The production and assay of segmental substitution lines in barley

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SUMMARY

The usual systems of assay using techniques of chromosome substitution demonstrated in *Drosophila* and wheat are not applicable to barley (*Hordeum vulgare*). Chromosomal material for assay may, however, be substituted from one variety into another by using translocations to mark and restrict crossing over in the chromosomes to be transferred. This paper describes the isolation and assay of lines derived in this way.

Seven substitution lines derived from the donor variety Maris Badger and the recipient variety Mars have been scored for quantitative characters in two field trials.

The results indicate that variation in flowering time and other associated characters is largely determined by genes on chromosome 2 and that chromosome 4 is involved in the control of plant height.

The feasibility of the technique as a method of assaying the contributions of chromosomes to qualitative characters by substitution in a diploid is discussed.

1. INTRODUCTION

The requirements for chromosome assay are twofold. First, the substituted chromosome must be suitably marked so as to be traceable throughout a crossing programme. Secondly, the chromosome or segment to be assayed must be kept 'intact' by the suppression of recombination. The methods of substitution and assay of single chromosomes which have proved so effective in *Drosophila* (Breese & Mather, 1957, 1960; Thoday, 1961) and wheat (Sears, 1953, 1954; Law, 1966, 1967) cannot be applied to barley (*Hordeum vulgare*, $2n = 14$). Suitable inversion stocks such as are used for marking and suppressing recombination in *Drosophila* are not available and, as a diploid, barley is intolerant of the aneuploidy upon which chromosome substitution in polyploid wheat depends. The aim of the present work was to produce segmental substitution lines for assay by using translocations (interchanges). By this means segments of 'pairs' of non-homologous chromosomes are transferred from donor to recipient parent. The translocations serve as markers and restrict recombination within interstitial segments of the substituted chromosomes.

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2. THEORY AND CROSSING PROGRAMME

(a) *Translocations and recombination*

Homozygous reciprocal translocations are usually indistinguishable from the normal genotype, but plants with heterozygous translocations are easily identifiable both cytologically and by the reduced seed set. Plate 1 shows mature ears of normal and translocation heterozygote plants (*a*) and typical MI configuration of normal (*b*) and translocation (*c*) genotypes. In the heterozygote, translocated chromosomes form the characteristic quadrivalent at MI and the relative frequencies of alternate and adjacent configurations determine the viability of gametes – in barley alternate configurations constitute about 70–75 % of the total and seed set of heterozygotes is about 70–75 % (Burnham, White & Livers, 1954).

The consequences of alternate disjunction is that normal and translocated chromosomes go to opposite poles at first anaphase of meiosis. Therefore the two types of chromosome, which in the experiment described below derive from the donor and recipient parents respectively, are kept separate in the resultant viable gametes. Two donor chromosomes are 'paired' with recipient translocated chromosomes and are then held heterozygous through a backcross programme.

In the translocation heterozygote: (*a*) independent segregation of genes on paired non-homologues is restricted by non-independent segregation of homologous centromeres, and (*b*) recombination between the centromeres and the translocation breakpoints is severely reduced. Hanson & Kramer (1950) give a theoretical maximum of 3 % recombination between the breakpoint and the centromere, and Kramer & Blander (1961) report data from experiments by Ramage showing that such recombination in chromosome 6 rarely exceeded 2–3 % even when the breakpoint was in the distally located satellite region of the chromosome.

In addition to the restriction of crossing-over due to translocation heterozygosity, chiasmata are generally restricted to the distal regions of chromosomes in the barley varieties used in this study. Cytological observations at early diplotene show that more than 70 % of chiasmata are distal. In the 20 % of arms with two chiasmata, one is usually in the proximal third of the arm, probably because pairing is initiated terminally (Kasha & Burnham, 1965) and the first chiasma strongly interferes with the second. There were rarely more than four chiasmata in the translocation quadrivalent. In barley, therefore, map units in proximal regions relate to very much greater cytological distances than do those in the distal regions.

(b) *The idealized crossing programme*

The crossing procedure used is shown in Text-fig. 1.

(i) A translocation homozygote (*T*-line) of the recipient genotype (R) is crossed to a normal donor genotype (D). The F_1 is heterozygous for both the translocation and the background genotype of the remaining five pairs of homologues.

(ii) This F_1 is backcrossed on to the recipient, translocation-carrying line. The resultant progeny will include two types: translocation homozygotes in which the donor homologues are excluded and translocation heterozygotes which will contain
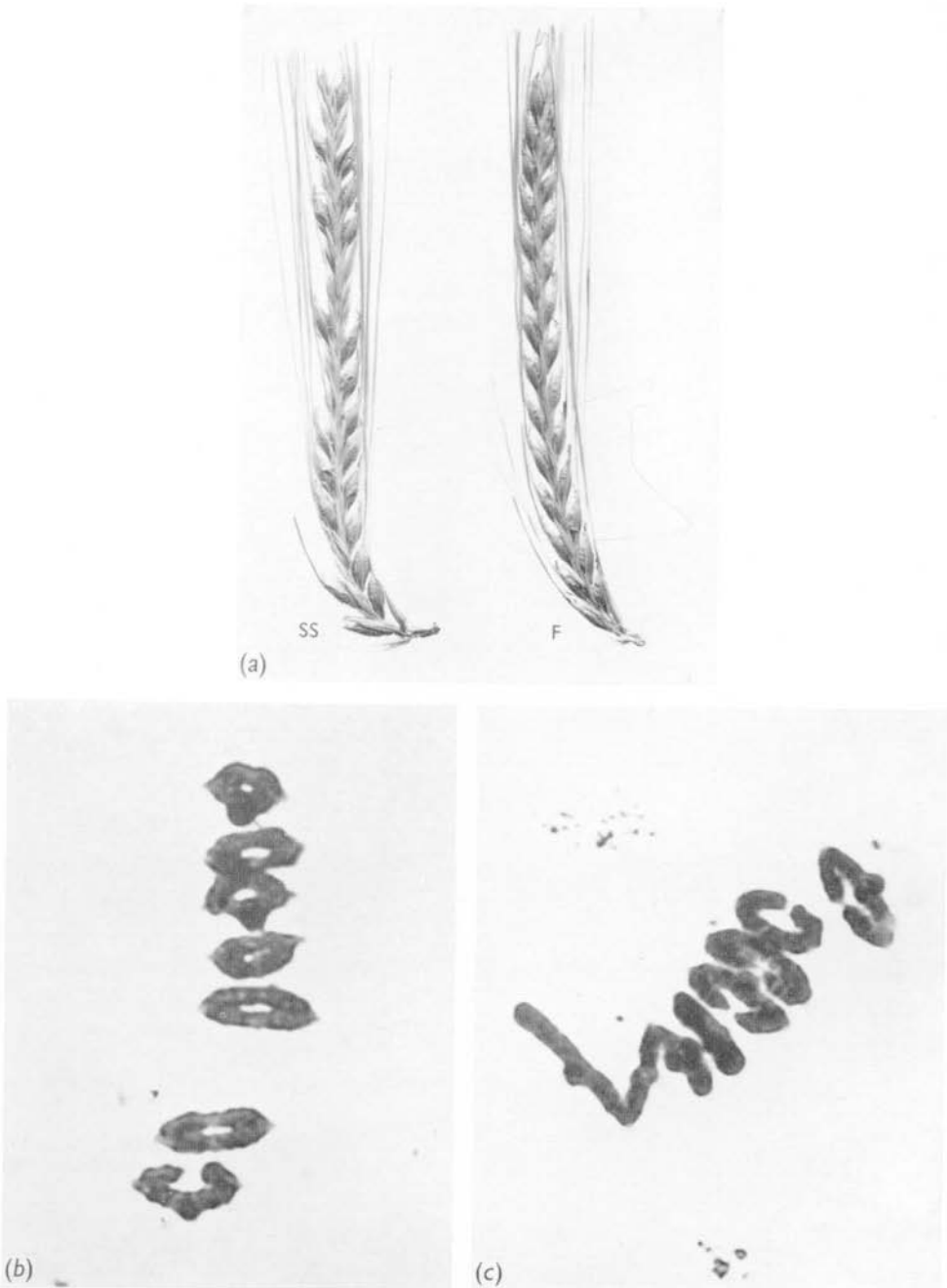
Normal (*F*) and translocation heterozygote (*SS*). (*a*) Mature ears, (*b*) first metaphase normal plant, and (*c*) translocation heterozygote.

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structurally normal donor chromosomes paired with recipient translocated chromosomes. These heterozygotes are selected for further backcrossing.

(iii) After n backcrosses to the recurrent, translocation-carrying parent, B_n progeny will include plants heterozygous for structurally normal donor and translocated recipient chromosomes. Ideally the background genotype will be that of the recipient parent.
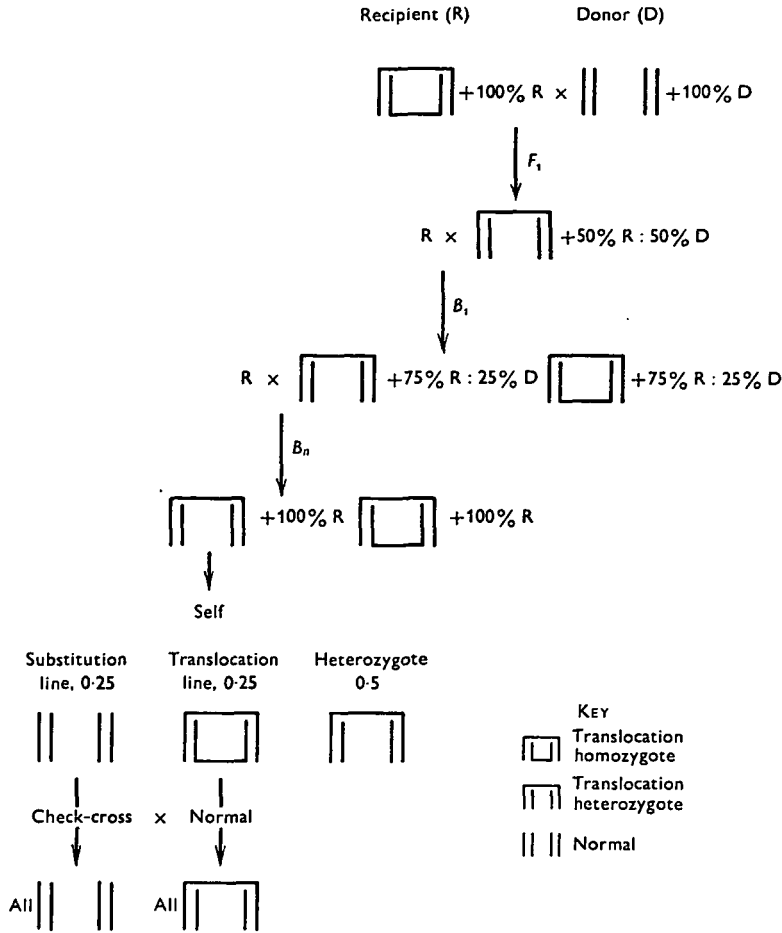
Text-fig. 1. The idealized crossing programme.

(iv) This B_n translocation heterozygote is then selfed to give equal numbers of structural heterozygotes and homozygotes in the progeny. The two types of homozygote are indistinguishable both cytologically and on the basis of fertility.

(v) Homozygotes for the normal (donor) or translocation (recipient) chromosomes can be identified from the meiotic behaviour of the progeny of a check-cross with normal plants. The two types of homozygote will be referred to as S-line and T''-lines respectively.

It will be noted that the T''-line will ideally be the reconstructed T-line and so can be used in a test for heterogeneity of the background genotype.

It was expected that segments of chromosomes would be substituted into the recipient background from the donor parent. The substituted segment would be composed of the interstitial segment, which can be regarded as a single locus in assay work, together with segments of indeterminate length on each side of it. The limiting value for these segments, genetic half-lengths, is $1/n$, where n is the number of backcrosses (Fisher, 1949), but for low values of n the limiting function is approached asymptotically (Hanson, 1959). The mean maximum value in these lines of barley, for two backcrosses and a selfing generation, can be calculated as $E(c)s_{2BX} = 21\cdot6$ map units, but in cases where the breakpoints are positioned distally this value will be an overestimate.

3. MATERIALS AND METHODS

(a) *Stocks*

Maris Badger, a normal line, was used as the donor parent. Mars, represented by homozygous translocation stocks, was used as the recipient. Nine T-lines of Mars were used during the experiment, two of the lines being removed from the programme following trials which showed that they differed greatly from normal Mars.

The translocations were selected to make a series of substitutions which involved each of the seven chromosomes twice, each time in association with a different chromosome. One of the lines, 2–6, was lost because of a crossing error at the second backcross.

The translocations used and the probable locations of the breakpoints are shown in Text-fig. 2. All the Mars stocks were obtained directly from Dr R. T. Ramage, University of Arizona, Tucson, U.S.A., or from the Welsh Plant Breeding Station, Aberystwyth.

(b) *The crossing programme*

The crosses made in the experiment are shown in Text-fig. 3. An F_1 and two backcrosses were made before the selfing generation. An extra generation, to multiply seed of the T'- and S-lines for the subsequent assay trials, was then carried out.

Duplicate lines for each substitution were produced separately to provide an estimate of the error in tests of the effects of substitution.

(c) *The analyses*

The substitution lines and the parental stocks allowed a number of comparisons to be made. An assay of the effects of the substituted chromosome segments on quantitative characters will have two sources of error over and above that contributed by environmental variation, namely variation in genetic background and variation in the amounts of donor chromosomes carried intact in the S-lines.

The differences between duplicates, $(T'_1 + S_1) - (T'_2 + S_2)$, will estimate this total error against which the effect of the substitutions, $(T'_1 + T'_2) - (S_1 + S_2)$, can be

tested. After two backcrosses the background genotype may be expected to contain $12 \cdot 5 \%$ of donor genetic material. The mean effect of genetic 'noise' in the S- and T'-lines can be estimated from the difference $2T - (T'_1 + T'_2)$.
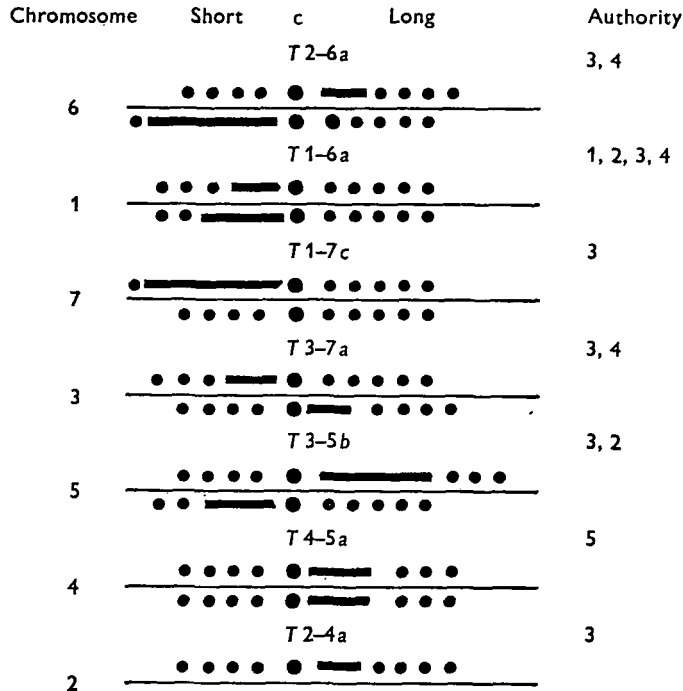

Text-fig. 2. The translocation stocks used in the crossing programme. The solid lines show the probable genetical lengths of the interstitial segments and the broken lines show the genetic half lengths calculated for two backcrosses. Authorities: (1) Burnham *et al.* (1954), (2) Burnham & Hagberg (1956), (3) Ramage, Burnham & Hagberg (1961), (4) Kramer & Blander (1961), (5) Hanson (1952).

A direct method of estimating the extent of the substitutions is possible where segregating major genes are available on the substituted chromosomes. With the varieties used in this experiment such major gene variation is available on chromosome 2, V/v (non-six rowed/six rowed) and on chromosome 5, Mla/mla (resistance to powdery mildew, *Erysiphe graminis*/susceptibility). These checks are discussed below.

4. THE EFFECTS OF HOMOZYGOUS TRANSLOCATION

It is important that the translocations themselves have, when in the homozygous state, little or no effect on the phenotype such as arose from position effects in *Oenothera blandina* (Catcheside, 1939) and *Zea mays* (Roberts, 1942).

The results of two field trials grown in successive years for three quantitative characters are shown in Table 1. The 1966 trial showed that $T\,2\text{--}3a$ was phenotypically very different from normal Mars. For this reason the translocation series was changed to include $T\,1\text{--}7c$ and $T\,3\text{--}5b$ which were grown in the 1967 trial.
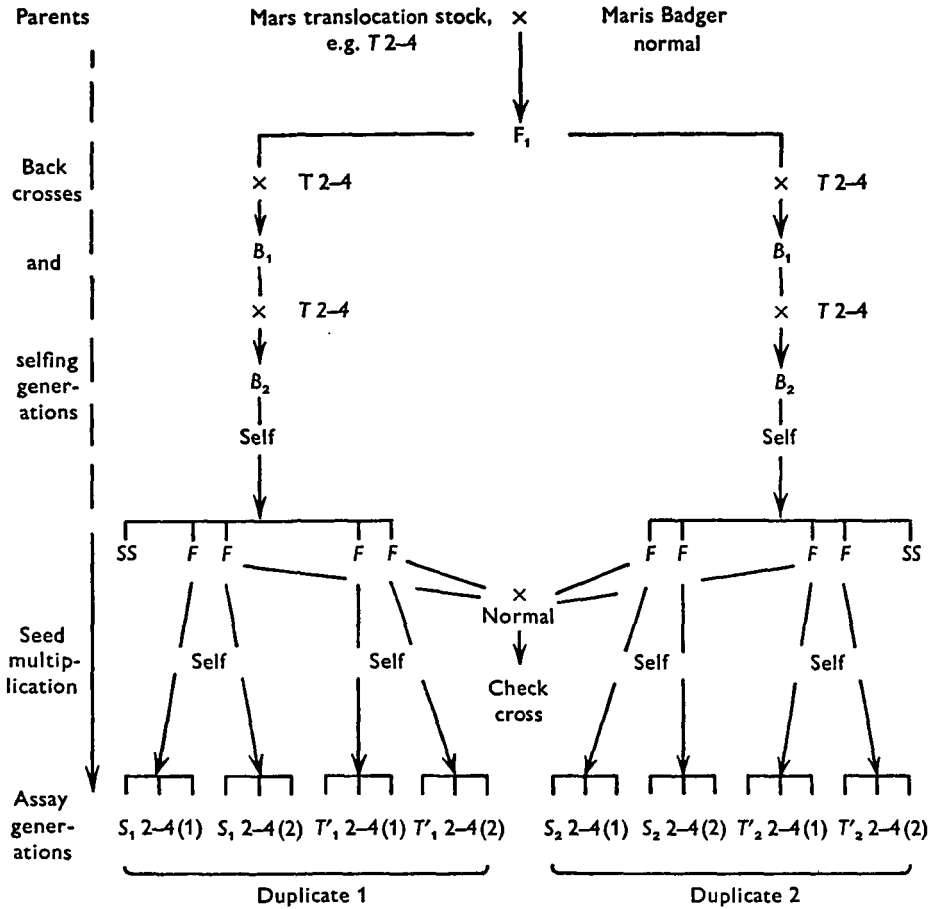
Text-fig. 3. The production of S- and T'-lines in duplicate. From two to four replicate lines within each duplicate S- and T'-lines were raised.

Table 1. *Line means for three characters scored in 1966 and 1967 trials*

	1966			1967		
Line	Ear emergence (days relative to Mars)	Tiller no.	Height (cm)	Ear emergence (days relative to Mars)	Tiller no.	Height (cm)
Maris Badger	+21·8	26·8	85·7	+18·6	35·3	98·8
T 1–6a	− 0·5	10·7	78·8	+1·4	11·7	106·4
T 2–3a	−2·9	1·1*	65·9	—	—	—
T 2–4a	+0·4	10·7	82·0	+1·1	12·6	100·7
T 3–7a	−1·0	14·1	85·1	+1·8	12·9	104·4
T 4–5a	−3·2	14·5	84·7	−0·5	13·2	105·9
T 2–6a	−3·5	14·3	82·4	+3·3*	13·4	103·5
T 3–7a	+5·6*	10·5	80·4	—	—	—
T 1–7c	—	—	—	−5·6*	8·1*	94·0*
T 3–5b	—	—	—	+0·8	12·2	93·4*
Mars	0	11·2	85·1	0	12·8	104·1

* Indicates significance when tested against the normal Mars mean ($P < 0.05$).

Both of these lines had different mean values from the normal Mars genotype in ear emergence time, tiller number and height.

These differences may have been due to position effects or other chromosomal mutations such as deletions. If the differences were caused by position effects then the *S*-lines derived from such stocks would not be expected to show them. However, if the differences were due to deletions on chromosomes other than those involved in the translocation then the aberration and its effect are likely to have been transmitted to the *S*-line. Further reference to the difference between these translocation lines and normal Mars appears in a later section.

5. ASSAY TRIALS

Two trials were grown of the *T'*- and *S*-lines in successive years. In both cases the normal parents and the parental *T*-lines were grown. In each trial 40 *T'*- and *S*-lines were represented, in 1968 each line was represented by five plants in rows randomized within four blocks and in 1969 each line was represented by twelve plants in rows randomized within six blocks.

In 1968 individual plants were scored for tiller characters when vegetative growth had ceased and in 1969 individual plants were scored for ear emergence times, height, spike length, dry weight, grain number, grain weight and yield.

6. RESULTS

In the two trials in successive years the characters scored cannot be directly compared. The 1968 trial was sown late in the season so that differences in ear emergence times and in vegetative characteristics were accentuated to the extent that Maris Badger and some of the later lines did not flower. In 1969 the trial was sown early so that the plants developed normally and all genotypes flowered.

(i) *1968*

(a) *Vegetative index*

The vegetative index, scored as the logarithm of the fraction of the total tillers that had flowered, is a useful general character of which tiller number and ear emergence times are components. The results are summarized in Table 2 and shown in histogram form in Text-fig. 4. The two substitution lines, *S* 2–4 and *S* 3–7, show significant *T'* − *S* values. Of the *T*-lines, only *T* 1–7 differs significantly from normal Mars ($P < 0.05$) and the high vegetative index scored for this genotype is reflected by the values for both *T'* 1–7 and *S* 1–7. The *T*-lines differ from the *T'*-lines on average by 0·30 or 12·3 % of the parental difference.

(b) *Flowering tiller number*

The results for this character are very similar to those for vegetative index which is to be expected since flowering tiller number is a major component of character discussed above. Again the *T'* − *S* differences for lines 2–4 and 3–7 are

Table 2. *Line means for three characters in 1968 and 1969 assay trials*

Line	Vegative index (1968) $\left(\log \dfrac{\text{Flowering tillers}}{\text{Total tillers}}\right)$				Ear emergence (1969) (days relative to Mars)				Height (1969) (cm)			
Mars (recipient) ...	2·61				0				94·4			
Maris Badger (donor) ...	0·29				14·8				103·8			
	T	T'	S	$(T'-S)$	T	T'	S	$(T'-S)$	T	T'	S	$(T'-S)$
1–6	2·67	2·48	2·54	−0·06	−0·4	+4·0	+14·4	−0·4	94·2	99·4	98·4	−0·8
2–4	2·81	2·55	1·07	+1·48**	0	+4·0	+12·8	−8·8*	94·6	108·8	119·5	−10·7*
3–7	2·81	2·28	1·99	+0·29*	+1·3	+5·3	+8·1	−3·8	97·5	103·6	103·4	+0·2
4–5	2·57	2·53	2·58	−0·05	−0·7	+1·7	+0·4	+1·3	95·7	96·2	105·8	−9·6*
1–7	2·95*	2·73	2·88	−0·15	−2·7*	−2·3	−2·3	0	83·2*	96·4	90·3	+6·1
3–5	2·70	2·47	2·60	−0·13	−0·5	+3·7	+0·8	+2·9	94·9	97·9	93·9	+4·1
Mean	2·75	2·45	—	—	−0·3	+2·8	—	—	93·3	100·8	—	—
Mean $(T-T')$	+0·30				−3·09				−7·5			
Parental difference (%)	12·3				28·8				79·8			

Values for the T'- and S-lines are the means of duplicate lines each of which is represented by two to four replicate lines.

Significant differences are shown in the T columns for T-lines differing from normal Mars and in the $T'-S$ column for the effects of substitution.

* $P < 0·05$ and ** $P < 0·01$.

significant and in the direction of the donor parent but, for flowering tiller number, unlike vegetative index, the differences associated with S 3–7 are almost as great as the difference due to S 2–4. Therefore, it may be that other factors transferred in the S 2–4 lines control different components of the complex character vegetative index.

(ii) *1969*

(a) *Ear emergence*

S 2–4 had a significantly later ear emergence time than T' 2–4. As another component of vegetative index this will clearly account for the differences in magnitude in the $T'-S$ values obtained for lines 2–4 and 3–7 for vegetative index and flowering tiller number in 1968. Again the parental T 1–7 differs from the normal Mars in being earlier and the difference is reflected in the T'- and S-lines produced from it. The mean difference $T-T'$ is 3·09 days or 28·8 per cent of the parental difference.

(b) *Height*

Two lines, 2–4 and 4–5, had significant $T'-S$ values for height. T 1–7 and its derivatives all show low values as with the characters discussed above. The mean $T-T'$ value is 7·5 cm or 79·8 % of the parental difference. This is high compared with the 12·5 % expected on a purely additive model after two backcrosses, but

if the donor parent's advantage in height derived from the interaction of a number of positive and negative factors then disruption of this interaction could account for this high figure.
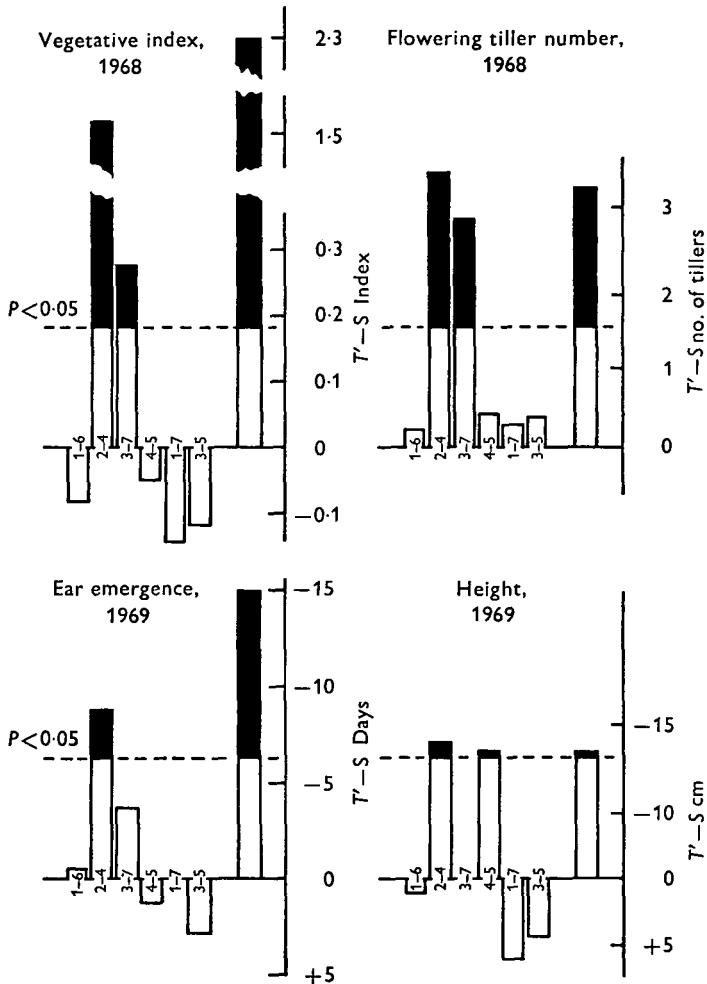
Text-fig. 4. The effects of substitution, i.e. the mean differences between the T'-lines and the S-lines $(T' - S)$ for four quantitative characters for the six substitution lines. The parental differences (Mars − Maris Badger) are shown on the right of each histogram. The shaded areas above the broken line indicate significant differences as tested by duplicate error $(P < 0.05)$.

(c) Other characters

Total tiller number in both trials and dry weight, grain number and yield in 1969, showed no significant $T' - S$ differences. Grain weight and spike length in 1969 both showed differences in the direction of the donor parent for line 2–4. The data for individual plants, however, showed that these differences merely reflected the substitution of the V allele (two-rowed spike) on the long arm of chromosome 2 in this line.

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(d) *Major gene markers*

The efficiency of the substitution technique could be evaluated directly where there were allelic differences between the parental lines with well-mapped loci. On chromosome 2, the locus V/v is located on the same arm as the breakpoint of the T 2–4 translocation. Both of the duplicate S-lines were two-rowed and did not segregate for this character, and the reconstituted T'-lines were homozygous for the recessive six-row allele. This is not evidence that the whole of chromosome 2 had been substituted, but it does not indicate that a substantial part of the donor chromosome had been transferred in two independent lines. A second major gene difference between Maris Badger and Mars is that the recipient is susceptible and the donor resistant to powdery mildew. This difference is controlled by the Mla/mla locus located near the centromere of chromosome 5. All T'-lines for both lines 4–5 and 3–5 were susceptible, the duplicate that was available for S 4–5 was resistant but both duplicates of S 3–5 were susceptible. These results indicate that the locus is on the short arm and that, while large segments of donor chromosomes were transmitted, there was more likelihood of transferring the arm containing the breakpoint for the translocation than the unmodified arm.

7. INTERPRETATION OF THE RESULTS

Before attempting to allocate the factors controlling the characters to individual chromosomes the positions of the translocation breakpoints and the genetic half-lengths, calculated for two backcrosses (Text-fig. 2), must be considered.

Factors affecting vegetative index and tiller number were transferred in substitution lines S 2–4 and S 3–7. The breakpoint on chromosome 4 is in the same position in both T 2–4 and T 4–5. Since the effects were not transmitted to S 4–5 it is most likely that the 'active' chromosome in S 2–4 was chromosome 2, where a large portion of the long arm was transferred. Neither S 3–5 nor S 1–7 showed the effects transmitted to S 3–7 and so the most likely locations of the second factor or factors are the long arm of chromosome 7 or the short arm of chromosome 3. Similarly, the ear emergence factors are likely to be on chromosome 2. The height factor which was transmitted to both S 2–4 and S 4–5 and affected both substitution lines to approximately the same extent, is most likely to be located on chromosome 4. A height factor has, however, been located on chromosome 2 (Mann, 1953) and there is, therefore, a possibility that this chromosome may be involved.

The differences involving T 1–7 were common to most characters scored and had been previously noted in trials of the T-lines (Table 1). The differences were consistently transmitted to both the T'- and the S-lines. This is strong evidence that the divergence of the T-line from the normal parent was not due to a position effect, but to a mutation, possibly a deletion, elsewhere in the complement.

8. DISCUSSION

The chromosome substitutions described in barley were less precise than in wheat and *Drosophila*. The checks on unwanted crossing-over were not absolute and the translocation method demands that chromosomes be transferred in pairs. This last difficulty cannot be overcome by using translocations with one break-point near the centromere since the genetic half-lengths transferred on either side of the centromere will not be insignificant except where many backcrosses are used. Several other factors including uncertainty in location of breakpoints, occasional chiasmata in proximal locations and a possible compensating increase in recombination outside the interstitial segments add to the imprecision.

Nevertheless, the results indicate that the method is viable and could well be improved to become a useful tool. Whole genotypes would be best explored using a series of translocations, similar to the 'all-arms' tester stocks in maize, which had been selected for distal breakpoints. Alternatively, individual chromosomes could be rigorously analysed using a number of interchanges involving that chromosome. The inclusion of markers is an obvious advantage both as a check on the effectiveness of the substitution procedure and to act as a control on the segregation of regions outside the interstitial segments. Since, however, the choice of recipient parent is in practice restricted to the two varieties in which adequate translocations are available, the single gene marker differences will usually be fortuitous. The raising of duplicate lines is necessary both as a further check that the substitution has occurred and as a measure of the heterogeneity of the background genotype. Assuming 2 % recombination in the interstitial segments, the chance of crossing-over in either of the interstitial segments resulting in the loss of that segment in both duplicates after five backcrosses is less than 5 %. Three or more replicates of substitution lines would provide adequate insurance against the loss of any such segments.

The number of backcrosses necessary in a programme of this type will vary with the precision required. Obviously, to obtain a completely isogenic background very many generations of backcrossing will be required, each of which will increase the chance of contamination by recombination. In the present experiment the transmission of differences in quantitative characters could be detected after only two backcrosses. There were, of course, relatively large differences between the two parent genotypes but probably not more than four or five generations would be needed for the detection of most genetic differences.

A further development of the technique might be to produce reciprocal substitutions from the same translocation lines. This could be carried out by backcrossing the same F_1 translocation heterozygote on to the former donor parent. At the selfing generation the T'-lines would be the substitutions of the former recipient into the former donor background and the S-lines would be the reconstituted former donor genotypes. Assays of both sets of reciprocal substitutions would then yield useful information on both the location and interactions of genes for quantitative characters.

17-2

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