

Inbreeding depression and heterosis of litter size in mice

By J. C. BOWMAN* AND D. S. FALCONER†

Institute of Animal Genetics, Edinburgh, 9

(Received 28 October 1959)

1. INTRODUCTION

The objects of the experiment described in this paper were the following:

1. To measure the rate of decline of litter size with inbreeding.
2. To see if the rate of decline was influenced by selection within lines.
3. To see what improvement in litter size could be made by crossing the best inbred lines.

The utilization of heterosis depends on selection applied either during the inbreeding or at the crossing of the inbred lines. In another experiment carried out with the same strain of mice, Roberts (1960) showed that the crossing of lines inbred without selection yielded no improvement in litter size; and that lines crossed at 50% inbreeding varied little in either general or special combining ability, so that selection applied to the crosses gave little promise of improvement. The experiment to be described here explores the possibility of improvement by selection applied to the lines during the inbreeding, first by selection of the best individuals within each line, and second by selection of the best lines judged on their average performance as inbreds.

Selection without inbreeding was applied in another experiment which ran concurrently with the inbreeding experiment described here. The results, of which a preliminary report was given by Falconer (1955), will be described in another paper.

2. PROCEDURE

History of stock used

The strain of mice used for all the experiments on litter size, known in the laboratory as the J-strain, was a heterogeneous one of mixed origin. In its past history there had been little inbreeding that might have eliminated deleterious genes, and it was about as close as one can get with laboratory mice to a 'natural' random-bred population. It had been made from crosses between Bateman's high-lactation line, Goodale's and MacArthur's large selected lines, and four mutant stocks with the C57BL inbred strain in parts of their ancestries. The following mutant genes were originally present: *a*, *a'*, *b*, *bt*, *c*, *c^{ch}*, *Ca*, *d se*, *ln*, *m*, *Re*, *si*. This stock was first used in another experiment (Falconer & Robertson, 1956), which however, resulted in no detectable genetic changes. The 'experimental' and

* Present address: Thornber Bros. Ltd., Mytholmroyd, Halifax, Yorks.

† Agricultural Research Council Unit of Animal Genetics.

'control' lines from that experiment were crossed after each had been maintained through ten generations by eight pairs of parents per generation with minimal inbreeding. Crossbred progeny drawn equally from twenty different matings were used to form the base populations for the inbreeding experiment here described and for the selection without inbreeding. This latter base population was continued without selection to act as a control for both experiments. It was designated JC and was maintained by ten single-pair matings per generation with minimal inbreeding, so that the effective population size was forty and the theoretical rate of inbreeding was 1.25% per generation.

Measurement of litter size

The mice were mated at the age of 6–9 weeks in harems of up to five females with one male. The females were isolated 18 days after mating and were subsequently examined daily for litters. The measure of litter size adopted was the number of live young present when the litter was found. Only first litters were recorded. Females that failed to produce any live young, even though previously noted as being pregnant, were excluded from all estimations of the mean litter size. The inclusion of still-born young or of zero-litters would have introduced an undesirable element of chance variation into the measure of mean litter size because the recording of still-born young depends on the mother not having eaten them before the litter is found, and because many abortive pregnancies would go unnoticed. Mean litter sizes therefore refer only to those females that produced at least one live young within about 6 weeks of mating.

Litter size as a 'character' for genetic analysis is complicated by having two components, one attributable to the fertility of the mother of the litter and the other attributable to the prenatal viability of the young in the litter. Furthermore, it is subject to an inverse maternal effect: mothers who were themselves reared in a large litter tend to have small litters because their body size is smaller (Falconer, 1955). For these reasons we shall not attempt to go much beyond a simple description of the results of this experiment. Further procedural details will be given in association with the results to which they are relevant. Inbreeding coefficients throughout the experiment were computed from the actual pedigrees, by the method of Cruden (1949).

3. INBREEDING PROGRAMME

Method of inbreeding

The inbreeding was started by matings between double first cousins, and continued thereafter by full-sib matings. The purpose of starting by cousin matings was to put in an intermediate step at 12.5% inbreeding, instead of jumping immediately to 25% as with full-sib matings. The inbreeding coefficients in the successive generations are given in Table 1. The matings between the mice of the base population were arranged so as to produce non-inbred progeny in families of which pairs were related as double first cousins. There were ten such pairs of families, and matings between them constituted the first inbreeding and divided the population into ten lines of

independent ancestry. The non-inbred mothers with 12.5% inbred litters were designated generation O.

Throughout the inbreeding programme each line was propagated from a single litter of the previous generation. All the females of the litter were mated to the same male, or if there were more than five females they were mated in two harems to two males of the same litter. The litters born of these matings provided the measure of the litter size of that line in that generation. One litter was reared for the continuation of the line and the rest were discarded. Propagation, and the measurement of litter size, were exclusively by first litters. Lines became extinct when no litter containing one surviving offspring of each sex was available.

Method of selection

At generation O each of the ten lines was divided into two, one to form the unselected series of lines, designated JU, and the other to form the selected series, JS. Thus each line of the selected series was related to one of the unselected series. Each line of the selected series was propagated always from the largest of the litters born in the previous generation of that line. The litters from which the unselected series of lines were propagated were not taken strictly at random, but were rather those of intermediate size and containing the highest proportion of females. This was done in order to reduce the chance effects of random sampling, and to provide as many litters in the next generation as was possible without any positive selection. The comparison of the selected with the unselected series of lines provided the test of the efficacy of artificial selection applied within the lines. Selection between lines was entirely 'natural': lines of both series became extinct, as already explained, as soon as they failed to produce a sib-pair for their propagation.

Results

The results of the first twelve generations of inbreeding are given in Table 1, and the first four generations are shown graphically in Fig. 1. The mean litter sizes given in Table 1 are the means of all litters irrespective of their line; but those plotted in Fig. 1 are the unweighted means of line-means. The means of the selected series were a little above those of the unselected series, but the difference is quite insignificant, and the selection quite failed to delay the loss of lines; indeed, the lines became extinct sooner than those in the unselected series. The selection differentials, given in Table 1, were however rather small, the selected litters exceeding the average by only about $1\frac{1}{2}$ mice. This amount of selection, though it was the most that could be achieved with the procedure adopted, could hardly be expected to show any effect. The failure of selection within lines to reduce the rate of decline is therefore to be attributed to the low intensity of selection and is not necessarily proof that the selection itself was ineffective.

The decline of litter size depicted in Fig. 1 is very regular and is linear with respect to the inbreeding coefficient. The rate of decline is 0.56 young per 10% increase of inbreeding. The comparable figure found by Roberts (1960) was 0.49 young. If the

Table 1. *Inbreeding coefficients (F) and mean litter sizes in the first twelve generations of inbreeding. The means are of all litters, irrespective of lines*

Gener- ation	F (%) of litters	Unselected Lines				Selected Lines				Selection differ- ential	
		No. of lines surviving	No. of ♀ mated	No. of litters	Litter size Mean s.e.	No. of lines surviving	No. of ♀ mated	No. of litters	Litter size Mean s.e.		
Base	0	—	30	30	7.77	0.48	—	—	—	—	—
0	12.5	10	45	43	6.88	0.43	—	—	—	—	1.88
1	31.3	10	29	28	6.32	0.55	10	37	33	5.58	0.46
2	43.8	10	29	27	5.04	0.51	10	34	34	5.68	0.41
3	54.7	9	19	18	5.00	0.57	10	35	28	5.32	0.46
4	63.3	6	16	12	4.58	0.60	4	17	8	4.38	0.60
5	70.3	4	11	8	5.89	0.99	1	1	0	—	—
6	76.0	3	10	8	6.63	0.80	0	—	—	—	—
7	80.6	3	19	14	7.71	0.54					
8	84.3	3	16	13	6.08	0.45					
9	87.3	3	23	19	5.21	0.56					
10	89.7	3	27	21	7.29	0.54					
11	91.7	2	28	11	5.18	0.77					
12	93.5	1	20	19	7.58	0.60					

linear decline in the present experiment had continued to higher levels of inbreeding, the litter size would have been reduced to two at 100% inbreeding. After generation 4, however, the mean litter size increased because the worst lines had become extinct.

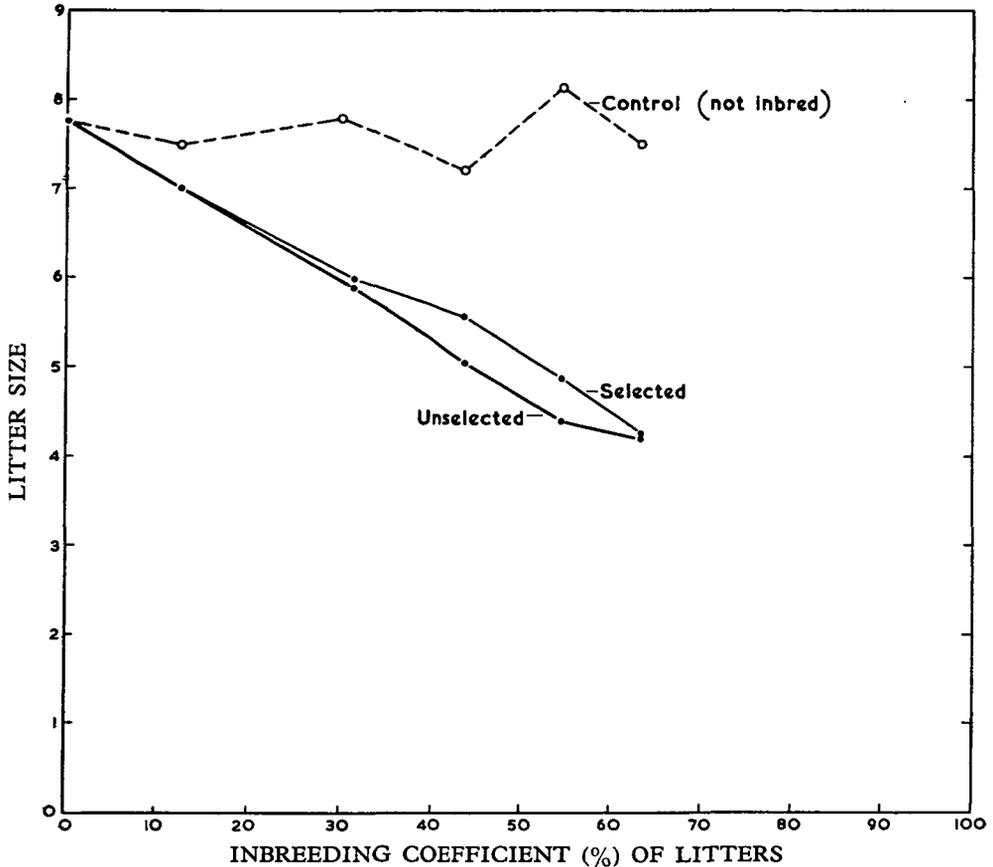


Fig. 1. Mean litter size plotted against the inbreeding coefficient of the litters. The points are the means of line-means.

Out of the whole twenty lines, one became extinct in generation 3 and all but one were extinct by generation 12. The distribution of losses was as follows:

Generation No.	2	3	4	5	6 . . . 10	11	12
Total no. of lines extinct	0	1	10	15	17	17	18

Most of the lines were lost at generations 4–5, i.e. when the inbreeding coefficient reached 60–70%. Three lines, however, survived to 90% inbreeding and one survived permanently. It is of interest to inquire into the individual histories of these three longest-surviving lines: did they start at high levels and decline like the others? Or, did they suffer less decline than the others? The individual histories, shown in Fig. 2, prove the latter: these three lines started below the average and suffered no decline up to about 80% inbreeding at generation 7 or 8. Then two of

them dropped and were lost at generations 10 and 11. The remaining line, which survived permanently, is shown in Fig. 2 up to generation 20, when the inbreeding coefficient was 98.8%, and its mean litter size was then fully equal to that of the non-inbred control.

In preparation for the crossing programme to be described below, the three surviving lines were expanded at generation 6 by the use of two litters for their continuation instead of one. All the litters of generation 7 were used for the crossing or the continuation of the lines, and thereafter all available litters (except unisexual ones) were used for the continuation. This expansion did not, however, prevent the

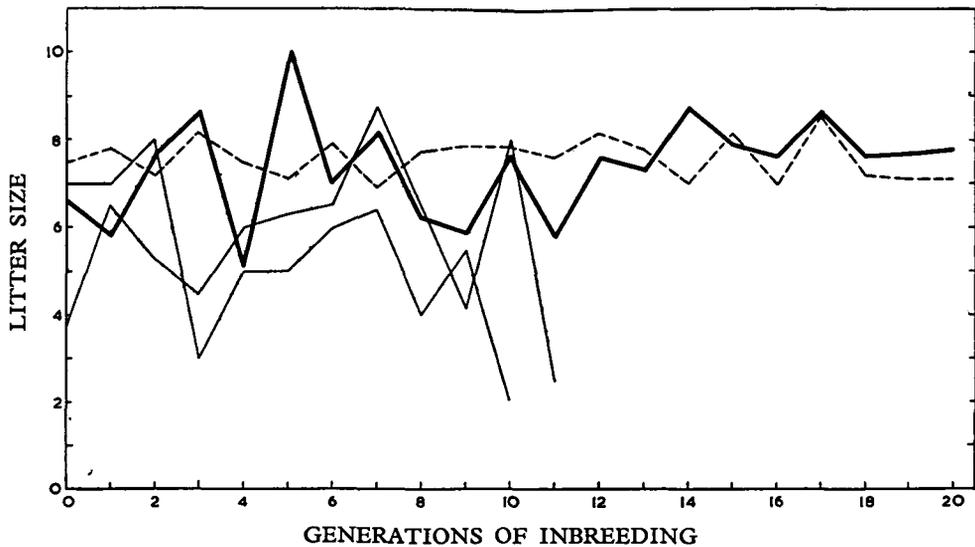


Fig. 2. Individual histories of the three lines that survived longest. The dotted line refers to the unselected, non-inbred control. The line means in the early generations are based on very few litters. The means of the permanently surviving line are based on about twenty litters in generations 12-16 and on about forty in generations 17-20.

loss of two of the lines, but the permanent survival of the remaining line was probably helped by the selection between sublines which was applied from generation 12 onwards, when the maintenance of the line became a matter of stock-keeping rather than of experiment.

4. CROSSING PROGRAMME

The crossing programme consisted of crosses between the best inbred lines followed by renewed inbreeding and crossing. Three such cycles of inbreeding followed by crossing were carried out. The procedure will be most easily understood if described step by step along with the results.

The results are given in Table 2 and the mean litter sizes are shown graphically in Fig. 3. This figure is divided into sections corresponding to the successive cycles of inbreeding and crossing. The horizontal axis in each section is marked out in

Table 2. *Inbreeding coefficients and mean litter sizes in the crossing programme*

Generation*	Inbreeding coefficient (%)		No. of litters	Litter size	
	Mothers	Litters		Mean	S.E.
8	80.6	84.3	13	6.08	0.446
X ₁	80.6	0	17	7.53	0.550
0	0	20.0	40	9.58	0.365
1	20.0	35.0	93	8.33	0.278
2	35.0	47.5	75	7.28	0.308
3	47.5	57.5	68	6.90	0.325
4	57.5	65.6	47	6.89	0.363
X ₂	65.6	25.7	59	7.80	0.315
0	25.7	26.5	57	7.90	0.283
1	26.5	37.8	54	7.15	0.268
2	37.8	50.8	68	8.47	0.233
3	50.8	59.7	54	7.37	0.324
4	59.7	67.7	35	6.00	0.467
X ₃	67.7	38.2	40	6.83	0.308
0	38.2	38.3	106	7.32	0.210

* Explanation of generation numbers:

X₁, X₂, X₃: crosses between lines after first, second and third cycles of inbreeding.

0: second generations of crosses, with minimal inbreeding of mothers and litters.

1, 2, 3, 4: first, second, etc. generations of inbreeding.

coefficients of inbreeding, and the generation means are plotted against the coefficient of inbreeding of the litters. The coefficient of inbreeding of the mothers is thus represented throughout by the previous point in the graph. The left-hand

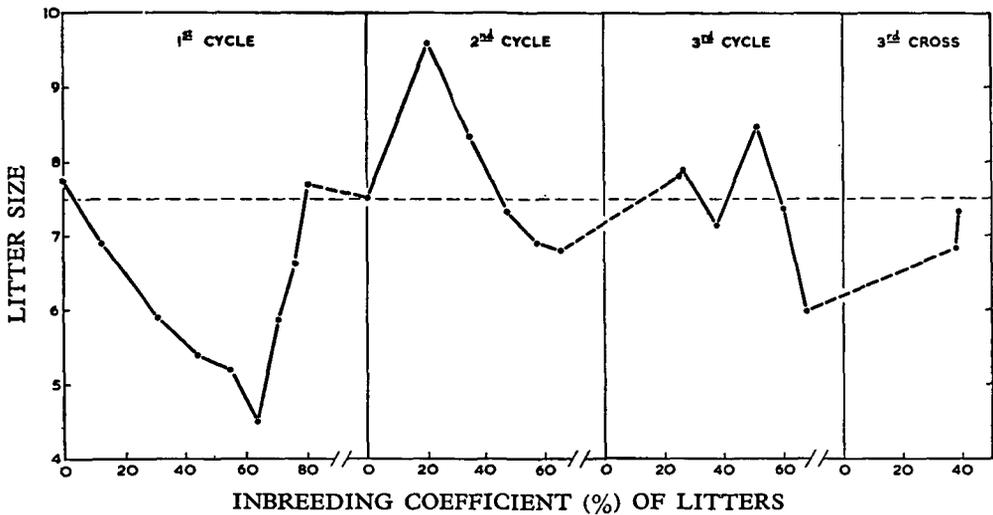


Fig. 3. Mean litter size in successive generations of the crossing programme, plotted against the coefficient of inbreeding of the litters. The horizontal broken line is the mean of the non-inbred control over the same period.

section of the figure represents the first cycle of inbreeding described in the preceding section. The litter sizes plotted here are the means of all litters in both the selected and unselected series. The litter size declined regularly, as we have already seen, up to 63% inbreeding. Then it rose again steeply as the worst lines became extinct till, at 81% inbreeding when only three lines remained, it was equal to the non-inbred control. At this point the three lines were crossed in all ways including reciprocals. The mothers of these crossbred litters were 81% inbred, and the litters themselves were zero-inbred. The point representing the cross on the graph is therefore shown against 0% inbreeding in the second cycle. There was no improvement of litter size. The crossbred progeny were then mated with others from a different cross, so as to produce litters with minimal inbreeding. The inbreeding coefficient of these litters did not, however, remain at zero, but increased to 20% because with only three lines in the cross, the mated pairs of F_1 's had always one parental line in common. The next point on the graph, then, refers to 20% inbred litters with 0% inbred mothers. There was now a marked improvement, the mean litter size increasing to 9.6. Thus inbreeding followed by crossing the best lines led to an improvement of two mice per litter in a space of ten generations. Selection without inbreeding, as will be shown in another paper, took twice as long to achieve the same improvement.

The next step was to subject the crossbred population to a second cycle of inbreeding and crossing to see if this would lead to further improvement. Twenty litters were kept, and inbreeding by full-sib mating was continued for four generations. The mean litter size among these twenty lines declined at almost exactly the same rate as in the first cycle of inbreeding. But since the lines started at a higher level they did not fall so low, and only three out of the twenty were lost. After four generations, when the litters were 66% inbred, the four best lines were selected on the basis of their performance as inbreds, and these four lines were crossed. The inbreeding coefficient of the crossbred litters was not zero as before, but 26%. This was the result of the initial restriction of the population to three lines in the first cross. The F_1 litters were again crossed to members of different F_1 's so that both mothers and litters would be minimally inbred; and, because this time there were four instead of three lines, the inbreeding coefficient of the litters was very little more than that of the mothers. The mean litter sizes of these crosses are shown by the first two points, close together, at about 25% inbreeding in the third cycle. It will be seen that there was some improvement from the heterosis in the litters, but no more in the next generation from the heterosis in the mothers. This second cycle of inbreeding and crossing brought the litter size to a little above the control level, but the level attained by the first cycle was not regained.

The third cycle of inbreeding was carried out in the same manner as the second, except that the first generation was not sib-mated. Matings with least relationship were made, with the intention of producing a second minimally inbred generation, but because of the previous restriction to four lines, the inbreeding coefficient increased from 26.5% to 37.8%. Three generations of full-sib mating followed. The mean litter sizes in the third cycle were rather erratic, but the average decline was at about the same rate as before, and by the time the litters were 68% inbred the

mean litter size had dropped to six. The four best lines were again selected and crossed. This time, because of previous restrictions of the population size, the inbreeding coefficient of the crossbred litters was nearly 40%. There was again some improvement from the heterosis in the litters. The crossbred mice were mated to others from a different cross and some further improvement in litter size was realized (this is the last point shown on the graph), but, even so, the litter size was still below the control level. The experiment was terminated at this point.

As a means for the improvement of litter size, the second and third cycles of inbreeding and crossing were conspicuously unsuccessful. There are two reasons for this. First, the restriction of the population to three lines in the first cycle and to four in the second and third cycles made it impossible to return to zero inbreeding in the second and third crosses, so that the full potentialities of heterosis could not be realized. The second reason, which has not yet been mentioned, concerns the selection between the lines. In the first cycle natural selection had eliminated all but three of the lines and there was no doubt that these were the best lines and that they were substantially above the others in performance. In the second and third cycles, however, where the selection was artificial and not natural, it was difficult to decide which were the best lines. The line means, generation by generation, were very irregular, and no lines stood out as being very clearly superior to the rest. For this reason the efficacy of the selection, on which any improvement must rest, is open to question, and the results indicate its ineffectiveness. If the graph is examined it will be seen that the mean litter size at corresponding levels of inbreeding is the same in the second and third cycles and the third cross which represents the beginning of a fourth cycle of inbreeding. For example, the litter size is about equal to the control level at about 40% inbreeding in the three cycles. Thus it appears that selection was effective only in the first cycle and that the improvement gained could not be maintained because the low inbreeding coefficient of the first cross could not be repeated.

5. COMPONENTS OF VARIANCE

The variance of litter size was analysed into two components, within and between groups of full-sister females. It will be remembered that during the inbreeding all the females of one litter were mated to one male (or occasionally to two males) and that this group of females represented the whole of one line in one generation. The analyses of variance were made on the sizes of litters produced by these groups of females. In the inbred generations the within-group component refers to the variance within lines, and the between-group component to the variance between lines. But the between-line variance is not purely genetic because it contains also the variance arising from maternal effects since all the litters in a line were produced by mothers who were litter-mates. In the crossbred generations the components are difficult to interpret because of the dual nature of the character, and there would be little gained from a detailed discussion of their content. A more meaningful analysis of the crossbred generations will be presented later.

The estimates of the two components of variance throughout the experiment are

depicted in Fig. 4. The within-group component remained substantially the same and was not influenced in a regular manner either by the inbreeding or by the crossing. The between-group component apparently started at a level considerably above the control and it declined during the first cycle of inbreeding. During the second and third cycles of inbreeding it may have increased a little but it did not rise much above the control level. The small amount of variance differentiating the lines accounts for the difficulty found in applying selection, which was mentioned at the end of the last section.

Neither of the components of variance described above behaved altogether as they might have been expected to. The genetic variance within lines might be expected to increase during the first stages of inbreeding from the effects of rare recessive genes (Robertson, 1952), but it should decline later. The environmental

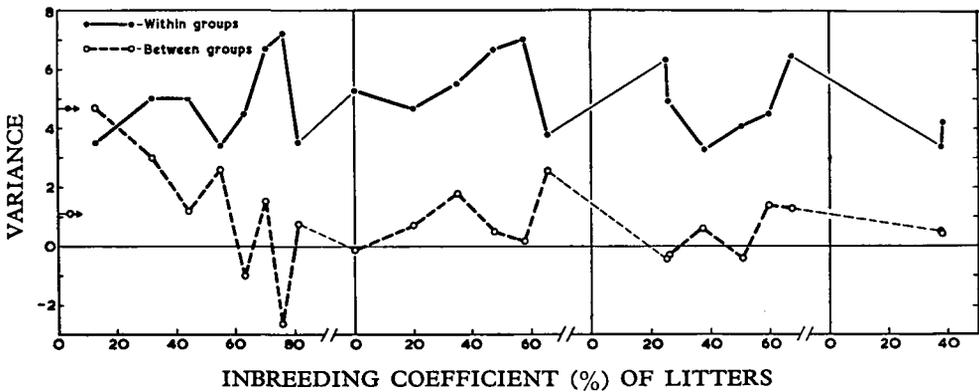


Fig. 4. Components of variance of litter size within and between groups of full sisters. The arrows on the left mark the components in the non-inbred control over the same period.

variance within lines might be expected to increase with inbreeding. The approximate constancy of the observed within-line variance may have been the resultant of these two more or less opposing underlying changes. The variance between lines should on theoretical grounds have increased with inbreeding. Its observed decline in the first cycle may have been due in part to a spuriously high initial level, but was probably due more to the elimination of the lines as inbreeding proceeded. The reliability of the estimates does not justify further comment on the changes of variance. It might, however, be pointed out that the sampling errors of the estimates of the two components are negatively correlated. If the within-group component is erroneously high, the between-group component will appear correspondingly too low. This accounts for much of the irregularity of the between-group estimates.

The variances of the crossbred generations were analysed in a different way, in order to see what evidence there was of general or specific combining ability of the lines crossed. The first cross was not analysed because the numbers were too small. The mean squares from the analyses of the second and third crosses are given in Table 3. The analyses separate three mean squares in addition to the error mean

square. The first and second, attributable to lines used as female parents and lines used as male parents, refer to general combining ability, and the third, attributable to interaction between lines, refers to special combining ability. The results are somewhat ambiguous. Each of the three mean squares referring to combining

Table 3. *Analyses of variance of the crosses*

	Parents inbred, litters crossbred		Parents crossbred, litters crossbred	
	d.f.	Mean square	d.f.	Mean square
<i>2nd cycle</i>				
♀ lines	3	12.45*	3	2.80
♂ lines	3	5.06	3	4.84
Interaction	5	5.68	5	10.44†
Error	47	5.50	45	4.01
<i>3rd cycle</i>				
♀ lines	3	6.93	3	2.40
♂ lines	3	12.26†	3	7.67
Interaction	5	0	5	3.00
Error	27	3.58	94	4.74

* $P < 0.10$. † $P < 0.05$

ability is significant in one of the four analyses but not in the others. These results give general support to the conclusions of Roberts (1960) in showing little evidence of differences in combining ability between the inbred lines.

6. CONCLUSIONS

There are two chief points of interest in the conclusions that can be drawn from this experiment. The first concerns the genetic situation underlying the inbreeding depression and heterosis of litter size, and the second concerns the utilization of heterosis in farm animals.

The decline of the mean value on inbreeding proves that there must be directional dominance of the loci concerned, but it does not discriminate between simple dominance and over-dominance. The behaviour of the individual lines, however, proves that in this case over-dominance cannot have been of paramount importance. If much of the original variation had been due to over-dominant loci it would hardly have been possible to find three lines that showed no inbreeding depression up to 80% inbreeding, and one that showed none up to 99% inbreeding. The behaviour of these lines is adequately accounted for by the hypothesis of simple dominance. Favourable dominant alleles, one may suppose, were fixed, or brought to high frequencies, at many loci and unfavourable recessives at a few loci. On balance there was no change of mean, but there was heterosis on crossing because the recessive alleles in each line were different. The decline of the mean observed in the second cycle of inbreeding must then be attributed principally to these loci that gave rise to the heterosis. That the decline was not slower than that of the first cycle is

understandable because the gene frequencies at these loci must all have been about one-third, whereas in the original base population the gene frequencies may well have been much lower, and the contribution of any locus to the inbreeding depression is dependent on its gene frequency.

The conclusions about the utilization of heterosis by cyclical inbreeding and crossing are not encouraging, if the experiment is regarded as an indication of what may be expected to happen with farm animals. The similarity between litter size in pigs and litter size in mice is probably very close, both genetically and physiologically, and the experiment seems to be very relevant to the improvement of productivity in pigs. Selection within lines during the inbreeding does not seem likely to be able to reduce the rate of decline of performance. This is in agreement with previous data from pigs, reported by Dickerson (1952). On the other hand, selection between lines on the basis of their performance as inbreds does encourage the hope that some useful heterosis may be gained by the crossing of the best lines. Since, however, crosses between the less-good inbreds were not tested we cannot exclude the possibility that these might have yielded better hybrids than the crosses between the best inbreds, as was found by Bell, Moore & Warren (1955) with egg-laying in *Drosophila*. With farm animals the maintenance of poor inbreds is impracticable, and if the lines are to be perpetuated for the continuous production of crossbreds, the selection would have to be made out of a very large number of lines if some are to be found that retain a high enough performance as inbreds for their maintenance to be practicable. There does not seem to be much hope of making further gains from the cyclical repetition of inbreeding and crossing, because of the difficulty of returning to a low level of inbreeding in the second and subsequent crosses. Each selection among the lines for crossing reduces the population size and so puts the population through a 'bottle-neck' from which it can never subsequently recover. Thus if the full amount of heterosis is to be achieved in a later cross, all the previous crosses would have to be made among a large number of selected lines. Success in a programme of cyclical inbreeding and crossing would require operations on a very large scale.

SUMMARY

1. A random bred population of mice was subjected to inbreeding and the changes of litter size, measured as the number of live young in first litters, were followed.

2. The mean litter size declined at a rate of 0.56 young per 10% increase of the inbreeding coefficient.

3. Selection for large litters within the lines during the inbreeding did not effectively reduce the rate of decline.

4. Out of twenty lines at the beginning of the inbreeding seventeen were lost by the time the inbreeding coefficient reached 76%. Two more were lost later and one survived indefinitely. The three lines that survived longest started at a level below the mean and did not decline in litter size. The one that survived indefinitely reached 99% inbreeding without dropping below the non-inbred control.

5. The three lines surviving at 81% inbreeding were crossed and the litters produced by the crossbred progeny were larger than the non-inbred control by about two young per litter. This gain from heterosis is attributable to selection among the lines on their performance as inbreds. A second and third cycle of inbreeding and crossing yielded no further progress, and the level of the first cross was never regained. This is attributable to the ineffectiveness of the selection applied and to the previous restrictions of the population size.

6. The behaviour of the lines in the inbreeding and crossing point to simple dominance rather than over-dominance at the loci causing variation of litter size.

7. This experiment suggests that, as a means of improvement of farm animals, cyclical inbreeding and crossing does not look very hopeful.

REFERENCES

- BELL, A. E., MOORE, C. H. & WARREN, D. C. (1955). The evaluation of new methods for the improvement of quantitative characteristics. *Cold Spr. Harb. Symp. quant. Biol.* **20**, 197-211.
- CRUDEN, D. (1949). The computation of inbreeding coefficients in closed populations. *J. Hered.* **40**, 248-251.
- DICKERSON, G. E. (1952). Inbred lines for heterosis tests? *Heterosis* (ed. J. W. Gowen). Iowa. Pp. 330-351.
- FALCONER, D. S. (1955). Patterns of response in selection experiments with mice. *Cold Spr. Harb. Symp. quant. Biol.* **20**, 178-196.
- FALCONER, D. S. & ROBERTSON, A. (1956). Selection for environmental variability of body size in mice. *Z. indukt. Abstamm.- u. VererbLehre*, **87**, 385-391.
- ROBERTS, R. C. (1960). The effects on litter size of crossing lines of mice inbred without selection. *Genet. Res.*, **1**, 239-252.
- ROBERTSON, A. (1952). The effect of inbreeding on the variation due to recessive genes. *Genetics*, **37**, 189-207.