The introduction of genetic material from inferior into superior strains

By H. EL SAYED OSMAN* AND ALAN ROBERTSON
Institute of Animal Genetics, Edinburgh

1. INTRODUCTION

At the moment many breeders of plants and animals face the possible exhaustion of useful genetic variation within highly selected strains. This is true for the breeder of broiler chickens who has selected intensely on growth rate in the past and for the corn breeder in the United States who is concerned how best to utilize exotic strains from other regions of the world. There are three possible ways of breaking through such selection limits. The first is the production of new genetic variation by irradiation, which has been valuable in some plant species, perhaps because of the high dosage levels and selection intensities possible, but which has as yet had no striking success in animals (Abplanalp, Lowry, Lerner & Dempster, 1964). The second, the crossing of different selected lines, is not always feasible because the breeder may have no other strain at all comparable to his own. The third is to introduce new genetic variation into the improved stock by crossing it with unimproved populations followed by further selection. It is with this last, which might be termed 'controlled introgression', that we shall be concerned in this paper.

We envisage a selected line, which has almost ceased to respond to selection, and another population far inferior to it in performance—in our case the random-breeding population from which the former was produced. There may, in the latter, be some alleles available which are superior to those fixed in the selected line. How do we disentangle them from the unwanted genes in the unimproved stock and insert them into the superior genotype? Clearly the programme must be based on a cross between the two, but on consideration there appear to be several variable factors and the optimum combination is not immediately obvious. We may pose these as a set of questions:

(i) Should the unimproved population be selected before crossing it with the selected line?

(ii) After crossing, should we wait for several generations before recommencing selection? After all, to insert genes from the unimproved population into the selected line we may be dependent on rare cross-overs and the more opportunity we leave for these the more chance of success.

(iii) Is there an optimum intensity of selection after crossing? Very intense

* Present address: Department of Animal Production, Faculty of Agriculture, Khartoum University, Sudan.

selection may merely reconstitute the selected line very quickly without giving enough time for crossing over. On the other hand, with mild selection we may never succeed in getting rid of the poorer genes introduced by crossing and may not even return to the level of the selected line.

(iv) Should we back-cross again to the selected line before recommencing selection? A potentially useful gene from the unimproved population may be only at a low frequency in the first cross. Each cross to the selected line halves its frequency and reduces the chance of eventually fixing it.

There are two criteria by which the success of the programme may be judged. The first is the extent by which the previous limit of selection is eventually exceeded. The second is the length of time before it is surpassed. It may be of as much practical value to exceed the previous limit by a small amount in, say, five generations as after fifteen generations with a line which eventually shows a very large improvement.

2. HISTORY OF THE SELECTED LINE

From our standard Kaduna population of *Drosophila melanogaster*, two lines had been selected downwards for sternopleural bristles with an intensity of 10/25 for 25 generations. They had then an average score of 12·4 bristles compared with the mean of 17·5 in the base population. The two selection lines were crossed together. The mean of the cross was close to that of the parental lines and it was still at this level when, after four generations of relaxation, selection was resumed with the same intensity. There was a gradual response so that, when the first part of this present experiment was started, the mean had reached 11·0 bristles after 30 generations of the renewed selection. Further progress downwards was slow, whilst there was no perceptible response in five generations of selection upwards. Analysis of salivary glands showed no inversions in the selected line nor in the base population.

During the present experiment, selection downwards was continued on the selected line, L, while a sample was also kept without selection. The continued selection produced a small but definite response and it was therefore decided to measure the genetic variance in the line. Because of the low expected heritability, the aim in the analysis was to have very large family groups. 4600 individuals were measured, the progeny of 46 males and 230 females. Significant differences were obtained between both full-sib and half-sib families and from these an estimate of the heritability in the selected line of 0.075 ± 0.02 was obtained. The realized heritability obtained from the response to the continued selection was 0.034 ± 0.005. A group of 10 inbred lines was produced by full-sibbing from the line and no significant differences were found between these in mean score. However, these observations, taken as a whole, suggest that some genetic variance in this character still remained in the selected line at the start of the experiment. This complicated the interpretation of the results but in a manner which might well be repeated in more practical situations.

3. THE EXPERIMENTAL DESIGN

After a preliminary experiment involving only one replicate within each treatment (Robertson, 1963), we decided to investigate the following factors: (1) selection before crossing, (2) waiting after crossing before restarting selection, and (3) intensity of selection after crossing. The structure of the experiment is summarized in Table 1. All flies were reared in half-pint milk bottles at 25 °C. In the first experiments, selection was of intensity 10/25, given the symbol M for mild. There were three sets of lines, one with no selection before crossing to the selected line L (with the symbol N for 'no selection'), and two sets with three and

Table 1. *The experimental design*

(The last three columns give the identification codes of the different groups of lines (N, no selection; M, mild selection; I, intense selection). There were three replicates in each group, identified by a figure in brackets after the code.)

Selection before crossing		Selection after crossing		Started at		
Genera-tions	Intensity	Genera-tions	Intensity	F_1	F_4	F_7
0	—	25	10/25	N1M	N4M	N7M
0	—	13	10/100	N1I	N4I	N7I
3	10/25	25	10/25	3M1M	3M4M	3M7M
6	10/25	25	10/25	6M1M	6M4M	6M7M
6	10/100	20	10/100	6I1I	6I4I	6I7I
6	10/100	20	10/25	6I1M	—	—

six generations of selection respectively before crossing and mild selection after. The three sets have codes NM, 3MM and 6MM. In each set, groups of lines were selected at the F_1, F_4 and F_7 generation after the cross, indicated by numerals *between* the two letters of a set. Within each group there were three replicates, indicated by the final numeral in brackets.

On the basis of these results, further crosses were made in which selection of intensity 10/100 (with the symbol I for 'intense') was used. There was no set of lines crossed after three generations of selection, but there was an extra set with six generations of intense preselection followed by mild selection, after crossing, from the F_1 generation. These then form the NI, 6II and 6IM sets. The identification of individual lines may be illustrated by two examples. N7M(2) is the second replicate of the group which was not selected before crossing and which was then selected at the F_7 generation with intensity 10/25. 6I1I(1) is the first replicate of the group with six generations of 10/100 selection and which was further selected with the same intensity at the F_1 generation.

4. RESULTS

(i) *The early generations of selection after crossing*

In the base population, the heritability of sternopleural bristle count is about 0·50. The crosses of this to the selected line, L, will have little genetic variation in the haploid set derived from the latter. On interbreeding the F_1, the genetic variation should increase owing to the segregation of whole chromosomes. On average there will be an excess of chromosome segments in the coupling phase between genes affecting the quantitative character in the same direction. Crossing-over in subsequent generations would then be expected to reduce the genetic variation as linkage equilibrium is to some extent re-established. Table 2 shows the trend

Table 2. *The mean square coefficient of variation in the lines after crossing*

(F_1, first cross; $F_{1.1}$, first cross selected for one generation; F_4 and F_7, F_1 kept without selection for 3 and 6 generations respectively.)

	Generation			
Set	F_1	$F_{1.1}$	F_4	F_7
NM	69·1	108·7	117·2	101·5
3MM	96·8	100·2	92·9	103·8
6MM	69·8	103·0	81·5	89·3
NI	63·0	112·5	107·6	104·8
6II	63·7	90·8	84·5	77·5

Table 3. *The mean of the lines on relaxation of selection after crossing*

	Generations of relaxation		
Set	0	3	6
NM	14·27	13·79	14·83
NI	13·67	14·15	14·12
3M	13·47	13·55	13·78
6M	12·91	13·04	13·17
6I	12·04	12·20	12·03

in the squared coefficient of variation. In NM and NI, for instance, where there was no selection before crossing and in which the differences between the parental lines were therefore greatest, the increase in the variation in the first generation after the cross would indicate that the genetic variation due to segregation made up some 40% of the phenotypic variation at this time. The realized heritability in the first two generations of selection in these F_1 lines was of this magnitude. The subsequent period without selection in the F_4 and F_7 groups seems to have had only a small effect on the phenotypic variability.

After crossing, the lines were kept in bottles under good conditions. Nevertheless, there would still be natural selection on male fertility and female egg-laying ability and the trend in the population mean is of some interest. Table 3 shows the mean of the three replicates, when artificial selection was restarted, in the five sets which

were relaxed for six generations. In the F_1 the mean is clearly lower in those sets with most selection before crossing. In four of the five sets, the F_7 mean is higher than that in the F_1 generation. However, the rate of regression is small, averaging only 0·05 bristles per generation, compared with the average deviation of four bristles of the F_1 from the mean value of the initial population.

(ii) *The selection limits*

The effect of selection before crossing can be studied in combination with both levels of selection intensity after crossing. With mild selection after crossing, NM, 3MM, 6MM and 6IM (the latter can be considered as having had the equivalent of 10 generations of mild preselection) give four different amounts of preselection. In NI and 6II we have different amounts of preselection with intense selection after crossing. There are two sets of comparisons of the effect of selection intensity after crossing, NM and NI without preselection and 6II and 6IM with the greatest amount of preselection.

As was mentioned earlier, there are two criteria of success in this programme. The first is the final limit of selection reached and the second is the length of time in generations from the start of the breeding programme before a line exceeds the selected line. The continued response of the selected line itself was a complication. We had, for instance, some replicates which temporarily exceeded the selected line, only in the end to be passed by it. The results at the limit are summarized in Fig. 1, which gives the average of the last five generations of selection for all the replicates, plotted as deviations from the final score of the selected line L. The mean level of L at the start of the crossing programme is also shown. For the second part of the experiment (sets NI, 6II and 6IM), selection was re-applied to a sample of L which had been kept without selection during the first part of the experiment. The figures below some entries are the number of generations required for the replicates to exceed the contemporary value of the selected line. Those in brackets refer to the anomalous cases in which the replicate exceeded the selected line only to be overtaken by it subsequently. Figures 2–4 present the selection curves for three of the sets. To reduce confusion, the five-generation running averages have been plotted. The relationship between different replicates should be noted. Each F_1 replicate has a corresponding F_4 and F_7. There is also a relation between the lines in the 6IM set and those in the 6II set, and also between the 3MM and 6MM sets. Such related lines do not always behave similarly. In the NM set, the second replicate shows a very large breakthrough at the F_7 but not at the F_1 and F_4 generations. Nevertheless, in the 6MM set, the first replicate in each set shows a significant breakthrough.

Variation between replicates is to be expected. Success will depend either on incorporating a gene which was rare in the base population or on a cross-over between two closely linked loci. However, various conclusions appear from examination of the results without a sophisticated statistical analysis.

(*a*) In Fig. 1 the sets with the greatest amount of selection before crossing (6II and 6IM) have exceeded the others.

(b) An examination of the horizontal differences in Fig. 1 shows no clear effect of a period of waiting after crossing before restarting selection. Of the four lowest lines, two, N7M(2) and 6I7I(1), had a waiting period of 6 generations before
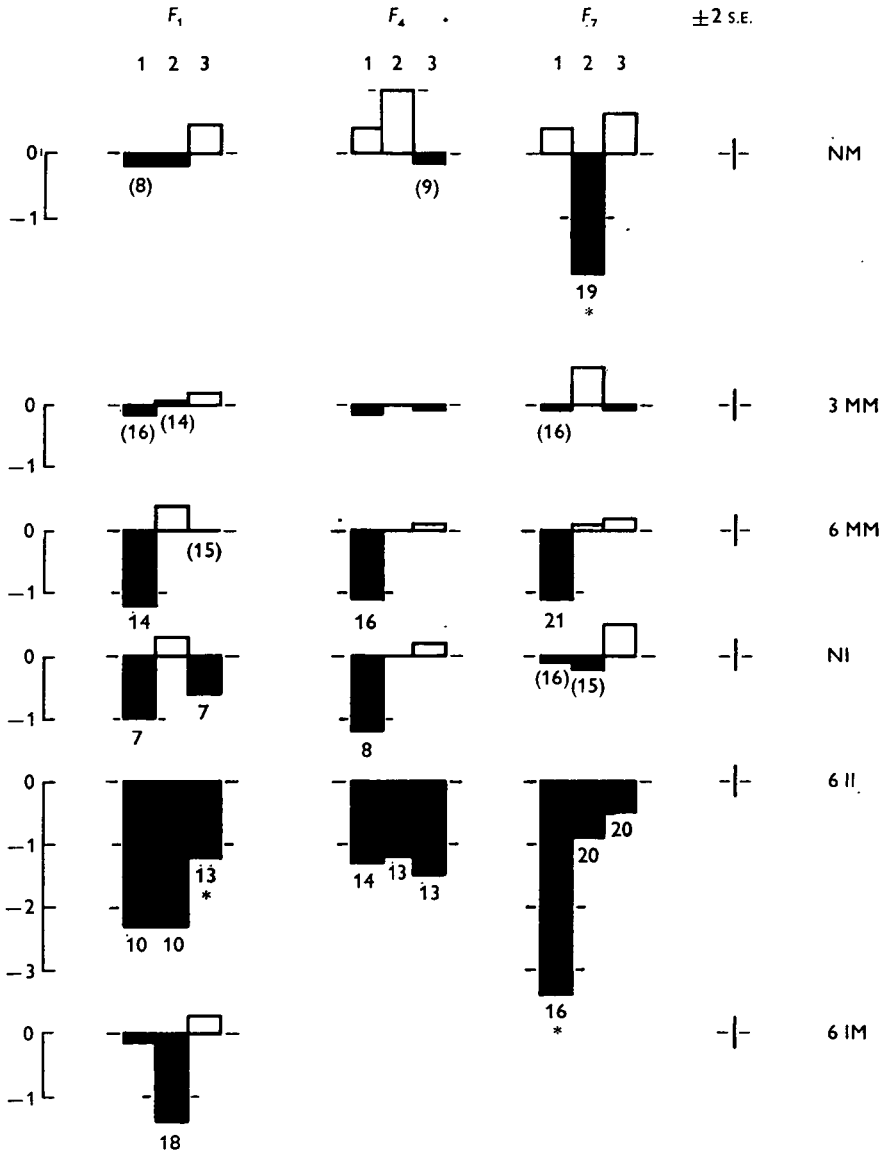

Fig. 1. The average of the lines in the last five generations of selection, rows corresponding to selection procedure and columns to time of resumption of selection. Scores are relative to the final value of the selected line. The vertical scale is shown in bristles and on the right is twice the standard error of the difference between two means. The numbers attached to each line show the total number of generations before the selected line was passed (those in brackets refer to lines in which the final difference was not significant). Asterisks indicate the three lines used in the chromosomal analysis.

reselection but there is evidence that in neither can the extra gain be ascribed solely to this. The response in the former accelerated after about 8 generations of selection (Fig. 2). The coefficient of variation then increased to a maximum at the 16th generation (by which time the mean was well below that of the contemporary selected line) and then declined. It seems unlikely that this response was due to
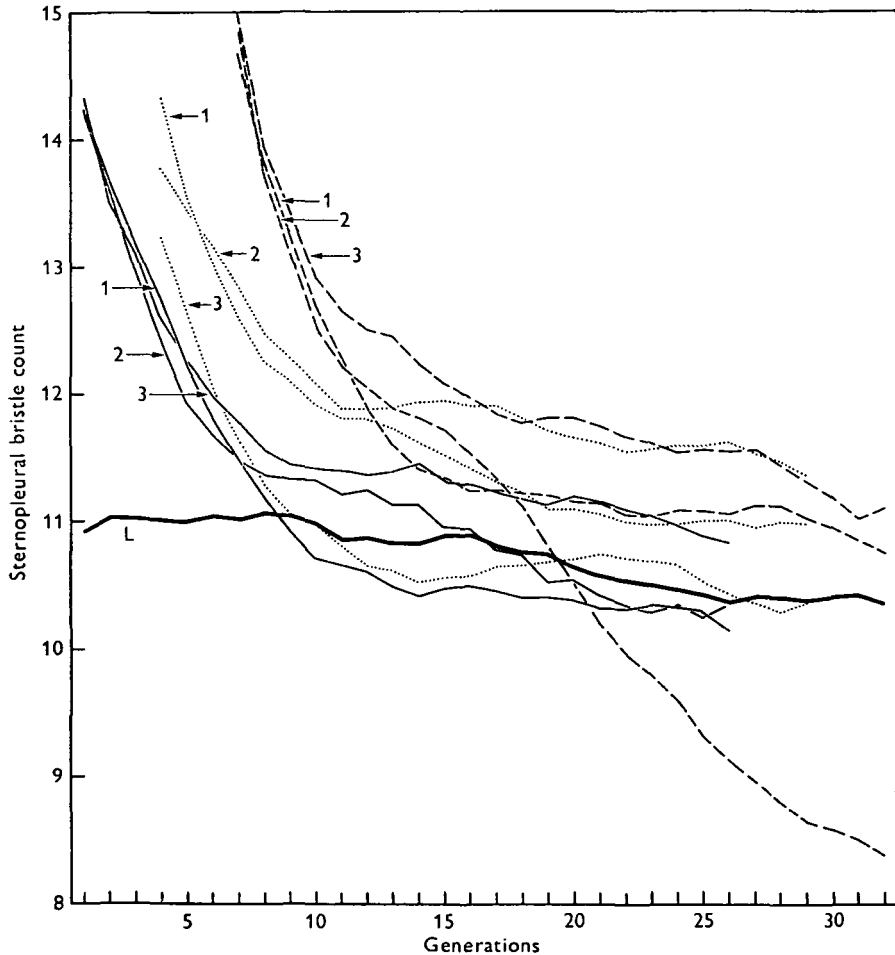

Fig. 2. The five-generation moving average score for the NM set of lines (no pre-selection, intensity 10/25 after crossing). Replicates are numbered; L is original selected line.

anything that had happened in the period of waiting. In 6I7I(1), much of the response is due to a gene with a recessive effect, reducing bristles, on the small fourth chromosome, on which crossing-over very rarely occurs. This can hardly be attributed to crossing-over during the waiting period.

(c) It was suggested earlier that there might be an optimum intensity of selection after crossing. This would be most probable when selection was started at the F_1 generation. Very intense selection then might reconstitute the selected
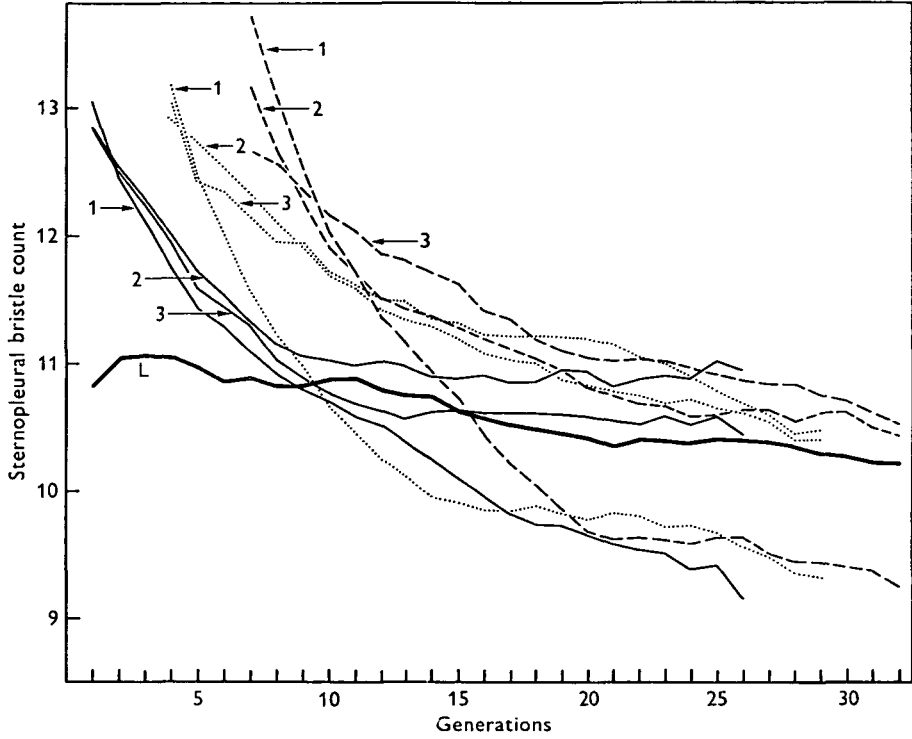
Fig. 3. The five-generation moving average score for the 6MM set of lines (6 generations preselection 10/25, intensity 10/25 after crossing). Replicates are numbered; L is original selected line.
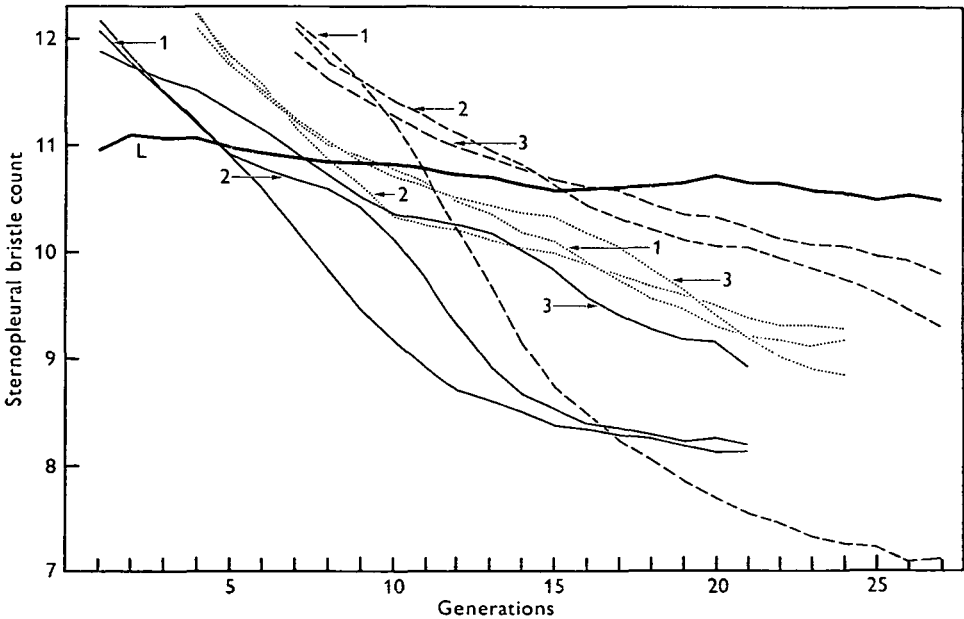

Fig. 4. The five-generation moving average score for the 6II set of lines (6 generations preselection 10/100, intensity 10/100 after crossing). Replicates are numbered; L is original selected line.

line without change and a slower response to less intense selection might allow the necessary crossing-over to take place during selection. Comparisons between NM and NI and between 6II and 6IM do not support the suggestion. The higher intensity in 6II is clearly better. In NM and NI with no preselection, where the effect might be more pronounced, the average response is slightly greater for the more intense selection although this is not significant.

The general trend of these results favours selection before crossing, and this is confirmed by a theoretical analysis presented later. But the gains for each additional generation of selection decrease as the selection continues and, in terms of the time taken to achieve success, too long a period of initial selection is harmful. It is possible to surpass the selected line in comparatively few generations by crossing without prior selection followed by immediate selection as in N1I(1) and N1I(3). The two criteria of success in such a programme would seem to be to some extent mutually incompatible. In a practical breeding programme it would perhaps be advisable to carry out alternative procedures simultaneously. To achieve a breakthrough quickly, a programme of immediate crossing followed by intense selection would seem to be called for while at the same time a programme with intense selection before crossing was being carried on to provide an increased response in the long term.

We have concentrated so far on those lines which have surpassed the selected line, but it must not be overlooked that there were several lines which were at the end significantly worse than the contemporary level of the selected line.

(iii) *The genetic analysis*

This was carried out in two stages. The first involved crosses between the selected line L and lines 6I7I(1), 6M7M(1) and N7M(2). For the first the cross was intermediate in bristle score between the parents, whereas for the other two the genes in L were apparently dominant. The introduction of marked autosomes into the last two lines showed that the second chromosome had little effect. Finally, introduction of homozygous third chromosomes from these lines into L showed that the third chromosome of N7M(2) had a large effect and was almost completely recessive to L. The third chromosome of 6I7I(1) had a smaller effect which showed an additive effect in the heterozygote.

The latter line, from now on given the symbol D, was subsequently used by A. R. and Dr J. H. Louw as the extreme low line in analysing genetic differences between extreme lines selected from the base population. It became clear that this line was homozygous for a recessive gene on the fourth chromosome, affecting sternopleural bristle score as well as the chitin melanin pattern in females. At 25 °C, the gene can easily be scored on melanin pattern (Robertson & Louw, 1966). The recessive must be rare in the base population as it has not been found in a sample of 50 fourth chromosomes. It decreases reproductive fitness (mostly through egg-laying) and, in competition with the dominant, rapidly declines in gene frequency.

Some two years later, line D was used in an analysis of some of the more extreme

lines. In this, a set of autosomes marked with dominants (Pm, Sb and ci^D on the second, third and fourth chromosomes respectively) was used. These were derived from a single heterozygous male and were always transmitted in males. All crosses involved males which were heterozygous for all the marked autosomes, the other set coming from one of the lines, and females from the same or other lines, e.g.

$$D_1 \; Pm/D_2 \; Sb/D_3 \; ci^D/D_4 \times A_1/A_1 \; A_2/A_2 \; A_3/A_3 \; A_4/A_4.$$

In such a cross, there are eight different kinds of progeny of each sex, and from these the effects of the separate autosomal substitutions and the interactions between them can be estimated. In the above cross, for instance, the difference in bristle score between genotypes Pm/A_2 and D_2/A_2 can be measured. By making all four crosses between the pairs of lines, the four differences

$$Pm/D_2 - D_2/D_2, \; Pm/D_2 - A_2/D_2, \; Pm/A_2 - A_2/D_2, \; Pm/A_2 - A_2/A_2$$

can be measured and the relative scores of the three wild-type genotypes can be estimated. It has to be remembered that these rest on the accumulation of differences—the estimate of $A_2/A_2 - D_2/D_2$ being the sum of four separate measurements, each of which have in our case a standard error of 0·15.

The lines 6I1I(1), 6I1I(2), 6I1I(3), 6M7M(1) and N4I(1) were first examined during the first cross only, which estimates only effects detectable in the heterozygote with D—all crosses have in common heterozygotes between the D and the marked chromosomes. These established that none of the lines contained the same fourth chromosome as D. All third chromosome heterozygotes were higher than D_3/D_3 but the second chromosome of 6I1I(3) produced a significant reduction. As a consequence a full set of crosses was made with 6I1I(3), N7M(2) (labelled B and A in Fig. 5), the selected line L, the base population K and the highest available line from the latter (H). The results are summarized in Fig. 5, in which the D/D homozygote is taken as an arbitrary zero. Interactions between chromosomes in bristle score were unimportant.

For the second chromosome, D_2/D_2 is a little below L_2/L_2, whereas the B_2/B_2 homozygote is more than two bristles below it. Gene action in the latter seems to be fairly additive. For the third chromosome, on the other hand, the two lines are similar. N7M(2) (whose second chromosome had been higher than that of the selected line) shows a considerable response which is recessive to the D chromosome, in agreement with the earlier evidence. D is the only line showing an effect on the fourth chromosome.

Reciprocal crosses were made between D and A, B and L respectively. Differences between the scores of males in the progeny did not indicate that much of the increased response was due to genes on the X chromosome. In that between D and L, for instance, the males with mothers from the former were lower than the reciprocal males, but not significantly so (0·17 ± 0·17).

A result of a more limited nature is specific to the character selected for, sternopleural bristles. It has often been assumed that three bristles on each side are different from the others and are almost invariant. In fact, we found no evidence in lines like D, which had average scores less than seven on occasions, that

there is a 'physiological barrier' at six. Flies with scores of four or five were not uncommon.
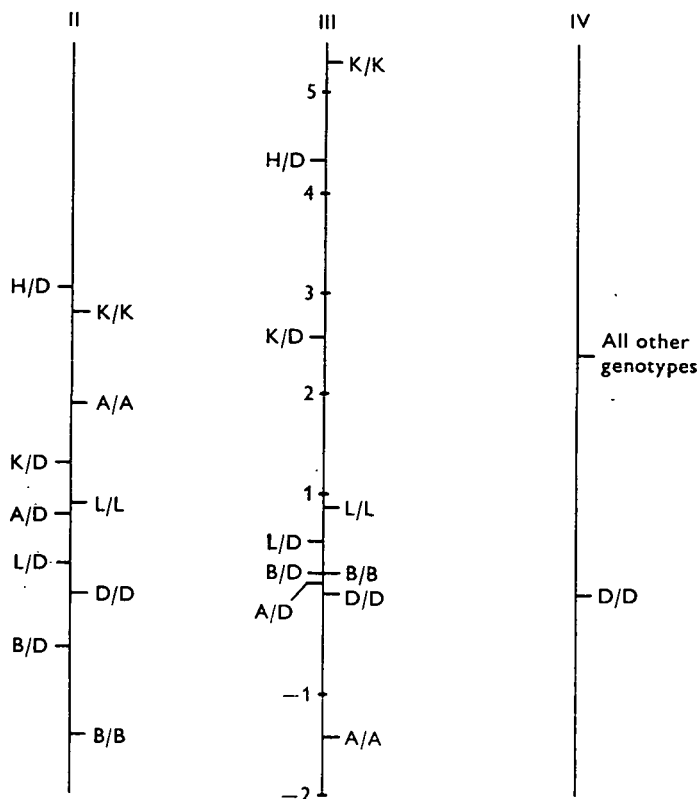

Fig. 5. The effect of chromosomal substitution into 6I7I(1). For each chromosome (II, III and IV), effects in heterozygotes are shown on the left of the vertical line and in homozygotes on the right. A, N7M(2); B, 6I1I(3); D, 6I7I(1); H, highest selected line from K (score ∼50 bristles); K, unselected Kaduna population; L, selected line L. D/D is taken as an arbitrary zero.

5. SOME THEORETICAL CONSIDERATIONS

We may get some theoretical indication of the probable outcome of these experiments if we assume independent segregation of the loci controlling the character. The basic formula is given by Kimura (1957) for the chance of final fixation, $u(q)$, of a gene with an additive effect on selective advantage:

$$u(q) = \frac{1 - e^{-2Nsq}}{1 - e^{-2Ns}},$$

where N is the effective population size, s is the difference in selective advantage between the two homozygotes and q the initial gene frequency. If the differences in selective advantage arise from artificial selection, we may write $s = ia/\sigma$, where i is the selection differential in standard units, a is the difference between the two homozygotes in the character under selection and σ is its phenotypic standard

deviation. If Ns is greater than 2, the denominator can be assumed equal to unity and the chance of fixation depends solely on Nsq.

In the present experiments we wish, by crossing a selected line to the base population, to fix desirable alleles which are not present in the selected line while retaining those which are. Consider first the crosses of the unselected populations to the selected line. Using subscript 1 for the selection procedure by which the selected line was produced and the subscript 2 for the selection programme after crossing, we have, for the over-all chance of fixation after crossing and reselection, the expression $u_1(q)\, u_2(\tfrac{1}{2}(q+1)) + (1 - u_1(q))\, u_2(\tfrac{1}{2}q)$.

The first term is the chance of fixing alleles before crossing and recovering them afterwards, when the gene frequency after crossing is $\tfrac{1}{2}(q+1)$, and the second refers to those lost before crossing but fixed afterwards, whose frequency after crossing is $\tfrac{1}{2}q$. There is a similar expression for the final chance of fixation after back-crossing followed by selection, except that the gene frequencies at the restart of selection are $\tfrac{1}{4}(q+3)$ and $\tfrac{1}{4}q$ respectively.

We may distinguish two classes of alleles which are not likely to have been fixed in the first selection. In the first, Nsq is low because s is low, the locus having a small effect on the character under selection. In the second class, the selection pressure is high, but the desirable allele has a low initial frequency.

In the first situation the chance of fixation is given approximately by

$$u(q) = q + Nsq(1 - q).$$

Using $(Ns)_1$ and $(Ns)_2$ to refer to the values of Ns by which the selected line was formed and that after crossing, respectively, we have for crossing followed by selection

$$u(q) = [(q + (Ns)_1\, q(1-q)]\left[\frac{q+1}{2} + (Ns)_2\frac{1+q}{2}\frac{1-q}{2}\right]$$

$$+ [1 - q - (Ns)_1\, q(1-q)]\left[\frac{q}{2} + (Ns)_2\frac{q}{2}\left(1 - \frac{q}{2}\right)\right]$$

$$= q + [\tfrac{1}{2}(Ns)_1 + \tfrac{3}{4}(Ns)_2]\, q(1-q) \text{ approx.}$$

so that the effective value of Ns for the whole process is $\tfrac{1}{2}(Ns)_1 + \tfrac{3}{4}(Ns)_2$. A similar expression is obtained for the chance of selection after a further cross to the selected line, except that the coefficients are now $\tfrac{3}{4}$ and $\tfrac{7}{16}$ respectively. From these expressions we may then draw the following conclusion.

(a) The expected values of the lines after crossing and reselection will be higher than that of the selected line if $(Ns)_2$ is greater than $\tfrac{2}{3}(Ns)_1$. For selection after back-crossing the corresponding factor is $\tfrac{4}{7}$.

(b) The expected gain in the back-crossing relative to that in the crossing programme depends on $\tfrac{1}{4}(Ns)_1 - \tfrac{5}{16}(Ns)_2$. If $(Ns)_2$ is less than $\tfrac{4}{5}(Ns)_1$, the expectation on selection after back-crossing is higher than that of selection after crossing and vice versa. Thus back-crossing before reselection will be most valuable when the chance of success in its absence is less than one-half.

When the desirable allele is not fixed because of a low initial frequency, we

can, in some cases, draw comparatively simple conclusions. If $(Ns)_2$ is so high that alleles fixed in the first selection are almost certain to be fixed again, the effective value of Ns for the whole process is $(Ns)_1 + \frac{1}{2}(Ns)_2$ for crossing and $(Ns)_1 + \frac{1}{4}(Ns)_2$ for back-crossing. No simple expressions have been obtained for the value of $(Ns)_2$ which gives an expectation equal to that of the first selection. It appears that, as $(Ns)_1$ increases, so does the value of $(Ns)_2$ necessary to equal the selected line after crossing but by no means in proportion. For instance, for a gene with an initial frequency of 0·02 and $(Ns)_1$ equal to 8, $(Ns)_2$ needs to be 2·2 to give an expected chance of fixation equal to that for the selected line. However, if $(Ns)_1$ is raised to 80, the necessary value of $(Ns)_2$ is only 5·0. The ratio of the two is then much less than for loci of the first type. The same generalization can be made about the relative values of crossing or back-crossing as when Ns is low, which may be summarized as follows: the expectation on selection after crossing is approximately equal to that on selection after back-crossing when both are equal to the expected value of the selected line. If $(Ns)_2$ is below this level, then back-crossing will be superior and vice versa.

The consequence of such a selection procedure depends on the value of Ns that we can reasonably impose in the reselection compared to that by which the selected line was produced. The more extreme the selected line, the more difficult will it be to surpass it on reselection after crossing and the greater the probability that back-crossing will be better than a single cross. One conclusion from these arguments as to the necessary ratios of $(Ns)_2$ to $(Ns)_1$ is that improvements to a highly selected line in such a programme are more likely to come from alleles which had not been fixed because they were rare, than from alleles which had not been fixed because their effects on the character under selection were small.

Similar conclusions apply to recessive genes where, for genes with small effects, we have $u(q) = q + \frac{2}{3}Nsq(1-q^2)$. If the expected changes of gene frequency are small, then the effective value of Ns over the whole programme is $\frac{1}{2}(Ns)_1 + \frac{7}{8}(Ns)_2$. To achieve an expected value greater than that in the first selection, $(Ns)_2$ must be greater than $\frac{4}{7}(Ns)_1$, a value slightly less than for an additive gene. If the gene is not fixed because q is small, even though $(Ns)_1$ is large, it appears, as in the additive situation, that the value of $(Ns)_2$ needed to regain the selected level may be much less than $(Ns)_1$. Further, the necessary ratio of $(Ns)_2/(Ns)_1$ may be much less for a recessive gene than for an additive gene. For instance, if $q = 0·05$ and $u_1(q) = 0·5$, then on crossing and reselection the necessary ratio is 0·31 for an additive gene and only 0·067 for a recessive. This happens because, when q is small and Ns high, $u(q) - q$ is proportional to Ns for an additive gene but to $\sqrt{(Ns)}$ for a recessive.

The selection line, L, had been produced by crossing two lines which had reached the limit at an intensity of selection of 10/25, followed by a further selection with a similar programme. The effective values of Ns by which the line was produced would then be rather less than twice that in the lines in which the selection was 10/25 after crossing. In those which were selected with an intensity of 10/100 after crossing, $(Ns)_2$ would be roughly equal to the effective value of $(Ns)_1$.

We have done similar calculations on the effect of selection before crossing on the final chance of fixation. Here the drift of gene frequency during selection in a finite population had to be taken into account, with the method of transition matrices used by Allan & Robertson (1964). It was found that, the more selection before crossing, the greater the expected final change in gene frequency. Such an increased gain was obtained at the expense of a greater time before the selection limit is exceeded.

It is difficult to discuss with any precision the value of waiting before applying selection pressure when we introduce the complication of linkage. Using the symbol + for an allele with a positive effect on the character, in the selected line the majority of sequences along a chromosome would be of the + + + kind. Some, however, will be + − + and in the crossing programme we are trying to insert a + allele from the base population into the sequence. The period of relaxation before reselection will clearly have an opposite effect on the two kinds of sequence. For the first, crossing-over which occurs in this period must reduce the chance of recovering the sequence on renewed selection, whilst, conversely, it will increase the chance of converting a + − + sequence into a + + + one. In the selected line, the majority of sequences will probably be of the first kind so than an increase in the amount of crossing-over will reduce the average gain on the resumption of selection. We are in practice concerned with the probability that some lines, after crossing and reselection, will exceed the selected line. If the expectation decreases continuously as crossing-over increases, but the variation between replicate lines increases, the chance of an individual line exceeding the selected lines might well have a maximum at an intermediate level of crossing-over. Even so, it is possible that the existing amount of crossing-over in the early generations of the selection may be greater than the optimum and that the chance of breaking through the limit might be increased if crossing-over were to some extent suppressed during selection. The better the selected line, the more sequences will be of the first kind and the greater the average reduction of gain by crossing-over. A resolution of these problems would seem to call for computer studies using Monte Carlo techniques.

6. DISCUSSION

The experiments that we have presented are relevant to a practical problem of fairly general interest. How general are the conclusions? The chances of success will depend on the nature of the selected line and of the unselected population from which we wish to draw useful genetic material. The theoretical discussion has been based on the assumption that this unselected population was that from which the selected line was initially derived. If the selected line is genetically unrelated to the unselected population, the latter might, nevertheless, contain some useful alleles at reasonably high frequencies with a large effect on the character being selected for. The results might also have been very different had we used a much more intensely selected line—if we used the line 6I7I(1), for example, we should probably have found it very much more difficult to break through the selection limit.

Success in such experiments will depend either on a rare cross-over event or the availability in the initial sample of a rare gene from the base population. There should therefore be more variation between replicates than are usual in selection lines. This we certainly find, so that the drawing of valid conclusions becomes rather difficult, even with reference only to our own material. In addition, the criterion of success is a dual one. For instance, both in practice and in theory, we have the highest chance of success with the greatest amount of selection before crossing, but such selection must increase the time necessary to achieve the breakthrough, since one half of the selection gain is lost on crossing. In fact, the most rapid breakthrough was achieved by crossing without preselection followed by intense selection immediately after crossing, whereas the set of lines which achieved the greatest final advance were those in which the selection before crossing had been most intense.

The importance of giving enough time for crossing-over can be evaluated from two aspects of the experimental results. The first is the effect of having several generations without selection after crossing. The variation between replicates makes a clear answer uncertain, but at least there is no strong evidence of any advantage in waiting before restarting selection. Admittedly some of the more extreme lines were in the group with six generations of waiting, but in some of these there was evidence to suggest that crossing-over had not played an important part. The other aspect of linkage comes from the comparison of the two intensities of selection applied immediately after crossing. The lines selected most intensely after crossing had a greater chance of breaking through than those selected less intensely. The explanation of this is probably that the limit was never reached so quickly that there was not enough opportunity for crossing-over to take place during the selection. It is by no means inconceivable that the optimum level of crossing-over should be below that achieved on selection immediately after crossing.

Drosophila melanogaster has effectively three chromosomes with no crossing-over in one sex. Linkage might then be more important in selection than in, say, maize, with ten pairs of chromosomes, or domestic animals with 30 or 40. The fact that measures designed to allow crossing-over to take place before selection was resumed had little effect here would suggest that they may be less important in organisms with a larger number of chromosomes.

It was already known that the response in the selected line relative to the base population had occurred on all three major chromosomes. The analyses of some of the lines which have broken through the limit show that useful variation was still available in that population on both the second, third and even on the very small fourth chromosome. It was interesting that the most extreme third and fourth chromosomes both proved to be recessive to the existing chromosomes in the selected line. These results emphasize the fact that the limit in the initial selection was an artefact of the selection programme itself, as many useful genes in the initial population had been lost during selection. Selection from crosses of different high lines, which in general leads to further response, supports this view.

SUMMARY

1. A series of experiments have been carried out with *Drosophila melanogaster* in order to improve further a population, highly selected for a quantitative character, by introducing genetic material from an inferior population. The effects of selecting the latter before crossing to the selected line, of waiting after crossing before restarting selection and of varying the intensity of selection after crossing have been studied. The inferior population used was the large random breeding population from which the selected line had been produced by selecting downwards for sternopleural bristles.

2. There proved to be some incompatibility between the conditions necessary to achieve the two criteria of success—the time to surpass the selected line and the extent by which it was eventually exceeded. The lines which surpassed it soonest had not been selected before crossing and had been selected intensely immediately afterwards. Those which surpassed it most had had the greatest amount of selection before crossing followed by the most intense selection afterwards.

3. Crossing-over did not appear to be a limiting factor after crossing. Neither a period of relaxation after crossing nor a lower intensity of selection after crossing increased the chance of success.

4. Genetic analyses were made of several lines which had exceeded the original selected line. In separate lines, second, third and fourth chromosomes were found which were superior to the chromosomes of the selected line. This and other evidence shows that the limits in such lines with a small number of parents are not in any way absolute but artefacts of the selection programme. The extreme third and fourth chromosomes were both recessive to that of the selected line in the effect on bristle score.

5. A theoretical discussion of such selection programmes is given, assuming that the character is controlled by independently segregating loci. It is shown that, if two alleles at different loci have an equal but small chance of fixation in the initial selection, one because it is rare and the other because, though frequent, it has little effect on the character, such a programme will tend to pick up the former rather than the latter. Of two such rare alleles with an equal initial chance of fixation, the one additive and the other recessive, the chance of fixation of the latter will be greater in a crossing programme.

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