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A LINKAGE STUDY INVOLVING TRANSLOCATIONS OF CHROMOSOME 6 IN BARLEY

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "A linkage study involving translocations of chromosome 6 in barley," submitted by Orest Martin Mylyk in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The translocations T1 - 6e, T2 - 6a, T3 - 6d, T4 - 6a, T5 - 6a, T5 - 6b, T5 - 6c, and T6 - 7b were crossed with two lines having markers previously associated with chromosome 6, one line recessive for $\underline{gp_2}$ and and $\underline{gl_4}$ and segregating for $\underline{gs_4}$, and the other recessive for $\underline{gp_2}$, $\underline{cu}$, and $\underline{o}$.

The genes $\underline{gs_4}$ and $\underline{o}$, which are located near the centromere, invariably showed strong linkage with the translocations, this being expected if little or no detectable recombination occurs in interstitial segments or if the translocation breakpoints are near the genes. The gene $\underline{gl_4}$ showed linkage only with the translocations T4 - 6a (5.1 $\pm$ 1.1% recombination) and T5 - 6b (26.8 $\pm$ 5.0% recombination), and $\underline{gp_2}$ and $\underline{cu}$ did not convincingly show linkage with any translocations.

The genes $\underline{gp_2}$, $\underline{gl_4}$, and $\underline{cu}$, if on chromosome 6, appeared to be distant from one another and from the centromere, but evidence from this study indicated that $\underline{gp_2}$ and $\underline{cu}$ may not be located on this chromosome. The possible sequence of the genes and translocations studied was suggested.

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INTRODUCTION

General

Barley has been the subject of genetic research since Tschermak's rediscovery of Mendel's laws (Smith, 1951). Early genetic studies dealt with the inheritance of naturally occurring variants. Cytological work was concerned largely with the determination of chromosome numbers and karyotypes of various species (Smith, 1941).

In recent years, genetic and cytological study has focussed largely on experimentally induced mutations and chromosome aberrations (Nilan, 1964). It has concerned (1) the induction process and modes of action of physical and chemical agents used, (2) the inheritance and cytological behavior of these variants, and (3) the agronomic application of these variants (see Smith, 1951, and Nilan, 1964, for extensive reviews on the genetics and cytology of barley). It is the second aspect with which the study reported here is concerned.

Genetic studies have led, in part, to the elucidation of linkage maps for barley (Robertson *et al.*, 1941, 1947, 1955, 1965). Translocations have facilitated these studies extensively, having permitted the association of linkage groups with chromosomes (Ramage *et al.*, 1961). Moreover, they have aided in the localization of centromere regions (Hanson, 1952; Ramage, 1964) and in the orientation of genes on chromosomes (Kramer and Blander, 1961). In addition they have aided in the cytological localization of some genes (Hagberg and Tjio, 1950; Nilan, 1964), although the inseparability of some phenotypes so localized from translocations suggests that they may be attributable to position effects rather than mutations.

Although seven linkage groups were established in early linkage studies (Robertson et al., 1941), two of these were subsequently associated with one chromosome (Kramer et al., 1954). It was found that the chromosome currently designated as chromosome 6 possessed no known markers. Since this discovery, a number of mutants and translocations have been associated with chromosome 6, but these are not abundant, and the sequence is not particularly well established. The work reported here is a linkage study involving eight translocations and five mutants associated with chromosome 6. It was carried out with the anticipation that localization of the translocation breakpoints in relation to genes would facilitate linkage studies in the future.

Chromosomes in Barley

Barley has seven pairs of chromosomes. Descriptions and designations are based largely on the karyotype studies, descriptions, and measurements of Tjio and Levan (1950) and Tjio and Hagberg (1951). Currently, chromosomes and linkage groups are designated by the Arabic numerals 1 - 7 (Ramage et al., 1961). Chromosomes 1 - 5 are the non-satellited chromosomes in order of length, chromosome 1 being the longest and chromosome 5 the shortest of this group. Chromosomes 6 and 7 are satellited, chromosome 6 being the shorter but having the larger satellite. Chromosome 6 is a strong, and chromosome 7 a weak nucleolus organizer. Other chromosomes occasionally exhibit nucleolar-organizing capacities (Nilan, 1964). Reports about tertiary constrictions observed in chromosomes vary, and some differences probably occur between varieties (Nilan, 1964).

Table 1 relates the present designations of chromosomes and linkage groups with key marker genes and previous designations commonly used in karyotype, linkage, and translocation studies.

Table 1. Current and previous designations of chromosomes and linkage groups in relation to key marker genes.[1]

	Designations							References
Current linkage group and chromosome numbers	1	2	3	4	5	6	7	Burnham and Hagberg 1956 Ramage et al. 1961
Previous designations								
Chromosomes								
from karyotype analyses	I	II	III	IV	V	VI	VII	Tjio and Levan 1950 Tjio and Hagberg 1951
from translocation studies	b	f	c	e	a	g	d	Burnham et al. 1954
Linkage groups	III and VII	I	VI	IV	II	-	V	Robertson et al. 1941
Key marker genes	N,n	V,v	Uz,uz	K,k	B,b	O,o	R,r	Robertson et al. 1965

1. This table is largely adapted from Nilan (1964); see ibid. for additional designations.

Translocation Studies

Both naturally occurring (Smith, 1941; Joachim, 1947; Ramage et al., 1961) and artificially induced translocations (Burnham et al., 1954; Burnham and Hagberg, 1956; Ramage et al., 1961; Ramage, 1964) have been used in genetic and cytological studies in barley. Most of these have been induced by a variety of physical and chemical means, including ionizing radiation, oxygen pressure, calcium deficiency, aging, and chemicals (Nilan, 1964).

Of particular interest have been the translocations resulting from exchanges of terminal segments of non-homologous chromosomes, referred to in the literature synonymously as segmental chromosomal interchanges, chromosomal interchanges, interchanges, reciprocal translocations, or translocations (Burnham, 1956; Ramage, 1964). The term "translocation" in this study refers to a rearrangement of this type.

Behavior of Translocations

Plants homozygous for a translocation usually have an appearance and chromosome behavior indistinguishable from normal plants (Burnham, 1956). Those heterozygous for a translocation, however, are usually characterized by the association at meiosis I of the chromosome pairs involved in the translocation and by semisterility.

Since pachytene chromosomes are usually clumped and knotted and have indistinguishable centromeres (Nilan, 1964), chromosome configurations and the degree of pairing have been largely inferred from the study of later stages of meiosis, and from the observation

of pachytene in other organisms. MacDonald (1961), however, was able to observe pachytene pairing and thus to locate one translocation breakpoint in a plant heterozygous for two translocations. Variable pairing in the region of the breakpoints has been observed in some instances in maize (Burnham, 1948) and may be of importance in linkage studies; but whether this occurs in barley is now known.

The metaphase I configuration resulting from the association of two pairs of chromosomes depends on the location of the breakpoints and on chiasma formation. A ring, a figure-8, a chain, or, if the exchanged segments are small, two bivalents are formed from the chromosomes involved in the translocation. Two alternate or two adjacent chromosomes usually become oriented towards the same pole, with the result that two chromosomes segregate to each pole. Occasionally one pair becomes oriented and the other pair remains uncooriented. Under these conditions two chromosomes move to each pole or three chromosomes move to one pole and one to the other (Hagberg, 1954; Burnham, 1956).

In the absence of crossing-over in the interstitial segments, only alternate segregation results in balanced meiotic products. A single crossover in one interstitial segment, followed by alternate segregation, results in balanced products containing the non-crossover chromatids. A single crossover followed by adjacent-1 segregation[1]. results in balanced products from crossover chromatids. Some multiple crossovers between centromeres (i.e. in the two interstitial segments combined) result in balanced crossover chromatids being recovered from

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1. Adjacent-1 segregation - chromosomes with homologous centromeres move to opposite poles.

alternate or adjacent segregation, depending on the position and numbers of the crossovers. Adjacent-2 segregation[1]. always results in unbalanced products (Hanson and Kramer, 1949; Burnham, 1956; Kramer and Blander, 1961; Ramage, 1964).

Only balanced meiotic products result in functional pollen and ovules, excepting where the resulting deletions and duplications are minute (in which cases, ovules probably function more frequently than pollen; Burnham, 1956). As a result translocation heterozygotes are characterized by pollen and ovule abortion. Hanson and Kramer (1949) found 1 - 12% ovule sterility in normal and translocation homozygotes of parental lines, with a modal class of 1 - 2%, whereas F_2's from crosses showed a bimodal distribution, with maximum-class intervals of 1 - 2% and 23 - 24%, suggesting approximately 25% sterility for translocation heterozygotes. Burnham et al. (1954) reported pollen sterility varying from 14.4 - 58.0%, with a mean of 28.8% in heterozygotes from 27 different lines of single translocations, and ovule abortion varying from 20.6 - 92.2% with a mean of 41.8% in heterozygotes from 26 of these lines (estimates for ovule sterility may not have been as reliable as those for pollen sterility). No translocations were cytologically detectable among fertile plants, but the number examined was small. Shih and Shebeski (1960) found ovule abortion ranging from 26.4 - 61.5% in heterozygotes of crosses from 19 lines of single translocations. The parental plants were fully fertile except for one plant believed to be heterozygous. Most studies have not shown a strong correlation between pollen and ovule fertility (Nilan, 1964).

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1. Adjacent-2 segregation - chromosomes with homologous centromeres move to the same pole.

Since ovule abortion of approximately 25% was found in translocation heterozygotes, Hanson and Kramer (1949) suggested that barley must have a high proportion of alternate segregation. Hence, since alternate segregation following crossing-over in the interstitial regions favors the production of balanced non-crossover spores, the actual frequency of crossing-over is masked (Hanson and Kramer, 1949; Hanson, 1952).

The following considerations concerning the relationship of segregation type and recombination have been derived by Kramer and Blander (1961). The 25% frequency of abortion restricts the frequency of alternate segregation in the absence of crossing-over in the interstitial segments to the range (0.75 - 1.0) and the frequency of crossing-over to (0.0 - 0.5). If alternate segregations with and without crossing-over occur with equal frequency, the maximum detectable recombination between genes at opposite ends of an interstitial segment is less than 3%. As adjacent-1 segregation becomes appreciable (the previous condition no longer holding), the maximum value increases. For example, for alternate segregation always occurring in the absence of crossing-over and alternate and adjacent-1 segregation occurring with equal frequency in the presence of crossing-over, the maximum value is 16.7%. (This, however, requires all crossing-over to occur in one interstitial segment.) Only a high frequency of alternate segregation without crossing-over accompanied by an excess of adjacent-1 segregation following crossing-over raises the maximum above this value. Even when types of segregation favor the recovery of crossover chromatids, the calculated detectable recombination is not particularly high. Furthermore, these values

assume no interference, which would have the effect of further decreasing the detectable recombination fraction.

For translocations involving chromosome 6 with breakpoints in the long arm, the short arm, and the satellite, Ramage found recombination of 1 - 2% in all but one case (this showed 8% recombination; Kramer and Blander, 1961; Ramage, 1964). This suggested that masking of crossing-over in interstitial regions is substantial, and that the lower values calculated by Kramer and Blander (1961) may be the more realistic. From crosses with 11 translocations involving chromosome 2, Ramage (1964) calculated recombination between the genes $\underline{ms}_2$ and $\underline{V}$. In nine cases the values were not significantly different from that found in normal stocks (26%). In two cases recombinations were 3% and 8%, suggesting that the region between $\underline{ms}_2$ and $\underline{V}$ is included, at least in part, in the interstitial segments of these translocations, and that little if any recoverable recombination occurs in these segments.

Translocations in Linkage Studies

The chromosomes involved in a translocation can be identified genetically on the basis of linkage studies. Genes carried on chromosomes involved in a translocation exhibit linkage with semi-sterility (Burnham, 1956, 1957), and, if they are on different chromosomes, show linkage with each other (pseudolinkage; ibid., 1956, 1957). The detection of such linkages depends, to some extent, on the positions of genes and breakpoints. Masking of recombination in the interstitial segments (Kramer and Blander, 1961) facilitates these tests.

The assignment of genes to chromosomes is aided by translocations. Linkage of a gene with semisterility indicates that the gene is probably on a linkage group carried by one of the chromosomes involved in the translocation (Joachim, 1947; Hanson and Kramer, 1950; Burnham, 1956, 1957; Ramage, 1964). Genes that show linkage with a group of translocations having one chromosome in common may be inferred to lie on the common chromosome (Burnham, 1957; Ramage, 1964). In addition, if a translocation shows linkage with at least two genes in a linkage group, and other genes linked with the translocation in the same test are in the same linkage group, the recombination values may be fitted to a linear map. If the genes are in different linkage groups, the recombination values can only be fitted to a T- or a +-shaped map (Burnham, 1957; Ramage, 1964). Because of a masking of recombination in the interstitial regions, linkage values from crosses with translocations are not necessarily indicative of the distance between loci.

Translocations are useful in the orientation of genes on chromosomes (Kramer and Blander, 1961; Ramage, 1964). Homozygotes indicate which genes in two linkage groups are still linked and which are not (Ramage, 1964). Masking of recombination in heterozygotes may be used as an indication of the relative proximity of genes to centromeres. Genes invariably showing strong linkage with translocations having breakpoints in different arms of a chromosome may be considered to be near the centromere. Genes away from the centromere show close linkage only if they are in the interstitial segment or close to it, and any appreciable amount of recombination between genes and breakpoints probably provides the indication that

the genes lie outside of the interstitial segment (Kramer and Blander, 1961; Ramage, 1964).

Designation of Genes and Translocations

Genes and translocations involved in this study are designated by currently accepted symbols (see Ramage et al., 1961; Robertson et al., 1965). To minimize the awkward use of mutant names, gene designations are used to refer to genes, alleles, characters, and phenotypes. Translocation designations refer to chromosome rearrangements or breakpoints. It is believed that the context in each case permits the correct distinction in interpretation.

MATERIALS AND METHODS

Crosses were attempted between eight translocation stocks and two mutant stocks carrying recessive genes associated with chromosome 6. F_2's were grown for linkage analyses. The mutant and translocation stocks are listed in Table 2, and successful crosses are listed in Table 3.

Description of Characters

Curly (cu). "Lemmas and awns are extremely curly; the rachis is usually bent, and the tillers curved or wavy" (Walker et al., 1963).

Glossy leaf-4 (gl_4). "Leaf blades are of a distinctive bright-green color, and lack the blue component of normal green foliage. The green color of the stem and sheath is normal" (Walker et al., 1963).

Glossy sheath-4 (gs_4). "Sheaths and stems at heading show the same color as the leaf blades of glossy leaf-4. The green color of the leaf blades of these mutants is normal" (Walker et al., 1963).

Grandpa-2 (gp_2). "Seedlings are slightly variegated but the plants become predominantly green before heading. At this time the terminal leaves show a high proportion of white tissue towards the tips, and the spike is entirely white" (Walker et al., 1963).

Orange lemma (o). The rachis and tips of lemmas and paleas proximal to the rachis are orange. At maturity stems are

Table 2. Genetic stocks and their origins.[1.]

Mutant stocks	U. of A. Acc. No.	Marker genes present	Designations of genes	Treatment of source material of genes	References
	845	grandpa-2 glossy leaf-4 glossy sheath-4	gp_2 gl_4 gs_4	rad.-beryll. aureomycin aldrin	Walker et al. 1963
	846	grandpa-2 curly orange lemma	gp_2 cu o	rad.-beryll.	Walker et al. 1963

Translocation stocks	U. of A. Acc. No.	Designations of translocations		Break positions in chrom. 6	Treatment of source material of translocations	References
		Present	Previous			
	1002	T1-6e	xT3 (b-g)	S[2.]	X-ray	Barley Genetics I 1964
	1003	T2-6a	c1433 (f-g)	L[2.]	X-ray	Ramage et al. 1961 Barley Genetics I 1964
	1004	T3-6d	xT13 (c-g)	L?[2.]	X-ray	Ramage et al. 1961 Barley Genetics I 1964
	1005	T4-6a	ϒkT2 (e-g)	L?	Chron.ϒ-ray	Ramage et al. 1961
	1006	T5-6a	xT9 (a-g)	L	X-ray	Barley Genetics I 1964
	1007	T5-6b	nT8 (a-g)	L?	neutrons	Ramage et al. 1961
	1008	T5-6c	ϒaT1 (a-g)	S	acute ϒ-ray	Ramage et al. 1961
	1009	T6-7b	xT14 (g-d)	L	X-ray	Ramage et al. 1961 Barley Genetics I 1964

1. Mutant stocks were produced from crosses at the University of Alberta reported by Walker et al. (1963). Translocation stocks were kindly provided by Dr. Ramage.

2. S = short arm; L = long arm; ? = probably in that position.

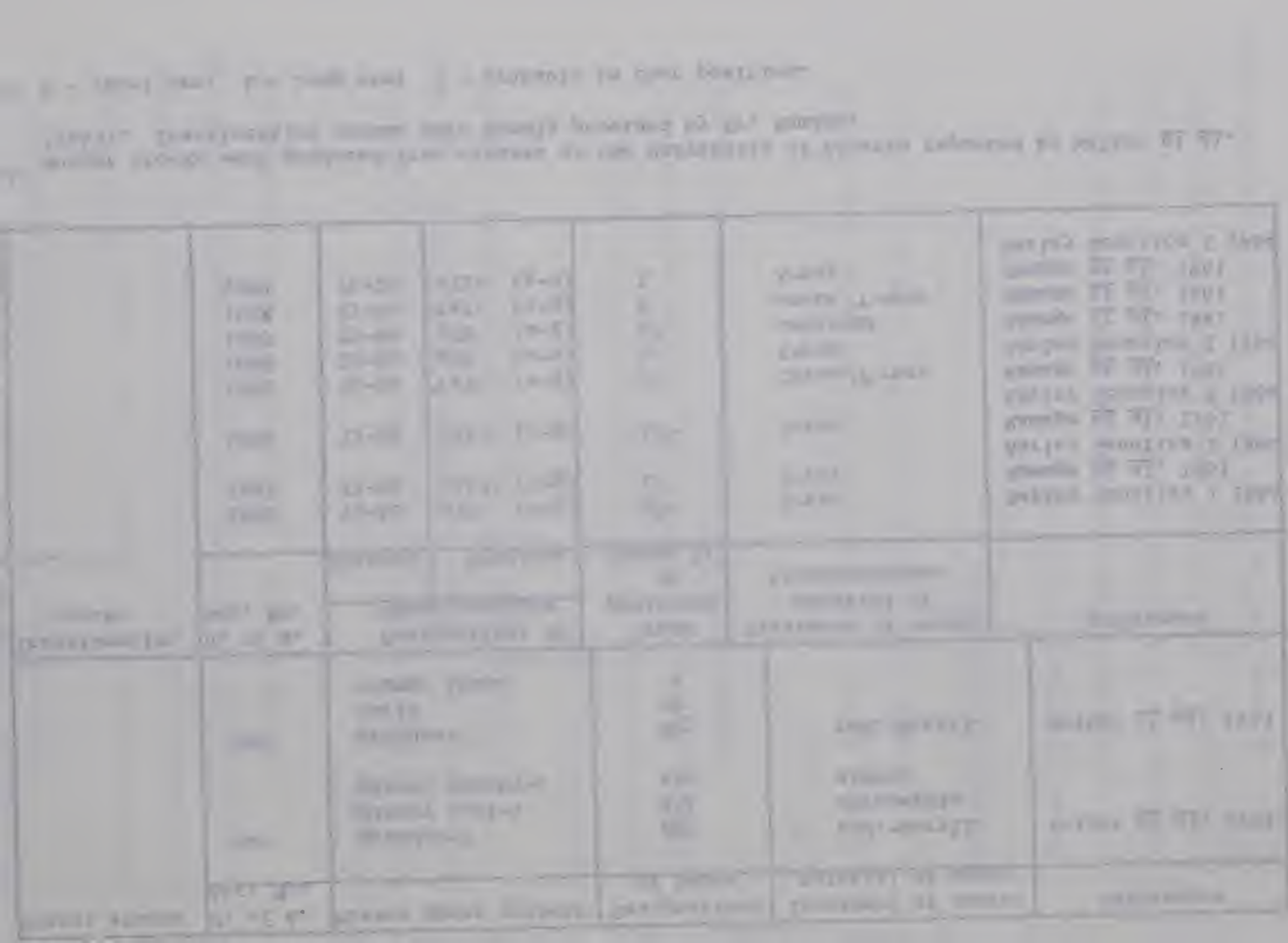


Table 3. List of crosses.

Data set No.[1]	Year F_2 planted	Female parent	Male parent
1	1964	T1 - 6e	gp_2 gl_4 (gs_4)[2]
2	1964	"	gp_2 cu o
3	1966	"	gp_2 gl_4 (gs_4)
4	1964	T2 - 6a	gp_2 gl_4 (gs_4)
5	1966	"	gp_2 gl_4 (gs_4)
6	1964	T3 - 6d	gp_2 gl_4 (gs_4)
7	1964	"	gp_2 cu o
8	1966	"	gp_2 gl_4 (gs_4)
9	1964	T4 - 6a	gp_2 gl_4 (gs_4)
10	1964	"	gp_2 cu o
11	1966	"	gp_2 gl_4 (gs_4)
12	1964	T5 - 6a	gp_2 gl_4 (gs_4)
13	1964	"	gp_2 cu o
14	1966	"	gp_2 gl_4 (gs_4)
15	1964	T5 - 6b	gp_2 gl_4 (gs_4)
16	1964	"	gp_2 cu o
17	1964	T5 - 6c	gp_2 gl_4 (gs_4)
18	1964	"	gp_2 cu o
19	1966	"	gp_2 gl_4 (gs_4)
20	1964	T6 - 7b	gp_2 gl_4 (gs_4)
21	1964	"	gp_2 cu o
22	1966	"	gp_2 gl_4 (gs_4)

1. For significance of this column refer to "Treatment of Data," page 17.

2. This line used as a male parent segregated for gs_4. The symbol for gs_4 is enclosed in brackets to denote uncertainty of the genotype and does not necessarily denote segregation in the F_2.

distinguishable from the normal by a characteristic brown discoloration of the base grading to an orange in the upper part of the stem.

Semisterility. Normal and translocation homozygotes are fully fertile (ff). Translocation heterozygotes are semisterile (ss).

Experimental Procedure

Parental lines were grown in the field at the University of Alberta Parkland Farm during the summer of 1963. Mutant stocks were seeded three times at intervals of approximately ten days. Translocation stocks were seeded twice at similar intervals. Planting parental stocks at intervals increased the chances of heads from mutant and translocation lines simultaneously reaching a stage of flower development appropriate for crossing.

The hand emasculation - pollination technique favored by Bonnett (1930) was employed for making crosses. Heads from translocation stocks were emasculated at a time judged to precede anther dehiscence by one to three days. Lateral florets from one side were removed to make central florets accessible. Lemmas were slit laterally with sharp forceps and the anthers removed. Following emasculation, heads were covered with glassine bags fastened with paper clips in order to prevent florets from drying out and to prevent fertilization by stray pollen.

One to three days following emasculation, when pollination would normally have occurred, glassine bags were removed from the heads, and the heads were pollinated using anthers from mutant stocks. Remaining lateral florets of six-rowed heads were usually removed as

a precaution against self-fertilization. Glassine bags were replaced over the heads until harvest, except for the occasional check for success of seed set.

F_1's were grown in the greenhouse during the winter of 1963-64. Wherever possible, crosses were confirmed cytologically by the observation of rings, figure-8's, or chains, formed from the chromosomes involved in the translocations, at metaphase I in aceto-carmine squashes of pollen mother-cells, or morphologically on the basis of semisterility. F_1 plants were threshed by hand and seeds were cleaned with the aid of an aspirator.

F_2's were grown in the field during the summer of 1964. Individual plants were spaced one foot apart in rows fifteen inches apart. This minimized competition between plants of different phenotypes and made the plants easily accessible for classification. Since crowding of F_1 plants and semisterility resulted in the production of a small number of seeds per plant, seeds from three or four plants were grown, to obtain approximately 350 - 400 plants per F_2. Seeds from different F_1 plants were grown in different plots so that any parental self-pollinations that escaped detection in the F_1 could be eliminated from the data.

F_2 plants were classified for $\underline{gl}_4$ and $\underline{gs}_4$ during or shortly prior to heading and for $\underline{gp}_2$ after heading. Plants exhibiting the recessive form of a character were marked with a tag of a given color to permit identification at a later time. The characters $\underline{cu}$, $\underline{o}$, and semisterility were classified when some plants were already ripe. At this time the complete phenotype for these characters and those classified previously was recorded for each plant.

Whenever F_2 plants that could not be confidently classified for semisterility produced seed, this was collected in the fall of 1964 and grown in the field in 1965. Wherever possible ten seeds per F_2 were planted with the anticipation that seven or more plants would be produced, permitting classification for full fertility versus semisterility with a confidence of 99%.

F_3 progeny were checked for translocation heterozygotes, either on the basis of semisterility or, less frequently, on the basis of metaphase I configurations of chromosomes in aceto-carmine squashes of pollen mother-cells.

Whenever all of seven or more F_3 plants showed full fertility or metaphase I configurations characteristic of normal or translocation homozygotes, the F_2 plant being tested was recorded as fully fertile. When one or more showed either semisterility or metaphase I configurations characteristic of a translocation heterozygote, the F_2 plant was recorded as semisterile.

Segregation ratios for individual characters and linkages involving genes and translocations were calculated from the F_2 results. The genes $\underline{gp_2}$, $\underline{gl_4}$, and $\underline{cu}$ usually showed very little or no linkage with translocation breakpoints. Since a large number of plants is essential for confirming the presence of linkage and estimating the recombination fraction if linkage is weak, additional F_2's from seeds not used previously were grown.

The additional F_2's were space-planted and classified in the field during 1966 in the manner described for 1964 F_2's. The results were incorporated into this report.

Treatment of Data

Since the F_2's from different F_1 plants were grown and classified separately, consolidation from each cross was desirable. A careful preliminary examination of the data for each translocation, involving either a comparison of the raw data or the segregation ratios among single-plant progenies, suggested that it might be best consolidated as follows:

(1) Translocation X Acc. 845 - F_2's grown in 1964

(2) Translocation X Acc. 846 - F_2's grown in 1964

(3) Translocation X Acc. 845 - F_2's grown in 1966

Since the 1966 F_2's were found to be more easily classifiable for semisterility than 1964 F_2's, these were treated separately. F_2 data from some single-plant progenies were eliminated because of conspicuous heterogeneity with data from other progenies. The data sets listed in Table 3 and used in subsequent tables refer to F_2's consolidated according to the above scheme.

Segregation ratios for individual characters, linkages for genes with translocations, and linkages for pairs of genes were tested for each data set. Only plants classified for all gene-controlled characters segregating were included in the data sets (very few plants were excluded due to lack of classifiability for some mutants).

Calculations for segregation ratios of gene-controlled characters and linkages for pairs of genes were performed separately for each of two subsets of each data set. Subset (a) consisted of

those plants clearly classifiable for full fertility versus semisterility; subset (b) consisted of the above plants plus those that could not be classified for full fertility versus semisterility with a reasonable degree of confidence. Significant differences between values calculated for subsets (a) and (b) were attributable to classification. Values for (b) were dependent largely on gene segregation.

Agreement of linkage tests for pairs of genes calculated for (a) and (b) increased the confidence of linkage tests for genes with translocations that could only be calculated on the basis of (a). Since the genes $\underline{gs_4}$ and $\underline{o}$ invariable showed close linkage with translocations, tests involving these genes were particularly helpful. In cases of disagreement between linkage tests on the basis of (a) and (b), the indication for linkage of any gene with $\underline{gs_4}$ and $\underline{o}$ based on (b) was likely a more accurate indication of linkage between the gene and the translocation than the test for linkage between the gene and the translocation itself.

Segregation for full fertility versus semisterility was tested against a 1:1 ratio. Segregation for other characters was tested against a 3:1 ratio of dominant versus recessive phenotypes. Fisher's X^2 described by Mather (1951) and Bailey (1961), with the table provided by Mather, was used. Linkage for genes and breakpoints and also for pairs of genes was tested using the partition of X^2 for linkage (Hanson and Kramer, 1950; Mather, 1951; Bailey, 1961) and the table provided by Mather. Wherever the indication for linkage was sufficiently strong, the recombination fraction was calculated by the product method (Immer, 1930; Joachim, 1947; Mather, 1951;

Bailey, 1961) facilitated by tables provided by Joachim (1947) for linkage of genes with translocations and by Immer (1930) for linkage of pairs of genes. Wherever results were available from several data sets, a X^2 was calculated for pooled data, and a X^2 heterogeneity test was performed.

RESULTS AND DISCUSSION

Plant Vigor and Viability

Some differences were observed in the vigor of plants carrying different traits or combinations of traits. Plants exhibiting only the o mutant were usually as vigorous as normals, and those expressing one of gl_4 and gs_4 were almost as vigorous, showing only a slightly slower rate of maturity. Those manifesting the gp_2 phenotype showed some reduction in vigor, especially in cases of extensive variegation during the seedling stage. (Some gp_2 plants died at this stage.) Those of the cu type were the least vigorous of the single gene mutants.

Combinations of mutant traits usually showed a cumulative effect on vigor, especially when the genes gp_2 and cu were involved. This was true of the non-translocation parents, as well as of F_2 plants. It was noted that the line carrying gp_2, cu, and o, in addition, exhibited very poor germination. This was probably due, in part, to the poor endosperm development that is a general feature of cu plants.

Classification of Mutants

The classification of mutant traits caused little difficulty, but the classification for semisterility was strongly affected by the reduced vigor of mutant types. Plants expressing mutant traits had to be left insufficiently classified for semisterility because their seed sets were so low that a distinction between chromosomal and physiological semisterility could not be made. This was despite the

fact that mutant plants were found with low vigor and yet with high enough seed-sets to be classified as fully fertile. Such a procedure would be expected to reduce the apparent frequency of semisterile recessives, and thus contribute materially to the significance of linkage X^2's; in some cases it may have led to a spurious diagnosis of linkage.

The trait cu was found to exhibit reduced fertility in the parental line. This is in agreement with findings of Walker (personal communication) that plants expressing the cu trait occasionally show partial sterility. Since this may be attributable to low vigor, cu plants having reduced seed set and particularly low vigor were classified for semisterility only where its presence could be confirmed by progeny tests.

Tests for Linkage

In this section data for different translocations are considered separately. In order to aid evaluation of data, all X^2's are retained, even though some heterogeneity tests indicates discrepancies. Recombination frequencies are presented (a) only where linkage is still strongly indicated after a consideration of various other factors contributing to significant X^2's, and (b) where appreciable distortion of a 1:1 ratio of full fertility versus semisterility, favoring linkage, was found in both dominant and recessive classes.

The data are presented in groups of three tables each, one group for each translocation studied. The first table of each group contains tests for the segregation ratios of individual

characters, the second for linkage of genes with translocations, and the third for linkage between genes. Although the segregation data from the first table are used in the evaluation of the linkage tests in the other tables, a discussion of their significance is reserved for the final collective discussion of the segregation data except where they have a particular significance in the interpretation of certain linkage tests.

T1 - 6e

Tables 4, 5, and 6 contain the data from crosses involving the translocation T1 - 6e. Table 5 provides evidence of strong linkage of the genes $\underline{gs_4}$ and $\underline{o}$ with the translocation; the genes $\underline{gp_2}$, $\underline{gl_4}$, and $\underline{cu}$ segregate independently of the breakpoint. Table 6 provides no evidence of linkage between pairs of genes. The significant X^2 for linkage between $\underline{gp_2}$ and $\underline{o}$ is associated with a shortage of parental in comparison with recombinant types, and is likely a result of lethality in double recessive types.

Table 4. Tests for segregation of characters in F_2's of crosses involving T1 - 6e.

Data set No.	Char. tested	Segregation in F_2 plants classified for semisterility				Segregation in total F_2			
		ff	ss	n	X^2 for 1:1				
1	ss	169	173	342	0.047				
2	ss	171	171	342	0.000				
3	ss	217	168	385	6.236*				
		tot. 557	512	1069	6.283				
		X^2 for total			1.894				
		X^2 het. (2 d.f.) =			4.389				
		A	a	n	X^2 for 3:1	A	a	n	X^2 for 3:1
1	gp_2	261	81	342	0.316	269	102	371	1.230
2	gp_2	266	76	342	1.407	284	90	374	0.175
3	gp_2	307	78	385	4.614*	311	82	393	3.584
		tot. 834	235	1069	6.337	tot. 864	274	1138	4.988
		X^2 for total			5.189*	X^2 for total			0.517
		X^2 het. (2 d.f.) =			1.148	X^2 het. (2 d.f.) =			4.472
1	gl_4	257	85	342	0.004	269	102	371	1.230
3	gl_4	299	86	385	1.455	305	88	393	1.426
		tot. 556	171	727	1.459	tot. 574	190	764	2.657
		X^2 for total			0.848	X^2 for total			0.007
		X^2 het. (1 d.f.) =			0.611	X^2 het. (1 d.f.) =			2.649
1	gs_4	82	22	104	0.821	86	25	111	0.363
2	cu	285	57	342	12.667**	303	71	374	7.219**
2	o	269	73	342	2.437	298	76	374	4.367*

* Significant at the 0.05 level.

** Significant at the 0.01 level.

Table 5. Tests for linkage between semisterility and genes in F_2's of crosses involving T1 - 6e.

Data set No.	Gene tested	F_2 segregation					$X_L^{2\,1.}$
		A ss (a)	A ff (b)	a ss (c)	a ff (d)	n	
1	gp_2	134	127	39	42	342	0.250
2	gp_2	136	130	35	41	342	0.561
3	gp_2	137	170	31	47	385	0.195
	tot.	407	427	105	130	1069	1.006
		X_L^2 for total					0.943
		X^2 het. (2 d.f.) = 0.062					
1	gl_4	133	124	40	45	342	0.561
3	gl_4	130	169	38	48	385	0.070
	tot.	263	293	78	93	727	0.632
		X_L^2 for total					0.103
		X^2 het. (1 d.f.) = 0.528					
1	gs_4	54	28	0	22	104	27.128**
		$p = 0.0 \pm 0.0^{2.}$					
2	cu	140	145	31	26	342	0.390
2	o	170	99	1	72	342	78.612**
		$p = 0.8 \pm 0.6$					

** Significant at the 0.01 level.

1. $X_L^2 = \frac{(-a + b + 3c - 3d)^2}{3n}$ (repulsion phase)

2. p = % recombination.

Table 6. Tests for linkage between genes in crosses involving T1 - 6e.

Data set No.	Genes tested X, x	Genes tested Y, y	Phenotypes of F_2 plants classified for semisterility XY (a)	Xy (b)	xY (c)	xy (d)	n	X^2_L [1.]	Phenotypes of total F_2 XY (a)	Xy (b)	xY (c)	xy (d)	n	X^2_L [1.]
1	Gp_2, gp_2	Gl_4, gl_4	193	68	64	17	342	0.812	198	71	71	31	371	0.779
3	Gp_2, gp_2	Gl_4, gl_4	233	74	66	12	385	1.801	235	76	70	12	393	2.552
			tot. 426	142	130	29	727	2.613	tot. 433	157	141	43	764	3.331
			X^2_L for total					2.543	X^2_L for total					0.796
			X^2 het. (1 d.f.) = 0.070						X^2 het. (1 d.f.) = 2.535					
1	Gp_2, gp_2	Gs_4, gs_4	58	17	24	5	104	0.427	59	17	27	8	111	0.001
1	Gl_4, gl_4	Gs_4, gs_4	60	17	22	5	104	0.154	62	18	24	7	111	0.001
2	Gp_2, gp_2	Cu, cu	217	49	68	8	342	1.249	226	58	77	13	374	1.142
2	Gp_2, gp_2	O, o	203	63	66	10	342	2.871	219	65	79	11	374	3.861*
2	Cu, cu	O, o	224	61	45	12	342	0.064	241	62	57	14	374	0.030

* Significant at the 0.05 level.

1. $X^2_L = \frac{(a - 3b - 3c + 9d)^2}{9n}$ (coupling phase)

T2 - 6a

Tables 7, 8, and 9 contain the data from crosses involving the translocation T2 - 6a. Table 8 indicates strong linkage of the translocation with $\underline{gs}_4$ and an apparent linkage with $\underline{gp}_2$ and $\underline{gl}_4$. Table 9 shows no evidence of linkage between the pairs of genes concerned.

Although linkage of $\underline{gp}_2$ with the translocation is indicated, a reappraisal of data sets casts some doubt on the strength of the indication. The classes exhibiting the dominant trait show approximately equal numbers of fully fertile and semisterile individuals, and it is the deficiency of semisterile (and the relative excess of fully fertile) recessives that contributes predominantly to the X^2 value for linkage. The type of distortion described earlier (under "Classification of Mutants") was found to apply to the $\underline{gp}_2$ segregation with semisterility, where weak plants were included in the data when fully fertile but often not when semisterile. Table 7 indicates that most of those plants remaining unclassified for semisterility in Data Set 4 were recessive for $\underline{gp}_2$. This data set yielded a significant X^2 for linkage of the translocation with $\underline{gp}_2$. In contrast, few plants remained unclassified for semisterility in Data Set 5, in which the X^2 for linkage of the translocation with $\underline{gp}_2$ is not significant. These observations support the suggestion that classifiability contributes strongly to the indication of linkage.

Since $\underline{gs}_4$ is closely linked with the translocation, it should show linkage with any other gene linked with the translocation. In Table 9 $\underline{gs}_4$ does not show a strong indication of linkage with $\underline{gp}_2$,

although it must be admitted that the number of plants in F_2's segregating for $\underline{gs}_4$ may be too small to indicate linkage between them.

The validity of the indication for linkage between the translocation and $\underline{gl}_4$ is open to question for similar reasons. Table 8 shows that only Data Set 4, where classification for semi-sterility was less successful, indicates linkage. Table 9 shows that $\underline{gs}_4$ may not be linked with $\underline{gl}_4$, and, consequently, linkage of the translocation with $\underline{gl}_4$ is not supported.

Table 7. Tests for segregation of characters in F_2's of crosses involving T2 - 6a.

Data set No.	Char. tested	Segregation in F_2 plants classified for semisterility				Segregation in total F_2			
		ff	ss	n	X^2 for 1:1				
4	ss	189	170	359	1.006				
5	ss	191	184	375	0.131				
		tot. 380	354	734	1.136				
		X^2 for total			0.921				
		X^2 het. (1 d.f.) =			0.215				
		A	a	n	X^2 for 3:1	A	a	n	X^2 for 3:1
4	gp_2	298	61	359	12.279**	304	78	382	4.276*
5	gp_2	287	88	375	0.470	292	90	382	0.422
		tot. 585	149	734	12.750	tot. 596	168	764	4.698
		X^2 for total			8.649**	X^2 for total			3.693
		X^2 het. (1 d.f.) =			4.101*	X^2 het. (1 d.f.) =			1.005
4	gl_4	276	83	359	0.677	289	93	382	0.087
5	gl_4	285	90	375	0.200	289	93	382	0.087
		tot. 561	173	734	0.877	tot. 598	186	764	0.175
		X^2 for total			0.801	X^2 for total			0.175
		X^2 het. (1 d.f.) =			0.076	X^2 het. (1 d.f.) =			0.000
5	gs_4	226	56	282	3.976*	230	58	288	3.630

* Significant at the 0.05 level.

** Significant at the 0.01 level.

Table 8. Tests for linkage between semisterility and genes in F_2's of crosses involving T2 - 6a.

Data set No.	Gene tested	F_2 segregation					$X_L^{2\,1.}$
		A ss (a)	A ff (b)	a ss (c)	a ff (d)	n	
4	gp_2	150	148	20	41	359	3.923*
5	gp_2	147	140	37	51	375	2.134
	tot.	297	288	57	92	734	6.057
		X_L^2 for total					5.902*[2.]
		X^2 het. (1 d.f.) = 0.155					
4	gl_4	141	135	29	54	359	6.092*
5	gl_4	141	144	43	47	375	0.072
	tot.	282	279	72	101	734	6.164
		X_L^2 for total					3.678
		X^2 het. (1 d.f.) = 2.485					
5	gs_4	143	83	2	54	282	55.149**
		p = 2.0 ± 1.2[3.]					

* Significant at the 0.05 level.

** Significant at the 0.01 level.

1. $X_L^2 = \left(\frac{-a + b + 3c - 3d}{3n}\right)^2$ (repulsion phase)

2. This value may not be valid because segregation for gp_2 was heterogeneous.

3. p = % recombination.

Table 9. Tests for linkage between genes in F_2's of crosses involving T2 - 6a.

Data set No.	Genes tested X, x	Y, y	Phenotypes of F_2 plants classified for semisterility XY (a)	Xy (b)	xy (c)	xy (d)	n	$X_L^{2\,1.}$	Phenotypes of total F_2 XY (a)	Xy (b)	xY (c)	xy (d)	n	$X_L^{2\,1.}$
4	Gp_2, gp_2	Gl_4, gl_4	224	74	52	9	359	1.649	227	77	62	16	382	0.615
5	Gp_2, gp_2	Gl_4, gl_4	221	66	64	24	375	0.655	224	68	65	25	382	0.727
			tot. 445	140	116	33	734	2.304	tot. 451	145	127	41	764	1.343
			X_L^2 for total					$0.102^{2.}$	X_L^2 for total					0.002
			X^2 het. (1 d.f.) = 2.201						X^2 het. (1 d.f.) = 1.340					
5	Gp_2, gp_2	Gs_4, gs_4	169	44	57	12	282	0.266	172	45	58	13	288	0.154
5	Gl_4, gl_4	Gs_4, gs_4	172	37	54	19	282	1.931	174	38	56	20	288	2.000

1. $X_L^2 = \frac{(a - 3b - 3c + 9d)^2}{9n}$ (coupling phase)

2. This value may not be valid because segregation for $\underline{gp_2}$ was heterogeneous.

T3 - 6d

Tables 10, 11, and 12 contain the data from crosses involving the translocation T3 - 6d. Table 11 shows strong linkage of the translocation with $\underline{gs}_4$ and $\underline{o}$, no linkage with $\underline{gl}_4$ or $\underline{cu}$, and a possibility of linkage with $\underline{gp}_2$. Table 12 shows linkage of $\underline{gp}_2$ and $\underline{cu}$. The significant X^2 for linkage of $\underline{cu}$ and $\underline{o}$ is a result of incomplete classifiability since total data favor the independent assortment of the two genes.

Table 11 shows that linkage of the translocation with $\underline{gp}_2$ is indicated most strongly by Data Sets 6 and 7 which Table 10 shows did not approach full classification for semisterility. Plants having the dominant phenotype expressed full fertility and semisterility in essentially equal proportions. In addition, Data Set 8, approaching full classification for semisterility, showed independent assortment of the translocation and $\underline{gp}_2$. Hence the suggestion of linkage with the translocation is subject to doubt.

It is not clear whether the linkage between $\underline{gp}_2$ and $\underline{cu}$ is a true linkage, a pseudolinkage, or a chance deviation.

Table 10. Tests for segregation of characters in F_2's of crosses involving T3 - 6d.

Data set No.	Char. tested	Segregation in F_2 plants classified for semisterility				Segregation in total F_2			
		ff	ss	n	X^2 for 1:1				
6	ss	189	178	367	0.330				
7	ss	202	176	378	1.788				
8	ss	189	184	373	0.067				
		tot. 580	538	1118	2.185				
		X^2 for total			1.578				
		X^2 het. (2 d.f.) =			0.607				
		A	a	n	X^2 for 3:1	A	a	n	X^2 for 3:1
6	gp_2	298	69	367	7.521**	302	90	392	0.871
7	gp_2	300	78	378	3.841*	309	98	407	0.184
8	gp_2	271	102	373	1.095	274	103	377	1.083
		tot. 869	249	1118	12.457	tot. 885	291	1176	2.138
		X^2 for total			4.438*	X^2 for total			0.041
		X^2 het. (2 d.f.) =			8.020	X^2 het. (2 d.f.) =			2.097
6	gl_4	282	85	367	0.662	293	99	392	0.014
8	gl_4	293	80	373	2.510	294	83	377	1.790
		tot. 575	165	740	3.172	tot. 587	182	769	1.804
		X^2 for total			2.883	X^2 for total			0.729
		X^2 het. (1.d.f.) =			0.290	X^2 het. (1 d.f.) =			1.075
8	gs_4	220	61	281	1.624	223	62	285	1.601
7	cu	301	77	378	4.321*	317	90	407	1.809
7	o	294	84	378	1.556	319	88	407	2.477

* Significant at the 0.05 level.

** Significant at the 0.01 level.

Table 11. Tests for linkage between semisterility and genes in F_2's of crosses involving T3 - 6d.

Data set No.	Gene tested	F_2 segregation					X_L^2 [1.]
		A ss (a)	A ff (b)	a ss (c)	a ff (d)	n	
6	gp_2	151	147	27	42	367	2.181
7	gp_2	148	152	28	50	378	3.390
8	gp_2	135	136	49	53	373	0.108
	tot.	434	435	104	145	1118	5.679
		X_L^2 for total					4.438*[2.]
		X^2 het. (2 d.f.) = 1.241					
6	gl_4	136	146	42	43	367	0.044
8	gl_4	144	149	40	40	373	0.022
	tot.	280	295	82	83	740	0.067
		X_L^2 for total					0.065
		X^2 het. (1 d.f.) = 0.001					
8	gs_4	147	73	2	59	281	71.204**
		p = 1.5 ± 1.0[3.]					
7	cu	148	143	28	49	378	2.966
7	o	174	120	2	82	378	76.222**
		p = 1.6 ± 0.9					

* Significant at the 0.05 level.

** Significant at the 0.01 level.

1. $X_L^2 = \left(\frac{-a + b + 3c - 3d}{3n}\right)^2$ (repulsion phase)

2. This value may not be valid because segregation for gp_2 was heterogeneous.

3. p = % recombination.

Table 12. Tests for linkage between genes in F_2's of crosses involving T3 - 6d.

Data set No.	Genes tested X, x	Genes tested Y, y	Phenotypes of F_2 plants classified for semisterility XY (a)	Xy (b)	xY (c)	xy (d)	n	$X_L^{2\,1.}$	Phenotypes of total F_2 XY (a)	Xy (b)	xY (c)	xy (d)	n	$X_L^{2\,1.}$
6	Gp_2, gp_2	Gl_4, gl_4	230	68	52	17	367	0.160	233	69	60	30	392	3.814
8	Gp_2, gp_2	Gl_4, gl_4	210	61	83	19	373	0.775	210	64	84	19	377	1.170
			tot. 440	129	135	36	740	0.935	tot. 443	133	144	49	769	4.984
			X_L^2 for total					0.118	X_L^2 for total					0.406
			X^2 het. (1 d.f.) = 0.817						X^2 het. (1 d.f.) = 4.578*					
8	Gp_2, gp_2	Gs_4, gs_4	158	47	62	14	281	0.731	160	48	63	14	285	0.861
8	Gl_4, gl_4	Gs_4, gs_4	169	49	51	12	281	0.209	170	49	53	13	285	0.141
7	Gp_2, gp_2	Cu, cu	247	53	54	24	378	5.927*	250	59	68	30	407	5.275*
			p = 40.0 $\pm$ 2.3[2].						p = 41.4 $\pm$ 2.3					
7	Gp_2, gp_2	O, o	238	62	56	22	378	1.976	244	65	75	23	407	0.262
7	Cu, cu	O, o	241	60	53	24	378	4.093*	254	63	65	25	407	2.464

* Significant at the 0.05 level.

1. $X_L^2 = \left(\frac{a - 3b - 3c + 9d}{9n}\right)^2$ (coupling phase)

2. p = % recombination

T4 - 6a

Tables 13, 14, and 15 contain the data from crosses involving the translocation T4 - 6a. Table 14 shows strong linkage between the translocation and genes $\underline{gl}_4$, $\underline{gs}_4$, and $\underline{o}$. Genes $\underline{gp}_2$ and $\underline{cu}$ segregate independently of the translocation. Table 15 indicates strong linkage of $\underline{gl}_4$ and $\underline{gs}_4$, which is expected since the translocation is strongly linked with each gene. The significant X^2 for linkage of $\underline{gp}_2$ and $\underline{gl}_4$, in Table 15 indicates a shortage of parental in comparison with recombinant types. However, this is partly due to incomplete classification for semisterility.

Table 13. Tests for segregation of characters in F_2's of crosses involving T4 - 6a.

Data set No.	Char. tested	Segregation of F_2 plants classified for semisterility				Segregation in total F_2			
		ff	ss	n	X^2 for 1:1				
9	ss	198	165	363	3.000				
10	ss	208	176	384	2.667				
11	ss	180	207	387	1.884				
		tot. 586	548	1134	7.550				
		X^2 for total			1.273				
		X^2 het. (2 d.f.) = 6.277*							
		A	a	n	X^2 for 3:1	A	a	n	X^2 for 3:1
9	gp_2	292	71	363	5.731*	296	85	381	1.471
10	gp_2	308	76	384	5.556*	316	89	405	1.976
11	gp_2	292	95	387	0.042	294	98	392	0.000
		tot. 892	242	1134	11.329	tot. 906	272	1178	3.447
		X^2 for total			8.100**	X^2 for total			2.292
		X^2 het. (2.d.f.) = 3.229				X^2 het. (2.d.f.) = 1.155			
9	gl_4	259	104	363	2.579	272	109	381	2.647
11	gl_4	284	103	387	0.538	287	105	392	0.667
		tot. 543	207	750	3.118	tot. 559	214	773	3.313
		X^2 for total			2.704	X^2 for total			2.971
		X^2 het. (1 d.f.) = 0.414				X^2 het. (1 d.f.) = 0.343			
9	gs_4	81	26	107	0.028	85	28	113	0.003
10	cu	326	58	384	20.056**	336	69	405	13.696**
11	o	286	98	384	0.056	305	100	405	0.021

* Significant at the 0.05 level.

** Significant at the 0.01 level.

Table 14. Tests for linkage between semisterility and genes in F_2's of crosses involving T4 - 6a.

Data set No.	Gene tested	F_2 segregation					$X_L^{2\,1.}$
		A ss (a)	A ff (b)	a ss (c)	a ff (d)	n	
9	gp_2	127	165	38	33	363	2.579
10	gp_2	144	164	32	44	384	0.222
11	gp_2	161	131	46	49	387	1.310
	tot.	432	460	116	126	1134	4.112
		X_L^2 for total					$0.001^{2.}$
		X^2 het. (2 d.f.) = 4.111					
9	gl_4	157	102	8	96	363	93.444**
11	gl_4	194	90	13	90	387	96.662**
	tot.	351	192	21	186	750	190.107
		X_L^2 for total					190.096**
		X^2 het. (1 d.f.) = 0.0108					
		$p = 5.1 \pm 1.1^{3.}$					
9	gs_4	52	29	0	26	107	31.779**
10	cu	145	181	31	27	384	2.000
10	o	175	111	1	97	384	102.556**
		$p = 0.6 \pm 0.5$					

** Significant at the 0.01 level.

1. $X_L^2 \left(\frac{-a + b + 3c - 3d}{3n}\right)^2$ (repulsion phase)

2. This value may not be valid because segregation for semisterility was heterogeneous.

3. p = % recombination.

Table 15. Tests for linkage between genes in F_2's of crosses involving T4 - 6a.

Data set No.	Genes tested X, x	Genes tested Y, y	Phenotypes of F_2 plants classified for semisterility XY (a)	Xy (b)	xY (c)	xy (d)	n	X_L^2 [1.]	Phenotypes of total F_2 XY (a)	Xy (b)	xY (c)	xy (d)	n	X_L^2 [1.]
9	Gp_2, gp_2	Gl_4, gl_4	205	87	54	17	363	1.293	209	87	63	22	381	0.539
11	Gp_2, gp_2	Gl_4, gl_4	208	84	76	19	387	2.929	209	85	78	20	392	2.834
			tot. 413	171	130	36	750	4.222	tot. 418	172	141	42	773	3.374
			X_L^2 for total					4.082*	X_L^2 for total					2.939
			X^2 het. (1 d.f.) = 0.140						X^2 het. (1 d.f.) = 0.434					
9	Gp_2, gp_2	Gs_4, gs_4	62	21	19	5	107	0.175	63	22	22	6	113	0.221
9	Gl_4, gl_4	Gs_4, gs_4	80	2	1	24	107	85.534**	84	3	1	25	113	86.735**
			p = 2.7 ± 1.1 [2.]						p = 3.2 ± 1.1					
10	Gp_2, gp_2	Cu, cu	259	49	67	9	384	0.018	262	54	74	15	405	0.046
10	Gp_2, gp_2	O, o	236	72	50	26	384	3.130	242	74	63	26	405	1.159
10	Cu, cu	O, o	241	85	45	13	384	0.296	249	87	56	13	405	1.089

* Significant at the 0.05 level.

** Significant at the 0.01 level.

1. $X_L^2 = \frac{(a - 3b - 3c + 9d)^2}{9n}$ (coupling phase)

2. p = % recombination

T5 - 6a

Tables 16, 17, and 18 contain the data from crosses involving the translocation T5 - 6a. Table 17 shows strong linkage of the translocation with o but no linkage with gp_2, gl_4, or cu. Table 18 shows no linkage between pairs of genes considered since any significant X^2's in this table result from shortages of parental in comparison with recombinant types. Lethality of double mutants probably constitutes the strongest contribution towards these shortages.

Table 16. Tests for segregation of characters in F_2's of crosses involving T5 - 6a.

Data set No.	Char. tested	Segregation of F_2 plants classified for semisterility				Segregation in total F_2			
		ff	ss	n	X^2 for 1:1				
12	ss	189	185	374	0.043				
13	ss	180	158	338	1.432				
14	ss	150	166	316	0.810				
		tot. 519	509	1028	2.285				
		X^2 for total			0.097				
		X^2 het. (2 d.f.) =			2.188				
		A	a	n	X^2 for 3:1	A	a	n	X^2 for 3:1
12	gp_2	290	84	374	1.287	294	109	403	0.901
13	gp_2	251	87	338	0.099	261	98	359	1.011
14	gp_2	233	83	316	0.270	236	86	322	0.501
		tot. 774	254	1028	1.656	tot. 791	293	1084	2.413
		X^2 for total			0.047	X^2 for total			2.381
		X^2 het. (2 d.f.) =			1.609	X^2 het. (2 d.f.) =			0.032
12	gl_4	283	91	374	0.089	301	102	403	0.021
14	gl_4	227	89	316	1.688	231	91	322	1.826
		tot. 510	180	690	1.777	tot. 532	193	725	1.847
		X^2 for total			0.435	X^2 for total			1.016
		X^2 het. (1 d.f.) =			1.342	X^2 het. (1 d.f.) =			0.831
13	cu	270	68	338	4.296*	280	79	359	1.717
13	o	256	82	338	0.099	275	84	359	0.491

* Significant at the 0.05 level.

Table 17. Tests for linkage between semisterility and genes in F_2's of crosses involving T5 - 6a.

Data set No.	Gene tested	F_2 segregation					$X_L^{2\,1.}$
		A ss (a)	A ff (b)	a ss (c)	a ff (d)	n	
12	gp_2	143	147	42	42	374	0.014
13	gp_2	110	141	48	39	338	3.318
14	gp_2	123	110	43	40	316	0.017
	tot.	376	398	133	121	1028	3.349
		X_L^2 for total					1.091
		X^2 het. (2 d.f.) = 2.258					
12	gl_4	137	146	48	43	374	0.513
14	gl_4	115	112	51	38	316	1.367
	tot.	252	258	99	81	690	1.880
		X_L^2 for total					1.739
		X^2 het. (1 d.f.) = 0.141					
13	cu	131	139	27	41	338	1.140
13	o	157	99	1	81	338	87.578**
		$p = 0.8 \pm 0.6^{2.}$					

** Significant at the 0.01 level.

1. $X_L^2 = \frac{(-a + b + 3c - 3d)^2}{3n}$ (repulsion phase)

2. p = % recombination

Table 18. Tests for linkage betweengenes in F_2's involving T5 - 6a

Data set No.	Genes tested X, x	Genes tested Y, y	Phenotypes of F_2 plants classified for semisterility XY (a)	Xy (b)	xY (c)	xy (d)	n	$X_L^{2\,1.}$	Phenotypes of total F_2 XY (a)	Xy (b)	xY (c)	xy (d)	n	$X_L^{2\,1.}$
12	Gp_2, gp_2	Gl_4, gl_4	212	78	71	13	374	4.137*	213	81	88	21	403	3.040
14	Gp_2, gp_2	Gl_4, gl_4	168	65	59	24	316	0.051	169	67	62	24	322	0.001
			tot. 380	143	130	37	690	4.187	tot. 382	148	150	45	725	3.041
			X_L^2 for total					1.809	X_L^2 for total					1.755
			X^2 het. (1 d.f.) = 2.378						X^2 het. (1 d.f.) = 1.286					
13	Gp_2, gp_2	Cu, cu	194	57	76	11	338	3.694	196	65	84	14	359	4.836*
13	Gp_2, gp_2	O, o	183	68	73	14	338	4.272*	192	69	83	15	359	5.150*
13	Cu, cu	O, o	208	62	48	20	338	1.106	217	63	58	21	359	0.572

* Significant at the 0.05 level.

1. $X_L^2 = \frac{(a - 3b - 3c + 9d)^2}{9n}$ (coupling phase)

T5 - 6b

Tables 19, 20, and 21 contain the data from crosses involving the translocation T5 - 6b. Table 20 shows strong linkage of the translocation with o, no linkage with gp_2 or cu, and loose linkage with gl_4. Table 21 shows no linkage between the pairs of genes considered.

Linkage between the translocation and gl_4 is not intense, but the ratio for full fertility versus semisterility in both dominant and recessive classes of plants favors linkage.

Table 19. Tests for segregation of characters in F_2's of crosses involving T5 - 6b.

Data set No.	Char. tested	Segregation in F_2 plants classified for semisterility				Segregation in total F_2			
		ff	ss	n	X^2 for 1:1				
15	ss	167	166	333	0.003				
16	ss	72	70	142	0.028				
		tot. 239	236	475	0.031				
		X^2 for total			0.019				
		X^2 het. (1 d.f.) =			0.012				
		A	a	n	X^2 for 3:1	A	a	n	X^2 for 3:1
15	gp_2	261	722	333	2.027	264	86	350	0.034
16	gp_2	107	35	142	0.009	110	38	148	0.036
		tot. 368	107	475	2.036	tot. 374	124	498	0.070
		X^2 for total			1.550	X^2 for total			0.003
		X^2 het. (1 d.f.) =			0.486	X^2 het. (1 d.f.) =			0.067
15	gl_4	251	82	333	0.025	262	88	350	0.004
16	cu	120	22	142	6.485*	121	27	148	3.604
16	o	115	27	142	2.714	119	29	148	2.306

* Significant at the 0.05 level.

Table 20. Tests for linkage between semisterility and genes in F_2's of crosses involving T5 - 6b.

Data set No.	Gene tested	F_2 segregation					$X_L^{2\,1.}$
		A ss (a)	A ff (b)	a ss (c)	a ff (d)	n	
15	gp_2	128	133	38	34	333	0.289
16	gp_2	53	54	17	18	142	0.009
	tot.	181	187	55	52	475	0.298
		X_L^2 for total					0.158
		X^2 het. (1 d.f.) = 0.141					
15	gl_4	134	117	32	50	333	5.046*
		p = 26.8 ± 5.0[2].					
16	cu	59	61	11	11	142	0.009
16	o	69	46	1	26	142	22.545**
		p = 2.5 ± 1.8					

* Significant at the 0.01 level.

** Significant at the 0.05 level.

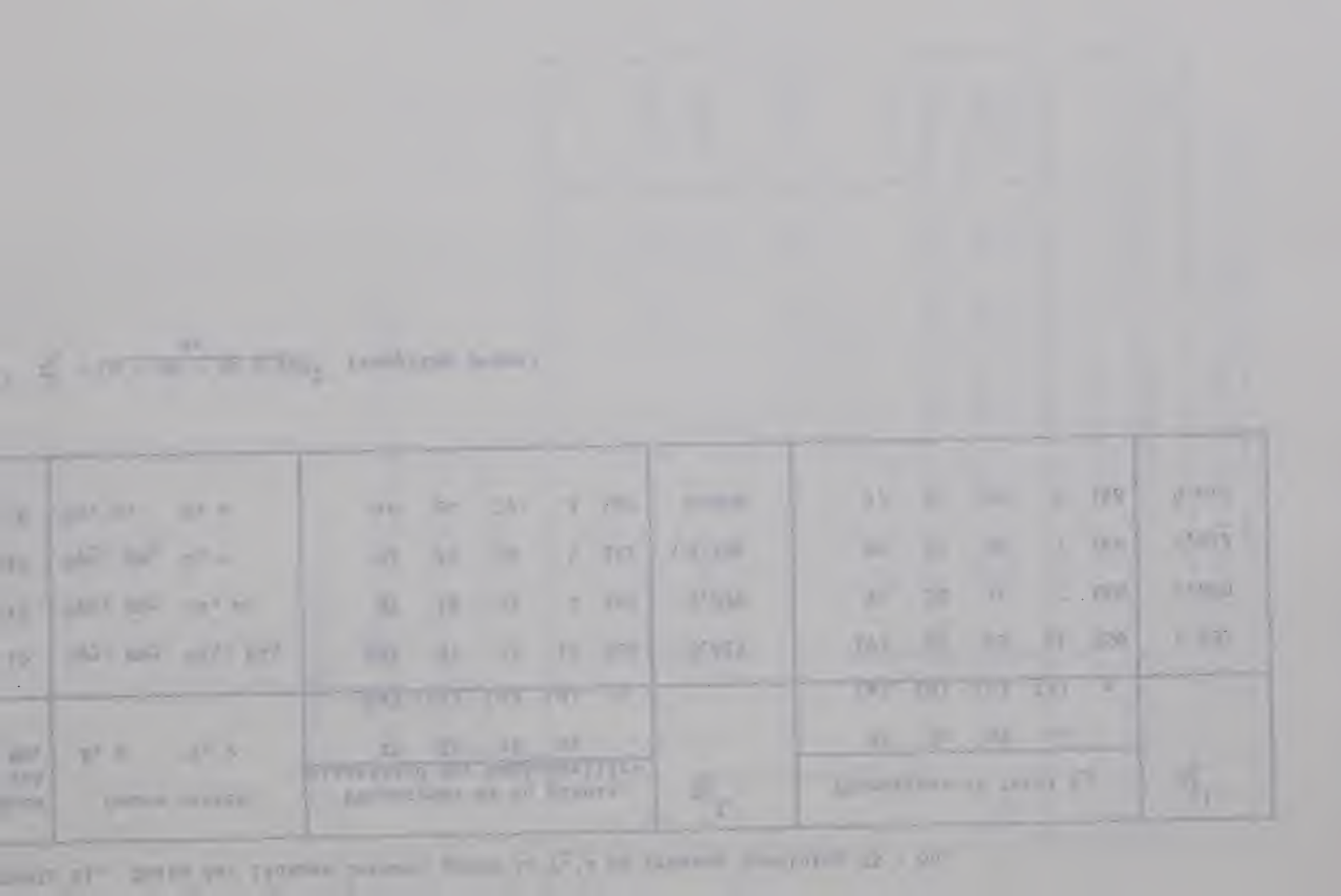
1. $X_L^2 = \frac{(-a + b + 3c - 3d)^2}{3n}$ (repulsion phase)

2. p = % recombination.

Table 21. Tests for linkage between genes in F_2's of crosses involving T5 - 6b.

Date set No.	Genes tested X, x	Genes tested Y, y	Phenotypes of F_2 plants classified for semisterility XY (a)	Xy (b)	xY (c)	xy (d)	n	$X_L^{2\,1.}$	Phenotypes of total F_2 XY (a)	Xy (b)	xY (c)	xy (d)	n	$X_L^{2\,1.}$
15	Gp_2, gp_2	Gl_4, gl_4	194	67	57	15	333	0.617	197	67	65	21	350	0.032
16	Gp_2, gp_2	Cu, cu	89	18	31	4	142	0.379	90	20	31	7	148	0.000
16	Gp_2, gp_2	O, o	85	22	30	5	142	0.529	89	22	30	7	148	0.012
16	Cu, cu	O, o	96	24	19	3	142	0.028	97	24	22	5	148	0.012

1. $X_L^2 = \frac{(a - 3b - 3c + 9d)^2}{9n}$ (coupling phase)



T5 - 6c

Tables 22, 23, and 24 contain the data from crosses involving the translocation T5 - 6c. Table 23 shows strong linkage of the translocation with gs_4 and o, but no linkage with gp_2, gl_4, or cu. Table 24 shows essentially no linkage between pairs of genes.

Table 22. Tests for segregation of characters in F_2's of crosses involving T5 - 6c.

Data set No.	Char. tested	Segregation in F_2 plants classified for semisterility				Segregation in total F_2			
		ff	ss	n	X^2 for 1:1				
17	ss	181	188	369	0.133				
18	ss	180	170	350	0.286				
19	ss	49	44	93	0.269				
		tot. 410	402	812	0.687				
		X^2 for total			0.079				
		X^2 het. (2 d.f.) =			0.609				
		A	a	n	X^2 for 3:1	A	a	n	X^2 for 3:1
17	gp_2	299	70	369	7.155**	306	84	390	2.492
18	gp_2	264	86	350	0.034	274	98	372	0.358
19	gp_2	76	17	93	2.240	76	17	93	2.240
		tot. 639	173	812	9.429	tot. 656	199	855	5.091
		X^2 for total			5.911*	X^2 for total			1.357
		X^2 het. (2 d.f.) =			3.518	X^2 het. (2 d.f.) =			3.734
17	gl_4	284	85	369	0.760	297	93	390	0.277
19	gl_4	71	22	93	0.090	71	22	93	0.090
		tot. 355	107	462	0.849	tot. 368	115	483	0.367
		X^2 for total			0.834	X^2 for total			0.365
		X^2 het. (1 d.f.) =			0.015	X^2 het. (1 d.f.) =			0.001
17	gs_4	162	42	204	2.118	171	45	216	2.000
19	gs_4	65	28	93	1.294	65	28	93	1.294
		tot. 227	70	297	3.412	tot. 236	73	309	3.294
		X^2 for total			0.324	X^2 for total			0.312
		X^2 het. (1 d.f.) =			3.087	X^2 het. (1.d.f.) =			2.982
18	cu	295	55	350	16.095**	308	64	372	12.057**
18	o	264	86	350	0.034	284	88	372	0.358

* Significant at the 0.05 level.

** Significant at the 0.01 level.

Table 23. Tests for linkage between semisterility and genes in F_2's of crosses involving T5 - 6c.

Data set No.	Gene tested	F_2 segregation					$X_L^{2\,1.}$
		A ss (a)	A ff (b)	a ss (c)	a ff (d)	n	
17	gp_2	156	143	32	38	369	0.868
18	gp_2	130	134	40	46	350	0.187
19	gp_2	37	39	7	10	93	0.176
	tot.	323	316	79	94	812	1.230
		X_L^2 for total					1.110
		X^2 het. (2 d.f.) = 0.120					
17	gl_4	146	138	42	43	369	0.109
19	gl_4	33	38	11	11	93	0.090
	tot.	179	176	53	54	462	0.199
		X_L^2 for total					0.026
		X^2 het. (1 d.f.) = 0.173					
17	gs_4	108	54	0	42	204	52.941**
19	gs_4	43	22	1	27	93	35.129**
	tot.	151	76	1	69	297	88.070
		X_L^2 for total					87.364**
		X^2 het. (1 d.f.) = 0.707					
		p = 0.7 ± 0.6[2].					
18	cu	141	154	29	26	350	0.461
18	o	170	94	0	86	350	106.244**
		p = 0.0 ± 0.0					

** Significant at the 0.01 level.

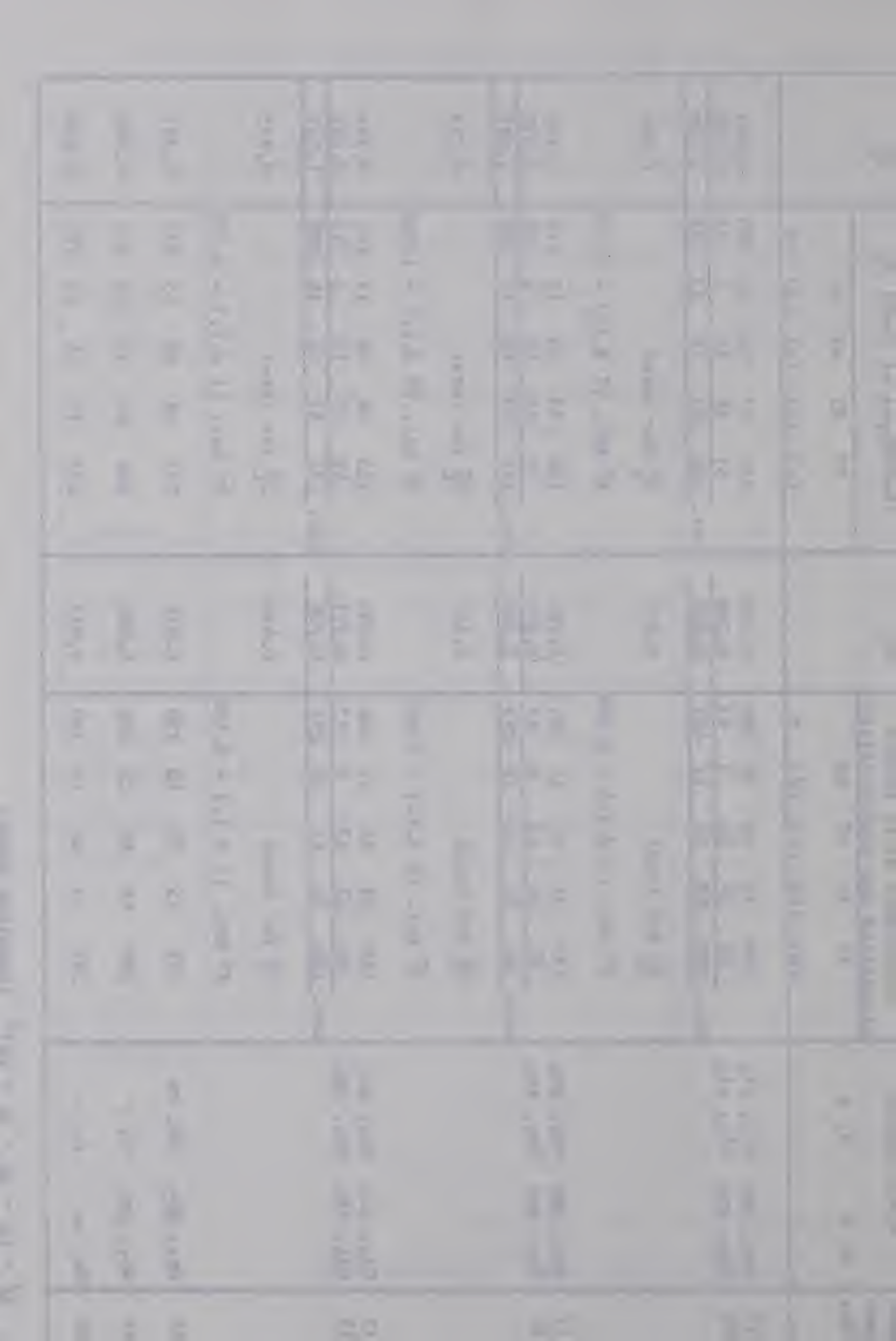
1. $X_L^2 = \left(\frac{-a + b + 3c - 3d}{3n}\right)^2$ (repulsion phase)

2. p = % recombination.

Table 24. Tests for linkage between genes in F_2's of crosses involving T5 - 6c.

Data set No.	Genes tested X, x	Genes tested Y, y	Phenotypes of F_2 plants classified for semisterility XY (a)	Xy (b)	xY (c)	xy (d)	n	$X_L^{2\,1.}$	Phenotypes of total F_2 XY (a)	Xy (b)	xy (c)	xy (d)	n	$X_L^{2\,1.}$
17	Gp_2, gp_2	Gl_4, gl_4	228	71	56	14	369	0.220	233	73	64	20	390	0.001
19	Gp_2, gp_2	Gl_4, gl_4	57	19	14	3	93	0.269	57	19	14	3	93	0.269
			tot. 285	90	70	17	462	0.488	tot. 290	92	78	23	483	0.270
			X_L^2 for total					0.424	X_L^2 for total					0.039
			X^2 het. (1 d.f.) = 0.064						X^2 het. (1 d.f.) = 0.231					
17	Gp_2, gp_2	Gs_4, gs_4	135	31	27	11	204	1.961	138	33	33	12	216	1.185
19	Gp_2, gp_2	Gs_4, gs_4	52	24	13	4	93	0.632	52	24	13	4	93	0.632
			tot. 187	55	40	15	297	2.593	tot. 190	57	46	16	309	1.817
			X_L^2 for total					0.512	X_L^2 for total					0.225
			X^2 het. (1 d.f.) = 2.081						X^2 het. (1 d.f.) = 1.592					
17	Gl_4, gl_4	Gs_4, gs_4	118	31	44	11	204	0.035	123	34	48	11	216	0.296
19	Gl_4, gl_4	Gs_4, gs_4	48	23	17	5	93	0.871	48	23	17	5	93	0.871
			tot. 166	54	61	16	297	0.906	tot. 171	57	65	16	309	1.167
			X_L^2 for total					0.458	X_L^2 for total					0.935
			X^2 het. (1 d.f.) = 0.488						X^2 het. (1 d.f.) = 0.232					
18	Gp_2, gp_2	Cu, cu	219	45	76	10	350	0.926	225	49	83	15	372	0.387
18	Gp_2, gp_2	O, o	200	64	64	22	350	0.062	209	65	75	23	372	0.005
18	Cu, cu	O, o	222	73	42	13	350	0.011	233	75	51	13	372	0.234

1. $X_L^2 = \frac{(a - 3b - 3c + 9d)^2}{9n}$ (coupling phase)



T6 - 7b

Tables 25, 26, and 27 contain the data from crosses involving the translocation T6 - 7b. Table 26 shows strong linkage of the translocation with $\underline{gs}_2$ and $\underline{o}$. The gene $\underline{gl}_4$ segregates independently and $\underline{gp}_2$ may do so as well although the data are heterogeneous. The gene $\underline{cu}$ shows linkage also, but the indication is not particularly strong. Table 27 indicates essentially no linkage between the pairs of genes considered.

The source of heterogeneity for linkage between the translocation and $\underline{gp}_2$ in Table 26 may be a result of classification for semisterility. In the three data sets the class of plants dominant for $\underline{gp}_2$ shows no shortage of fully fertile plants that would characterize linkage. Only the shortage of semisterile recessives in Data Set 22 suggests linkage. Hence independent assortment is favored.

Data Set 21, in Table 26, indicates linkage of $\underline{cu}$ with the translocation. Table 27, however, shows no linkage of $\underline{o}$ with $\underline{cu}$ which would be expected if $\underline{cu}$ were closely linked with the translocation. Table 25 indicates distorted segregation ratios for semisterility and for $\underline{cu}$, while the segregation for $\underline{o}$ is good. The treatment of distorted segregations for semisterility and $\underline{cu}$ as possible causes for the apparent contradiction is pursued below.

> Suppose that plants were added to the F_2 to restore good segregation ratios for individual characters. They would usually have to be semisterile

in order to approach a 1:1 ratio for full fertility versus semisterility, and would usually have to be cu in order to restore a 3:1 ratio of Cu versus cu. These plants could seldom be o because there is already a slight excess of that phenotype over the expectation. This would therefore mean an increase in the proportion of semisterile cu plants, and hence the weakening of the linkage indication between cu and the translocation. Similarly the indication for linkage between cu and o, which is not significant, would be further weakened.

Since the shortage of cu plants was highly significant, it appears that the apparent contradiction discussed above is a result of this shortage. The indication of independent segregation between cu and o, although being hampered by distorted segregation of cu, is not hampered by other factors. Consequently, independent assortment of cu and the translocation is favored by the independent assortment of this pair of genes, on the basis of plants classified for semisterility, and on the basis of the total F_2.

Table 25. Tests for segregation of characters in F_2's of crosses involving T6 - 7b.

Data set No.	Char. tested	Segregation in F_2 plants classified for semisterility				Segregation in total F_2			
		ff	ss	n	X^2 for 1:1				
20	ss	196	181	377	0.597				
21	ss	196	158	354	4.079*				
22	ss	148	126	274	1.766				
		tot. 540	465	1005	6.442				
		X^2 for total			5.597*				
		X^2 het. (2 d.f.) =			0.845				
		A	a	n	X^2 for 3:1	A	a	n	X^2 for 3:1
20	gp_2	296	81	377	2.484	299	87	386	1.247
21	gp_2	296	58	354	14.015**	306	68	374	9.273**
22	gp_2	196	78	274	1.757	202	84	286	2.914
		tot. 788	217	1005	18.255	tot. 807	239	1046	13.433
		X^2 for total			6.225*	X^2 for total			2.581
		X^2 het. (2 d.f.)=			12.030**	X^2 het. (2 d.f.)=			10.852**
20	gl_4	285	92	377	0.072	293	93	386	0.169
22	gl_4	193	81	274	3.041	201	85	286	3.399
		tot. 478	173	651	3.113	tot. 494	178	672	3.568
		X^2 for total			0.861	X^2 for total			0.794
		X^2 het. (1 d.f.) =			2.252	X^2 het. (1 d.f.) =			2.774
20	gs_4	302	75	377	5.242*	309	77	386	5.254*
22	gs_4	138	46	184	0.000	144	47	191	0.016
		tot. 440	121	561	5.242	tot. 453	124	577	5.270
		X^2 for total			3.523	X^2 for total			3.790
		X^2 het. (1 d.f.) =			1.719	X^2 het. (1 d.f.) =			1.480
21	cu	304	50	354	22.331**	317	57	374	18.998**
21	o	263	91	354	0.094	281	93	374	0.004

* Significant at the 0.05 level.

** Significant at the 0.01 level.

Table 26. Tests for linkage between semisterility and genes in F_2's of crosses involving T6 - 7b.

Data set No.	Gene tested	F_2 segregation					X_L^{2} [1.]
		A ss (a)	A ff (b)	a ss (c)	a ff (d)	n	
20	gp_2	136	160	45	36	377	2.300
21	gp_2	131	165	27	31	354	0.456
22	gp_2	99	97	27	51	274	6.662**
	tot.	366	422	99	118	1005	9.417
		X_L^2 for total					0.000 [2.]
		X^2 het. (2 d.f.) = 9.417**					
20	gl_4	138	147	43	49	377	0.072
22	gl_4	89	104	37	44	274	0.044
	tot.	227	251	80	93	651	0.115
		X_L^2 for total					0.115
		X^2 het. (1 d.f.) = 0.000					
20	gs_4	180	122	1	74	377	67.842**
22	gs_4	82	56	1	45	184	45.225**
	tot.	262	178	2	119	561	113.066
		X_L^2 for total					112.433**
		X^2 het. (1 d.f.) = 0.633					
		p = 1.1 $\pm$ 0.6 [3.]					
21	cu	146	158	12	38	354	4.102*
21	o	157	106	1	90	354	95.220**
		p = 0.7 $\pm$ 0.5					

* Significant at the 0.05 level.
** Significant at the 0.01 level.

1. $X_L^2 = \frac{(-a + b + 3c - 3d)^2}{3n}$ (repulsion phase)

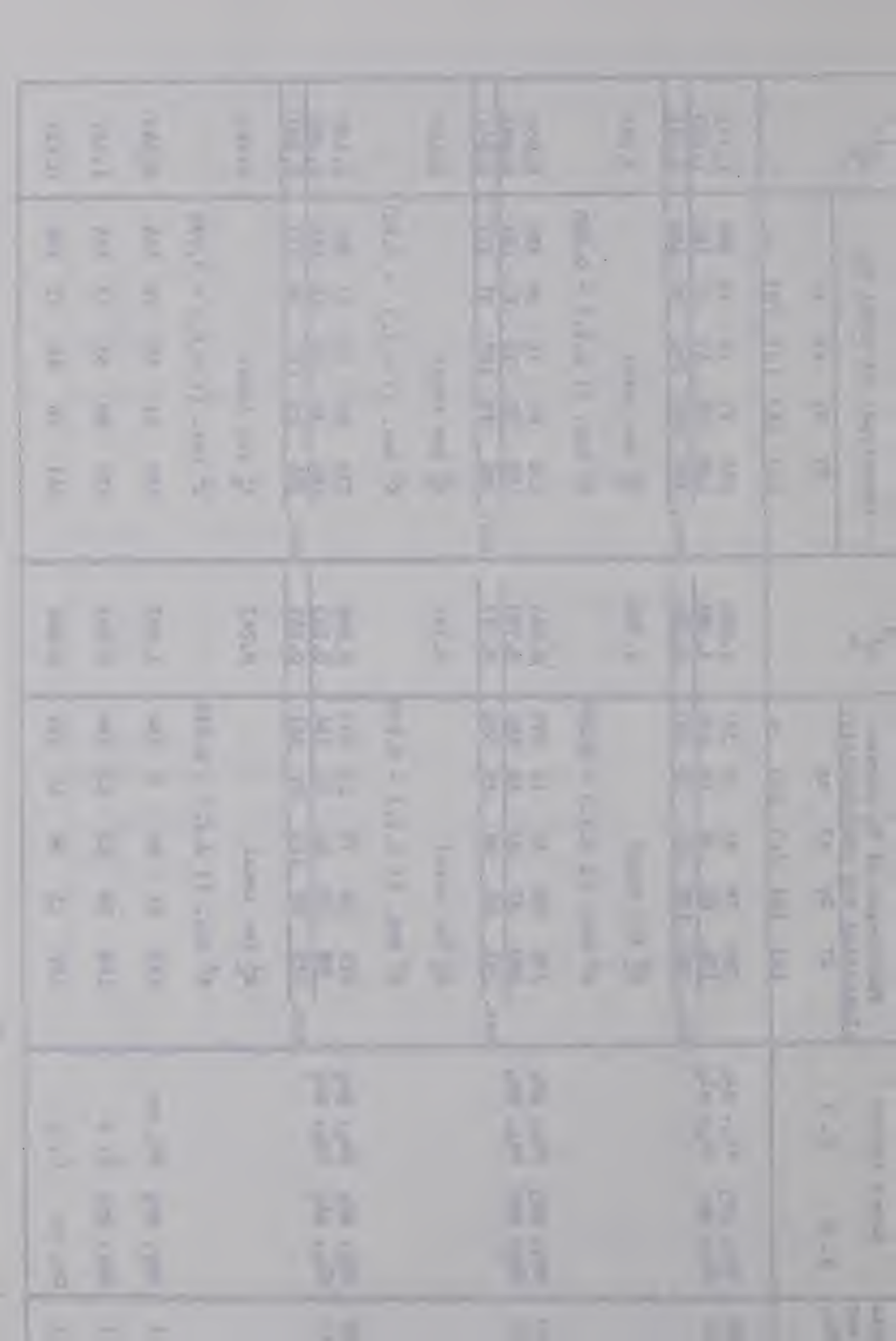
2. This value may not be valid because segregation for gp_2 was heterogeneous.

3. p = % recombination

Table 27. Tests for linkage between genes in F_2's of crosses involving T6 - 7b.

Data set No.	Genes tested X, x	Genes tested Y, y	Phenotypes of F_2 plants classified for semisterility XY (a)	Xy (b)	xY (c)	xy (d)	n	X_L^2 [1.]	Phenotypes of total F_2 XY (a)	Xy (b)	xY (c)	xy (d)	n	X_L^2 [1.]
20	Gp_2, gp_2	Gl_4, gl_4	220	76	65	16	377	1.026	222	77	71	16	386	1.751
22	Gp_2, gp_2	Gl_4, gl_4	134	62	59	19	274	1.364	138	65	63	21	282	1.280
			tot. 354	138	124	35	651	2.390	tot. 360	142	134	37	668	3.031
			X_L^2 for total					2.336	X_L^2 for total					3.031
			X^2 het. (1 d.f.) = 0.054						X^2 het. (1 d.f.) = 0.000					
20	Gp_2, gp_2	Gs_4, gs_4	236	60	66	15	377	0.014	238	61	71	16	386	0.056
22	Gp_2, gp_2	Gs_4, gs_4	105	32	33	14	184	0.783	108	32	36	15	191	0.885
			tot. 341	92	99	29	561	0.797	tot. 346	93	107	31	577	0.941
			X_L^2 for total					0.167	X_L^2 for total					0.120
			X^2 het. (1 d.f.) = 0.630						X^2 het. (1 d.f.) = 0.821					
20	Gl_4, gl_4	Gs_4, gs_4	227	58	75	17	377	0.106	233	60	76	17	386	0.139
22	Gl_4, gl_4	Gs_4, gs_4	98	34	40	12	184	0.155	101	35	43	12	191	0.364
			tot. 325	92	115	29	561	0.261	tot. 334	95	119	29	577	0.503
			X_L^2 for total					0.243	X_L^2 for total					0.425
			X^2 het. (1 d.f.) = 0.018						X^2 het. (1 d.f.) = 0.078					
21	Gp_2, gp_2	Cu, cu	255	41	49	9	354	1.367	259	47	58	10	374	0.343
21	Gp_2, gp_2	O, o	218	78	45	13	354	0.363	226	80	55	13	374	1.142
21	Cu, cu	O, o	229	75	34	16	354	0.664	241	76	40	17	374	0.629

1. $X_L^2 = \frac{(a - 3b - 3c + 9d)^2}{9n}$ (coupling phase)



Distorted Character Segregation

Tentative explanations given earlier regarding the cause of distorted F_2 segregations have a bearing on the possibility of accommodating the data for these to provide more valid estimations of linkage. Some generalization of these explanations is possible. In many progenies concerned the distortions occurred only in the portion that could be reliably classified for semisterility, and hence are probably to be attributed to this deficiency in classification. Others, especially those for gp_2 and cu, are characteristic of total progenies; and since the viable plants showing these characters showed lower vigor than did normal plants, the most likely explanation for the distorted ratios is differential viability.

Mather (1951) and Bailey (1961) discuss modifications of X^2_L where two characters show differential viability. However, these modifications assume that viability effects operate independently. Since multiple mutants often have very low vigor, the assumption is not necessarily justifiable in this study. Most distortions of two characters are, moreover, in F_2's classified for semisterility, and hence in these cases the modification for differential viability would not be valid.

Orientation of Genes and Translocations

The gene o is close to the centromere of chromosome 6 (Kramer and Blander, 1961; Ramage, 1965). The gene gs_4 is close to o (Walker et al., 1963). In this study gs_4 or o invariable show close linkage with each translocation. On the basis of these genes

alone, it is not possible to make assertions regarding the proximity of each translocation to them or to the centromere. The genes $\underline{gl}_4$, $\underline{gp}_2$, and $\underline{cu}$, moreover, cannot unquestionably be assigned to chromosome 6 on the basis of the data from crosses reported here. Consequently various possibilities have to be considered in regard to orientation.

Walker *et al.* (1963) found 29.4 ± 2.6% recombination between $\underline{gl}_4$ and $\underline{gs}_4$. In crosses reported here involving six translocations, five cases show no linkage between these genes. The remaining case shows strong linkage, probably attributable to masking of recombination in the interstitial segment (recombination was not tested between these genes in crosses with the remaining two translocations because $\underline{gs}_4$ did not segregate). The data from these crosses suggest that if $\underline{gl}_4$ and $\underline{gs}_4$ are on the same chromosome, they are not close together.

The gene $\underline{gl}_4$ shows 5.1 ± 1.1% recombination with T4 - 6a and 26.8 ± 5.0% recombination with T5 - 6b, and may possibly be linked with T2 - 6a, although the indication is not strong. The three translocations cited here likely have breakpoints located in the long arm of chromosome 6, although the evidence for this with regard to the first two is not conclusive (see Table 2). The data found here indicate, at least, that the two are both in the same arm.

If the above translocations are all in the long arm, $\underline{gl}_4$ must be in the same arm and distant from the centromere. The translocations T3 - 6d and T6 - 7b, believed to be in this arm as well (see Table 2), in order not to show linkage with $\underline{gl}_4$, must then

have breakpoints near the centromere. This is consistent with locations suggested in Barley Genetics I (1964). T5 - 6a, believed to be in the long arm (see Table 2), must also have a breakpoint near the centromere since it shows no linkage with $\underline{gl}_4$. The possibility has not definitely been excluded, however, that $\underline{gl}_4$ is in the short arm[1]. In this case T4 - 6a and T5 - 6b would also have to be in the short arm, and T1 - 6e and T5 - 6c, believed to be in the short arm, would have to be close to the centromere in order to show no linkage with $\underline{gl}_4$.

In any of the data sets studied, the genes $\underline{gp}_2$ and $\underline{cu}$ do not convincingly show linkage with the translocations involved. Only in one case (involving T3 - 6d) is linkage between $\underline{gp}_2$ and $\underline{cu}$ found (in which case it cannot be clearly explained, see page 32), and in no cases is it found between $\underline{gp}_2$ and $\underline{gl}_4$. In addition $\underline{cu}$ and $\underline{gl}_4$ cannot be close together since $\underline{cu}$ does not show linkage with the translocation with which $\underline{gl}_4$ shows linkage. Consequently the genes $\underline{gp}_2$, $\underline{gl}_4$, and $\underline{cu}$, if on chromosome 6, must lie at a considerable distance from one another and from the centromere region, and $\underline{gp}_2$ and $\underline{cu}$ must be distant from the breakpoints of all of the translocations studied.

Assuming $\underline{gl}_4$ to be in the long arm of chromosome 6, then $\underline{gp}_2$ or $\underline{cu}$, if on this chromosome, must be in the short arm, distant from the centromere, or in the long arm, beyond $\underline{gl}_4$. If $\underline{gl}_4$ is in the short arm, $\underline{gp}_2$ or $\underline{cu}$ must be in the long arm, distant from the centromere, or in the short arm, beyond $\underline{gl}_4$. Each of these

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1. Short arm, in this discussion, includes the satellite.

possibilities requires the location of breakpoints of most other translocations used in this study to be near the centromere.

A possibility, although not a very strong one, is that $\underline{gl_4}$ is not on chromosome 6. Since T4 - 6a is strongly linked with $\underline{gl_4}$, $\underline{gl_4}$ may be on chromosome 4. Such a possibility increases the possibility that $\underline{gp_2}$ and $\underline{cu}$ are both on chromosome 6.

Since $\underline{gp_2}$ shows a possibility of linkage with several translocations involved in the crosses, and since it shows independent assortment with markers on all chromosomes except 4 and 6 (Walker *et al.*, 1963), it may best be suggested to be on chromosome 6. If this is the case it is probably in the short arm, and translocations T1 - 6e and T5 - 6c are near the centromere. The location of T1 - 6e, in this case, is consistent with the location suggested in Barley Genetics I (1964). Another possibility, although not a particularly strong one, is that $\underline{gp_2}$ is on chromosome 2, since T2 - 6a probably shows as strong a linkage with $\underline{gp_2}$ as does any other translocation tested.

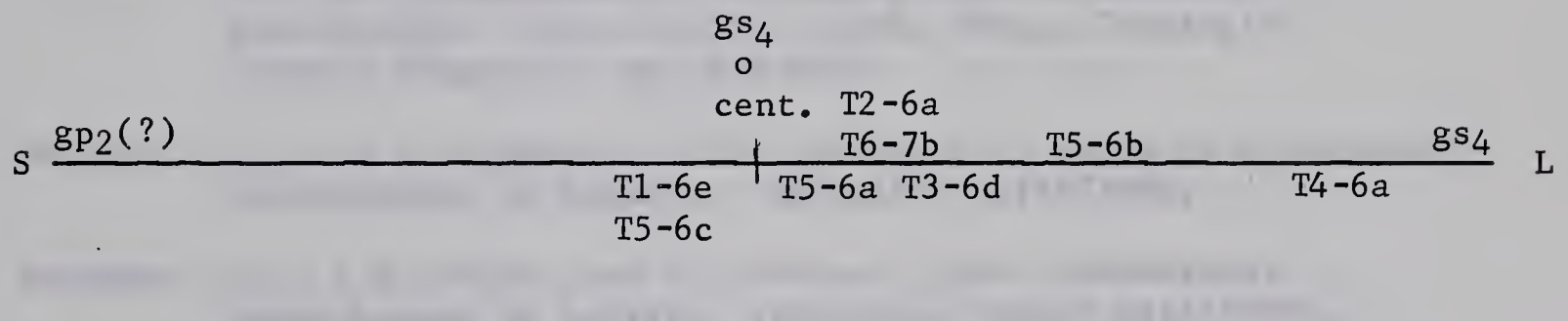
The gene $\underline{cu}$ appears most likely to be on a chromosome other than chromosome 6. If the linkage of $\underline{cu}$ and $\underline{gp_2}$ in the cross involving T3 - 6d is a pseudolinkage, and if $\underline{gp_2}$ is located on chromosome 6, $\underline{cu}$ may be on chromosome 3.

The gene $\underline{gl_4}$, probably on chromosome 6, should be checked for location in the long or short arm. The gene $\underline{gp_2}$, if on chromosome 6, should be checked for location in the short arm. The gene $\underline{x_n}$ and the translocation T1 - 6a in the long and short arm, respectively, may serve this purpose.

The gene cu should be tested for linkage with widely-separated markers on chromosome 3, and, if no linkage is found, with widely separated markers on other chromosomes.

Figure 1 contains the possible sequence of genes and translocations used in this study. It contains information obtained from previous studies (Ramage et al., 1961; Barley Genetics I, 1964) as well as this one, and assumes gl_4 and gp_2 to be in the long and short arms of chromosome 6 respectively. Loci known to be closely linked or believed to be in approximately the same region are grouped.

Fig. 1. Sequence on chromosome 6 of genes and translocations involved in this study, based on the information obtained in this and previously reported studies.



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