pairs were allowed to breed, each in a single vial, and the fertility or sterility was recorded according to whether offspring were produced or not. The table shows that all 9 females in the M class gave offspring, while only 5 from each of the H and L classes did so. The M males had one more fertile mating than the H or L males. There is thus an indication, though hardly significant evidence, that females with medium chaeta numbers were more often fertile than their extreme fellows. In the case of the males the differences between the three classes were too small to be regarded as even suggestive. The 18 fertile matings averaged 38 progeny each.

The second test was made by mating 15 females and 20 males individually to single mates from the Or line, after counting their chaetæ (table 5). Fertility of the line 1 females was judged by the presence of larvæ in the culture vials, and of the males by the presence of live sperm in the receptacles of their mates after two days. There

is a suggestion that the females with low chaeta numbers are more likely to be sterile than those with higher ones, but the difference in

TABLE 4
Chaeta number and fertility of matings in the low mass line (S-75)

		Males					
		H	M	L			
Females	H	36×36 36×35 38×33 1f+2s	39×32 41×33 36×31 2f+1s	37×30 36×30 37×27 2f+1s	5f+4s		
		35×35 35×34 34×33 3f+os	35×32 35×32 35×32 3f+os	35×29 35×26 35×29 3f+os		9f+os	
		34×33 31×35 33×33 2f+1s	32×32 34×31 34×31 2f+1s	34×30 32×30 33×30 1f+2s			5f+4s
			6f+3s	7f+2s	6f+3s		

The numbers are the chaeta numbers of the parent flies
f = fertile and s = sterile culture

TABLE 5
Fertility of single flies of low mass line in crosses to Oregon
Females—Fertility judged by presence of larvæ after mating with Oregon male

Chaeta number	.	.	.	.	33	34	35	36	37	38	39
Fertility	.	.	.	.	f s	f s	f s	f s	f s	f s	f s
Number of flies	.	.	.	.	1 1 ...	2 4 ...	2 1	3 ...	...	...	1 ...

Mean chaeta number of
Sterile females 34.25 } Difference not significant
Fertile females 35.91 }

Males.—Fertility judged by presence of live sperm in Oregon female

Chaeta number	.	28	29	30	31	32	33	34	35	36
Fertility	.	f s	f s	f s	f s	f s	f s	f s	f s	f s
Number of flies	.	... 1 ...	3 2 1	2 ...	4 1	3 ...	2 ...	...	...	1 ...

Mean chaeta number of
Sterile males 29.50 } Difference significant (P 0.01-0.001)
Fertile males 32.36 }

mean chaeta number of the sterile and fertile females is not significant. It might, however, be noted that, as in the first test, no female with 35 chaetæ proved sterile. The test of males proved more conclusive.

Of these 6 were sterile, including 5 of the 6 flies with the lowest chaeta numbers. The mean chaeta number of sterile males was 2.86 below the average of their fertile counterparts, a difference which is significant with a probability only just above 0.001.

The third test was made on four pair matings in S-57, both males and females coming from line 1. The paired flies were transferred to new vials daily, to permit counts to be made of the eggs laid (table 6). The average daily output of the 4 females was only 18.4

TABLE 6
Fertility of eggs from four matings in low mass line (S-57)

Mating	Eggs laid	Progeny obtained
37 × 35	68	0
33 × 27	19	0
32 × 35	156	54
31 × 26	125	0

Chaeta number of female parent given first

eggs, with 63 as the most laid by any one fly on any one day. Progeny were obtained from only one mating and then to the extent of only about $\frac{1}{3}$ of the eggs laid. This fertile pair consisted of a female with a moderately low and a male with a moderately high chaeta number.

The evidence from these tests can hardly be regarded as conclusive. They do show, however, that the flies of line 1 had a fairly high incidence of sterility, and that the output of progeny by even a fertile pair was low. There is also an indication from the first test that the females with more extreme chaeta numbers were less often fertile than those with central counts; and from the second test that males with low chaeta numbers failed to inseminate their mates more often than those with higher numbers.

So far as they go, therefore, these tests agree with the view that the low mass line had developed a balanced sterility system within itself. Of how it came to be so sharp in its action we have no evidence except that, from the fates of the successive attempts to select from the mass, the rise of system would seem to have been gradual. A further investigation of the system would clearly have demanded very elaborate experiments, more costly in both time and space, and less informative on the questions with which the work was started than those which had become necessary in the high counterpart of the low selection line and its derivatives. The investigation of the line 1 was therefore abandoned and attention concentrated on the high selection and its derivatives, to which we must now turn.

(b) *The high selection line and its derivatives*

The high selection line was started at the same time and from the same F_2 as its low counterpart. Its progress in chaeta number

was both more rapid and more smooth than that of the low line, without any suggestion of a prolonged pause after the first few generations (fig. 1). By generation S-5 it had risen from the initial chaeta number of 36 to $41\frac{1}{2}$, by S-10 to 45, and by S-15 to $49\frac{1}{2}$. It would thus appear that recombination within chromosomes must have begun to release variability, so as to permit further response to selection, as soon as, or even before, the response due to the reassortment of whole chromosomes came to an end. The effect of the change in counting after S-13 is not particularly noticeable, perhaps because it served to mask a period of reduced progress. However this may be, the rise in chaeta number continued, though a little erratically in the last 4 generations, up to S-20 when the chaeta number of 56 was attained, representing an increase of 20 over the initial number.

FIG. 2.—As fig. 1, but covering generations S-56 to S-112. Line 4 appears for the first time at S-60 as the mass taken from line 6.

As in the low line, fertility fell in the later stages of the advance in chaeta number. The fall was, as might be expected from the rates of change in chaeta number, more rapid in the high line than in the low, and indeed a total of only 8 flies was obtained from the two cultures of S-20. To continue with selection appeared therefore likely to result only in the total loss of the line in S-21 or soon after, and resort was made to mass culture. In this mass line (line 3), which replaced the high selection line, the average chaeta number immediately began to fall very sharply, and by S-25 was down to little more than 39, a loss in 5 generations of over 80 per cent. of

the gain which had taken 20 generations to achieve. Indeed, in view of the change in counting at S-13 it may well be that the whole of the selective advance had been lost in these 5 generations of mass culture.

The fall in chaeta number checked at S-25 and an erratic rise from a mean of 39 to one of 46 seems to have occurred during the next 4 generations of line 3. It then fell back to 41 by S-31, after which it fluctuated much as the line 1 did. Line 3 suggests, however, a number of longer and slower changes superimposed on the erratic, non-heritable and chance fluctuations which formed the only variation in the line 1. There seems to have been a slow rise towards 44 chaetæ between generations S-42 and S-55, followed by a period of general stability until just after S-70 (fig. 2), when a small rise took place, only to be followed in its turn by a steady decline to a general level of 40 chaetæ at S-100. A further rise then appears to have occurred to S-106, but some doubt is cast on this by the more or less parallel behaviour of the three other mass lines of fig. 2. Possibly the environment, common to all the lines, was changing at this point. In any case there came a fall to S-109, after which the mean chaeta number was stable (leaving aside the customary small fluctuations) till the end of the experiment (fig. 3.).

Even if their occurrence be taken as real, these long, slow and not very large changes in chaeta number are no more than might be expected in what, as we shall presently see, must have been genetically a heterogeneous population, and one whose degree of heterogeneity was itself changing. It is the rapid fall in chaeta number between S-20 and S-25 which must attract our main attention, rather than the near stability which followed.

The fall in fertility of the high selection line is the customary correlated response to selection for changed chaeta number. The recombinant chromosomes picked out by their changed, and, from the standpoint of the original stocks, unbalanced combinations governing chaeta number, are by virtue of the same recombination also unbalanced for the polygenic system governing fertility. And genic unbalance in respect of fertility can only mean one thing—increased sterility.* Thus these recombinant chromosomes, giving the higher chaeta numbers which artificial selection favours, also change fertility in a way which natural selection must disfavour. They are maintained in the line only because the artificial selection is stronger than the natural selection.

Should the effect on fertility not be too disastrous, the selection for chaeta number will eventually fix the chromosomes with the

* In these experiments sterility of the parents is generally inseparable from inviability of the offspring. Both give sterility of the line. The single term sterility will therefore be used to cover both phenomena. The arguments relating to recombination, unbalance and correlated response will obviously apply as much to viability as to true sterility, and so of course also to sterility used in the present sense to cover both phenomena simultaneously.

most changed combinations by rejecting all the ancestral and less changed types in the population. Where the flies contain only the combinations giving reduced fertility, relaxation of selection for chaeta number will merely result in the line maintaining the level that it has attained in chaeta number, for there will be nothing to change it. But if the more changed combinations have not fully ousted the

FIG. 3.—As figs. 1 and 2, but covering generations S-96 to S-138. Lines 2 and 9 are also included: their origins and earlier behaviours are shown in figs. 6 and 5 respectively. The wavy lines show where selection had to be relaxed in line 9 owing to infertility.

parental and less changed ones, owing to an overdrastic reduction in fertility when homozygous, this relaxation of the selection for chaeta number will expose the more changed combinations to competition from the less changed. The latter give the greater fertility and so, when the inability to bring about high chaeta number is no longer an agent in determining their fate, they will multiply at the expense of the changed types, and chaeta number must fall.

We can see in fact the reverse side of the correlated response to selection in this situation.

This seems to be exactly what has happened in the high line. So long as artificial selection made chaeta number the capital character and fertility the subordinate, chaeta number rose even though fertility was falling. The line failed, however, to lose all representatives of the chromosomes having parental or at least less changed effects on chaeta number and fertility, doubtless because flies carrying nothing but the most drastically altered combinations were sterile or inviable or nearly so. Thus when artificial selection was relaxed another type of selection took charge. Owing to the natural selection of competition under the conditions of mass culture, fertility became the capital character and chaeta number the subordinate. The less changed combinations multiplied so that fertility rose and chaeta number fell, just as chaeta number had risen and fertility fallen under the artificial selection. Thus the two compound responses to the two types of selection owed their occurrence to the same phenomenon. Intermingling of the two polygenic systems along the chromosomes resulted in recombination causing them to change simultaneously. The high chaeta combinations of necessity came to be associated with lowered fertility, so that no matter which was rising under selection, chaeta number or fertility, the other must fall.

There must, however, be a difference as well as a resemblance in the two aspects of the correlated response as revealed by the changes under the two types of selection. When chaeta number was rising and fertility falling, they were doing so only as recombination released the hidden variability by producing changed polygenic combinations. The advance under selection depended on the building up of new systems of genes, and the speed of advance could only be the speed at which crossing-over could reassociate the effective genes. Several, if not many, cross-overs might be needed to do this within one chromosome. When natural selection for fertility took charge, on the other hand, its discriminative effect would be exercised between chromosomes carrying widely divergent combinations of genes already in existence. The rise in fertility and fall in chaeta number in the mass line had not to wait on recombination by crossing-over, so that the change was much more rapid than its opposite which had occurred under the artificial selection of the high line.

Thus once obtained, the combinations built up slowly through the agency of crossing-over behave virtually as units in inheritance; they are, and indeed must be, as easy to maintain as they were difficult to make, because they can break down only by the same process that built them up. We have seen that the fall in chaeta number of the line 3 was much faster than the previous rise in the selection line for this reason. Further rises in reselection lines taken off the mass line should, however, be as fast as the fall had been, because they would depend on the utilisation of the high chaeta number combinations

already available, though with diminishing frequency, in the mass line. Reselection should in fact reverse the process whose effects had been seen in the rapid fall of the line 3, rather than repeat the process which had given the slow rise of the first selection.

Eight reselection lines were taken off the line 3 at various times. The first (line 8) was begun at S-24, a generation before line 3 reached its low level, and rose so rapidly that in 4 generations it had reached within about one chaeta of the peak recorded at S-20 in the first high selection. Here it became effectively stable in chaeta number and remained so until S-80 even though selection was continued. Its later behaviour will be described in section 3 (*f*). The important point for our present purpose is that this line recovered the high chaeta number as rapidly as the line 3 had lost it. The high chaeta combinations, which as units had dwindled in frequency under the natural selection of the mass culture, increased as units once again when artificial selection was reapplied. The slow process of building them up by crossing-over had not to be repeated.

One further point remains to be noticed about this reselection. Unlike the first selection line, the high chaeta number was attained without an overdramatic loss of fertility. The number of flies never fell so low as to endanger the stock. Furthermore, when a second mass line (line 7) was made from this reselection at S-34 its mean chaeta number did not fall in the way that that of line 3 had done. The selection must have been effective in making the line homogeneous for high chaeta number combinations, so that relaxation of the selection for chaeta number did not result in its fall. There must have been between S-20 and S-34 a sufficient reassociation of the polygenes governing chaeta number and fertility for the high chaeta number combinations to acquire sufficient fertility to give a stable line. That this line was only homogeneous for high chaeta combinations in the various chromosomes, and not homozygous for any of them, will become clear when we consider its subsequent behaviour in section 3 (*e*).

The second reselection was taken from line 3 at S-27. By S-30 it was so obviously repeating the behaviour of its fellow, begun 3 generations earlier, that it was deliberately terminated. Line 3 then fluctuated upwards in chaeta number for 2 generations before it fell again, and during this fluctuation its genetical constitution must also have changed, for the next 5 reselections, taken off at S-30, S-33, S-36, S-39 and S-44, rose each in 2 generations to a mean chaeta number of 45 or 46 at which level the rapid advance ceased. They had gone up rather less than half-way to the level reached by the reselections of S-24 and S-27. The change in the line 3 between S-27 and S-30 had evidently permanently reduced the variability available for response to selection by rather more than half, just as would happen if the high chaeta number combinations in at least one and at most two of the three major chromosomes had finally

dwindled in frequency so far under the conditions of competition in mass culture that they had vanished altogether. Having been relatively rare before, their total loss had no detectable effect in lowering the mean chaeta number of line 3 : it could only be detected by the loss of half the capacity to respond to reselection.

The last of the 5 reselection lines (line 6) which rose rapidly only to the halfway mark, *viz.* the one begun at S-44, was maintained under selection for some 80 generations. It showed no further rapid advance, though it rose slowly by 4 chaetæ in the 12 generations after S-49. It then became stable until about S-93, when it began another slow rise ending some 14 generations later. A mass culture line (line 4) begun from it at S-60 remained as stable as a mass line could be expected to do until the end of the experiment (figs. 2 and 3). Thus like line 8 at the top level, developed by the first reselection, line 6 must have been homogeneous for high chaeta combinations in such chromosomes as had retained in line 3 the variability upon which response to selection depended ; but it could not have been homozygous for any of them. Its power of rapid advance, which depended on the rise in frequency of existing high chaeta number combinations, was expended in the first 2 generations, but the line could and did advance further as new combinations giving even higher numbers were built up slowly by crossing-over just as in the first 20 generations of the experiment.

The change in line 3, whose effect we have just seen, was not the last to occur. At S-72 the eighth high reselection was begun from this mass by Mr S. H. Revell, to whom we are indebted for permission to make use of his results. He failed to obtain any advance in chaeta number in the 4 generations during which he practised selection (fig. 2). The fate that had overtaken the high chaeta combinations of one, or possibly two, of the three major chromosomes between S-27 and S-30 had extended to those of the remaining chromosomes. None of those combinations producing high chaeta numbers built up by the selection of the first 20 generations of the experiment had survived in the mass line up to S-72. Any further increase in chaeta number under selection must thus depend once again on the slow process of recombination within the chromosomes.

(c) *The chromosome assays*

The responses to selection enable us to see how high chaeta number combinations are built up slowly and piecemeal by crossing-over under selection ; how they are associated with reduced fertility ; how, once in being, they act as units when they are decreasing in frequency by virtue of natural selection for fertility, or increasing in frequency under reselection for high chaeta number ; and how they are even finally lost as units in the mass line. These results do not, however, enable us to specify which particular chromosomes have

been affected by these changes, or how much each chromosome has contributed to the responses to selection at the various stages of the experiment. This information can be gained in a large measure, and the general inferences drawn from the results of selection verified, through the use of the technique of chromosome assay described by Mather (1942).

The method of assay depends on comparisons of the chaeta producing power of the chromosomes from the various lines, developed in the experiment, with their homologues from a common tester stock. The homologous chromosomes of the various lines can then be compared with one another by means of this common standard of reference. Direct comparison of the chromosomes from the various lines is of course impossible because the lines all being wild-type, their chromosomes cannot be followed in segregation. The chromosomes of the tester are, however, marked with dominant major genes so that their segregation from their homologues can be followed. Inversions are used in the tester chromosomes to reduce their recombination in the heterozygotes, so that they segregate sufficiently well as whole units, the marker genes enabling these units to be traced in transmission.

The tester used was a stock of *ClB/+ ; Cy L⁴/Pm ; H/Sb In 3(R)Mo*. Females from it were crossed with males of the line to be assayed ; and females of the type *ClB/+ ; Pm/+ ; Sb/+* were taken from the F_1 and crossed back to males of the experimental line. In this backcross 8 classes of female progeny are to be expected, in all of which one representative of each chromosome will have come through the father from the line under assay. The other representative, that from the mother, will sometimes be derived ultimately from the line (L) and sometimes from the tester (T), the origin being indicated by the presence or absence of the marker gene, as shown in table 7. The same will be true of the autosomes in the males from the backcross, but not of the X. Males have, therefore, not been used in these assays, and the data given in this section refer always to females unless otherwise stated.

The chaetæ were counted on a maximum of 5 females of each of the 8 classes in each of the replicates of the backcross. Usually 8 (though occasionally 10) replicates were used and the class averages thus determined from about 40 flies. Now classes 1 and 5 in table 7 differ only in that 1 has an X chromosome each from the tester and the line, while 5 has both X's from the line. Thus the difference in average chaeta number of these classes affords an estimate of the excess in chaeta producing power of the X from the line over its homologue from the tester. The same is true, however, of classes 2 and 6, 3 and 7, and 4 and 8. So we can obtain a pooled estimate of the excess by finding $\frac{1}{4} (\bar{8} + \bar{7} + \bar{6} + \bar{5} - \bar{4} - \bar{3} - \bar{2} - \bar{1})$ where $\bar{8}$ is the average number of chaetæ carried by females of class 8 and so on.

The data can be made to yield estimates of the excess chaeta producing power of chromosomes II and III from the line over their homologues from the tester by adding and subtracting according to the signs in columns 7 and 8 of the table. Since the experiment is

TABLE 7
The method of chromosome assay

Class	Phenotype	Source of chromosomes			Combinations measuring							
					Main effects			Interactions				
		X	II	III	X	II	III	X-II	X-III	II-III	X-II-III	
1	<i>B Pm Sb</i>	T, L	T, L	T, L	—	—	—	+	+	+	—	
2	<i>B Pm</i>	T, L	T, L	2L	—	—	+	+	—	—	+	
3	<i>B Sb</i>	T, L	2L	T, L	—	+	—	—	+	—	+	
4	<i>B</i>	T, L	2L	2L	—	+	+	—	—	+	—	
5	<i>Pm Sb</i>	2L	T, L	T, L	+	—	—	—	—	+	+	
6	<i>Pm</i>	2L	T, L	2L	+	—	+	—	+	—	—	
7	<i>Sb</i>	2L	2L	T, L	+	+	—	+	—	—	—	
8	wild-type	2L	2L	2L	+	+	+	+	+	+	+	

T, L = 1 chromosome each from the tester and the line

2L = both chromosomes from the line

factorial in type these estimates will be independent of one another and of the estimate of the effect of the X (see Fisher, 1947 ; Mather, 1946). The analysis can be completed by finding the so-called interactions in effect of the chromosomes taken two at a time and all three together, using the functions in columns 9-12. These interaction items measure the departure from simple additiveness of the effects of the various chromosomes in their different combinations. All the totals arrived at by using these functions are divided by 4 as in the case of the X. The functions are orthogonal to one another and the values obtained by their use are independent, so that each of the seven (three main effects, three first-order interactions, and one second-order interaction) can be manipulated and interpreted as a separate entity.

Before turning to the consideration of the assay data themselves, two limitations to the technique must be noted. Since the comparison for each chromosome is between the heterozygote T/L and the homozygote L/L, the effects of the genes from the line, L, will escape detection where these genes are completely dominant to their allelomorphs from the tester, T. Only fully recessive genes from L will exert their full effects in this comparison, those of intermediate dominance exerting corresponding fractions of their effects. If dominance is absent or is balanced in the sense that the total dominance of genes from L is equalled by that of the genes from T, the assays

will account for half the genetic difference in chaeta producing power. Or to put it another way, they will be 50 per cent. efficient.

The second limitation is that the inversions used, especially those in chromosomes X and III, are known not to suppress all effective recombination. The comparisons will thus not always be between the homologous chromosomes of T and L as units. Some of the genetic differences will escape detection, or will appear as having a spuriously low effect. The loss of efficiency of the assay due to this cause should not, however, be great because, even though not eliminated, recombination is very materially reduced. This expectation is borne out in practice. The flies of the wild-type and the *Cib/+* ; *Pm/+* ; *Sb/+* classes in the backcross should differ in average chaeta number by as much as the flies of the line itself and of the F_1 between line and tester, apart from loss due to recombination. Table 8

TABLE 8

Loss of efficiency of assays through recombination

The entries in this table are the differences in chaeta number between wild-type flies and *Cib/+* ; *Pm/+* ; *Sb/+* when these are

A = from the line under assay and the F_1

B = segregant classes from the backcross

Line	A	B	Flies for assay taken from
Sk inbred	-9.1	-5.6	...
Sk mass	-8.8	-5.5	...
Line 1	-6.3	-4.9	S-110
Line 2	-6.6	-6.0	S-109
Line 3	-2.1	-1.0	S-108
Line 4	0.6	3.5	S-109
Line 5	2.3	3.5	S-109
Line 6	2.0	3.7	S- 97
Line 7	5.0	6.0	S-103
Line 8	8.8	7.0	S-108
Line 9-1	6.6	6.2	S-107
Line 9-2	13.4	11.6	S-124
Average neglecting sign	5.97	5.38	...

compares these differences in 12 different assays. Those in the backcross (column B) are generally speaking a little lower than those between line and F_1 (column A) ; but this loss is on the average only 10 per cent. of the column A difference. The loss of efficiency due to recombination, with which of course is confounded the loss due to failure to assay chromosome IV, is thus insufficient to cause any serious misjudgment of the situation. There is a suggestion that the loss is greater in the lines with lower chaeta numbers, particularly the parental Sk line, but the data are not fully consistent in regard to this relation between chaeta number and loss.

The parental Or line was assayed in 11 tests, at least one such being undertaken whenever other lines were being tested. Since the

Or line was inbred and so genetically uniform, or nearly uniform, throughout the experiment, these 11 assays afford a measure of the consistency of the method, or looked at the other way of its error variance. Their results are set out in table 9. The 11 values for

TABLE 9
The eleven assays of the Oregon parent

	Main effects			Interactions				
	X	II	III	X-II	X-III	II-III	X-II-III	
	0.83	0.44	-3.05	0.07	0.46	-0.15	-0.16	
	0.52	0.12	-2.12	1.12	0.58	0.78	0.58	
	1.68	0.46	-2.44	0.21	-0.12	0.79	-0.24	
	1.08	0.26	-0.79	-0.32	-0.57	0.67	0.01	
	1.03	0.97	-3.45	-0.81	-0.21	0.71	-0.70	
	0.40	0.94	-2.90	-0.30	-0.19	1.22	-0.46	
	0.75	0.47	-1.22	-0.04	-0.26	0.47	0.13	
	0.49	0.47	-2.48	0.21	-0.68	1.08	-0.03	
	0.39	0.57	-2.09	-0.93	-0.92	-0.10	1.31	
	0.52	0.47	-3.43	-0.63	0.66	0.78	-0.02	
	0.98	0.73	-2.87	-0.24	-0.23	0.16	0.03	
Average	0.79	0.54	-2.44	-0.15	-0.14	0.58	0.04	
<i>Analyses of variance—mean squares</i>								N
Grand deviation	6.81	3.18	65.50	0.25	0.20	3.75	0.02	1
Heterogeneity	0.15	0.07	0.72	0.33	0.26	0.20	0.28	10
Pooled heterogeneity	0.2869 for N = 70							

each of the 7 independent comparisons (table 7) are given, together with their averages. The variance of each of the 7 comparisons can be analysed into two parts, one measuring the grand deviation from 0 and based on 1 degree of freedom ($N = 1$), and the other measuring the heterogeneity of the 11 corresponding estimates and based on 10 degrees of freedom ($N = 10$). The 7 heterogeneity mean squares are consistent with one another in value except possibly that for the main effect of chromosome III. Even this is significantly greater than the pool of its fellows only at the 1 per cent. level, and when we remember that it was picked out as the largest value of seven, it seems that we should not regard it as inhomogeneous with the rest. The 7 heterogeneity mean squares may therefore be pooled to give a joint error mean square of 0.2869 based on 70 degrees of freedom. Thus single estimates of the various comparisons will have a standard error of $\sqrt{0.2869}$ or 0.5356, while averages of two estimates will have a standard error of $\sqrt{\frac{1}{2} \times 0.2869}$ or 0.3787, and averages of n estimates a standard error of $\sqrt{\frac{1}{n} \times 0.2869}$.

The grand deviations of all three main effects are significant, so showing that Or differs from the tester in all 3 chromosomes. It will be observed from the average comparisons that in both X and II Or has a greater chaeta producing power than the tester, but that the joint effect of these 2 chromosomes, $0.79 + 0.54$, is more than offset by the lower chaeta producing power of chromosome III, *viz.* -2.44 . Thus the Or chromosomes have overall a lower chaeta producing power than the particular chromosomes used from the tester, though the differences are partly balanced.

There is no evidence of interaction between the genes in the different chromosomes except between II and III. This interaction is in the positive direction, *i.e.* the combinations found together in the line and the tester have greater effects than would be expected from their individual behaviour taken over the segregating families as a whole.

Twelve other lines were assayed (table 10) six of them in duplicate. Furthermore two of the twelve were effectively duplicates, since one was the Sk mass line from which the parents of the original cross were taken, and the other was the Sk inbred line derived from this mass. Each of these two was done in duplicate, so that in all we have 4 assays of Sk. The homogeneity of the various duplicate assays, and of the quadruplicate assay of Sk, was tested by comparison with the pooled error mean square obtained from the Oregon tests. All that need be said is that with one exception, line 7, no difference could be detected between replicates, whether in main effects or interactions, which could not be referred to error variation. The exception, line 7, gave a mean square of 0.9038 for differences between duplicates in all 7 components of the analysis taken together. When compared with the pooled error from the Or tests this is significantly high at the 1 per cent. level; but since this was chosen as the most extreme mean square out of the five (from the 5 lines tested in replicate) it is doubtful whether its significance should be rated too highly. If pooled with the other four all significance is lost. The replicates therefore gave no reliable evidence of differing genetically. In what follows we shall therefore pool all replicates. Only in line 7 is there any possibility that this could lead to misjudgment.

In table 10 are set out the results of assay of all the lines tested including Or. The number of replicate assays is shown for each of them. The origins of some of these lines have not yet been described, but they will be included in the present consideration wherever it is necessary to complete the analysis. The analyses of variance show, as we should expect, that the lines are inhomogeneous in regard to the main effects of the 3 chromosomes. These results will be made the subject of more detailed examination below. The data on the second order interaction, X-II-III, give no indication that this is either significant when taken over all the lines or anything but the same in all of them. We can therefore neglect the possibility of any complex interaction involving all 3 chromosomes at once.

The three first order interactions are, however, more interesting. The lines taken together confirm the evidence from the Or analysis of a positive interaction between II and III. They also suggest that there is a negative interaction between X and II. Both these interactions seem to be homogeneous over all the lines when tested against the error variance from the Or assays, despite the failure to

TABLE 10
Results of chromosome assays on the twelve lines of the experiment

Line	Number of assays	Main effects			Interactions				
		X	II	III	X-II	X-III	II-III	X-II-III	
Or . . .	11	0.79	0.54	-2.44	-0.15	-0.14	0.58	0.04	
Sk . . .	4	-1.06	-1.30	-3.24	-0.67	-0.83	0.53	-0.00	
Line 1 . . .	1	0.25	-1.05	-3.42	0.54	0.60	0.42	-0.69	
Line 2 . . .	1	-2.30	-1.13	-3.25	0.10	0.21	0.34	-0.60	
Line 3 . . .	1	0.94	0.33	-2.20	-0.64	-0.29	0.27	-0.06	
Line 4 . . .	1	2.94	-0.03	0.77	-0.42	0.82	-0.20	-0.15	
Line 5 . . .	1	2.11	0.40	0.56	-0.06	0.67	-0.03	0.45	
Line 6 . . .	2	2.96	0.54	0.36	-0.26	0.95	-0.10	-0.15	
Line 7 . . .	2	2.75	3.01	0.66	-0.62	0.47	0.72	-0.42	
Line 8 . . .	1	2.62	3.23	1.72	-0.86	1.36	0.60	-0.58	
Line 9-1 . . .	2	1.94	3.18	1.18	-0.82	0.44	0.12	-0.02	
Line 9-2 . . .	2	2.96	2.76	5.65	-0.37	1.13	0.12	0.23	
<i>Analyses of variance—Mean squares</i>									N
Grand deviation . . .		35.67*	15.86*	30.83*	3.32*	0.71	4.67*	0.02	1
Heterogeneity between lines . . .		5.41*	5.57*	15.95*	0.26	0.99*	0.19	0.17	11

N = Degrees of freedom

* = Significant when compared with the error variance of 0.2869 from the Or analysis (table 9).

find that between X and II in the Or tests themselves. We may therefore describe the situation in another way. All the lines assayed show the same X-II and II-III interactions when compared with the tester. It would thus be more reasonable to say that in these lines X-II and II-III show no interaction but that in the tester they do so. In other words the interactions are the properties of the tester, possibly because of the marker genes the chromosomes carry there.

The interaction between X and III behaves differently. Unlike the other two there is little evidence to be obtained for this interaction from the grand deviation of all lines from the tester. But the lines themselves are not homogeneous in respect of it. Some of them must show it in one direction and some in the other. This interaction cannot therefore be referred wholly to the markers or indeed to any other genes of the tester.

Now the 11 lines can be divided into 5 groups according to the effects of selection. The first of these includes the parents, Or and Sk,

upon which selection has not been practised. The second includes line 1 and 2 which are both the outcome of low selection, and average about 34 chaetæ when both males and females are counted. The third contains only line 3, the original high mass line, which must be virtually at the Or level when allowance is made for the change in counting at S-13. The fourth group includes lines 4, 5 and 6, all of which come at the intermediate level of response to high reselection as described in the previous section, though line 6 approached the highest level of reselection in chaeta number by the time the assays were made. Finally lines 7, 8 and 9 (assayed at two different levels) were at the highest level of all, averaging for males and females between 52 and 56 chaetæ. If we consider the X-III interaction as it is distributed amongst these groups we find that the differences between the groups taken as wholes account for all but an insignificant residual of heterogeneity (table 11). The level of interaction is thus related to selection.

TABLE 11
Interaction of chromosomes X and III

Group	Average interaction (weighted)		
Parents	.	.	-0.32
Lines 1 and 2	.	.	0.40
Line 3	.	.	-0.29
Lines 4, 5 and 6	.	.	0.85
Lines 7, 8 and 9	.	.	0.78
Analysis of variance		N	Mean squares
Heterogeneity between groups	.	4	2.2698 *
Heterogeneity within groups	.	7	0.2672

* Significant when tested against error variance from the Or analysis (table 9).

We can go even further. The parents have a negative interaction, and so does line 3 which has receded virtually to the parental level. The low lines have a high positive interaction and the two groups of high lines an even greater one. The negative interactions of Or and line 3 could be regarded as a positive interaction of the L as opposed to the T chromosomes for in these cases L departs from T in opposite directions in chromosomes X and III. The negative interaction of Sk cannot, however, be so interpreted as the departure from the tester is in the same direction in both X and III. It must thus be regarded as a genuine negative interaction; and we may observe that it is higher than the more doubtful interactions of Or and line 3. There is thus some evidence that effective selection, whether for low or high chaeta number, appears to have produced a change towards positive interaction between X and III. That selection in either direction should do this is not surprising, for a positive interaction implies that the two sets of downward allelomorphs produce disproportionately low chaeta numbers when together as well as that the two sets of high allelomorphs jointly produce a disproportionately high chaeta number.

Nor is the lack of difference between these two high groups in the level of interaction surprising when we see that the difference in chaeta number between them depends largely on a change in chromosome II whose interaction is not in question. We are therefore led to conclude that selection may pick out sets of genes to some extent by their interactions as well as by their individual effects. The negative interactions of the parents, tending to reduce the sum of the individual effects of the genes on chaeta number, would thus be replaced by positive interactions tending to enhance the joint effects.

One last word about these interactions. If the scale upon which to represent the metric, here the chaeta number, is ill chosen interaction would appear for this reason alone (Mather, 1949a). Such interactions would, however, be uniform for all the pairs of chromosomes and would change uniformly as the metric changed in value. Neither of these criteria is satisfied in the present case: some pairs of chromosomes have interactions where others do not: and the X-III interaction is negative with the central values and positive with both extremes of the metric, low as well as high. Rescaling would not remove our interactions. It could only at most replace them by others requiring the same general interpretation. We have no reason to believe that the simple scale of chaeta number is not adequate to give a true picture of the genic interactions and their changes under selection, as well as of the main effects of the chromosomes, with which will of course be included the interactions between genes within the same chromosome.

So far we have barely touched upon what was the avowed purpose of the assays, to discover in which chromosomes the changes inferred from the responses to selection and reselection had occurred. The analyses of variance of table 10 show us that all three major chromosomes in the various lines differ in their main effects not only from the tester but also from one another. That they differ from one another is the important observation, for such differences in chaeta producing power cannot be ascribed to the major marker genes in which the tester differed from all the lines, but in which the lines, being wild-type, were all alike.

The effects of the major genes used as markers can be eliminated from the analysis by a further process of differencing. The Or X showed an excess of 0.79 in chaeta producing power over its counterpart from the tester, marked by *CLB*. The Sk X showed a corresponding deficiency of -1.06, the fact that it is a deficiency being indicated by the -ve sign. The Sk X can be compared directly with the Or and the effect of the common tester eliminated by subtraction. Thus if the chaeta producing power of the tester X is A, that of the Or O and that of the Sk S, we already know that

$$O - A = 0.79 \quad \text{and} \quad S - A = -1.06.$$

Then to express the power of the Sk X directly as an excess over

that of Or, so eliminating the effects of all genes peculiar to the tester, we need

$$\begin{aligned} S-O &= (S-A) - (O-A) \\ &= -1.06 - 0.79 = -1.85. \end{aligned}$$

The values so reached for all the chromosomes of all the lines are shown in table 12, which thus includes only comparisons between

TABLE 12

Effects of the individual chromosomes on chaeta number in females, expressed as excesses over those of the Oregon chromosomes

Line	Chaeta number of line females	Excess over Oregon (43.53)	Effects of			Total	Efficiency percentage
			X	II	III		
Sk	37.63	-5.90	-1.85	-1.83	-0.80	-4.48	76
1	36.00	-7.53	-0.54	-1.59	-0.98	-3.11	41
2	37.04	-6.49	-3.09	-1.67	-0.81	-5.57	86
3	44.35	0.82	0.15	-0.21	0.23	0.17	21
4	50.65	7.12	2.15	-0.57	3.21	4.79	67
5	52.26	8.73	1.33	-0.14	3.01	4.20	48
6	52.60	9.07	2.17	0.01	2.80	4.98	55
7	59.53	16.00	1.96	2.47	3.10	7.53	47
8	63.35	19.82	1.83	2.69	4.16	8.68	44
9-1	59.19	15.66	1.15	2.65	3.62	7.42	47
9-2	70.25	26.72	2.17	2.22	8.09	12.48	47
	Sums neglecting sign	123.86				63.41	51

wild-type chromosomes, taking the Or chromosomes as the standard of reference in all cases. The changes in chaeta producing power under selection are thus not to be referred to any known major genes. They depend on alterations in a polygenic system which involves at least all three large chromosomes.

Table 12 tells us a number of things. The Sk parent has a lower chaeta number than the Or because of the action of genes in all chromosomes. The result from the low mass line (line 1) whose origin was described in section 3 (*a*) is a little disappointing since its chromosome appears to have much the same effects as those of the Sk parent, except in the X where the effect seems to be to produce more chaetae in line 1 than in Sk. Caution is, however, engendered when we look at the total effect accounted for by all the three chromosomes together. In Sk this is -4.48 or 76 per cent. of the observable difference in mean chaeta number between Sk and Or females (table 12); but in line 1 it is only 41 per cent. For some reason the efficiency of the assay of line 1 is only half that of Sk, so that comparisons of the effects directly traceable to the chromosomes by the assay must be somewhat unreliable. This question of efficiency will be discussed further below.

Most of the assays show a reasonably uniform efficiency. The assay of line 2, whose origin is yet to be described, is of remarkably high efficiency with 86 per cent., but we are not specifically concerned with this line at present. We need only note that at least its X has a lower chaeta producing power than those of the two parents. Line 3, the first high mass line of the previous section, has a low assay efficiency of only 21 per cent. ; but this is doubtless merely a reflection of the fact that both its overall chaeta number and the summed effects of its 3 chromosomes approximate closely to those of Or. With such small differences sampling variation will sway the percentage to a very marked degree. The chief point to notice about this line is that so far as the assays go they agree well with the inference, made in the last section, that its chromosomes must closely resemble those of Or in effect on chaeta production. That they are not, however, genetically identical will become clear in section 4 (a).

The rest of the assays with which we are concerned at present, those of lines 4, 6, 7 and 8, have reasonably similar efficiencies and comparisons can therefore be made with some confidence. Lines 8 and 7, the first high reselection and the mass line from it, exceed even the higher of the two parents, Or, in all 3 chromosomes. Furthermore they are much like one another, line 8 only exceeding line 7 by a little in chromosome III. This excess may be due to a delayed response which had occurred in line 8 prior to the assays, and by which it differed a little from line 7 after S-84. Lines 4 and 6, the seventh reselection which went up only half way and the mass line from it, closely resemble line 7 in chromosome X and III, but in chromosome II they are like line 3 and the Or parent. Thus the change in the high mass line between S-27 and S-30 was in chromosome II. The high chaeta combinations of this chromosome had vanished so that reselection could only pick out those in X and III from S-30 onwards. The advance under reselection must thus be smaller than the advances obtained earlier, though the reduction in the advance obtained was perhaps a little greater than would be expected from consideration of the assays alone. We do not, however, know the efficiency of the assays for the individual chromosomes, and it may be that it is lower for II than for X and III, so that the difference seen in the assay for this chromosome would represent a smaller fraction of that which was actually being produced between the lines themselves. However, this may be, the assay results bear out the conclusions reached from the consideration of the results of selection and reselection as well as could be expected.

One last point about the assays requires comment. We have already seen that their efficiencies will depend on the dominance relations of the genes involved relative to the tester, and on the amount of residual recombination with the tester. The effect of the latter factor has been estimated as an average loss of 10 per cent. in efficiency. The remaining loss must be due to dominance, either by it being

incomplete in the individual tester genes as compared with their allelomorphs from the lines, or by it being balanced in that it goes one way in some of the tester's genes and the other way in the rest. Low efficiency would mean low dominance of the tester's genes. With absence of dominance, or with fully balanced dominance, the loss from this cause would be 50 per cent., which taken with the recombinational loss would give 45 per cent. efficiency in the final results.

It is impossible to test the efficiency of the assays by direct comparison with the tester as we cannot obtain flies homozygous for the actual tester chromosomes used. There is thus no means of measuring the overall difference in chaeta number between tester and line flies. The indirect comparisons of the various lines with Or suggests, however, that in the assays of both Or and the majority of the lines efficiency must have been near to this 45 per cent. A few lines, notably Sk and line 2, depart somewhat widely from this expectation and there is consequently some ground for thinking that the dominance relations of their genes relative to the tester's were different from those of the other lines. On this view the Sk and line 2 genes must have been recessive to their allelomorphs from the tester more often than not.

(d) *Crosses between the levels*

The high mass line (line 3), and the first (lines 7 and 8) and seventh (lines 4 and 6) reselections from it, establish three levels of response to selection for high chaeta number, at which the lines can remain stable or virtually so in respect of chaeta number even under the conditions of mass culture. These levels, which can be seen clearly in figs. 1 and 2, will be designated as the upper (U), middle (M) and lower (L). The chromosome assays have shown us that U differs from M in respect of the polygenic combinations carried in chromosome II, and from L in respect of the combinations of all three large chromosomes. M differs from L in the combinations of chromosomes X and III. The properties of these combinations in potence, *i.e.* the integrated dominance of all the genes in the combination (Wigan, 1944), and segregation were further investigated in crosses between the levels.

The first of these crosses, between U (line 7) and M (line 6), was made with flies taken from S-58. The flies used as parents for this cross, and indeed for all the crosses between levels, were taken as having chaeta numbers closely approximating to the average for the sex in question in the particular culture. The cross was made reciprocally, two matings being made each way. The average number of chaetæ for the two sexes taken over all four F_1 cultures, was 50 which is almost exactly midway between the parent values (fig. 4), and the average of the sexes taken over both F_2 's, from the

reciprocal F_1 's, was $51\frac{1}{4}$. It will be seen from fig. 4, however, that the chaeta numbers of both the parental lines were higher in S-60,

FIG. 4.—The level crosses between lines 6 and 7, and lines 3 and 6, and their behaviour under selection. The brackets join the lines at the generations from which the parents are taken. The F_1 means are at the beginnings of the heavy lines near the points of the brackets. The heavy lines proceed to the F_2 means, after which they diverge into the three selected lines, for high (*h*), mid (*m*) and low (*l*) chaeta numbers. These lines were all terminated deliberately. The large dots indicate the means of the backcrosses. The parental lines are shown faintly.

when the F_2 was raised, than in S-59, due doubtless to non-heritable changes. Thus the F_2 mean lies midway between those of the parent

lines, so that it resembles the F_1 in mean chaeta number as closely as would be expected. The cross between U and M was repeated at S-70 for reasons which will be given later, and the behaviour of F_1 and F_2 was the same as in the first cross. There is thus no evidence of the potency of the set of genes from one parental line exceeding that of the genes from the other.

This was tested further by backcrosses of the F_1 to the parental lines. These were made using both males and females from the reciprocals of F_1 . Their overall average chaeta numbers are shown as dots at S-60 in fig. 4. These averages lie rather nearer to the F_2 mean than to the values for the parental lines; but since this is true for both of them the results cannot be taken as indicating any greater potency one way or the other. Such departures as these from the points midway between the F_2 and parental averages must be due to sampling variation or to elimination of some genotypes in the backcross progenies. The backcross results from the other two level crosses, U with L and M with L, give little indication of similar regular departures from the values half-way between F_2 and parental averages so that sampling variation seems to be the most likely explanation in the present case.

The cross between M and L was made with parents taken from S-60. Once again the average chaeta numbers of F_1 and F_2 fell as nearly as could be expected midway between those of the two parental lines in S-61 and S-62 respectively. The backcrosses confirmed the lack of potency of the sets of genes from the two parents (fig. 4). The reciprocal F_1 's are of interest in this cross between L and M. We have already seen that U differs from M in chromosome II, but that M differs from L in chromosomes X and III. Thus, while the females will be genetically alike in the reciprocal crosses, whether of U and M or of M and L, the males should be alike in the former but not in the latter. Provided that the Y chromosome is not interfering, the cross $M \times L$ should give males with a higher average chaeta number, other things being equal, than $L \times M$, because the former will have an X chromosome from M and the latter one from L.

The comparison is made between the reciprocal crosses in table 13. In order to minimise the effects of differences in conditions between different cultures, the data have been expressed as differences between the mean chaeta numbers of the sexes within each culture, the male average being deducted from the female. Any effect common to the flies of one culture will thus be eliminated. Since several cultures were raised of each reciprocal of each cross, the excesses of females over males were found for each culture and then averaged over all like cultures. The figures so obtained are given in the table. Where the X chromosomes of the parents differed in chaeta producing power the excess of the female chaeta number over the male should be lower where the female parent had the higher chaeta number. Thus the value in table 13 for $M \times L$ should be lower than that for $L \times M$ by

an amount reflecting the difference in effect on chaeta number of the X chromosomes of M and L.

TABLE 13

Comparisons between the reciprocal crosses between the levels

The entries in the table are the sex differences in chaeta number (female—male) averaged over all cultures of the given types.

Cross	Sex difference in chaeta number
U × M	8.16
M × U	8.31
M × L	5.92
L × M	6.21
U × L	6.00
L × U	7.55

The difference between the two reciprocals is in the direction anticipated, but it is much smaller than might have been expected. In the assays the difference between lines 3 and 6 was about 2 chaetæ in the X, and as we saw the assays will not generally measure the full effect of the chromosome. Instead of a value of this order we find a difference of only $6.21 - 5.92 = 0.29$. True it is larger than the value obtained from the reciprocals $U \times M$ and $M \times U$, where no difference is expected to arise from the X chromosomes; but it is nevertheless too small.

A further estimate of the effect of this difference in the X chromosomes can be obtained from the cross between U and L levels. These differ in all 3 chromosomes, the difference in the X being the same as that between the X's of M and L (table 11). This cross between U (line 7) and L (line 3) was made twice, with parents from S-64 and S-74. Again the mean chaeta numbers of F_1 and F_2 fall midway between those of the parental lines on both occasions; and back-crosses made from the first cross confirm the lack of potency of the two sets of genes (fig. 5). We may note that, just as with the crosses between U and M and between M and L, these results will be unaffected by any effects of the X in reciprocal crosses, for the values shown in fig. 5 are the averages of cultures among which the various reciprocals were equally represented.

The effect of the difference between the X's on the reciprocal F_1 's from the cross U and L levels is shown in table 13. It is 1.55, a value much larger than the estimate obtained from the crosses between L and M, and one more in keeping with the difference of two found in the assays. It would thus appear that the low difference found from L and M was in part due to the vagaries of sampling variation. The sex difference fluctuates sufficiently between cultures

of comparable crosses easily to make possible a difference as great as that between these two estimates of the effect of the X. Nevertheless even the higher estimate is somewhat below that obtained from the assays, which were themselves very unlikely to have revealed the whole of the difference.

FIG. 5.—The level crosses between lines 3 and 7 (shown faintly). The symbols are as in fig. 4. Line 10 died out through sterility. The *l* and *m* lines were terminated deliberately.

Thus the comparison of reciprocal crosses shows differences in the places where they would be expected, but of a size less than would be expected. They suggest that the Y chromosome may have been acting to obscure part of the effect of the X. If line 3 had a Y chromosome as well as an X whose effect was to determine a lower chaeta number than did the corresponding chromosome of line 6, or of course of line 7, the difference between the reciprocals would

represent not the full effect of the difference between the X's but only the excess in effect of the difference in the X's over that of the Y's. That the Y can affect the manifestation of chaeta characters is known from earlier work (Mather, 1944), and it also seems likely that its differences will generally be smaller than those of the X (Wigan, 1949), in the way that the present data would require.

The data from the F_2 and backcrosses of these crosses between the three levels can be put to use in another way. They can be made to yield estimates of the number, not of genes but of effective factors, *i.e.* groups of genes effectively behaving as units in transmission (Mather, 1949*a* and *b*), in which the lines at the various levels differed. Four assumptions must be made in order to obtain the estimates (Wright, 1934). The factors must be assumed: (i) to be isodirectionally distributed between the parents of the cross, *i.e.* for the + or increasing allelomorphs of all of them to be in one parent and the — or decreasing allelomorphs in the other, (ii) to be of equal action, (iii) to show no dominance, and (iv) to be unlinked. Failure in any of these respects must lead to an underestimate.

Using the notation of Mather (1949*a*), let the difference in manifestation of the character, *i.e.* in chaeta number, between individuals of the two genotypes which differ only in being homozygous for the allelomorphs of factor $A-a$ be $2d_a$ and let the chaeta number of the corresponding heterozygote, Aa , depart from the average of the values of the two homozygotes by h_a , which can take a + or a — sign according to the direction of departure. Then this gene contributes $\frac{1}{2}d_a^2 + \frac{1}{4}h_a^2$ to the variance of F_2 . In the absence of linkage we can put $D = S(d^2)$ and $H = S(h^2)$, summation proceeding over all the factors which contribute to the difference between the parents, and the variance of F_2 becomes

$$V_{F_2} = \frac{1}{2}D + \frac{1}{4}H + E$$

where E is the non-heritable component.

Now if $d_a = d_b = \dots = d_k = d$ for all the k factors concerned in the segregation, the parental difference in chaeta number will be

$$\bar{P}_1 - \bar{P}_2 = 2S(d) = 2kd$$

where the factors are distributed isodirectionally. At the same time

$$D = S(d^2) = kd^2$$

whether distribution is isodirectional or not. Then with isodirectional distribution

$$\frac{\frac{1}{2}(\bar{P}_1 - \bar{P}_2)^2}{D} = \frac{(kd)^2}{kd^2} = k$$

But when the distribution is not isodirectional

$$\bar{P}_1 - \bar{P}_2 < 2S(d)$$

and k must be underestimated.

When the various d increments are not equal and we put $kd = S(d)$ so that

$$d_a = d(1 + \alpha_a), d_b = d(1 + \alpha_b), \text{ etc.,}$$

we find

$$\frac{\frac{1}{2}(\bar{P}_1 - \bar{P}_2)^2}{D} = K = \frac{k}{1 + V_\alpha}$$

where V_α is the variance of α and K must thus be less than k .

The calculation as shown requires the separation of the D and H components of V_{F_2} ; but this cannot be undertaken from F_2 data alone. If we estimate E directly from the parents and F_1 , and deduct it from V_{F_2} we are left with $\frac{1}{2}D + \frac{1}{4}H$. This will afford a consistent estimate of D only where H is 0, *i.e.* in the absence of dominance. Otherwise $\frac{1}{2}D + \frac{1}{4}H > \frac{1}{2}D$, and the use of twice the heritable variance of F_2 as an estimate of D must lead to underestimation of k by an amount which will reflect the dominance relations of the factors as these are expressed in H .

With isodirectional distribution of the factors between the parents any linkage must be in coupling. In that case $D > S(d^2)$ by an amount depending on the recombination values and relative magnitudes of the gene effects (Mather, 1949*a*). This must again lead to an under-estimate of k .

A test of dominance is possible where, as in the present case, backcrosses are available as well as F_2 's. The summed variances of the two backcrosses, one to each parent, are (Mather, 1949*a*)

$$V_{B_1} + V_{B_2} = \frac{1}{2}D + \frac{1}{2}H + 2E$$

Then after deducting the non-heritable components as estimated directly from the variances of the parents and F_1 , the heritable portion of

$$(V_{B_1} + V_{B_2}) - V_{F_1} = (\frac{1}{2}D + \frac{1}{2}H) - (\frac{1}{2}D + \frac{1}{4}H) = \frac{1}{4}H.$$

In the present case taking all three level crosses into account, the sum of the summed backcross heritable variances is actually less than the sum of the heritable variances of the three F_2 's, *i.e.* our estimate of the summed values of the three H 's is negative. Now H , being the sum of quadratic quantities, cannot be negative, so that we must take 0 as the best estimate of the summed H values. Or to put it another way, we can safely assume that dominance is absent, or at least on the average so small as not to be a disturbing factor in the calculations. The lack of potence, which is dominance integrated over all the factors in question, as already observed agrees with this conclusion.

Now if we take $H = 0$, then

$$V_{B_1} + V_{B_2} + V_{F_1} = \frac{1}{2}D + 2E + \frac{1}{2}D + E = D + 3E.$$

Deduction of three times the average variance of parents and F_1 ,

which affords the best available estimate of E , will give an estimate of D . This is done for all three level crosses in table 14.

TABLE 14

Estimation of the numbers of effective factors in the level crosses

Cross	Average variance of parents and F_1 (= E)	Sum of variances of two backcrosses and F_2 (= $D+3E$)	D	$\frac{1}{2}(\bar{P}_1 - \bar{P}_2)$	K
U $\times$ M	9.82	33.11	3.65	2.5	1.71
M $\times$ L	7.93	27.07	3.28	2.5	1.91
U $\times$ L	8.00	34.28	10.28	5.0	2.43

The estimates of E were found as the average variances of the chaeta number in the two parental lines, in the generations from which the parents of the F_1 's and of the backcrosses were taken, and in the F_1 . In so far as the parental lines were not fully homogenic, this estimate will include a small heritable component; but one which, we may observe, has nothing to do with the differences between the levels, and which may therefore be fairly eliminated with the non-heritable component of variation. In the case of the crosses of U and M and of U and L, which were made twice, the average was taken over the various families of both crosses. These estimates of E appear in the second column of table 14. The third column of the table gives the sums of the variances of the two backcrosses and F_2 in the various level crosses. In all cases the variances of each backcross, and of F_2 , were averaged over all families of the particular type, so removing any effect of differences between reciprocal crosses. It should also be noted that in all cases the variances were found for males and females separately within each culture, the two then being averaged so as both to eliminate the inflationary effect of the difference in sex means and to make equal adjustment in all types of family for any small differences there might be between the sexes in variance. D was then found by subtracting three times the entry in column 2 from that in column 3 of the table. Thus for U $\times$ M,

$$D = 33.11 - 3 \times 9.82 = 3.65$$

and so on.

The differences in mean chaeta number between the parental lines were not found using the mean chaeta numbers of these lines in the generations from which the actual parents were taken, because presumably under the action of the environment these means fluctuate from generation to generation sufficiently to introduce error into the calculation. Instead the average chaeta numbers of the three parental lines were estimated over the relevant ranges of S-56 to S-80 by eye from fig. 4, as 53.25, 48.25 and 43.25. Then half the parental differences are as shown in column 5 of table 14. Each of these

values squared and divided by the corresponding value of D gives K , the estimate of the number of effective factors k , as shown in the final column of the table.

What do these values of K tell us? We have already discovered from the assays that U differs from M in chromosome II and from L in all three chromosomes, while M differs from L in chromosomes X and III. If these chromosomes or the parts of them carrying the differences were themselves behaving as effective factors we should expect $K = 1$ for $U \times M$, $K = 2$ for $M \times L$, and $K = 3$ for $U \times L$. The values obtained are in fact of this order of size and they fall in the same sequence. That of 1.91 for $M \times L$ is a very close approximation to the expected 2, but at 1.71 that for $U \times M$ is somewhat high, and at 2.43 that for $U \times L$ is somewhat low. When, however, we recall that even in the cross of U and L the heritable portion of $V_{B_1} + V_{B_2} + V_{F_2}$ is less than half the non-heritable, so making the error variance of D and hence of K disproportionately high, and that error variance is made all the larger by the experiment not being designed and conducted in the way best suited to yield data for the calculation of K , these differences between the values observed and those expected are set in their proper perspective. The overall agreement in magnitude and sequence is very good, and the departures in detail are reasonably ascribable to error variation. It would thus appear that just as, once built up, the linked polygenic combinations within the chromosomes behave as effective units in selection and in loss within the lines, so they also behave as effective units in the segregation which follows crossing between lines. It would further appear that the combinations cannot cover a great genetic length within the chromosomes, for if they did it would be difficult to see how they could behave as units, at least in transmission through females.

On this view, that the differences within each chromosome are behaving as one unit in segregation, all the assumptions made in regarding K as a consistent estimate of k will be satisfied. Distribution must be isodirectional—selection has seen to that. The factors must also, of course, be unlinked, for they are in different chromosomes. And the effects of the different chromosomes, as measured by the assays, are approximately equal. Table 12 shows us that the difference in the X is about 2, that in II about 2.5, and that in III about 3. Then in the cross of U and L the average of d is 2.5, and $\alpha_X = -0.2$, $\alpha_{II} = 0$ and $\alpha_{III} = 0.2$. Thus $V_\alpha = \frac{1}{2}(0.08) = 0.04$, which will cause little disturbance in the estimation of the number of effective factors. With the effects of dominance we have already dealt. Everything points to the operative parts of the chromosomes having behaved as effective factors in the segregation following the level crosses.

If the chromosomes behave as effective units in segregation, they should, in the absence of disturbing agencies, also behave as units in selection from the F_2 's. Such selection tests were in fact made

(figs. 4 and 5). Three lines were taken off each F_2 , one (h) in which the parents were selected in each generation for high chaeta number, one (l) in which they were selected for low chaeta number, and a third (m) in which they were selected as having chaeta numbers near the mean. The first such test was made from the F_2 of the cross between U and M at S-60. In two generations the h line had gone far towards recovering the chaeta number of the U parent. The other two lines, however, behaved strangely in the first generation of selection, l staying in the middle, as m was expected to do, and m falling as l was expected to do. Continuing the putative m line, by breeding from flies with central chaeta numbers, resulted in its average number rising almost back to the F_2 mean in the next generation (fig. 4). The suspicion that l and m had been accidentally interchanged in the first generation of selection thus became too strong for any reliance to be placed in the results, and the test was abandoned in favour of starting again with a fresh cross of U and M.

This was made at S-70 and the three selection lines were once more taken off the new F_2 . The mean chaeta number of the m line so obviously stayed near the F_2 mean that this line was terminated after two generations. Meanwhile both h and l had regained, in fact in the latter case transgressed, the upper and lower parental levels respectively. After the first two generations they each maintained a constant relation with the corresponding parental line, following the vagaries of these lines in a most striking fashion. Clearly, as would be expected with only one effective unit of segregation, selection had quickly restored the parental genotypes. That l fell below the lower parent is in no way surprising because, as we shall see in the next section, line 6 was not wholly homogenic, so that its chaeta number represents the mean action of the combinations it carries. Any particular sample of these such as would be recovered in a selection from an outcross might well show a small constant departure from the average of the full group.

Selection from the cross between M and L gave basically similar results (fig. 4). Again the m line stayed near the F_2 in mean chaeta number, while h and l quickly reached mean chaeta numbers approximating to those of the M and L parents, after which they maintained a constant relation with those lines. As in the cross of U and M, the means of the h and l lines departed a little from those of the parental lines in the way we might expect. One point is, however, worthy of note. In selecting from the cross of U and M, h and l nearly recovered the parental levels after one generation's selection, and passed them slightly after two. Following the cross of M and L, although h achieved its full advance in two generations, the l line took three to reach the lower parental level. Now the total response was much the same after both crosses, but in that of M and L it had to be achieved by the joint selection for two effective factors (one sex-linked) as opposed to one. Instead of $\frac{1}{4}$ at most $\frac{1}{8}$ of

the flies would be of the correct genotype. Thus with 10 per cent. of the flies selected as parents and the expressions of the genotypes obscured by a high measure of non-heritable variation, progress under selection is likely to be slower from the cross between M and L than from that between U and M.

This statement is, however, one of probability only. If the extreme genotype should be picked up in selection in spite of its relative rarity, then progress must be rapid. This seems in fact to have happened in the first cross between U and L (fig. 5). Despite the fact that three effective factors were segregating, so that at most $\frac{1}{32}$ of the flies would have the extreme low genotype in F_2 , most of the progress in the l line was already accomplished after one generation's selection, and it was complete after two. The h line, on the other hand, progressed very slowly; so slowly in fact that it took 7 generations to approach the upper parental level at all closely, and 10 generations to pass it. Such progress is much slower than would be expected even where three effective factors have to be sorted out. Two steps were therefore taken to see further into the matter. The h line was itself kept going as line 9, and the whole cross was repeated.

In the repetition of the cross the new l line displayed the same slow response that the h line did in the first. Evidently the fortunate chance of early selection of the extreme low genotype, responsible for the rapid advance in the first l line, had not repeated itself in the second. This second l line was, however, discontinued once its general behaviour had become clear. The h line (maintained as line 10) from the second cross also showed a slow advance, crossing the parental level after 8 generations' selection—a result sufficiently well in keeping with the behaviour of line 9.

The subsequent behaviour of lines 9 and 10 is of great importance for our interpretation of their early progress. Neither of them stabilised near the parental level in the way that the selection lines showing rapid early advance had done. Line 9 hovered between 1 and 2 chaeta above its parent line 7 for 5 generations under selection but then owing to sterility causing a complete failure of the cultures in one generation it had to be recovered without selection from the old culture at S-83. This resulted in a decline of the chaeta number to the parental level, after which, however, it rose again under selection till it exceeded the parental line by about 2 chaetæ. Here it remained constant for some 25 generations in spite of a somewhat low fertility. Line 10 progresses fairly steadily, apart from 4 generations near stability at about the parental level, until it eventually came to exceed the parent line by 4 chaetæ. At this point its fertility became so poor that it died out in S-94.

Thus not only did line 9 and 10 progress more slowly than expected under selection; they progressed well beyond the parental level, and they developed a sterility which was foreign to the parent. They appear in fact not to represent mere recoveries of the parental genotype,

but to owe their high chaeta numbers to the building up of new combinations by crossing-over. These would take time to develop, so that progress under selection would be slow; they would not exactly match the old ones in effect on chaeta number, so that the parental level could be exceeded; and they would carry with them the customary correlated response of lowered fertility. If this were all the evidence, it might still be considered that the data were not conclusive. Fortunately a second correlated response to selection for chaeta number was discovered in the form of changes in the number of spermathecae of the females; and, as we shall see in section 4 (a), lines 9 and 10 both showed spermathecal types not found in their parent lines. There can thus be no doubt that they carried new combinations, and that their tardy progress under selection was due to the slow building up of these by recombinations within the chromosome, just as had occurred in the original high selection line before S-20.

Line 10 died out by reason of its sterility before its chromosomes could be assayed; but assay results are available from line 9. Those given under the heading of line 9-1 are the more relevant set, line 9-2 covering assays made after a delayed rise which occurred approximately at S-112 (fig. 3). So far as can be judged from the assays, chromosomes X and II had much the same chaeta producing power in line 9 as in lines 4-8. Line 9-1 is rather low in the X but the results from 9-2 suggest that this may have been due, in part at least, to error variation (table 12). In chromosome III however 9-1 exceeds all the earlier lines except 8, which had itself shown, before it was assayed, a delayed rise above the level which it had earlier shared with line 7. It would appear therefore that line 9 mainly owed its difference in chaeta number from its parent line 7 to chromosome III, there being also a possible smaller difference, in the other direction, in the X. It is thus of interest that, as we shall see, chromosome III is most likely responsible for the altered effect on the spermathecae. Evidently new combinations have been built up by selection at least in chromosome III.

As to why high selection from the crosses between U and L should have resulted in the slow building up of new combinations rather than the speedy recovery of the high parental genotype we can only conjecture. Possibly individuals having this extreme genotype, though viable in isolation, were at so marked a disadvantage in competition with those having the less extreme and more heterozygous genotypes in the F_2 that the extreme type persistently escaped selection. Progress could then be made only as new combinations were built up from the heterozygotes. Against this view is the fact that the ultimate outcome of the selection was less viable or less fertile than the individuals of the parental type, though again this might not be so serious a difficulty for the argument if the decline in fertility could have been both gradual and matched by an equally gradual replacement of the more vigorous types. However this may be, the results

are clear in the main respect. Selection can at times fail to seize on a favourable type which segregation must have been capable of producing, and which is known to be sufficiently viable and fertile to persist in the absence of competition. Progress must then depend on building up new combinations once again, with all the attendant complications and disturbances from correlated responses.

(e) *The relation between chaeta number and fertility*

A number of facts about the relation between chaeta number and fertility have emerged from the account already presented. Fertility fell in both the high and low selection lines taken off the original F_2 . The fall was reasonably steady in the high line, just as was the rise in chaeta number. In the low line the fall in chaeta number was more erratic, there being something of a pause in the response before S-20, after which the fall was resumed and accelerated. The corresponding behaviour of fertility can be seen in fig. 7. Here too there was a pause, even a recovery of fertility, before S-21, at which generation the downward trend was resumed and became very rapid until the line died out at S-35.

In the high selection line the relaxation of selection resulted in fertility taking charge as the capital character, so that the chaeta number of the mass line fell back to an average of little more than 40, at which it became more or less stable. In all seven of the reselections from this mass (line 3) the fertility was lower after three generations of selection than it was in the first generation. It would thus appear that the first effect of reselection was to cause something of a fall in fertility within the line, though the 7 lines differed widely in fertility among themselves in no apparent order.

Only two of the reselections were continued over a sufficiently long period for their later behaviour to appear, *viz.* the first one which became line 8 at the upper level, and the seventh which became line 6 at the middle level. In line 8, which recovered the high chaeta number attained in the first high selection only at the expense of almost complete sterility in S-20, fertility was, apart from a peak at S-28, poor right up to S-31, dangerously poor in fact on several occasions (fig. 7). After S-31 however a rise set in, unmatched by any corresponding change in chaeta number, until S-41. After this there was again a decline, followed in its turn by a fluctuating behaviour which lasted more or less throughout the experiment. A small general decline at about S-83 probably has little genetical significance as it seems to have been shown by all lines. The important feature about line 8 is that its fertility did recover sufficiently for the line to be maintained at a time when chaeta number was itself stable. The fertility from S-43 onwards was in fact on the average at least $\frac{2}{3}$ that of the original high selection in its early generations, and was above that of generations S-15 to S-20.

Fertility in line 6 fluctuated somewhat, but it was never dangerously low. Up to S-70 its average was higher than that even of the early generations of the initial high selection, when chaeta number was well below the middle level upon which line 6 fell.

As already recorded, mass lines were made up from these two reselection lines. Mass line 7 was taken off selection line 8 at the upper level, and mass line 4 from selection line 6 at the middle level. These two mass lines were stable from the outset in their average chaeta numbers, hardly changing in a hundred or more generations. They did not repeat the early behaviour of line 3, only copying its later stability. Such immediate stability could come about in two ways. It might be because the selection line, from which the mass was begun, was itself homogenic, so that no matter what system of breeding was adopted change could come about only as mutation gave rise to new variability. Or it might be because, though heterogenic, the combinations giving the chaeta number in question had a selective advantage in mass culture over any which would bring about a change in chaeta number. Such an advantage must lie in a higher fertility, in the broad sense in which we are using this term.

A decision has been reached between these two alternatives by back-selection tests. Selection lines for lowered chaeta numbers were taken off lines 3 and 6 at S-47 and S-48 respectively (fig. 1). In both cases a response was obtained to the selection, that of the back-selection from line 6 being the greater. These lines were terminated at S-59. Thus the mass line 3 had within it genes capable when recombined of giving combinations reducing chaeta number. Mass line 4 was not itself tested, but since line 6, from which it was taken, also carried genes capable of building up low chaeta combinations, there is little reason to doubt that line 4 too would carry the same heterogenicity.

The test of mass line 7 did not admit even of this slight doubt, for the back-selection (line 2) was taken from the mass itself at S-56, 7 generations after it had been made up from the selection line 8. As fig. 6 shows, the response to low selection was immediate and steady up to S-67, by which time the average chaeta number had fallen to the middle level as a result of change in chromosome II (table 12). There it stayed until S-83 when another and longer fall began so that by S-90 line 2 was nearly down to the level of the low mass line 1, from which it finally became indistinguishable in chaeta number at S-107. All 3 large chromosomes had changed in their chaeta producing power by this time. A high selection (line 5), taken from line 2 at S-81, failed to advance, maintaining the middle level until its termination at S-110. A further low selection, taken from line 5 at S-100, showed no change for 6 generations, after which it was terminated.

The mass line 7 was thus stable in chaeta number in spite of its containing all the genes necessary to reduce the chaeta number below

the original parental level, when they were brought together by recombination under selection. Combinations tending to reduce the number must continually have been occurring in the mass, yet they never obtained a hold in it. This failure cannot have been due to the method of maintaining the mass line. In both mass and selection line 20 flies of each sex were taken for counting. In the first selection 4 of each sex, and in later selection generations 2 of each sex from each of 2 cultures, were chosen to be the parents of the next generation. For the mass, 10 of the 20 were taken without selection from each sex for use as parents. Thus any fly which could be chosen in starting

FIG. 6.—The origin of line 2 by low selection from line 7, and its subsequent behaviour. Line 5 is a high selection from line 2. Line 5 was separated into high and low selection lines at S-100 these being terminated deliberately at S-110 and S-106 respectively. The letter M indicates that at S-111 line 2 was mass cultured and continued as such in later generations (see fig. 3).

the selection line would have a half chance of being used in the mass line. Since flies with genotypes capable of giving lowered chaeta numbers obviously occurred in the mass line, for selection would have been ineffective in their absence, they could hardly have been missed as parents generation after generation in the mass itself. It can only be supposed therefore that in line 7 the low chaeta combinations were at a selective disadvantage under the conditions of mass culture as compared with those giving what may be termed the standard range of chaeta numbers.

Such a disadvantage of the low chaeta combinations need of course be neither unconditional nor final. It only need be sufficiently enduring to prevent the immediate combinations from obtaining a foothold.

It is not necessary to suppose that combinations built up by recombination from these, and giving still lower chaeta numbers (and the slow rate of fall as well as the chromosome assays show that change did depend on recombination), had an equal incubus of poor fertility; for by virtue of the selective elimination of their progenitors these

FIG. 7.—Fertility as measured by mean output of flies per culture in the original high and low selection lines and in lines 6 and 8 up to S-56. Note that all these are selection lines: the broken lines do not indicate mass mating as in earlier figs. Ordinate is mean number of flies per culture.

FIG. 8.—Fertility in lines 2 and 8 between S-56 and S-110. Both were selection lines. Line 8 is here shown solid, whereas it was broken in fig. 7. No change in selection is implied by this alteration of style.

latter would never come into being within the mass line itself. The fertility of line 2 suggests that this may have been the situation. A direct comparison with line 7 is of course impossible, owing to the difference in method of keeping the mass and selection lines. But line 2 had at first a lower fertility than the average of line 8, and one

which was indeed itself falling for 5 generations. Thereafter it rose and stayed above the average of line 8 until S-90, a period of some 35 generations (fig. 8). The later change in chaeta number of line 2 was unaccompanied by any noticeable change in fertility for lines 5 and 2 had much the same average fertility.

The conditional nature of the selective disadvantage of the low chaeta combinations is suggested by the low selection from line 3. In this selection the fertility, as we measure it by average number of offspring per culture, rose for the first 5 generations. Thus the impediment, which was sufficient to prevent the low chaeta combinations obtaining a foothold in the mass, was not such as to lower fertility when measured in the cultures of a selection line. The precise difference between the conditions of mass and selection lines can only be conjectured ; but competition among the growing flies in a mass culture must be more severe and involve a wider range of genotypes. There must also be a keener competition among the males for mates (see Bateman, 1948), and among the females for early laying, in a mass than in a selection line.

The response of fertility to selection for chaeta number shows that a relation must exist between these characters, but it is clear from these various findings that relation is not a simple one. A fall in fertility can accompany both increase and decrease in chaeta number under selection, as in the original high and low selection lines. Furthermore, in spite of heterogenicity, the average chaeta number of a mass line may, as a result of the advantage in what must be fertility (using the term in a broad sense) of the combinations giving central chaeta numbers, be stabilised at levels differing as widely as those of lines 2, 3, 4 and 7. Thus no chaeta number is associated with an unconditional advantage in fertility. Finally, fertility may change and recover without any corresponding change in chaeta number (line 8), while chaeta number may also change without fertility altering (lines 5 and 2).

It need hardly be said that such behaviour finds a complete basis in the theory of correlated response. Selection of recombinant chromosomes through the change they show in the balance of the polygenic combinations affecting one character must often, though not necessarily always, mean the unbalance of the intermingled polygenic combinations affecting other characters. The direction of the correlated response in the subordinate character, like its very occurrence, will depend on the detailed organisation along the chromosome of the different polygenic combinations with respect to one another ; but where the subordinate character is one which like fertility depends on a delicate genic balance, the alteration must generally be for the worse. The same process of recombination, if continued sufficiently long, will permit the subordinate character to be readjusted at some new level of the capital one ; but the attainment of full readjustment is likely to be a lengthy process if the

intermingling is complex, so that for a long time any further change of the subordinate character induced by back-selection for the capital one is likely not to be so disastrous as that induced by the first selection.

No simple scheme of pleiotropic action, on the other hand, can account for these findings. They would require four types of genes : (i) those in which the allelomorph associated with raised chaeta number also gives lowered fertility, (ii) those in which the allelomorph associated with lowered chaeta number also gives lowered fertility, (iii) those which affect chaeta number but have no effect on fertility, and (iv) those which have no effect on chaeta number but affect fertility. These four types represent all the possible associations of gene differences affecting chaeta number and fertility taken either singly or jointly. Such an interpretation differs in fact from the theory of correlated response through linkage only in that the latter provides an account of the mechanism by which the various associations come into being, and are changed or lost. If we consider correlated responses in terms of the effective factors, the segments of chromosome which are the units of immediate segregation and response to selection, we can see that these units must be pleiotropic in their action. Theirs, however, is a pleiotropy which, like their action as expressed in any single character, will depend on their internal organisation, and will be capable of being altered and built up by recombination within them.

(f) *Delayed responses to selection*

Three responses to selection observed in the later stages of the experiment call for special comment. Line 8 responded sharply to selection between S-24, when it was begun by reselection from line 3, and S-28 when it had reached (and indeed for one generation just passed) the general level of 53 or 54 chaetæ. In spite of the continued selection it was stable at this level, apart from temporary fluctuations, up to S-82. At S-82, however, its chaeta number began to rise once more and had become 57 by S-84. It then fluctuated a little, but settled down to hover about the general level of 56 for the rest of the experiment. Comparison with line 7 in the chromosome assays suggests that the increase was due to a change in chromosome III. Selection was thus ineffective for over 50 generations ; it then produced a response for 2 generations ; and finally became ineffective again for the remaining 50 generations over which its action has been observed.

In line 9 the first period of stability was shorter. The chaetæ reached 54 at S-87, rose above this figure for 2 generations and then settled down to fluctuate round it until S-110, in spite of the continued selection. It was during this period that the assay termed 9-1 was made. At S-110 generation a response began which was not completed until S-116. Two of these 6 generations were, however, wasted by the necessity of relaxing selection to cope with the difficulty of low fertility. Apart from a further period of relaxation between

S-121 and S-123 the line has maintained its new level of about 63 chaetæ since S-116. The assay termed 9-2 was made when the line had reached this new level. The chromosome assays show that the increase chaeta number is due, at least mainly, to a change in chromosome III. Though the first period of stability was only about 23 generations as compared with over 50 in line 8, the pattern of behaviour was the same as in that line—a long period of failure to respond to selection, a sharp response, and then stability again.

Line 2 showed similar behaviour under continuous selection for low chaeta number. Begun by back-selection from line 7 at S-56, it had fallen to the middle level of chaeta number by S-65 and fluctuated round 46 or 47 until S-81. A further and larger response began somewhere between S-81 and S-83, the data not permitting a more exact timing of the response (fig. 6). The main fall, which comparison of the chromosome assays of lines 2 and 5 shows to have been due to changes in all chromosomes, lasted until S-90, but there is evidence of a much slower continuation merging insensibly into stability which then lasted until selection was discontinued at S-111. In this line the first period of stability was shorter even than that of line 9, a mere 15 generations or so, and the succeeding fall was longer; but again we can recognise the same pattern of behaviour.

We can also see this pattern in two of the lines *Sismanides* (1942) selected for increased number of scutellar bristles. These two lines, C and D, originated from common parents and their remarkable similarity in behaviour can leave little doubt that they were genetically like one another, constituting, one might say, a duplicate experiment. Line C showed a first major response between the second and third generations of selection. In D the response came 4 generations later, but it was both of exactly the same size and due to recombination within the same chromosome (II). After the first response C was stable or almost so, under continued selection, until generation 14 when it once more began to change, reaching a new level and near stability at generation 17. Line D was again a little slower, beginning its second response at generation 16 and continuing until 19. Like the first responses, these second ones were due to changes of the same effect in the same chromosomes (II and III) in both lines.

Here then we have 5 cases where periods of stability, the shortest of which was 9 generations (in *Sismanides'* line D) and the longest 55 (in our line 8), were followed by marked responses to the same selection that had earlier been ineffective. To what must these delayed responses be attributed? Certainly not to any change in the mode of selection, for, with rare exceptions not associated with the onset of the delayed rises, this was kept constant throughout the history not merely of each line but of the whole experiment. We must look for the cause on the sudden appearance of variability after a long period when none was available for selective response.

The polygenes affecting chaeta number are known to mutate

(Mather and Wigan, 1942), but there are several obstacles in the way of regarding these delayed responses as due to what is normally implied by point mutation. In the first place the responses which were obtained in Sismanides' two lines, each maintained as an independent breeding unit, depended on changes in the same chromosomes (II and III) and were also of almost the same magnitude. Line C advanced from a mean of about 4.65 scutellar bristles to one of about 5.15. Line D rose from much the same initial value to about 5.25. Admittedly there is a difference of 0.1 in the sizes of these responses, but this is only 20 per cent. of the smaller of them.

The second obstacle is that the responses in Sismanides' lines occurred within two generations of one another in spite of their periods of stability under selection having been 11 and 9 generations respectively. The two lines, and even the two chromosomes within each line, failed to change over much the same lengthy period and then changed within two generations of one another. There is a determinacy about the changes in lines, chromosomes, time and size of total response, which we should hardly expect from point mutation.

The same simultaneity is shown even more strikingly in our own experiments. Line 2 was stable for about 15 generations, but when it did change all chromosomes showed the effect. The change in this line came between S-81 and S-83, while line 8 which was stable for 55 generations also began its change at S-82. The two changed within a generation of one another; that is to say, since the different generations of the various lines were kept in step, within two weeks of one another. Again we have an appearance of determinacy in time difficult to reconcile with the idea of point mutation.

Finally, the responses in chaeta number were not the only signs of change in lines 2 and 9. After it had stabilised at the lowest level, line 2 was discovered to show a characteristically anomalous pigmentation in all its males. In line 9 the males also showed anomalous pigmentation for the first time after the delayed response, this being a different type of anomaly and one which occurred in only a proportion of the flies. These pigmentary variants will be described more fully in section 4 (c); it is sufficient for our present purpose to observe that at least in line 9 the evidence points to the altered pigmentation having arisen at the same time as the delayed response occurred in chaeta number. In line 2 the evidence is less clear but would agree with this view. Line 9 also showed a change in the spermathecal content of the females about the time of the delayed response (section 4 (a)). All these changes seem to be correlated responses.

The observations all fall into place if the changes are regarded as due, not to point mutation in the strict sense, but to recombination within the chromosomes. In all the lines responses to selection had preceded the first periods of stability, responses which assays show to have depended at least in part on recombination in the very chromosomes to which the delayed responses were later traceable. Thus the

full harvest of response from those recombinations which occur regularly within the chromosomes must have been gathered in these early changes. During the period of stability the lines must therefore have been either homogenic or, more likely, balanced so that heterozygotes were always favoured by selection in regard to those parts of the chromosomes which showed this regular recombination. But we know that there are sizeable portions of the chromosomes, lying near the centromeres, in which crossing-over and recombination are rare. The first response to selection need not have involved any alteration in these regions. We also know that the occurrence of crossing-over within these regions is particularly susceptible to the action of non-heritable, external agencies such as temperature. A chance environmental change, possibly of only temporary occurrence, could therefore bring about a redistribution of crossing-over such as would cause recombination of genes hitherto virtually immune to it. All lines and all chromosomes would be affected simultaneously, but only those would show a release of variability in which the organisation of the polygenic combinations was such that the recombination could be effective in leading to an upset of balance. The occurrence of correlated responses would also, of course, depend on the organisation and intermingling of the polygenic combinations. If two lines, like those of Sismanides, were genetically almost duplicates the results of the changes in distribution of crossing-over, in particular the responses to which they gave rise, must be closely alike. And once the immediate release of variability had been used up in response to selection each line must stabilise again until such time as an even more drastic redistribution of crossing-over was induced by environmental action, for a mere repetition of the first upset must obviously be without effect.

Thus all the special features of the delayed responses to selection, their determinacy in time, in size and in chromosomes responsible, as well as their correlated responses, can be understood if they are referred to new recombination arising from the redistribution of crossing-over which many experiments have shown to follow changes in external conditions. As to what environmental changes could most successfully and most often bring about such release of variability only further and specially designed experiments can show. Such experiments, if successful, would offer a powerful tool for the furtherance of improvement by selection of crops and stock, for they would open the way to the utilisation of hitherto largely untapped reservoirs of variability.

(To be concluded)