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High recombination between the breakpoint of a reciprocal translocation in rye (*Secale cereale* L.) and an interstitially located gene

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Summary. The recombination fraction between the interstitially located gene *an* and interchange 303 of rye was found to be 0.244 ± 0.038 in a test cross using the translocation as the male parent. In first metaphase translocation configurations in pollen mother cells of the same plant, the chiasma frequency between *an* and the translocation breakpoint was found to be significantly more than twice the recombination fraction. Recombination was concluded to be masked by a difference in the alternate frequency between configurations without interstitial chiasmata and configurations with interstitial chiasmata, the effect of the first type being of major importance. Random centromere orientation of translocation multivalents with interstitial chiasmata was concluded to be a realistic assumption. The exceptionally high recombination between *an* and translocation 303 is discussed. Consideration is also given to the use of interchanges in the establishment of a marker's chromosomal position, and to the use of translocation chromosomes in balanced systems for hybrid breeding purposes.

Key words: *Secale cereale* L. – Translocations – Interstitial chiasma formation – Centromere orientation – Recombination

Introduction

In interstitial segments of interchange heterozygotes, chiasma formation and consequently recombination are usually reduced due to disturbed pairing around the translocation breakpoint. In addition, observed recombination is affected by the orientation – alternate or adjacent – of the translocation configuration (Lamm 1948). Kramer and Blander (1961) presented formulae describing the influence of centromere orientation and interstitial chiasma frequency on the observed recombination fraction between interstitially located genes and the translocation breakpoint. In their report the frequencies of alternate orientation and interstitial chiasma formation were based on the level of semisterility, while no further cytological observations were made.

The present article deals with the recombination between a reciprocal translocation of rye (*Secale cereale* L.) and an interstitially located morphological marker with monogenic recessive inheritance. The relation between observed recombination, the observed frequencies of first metaphase (MI) configurations formed by the interchange and their orientation is analyzed. In addition, some consideration is given to the interpretation of recombination fractions between genes and translocations in determining the chromosomal positions of marker genes, and to the use of translocations in balanced systems, set up for the maintenance of genetically male sterile seed stocks for hybrid breeding purposes.

Model: relation between meiotic configuration frequencies and recombination fraction

In karyotypically normal diploids, with maximally one chiasma between two loci, the chiasma frequency is twice the recombination fraction. For a gene located interstitially in an interchange heterozygote (Fig. 1), the degree in which the chiasma frequency between the gene and the breakpoint is reflected in the recombination fraction is determined by the centromere segregation (alternate, adjacent-1 or adjacent-2). Considering

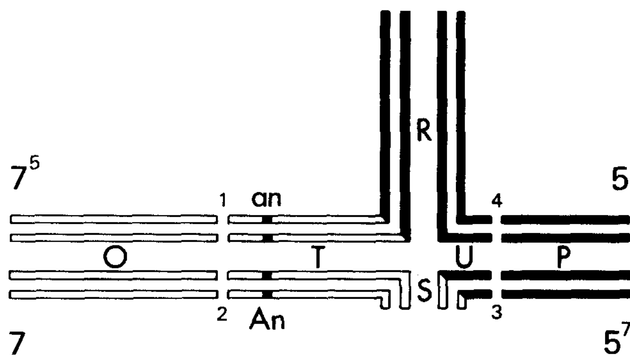

Fig. 1. Pairing diagram of translocation heterozygote 303 between 5RL and 7RL. *O* and *P:* unchanged arms, *R* and *S:* exchanged segments, *T* and *U:* interstitial segments. Locus *An – an* (anthocyanin vs. anthocyaninless) located in T, i.e. on 7RL, not far from centromere. Recessive allele on translocation chromosome. With alternate disjunction, centromeres 1 and 3 move to same pole, 2 and 4 to opposite pole. With adjacent-1 disjunction, centromeres 1 and 4 move to one pole, 2 and 3 to the other pole. With adjacent-2 disjunction, homologous centromeres (1 and 2, and 3 and 4 respectively) move to same poles

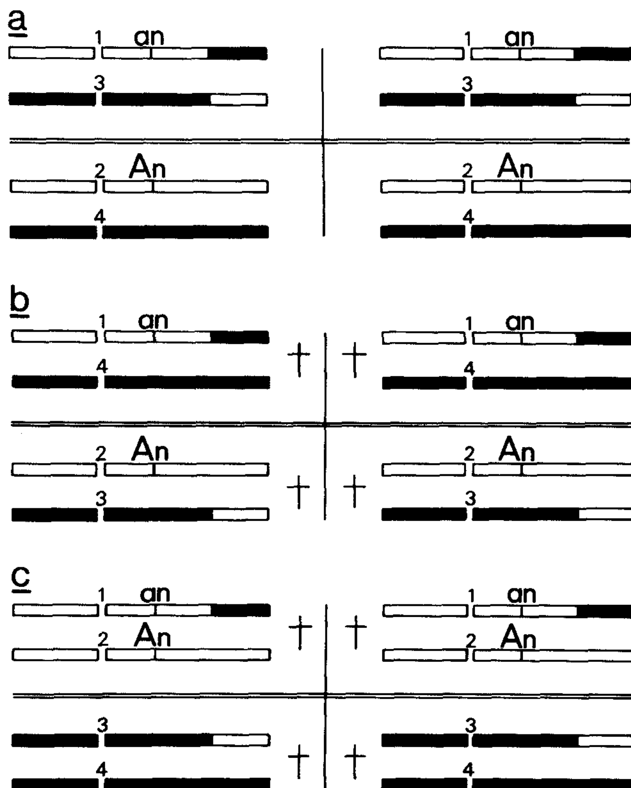

Fig. 2. Tetrads from alternate (**a**), adjacent-1 (**b**) and adjacent-2 (**c**) disjunction after chiasma formation in at least 3 out of 4 end segments *O, P, R* and *S* (Fig. 1). Chromatids are not drawn proportional to length. *Double line:* reductional cell wall, *single line:* equational cell wall. Abortive cells are marked with cross. There is no recombination between marker and translocation breakpoint

the reduced pairing around translocation breakpoints, it is realistic to assume that a maximum of one chaisma is formed in the interstitial segment. Numerical non-disjunction is assumed to be infrequent and will not be considered.

With zig-zag (alternate) disjunction, the two alternate (non-homologous) centromeres move pairwise to the same pole. With adjacent-1 disjunction two adjacent non-homologous centromeres move to the same pole. Homologous adjacent centromeres move to the same pole with adjacent-2 disjunction (Fig. 1). The frequency of alternate orientation among translocation multivalents without interstitial chiasmata (ring-of-4, chain-of-4, chain-of-3 with univalent), is z. For multivalents with interstitial chiasmata (closed-figure-8, frying-pan, O. K.- and Y-shaped multivalents), the alternate orientation frequency is z'. The frequency of multivalents in which either one or both exchanged segments R and S (Fig. 1) have chiasmata, in addition to at least one of the segments O, P, T or U, is e, and $1-e$ is the frequency of configurations without chiasmata in the exchanged segments. The chiasma frequency in interstitial segment T (Fig. 1) is t, that in U is u. The fraction of the chiasmata in segment T formed between interstitial locus *An-an* (Fig. 1) and the translocation breakpoint is f, whereas that between the locus and the centromere is $1-f$.

A formula can be derived which relates the recombination fraction (r) between gene and breakpoint to the chiasma frequency in this chromosome segment ($f \cdot t$) in dependence of the orientation frequencies. Table 1 lists the contribution of each group of translocation configurations to the four classes of reproductive gametes (translocation or normal, recombinant or non-recombinant). Multivalents (frequency e) without interstitial chiasmata (frequency $(1-t)$ $(1-u)$) only contribute to the class of non-recombinant spores (Table 1). Since gametes originating from either adjacent-1 or adjacent-2 orientation are unbalanced, this contribution is directly proportional to the frequency of alternate orientation (z), from which four balanced gametes per configuration result (Fig. 2). The contribution to r of multivalents having a chiasma in one interstitial segment depends on whether the chiasma is formed in T or U, on the fraction of the chiasmata in T between marker and translocation breakpoint (f) and on the type of orientation. The assumption is made that adjacent-2 orientation, giving only unbalanced gametes, may be excluded, as co-orientation of homologous centromeres is very unlikely here. Absence of adjacent-2 is a reality in translocations between (sub-)metacentric chromosomes having at least one relatively large interstitial segment (Burnham 1950; Vosselman 1981). Two non-recombinant and two unbalanced gametes result from alternate disjunction (frequency z',

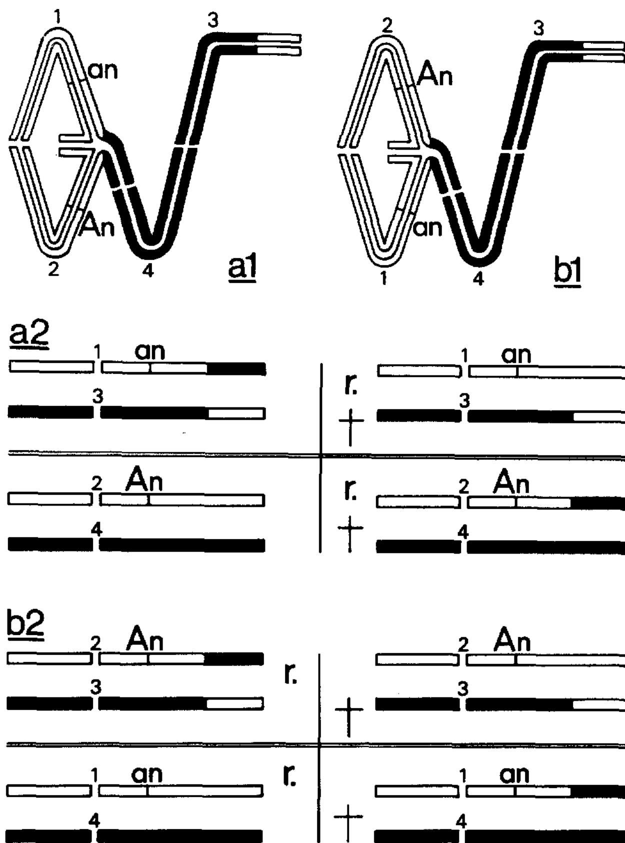

Fig. 3. Diagrams of MI-configurations (**a 1, b 1**) resulting from chiasma formation in *O, P, R* and in *T* between *An – an* and translocation breakpoint (chromosomes not drawn proportional to length; Fig. 1). Recombinant tetrad cells (**a2, b2**) are indicated with "*r*". For further legend see Fig. 2. *Note:* alternate (**a1**) and adjacent (**b1**) "frying pans" have identical morphology, but in tetrads from alternate disjunction (*a2*) recombinant chromatids are included in abortive cells, while recombinant tetrad cells from adjacent-1 disjunction (**b2**) are viable. Frying pans with chiasma in U (not in T) have smaller ring and larger "handle" than frying pans with chiasma in T (not in U) (Fig. 1)

Fig. 3), when a chiasma is formed between marker and breakpoint (fraction f), whereas adjacent-1 disjunction (frequency $1-z'$) gives two recombinant and two unbalanced spores. Irrespective of orientation type, only non-recombinant gametes result from chiasma formation between marker and centromere (fraction $1-f$) as well as from chiasma formation in U (frequency $(1-t)\,u$), 50% of the gametes being unbalanced again. Only as long as $z'=0.5$, equal proportions of recombinant and non-recombinant spores originate from multivalents having a chiasma between the gene and the breakpoint without a chiasma in U (Fig. 3). Irrespective of orientation type, multivalents always give rise to equal proportions of recombinant and non-recombinant gametes when a chiasma in U is formed simultaneously (Fig. 4). When both T and U have chiasmata, the contribution to each class of gametes, therefore, only depends on f (Table 1).

In Table 1, the production of viable gametes by cells with multivalents includes both trivalents with one univalent and quadrivalents. However, due to the loss of the univalents in the first meiotic division, the gamete production resulting from trivalents is half of that from quadrivalents. With considerable univalent frequency, this should be taken into account.

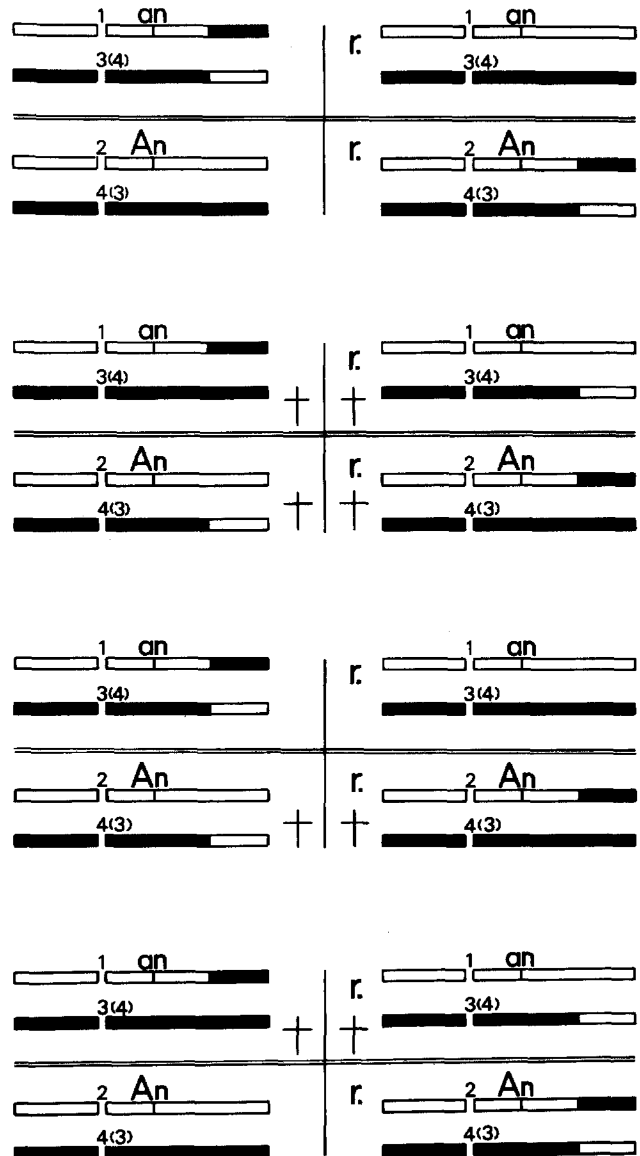

Fig. 4. Tetrads from chiasma formation in T between *An – an* and translocation breakpoint, and in U. For legend, see Figs. 2 and 3. Half of balanced tetrad cells is recombinant, irrespective of orientation

Translocation bivalents are assumed to result from chiasma formation between chromosomes with homologous centromeres. This is true for configurations consisting of two ring bivalents, or one ring- and one open bivalent. Part of the open translocation bivalent pairs, however, may also originate from crossing over in the exchanged segments, linking non-homologous centromeres. Adjacent-2 orientation is the result, and there is no contribution to any of the four gamete classes. Simultaneous absence of chiasmata in both unchanged arms is required, which is highly improbable for the (sub-)metacentric rye chromosomes with frequencies of arms being bound often approaching 1. Therefore, the error introduced by assuming that all translocation bivalents are the result of chiasma formation between chromosomes with homologous centromeres is limited, the more so when the frequency of cells with multivalents is high relative to that of cells without. Due to independent orientation of the translocation bivalents, an alternate to adjacent-1 ratio of 1 : 1 is realized, and $z=z'=0.5$ is substituted in the terms for the multivalent contributions (Table 1) to obtain the frequencies in which configurations without chiasmata in the exchanged segments (frequency 1-e) contribute to the four gamete classes, since the same rules apply to these configurations and multivalents. The term 1-e includes translocation configurations with two or four univalents, which do not contribute to any class of reproductive gametes. This should be taken into account, when their frequency cannot be neglected.

The fraction of recombinant gametes in the total gamete pool now is (Table 1)

$$r=\frac{1-(1-u)\,e\,(2z'-1)}{2[1+(1-t)\,(1-u)\,e\,(2z-1)]}\cdot f\cdot t \qquad \text{(A)}$$

In formula A, the term $[1-(1-u)\,e\,(2z'-1)]\cdot[2[1+(1-t)\,(1-u)\,e\,(2z-1)]]^{-1}$ determines the degree in which the chiasma frequency between marker and translocation breakpoint ($f\cdot t$) is recovered in the recombination fraction (r). In the following, this term will be referred to as d. As long as alternate and adjacent orientations are equal, no effect on the recombination fraction – other than that due to the overall disturbance of pairing and chiasma formation around the translocation breakpoint – should be observed (Kramer 1954). As in a normal diploid, the chiasma frequency between the two factors under consideration should then be twice their recombination fraction: $d=0.5$. When $z=z'=0.5$ is substituted in formula A, it is indeed found that $r=0.5f\cdot t$.

The term d also equals 0.5 when $e=0$, i.e. when only translocation bivalents are formed between chromosomes with homologous centromeres, giving random orientation of the non-homologous centromeres, or when $u=1$, i.e. when the interstitial segment without the gene has always a chiasma, resulting in the inclusion of recombinant and non-recombinant chromatids in the abortive spores in equal proportion, irrespective of orientation type (Fig. 4).

With random orientation in open translocation multivalents, the ratio of alternate to adjacent-1+2 is expected to be 1 : 1 (Sybenga 1975). This ratio has been observed by Burnham (1950) in several interchanges of

Table 1. Contribution to the recombinant and non-recombinant gametes for each group of translocation configurations. Maximum of one chiasma per interstitial segment. Chiasma frequency in T (Fig. 1): t; in U: u. Multivalent frequency: e (see text). Fraction of chiasmata in T between gene and breakpoint: f. Alternate frequency among multivalents without interstitial chiasmata: z; with interstitial chiasmata: z'. Half of the gametes are normal, the other half have the translocation karyotype

Configurations	Contributions to non-recombinant spores	Contributions to recombinant spores
Multivalents		
without interstitial chiasmata	$4(1-t)(1-u)e\cdot z$	–
chiasma in one interstitial segment	$2\cdot f\cdot t(1-u)e\cdot z'+$ $+2(1-f)t(1-u)e+$ $+2(1-t)u\cdot e$	$2\cdot f\cdot t(1-u)e(1-z')$
chiasma in both interstitial segments	$f\cdot t\cdot u\cdot e+$ $+2(1-f)t\cdot u\cdot e$	$f\cdot t\cdot u\cdot e$
Bivalents		
without interstitial chiasmata	$2(1-t)(1-u)(1-e)$	–
chiasma in one interstitial segment	$f\cdot t(1-u)(1-e)+$ $+2(1-f)t(1-u)(1-e)+$ $+2(1-t)u(1-e)$	$f\cdot t(1-u)(1-e)$
chiasma in both interstitial segments	$f\cdot t\cdot u(1-e)+$ $+2(1-f)t\cdot u(1-e)$	$f\cdot t\cdot u(1-e)$

maize with two short interstitial segments. In rye, alternate orientation predominates among translocation multivalents without interstitial chiasmata (Sybenga 1968): $z > 0.5$. Apparently chance coordination is not realized here, probably for reasons of centromere reorientation during prometaphase and greater stability of the alternate than of the adjacent orientation (Sybenga 1975). In translocation multivalents having either one or both interstitial segments associated by chiasmata, alternate and adjacent-1 cannot be microscopically distinguished in first metaphase configurations of pollen mother cells (PMCs) without morphological markers in the centromeres. There is, however, no a priori mechanical reason why the frequencies of alternate and adjacent-1 would be different (Fig. 3), so that a 1 : 1 ratio of both orientations ($z' = 0.5$) would be expected. For any translocation, with given values of t, u and e ($u \neq 1, e \neq 0$), the term d becomes smaller with increasing values of either or both z and z'. The denominator of d is at its maximum for $z = 1$, and for $z' = 1$ the numerator reaches its minimum. Thus, with predominant alternate orientation in the open multivalents ($z > 0.5$), and random orientation among closed multivalents ($z' = 0.5$), part of the interstitial chiasma frequency will be "masked" and not expressed in the recombination fraction. When $z' = 0.5$, the degree of masking is limited: d reaches a minimum of value of 0.25 when $z = 1$, $e = 1$, $u = 0$ and t approaches 0, in which case of course hardly any recombination will be observed. With increasing values of t, d becomes larger than 0.25, and will be 0.5 again when $t = 1$: with more configurations having interstitial chiasmata, the contribution to the total pool of reproductive gametes orginating from random centromere orientation ($z' = 0.5$) increases at the cost of that resulting from nonrandomness ($z > 0.5$), so that interstitial chiasma formation is more fully expressed. Only when $z' > 0.5$, can d become smaller than 0.25.

Values of e, t, u and z are obtained by meiotic observations, while r is estimated in backcrosses or F'2s segregating for the marker and the translocation (de Vries and Sybenga 1983). Substitution in formula A together with the expected value of 0.5 for z' results in an estimate of f. f can also be estimated from the interstitial chiasma frequency t when the genetic distance between marker and centromere is known. Then, using formula A it can be checked whether the assumption that $z' = 0.5$, is realistic.

When p is substituted for r, x for t, y for u, a' and a for z' and z respectively, and $(1-P)$ for $(1-t)(1-u)$, and when $e = 1$ and $f = 1$, formula IV in the report of Kramer and Blander (1961) is found. The validity of their formula is, therefore, limited to recombination between centromere and translocation breakpoint ($f = 1$), in translocations in which either one or both exchanged segments in addition to at least one of the other segments are practically always bound ($e = 1$).

Materials and methods

The translocation investigated in this study (Fig. 1) carries code number 303 and is part of the Wageningen translocation tester set (Sybenga and Wolters 1972; de Vries and Sybenga 1976; Sybenga 1983). Translocation 303 involves chromosomes 5R and 7R, with the breakpoints proximally in 5RL and distally in 7RL (de Vries and Sybenga 1983; Sybenga, in preparation). Due to the difference in length of the exchanged segments (R and S, Fig. 1), both translocation chromosomes are easily recognized in mitotic metaphase. In the first metaphase of meiosis, the short translocation chromosome 5^7 is markedly small when appearing as a univalent. Open bivalents in which chromosome 5^7 is involved are identified by their heteromorphic appearance, whereas a ring bivalent formed by chromosomes 5^7 and 5 is about half the size of an average normal ring bivalent, showing a large projection which represents the long exchanged segment.

The gene *an*, determining the presence of anthocyanin in the caryopsis (xenia), coleoptile and nodes of rye seeds and plants, is located on chromosome 7RL (Sybenga and Mastenbroek 1980; de Vries and Sybenga 1983), interstitially in chromosome 7^5 of translocation 303 (Fig. 1). Sybenga and Mastenbroek (l.c.) established a genetic distance of 5 cM between *an* and the centromere in translocation heterozygote 282, in which the breakpoint is in the other arm of chromosome 7R.

One plant, heterozygous for translocation 303 and for *an*, was used in a reciprocal test cross with karyotypically normal, anthocyaninless (*an an*) plants. In the translocation heterozygote, the dominant allele *An* was located on the normal chromosome 7R (N), while translocation chromosome 7^5 (T) carried the recessive allele *an*. The test cross is symbolized as $\frac{an}{T}\frac{An}{N} \times \frac{an}{N}\frac{an}{N}$. A total of 327 seedlings of the test cross progeny was karyotyped and scored for *an*, providing data for the estimation of the recombination fraction r between *an* and the translocation breakpoint. Configuration frequencies at first metaphase were scored in 1,000 PMCs of the plant which was also used in the test crosses. In translocation multivalents without interstitial chiasmata (rings-of-four, chains-of-four, chains-of-three with univalent) the type of orientation – alternate or adjacent – was established simultaneously. The PMCs were in early MI, since a few cells at diakinesis and none at first anaphase were observed. This implies that final orientation had not been reached and the frequency of alternate orientation was underestimated (Sybenga 1968).

All plants were grown in a greenhouse at 18°–20 °C. Karyotypes were classified in root tip mitoses after pretreatment in a saturated aqueous alpha bromonaphthalene solution for 2 h at 24 °C, fixation-maceration in 1 N HCl at 60 °C for 12 min, and Feulgen staining. Anthers were fixed in 1 : 3 acetic alcohol and stored at –10 °C for two years. First metaphase chromosomes were stained with 2% aceto carmine, and the preparations were mounted in Euparal for cytological analysis.

Results

The segregation of translocation 303 and *an* in the offspring of the test cross $\frac{an}{N}\frac{an}{N} \times \frac{an}{T}\frac{An}{N}$ (TN 303 as ♂)

Table 2. Segregation of translocation 303 and marker gene *an* in the testcross progeny of $\frac{an}{N}\frac{an}{N}\times\frac{an}{T}\frac{An}{N}$ (a), and of the reciprocal (b); *r:* estimated recombination fraction between *an* and the translation; s_r: standard deviation of *r*

a

Phenotype \ Karyotype	NN	TN	Total	
An	58	16	74	
an	16	41	57	
Total	74	57	131	$r \pm s_r = 0.244 \pm 0.038$

Segregation of TN 303 and *an* fit their 1 : 1-expectation at the 5% level ($\chi^2 = 2.206$)

b

Phenotype \ Karyotype	NN	TN	Total	
An	85	14	99	
an	24	73	97	
Total	109	87	196	$r \pm s_r = 0.194 \pm 0.028$

Segregation of TN 303 ($\chi^2 = 2.469$) and *an* ($\chi^2 = 0.020$) fit their 1 : 1-expectation at the 5%-level

is presented in Table 2a; the reciprocal cross in Table 2b.

The Tables 3–6 contain the classification of MI translocation configurations in 1,000 PMCs. In 980 cells, the translocation configurations could be distinguished from the configurations originating from the 5 pairs of normal chromosomes (group A). In 943 of these, multivalents occurred (configurations 1–9, Table 3). No multivalents were observed in 37 cells, but the translocation bivalent formed by chromosomes 5 and 5^7 could be morphologically identified. In addition, 6 ring bivalents were found in these same cells. In 6 of the 37 cells with bivalents only, 7 ring bivalents were observed, of which one was small and had a large projection so that the translocation configuration could be concluded to consist of two ring bivalents (conf. 10). A heteromorphic open bivalent formed by chromosomes 5 and 5^7 next to 6 ring bivalents was found in 30 PMCs, which means that in these cells the chromosome pair 7–7^5 formed a ring (conf. 11). Finally, 1 cell carried a pair of univalents originating from chromosomes 5 and 5^7 next to 6 rings, and consequently a ring bivalent must have been formed by chromosomes 7 and 7^5 in this cell (conf. 12, Table 3).

To establish the translocation configuration in the remaining 20 PMCs in which no direct distinction

Table 3. First metaphase translocation configurations in 1,000 PMCs of the plant of Table 2. Configuration frequencies of no. 10–15 partly based on Tables 4–6 (see text)

Type no.	Configuration		Orientation	No. of cells	Notes
1	ring-of-4		alternate	10	
2	chain-of-4		alternate	196	
3	ring-of-4		adjacent	2	
4	chain-of-4		adjacent	43	
5	chain-of-3 with univalent		alternate	12	[a]
6	closed-figure-8			1	
7	frying pan quadrivalent			643	
8	Y-shaped quadrivalent			2	
9	frying pan trivalent with univalent			34	[a]
			subtotal	943	
	Bivalents of chromosomes				
	7–7^5	5–5^7			
10	ring	ring		9	
11	ring	open		33	
12	ring	2 univalents		1	
13	open	ring		7	
14	open	open		6	[b]
15	open	2 univalents		1	[b]
			total	1,000	

[a] Univalent is chromosome 5^7

[b] Configurations 14 and 15 are assumed to have originated from chiasma formation between chromosomes with homologous centromeres (see text)

Table 4. Bivalent configurations for the 5 pairs of chromosomes not involved in the translocation complex, in 980 PMCs in which the translocation configuration can be distinguished directly (group A)

Ring bivalents	Open bivalents	Pairs of univalents	No. of cells
5	0	0	696
4	1	0	254
3	2	0	29
2	3	0	1
			980

Total number of configurations: $5 \times 980 = 4{,}900$
Total number of open bivalents: 315 (6.43%)

could be made between configurations originating from the translocation complex and the normal chromosome pairs (group B), a procedure outlined by Sybenga and Mastenbroek (1980) was followed. Table 4 presents the distribution of bivalent configurations originating from the 5 pairs of normal chromosomes in group A. The configurations found in the 20 PMCs of group B are listed in Table 5. In 8 cells a heteromorphic open bivalent formed by chromosomes 5 and 5^7 was observed, next to 5 ring bivalents and 1 morphologically normal open bivalent (type I, Table 5). Of the $8 \times 5 = 40$ configurations formed by the 5 pairs of normal chromosomes, 6.43% (Table 4) or 3 bivalents are expected to be open bivalents. Chances are small that these were not formed in three different cells. Thus in 3 of the 8 PMCs of type I, a ring must be assumed to have been formed by chromosomes 7 and 7^5 (although not recognized at meiosis), while in the remaining 5 cells these chromosomes must have formed an open bivalent. The expected translocation configurations in the other PMC types of Table 5 are derived in a similar way, leading to the configuration frequencies of Table 6. When added to the configurations of group A, the totals of Table 3 are found.

Table 5. Configurations in the 20 PMCs in which the translocation configuration is not a multivalent and cannot be directly distinguished (group B)

PMC-type	Ring bivalents	Heteromorphic open bivalents (chr. $5-5^7$)	Open bivalents (not heteromorphic)	Heteromorphic pairs of univalents (chr. $5-5^7$)	Pairs of univalents (not heteromorphic)	No. of cells
I	5	1	1	0	0	8
II	5	0	1	1	0	1
III	4	1	2	0	0	1
IV	6	0	1	0	0	6
V	5	0	2	0	0	2
VI	4	0	3	0	0	1
VII	6	0	0	0	1	1
						20

Table 6. Translocation configurations in the 20 cells of group B, estimated from Tables 4 and 5

Type no. of Table 3	Bivalents of chromosomes		Contribution of PMC types listed in Table 5							No. of cells included in Table 3
	$7-7^5$	$5-5^7$	I	II	III	IV	V	VI	VII[a]	
10	ring	ring	–	–	–	2	–	–	1	3
11	ring	open	3	–	–	–	–	–	–	3
13	open	ring	–	–	–	4	2	1	–	7
14	open	open	5	–	1	–	–	–	–	6
15	open	2 univ.	–	1	–	–	–	–	–	1
Total			8	1	1	6	2	1	1	20

[a] The pair of univalents in this PMC is arbitrarily attributed to one of the pairs of normal chromosomes

Discussion

Interstitial chiasmata

Chiasma formation in both interstitial segments simultaneously is scarce among multivalents, as can be concluded from the fact that only one configuration with such origin, a closed-figure-8, was observed (configuration 6, Table 3). The frequency is much higher among the translocation bivalents: 9 cells were found with configuration 10, whereas cells with configurations 11, 13 and 14 may also have originated from chiasma formation in both T and U. The number of these cells cannot be established microscopically, but from Table 7 it is seen that all open bivalents consisting of chromosomes 7 and 7^5 must have originated from chiasma formation in 0 ($o=1$), while of the heteromorphic open bivalents formed by chromosomes 5 and 5^7 a fraction of $\frac{(1-p)\,u}{p\,(1-u)+(1-p)\,u}=0.022$ (Table 7) must have originated from crossing over in U. Consequently, only 1 cell, out of the 33 with configuration 11, can be added to those with 6 or 10 (Table 8).

The remaining 32 cells with configuration 11 have a chiasma in T but not in U, which also applies to cells with configurations 9 or 12 in which 5^7 appeared as a univalent. Configuration 13 has a chiasma in U but not in T, while in cells with configurations 1–5, and 14 and 15 no chiasmata have been formed interstitially (see above).

This leaves the configurations 7 and 8, which may arise from crossing over in either T or U. When the frequency of all configurations (translocation bivalents as well as multivalents) without interstitial chiasmata is $(1-t)\,(1-u)$, and that of configurations with chiasmata in both interstitial segments is $t\cdot u$, it follows from Table 3 that

$(1-t)\,(1-u)=0.270$ (conf. 1–5, 14 and 15), and

$t\cdot u=0.011$ (conf. 6, 10 and 11 (1 cell)).

The largest root of these equations represents the chiasma frequency in the larger segment. Thus, $t=0.725$ and $u=0.015$ (chiasma interference between interstitial segments is not taken into account). A chiasma in U has actually been formed in configurations 6, 10, 11 (1 cell) and 13, i.e. in 18 out of 1,000 PMCs (Table 3). This is close to $u=0.015$ and it is improbable that additional configurations have resulted from chiasma formation in U. Therefore, configurations 7 and 8 are concluded to have a chiasma in T, and not in U. This is supported by the fact that, although multivalent types 7 and 8 originating from chiasma formation in U are expected to be morphologically different from configurations having a chiasma in T (size of ring and "handle" of the frying pan, Fig. 3), no such differences were noted. No frying pans were detected with a ring notably smaller than normal ring bivalents, although ring bivalents formed by chromosomes 5 and 5^7 are easily recognized. The conclusion, therefore, must be that most – if not all – multivalent types 7 and 8 have originated from chiasma formation in T.

Table 8 summarizes these conclusions. It is seen that in 723 of the 1,000 PMCs a chiasma has been formed in T ($t=0.723$), while the chiasma frequency in U (u) is 0.018. For bivalents alone (Table 7), t and u are 0.754 and 0.295 respectively. The difference indicates strong positive interference between exchanged and interstitial segments, for which obviously the short segment U is the most susceptible. Strong positive interference between interstitial and exchanged segments has been frequently observed in rye interchange heterozygotes (Sybenga 1970; Sybenga and Mastenbroek 1980).

Estimates of e, z and r

As seen from Table 3, the multivalent frequency (e) equals 0.943. In 218 of 263 PMCs having multivalents without interstitial chiasmata, alternate orientation was

Table 7. Frequencies of bivalent configurations formed by chromosome pairs 7–7^5 and 5–5^7 respectively among 57 PMCs with configurations 10–15 (Table 3) and estimated frequencies o, p, t and u in which segments O, P, T and U respectively are bound among configurations without chiasmata in R and S (Fig. 1). Chiasma interference is not taken into account

Configuration	Chromosome pair 7–7^5		Chromosome pair 5–5^7	
	Frequency	Observed	Frequency	Observed
Ring bivalents	$o\cdot t$	0.754	$p\cdot u$	0.281
Open bivalents	$o(1-t)+(1-o)t$	0.246	$p(1-u)+(1-p)u$	0.684
Pairs of univalents	$(1-o)\cdot(1-t)$	0.0	$(1-p)\cdot(1-u)$	0.035
	$o=1.0$		$p=0.950$	
	$t=0.754$		$u=0.295$	

Table 8. Origin of translocation configurations (Table 3) with respect to interstitial chiasma formation, as concluded from Tables 3 and 7

Configuration type	Chiasma formation		No. of cells
	in T	in U	
6, 10 and 11[a]	+	+	11
7, 8, 9, 11[b] and 12	+	–	712
13	–	+	7
1, 2, 3, 4, 5, 14 and 15	–	–	270
			1,000

[a] 1 of 33 cells
[b] 32 of 33 cells

Total of configurations with chiasma formation in T: 723 ($t=0.723$)
Total of configurations with chiasma formation in U: 18 ($u=0.018$)

observed: $z=0.829$. This is considered a minimum estimate because the PMCs were in early metaphase, while Sybenga (1968) observed an increasing frequency of alternate orientation – up to 95% – with progressing MI. A value of 0.9 for z is considered more realistic and will therefore be used in further calculations.

For the recombination fraction, the value of Table 2a may be taken ($r=0.244 \pm 0.038$), because the meiotic data of Table 3 were obtained from PMCs of the same male parent.

Estimates of f

Two approaches are given to estimate the fraction of chiasmata in T formed between the *an*-locus and the translocation breakpoint (f). The first is based on the assumption that centromere orientation is random among configurations having either one or both interstitial segments bound by chiasmata ($z'=0.5$), the second makes use of the genetic distance between *an* and the centromere of chromosome 7R, which is 5 cM according to Sybenga and Mastenbroek (1980).

1. By substituting $z'=0.5$, $z=0.9$, $t=0.723$, $u=0.018$, $e=0.943$ and $r=0.244 \pm 0.038$ in formula A, f becomes 0.813 ± 0.127. Reliable confidence limits for f cannot be given, since the statistical distributions of t, u and e are not known.

Univalent formation causes a reduction in the production of viable gametes. Due to configuration 9 (frequency 0.034, Table 3) the contribution to the class of recombinant spores is reduced by $0.034 f(1-z')$, and the contribution to the non-recombinant class by $0.034.(f \cdot z'+1-f)$, whereas the reduction of the contribution to the latter caused by configuration 5 (frequency 0.012) equals $0.024\,z$. Introducing these corrections gives $f=0.814 \pm 0.127$, and it appears that their effect is negligible. No consideration will be given to the effect on the gamete productions of configurations 12 and 15, because their frequencies are extremely low (0.001 for each, Table 3).

Configurations 5 and 9 only contribute to the karyotypically normal spores, since the univalent is always the short translocation chromosome 5^7. It is interesting to note, that the preferential loss of univalent 5^7 results in a shortage of translocation heterozygotes in both test crosses (Table 2a, b), which becomes significant when the two populations are pooled ($\chi^2=4.65$; $P<0.05$).

2. A genetic distance of 5 cM between *an* and the centromere of chromosome 7 corresponds with a chiasma frequency of 10% in this segment, or a chiasma in 100 out of the 1,000 PMCs analyzed. With 723 PMCs having a chiasma in T (Table 8), there would be 623 with a chiasma between *an* and the translocation breakpoint, and f is then estimated as $\frac{623}{723}=0.862$. This is within the limits established for $z'=0.5$ (0.813 ± 0.127, see above) and relatively close to the average value, in spite of the fact that the genetic distance between *an* and the centromere was established in translocation heterozygote 282 (Sybenga and Mastenbroek 1980), in which the gene is located in the unchanged arm and not interstitially like in translocation 303.

Difference between z and z'

With all chiasmata in T between *an* and the translocation breakpoint ($f=1$), a maximum of 0.601 ± 0.068 is found for z'. It is evident that the alternate orientation frequency among translocation multivalents with interstitial chiasmata (z') never reaches the level of that among multivalents without (z). However, f is smaller than 1 (see above) and a realistic estimate for z' of 0.530 ± 0.080 is obtained with $f=0.862$. Thus, it is very reasonable to conclude that $z'=0.5$, which corresponds with the view that there are no a priori mechanical reasons for a preference of alternate orientation among translocation multivalents having interstitial chiasmata. For $f=0.813$ and $r=0.244 \pm 0.038$, z' becomes 0.500 ± 0.084.

Degree of "masking"

The degree in which the actual chiasma frequency between gene and breakpoint is masked in the observed recombination fraction is determined by d: $r=d \cdot f \cdot t$ (formula A). In the present case, $d=\frac{0.244}{0.813 \cdot 0.723}=0.415$ whereas with random overall orientation ($z=z'=0.5$) d would be 0.5. When $z'=0.5$, the numerator of d (see formula A) becomes 1. Thus if the conclusion that $z'=0.5$ is correct, masking of the recombinational effect of interstitial chiasmata can only be attributed to an alternate orientation frequency among multivalents without interstitial chiasmata (z) larger than 0.5, resulting in an excessive contribution of

viable (non-recombinant) spores to the total gamete pool (Fig. 2).

When $z'=0.500+0.084$ (see above), d becomes 0.350: masking of interstitial recombination becomes stronger due to the preferential inclusion of recombinant chromatids in the non-reproductive gametes caused by values of z' larger than 0.5 (Fig. 3). The minimum of d in the present case would be 0.285, i.e. when $f=1$ and $r=0.244-0.038$, giving $z'=0.669$ (see above).

Lamm (1948) was the first to present a formula quantifying the effect of centromere orientation and interstitial chiasma formation on the observed recombination fraction for interstitial loci. Identity of z and z' was assumed, but Lamm pointed out explicitly that no proper allowance was made for the possibility that interstitial chiasmata act upon the orientation of the configuration. Lamm's formula was subsequently refined by Hanson and Kramer (1949), who concluded that barley interchanges, on the basis of an average sterility of 25%, should at least show an alternate frequency of 0.75. Implicitly they assumed identity of z and z' and, therefore, a value for z' of at least 0.75. Apart from overall disturbed crossing over around the translocation breakpoint, the preferential inclusion of recombinant chromatids in the abortive gametes which results from $z' > 0.75$ (Fig. 3) was used to explain reduced recombination, without paying attention to the effect of the excessive contribution of viable, non-recombinant spores caused by values for z of at least 0.75. Similar incorrect explanations for masking of interstitial recombination are found in the reports of Burnham (1950), Hanson (1952), Kramer (1954), Burnham and Hagberg (1956), Ramage (1964), Kasha and Burnham (1965), Persson (1969) and Künzel (1982). The only parameter used to determine the frequency of alternate disjunction, implicitly assuming that z and z' are the same, is semi-sterility, but in none of these reports are cytological or other arguments found to support identity of z and z'. Kramer and Blander (1961) distinguished between z and z' (a and a' respectively in their report) and demonstrated that 25% sterility and a low recombination fraction between an interstitial marker and a translocation may very well be compatible with $z'=0.5$ and considerably higher values of z at the same time. On the basis of combined recombination and cytological data, the present report provides evidence that z can indeed greatly exceed z', and that the assumption of random centromere orientation among translocation multivalents having interstitial chiasmata ($z'=0.5$) is realistic. The reduction of recombination should, therefore, be attributed to the effect of a high value of z, z' being of minor or no importance. Since the effect of z is, in turn, limited (d can only reduce from 0.5 to a minimum of 0.25 when $z'=0.5$, see above), disturbance of pairing and crossing over in the region of the interchange breakpoints will in most instances be the main explanation of low recombination between interstitial markers and translocation breakpoints.

Use of interchanges in gene localization and balanced chromosomal systems

Reciprocal translocations can be used to locate marker genes on specific chromosomes (de Vries and Sybenga 1983). To determine a marker's chromosomal position, the measurement of linkage values between the marker and a series of reciprocal interchanges involving the chromosome which carries the marker, with breakpoints at various and known positions (Hanson 1952; Ramage 1964; Künzel 1982, and others) has proven to be a fruitful approach, provided that a fair number of such interchanges is available. Chiasma formation among the chromosomes involved often follows patterns which are not predictable, giving recombination values which may be misleading. The extremely high recombination fraction between *an* and translocation 303 is an illustrative exception to the rule that recombination in interstitial segments is very low. Sybenga and Mastenbroek (1980) established the arm position of *an* on the basis of the recombination fraction between *an* and translocation 282, the absence of linkage between *an* and the gene *br* (brittle) in a translocation 282 homozygote and a quantitative analysis of first metaphase configurations of the translocation heterozygote. It is obvious that the methods described above are rather laborious and that, therefore, when available, the use of telocentric and several types of translocation-derived trisomics (de Vries and Sybenga 1983) is to be preferred in determining the chromosomal position of a marker.

Several balanced chromosomal systems have been worked out to make use of genetic male sterility in hybrid breeding (Ramage 1965, for barley; Patterson 1973, for maize). Very low recombination between the male sterility gene, a selection marker and the breakpoint is an essential condition to make these systems operational. It has been suggested that interstitial location of the genes would guarantee low recombination. The present report demonstrates, that this is not necessarily true.

Other regions may appear to be favourable as well. For instance Künzel (1982) in barley studied two balanced tertiary trisomics, both with an *ms* gene in the exchanged segment of the extra translocation chromosome. No recombinants were observed among the selfed offsprings (433 and 293 individuals respectively). Similar results for exchanged segments as well as unchanged arms are obtained in studies concerning balanced trisomic systems in rye (de Vries et al., in preparation).

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