

Improvement of litter size in a strain of mice at a selection limit

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SUMMARY

A strain of mice that had ceased to respond to selection for high litter size was inbred with continued selection. Depression of the mean proved the existence of residual genetic variance. Four lines survived the inbreeding, and one reached 20 generations with a mean equal to the original strain, thus disproving overdominance as a major cause of the residual variation. The four selected inbred lines were crossed and a new strain derived from the cross was maintained in parallel with the original strain. The new strain showed an improvement of 1.5 mice per litter over the original strain. Thus selection with inbreeding was able to achieve an advance beyond the limit attained by the original selection.

The hypothesis that the residual variation was due to genes with simple dominance was tested by seeing if it could account for the observations with reasonable values of the relevant parameters. The improvement made by the inbreeding and crossing required the elimination of about 30 recessive genes with effects (homozygote difference) of 0.5 phenotypic standard deviations and gene frequencies of 0.2. Consideration of the mean levels of the selected inbred lines in conjunction with the rate of depression found on inbreeding without selection showed that the selection with inbreeding had eliminated about 75% of the segregating recessives. The number of genes contributing to the residual variance was therefore about 40. The additive variance generated by these genes was just consistent with the estimate of zero from the realized heritability. Consideration of the original selection showed that about half the genes could have been still segregating when the response ceased. The hypothesis therefore requires the number of genes in the base population to have been about 80. The number of genes required, though large, does not seem impossible, and the hypothesis of genes with simple dominance can account for all the observations.

1. INTRODUCTION

When a closed population under artificial selection has reached a selection limit it may nevertheless contain genetic variation affecting the character selected. The presence of this residual genetic variation has often been proved by a response to reversed selection, or by a regression toward the unselected level on suspension of the artificial selection, or by a change of mean on inbreeding. The precise nature of

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the residual genetic variation, however, is not well understood (see, for example, Roberts, 1966*b*). If genetic variation of the character selected remains in the population it should be possible to obtain further improvement beyond the level of the selection limit by an appropriate breeding method. But without an understanding of the nature of the variation there is no rational basis for deciding what would be an appropriate breeding method, and any attempt to improve on the selection limit must be essentially a matter of trial and error.

The work described here started in this way, as an empirical attempt to obtain further improvement of a strain of mice that had ceased to respond to selection for high litter size. The only method that had proved successful in improving the body weight of mice after selection limits had been reached was making a new population from a cross between two or more such selected strains (Roberts, 1967*a, b*). This method was not possible because only one line selected for litter size was available. The method applied, which as far as I know had not been tried before, was inbreeding with continued selection during the inbreeding, and the subsequent crossing of the four best inbred lines. This procedure served also to show whether any genetic variation of litter size remained in the selected strain. The method proved to be remarkably successful, but the reasons for its success were not at all apparent. The subsequent work was therefore directed toward finding an acceptable genetic interpretation of the results of the inbreeding and crossing, and at the same time finding an explanation of the residual genetic variation that was consistent with all the experimental observations.

2. HISTORY OF STRAIN

The strain of mice used, designated JH, was selected for high litter size, the criterion of selection being the number of live young born in the first litters. Selection for low litter size (JL) was also applied, and an unselected control (JC) was maintained. The selection was applied within families of full-sib sisters. All three strains were maintained by minimal inbreeding with 10 single-pair matings per generation. The results of the selection up to generation 42 have been described in previous publications (Falconer, 1960, 1963, 1965). The generation means over the whole experiment are shown in Fig. 1. In the calculation of mean litter sizes, infertile matings and 'zero-litters' (i.e. litters containing only dead young) were always excluded.

The control line showed no systematic change up to about generation 30, the mean level over this period being 7.60. The absence of any inbreeding depression in the control line was surprising, since by generation 30 the theoretical inbreeding coefficient was at least 30%, and from estimates based on rapid inbreeding in this strain (Bowman & Falconer, 1960) a decline of 1.7 mice per litter was to be expected at this level of inbreeding. The selected lines both responded to selection, with realized heritabilities, up to generation 20, of 7.6% upwards, 25.0% downwards and 13.6% overall. The low line reached a selection limit after about 20 generations, and the mean level from generation 21 to the end was 6.03, giving a final response

of 1.57 mice per litter. The selection limit in the high line is less clear. The limit first appeared to have been reached at about generation 20, as in the low line, since there was no response during the next 15 generations. But after generation 35 some further improvement became apparent and the mean rose from 9.04 in generations 21–35 to 9.71 in generations 36–44. Between generations 44 and 45 there was a large increase in litter size. This was probably caused by a modification of the diet which was made at this point; the riboflavin content was increased from 2.5 to 4.5 ppm, and wheat meal (20%) was substituted for miller's offals. After the change of diet there was no further improvement over the 20 generations to the end of the experiment, at generation 64, the mean during this period being 11.09. Thus there was no evidence of genetic improvement after generation 36 and the level of the limit under the original diet can be taken as 9.71, the level between generations 36 and 44. This makes a final response to upward selection of 2.11 mice per litter, and the total response measured as the difference between the high and low selection limits was 3.68.

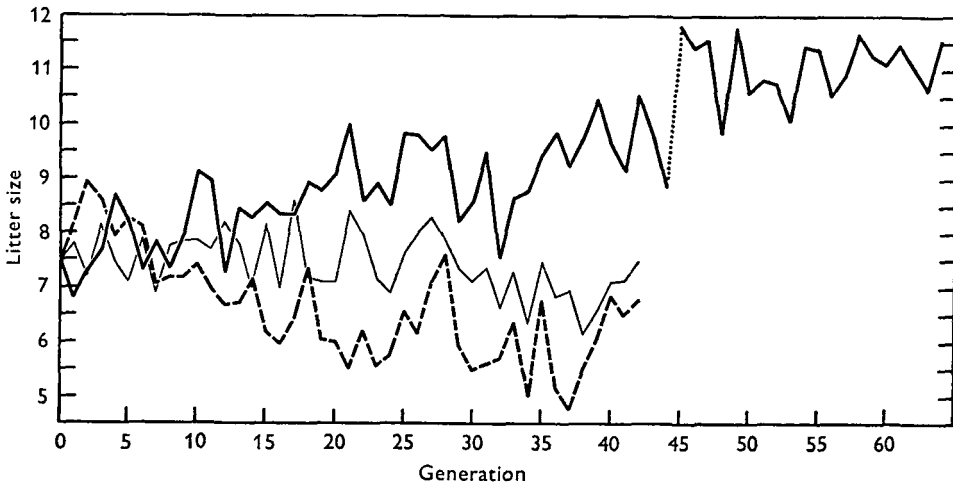


Fig. 1. Mean litter sizes in the strains selected for high litter size (JH, thick continuous line), for low litter size (JL, thick broken line) and the unselected control (JC, thin continuous line). The composition of the diet was modified between generations 44 and 45.

It is worth noting that this total response amounted to only 1.8 phenotypic standard deviations, or 3.8 additive genetic standard deviations, which is much less than has commonly been found in selection experiments.

After generation 42 the low line and control were discontinued, but the high line was continued up to generation 64 in parallel with the inbreeding experiment which started at generation 43 by which time the selection limit, as noted above, had been reached.

3. INBREEDING WITH SELECTION

As already stated, the JH strain was continued during the course of the inbreeding experiment. There were, however, some small changes in the system of mating and selection which should be mentioned. Up till generation 42 all the young females of each full-sib family were mated at 6 weeks of age, usually all to the same male in one cage. The females were separated when pregnant, and the largest litters in each sibship were selected. The number of females per sibship averaged about 4-5, so the selection intensity was about 1 in 4 or 1 in 5. With ten sibships per generation, altogether about 45 litters were born from which the generation mean was estimated. The number of females from each sibship that were mated was reduced to three at generation 43 and to two at generation 54. Thus the intensity of selection was reduced. From generation 46 onwards each female was mated separately in single-pair matings, in order to make the JH strain comparable with the inbred lines. None of these changes had any detectable effect on the mean litter size. Finally, from generation 57 the number of sibships and females selected was increased from 10 to 16 in order to provide a larger number of litters from which to estimate the mean at the time when the critical comparison was being made.

The inbreeding was started from the surplus litters of generation 42 of the JH strain. There were 38 litters, and their mothers came from nine of the ten full-sib families of the previous generation. Thus the inbred population consisted of nine lines of independent origin. Generation I-0, with no inbreeding in either mothers or young, was a part of generation JH-42. Eighty females from the 38 litters of I-0 were mated each to a sib male, to make I-1, in which the mothers were non-inbred and the litters were 25% inbred. The inbreeding was continued by full-sib matings of between 80 and 83 females in each generation. From I-3 onwards single-pair sib-matings were made. Throughout the inbreeding, selection for large litter size was applied. About 20 selected litters were required to provide the 80 females for the next generation, so the intensity of selection was about 25%. At first the selection was applied on an individual basis, without regard to the subdivision into lines. This led to the elimination of three of the nine lines in the 2nd generation of inbreeding (I-2), and another one at the 3rd generation. At I-7 another line dropped out and the population was reduced to four lines. Since it was important to retain four lines for the crossing, the selection was then changed and applied within lines. Thus, from generation I-7 onwards, 20 new matings were made in each line, selected for the largest litters in the line.

The inbred and JH generation means are shown in Fig. 2, and the differences between the two in Fig. 3. The change of diet, and the selection with the consequent elimination of lines, made it difficult to assess quantitatively the effect of the inbreeding. It is clear, however, that inbreeding did reduce the mean, and that the JH strain was therefore not fixed for all genes affecting litter size. The rate of inbreeding depression will be discussed later, in connexion with another experiment.

The most important conclusion from the inbreeding is that three of the four surviving lines showed little or no inbreeding depression, at least from generation

I-3 onwards, i.e. after the change of diet. The means of the four lines, averaged over five-generation intervals, are shown in Fig. 4. The best line (JY) maintained a level equal to JH. The second best line (JW) improved after I-3 and from I-8 onwards averaged about 0.5 mice per litter below JH. The third line (JZ) declined

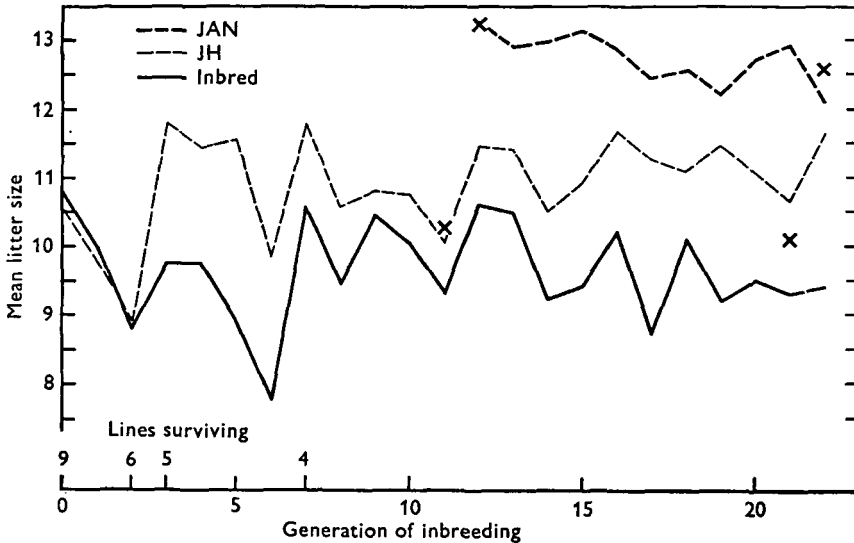


Fig. 2. Generation means over the period covered by the present experiments.

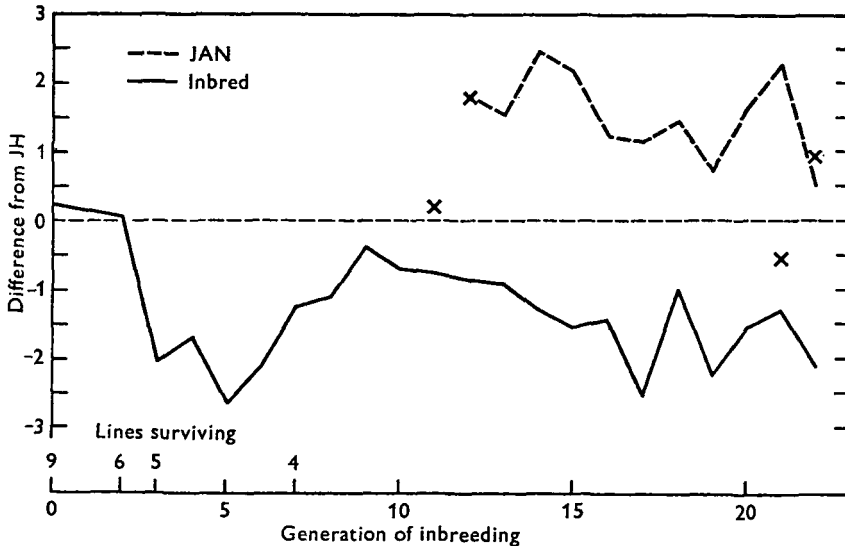


Fig. 3. Generation means as deviations from the JH strain.

slowly and ended at about 1.5 below JH. The fourth line (JX), which was the best at the beginning, showed the greatest inbreeding depression and ended at 4 mice per litter below JH. The fact that one line reached a high level of inbreeding without falling below the outbred mean proves that the residual genetic variance in the JH strain cannot be attributed to overdominance.

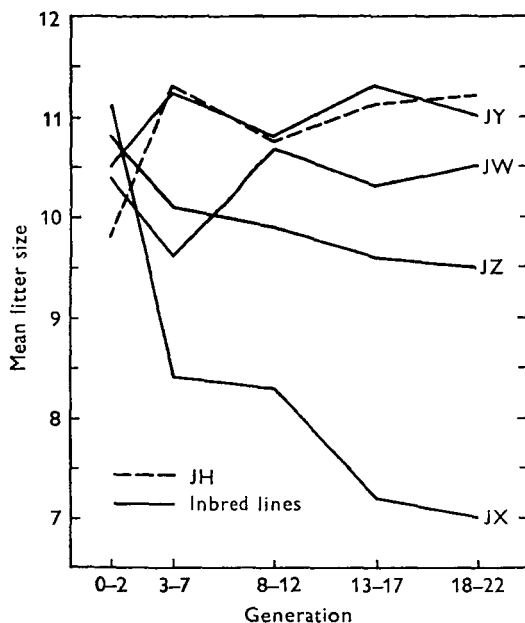


Fig. 4. Means of the four selected inbred lines, averaged over 5-generation periods.

4. CROSSING

The four surviving inbred lines were crossed at generation I-11, when the inbreeding coefficient of the parents used in the cross was 89%. (The crossing was repeated at I-21, but the description of this will be deferred till later.) The 1st cross, to produce F_1 litters from inbred mothers, was made in a full diallel, with reciprocals and the diagonal. Matings forming the diagonal were used to continue the inbred lines. Eight matings were made in each of the 12 crosses, from which a total of 75 litters were born, but these were not equally distributed between the crosses. Twelve matings were made in each of the inbred lines (diagonal), from which a total of 37 litters were born, again not equally distributed. The 2nd cross, to produce F_2 litters from F_1 mothers, was made from the progeny of the 1st cross in the normal manner of a 4-way cross, so that all four inbred lines were equally represented in the ancestors of the F_2 progeny, and the coefficient of inbreeding both of the mothers and of their litters was zero. A full diallel was made, with reciprocals, but excluding the diagonal. The means, calculated as the mean of line- or cross-means, are given in Table 1, and are marked by crosses in Figs 2 and 3. The F_1 mean shows a small gain, of 0.97 mice per litter, from heterosis in the litters. The F_2 mean was 13.23, which was better than the contemporaneous JH (11.45) by 1.78 mice per litter, and better than the inbred level by 2.66.

The improvement over the selection limit of JH was very substantial. In order to see whether the high level of the F_2 was partly a chance deviation or would be maintained, a new strain was established from the F_2 litters. This strain, designated JAN, was maintained for ten generations by minimal inbreeding with 16 pairs.

Two females from each of the 16 families were mated for the measurement of litter size, one was chosen for the continuation of the strain. Weak directional selection was applied, the female chosen being the one with a litter size nearest to 14. The object of selecting litters of nearly intermediate size was to reduce the variation caused by maternal effects, though the reduction expected was not great; about 2% of the phenotypic variance is attributable to variation in the size of the litter in which the female was reared, i.e. the mother's litter size (Falconer, 1965). The

Table 1. Mean litter sizes in crosses and contemporary inbreds

(The means are calculated as means of cross-means or line-means.)

	First cross (JH-53, I-11)		Repeated cross (JH-63, I-21)	
	No. of litters	Mean \pm s.e.*	No. of litters	Mean \pm s.e.*
Contemporary with F_1				
JH	18	10.06 \pm 0.74	30	10.36 \pm 0.65
Inbred	37	9.30 \pm 0.47	35	9.31 \pm 0.50
F_1	75	10.27 \pm 0.37	102	10.08 \pm 0.30
Heterosis†		0.97 \pm 0.60		0.77 \pm 0.58
Contemporary with F_2				
JH	20	11.45 \pm 0.61	31	11.58 \pm 0.54
Inbred	41	10.57 \pm 0.50	37	9.40 \pm 0.53
F_2	91	13.23 \pm 0.26	93	12.54 \pm 0.30
Heterosis†		2.66 \pm 0.56		3.14 \pm 0.61

* Sampling variance of mean of group means, calculated as $(1/N^2) \sum s_x^2$, where N is the number of groups and s_x^2 the sampling variance of the group mean.

† Difference between cross-bred and inbred.

mean litter sizes are shown in Fig. 2, and the differences from the contemporary JH means in Fig. 3. A reduction of the mean was expected in the first two generations after the cross, for two reasons. The first generation after the F_2 (i.e. JAN-1) would have been subject to an adverse maternal effect from the high litter size of the mothers (F_2 litters); and the progeny of JAN-1 were 25% inbred relative to the F_2 litters, from which a loss of about one quarter of the heterosis was expected. Only a very small reduction was observed, and the JAN strain maintained a level of 13 mice per litter for four generations after the F_2 . The gain from the inbreeding and crossing was thus proved to be neither a transient nor an accidental phenomenon. Over the ten generations, however, the JAN strain did decline a little, the regression of mean on generation number being -0.08 ± 0.026 mice per litter per generation. The difference between the JAN strain and the contemporary JH generations averaged 1.54 ± 0.19 mice per litter over the ten generations.

As a final check on the reality of the gain from inbreeding and crossing, the 4-way cross was repeated at generation I-21, when the inbreeding coefficient was 99%. The crosses were made in the same way as before, except that the number of females mated for each cell of the table was ten in the F_1 and eight in the F_2 . The

results are shown in Table 1 and Figs 2 and 3. On the whole they confirm the previous results, but the F_2 was not as high; in the first crossing the F_2 mean was 13.2, whereas in the repeated cross it was 12.5, and about equal to the contemporary level of the JAN strain. With a standard error of 0.40 the difference between the two F_2 s is not significant. The repeated cross clearly confirmed the reality of the improvement gained from inbreeding and crossing.

It is not easy to see which of the values given in Table 1 provide the best estimate of the heterosis and of the gain over the JH level. From Fig. 2 it can be seen that the inbred mean and the JH strain fluctuated closely in parallel from generation to generation. For this reason the heterosis shown in Table 1 is calculated from the difference between contemporary groups. On the other hand, the between-generation variation of the JAN strain was clearly not in parallel with that of the other strains; in fact the change from one generation to the next was nearly always in the opposite direction to that in the JH strain. Since the F_2 generations would be expected to behave in the same way as the JAN strain, the difference between contemporary groups does not seem to provide the best estimate of the heterosis in the F_2 and the gain over the JH level. Perhaps the best estimate of the gain would be from the means of the JAN and JH strains over the last five generations. Over the last five generations the JAN mean was 12.5 and the JH mean was 11.2, making the gain 1.3 mice per litter. The gain from selection in the JH strain up to the change of diet was 2.1, so the gain from inbreeding and crossing amounted to 60% of the gain from selection.

It is not profitable to analyse the crosses in detail because the means of the individual crosses were not estimated sufficiently accurately. In both crosses the F_1 means followed fairly closely the mean of the inbred line used as a female parent, as was to be expected since the female parents of the litters were inbred. The F_2 means showed no relationship with the performance of the inbred parents of the mother, and there were no striking differences in general or in special combining ability.

5. DISTRIBUTION OF LITTER SIZE, AND EMBRYONIC LOSSES

The effect of the crossing on the distribution of litter size is interesting. The distributions, pooled over the generations contemporaneous with JAN 0-10, are shown in Fig. 5. The JH and JAN strains have the same modal value of 13, and the higher mean of JAN results from a reduction in the number of small litters. The JH distribution is asymmetrical, with a larger proportion of litters below the mode than above it, and this asymmetry virtually disappears in the JAN strain. Consequently the variance is greater in the JH than in the JAN strain. The distributions of the four inbred lines show that the small amount of inbreeding depression in the JW and JZ lines was mainly due to an increased frequency of small litters rather than a reduction in the modal value, while the larger depression in the JX line was due to a much reduced modal value. The best inbred line, JY, has a distribution resembling that of JAN. Fig. 5 shows also the proportion of infertile

matings and 'zero-litters', i.e. litters born but with no live young. The frequency of infertile matings and zero-litters amounted to 5.4% in JH and 3.3% in JAN; the difference, however, is not significant. The inbred lines, particularly two of them, and the F_1 showed much higher frequencies - up to 29% in the JX inbred line.

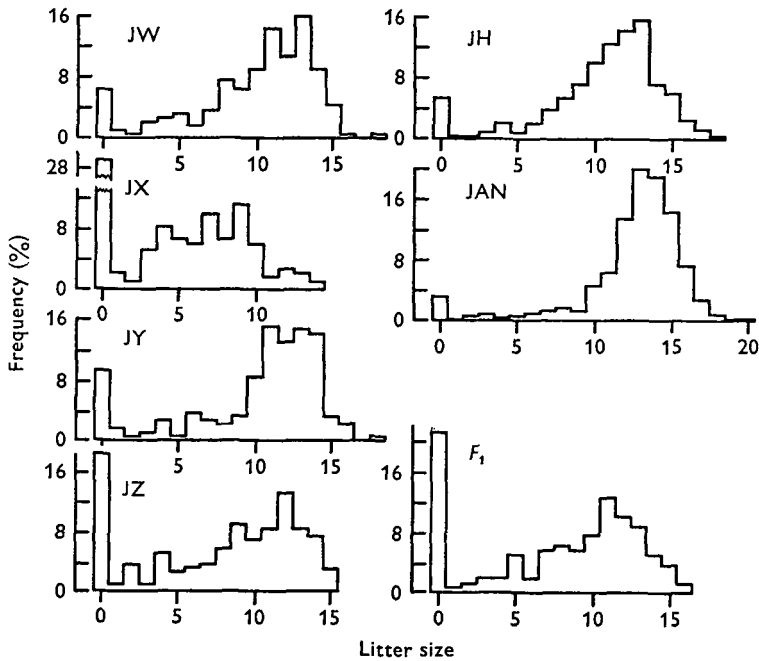


Fig. 5. Distributions of litter size, including sterile matings and 'zero-litters', based on the following generations and numbers of litters (in parentheses): Inbred lines, 12-22, JW (187), JX (182), JY (192), JZ (192); F_1 of first crossing (160); JH 54-64 (331); JAN 0-10 (528).

The shape of the distribution of litter size in the JH strain suggests that the lower litter sizes may have resulted from embryonic losses and that the effect of crossing had been to reduce the embryonic losses in the JAN strain, rather than to increase the ovulation rate. This supposition was fully confirmed by counts of corpora lutea and implantation sites, which were kindly made by Dr R. C. Roberts and analysed by Mr J. Toop. The counts were made on 17-day pregnant females which were the progeny of the last generations of the JH and JAN strains. The results are given in Table 2. The mean number of corpora lutea (estimating the number of eggs shed) is virtually the same in the two strains, but the number of losses per litter is less than half as great in JAN as in JH litters, the difference being mainly in the losses before implantation. The difference in the number of embryonic losses identified in these counts fully accounts for the difference in litter size at birth. The variance of the number of losses was also less in the JAN than in the JH strain, and this difference fully accounts for the difference of the variance of litter size at birth.

It is interesting to compare the embryo counts made at the end of the experiment with those made earlier. The results of counts on the JH strain at generation 33 (Falconer, 1963) are given in Table 2. The increase of about 2 in the mean litter size resulted almost entirely from a reduction in the embryonic mortality and not from an increased ovulation rate. The total embryonic mortality was reduced from 33 to 20 %.

Table 2. *Corpora lutea and embryonic losses in contemporary generations of the JAN and JH strains, and at an earlier stage of the JH strain (JH-33, data from Falconer, 1963)*

	(Means per litter \pm standard error.)		
	JAN-11	JH-65	JH-33
No. of mice ...	49	32	38
No. of corpora lutea	13.2 \pm 0.26	13.6 \pm 0.36	13.1
Losses, pre-implantation	0.33 \pm 0.19	1.59 \pm 0.42	2.5
Losses, post-implantation	0.90 \pm 0.13	1.19 \pm 0.22	1.8
No. of live embryos	11.98	10.81	8.8
Comparable litter size	12.48*	11.17*	9.2†
	Percentage losses		
Pre-imp. % of corpora lutea	2.5	11.7	18.5
Post-imp. % of implantations	7.0	9.9	16.4
Total, % of corpora lutea	9.3	20.5	32.8

* Mean of preceding five generations.

† Mean of contemporary group.

6. INBREEDING WITHOUT SELECTION

The results of inbreeding and crossing prove that genetic variation remained in the JH strain despite the cessation of response to selection. There seem to be only two possible explanations of this residual genetic variation, overdominance and recessive genes at low frequencies, both of which might be complicated by epistatic interactions. Overdominance seems to be ruled out as the main cause of the variation by the fact that one inbred line reached a high level of inbreeding without falling below the JH level, and two others fell only a little below it. Recessive genes at low frequencies seem to be a more plausible explanation. Recessive alleles reducing litter size would be eliminated only slowly by the selection, and when they had reached low frequencies the strain would appear to be at a selection limit. Selection with inbreeding would be more effective in eliminating the recessives since the variance due to recessive genes increases with inbreeding up to about 50 % (Robertson, 1952). Crossing the selected inbred lines would then produce a population from which some of the recessives had been eliminated, and the mean would thus be increased.

As a means of discriminating between the two hypotheses it seemed important to estimate the rate of depression resulting from inbreeding without selection in the plateaued JH strain, for comparison with that in the base population. If the residual variation in the JH strain were due to overdominance, the rate of inbreeding depression might be expected to be as great or perhaps greater than in the

base population. If, on the other hand, the residual variation were due to recessives, it would be less, since the gene frequencies of the recessive alleles would have been reduced by the selection from the base population. The rate of inbreeding depression in the JAN strain might also have some diagnostic value. If all, or nearly all, the recessives had been eliminated by the inbreeding with selection then the inbreeding depression in the JAN strain would be reduced. But, on the other hand, the gene frequencies of any recessives that remained in the JAN strain might be increased, since they could not be less than 0.25, and the inbreeding depression due to these genes might be increased. Consequently a reduced rate of inbreeding depression in the JAN strain would argue in favour of the recessive gene hypothesis, but an equal or increased rate would be inconclusive.

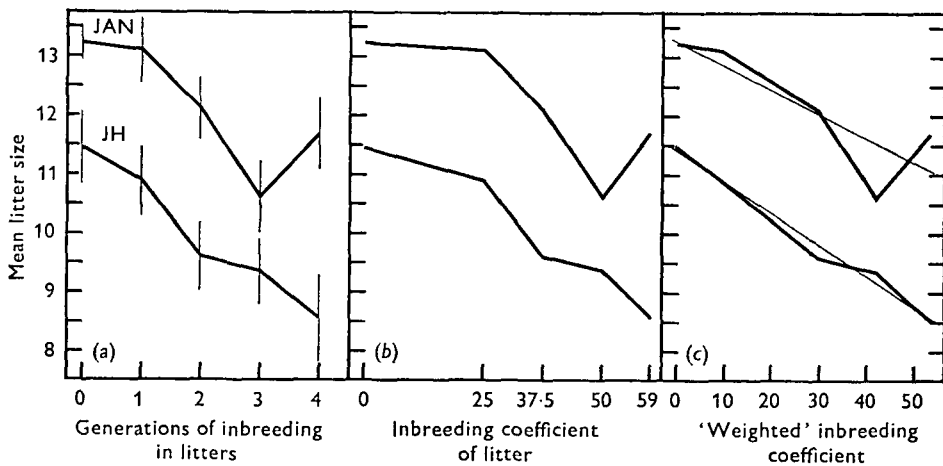


Fig. 6. Generation means on inbreeding without selection from JH-54 and JAN-0. Vertical lines in (a) extend to \pm one standard error. Thin lines in (c) are fitted linear regressions. Further explanation in the text.

For these reasons the JH and JAN strains were inbred without selection. The inbreeding was started by sib-mating 20 JH females of generation JH-54, and 32 JAN females of the F_2 of the cross. Full-sib inbreeding was continued for four generations by taking one pair at random from each litter, without replacement of litters that failed to produce a pair. At the last generation, when the mothers were 50% inbred and the litters 59%, 13 litters were born in the JH lines and 20 in the JAN lines. The mean litter sizes, plotted against the generations of inbreeding, are shown in Fig. 6(a). Both strains declined on inbreeding, and at roughly the same rate. Unfortunately the decline in the JAN strain was not regular and any estimate of the rate of decline must have a wide margin of error. The decline in the JH strain, however, is more regular and the rate can be more reliably estimated.

A difficulty in relating the inbreeding depression to the coefficient of inbreeding is to know how to partition the depression between inbreeding in the mothers and inbreeding in the litters. The earlier work on the JC strain (Bowman & Falconer, 1960) showed empirically that the mean litter size was linearly related to the

inbreeding coefficient of the litters, and the rate of depression was 0.56 young per 10% of inbreeding in the litters. The rate of decline in the JH and JAN strains, calculated in the same way, was 0.50 ± 0.08 in JH and 0.38 ± 0.15 in JAN. These rates differ in the manner expected from the recessive gene hypothesis, but none of the differences is significant.

Plotting the litter size against the coefficient of inbreeding in the litters, as in Fig. 6(b) shows, however, that in the JH and JAN strains the decline was not linearly related to the coefficient of inbreeding of the litters. Inbreeding in the litters, which shows in the first generation, had relatively less effect than inbreeding in the mothers, which shows in the second generation. Thus both the JH and the JAN strains appeared to differ from the base population in the relative effects of inbreeding in the mothers and the litters. The standard errors marked in Fig. 6(a) show that the small amount of depression in the first inbred generation could have been due simply to sampling error. But it occurred in both strains and, furthermore, the difference between JH and JC appeared also in the heterosis displayed by the F_1 and F_2 generations of the crosses. The apparent difference in the relative effects of inbreeding in the litters and in the mothers therefore seems worth looking into farther.

The inbreeding depression and the heterosis were partitioned between mothers and litters in the following way. The observed inbreeding depression, D , was estimated as the difference between the first or the second inbred generation and the zero inbred generation from which the inbreeding started. The observed heterosis, H , was estimated as the difference between the F_1 or the F_2 generation of the cross and the mean of contemporaneous inbreds. If the effects of inbreeding in the mother and in the litter are assumed to be independent of each other, the observed inbreeding depression is

$$D = F_M D_M + F_L D_L.$$

F_M and F_L are the inbreeding coefficients of mothers and litters respectively; D_M and D_L are the inbreeding depressions resulting from 100% inbreeding in the mothers and in the litters respectively. D_L is estimated from the first inbred generation, where $F_M = 0$, and D_M is estimated from the second inbred generation by substitution of the already calculated value of D_L . The total inbreeding depression expected if both mothers and litters were 100% inbred is $D_T = D_M + D_L$. Similarly the observed heterosis is $H = F_M H_M + F_L H_L$, where F is here the reduction of the inbreeding coefficient on crossing. H_L is estimated from the F_1 and H_M from the F_2 . The total heterosis expected in an F_2 following crossing of 100% inbred lines is $H_T = H_M + H_L$.

The data on inbreeding in the JC strain comes from two sources - the inbreeding from generation 0 described by Bowman & Falconer (1960), and the inbreeding from generation 11 described by Roberts (1960); the partitioning in the first has been calculated from the means of line-means and in the second from the original data kindly supplied by Dr Roberts. The partitioning of the heterosis in JC, given in Falconer (1960), has been recalculated from comparisons of contemporary generations.

The results of the partitioning are given in Table 3. The heterosis is consistently less than the inbreeding depression. This is to be expected in the JH crosses because the inbred lines were selected. It may result also partly from the fact that the inbreeding effects in mothers and litters are not independent, as was shown by Roberts (1960). Though the estimates of inbreeding depression and heterosis are clearly not reliable individually, the results as a whole are entirely consistent with the recessive gene hypothesis. Thus both inbreeding depression and heterosis are less in JH than in the base population, and less in JAN than in JH. Moreover the differences are much more marked in the litter-effect than in the mother-effect. Whereas in the base population the effect of inbreeding in the mothers was equal to or less than that of inbreeding in the litters, in the JH and JAN strains it is about three times as great.

Table 3. *Partitioning of inbreeding depression and heterosis between mothers (M) and litters (L)*

	Depression or heterosis per 100 % of inbreeding (mice per litter)			Percentage of total	
	M	L	Total	M	L
Inbreeding depression					
JH	4.01	2.28	6.29	64	36
JAN	3.70	0.55	4.25	87	13
JC-0	6.66	6.00	12.66	53	47
JC-11	1.84	4.96	6.80	27	73
Heterosis					
JH, 1st crossing	1.83	1.07	2.90	63	37
JH, 2nd crossing	2.39	0.78	3.17	75	25
JC	1.80	2.57	4.37	41	59

The partitioning of the inbreeding depression can be used to formulate a 'weighted inbreeding coefficient' with which the mean will be linearly related if the weighting holds good also at the higher levels of inbreeding. This will allow a more accurate comparison of the rates of inbreeding depression. The weighting factors applicable to JH and JAN are taken from the heterosis in the first crossing since these were likely to be the most reliable, and the weighted inbreeding coefficient was calculated as

$$F_W = 0.63F_M + 0.37F_L.$$

The mean litter sizes are plotted against the weighted inbreeding coefficient in Fig. 6(c). The JH means fit very closely to a straight line in all generations, and the JAN means do so in the first three generations. Regression coefficients fitted to the weighted inbreeding coefficients give estimates of the rate of inbreeding depression per 10 % of inbreeding of 0.53 ± 0.04 in the JH strain and 0.41 ± 0.14 in

the JAN strain. Though the standard errors are reduced the difference is still not significant.

The difference between JH and JC in the partitioning of the inbreeding depression is readily understandable as a consequence of the selection for litter size. Litter size is a composite character measuring the fertility of the mother and the viability of the young. The selection of whole litters applies individual selection to the fertility of mothers, but family selection to the viability of young. Family selection would be expected to be more effective than individual selection in eliminating recessive genes. Consequently a greater reduction would be expected in the inbreeding depression due to the viability of the young than in that due to the fertility of the mothers.

7. THEORETICAL POSSIBILITY OF RECESSIVE GENE HYPOTHESIS

The experimental results point to the continued segregation of recessive genes as the most likely explanation of the residual genetic variation in the JH strain, and the elimination of some of the recessive alleles as the cause of the superiority of the JAN strain. Is this explanation possible on theoretical grounds? This question involves consideration of three parameters – the gene frequencies, the magnitude of effect of the genes, and the number of genes – and seeing whether a set of values for these can be found that is consistent with the observations, and that can be accepted as ‘reasonable’ values.

In order to make the problem manageable it will be necessary to consider a set of equivalent genes, i.e. having the same gene frequencies and magnitude of effect. Furthermore, only completely recessive genes will be considered. The gene frequency of recessive alleles, q , has a lower limit of 0.025 imposed by the number of parents, and values higher than about 0.3 seem unlikely. The magnitude of effect, a , will be taken as the difference in litter size between the two homozygotes and will be expressed as a/σ , where σ is the phenotypic standard deviation. There is no restriction on the gene effects, but values greater than $a/\sigma = 1$ seem unlikely. The critical parameter for which a reasonable value has to be found is the number of genes, n , eliminated by the selective inbreeding and therefore causing the differences between JH and JAN. There is no rational basis for deciding what a ‘reasonable’ number would be, and one can only rely on intuition. Let us say, more or less arbitrarily, that a number of the order of tens would be acceptable but a number of the order of hundreds would not. To find out if the hypothesis is possible, the following questions must be answered. (1) How many genes with specified frequencies and magnitude of effect would be needed to account for the difference between JH and JAN; and would the additive variance generated by them be compatible with the cessation of response? (2) Could these genes reasonably be expected to be still segregating after 40 generations of selection, and (3) could they be expected to have been eliminated from all four lines during the inbreeding with selection?

(i) *Difference between JH and JAN*

The expected difference of mean, in terms of the gene parameters, is naq^2 . The observed difference, calculated as the mean difference between the JH and JAN strains over the ten generations during which the JAN strain was maintained, was 1.54. The phenotypic standard deviation in JH was 2.94, so the observed difference of mean was 0.52σ . By equating the observed and expected difference of mean, the number of genes required (n) was calculated for three values of q (0.1, 0.2, 0.3) and three values of a/σ (1.0, 0.5, 0.2). The values of n so obtained are given in Table 4. There is a wide range of parameter values that could account for the difference between JH and JAN.

Table 4. *Number of genes (n) with given gene frequency (q) and effect (a/σ) required to produce the observed difference of mean, with the consequent heritability (h^2) due to these genes*

q	$a/\sigma = 1.0$		$a/\sigma = 0.5$		$a/\sigma = 0.2$	
	n	$h^2(\%)$	n	$h^2(\%)$	n	$h^2(\%)$
0.1	52	9	104	5	260	2
0.2	13	17	26	8	65	3
0.3	6	23	12	11	29	4

Table 4 shows also the heritabilities that would result from the segregation of these genes, calculated from the expectation $h^2 = 2n(a/\sigma)^2q^3(1-q)$. It ranges from 2% with the largest number of genes to 23% with the smallest number. We have to consider what level of the heritability would be compatible with the apparent cessation of response. The realized heritability in the JH strain from generation 45 to 64 was calculated from the regression of response on selection differential. The regression coefficient with its standard error was -0.0014 ± 0.0261 . This was converted to an estimate of -0.002 ± 0.046 for the overall, individual, heritability. Adding two standard errors to the estimate gives about 10%, as the upper limit compatible with the data. Hill (1971) has shown, however, that the standard error of the regression coefficient underestimates the sampling variance of the realized heritability, so the upper limit could be set a little higher.

The heritabilities given in Table 4 are those arising from the genes that have been eliminated by the selective inbreeding. The JH strain, however, must have contained other segregating genes besides these because the JAN strain showed inbreeding depression. Therefore an acceptable set of parameters for the genes eliminated must give a heritability of less than 10%. It will be shown later that the genes eliminated by the selective inbreeding represented probably about 75% of the genes segregating in the JH strain. Consequently the upper limit of the heritability attributable to the genes eliminated must be reduced to about 7.5%, but, in view of the underestimation of the standard error of the observed heritability a value up to about 10% might be acceptable.

Consideration of the heritability generated still leaves a fairly wide range of acceptable parameters, and so the recessive gene hypothesis seems to be possible. The range of possible gene numbers and gene effects are roughly between 30 genes with effects of $a/\sigma = 0.5$ and 60 genes with effects of $a/\sigma = 0.2$, the gene frequencies being around 0.2. The calculations that follow will be based on these values of the parameters.

(ii) *Effect of original selection*

The effects of the original selection will now be examined, in order to find out whether recessive genes could still have been unfixated in the JH strain. The parameters required are the effective population size, N , the coefficient of selection, s , the initial gene frequencies, q , and the gene-effects, a/σ .

The original selection was carried out over 40 generations with as far as possible 10 pairs of parents equally represented in the next generation. The effective size was therefore ideally 40, but because of sterile matings it was probably nearer 32. The average selection differential on females was about one standard deviation, so the net selection differential in standard deviation units was $i = 0.5$. For individual selection, the coefficient of selection is given by $s = ia/\sigma$. For selection within families, it is $s = ia/2w\sigma$ (Roberts, 1966a), where w is the ratio of the within-family phenotypic variance to the total. In this case w was 0.9. The previous calculations showed the possible values of a/σ to lie between 0.2 and 0.5. The value of s then works out to be between 0.056 and 0.14. The parameters N and s enter the calculations as the product, Ns , which thus lies between about 2 and about 4.

The probability that a recessive gene is still segregating is not obtainable, as far as I know, from any publication, but was kindly provided by Dr W. G. Hill from calculations made in connexion with the paper by Hill & Robertson (1968). The probability of a gene being unfixated in a population of size $N = 32$ after 40 generations with $Ns = 2$ is 0.54 if the initial gene frequency was $q = 0.2$, and 0.85 if $q = 0.5$; and with $Ns = 4$ it is 0.48 with $q = 0.2$ and 0.79 with $q = 0.5$. Thus, over the range of parameters considered, at least half of the genes that were originally segregating are expected to have been still segregating in the JH strain when the inbreeding experiment started. It therefore seems possible that the number of genes segregating was 30 or more, as required by the hypothesis. The expected frequencies of the genes still segregating can be obtained from fig. 6 of Hill & Robertson (1968), which shows the expected mean gene frequency to be 0.1. The frequency of 0.2 deduced from the previous calculation seems not impossible.

(iii) *Effect of inbreeding with selection*

The next question to be considered is the efficacy of the inbreeding with selection in eliminating recessive alleles. We need to know the probability of a favourable dominant allele being fixed in any one line, and simultaneously in all four of the lines used in the crossing. If there had been no selection the probability of fixation in any one line would have been simply the frequency of the dominant allele, i.e. about 0.8, and the probability of fixation in all four lines would have been 0.41. If

the selection had been applied within lines only, the probability of fixation can be obtained from equation 8 of Robertson (1960) or equation 11 of Hill (1970). For $q = 0.2$ it works out to be 0.82 with $a/\sigma = 0.2$ and 0.84 with $a/\sigma = 0.5$. The probability of fixation in all four lines would then be 0.44 or 0.50. There is, however, no theory that allows selection between lines to be taken into account, and a different approach must be made. The following partly empirical solution is based on the observed means of the selected inbred lines and the observed rate of depression on inbreeding without selection.

Let k = the number of genes segregating in the JH strain; q = the frequencies of the recessive alleles; a = the homozygote difference; r = the proportion of genes fixed for the recessive allele in an inbred line. As before, all genes are assumed to have the same q and a . The mean of the JH strain is then $\frac{1}{2}ka(1 - 2q^2)$. An inbred line has rk genes with frequency $q = 1$, and $(1 - r)k$ genes with frequency $q = 0$. The mean of the inbred line is therefore $\frac{1}{2}rka(-1) + \frac{1}{2}(1 - r)ka = \frac{1}{2}ka(1 - 2r)$. The difference in mean between JH and an inbred line is

$$C = ka(r - q^2).$$

The total inbreeding depression, when inbreeding is complete and no selection has been applied, is given by

$$D = kaq(1 - q).$$

The ratio of the change of mean on inbreeding with selection (C) to the change of mean on inbreeding without selection (D) is independent of the unknown parameters k and a . Thus

$$\frac{C}{D} = \frac{r - q^2}{q(1 - q)},$$

whence

$$r = \frac{C}{D}q(1 - q) + q^2,$$

and the probability of fixation of the favourable dominant is given by $1 - r$.

For the estimation of the probability of fixation, the means of the selected lines were taken from the five-generation averages shown in Fig. 4. Calculations were made for the two periods, generations 8-12 and 18-22, corresponding to the first and the repeated crosses. The values of C were taken as the difference between the inbred line and the mean of the JH strain over the same period. The total depression of the JH strain when inbred without selection was calculated from the regression of mean litter size on the 'weighted' inbreeding coefficient, as explained earlier, the value being $D = 5.3$. The probability $(1 - r)$ of a recessive allele being eliminated from each selected inbred line separately was calculated for three gene frequencies, 0.1, 0.2 and 0.3. The probability that any particular recessive allele was eliminated from all four lines is then the product of the four separate probabilities. It is not necessary to give all the results. Table 5 gives the values of C for each line, the separate probabilities for $q = 0.2$, and the combined probabilities for all three gene frequencies. If we take $q = 0.2$ as the gene frequency indicated by the previous calculations, the conclusion is that about 75% of the recessive genes segregating in JH were eliminated by the selective inbreeding and were not

present in the JAN strain. Virtually all of the remaining 25% will have been eliminated from three of the lines and fixed in the fourth, so that nearly all the genes still segregating in the JAN strain will have had frequencies of 0.25. If the number of genes eliminated by the inbreeding with selection was between 30 and 60, as indicated by the previous calculations, it follows that the number of genes segregating in the JH strain was between 40 and 80.

Table 5. *Differences of mean (C) between selected inbred lines and JH, and probability (P) of elimination of a recessive allele*

Line	At first crossing		At repeated crossing	
	C	P	C	P
JW	0	0.96	0.9	0.93
JX	2.4	0.89	3.5	0.85
JY	0	0.96	0.1	0.96
JZ	0.8	0.94	1.4	0.92
All four				
$q = 0.1$		0.91		0.87
$q = 0.2$		0.77		0.70
$q = 0.3$		0.60		0.49

(iv) *Inbreeding depression and variance in JAN*

The conclusions of the last section make it possible to test for consistency the two observations on the JAN strain that have not yet been used. The first is the rate of depression when the JAN strain was inbred without selection. If the number of genes segregating in JAN was one quarter of those segregating in JH, and the gene frequencies were 0.25 in JAN and 0.2 in JH, then the expected ratio of the inbreeding depression in JAN to the inbreeding depression in JH works out to be 0.29. The observed ratio, derived from the regressions on the 'weighted' inbreeding coefficient, was $4.1/5.3 = 0.77$, with a standard error of 0.28. The consistency is not very good, the observed depression in the JAN strain being about $2\frac{1}{2}$ times the expected depression. The difference, however, is not significant, even on the assumption that the expectation is precise, which it is not, and so this discrepancy does not seriously weaken the hypothesis.

The second observation is the reduced phenotypic variance, seen in the frequency distributions in Fig. 5. The total genetic variance caused by k equivalent recessive genes is $ka^2q^2(1-q^2)$. Putting the number of genes as k in JH and $0.25k$ in JAN, and the frequencies $q = 0.2$ in JH and $q = 0.25$ in JAN, the difference of variance between JH and JAN reduces to $0.024ka^2$. Assuming 40 genes in JH with $a/\sigma = 0.5$ yields 2.1 for the expected difference of variance, and assuming 80 genes with $a/\sigma = 0.2$ yields 0.7. The observed phenotypic variances were calculated from the within-generation variance pooled over the last 10 generations, and the values obtained were $JH - JAN = 8.8 - 6.7 = 2.1$. This agrees perfectly with the expectation based on 40 genes. The observed difference of variance thus tends to support the smaller number of genes.

8. DISCUSSION

This experiment is of both practical and theoretical interest. An attempt was made to improve on what appeared to be a selection limit by a method with no precedent and little promise, and it worked. A method that worked with litter size in one particular strain of mice, however, could not be applied with much confidence to other characters or to other animals without an understanding of why it succeeded. This is one reason for having given a good deal of space to examining its genetic interpretation.

There was nothing unusual about finding residual genetic variation in the strain at the selection limit. Overdominance is a simple and attractive mechanism to postulate for the maintenance of genetic variation after a strain has ceased to respond to selection. In this case, however, overdominance was disproved, at least as a major factor. The only alternative was the hypothesis that the genes affecting the character selected were in the main genes with simple dominance in the direction of selection, i.e. for high litter size, and that some of these genes were still segregating. The contradiction between the segregation of genes with simple dominance and the lack of response at the selection limit can best be explained by the chances of random drift having counteracted the continued selection pressure, or possibly by a progressive fall in the environmental level having masked a real response. It follows, therefore, that the selection limit was more apparent than real. Presumably, if the selection had been continued, some further progress would eventually have been made.

The hypothesis of genes with simple dominance was not in any strict sense proved, unless by default of a better one. The evidence in its favour is that it provides a reasonably consistent interpretation of all the experimental results. The aspects of the experiment with which it was found to be consistent were: the original selection applied, the lack of response at the selection limit, the mean level of the selected inbred lines, the gain from crossing the selected inbred lines, the rate of depression on inbreeding both before and after the crossing, and the reduced variance in the strain derived from the crosses.

The number of genes required by the hypothesis may be thought to be rather large. The improvement made by the crossing of the selected inbred lines could be accounted for by a few genes with large effects or by many genes with small effects. But a heritability low enough to be consistent with the lack of response to selection requires the second alternative, many genes with small effects. The smallest number required to account for both observations is about 30, if all had the same effects and the same gene frequencies. This means that the number segregating in the strain at the selection limit was at least 40, and the number in the base population about 80 or more, again if they had equal effects and frequencies. Recent work has tended to point to much smaller numbers of genes being responsible for selection responses (Spickett & Thoday, 1966; Roberts, 1966*a*; Robertson, 1967). But, since litter size is a complex character made up of the fertility of the mother and the viability of the young, it is not unreasonable to expect it to be influenced by

a large number of genes; indeed, one might expect almost all segregating genes to influence one or other of its components in some degree. It must be remembered, also, that the number of genes is deduced from calculations that are very rough and are based on several simplifying assumptions that are certainly not true. The number of genes arrived at serves mainly to show that the hypothesis is a possible one. The other parameters deduced along with the number of genes are entirely acceptable: recessive allele frequencies of about 0.2 and gene effects of between 0.2 and 0.5 standard deviations.

The nature of the genetic control of litter size that can be deduced from this experiment and previous ones is as follows. First, it is almost entirely a character of the mother and the young; males have very little influence on the size of litter sired (Falconer, 1960), except in so far as they contribute genes to the young. The fertility of females is influenced by two sorts of gene. There are genes affecting ovulation rate. These are mainly additive, or at least are not dominant in one direction rather than the other, because ovulation rate did not decline on inbreeding (Falconer & Roberts, 1960). It was mainly these genes that gave rise to the response over the first 30 generations of selection (Falconer, 1963). There are also genes affecting fertility through the loss of eggs or embryos before implantation and to a lesser extent after implantation. These are recessive, i.e. the recessive alleles increase the losses, and the maternal component of inbreeding depression is due to them. They gave rise to the response to selection for low litter size (Falconer, 1963). They contributed only a little to the response to selection for high litter size, and were the main cause of the residual genetic variation when the response ceased. Roughly speaking, each of these genes when homozygous in a female causes a loss of about $1\frac{1}{2}$ mice per litter. Finally there are genes affecting the prenatal viability of the young. These are recessive and give rise to the litter component of the inbreeding depression. Selection for litter size applies family selection to these genes. For this reason it acted more effectively on them than on the genes affecting maternal fertility, and the litter component of inbreeding depression was reduced more than the maternal component in the selected line.

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