The limits to artificial selection for body weight in the mouse

III. SELECTION FROM CROSSES BETWEEN PREVIOUSLY SELECTED LINES

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1. INTRODUCTION

Previous papers in this series (Roberts, 1966*a*, *b*) examined the limits to artificial selection for body weight in the mouse, and the genetic nature of those limits. It was found that, in a line selected for large size, the additive genetic variance had, effectively, been exhausted through the fixation of alleles contributing to large size. A line selected for small size, on the other hand, displayed a surprising amount of residual genetic variance at the limit, and it responded readily to reversed selection. However, this reversed response, as it tailed off, fell well short of the initial level of the base population, which established that the small line also had undergone a considerable amount of fixation, as indeed would be expected. Some evidence was adduced that these results may be representative of five other selected lines, three large and two small, that had been developed in this laboratory. This leaves open the question whether different lines selected in the same direction are fixed for the same alleles at the various loci affecting body weight. If they are not, then crosses between such lines ought to contain some genetic variance, and a response to further selection from the crosses may be expected. The limit to this second cycle of selection will depend on the extent of genetic differentiation between the lines at their original limits.

A precedent for this approach, with encouraging results, is reported by Falconer & King (1953). They obtained samples of two strains of mice selected for high 60-day weight, one by Goodale (1938, 1941) and one by MacArthur (1944, 1949). By the time the samples of these strains were procured, both had apparently reached a limit to selection, corresponding to a 6-week weight of about 29 g. in each case. Falconer & King noted that whereas Goodale's strain was large-bodied and not very fat, MacArthur's strain was smaller in linear dimensions but was very fat. From this observation, Falconer & King argued that a cross between them should provide new genetic variance upon which continued selection could act. This expectation was realized in practice, and over the nine generations of further selection which they reported, the mean weight rose by almost 3 g. to 32 g.

The work reported in this paper is an extension of Falconer & King's approach. The intention was to examine in more detail the potentiality of crossing selected lines to provide material for further selection, and to determine by how much the original limit to selection might be transcended.

2. MATERIALS AND METHODS

The experimental work described here stems from two base populations that were constructed from lines of mice that had been selected to the limit either for high or for low 6-week weight. The first population derived from four lines selected for high 6-week weight, and the second from three lines selected for low 6-week weight. A description of these seven original lines, and a report of the limits to selection which they had reached, is given in the first paper of this series (Roberts, 1966a).

The two base populations for the present studies were constructed as follows. As the scheme differed somewhat for the two, they are described separately.

Combining the four large lines presented no problem. They were first completely intermated according to a 4 by 4 diallel scheme, the 'pure lines' being included for comparison with the crosses. With the one exception noted below, the 'pure lines' were then discarded and a sample of each cross was mated to its complement, i.e. to a cross between the other two lines. Reciprocal crosses were included in all possible combinations. Each individual progeny of this generation thus had each of the four original large lines represented in its ancestry in equal proportions. This means that the gene frequencies at segregating loci had values of 0·25, 0·50 or 0·75. From the 120 matings that had been set up, fifteen fertile ones were chosen at random to provide a litter for continuing the stock, the random choice being disturbed only to ensure that different maternal combinations (and by reciprocity, the paternal ones) were represented as equally as possible. The fifteen litters so selected were designated the zero generation of the LX stock (L for 'large', and X for 'crosses').

Combining the three small lines was slightly more cumbersome; it is a consequence of diploidy that it is easier to combine four strains equally than three. The first step was exactly as before, and two-line crosses were extracted from a 3 by 3 diallel. Again, one 'pure line' was continued, while the other two were discarded. A random sample of each cross was then mated to a cross involving the third line, in all possible combinations. This, however, meant that mated animals shared one parental line in common. In the next generation, matings were between three-line cross animals, with the restriction that the common parental line should differ in the two mates. The progeny of this generation therefore had the three original lines represented in their ancestry in the proportions of 3:3:2, as far as an individual progeny was concerned. But as the crossing had been done comprehensively and schematically, in the population as a whole the three original lines were represented equally. Gene frequencies at segregating loci were thus either 0·33 or 0·67. From the seventy-two matings that had been set up, fifteen were chosen to provide the litters that

were designated the zero generation of the second base population constructed, SX (S for 'small', and X for 'crosses'). The choice of litters was at random from within subgroups, care being taken to maintain the equal representation of the original three small lines.

Having thus constructed the two base populations, they were thenceforth treated similarly. From the zero generations, the LX line was selected for high 6-week weight whereas the SX line was selected for low 6-week weight. Each line was maintained on fifteen pair matings, and the within-family method of selection was practised in both cases.
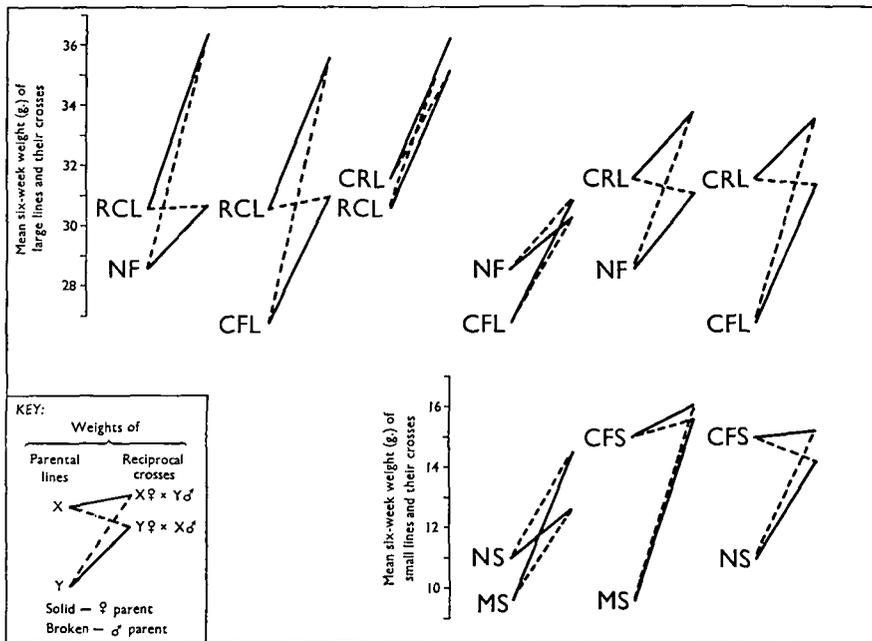

Fig. 1. Heterosis shown in crosses between selected lines of mice. Strain designations as given by Roberts (1966a).

For purposes of comparison with lines selected from crossbred material, one large line (CL) and one small line (CS) were maintained. These are reproduced in some of the figures with little further comment; they have been described fully in an earlier paper (Roberts, 1966b).

3. RESULTS

(i) *Differentiation between selected lines*

The detailed results of the line-crossing undertaken to form the base populations are not of great relevance in the present context. The most pertinent feature concerns the first stage of the crossing, and the results are summarized in Fig. 1. This shows the mean weights of the two-line crosses, the reciprocals being shown separately, compared with their 'pure-line' contemporaries from the two diallels. The

designations of the lines are those given by Roberts (1966 a). For the large lines and their crosses, each point represents the mean of (usually) some twenty to fifty individuals, and have standard errors of somewhere between one-half and three-quarters of a gramme, depending on the number. The means for the small lines and crosses, by virtue of the lower variance of small mice, have standard errors about half as great. The important point for the present is that *all* crosses displayed considerable heterosis in body weight, at least one (and usually both) of the reciprocals exceeding the better parental line. The fact that all crosses did this means that all of the lines crossed were genetically differentiated to some degree with respect to body weight, for heterosis can result only from dominance or epistatic relationships between differing alleles. The increase in weight on crossing, even among the small lines, confirms the well-known fact that directional dominance favours a higher body weight in the mouse. In other words, genes for low body weight tend to be recessive.

The higher mean weight obtained when the lines were first crossed was not increased any more by further crossing, as can be seen from the summary of mean weights at different stages of the crossing shown in Table 1. However, some increases may have been obscured because the fertility of the two-line crosses (also shown in Table 1) was much higher than that of the parental lines, especially in the case of

Table 1 *Mean body weights and litter sizes of crosses between selected strains*

Stage of crossing (see text)	LX population (from large lines)		SX population (from small lines)	
	Litter size	6-week weight	Litter size	6-week weight
2-line cross	7·03	32·75	4·36	14·76
3 or 4-line cross	10·59	32·04	5·31	15·14
Further cross	—	—	5·56	14·66

the large mice. Although I have argued earlier in this series of papers (Roberts, 1966 b) against the adjustment of generation mean weights for litter size differences, it is possible that an increase of 50% in fertility (as in the large mice) should not be ignored; it may have depressed the mean weight by, perhaps, 2 g.

During the formation of the base populations, there was therefore *a priori* evidence that new genetic variance would be available, because the selected lines that were employed for the crossing were differentiated genetically at loci contributing to variance in body weight. Furthermore, it appeared subjectively that this differentiation was widespread and pronounced. Even the two closely related large lines, *CRL* and *CFL*, drawn initially from the same source, showed the usual amount of heterosis on crossing. However, *CRL* was originally selected on a restricted diet, while *CFL* was selected on a full diet (Falconer, 1960), and as shown by Falconer, the genetic correlation between growth on the two planes, taking the average of four estimates, is only about 0·5.

The crossbred populations were formed to provide bases for further selection for large or small size, as appropriate. The results from these two operations are given separately.

(ii) *Further selection for large size*

From the zero generation, the *LX* line was selected for a further eighteen generations for high 6-week weight, after which it became extinct through infertility. The cause of the infertility appeared to be excessive fatness in females, few of whom ever gave birth to a second litter in the later stages of the experiment, and many of whom failed to produce even one litter. Males, on the other hand, when mated to females of more normal body size, were fertile for at least a few months. The trouble in the *LX* line arose when mating had to be delayed until sufficient animals reached 6 weeks of age, by which time the older females were 8 to 10 weeks old and were already too fat to breed. A later derivative of *LX*, which is not described further in this paper, was mated at 5 weeks of age, which did not permit an excessive accumulation of fat before mating. The early mating overcame the fertility problems in the line completely.

Before it became extinct, the *LX* line as a result of the selection reached a mean weight of 40 g. over its last six generations, and represents a considerable improvement over the original lines at their limits. Its progress is summarized in Fig. 2. The weights of the largest of the original lines (*CL*), over approximately the same period, are also shown in Fig. 2 for comparison. *CL* had reached a limit at 32 g., and the *LX* line eventually yielded an increase of 25% over this limit. Even compared to a later increase in the *CL* line, most likely due to a recombinational event (Roberts, 1966*b*), the *LX* line still shows a substantial improvement which must be attributed to the infusion of genes from the other selected large lines. In empirical terms, the *LX* line indicates clearly that crosses between the original lines at their limits yielded sufficient genetic variance for an appreciable further advance under selection.

The details of the response, however, are less clear. Some 18 months after the base population had been formed, it was by no means obvious from the 6-week weights of the 6th generation that any progress had been made. A promisingly high weight at the 4th generation had vanished as mysteriously as it had appeared. But after the 6th generation, there was a good response until a steady phase was reached by about the 13th generation. Though a linear fit would probably be an adequate description of the response retrospectively, a very different impression was formed as the data were collected. It seemed as if the response could be divided into three phases—an initial lag, a rapid response period, and a final limit. If this is so, then it is not at all typical of the asymptotic response curve classically expected of a selection programme. The initial lag differs also from what Mather & Harrison (1949) called 'delayed responses', which occurred after long periods of stability under selection.

If the suggested sigmoid shape of the response curve is real, one factor which

could explain it is linkage. If alleles that differed between lines were at loci that were linked, they would of course appear predominantly in the repulsion phase during the early generations, and progress under selection would depend on a sufficient number of cross-overs becoming available. If the postulated linkage were tight, this process would take a little time, though some progress would be expected from the start. Somewhat fortuitously, a partial check of the linkage hypothesis
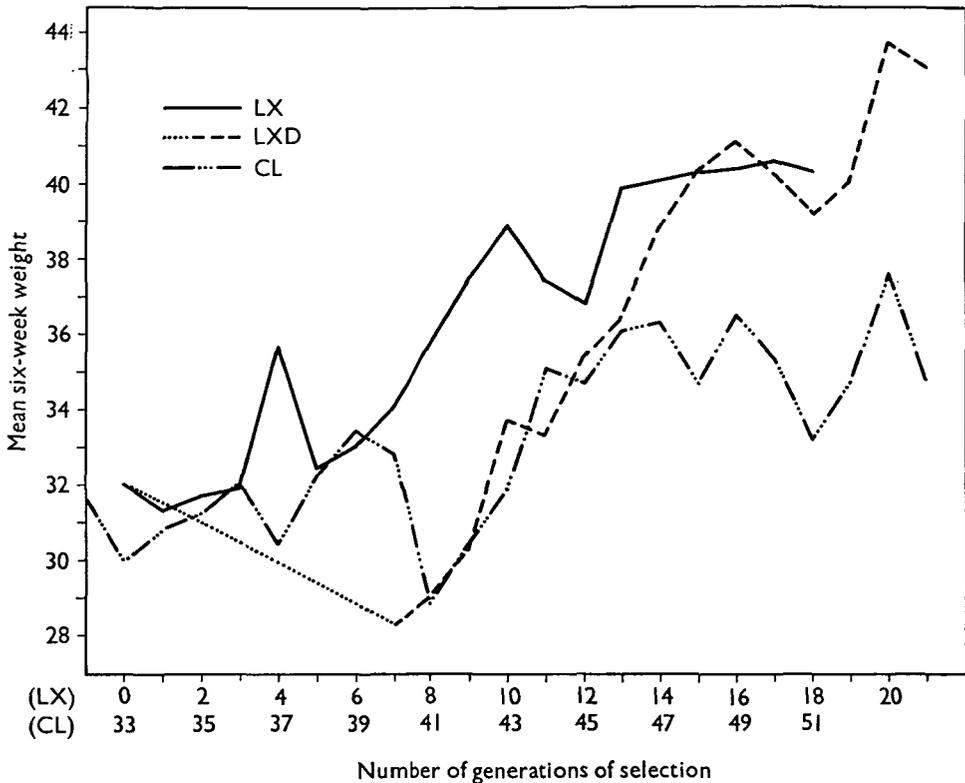

Fig. 2. Response to selection of *LX* and *LXD* lines. *CL* line shown for comparison.

was available when, by the 10th generation, the results suggested some such phenomenon. Thirty-five surplus litters from the LX_0 generation had been acquired by Dr Joyce Bloom for lung-tumor studies, described by Bloom (1964) and Falconer & Bloom (1962, 1964). From these litters, a control stock had been formed, which in the meantime had undergone six generations of random mating. Dr Bloom kindly allowed me to recover fifteen pairs of mice from different litters of her control stock, and these animals were mated appropriately to give a 7th generation of random mating. This formed a base population from which a second line, *LXD* (*D* for 'duplicate'), was selected for high 6-week weight. The mean weight of the base population of the *LXD* line is marked opposite the 7th generation of *LX* in Fig. 2. It can be seen that the random mating, or relaxed selection, had resulted in a drop

of about 4 g. since the zero generation. This, however, for present purposes, is inconsequential. The hypothesis to be tested was that, if linkage had impeded initial progress in the *LX* line, then the random mating ought to have allowed such linkage to break up, and that therefore the *LXD* line ought to give an immediate response when selection was applied to it.

The results, summarized also in Fig. 2, are easily compatible with this hypothesis. The response was indeed immediate, and despite its lower starting point, the *LXD* caught up with *LX* after eight generations of further selection. The relative rates of responses are seen more clearly in Fig. 3, which shows the generation means plotted against cumulated selection differentials. Over the period of the response, the
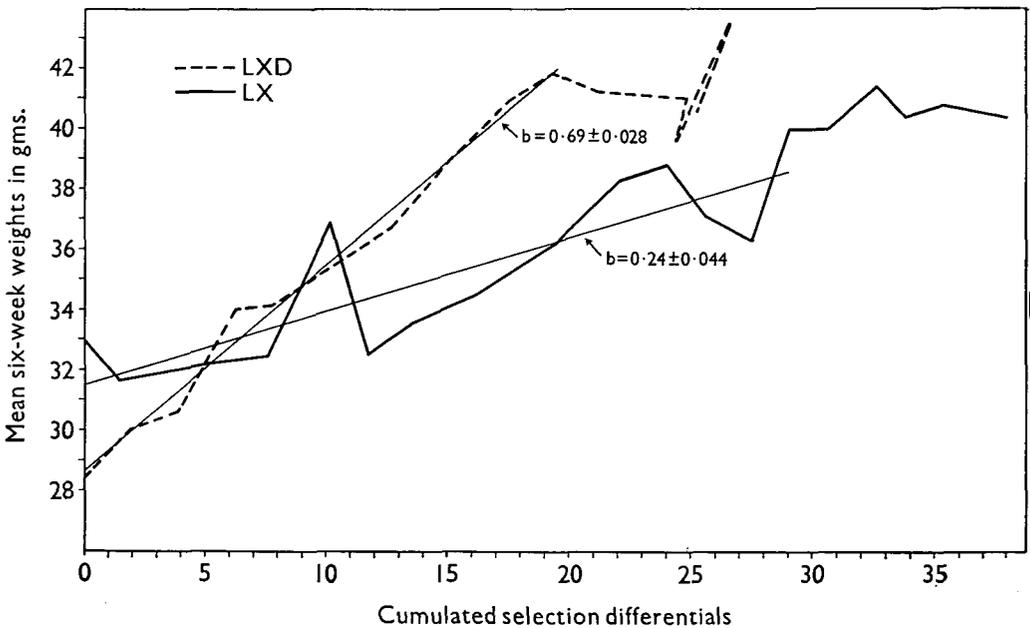

Fig. 3. Realized heritabilities in *LX* and *LXD* lines.

regression of generation means on cumulated selection differential, measuring the realized heritability, is much greater in *LXD* than in *LX*, the difference being significant beyond the 0·1% level (see Fig. 3). While this does not conclusively establish the linkage of genes affecting body weight in this population, no other explanation satisfies the facts with anything like the same facility.

If linkage did impede progress initially, then it is also possible that segments of chromosomes might have been fixed in the *LX* population while they were still in the repulsion phase, *i.e.* that some less favourable alleles, among those initially available, might have been fixed. This might be especially true of favourable alleles that had an initial frequency of 0·25, or of alleles linked to another with a greater effect on the character. If such were the case, then selection following a period of random mating might be expected to yield a further total advance under selection

than when the selection was applied from the start. The last two points of LXD are considerably higher than the final level of LX, suggesting that it had been advantageous to allow linkage to break up before selection was applied. However, these two points are based on the means of animals drawn from only seven and four litters, respectively, so that not a great deal of reliance can be placed on them. The LXD line (like LX before it) was approaching extinction through infertility by this time. Though tantalizingly suggestive, the results are therefore inconclusive on the question whether selection from crosses should be preceded by a period of random mating. In terms of applications to animal breeding, this is an important question which would merit further experimental investigation. For unless a greater advance is ultimately obtained, it is obviously inadvisable to delay the response by deliberately avoiding selection. An additional reason why selection should not be delayed for too long is that the LXD line regressed during the period of random mating. Though it obviously had not happened in this case, this could have meant that some alleles, or combinations of alleles, favouring large size might eventually have been eliminated from the population by natural selection acting against them.

The conclusions from this section are therefore that the original four large lines, at the limit, each lacked some genes contributing to large body size that were contained in one or more of the other three lines. It is also suggested strongly that, when the original lines were crossed, favourable alleles from different lines were put in the repulsion phase of linkage, and that this impeded the initial rate of advance if not the final limit.

(iii) *Further selection for small size*

The population, SX, formed by crossing three small lines at their limits, was subjected to continued selection for low 6-week weight. The results of this selection are summarized in Fig. 4. For comparison, the weights over the period of study of the CS line, the largest of the original small strains, are also shown in the figure.

For a long time, certainly up to generation 15, there was little if any evidence that the SX line had responded to selection at all. Since then, it has become more apparent that some progress has been made, though much of this impression stems from the last two points. The linear regression of generation means on cumulated selection differential was -0.124 ± 0.035, which constitutes evidence of a significant response, albeit small. It is fair to add that this slope was increased from -0.083 by the addition of the two final points.

The final points of the SX and CS lines shown in Fig. 4 were roughly contemporaneous, so it can be seen that the mean weights of SX have been lower than those of CS for several generations. This response, however, is much less than expected; the CS line had reached a limit to selection at around 14 g. (Roberts, 1966a) when it was crossed to the two other small lines, whose limits were about 11 and 10 g. Since genes from these smaller lines were at a frequency of at least 0.33 in the SX population, there is no obvious reason why the low weights of the smaller lines should

not have been regained by selection. The fertility of the *SX* line was consistently good and there is no likelihood that these genes were lost through drift. But the possibility that the lower limits found in previous experiments should be transcended, or even recovered, appears to be remote.

To what, then, must we ascribe the relatively poor response of the *SX* line? In an earlier paper (Roberts, 1966*b*) it was argued that the limit to selection for low 6-week weight in the *CS* line could be attributed to the opposing effect of natural selection acting on viability. In the case of *SX*, such an argument does not seem to apply. Some 95% of all matings were fertile, and viability over the critical period
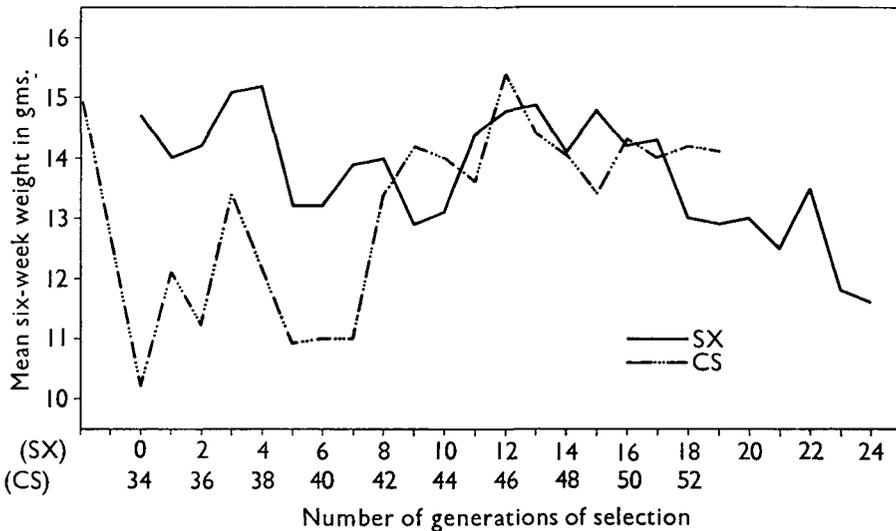

Fig. 4. Response to selection of *SX* line. *CS* line shown for comparison.

from birth to weaning was 96%, which is an extraordinarily good performance. Unless there was a great differential mortality of small mice *in utero*, it is difficult to see where any natural selection could have applied.

Another possible reason for the low response could be maternal effects. It is well known that in the mouse, a decrease in body weight leads to a reduced ovulation rate, and the mice gestated and reared in the consequently smaller litters have an advantage in body weight over mice from larger litters. (Selection for large size would equally lead to larger litters and a depressing effect on body weight.) However, these considerations, either, do not seem to apply to the *SX* line, as the mean litter size showed no evidence of any trend over the course of the experiment.

About the only remaining possibility is, again, linkage. It was seen from Fig. 1 that when the original small lines were crossed, body weight increased. This was not unexpected, for directional dominance is known to favour large size in the mouse. It does mean, however, that alleles for small size that differed in the three lines were put in repulsion on crossing, and that furthermore they would be masked

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by the dominant alleles for larger size. If linkage is important, selection for small size would thus be expected to be ineffective in the early stages of the experiment. However, with crossing-over, coupling homozygotes should soon begin to appear, and selection for recessive genes, which is an efficient procedure, should yield a pronounced response once it began. This, obviously, did not happen, which means that if linkage is to be invoked as the full explanation of the poor response, we must stipulate that the linkage was very tight—far tighter than that which seemed to affect the loci controlling large size, discussed earlier. It did not even begin to break up until the 15th generation, and then only very slowly. To the extent that this is improbable, the linkage hypothesis lacks conviction as an adequate explanation of the slow response in the SX line. While linkage, almost certainly, impeded the response, it seems likely that it was augmented by some unidentified factor.

Whatever the full explanation may be, experience with the SX line provides a clear warning for those animal breeders concerned with the preservation of genes from declining breeds of livestock. It constitutes a strong empirical argument against tipping all these breeds into one gene pool. Even though desirable alleles are not lost through drift, they may not be easily recoverable from the pool—at least, not without more generations of selection than any breeder of large animals could cheerfully contemplate.

4. DISCUSSION

In as much as the crossing of selected strains generated new genetic variance and led to further responses to selection the results described above lend qualitative support to Falconer & King's (1953) procedure, quoted earlier. But the details are quite different. The heterosis found by Falconer & King when they crossed their two lines was only 5%; in the crosses reported here, the heterosis ranged from 8% to 32%, with an average of 16%. But the important difference is that Falconer & King did not find it necessary to suggest that progress had been impeded by linkage in their crossbred population. However, when their results are re-examined, the possibility of linkage cannot entirely be discounted. From their cross of the two large lines, they selected further for both high and low 6-week weight. After two generations by which time the cumulated selection differential was about 8 g. for the divergence, the high and low lines had failed to separate. The low line then came down, but their high line did not increase at all for another two generations. If we were now to wish to interpret these results in terms of linkage, we should obviously have little difficulty in doing so.

The relatively good response found by Falconer & King for downward selection from a cross of large lines has no bearing on the poor response reported here for the SX population, which was a cross of small lines. The two studies represent quite different situations.

The interpretation of the responses reported in this paper leans heavily on the hypothesis that linkage of loci affecting body weight was a prominent feature of the

crossbred base populations. There is, of course, no novelty in this suggestion. The influence of linkage on polygenic systems has long been discussed by Mather (see, for instance, his review, 1943), and a particularly clear case of linkage affecting sternopleural chaetae number in *Drosophila* was analysed by Thoday, Gibson & Spickett (1964). However, linkage in *Drosophila* is one thing; it would not necessarily lead one to expect the same phenomenon in an organism like the mouse, with twenty pairs of chromosomes. Now, the total number of genes, or effective factors, affecting body weight in the mouse is also of this order of magnitude (Roberts, 1966*a*). If these genes are linked to any important extent, it must mean that there is a considerable concentration of similar genes in certain segments of a few chromosomes. The phenomenon of clustering of functionally related genes is now well known in certain micro-organisms, although even among bacteria, it is by no means universal (Fargie & Holloway, 1965). As the clustering appears to be more widespread in bacteria than in higher organisms (Bodmer & Parsons, 1962), it would be most unexpected if close linkage were a basic feature of loci controlling body weight —a trait composed of diverse components—in the mouse. The linkage found, or suggested, in the experiments reported in this paper is much more likely to be the product of a special situation, as follows.

It is shown by Hill & Robertson (1966) that linkage affects the chance of fixation of alleles under selection. An unfavourable allele at a locus is more likely to become fixed if it is linked to another locus with a greater effect on the character. If the effects of the two loci are approximately equal, the chance of fixation of the more favourable allele is reduced at both loci. All this occurs even if the initial population is in linkage equilibrium.

Now, turning the argument around, this would suggest that under certain conditions, the only loci where an unfavourable allele is fixed are those that are linked to other loci affecting the character under selection. Loci that are unlinked would all be fixed for the more favourable allele, given those conditions. The conditions are the ones that exclude chance fixation, spelled out by Robertson (1960) and discussed by Roberts (1966*a*), who showed that these same conditions applied to all of the seven selected lines employed to form base populations for the studies described here. Therefore, when these lines were crossed, loci that were linked had sometimes been fixed for unfavourable alleles; and where the loci were of roughly equal effects, the allele fixed at a particular locus need not be the same for all the lines. Unlinked loci on the other hand, were largely fixed for the same alleles; the probability of this occurring was enhanced by some overlap in the origins of the various lines, as mentioned in an earlier paper (Roberts, 1966*a*). Genetic variance in the two crossbred populations would therefore be dominated by linked loci; unlinked loci would tend not to segregate and therefore contribute no variance.

If all this is correct, then the apparent importance of linkage in the *LX* and *SX* populations is largely an artefact of the method of construction of those populations. It does not necessarily mean that linkage generally affects the genetic variance of body weight in an unselected outbred population to anything like the same extent.

The relative importance of linkage will be the amount of genetic variance due to loci that are linked, as a proportion of the total variance in the character. It is suggested that this ratio is maximized in populations derived from crosses between lines that have previously been selected in the same direction.

SUMMARY

1. Four lines selected for large size were crossed to form a base population for further selection for high 6-week weight; three small lines were crossed similarly, and the crossbred population was selected for low 6-week weight.

2. In every case, a cross between two selected lines resulted in heterosis increasing body weight. This shows that all of the selected lines were differentiated with respect to genes affecting body weight.

3. Further selection for large size produced a stock whose mean weight was 25% higher than the largest of the original lines at its limit. But the response to selection for small size was slow, and after twenty-four generations of selection, the low weights of two of the original lines had not been recovered.

4. The evidence points to linkage of genes affecting body weight in the mouse. It is suggested that this is a particular feature of crosses between previously selected lines, rather than a general feature of mouse populations.

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