AN EXPERIMENTAL CHECK ON QUANTITATIVE GENETICAL THEORY

II. THE LONG-TERM EFFECTS OF SELECTION

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(With Eight Text-figures)

(*Received* 29 *March* 1956)

The existing theory of quantitative variation, in the form generally applied to animal breeding, is essentially descriptive in the sense that it describes the variation in a population in terms of certain statistical parameters. From these parameters, it is theoretically possible to predict how the mean of the population will change as a result of continual selection. But as we know that selection will change gene frequencies and that these control the values of the descriptive parameters, this prediction of change in mean is of limited value because we cannot predict the change in gene frequency. The problem of how long the response will continue is therefore only to be solved by experiment.

In an earlier paper (Clayton, Morris & A. Robertson, 1956), we have described the effects of selection for abdominal bristles in a population of *Drosophila melanogaster*. We were there concerned with the early generations. The response of the population to different methods of selection (individual, half-sib and full-sib) was in reasonable agreement with predictions based on parameters derived from the base population. One of the experiments, that involving selection of the extreme twenty individuals out of 100 of each sex in five replicates in each direction, was carried on for twenty generations and in some of the lines for up to thirty-five, and it is these long-term effects of selection, predictable only in a very general sense, that we wish to discuss here.

It was remarked in the paper on short-term response that the repeatability of the replicates in response was not high in the sense that the different replicates soon established a definite order between themselves, the differences in the high lines being greater than would be expected on any simple genetic sampling explanation. This individuality of the lines in respect to mean in the later generations spread to other aspects such as variation (Table 3, Fig. 4), ratio of scores in males and females (Fig. 3), and lethal genes present (Table 1). Another interesting feature was the lack of predictability of the behaviour of a given line in future generations. At generation 20, it was decided to discontinue two of the replicates in each set. This decision was taken on the basis of the mean response in the line. In each set, the 'best' two and the 'worst', in terms of response, of the lines were kept. At the 33rd generation, when selection finally ceased, it was surprising that in both directions, the line which had only been retained because of its early slow response was now leading the field. The approach to final stability, which might on general grounds be expected to be a gradual deceleration of progress as the limit was reached, was in many

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cases quite sudden. The line H1, for istance, had risen unchecked for nineteen generations when response suddenly ceased, and in the subsequent fourteen generations it barely exceeded the 19th generation level (see Fig. 6). We shall concentrate first on the details of the separate phenomena and then consider their interrelationships by considering the characteristics of each line separately.

RESPONSE OF THE MEAN VALUE OF THE SELECTED CHARACTER

The response to selection of the different replicates is shown in Fig. 1 in which the female score is given as the average of measurements of 100 individuals. The maintenance of a definite order in the lines over periods of several generations is shown most definitely in the 'high' set. After twenty-four generations the 'high' lines averaged 75 bristles in females and 60 bristles in males. This represents a change in the mean of the character of about ten phenotypic standard deviations in the base population. Line H1 reached a

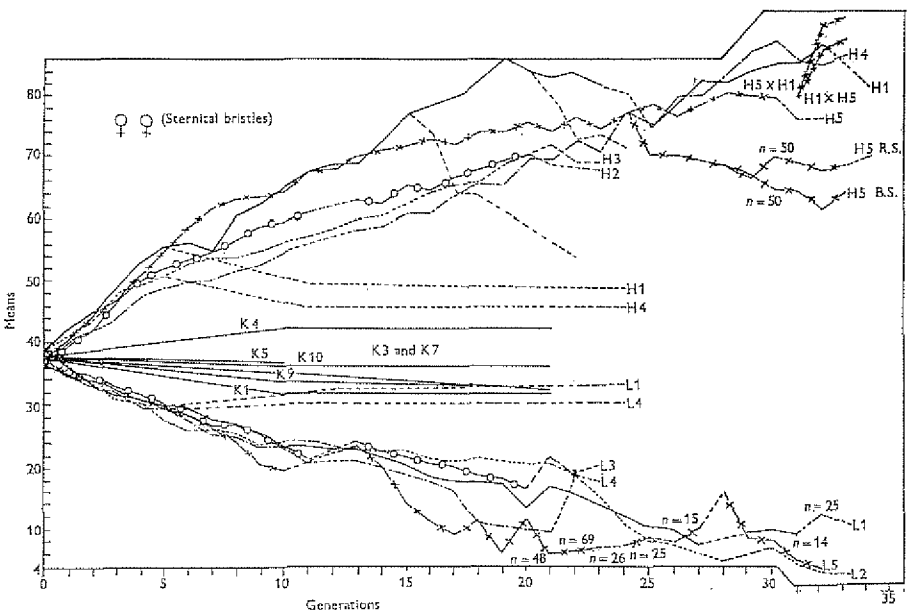

Fig. 1. Response to selection for abdominal bristles in females. Each point is the mean of 100 observations. Dashed lines indicate relaxation. Note that all lines were relaxed at generation 5, but only two in each set are shown. The other relaxed lines do not deviate much from the range indicated. The solid lines labelled K 1–K 10 show the divergence of a series of inbred lines discussed in the text. B.S. indicates back-selection. R.S. indicates re-selection.

value of 83 bristles in females at generation 19 at which time H4 had an average score of 64. Nevertheless, when selection finally ceased at generation 33, H4 was higher than H1 by some two bristles. The response in H4 was almost linear from the 5th to the 30th generations with, if anything, a slight acceleration after the 20th. Another illustration of differences between replicates is shown by the fact that fifteen generations of selection in H1 achieved as much as thirty generations in H5.

The effect of the selection is shown strikingly in Fig. 2. which gives the distributions of female scores in the initial population and in the most extreme high and low lines. The figure illustrates too the high residual variability in the selected lines.

The low lines again established a definite order in the early stages, but it was not by any means as marked as in the high set, and it is certainly much less obvious in the figures because of the compression of scale in the downward direction. The early response was less in this direction partly because of the effect of scale and partly because of a real decline in heritability after two or three generations of selection. However, in all the low lines there appeared, at different times of onset, the same peculiar phenomenon—a sudden increase in the variation in females followed by a rapid response in that sex. In some cases there was no corresponding change in the male score and in others the males showed the same kind of response (most markedly in L2), but in all cases the ratio of counts in males to that in females became greater than 1·0 compared to the value of 0·80 in the base population (Fig. 3). L5 first developed an increasing variability in females at generation 7, L1 at generation 15, and L2 at generation 19 (Figs. 7, 8). When

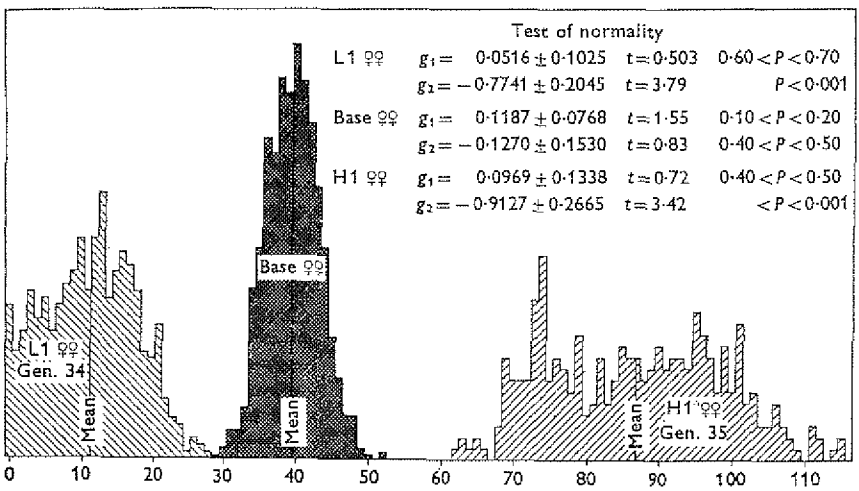

Fig. 2. Frequency distribution of females in a high and low line after extensive selection compared with base population.

this occurred, L5 averaged 26 bristles in females, L1 averaged 23, and L2 averaged 19. The later generations were marked by extreme variation and extremely low scores in females. The lowest average we recorded was in L2 at generation 33 when the value was 2·7 bristles. Many of these females showed gross defects in the sclerotinization of the abdomen. Similar results have been obtained by Rasmuson (1955). We have no explanation of this phenomenon, but feel that any adequate treatment would be in physiological rather than genetic terms. The simplest rationalization of the situation is to say that below a certain threshold the effects of the segregating genes become greatly magnified. In other terms, the developmental buffering has broken down. Although no detailed experiments were carried out, it is probable that the lines were also more susceptible to environmental influences, as it was noted that generation means were much less repeatable than in the base population.

Although the change of the ratio of scores in the two sexes was most marked in the low lines, similar differences between lines, though much smaller in magnitude, were found in the high lines. Compared to the initial ratio of 0·803, H1 showed a mean value at the end of selection of 0·848 and H5 a mean of 0·775 (Fig. 3).

The lethal analysis

At the 7th generation, lines H1 and L1 were tested for lethals on the 2nd chromosome. The lethal testing technique employed was the usual one using dominant markers with cross-over suppressors. Reeve & Robertson (1953) provide an example of the method. In the L1 line, 10 out of 32 chromosomes were lethal when homozygous, and in H1, 3 out of 25. The 10 lethal chromosomes in the line were cross-tested and 9 proved to be 'identical'. This means that there was a lethal gene or chromosome segment in this line with a frequency of about 30%. This was found to persist in the later generations.

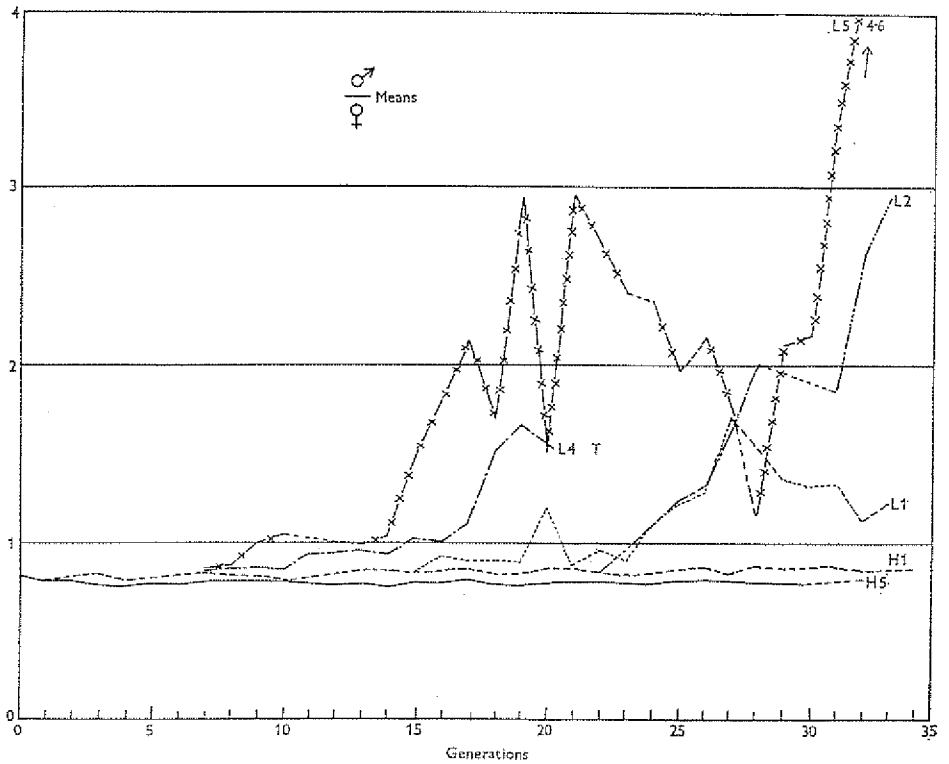

Fig. 3. The ratio of male to female means. Only two high lines are plotted. The others fall approximately between the limits set by H1 and H5.

Subsequent analyses done at generation 14 and at generation 20 confirmed that the situation was similar in the other lines. It was not possible to cross-test all the lethals thoroughly because this was added to the burden of the selection work which was still being carried on. However, the results are summarized in Table 1, in which a + indicates that a lethal gene has been found at appreciable frequency, i.e. 20–30%, the expected frequency if the selected parents contained a high proportion of heterozygotes.

Various tests were made between lethal chromosomes isolated from different lines. In no cases were lethals from high lines 'identical' with those from low. But the same lethals were found several times in lines from the same group. The lethals in H2 and H5 were identical on both 2nd and 3rd chromosomes. The 3rd chromosome lethal

appeared also in H1, but only at a low frequency. Similarly the same 3rd chromosome lethal was found in both L2 and L5.

The 2nd chromosome lethal in H2 and H5 occasionally emerged in the homozygote and proved to have brown eyes, shortened wings with no posterior cross-vein, and shortened legs. Linkage tests showed it to be in the neighbourhood of the 'brown' gene, but cross-tests showed that they were not alleles. The 3rd chromosome visible gene, veinlet, affecting wing venation, was noted several times in H4. It was known to have been present in the initial population before the experiment started, and recent tests have shown that its present gene frequency is about 11%. It has probably little or no effect on bristle number, as in the other individually selected lines it was noted in two up lines and one down line.

Table 1. *Lethal analysis of the selected lines, +indicates lethal chromosome found at appreciable frequency*

	2nd chromosome	3rd chromosome
H1	−	+
H2	+	+
H3	−	−
H4	−	−
H5	+	+
L1	+	−
L2	−	+
L3	+	−
L4	−	?
L5	−	+

It is obvious from the above table that the maintenance of heterozygosity was an essential feature of the genetic situation. It was natural that we should measure the effect of the 'lethal' chromosome on bristle score. When we had time to do this, we found that the heterozygotes were more extreme in the direction of previous selection than were flies not carrying the lethal gene. An example of this is given in detail in the description of the line H2. In other lines, in which direct tests were not done, other observations provided evidence that a lethal was being maintained in the line because of its effect on bristle score.

It was of great value to us in this work that we were dealing with an organism in which lethal chromosomes could be isolated easily. The lethals provided the key to several puzzling situations which will be dealt with when the lines are treated separately.

Variability

Before we embark on a detailed discussion of the effects of long-term selection on variability, we need to know in what framework we can deal with it most satisfactorily—or, in other words, what scale we should use. Wright (1952) has described several criteria for the evaluation of scale transformations which need not necessarily agree in the conclusions they lead to. As we are here concerned with changes in variability, perhaps the best scale is that on which variance is most constant. In the early generations we may presume that we are dealing only with quantitative changes and we will therefore use the results obtained early to establish a standard of reference for the later generations. A comparison of variances in the initial population and in the 4th generation of 20/100 selection are given in Table 2. The table gives the variance σ_P^2 of the total score (which is the sum of scores on the fourth and fifth sternites) and σ_D^2 the variance of the difference between the

two scores which, on evidence presented in the earlier paper, is in the base population almost entirely due to accidents of development affecting each sternite separately. CV_P and CV_D are the respective coefficients of variation. The effect of selection in the first four generations has been to change the variance as well as the mean. However, it will be noted that the decline in σ_D^2 in the low lines is not as great as that in σ_P^2. The ratio σ_D^2/σ_P^2 which is the proportion that this non-genetic part makes of the total, is therefore higher in the low lines. This is in agreement with the smaller values of heritability found in the low lines after a few generations of selection.

Table 2. *The effect of selection for four generations on different measures of variation*

Males						Females					
Mean	σ_P^2	σ_D^2	CV_P	CV_D	σ_D^2/σ_P^2	Mean	σ_P^2	σ_D^2	CV_P	CV_D	σ_D^2/σ_P^2
High lines											
39·6	13·61	4·46	0·093	0·053	0·33	50·0	16·87	5·84	0·082	0·048	0·35
Base population											
31·4	9·20	3·47	0·096	0·059	0·38	39·2	13·49	4·72	0·094	0·055	0·35
Low lines											
25·2	6·64	2·99	0·102	0·069	0·45	30·9	9·63	4·13	0·100	0·066	0·43

The coefficients of variation show a smaller change after selection although the trend is reversed, the low lines showing a higher figure than the high group. The coefficient of variation is equivalent to the standard deviation which we should find after transformation of the observed scores to logarithms. It follows that the log transformation would be a slight over-correction, but would nevertheless be closer to constancy in the early generations than is the simple score. In considering the later results, we shall therefore express them both as standard deviations and as coefficients of variation.

The pattern of variability within lines in the later generations is difficult to discuss because of the tremendous differences between lines. However, in order to draw some general conclusions, we have presented in Fig. 4 the results at five-generation intervals for some measures of variability. We had noted earlier that there was an apparent discontinuity of behaviour, perhaps physiological in nature, in the response of females (and to a lesser extent males) in the low lines. As might be expected, the high lines show a much more regular pattern of behaviour in the changes of variation as selection proceeds. CV_D, a measure of developmental error within the animal, declines only slightly during the twenty generations of selection. The total coefficient of variation CV_P declines a little more, but the behaviour is fairly regular. As a result, the ratio σ_D^2/σ_P^2 changes little in the course of selection, so that here we have no indication that genetic variation is being exhausted.

The low-line females show a very considerable increase in variance. On the absolute scale, the average value for the five lines at generation 20 was 24·2 units, considerably higher than in the base population and in fact higher than the high-line males at the same time. This is even more pronounced in the coefficient of variation where the average value for low-line females is over three times that in the base population. It will be seen that CV_D has increased, though not to the same extent, perhaps reflecting the breakdown in some important process so that the organism is now not so well buffered. However, σ_D^2/σ_P^2 has a value below that in the base population, so that here again most of the

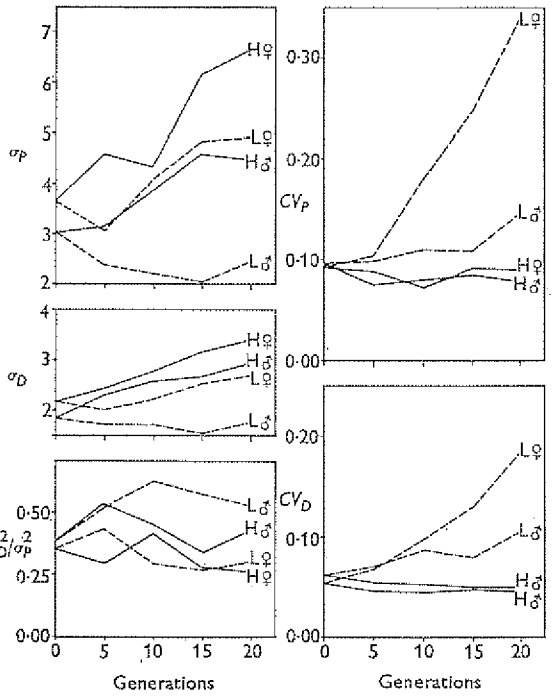

Fig. 4. Measures of variability at different stages of selection, averaged over five replicates. σ_P and σ_D are standard deviations of the sum and the difference respectively of the scores on the 4th and 5th sternites.

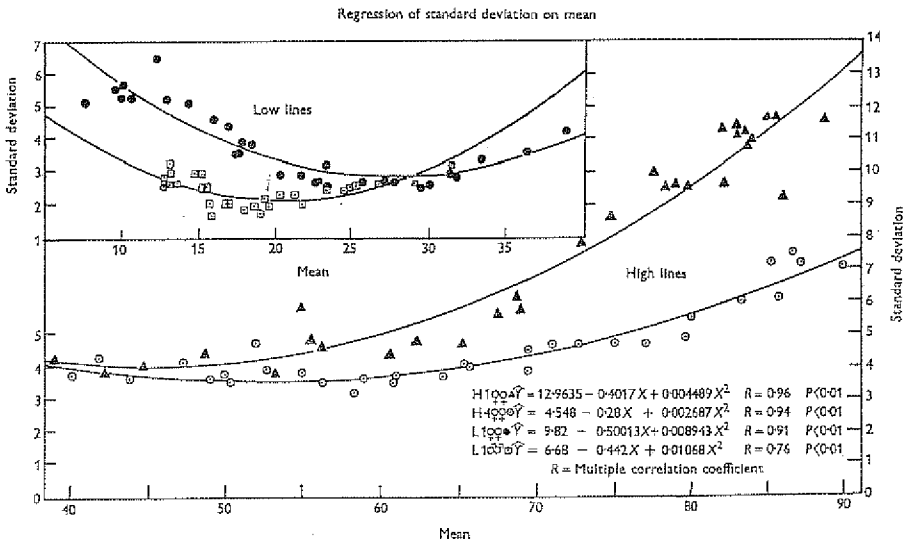

Fig. 5. The curvilinear relation found between standard deviation and mean in some selected lines.

variation is probably still genetic. The low-line males are more restrained than the females, but the same tendencies can be distinguished.

Another view of the effect of selection on variability is shown in Fig. 5, in which the standard deviation is plotted against the mean for several lines. All lines analysed (three high and three low) showed significant curvilinear regressions of standard deviation upon mean. The greater variability of the low-line females is also strikingly shown up in the diagram.

The general trend in these experiments shows that selection can very frequently reach a balanced state in which considerable variability is maintained. From other evidence, we can be fairly sure that in all such cases a high proportion of the total variation is genetic. Selection has thus by no means led to uniformity, but in some cases has even magnified the total variation.

Having discussed the separate phenomena in fairly broad outlines, we shall now deal in detail with each line separately, integrating the separate phenomena as they occur.

THE BEHAVIOUR OF THE INDIVIDUAL LINES

In this section we shall attempt to show the integration of the different phenomena in the separate lines. The various parameters describing the lines towards the end of the selection are summarized in Table 3.

Table 3. *The effect of long-term selection on mean bristle number and variation*

		Males			Females			
Line	Generation	Mean	σ_P^2	σ_D^2/σ_P^2	Mean	σ_P^2	σ_D^2/σ_P^2	♂/♀ means
H1	20	70·6	49·9	0·27	83·6	116·0	0·15	0·844
	30	72·2	73·3	0·19	84·9	135·7	0·15	0·851
H2	20	53·7	12·3	0·56	70·4	29·0	0·39	0·763
H3	20	55·9	11·9	0·69	70·3	20·4	0·53	0·795
H4	20	55·7	11·8	0·69	69·5	20·3	0·39	0·801
	30	72·5	31·5	0·43	88·9	50·0	0·34	0·815
H5	20	57·4	15·4	0·34	75·4	34·7	0·31	0·761
	30	60·5	21·4	0·53	79·6	30·7	0·39	0·760
L1	20	15·5	6·3	0·39	13·0	28·1	0·22	0·187
	30	13·3	8·8	0·37	10·1	32·8	0·16	1·308
L2	20	16·7	4·8	0·59	20·7	9·0	0·29	0·809
	30	9·4	5·4	0·55	7·1	12·8	0·32	1·327
L3	20	17·6	4·2	0·72	16·6	27·5	0·30	1·063
L4	20	15·9	10·5	0·40	10·2	20·8	0·35	1·557
L5	20	17·8	4·3	0·75	11·7	35·5	0·25	1·514
	30	18·4	4·5	0·78	8·5	23·6	0·17	2·171

At the start of the discussion of each line, we shall give a brief summary of the main phenomena observed in it.

H1. Rapid early responses ceasing suddenly at generation 19—thereafter little change. Early, most variable high line—sudden increase in variance at generation 14, remaining high to end, with σ_D^2/σ_P^2 low. Lethal balanced against selection on chromosome 3 (Fig. 6).

We have here the most extreme case of selection of the heterozygote for a lethal gene. The effect was not measured by isolation of the lethal chromosomes, but the following experiment provided a striking illustration of the situation. In generation 29 the measured flies were split into five groups of twenty pairs each, in order of bristle score, and each group was bred from with the results shown in Table 4. Group 1 is, of course,

the selected line and 100 flies of each sex were measured, whereas only twenty-five were measured in the other groups. The table shows clearly the asymmetry of the response to selection in the different directions. The upward selection (group 1) shows no response, in agreement with the previous ten generations. The distribution (Fig. 2) shows clearly the 2:1 segregation of heterozygotes and non-lethal homozygotes. The downward selection (group 5) shows a response of such magnitude that the offspring have about the same score as their parents. The offspring of group 1 are very variable as the result of the lethal

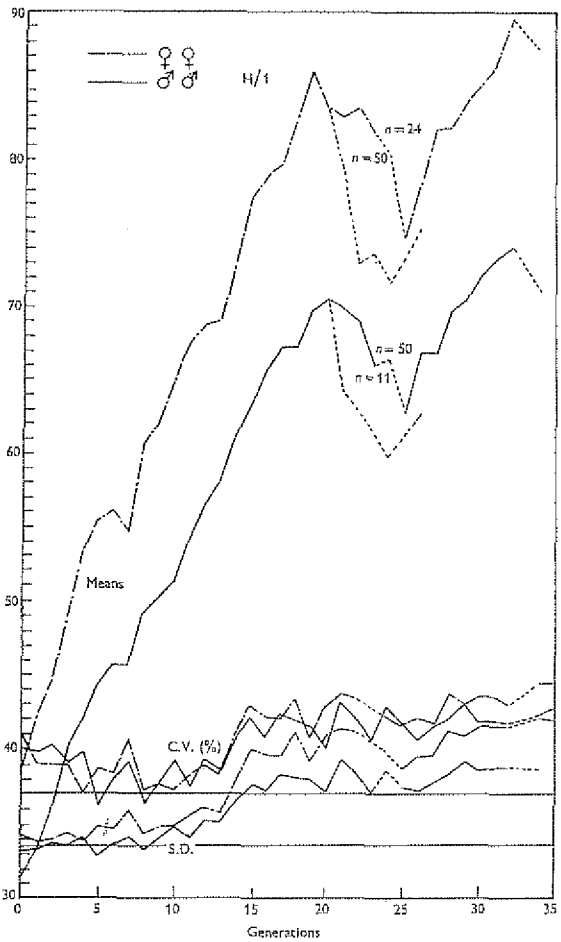

Fig. 6. Response and variation in H 1.

Table 4. *Mean and variance of the progeny of parents selected in order of bristle number (H 1)*

Group	Parents Mean	Offspring Mean	Offspring Variance
1	97·80	84·92	135·71
2	91·60	87·28	101·96
3	84·50	80·40	67·75
4	75·60	76·48	50·67
5	68·40	69·56	29·70
All	83·89	79·73	

segregating. The offspring of group 5 are comparatively uniform, as they are the progeny of individuals without any lethal genes. In terms of the normal heritability formulation, the heritability is 50% as calculated by mid-parent offspring regression. However, the realized heritability upwards is zero and downwards it is 100%. Obviously the usual heritability approach breaks down completely when, because of a lethal gene, Mendelian ratios are not found in the adult population. It is interesting that the effect of the lethal 'gene' on bristle number in the heterozygote is to increase it by 22. This raises a problem concerning the existence of the gene in the base population in which the standard deviation was 3·7. This will be discussed later. These results are very similar to those obtained by Reeve & Robertson (1953) on a strain of flies selected for long wings.

At generation 35 a genetic analysis was done by the measurement of full-sib and half-sib groups, there being 64 sires and 120 dams with 6 offspring per family. At this point, the variance was some ten times that in the base population and the distribution was markedly flattened (see Fig. 2 for the female distribution). The sire and dam components made up 10·9 and 4·8% respectively of the total variance. If we assume that all the variance in a population is due to a lethal gene, which is being selected in the heterozygote, we can, by preparing a table of the frequencies of the various types of mating and of the means and variances of their progeny, calculate the expected variance components between sires and dams as making up 6·1 and 8·9% of the total in fair agreement with the observed figures.

H1 was one of the weakest selected lines, and after generation 19 was several times relaxed to save it from extinction. In the end it was lost from female infertility.

H2. Medium response and variability. Lethals on both chromosomes 2 and 3. Discarded at generation 20 because of similarity to H5.

Here the lethal chromosomes were isolated in the genetic background of the line. In both cases the lethal chromosomes derived from females with an average score above the general mean. The different lethal and non-lethal chromosome sets were then used to synthesize groups of flies of different types and these were scored. The results are given in Table 5 in which N2 L3 signifies a group not carrying the 2nd chromosome lethal but heterozygous for that on the third.

Table 5. *Mean scores for flies of different constitution (H 2)*

	N2 N3	L2 N3	N2 L3	L2 L3
Female	62·4	63·6	70·0	73·1
Male	48·4	47·8	52·4	54·2

(25 scored—standard error of each group approximately 0·6 unit.)

The results show that the 2nd chromosome lethal is having a small effect of the order of 2–3 bristles, and that on the 3rd chromosome about 5 in males and 8 in females.

H2 had a high frequency of females with abnormal spermathecae which proved to be associated with the 3rd chromosome lethal. This will be discussed in more detail in a succeeding paper.

H3. Moderate response—low variability. No lethals present. Discarded at generation 20.

H4. Slow initial response and low variance. Slight increase in response after generation 20 with increase in variance. Highest line. No lethals.

The responses in females in the three successive intervals of ten generations were

18·2, 14·6 and 19·4 bristles respectively. In the last three generations, there were suggestions that response had ceased. Nevertheless, the ratio σ^2_D/σ^2_P indicated that considerable genetic variance remained. A genetic analysis confirmed this, giving no significant difference between sires but a dam component of 15% of the total variance. Back selection at generation 34 was ineffective.

Here again we have a period of increased variance followed by a sudden stop. However, in H4 selection seems ineffective in either direction and a situation involving lethals can be ruled out. The simplest rationalization of the facts would be an overdominant situation in which the heterozygote is being selected for and where both homozygotes are roughly equal in value. We should then expect to find as we do a significant dam component of variance but not one due to sires. However, it must be emphasized that the over-dominance hypothesis is not proven.

H5. Rapid early responses falling off slowly. Variance slowly increasing till end. Same lethals on chromosomes 2 and 3 as in H2.

Here again we have maintenance of genetic variance in spite of the lack of response. This line was back selected at generation 24 with results consistent with the effect of the two lethals, measured in H2. There was an immediate response to back-selection and the variance also dropped sharply.

After four generations of back-selection, selection upwards was restarted but met with little response in five generations. Of all the selected populations that we had dealt with, this back-selected line in H5 was the only one in which the genetic variation appeared much reduced. In the last four generations of back selection, the developmental error appeared to make up 90% of the total variance.

Here again we have found the same phenomenon. The cessation of response to selection has not meant the exhaustion of genetic variance but the maintenance of even more than there was in the base population by the continual selection of heterozygotes for lethal genes.

In three of the high lines we have thus found evidence of selection for heterozygotes for lethal genes, some with strikingly large effects on bristles. Certainly, selection has not led to genetic uniformity.

The low lines

All the low lines behave similarly in the early generations, the response falling off and the variance declining, so that we expected an early end to the response. In one line after another, however, there appeared the same phenomenon, increased variance and response in the females sometimes paralleled by the males and sometimes not.

L1. Increase in female variance and response at generation 15—followed by males to a lesser degree. Lethal on chromosome 2. Heterozygous for small inversion in 3R (Fig. 7).

Here again we have stability without uniformity, most strikingly in the females. At generation 34, a genetic analysis was done in which two offspring of each sex were measured per dam. There were two dams per sire and 100 sire groups. The results are as follows:

	Components of variance	
	Male progeny	Female progeny
Sires	0·09	11·9
Dams	0·16	7·7
Within full-sibs	6·19	21·2
Total variance	6·44	40·8

Son-dam correlation, 0·10. Daughter-dam correlation, 0·35.

In the males there seems to be little remaining genetic variation, confirmed by the fact that the ratio σ^2_D/σ^2_P was 0·70. There is plenty of genetic variation in the females—in fact, the heritability estimate from the sire component is $117 \pm 19\%$. In this analysis, it was possible to measure some of the dams, and of these the 82 which produced offspring averaged 11·48 bristles and the 36 which did not averaged 9·31. This difference is not significant but suggests that female fertility is playing some part. This might perhaps explain the higher sire component than dam component if the genetically more extreme dams were not fertile. When the 2nd chromosome lethal was examined in earlier generations, its effect was small (~ 2 bristles), so that it may not play an important part in the final situation. Again we have high genetic variability after response has ceased, but the

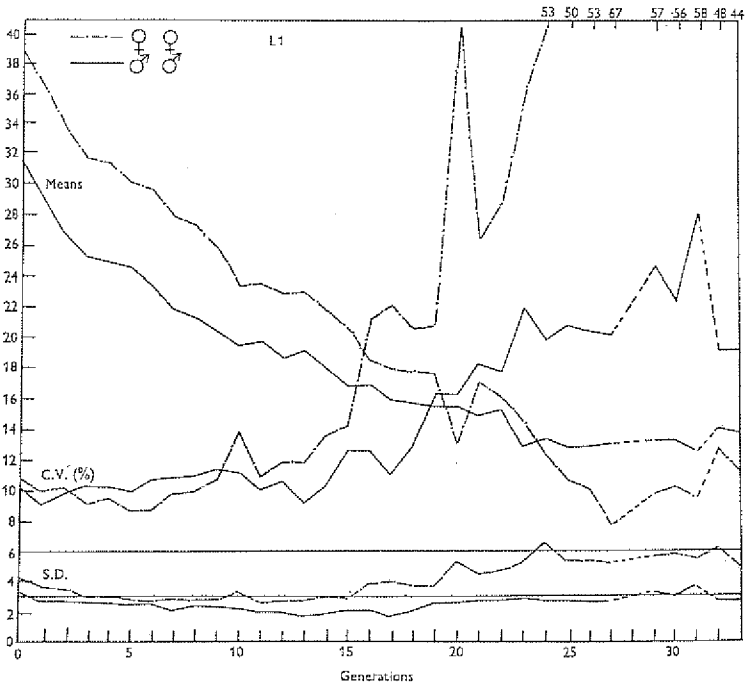

Fig. 7. Response and variation in L 1.

explanation is by no means so simple in this case. Perhaps the balance is between artificial selection and natural selection for female fertility. This was one of the most infertile lines, and the number of adult flies produced in later generations was only 2–3% of those obtained from the base population from the same number of parents.

L2. Rapid early response falling-off considerably. Sudden increase in variance at generation 22, followed by response in both sexes. Lethal on chromosome 2 (Fig. 8).

As in the up lines, we have the odd happening that the line which we had chosen to retain as the laggard eventually became the winner. In the last generation, 10% of females had no bristles on either sternite. In L2, in contrast to the other lines, the variation did not remain high at the end. The sudden response and rise in variance followed by a decline in the latter suggests the occurrence of a single event leading to a fixable variant, which perhaps led to the uncovering of new variability by a change of the genetic background in which the other genes were acting. The single event might have

been a mutation or, perhaps more likely, an unusual cross-over. The 3rd chromosome lethal in this line appeared to play little role in the behaviour of the line as the variance at the end was low. This line became infertile and selection had to be relaxed for one generation at generation 29.

L3. Similar to L1. Increase in female variance at generation 9. Sexual dimorphism not marked. Lethal on chromosome 3. Discarded at generation 20.

L4. Similar to L1. Increase in female variance at generation 13, remaining high. Males little affected. Lethal situation uncertain, possible balance on chromosome 3. Discarded at generation 20.

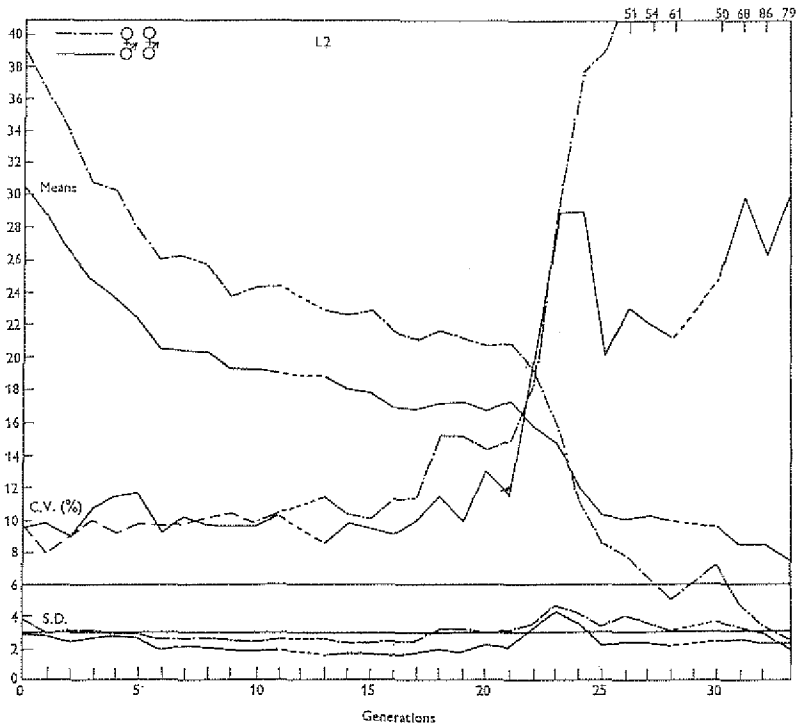

Fig. 8. Response and variation in L2.

L5. Increase in female variance at generation 5 when mean was 24, to maximum at generation 15—thereafter declining. Final female score 3·7. Little response in males, high male/female ratio. Lethal on chromosome 3. Heterozygous for small inversions in 3R.

This was the most interesting of the low lines, as on it we had more evidence than on the others and the situation was obviously complex. The additional evidence to that presented above was as follows:

(i) a genetic analysis at generation 33 gave the following results:

		Components of variance	
	D.F.	Male progeny	Female progeny
Sires	99	1·28	5·75
Dams	200	0·67	2·28
Within full-sibs	600	4·46	17·64
Total		6·41	25·67

As in L1, we have a higher sire component than a dam component, though this time in both sexes. At its face value the sire term indicates a heritability of 80–90% in both sexes.

(ii) At generation 35, chromosome sets from females scoring zero were made homozygous. Thirteen out of the eighteen sets showed lethality on chromosome 3, but pure breeding stocks could not be obtained from the non-lethal sets because of female infertility.

(iii) The inversion balance appeared to be independent of the lethals as lethal chromosomes of both types appeared. In addition, homozygotes for both inversions were found as adult animals.

(iv) At generation 35, 100 scored females were allowed to produce offspring in separate vials, after mass mating. The results are summarized in Table 6.

Table 6. *The offspring of groups of scored females (L5)*

Dam score	No.	Av. no. of progeny/dam	Males score	Females score
0–2	25	23·6	14·5	7·9
3–5	30	32·6	16·0	8·5
6–9	22	30·5	16·1	9·3
10–18	20	49·1	16·6	8·6

The son-dam correlation is significantly positive ($0{\cdot}20 \pm 0{\cdot}07$), though the daughter-dam correlation is not. The regression of number of offspring on dam score is significant, so that natural selection for female fertility is probably in some way responsible for the genetic situation and may account for the sire component being higher than that of the dam.

To probe a little further, three more flies of each sex in each family were counted and matings were then made within families with the highest and lowest female scores. The results are summarized in Table 7 when in each family ten of each sex were scored in the second generation.

Table 7. *Parents and offspring of families ranked on female score (Table 6)*

			Progeny	
Male	Female	Dam score	Male	Female
High female families				
18·4	16·2	6	18·1	4·4
15·2	13·2	4	16·9	4·8
16·8	12·8	10	16·4	9·3
15·2	11·2	4	15·5	4·1
Low female families				
18·0	4·0	0	17·7	5·8
17·4	4·0	4	15·1	3·3
11·4	2·8	3	10·8	5·8
13·4	1·2	16	12·9	4·4
13·6	0·4	12	12·4	3·0

These results are a little bewildering. The low female families can have dams at either end of the distribution, as would be expected from the low daughter-dam regression. But, with one exception, the high female families gave in the next generation inbred progeny with the same mean as did the low families.

This line remains a puzzle and it is doubtful if any simple explanation can be given. Several different factors seem to be playing a part—balanced inversions, natural selection

against lethals and against female infertility. We feel that the latter may be the most important single factor.

The low lines do not seem to be as simple in their behaviour as the high, although we have again the maintenance of genetic variance with no response to selection. Perhaps in these lines we are dealing with an opposition of artificial selection and natural selection for fertility of the kind described by Lerner & Dempster (1951) in the selection of poultry for shank length. With one exception, these selected lines are still in existence after about twenty generations of relaxation and some of the low lines would probably repay further study.

Discussion

After the comparative order of the earlier paper on the short-term effects of selection, in which results were in fair agreement with expectation, the long-term behaviour of these lines is bewilderingly complex. We found that fairly early in the selection the lines developed different patterns of response. Later on, the separate lines became strikingly different in many different facets of behaviour.

The period of response is of some interest, not only from the practical point of view but because it throws some light on the action of the individual genes concerned. As we saw earlier, in the first five generations the response was in fair agreement with predictions based on the analysis of the initial population. The response then slowed somewhat and in most of the lines was almost over after twenty generations, although in one, H4, it continued until the 30th generation. Falconer (1953) has shown that the period of response depends on the magnitude of the effects of the individual genes responsible. In such a case as this he showed that the main part of the variance should be due to genes having an effect (as measured by the difference between the two homozygotes) of about one-fifth of the observed standard deviation—here an effect of one-half to one bristle.

However, the actual cessation of response to selection in these lines is quite different from that to be expected on the simple classical model, i.e. a gradual diminution of genetic variation as gene frequencies approach extreme values and an asymptotic approach to a final plateau. On the contrary, we find that almost invariably the final situation is one of unstable equilibrium with a considerable amount of unfixable genetic variation, and in several cases the cessation of response has been abrupt and surprising. Although the causes of these phenomena have not been thoroughly explored in all cases, in many lines the explanation of the situation is that we are continually selecting heterozygotes of genes which are lethal when homozygous. As we then reach equilibrium with intermediate frequencies of such genes, this presents a reason for the maintenance of the genetic variation and also for the sudden cessation of improvement.

The maintenance of genetic variation in spite of continued selection has been demonstrated in *Drosophila* before by Reeve & Robertson (1953). Presumably many of the genes, which are lethal when homozygous, are not completely recessive but have some effect in the heterozygote, either on quantitative characters or on viability, as has been shown by Stern (1948).

Operationally speaking, this situation can be said to involve over-dominance, in the sense that heterozygotes have an overall selective advantage. But this advantage derives from both artificial and natural selection—from the effects of the gene on both bristle count and fitness. It seems to us that we should distinguish between two extreme

situations. In the one, the gene has no effect on natural fitness, but the heterozygote is more extreme for the selected character than either homozygote. In the other, the gene is additive for the selected character but in the homozygote is inferior in natural fitness—in our case, the homozygote does not survive. In both situations, we will see the selected population in equilibrium with genetic variation being maintained in the selected character. But the analysis of this genetic variance in the selected character will give quite different results in the two situations. It is proposed to give such a theoretical analysis in a later publication. We have some evidence that the first situation (true overdominance for the measured character) did exist in at least one of our lines, whereas the second (artificial selection for the heterozygote balanced by natural selection against the homozygote) existed in seven of them.

In distinction to the fair success of the available theory in predicting response to selection in the earlier generations, we have found that the theory in its simple form is quite inadequate in the later generations when response to selection has almost ceased. Different estimates of heritability have given wildly different results—the response to selection has been zero in one direction and complete in the opposite direction and so on. But it would be a mistake in this situation to take too black a view and dismiss the classical approach as useless. We may reasonably ask why it is apparently of so little value here—can it in any way be modified to fit these situations? The most obvious cause for its breakdown is that it assumes that the Mendelian laws of segregation will hold. If we have lethals segregating, their homozygotes will not be observed—the basic mechanism of heredity will still operate, but some genotypes will be absent when the adults are measured. The up line with the most striking behaviour (H 1) can be completely explained by the assumption of the segregation of a lethal gene with a large effect on bristles in the heterozygote. In such cases it is questionable whether such concepts as additive genetic variance or heritability have any meaning. All we can do is to observe the reaction of the population to analysis of various sorts and do our best to make sense of the results. Until we have done so, we are not justified in throwing away the concept of heritability as valueless in all cases. Certainly we have more hope of detailed understanding of complex situations in *Drosophila* than we have in other organisms lacking the special techniques developed for this species.

In our selected lines at equilibrium, we have found different lethals segregating, some with considerable effects in the heterozygote. But the word 'lethal' is misleading in this context—all we can really say is that the whole chromosome behaves in this fashion. This is the aspect of the work in which gaps in our knowledge are most obvious, but the addition of the problem of testing for lethals and also the crossing of the lethals was rather too much when the selection was still being carried on. Where did these lethals come from—were they present in the base population? Here, again, the work leaves something to be desired, but chromosomes which appeared 'identical' on crossing with three of the lethal chromosomes (2 in the high lines and 1 in the low) have been found in the base population. The only lethal gene which has been roughly located and examined cytologically was not associated with an inversion.

It seems most unlikely that these lethals have had, in the base population, an effect on bristles in heterozygotes of the magnitude they had in the selected lines. In H 1, for instance, the lethal chromosome had an effect of 22 bristles in females in which the standard deviation of the base population was only 3·8. It was observed in this case that

the increase in variance was quite sudden. We may perhaps postulate in this case that a lethal gene with a small effect in the heterozygote has been segregating in the population up to this point. Then a mutation, or more likely a rare cross-over, has occurred so that a new gene enhancing the effect has come into the chromosome close to the lethal gene so that now the two in selection behave as one unit. It is indeed likely that such unfixable genetic variation should be magnified by selection of modifiers affecting the heterozygote alone, as Robertson & Reeve (1952) have pointed out.

The other surprising phenomenon, the sudden appearance of additional variation and rapid response in females in the low lines, can hardly be profitably discussed as it seems to be mainly of physiological rather than genetic interest. Having said that there is a threshold below which the gene effects in females are greatly enhanced, almost all that is useful has been said. These lines will probably repay further investigation from the genetic point of view, as in them the bristle character has been moved from its neutral position to one of major importance for fitness. It then reaches a position of equilibrium, with the maintenance of genetic variation, as do the major components of fitness. Can we then use the low lines to investigate a character which is, and has been for many generations, a component of fitness and which is very easy to measure, which fitness components generally are not? We propose to do some further work along these lines and further evidence on the peculiar behaviour of the females may then accrue.

Before we lay too much emphasis on these results, we must enquire to what extent they may be peculiar to *Drosophila*. The two most important aspects of this are the enormous reserve of reproductive ability (enabling the preservation of much harmful variation in a population quite capable of reproducing itself) and the small number of genetic units (deriving from the small chromosome number and the lack of crossing-over in the male). The high reproductive ability means that selected lines, such as L5, may accumulate balanced genetic situations affecting different aspects of reproductive fitness in a way that a population, say, of dairy cattle could not. The small number of genetic units is of importance in enhancing the importance of linkage and hence the possibility that the factors we are really dealing with are chromosome segments rather than individual genes (if this distinction is really meaningful). The lack of crossing-over in males is a means of preserving in *Drosophila* considerable chromosomal polymorphism which could have been the basis of some of our balanced situations. In species in which there is crossing-over in the male, inversion heterozygotes are at a disadvantage (if the inversions are long) because of the inviable products of single cross-overs within the inversion (see Sturtevant & Beadle, 1936). It is noteworthy that cytological examination of mouse stocks has not produced one example of an inversion, although they are ubiquitous in *Drosophila* populations.

The behaviour of the lines after many generations of selection has been in complete contrast to their early behaviour. The divergence between replicates and the sudden alterations of rates of response has meant that any prediction of future happenings whether of lines in general or of any line from its own past behaviour has been hazardous in the extreme.

An awkward question can be raised here by the geneticist concerned with practical animal improvement, assuming that our results have some general relevance. He may reasonably object 'It is very well to say that the heritability approach gave satisfactory results at the beginning of the experiment and crazy ones at the end. But how am I

to know which situation I am in when dealing with milk yield in cattle or litter size in pigs?' There would seem to be two methods of making some decision about this. The first would be to examine the situation for signs of internal consistency. Admittedly it is difficult enough in some cases to get any estimates of heritability at all—without having to decide definitely whether or not different estimates agree with each other. The other method would be to look for signs of natural selection, especially in a form due to the segregation of single genetic units. The causes of the maintenance of genetic variation in a selected population can be either opposing natural selection or true over-dominance. The former situation may give positive values for heritability estimates, the latter will not, except in the case of a full-sib estimate. These approaches are perhaps not very precise, but a fuller theoretical investigation of the possibilities might give clues as to how to approach the problem.

Summary

1. The results of continued selection for abdominal bristles in a large population of *Drosophila melanogaster* are presented and discussed.

2. Response had slowed down considerably in many lines after twenty generations, although in some it continued until the 30th generation. In many of the lines, the cessation of response was abrupt and lack of response did not mean exhaustion of genetic variability.

3. In three of the high lines, the high variability was apparently due to continued selection of heterozygotes for a lethal gene. In two high lines, such genes were present on both 2nd and 3rd chromosomes.

4. In the low lines, a striking phenomenon was a sudden increase of variation in females followed by a rapid response in that sex. This appeared in all lines with different times of onset. The ratio of male to female score in all low lines was greater than unity, compared to 0·8 in the initial population.

5. Genetic variation was maintained in many of the low lines after response had ceased. The situation appeared to be complex in that lethal genes, infertility of extreme females, and heterozygosity for inversions all played some part.

6. In such situations, the classical heritability approach appeared to break down completely. In some cases, the reason for the breakdown could be given in terms of the other phenomena observed, but in some of the low lines the full explanation was not clear.

7. The relevance of these results to practical problems of animal breeding is discussed.

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