

Metaphase I centromere co-orientation in interchange heterozygotes of pearl millet

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SUMMARY

Using interchanges T(1, 3) and T(3, 6) of *Pennisetum americanum* orientation types of the interchange multiple at M I were studied in different genetic backgrounds. Orientation types alternate 1 and alternate 2, in addition to adjacent 1, adjacent 2 and indefinite could be identified for both the interchanges. The relative frequencies of various orientation types were influenced by a change in the genetic background.

For these interchanges, homologous centromeres do not seem to play a predominant role in the co-orientation of interchange multiple. The non-homologous co-orientation types were more subjected to genetic regulation than the homologous co-orientations.

1. INTRODUCTION

Burnham (1934, 1962) by cytological and genetic evidence proved the existence of adjacent 1 (homologous centromeres moving to opposite poles) and adjacent 2 (homologous centromeres moving to the same pole). Hagberg (1954) recognized a third type of orientation in which two chromosomes are directed to the opposite poles while the other two do not show any orientation, and this was called indefinite orientation.

Burnham (1934, 1956, 1962) and John & Lewis (1965) on the basis of co-orientation of centromeres postulated the existence of two types of alternate orientations corresponding to the two types of adjacent orientations. Cytologically if the components of the interchange multiple are identifiable by the morphological difference between the chromosomes or by differences in the sizes of the four units at M I, it is possible to distinguish the various disjunction types of interchange heterozygotes. The existence of two types of alternate and two types of adjacent orientations was proved cytologically for interchanges in cotton (Endrizzi, 1974), in the German cockroach (Cochran, 1976, 1977) and in rye (Lacadena & Candela, 1977). Further in German cockroach, Cochran (1977) showed that the disjunction pattern of a given interchange can be modified by varying the genetic background.

The present report documents the cytogenetic behaviour of two spontaneously occurring interchanges of *Pennisetum americanum* (L.) Leeke ($2n = 14$) in different genetic backgrounds.

2. MATERIAL AND METHODS

The spontaneous occurrence of heterozygosity for a chromosomal interchange was observed in the inbred line IP 457 of *Pennisetum americanum* from which a line homozygous for the interchanged chromosomes was developed. The homozygosity for the interchange was found to be associated with a semi-dwarf phenotype. By the study of pachytene chromosomes the interchange was found to involve chromosomes 3 and 6, with the break points situated near to the centromeres in the long arms in both the chromosomes. This interchange homozygote was designated as T(3, 6). IP 457 standard line, on crossing to any one of the four inbred lines – Vg 212, Vg 257, IP 2361 and Tift 23DB – gave rise to F₁ plants with 7_{II} and normal fertility; evidence for chromosomal alterations was not found in the subsequent generations. Thus IP 457 standard line and the four inbred lines have standard homozygous complement, and F₁ hybrids of T(3, 6) with the above five inbred lines constituted the material for the study of this interchange in different genetic backgrounds.

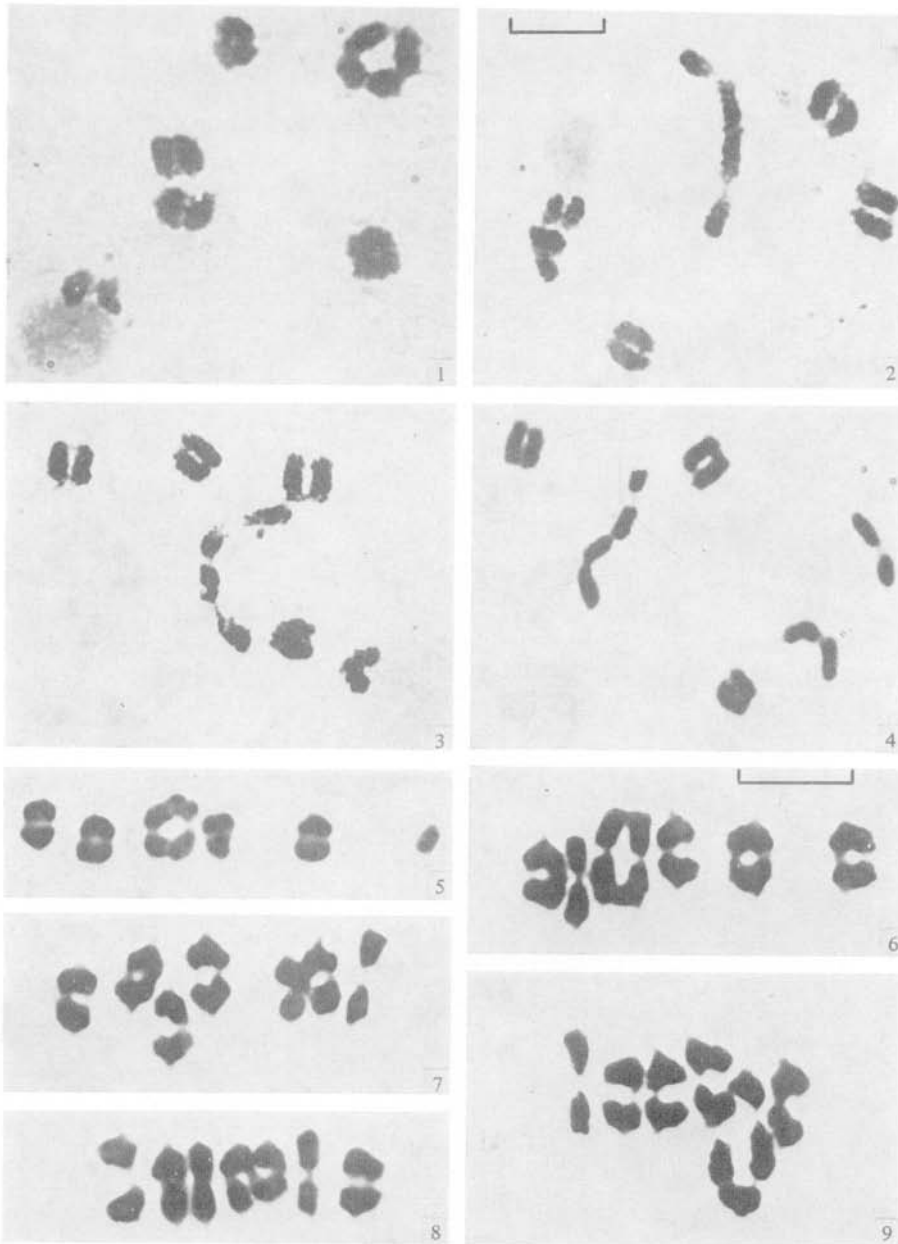
Inbred line Vg 268 on crossing to IP 457, Vg 212, Vg 257, IP 2361 and Tift 23DB resulted in F₁ hybrids with heterozygosity for another chromosomal interchange. By pachytene study chromosomes 1 and 3 were found to be involved in the interchange with the break points situated near to the centromere in the short arm of chromosome 1 and in the long arm of chromosome 3. Thus, Vg 268 represents the homozygous line for the interchange T(1, 3) and the F₁ hybrids of Vg 268 with the five inbred lines constituted the material for the study of this interchange in varied genetic backgrounds.

Chromosomes 1 (52.38 μm) and 3 (40.71 μm) at pachytene differ by about 12 μm in length and chromosomes 3 (40.71 μm) and 6 (26.14 μm) differ by about 14 μm . Thus conspicuous size differences exist between the two chromosomes involved in either of the interchanges. Moreover, in each case, the size of the exchanged segment between the non-homologous chromosomes was almost of equal length; therefore the interchanges more or less preserved the original size differences between the chromosomes.

Material was fixed in 1:3 acetic alcohol for 24 hours and stored in 70% alcohol. Acetocarmine-stained PMCs were examined.

3. RESULTS AND DISCUSSION

At diakinesis both the interchanges formed an open ring of four chromosomes in more than 90% of the PMCs in all the genotypes (Table 1). The interchange multiple consisted of two small and two big chromosomes in both the cases (Plate 1, fig. 1) where the centromeres of the two small chromosomes were homologous; likewise centromeres of the two big chromosomes were homologous. Depending on the location of the small and big chromosomes, the chain multiples were classified into three types: (1) those with the two small chromosomes one at each end of the chain (Plate 1, fig. 2), (2) those with the two big chromosomes one at each end of the chain (Plate 1, fig. 3) and (3) those with the big and small



Bar represents 10 μ m.

Figs. 1-4. Cytological behaviour of T(3, 6) at diakinesis.

- Fig. 1. The ring composed of two large and two small chromosomes.
- Fig. 2. Type I chain with the two short chromosomes at the ends.
- Fig. 3. Type II chain with the two short chromosomes in the middle.
- Fig. 4. Type III chain with the two short chromosomes at one end (top end).

Figs. 5-9. Co-orientation types of interchange complex of T(3, 6) at M I.

- Fig. 5. Adjacent 1.
- Fig. 6. Adjacent 2.
- Fig. 7. Alternate 1.
- Fig. 8. Alternate 2.
- Fig. 9. Indefinite.

chromosomes at the end positions (Plate 1, fig. 4). These types represent the failure of chiasma formation in the different arms of the pachytene cross. On a random basis of failure of chiasma formation in the four arms of the pachytene cross, these three chain types should appear in a frequency of 1:1:2 respectively at diakinesis. Though this expected frequency was not realized for the two interchanges studied now, the deviation was significant for T(1, 3) only (Table 2). In both, type 2 chains were less frequent, indicating a low frequency of chiasma failure in the arm of the cross adjoining the homologous centromeres of the chromosomes 3 in the case of T(3, 6) and those of chromosomes 1 in the case of T(1, 3). The reasons for this differential behaviour are not known.

Table 1. *Frequency of ring- and chain-type associations at diakinesis in the interchanges T(1, 3) and T(3, 6) in different genetic backgrounds*

Parents	Total no. of cells	Cells with rings	Cells with chains	Percentage of cells with rings
Vg 268				
T(1, 3) × IP 457	172	160	12	93.02
T(1, 3) × Vg 212	133	124	9	93.23
T(1, 3) × Vg 257	135	125	10	92.59
T(1, 3) × IP 2361	97	90	7	92.78
T(1, 3) × Tift 23DB	145	136	9	93.79
IP 457				
T(3, 6) × IP 457	126	120	6	95.24
T(3, 6) × Vg 212	153	144	9	94.12
T(3, 6) × Vg 257	116	110	6	94.83
T(3, 6) × IP 2361	168	158	10	94.05
T(3, 6) × Tift 23DB	156	146	10	93.59

Table 2. *Frequency of various chain types at diakinesis*

Inter-change	Total no. of chains	Chain type			χ^2 (1:1:2)	P (D.F. = 2)
		1	2	3		
T(1, 3)	47	15(31.92)	3(6.38)	29(61.70)	8.71	0.05-0.01
T(3, 6)	41	12(29.27)	5(12.20)	24(58.54)	3.59	0.2 -0.1

Figures in parentheses represent percentages.

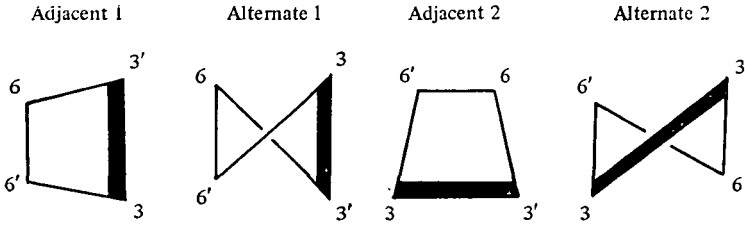
For both the interchanges T(3, 6) and T(1, 3) four co-orientation types – adjacent 1, adjacent 2, alternate 1 and alternate 2 – were noticed at MI of meiosis (Plate 1, figs. 5–8) with frequencies of 92.78–99.50% in different hybrids, and the remainder were of indefinite type (Plate 1, fig. 9). The distinction between the various disjunction types is possible because there were size differences in the components of a ring. The pattern of various disjunction types was in accordance with the scheme proposed by Endrizzi (1974, Text-fig. 1).

There is thus evidence for the existence of alternate 2 configurations at meta-phase I of meiosis of interchange heterozygotes of *P. americanum*. Previously

Endrizzi (1974) in cotton, Cochran (1976, 1977) in *Blattella germanica* and Lacadena & Candela (1977) in *Secale cereale* reported on the alternate 2 configuration in the interchange heterozygotes.

Interchanges T(3, 6) and T(1, 3) behaved differently in various cytological features.

In both the interchanges the orientation behaviour of the interchange multiple was influenced by a change in the genetic background (Table 3). Thompson (1956)



Text-fig. 1. Diagrammatic representation of the pattern of the disjunction types of the interchange complex at M I (after Endrizzi, 1974).

Table 3. *Frequencies of orientation types at M I of interchange association of the two interchanges T(3, 6) and T(1, 3) in different genetic backgrounds (ring and chain types pooled)*

Parents	Total no. of cells	Adjacent 1	Alternate 1	Adjacent 2	Alternate 2	Percentage alternate
IP 457						
T(3, 6) × IP 457	371	82 (22·10)	106 (28·57)	110 (29·65)	73 (19·68)	48·25
T(3, 6) × Vg 212	271	61 (22·51)	81 (29·89)	80 (29·52)	49 (18·08)	47·97
T(3, 6) × Vg 257	260	57 (21·92)	62 (23·85)	70 (26·92)	71 (27·31)	51·15
T(3, 6) × IP 2361	216	51 (23·61)	52 (24·07)	77 (35·65)	36 (40·74)	40·74
T(3, 6) × Tift 23DB	302	67 (22·19)	82 (27·15)	103 (34·11)	50 (16·56)	43·71
Vg 268						
T(1, 3) × IP 457	301	68 (22·59)	80 (26·58)	45 (14·95)	108 (35·88)	62·46
T(1, 3) × Vg 212	334	80 (23·95)	87 (26·05)	66 (19·76)	101 (30·24)	56·29
T(1, 3) × Vg 257	336	74 (22·02)	79 (23·51)	43 (12·80)	140 (41·67)	65·17
T(1, 3) × IP 2361	198	37 (18·69)	44 (22·22)	92 (46·46)	25 (12·63)	34·85
T(1, 3) × Tift 23DB	342	80 (23·39)	86 (25·15)	62 (18·13)	114 (33·33)	58·48

Figures in parentheses represent percentages.

showed that in rye interchanges the frequency of disjunction to non-disjunction types is genotypically controlled, while in *Culex*, Dennhöfer (1975) reported that the disjunction frequencies in interchange heterozygotes were controlled by a series of alleles of a Mendelian gene. Changes in disjunction frequencies of interchange multiples in different genetic backgrounds were also observed earlier in insects (Sakai *et al.* 1972; Dennhöfer, 1975; Robinson, 1976; Cochran, 1977).

Interchange T(3, 6) showed 50% alternate in three genotypes but showed a directed disjunction with significantly higher adjacent orientation in two genotypes. Interchange T(1, 3) had directed disjunction in all genotypes with significantly higher than 50% alternate in four genotypes and more than 50% adjacent orientation in one genotype (Table 3).

Among the three genotypes in which T(3, 6) showed 50% alternate orientation, there was complete randomness of adj. 1, alt. 1, adj. 2 and alt. 2 types in only one genotype but in the other two the four types showed 1:1.5:1.5:1 ratio respectively. Thus in all the three cases a numerical equivalence among cell pairs adj. 1, alt. 2 and adj. 2, alt. 1 was present (Table 3). Similar relationship was observed among three interchanges showing 50% alternate orientation in the German cockroach (Cochran, 1977), though the ratio was 2:1:1:2; however, the significance of this numerical equivalence in pairs of cell types is not understood. In the two genotypes of T(3, 6) and for all the genotypes of T(1, 3) with directed disjunction no such pattern in the frequencies of the four classes was observed. In the two genotypes of T(3, 6) and in the genotype T(1, 3) × Vg 212 the χ^2 calculations show that the proportions of the four classes would fit 1:1.5:1.5:1 for T(3, 6) and 1:1:1:1 for T(1, 3) but these proportions must be considered as fortuitous because there is significant deviation from the 1:1 ratio of adjacent:alternate types which would be expected if the ratios were correct (Table 3). It is interesting to note that there is a similar lack of pattern in the orientation frequencies in German cockroach (Cochran, 1977) for interchanges showing directed disjunction and for the three interchanges of rye reported by Lacadena & Candela (1977) which also showed directed disjunction.

In the co-orientation of centromeres of an interchange heterozygote John & Lewis (1965) and Sybenga (1975) consider that the homologous centromeres do not show preference to non-homologous centromeres. Therefore type I (adj. 1 + alt. 1) and type II (adj. 2 + alt. 2) co-orientations were expected with equal frequency. Lacadena & Candela (1977) observed in all the three structural heterozygotes of rye a higher frequency of type I co-orientations than type II, which was considered as a proof for the predominant role of homologous centromeres in the co-orientation of the ring configuration. Cochran (1977) observed for two interchanges T(7, 12) and T(9, 11), showing 50% alternate configuration, that type II cells were 50% of the total. However, Lacadena & Candela (1977) contended that there was a risk of misclassification of type I and type II when scoring alternate chain configurations, which were included by Cochran (1977) in the scorings. For the two interchanges T(3, 6) and T(1, 3) studied here in all genotypes except T(1, 3) × IP 2361, irrespective of random or directed disjunction

Table 4. *Frequencies of type I and type II cells at metaphase I of interchanges T(3, 6) and T(1, 3)*

Parents	Total no. of cells	Type I*		Type II†		% alt. disjunction	% of cells as type II	
		Freq.	% alt.	Freq.	% alt.		‡	§
IP 457								
T(3, 6) × IP 457	371	188	56.38	183	39.99	48.25	49.33	49.14
T(3, 6) Vg 212	271	142	57.04	129	37.98	47.97	47.60	46.88
T(3, 6) × Vg 257	260	119	52.10	141	50.35	51.15	54.23	54.10
T(3, 6) × IP 2361	216	103	50.49	113	31.86	40.74	52.31	52.22
T(3, 6) × Tift 23DB	302	149	55.03	153	32.68	43.71	50.66	50.35
Vg 268								
T(1, 3) × IP 457	301	148	54.05	153	70.59	62.46	50.83	50.00
T(1, 3) × Vg 212	334	167	52.10	167	60.48	56.29	50.00	49.51
T(1, 3) × Vg 257	336	153	51.63	183	76.50	65.17	54.46	53.25
T(1, 3) × IP 2361	198	81	54.32	117	21.37	34.85	59.09	58.79
T(1, 3) × Tift 23DB	342	166	51.81	176	64.77	58.48	51.46	51.75

* (adj. 1 + alt. 1); † (adj. 2 + alt. 2); ‡ ring and chain types combined; § ring types alone.

Table 5. *Frequencies of chain-type associations of T(3, 6) and T(1, 3) in the four orientation types at M I*

Parents	Total no. of chains	Orientation types				% alternate
		Adja-cent 1	Alter-nate 1	Adja-cent 2	Alter-nate 2	
IP 457						
T(3, 6) × IP 457	21	4	6	7	4	47.62
T(3, 6) × Vg 212	15	2	4	6	3	46.67
T(3, 6) × Vg 257	16	4	3	4	5	50.00
T(3, 6) × IP 2361	13	3	3	5	2	38.46
T(3, 6) × Tift 23DB	20	4	5	8	3	40.00
Vg 268						
T(1, 3) × IP 457	25	6	5	5	9	56.00
T(1, 3) × Vg 212	27	7	5	5	10	55.56
T(1, 3) × Vg 257	28	5	4	5	14	64.29
T(1, 3) × IP 2361	16	3	3	7	3	37.50
T(1, 3) × Tift 23DB	27	6	8	5	8	59.26

type II cells constituted 50% of the total in each case (Table 4); the proportion did not differ when either ring configurations alone or both ring and chain configurations were considered (Tables 4, 5). In the one exceptional case of T(1, 3) type II rather than type I was predominant. Thus for these interchanges homologous centromeres do not seem to play a predominant role in the co-orientation of interchange multiple. Thus in the four materials so far studied, rye (Lacadena & Candela, 1977) and cotton (Endrizzi, 1974) seem to be similar in having predominance of type I cells, whereas German cockroach (Cochran, 1976, 1977) and *Pennisetum* do not show such preference.

Table 6. *Relationship between percentage alternate cells, percentage pollen stainability and percentage seed set in different genotypes of T(3, 6) and T(1, 3)*

Parents	% alternate orientation	% pollen stainability	% seed set
IP 457			
T(3, 6) × IP 457	48.25	45.30	46.50
T(3, 6) × Vg 212	47.97	47.30	43.10
T(3, 6) × Vg 257	51.15	55.60	48.20
T(3, 6) × IP 2361	40.74	39.40	40.10
T(3, 6) × Tift 23DB	43.71	39.50	40.00
Vg 268			
T(1, 3) × IP 457	62.46	65.50	57.30
T(1, 3) × Vg 212	56.29	56.50	49.00
T(1, 3) × Vg 257	65.18	67.40	62.10
T(1, 3) × IP 2361	34.85	37.00	32.40
T(1, 3) × Tift 23DB	58.48	60.50	55.10

For five interchanges of German cockroach with directed disjunction in which alternate types constituted more than 50% of the total, Cochran (1977) observed an increase in the frequency of cell type II (adj. 2 + alt. 2) as well as an increase in the frequency of alt. 2-type cells. From the data of Lacadena & Candela (1977) it appears that the directed disjunction they observed for three interchanges in rye was due to predominance of alt. 1 cells. However, in the present study it was observed that irrespective of random or directed disjunction the ratio between cell type I and II was unaltered. Further, the ratio of adj. 1 to alt. 1 among type I cells also did not alter, but the ratio of adj. 2 to alt. 2 among type II cells showed variation, and that whenever there was directed disjunction adjustment between adj. 2 and alt. 2 frequencies was noticed (Table 4). Thus when the direction is towards overall alternate high, the frequency of adj. 2 decreases, with a simultaneous increase in the frequency of alt. 2; and in cases where the direction is towards overall low of alternate types the reverse situation is true. Therefore, the directed disjunction observed for the two interchanges, T(3, 6) and T(1, 3), completely rests upon the fluctuations of the two types of cell type II. From these data it may be concluded that the non-homologous co-orientation types were more subjected to genetic regulation than the homologous co-orientations.

Since alternate disjunction in the absence of chiasmata in the interstitial

segments results in fully balanced gametes good correlation is expected between percentage alternate orientation and percentage seed set (Burnham, 1956; John & Lewis, 1965; Sybenga, 1968). Though very rarely the 'figure-of-eight' type of interchange rings were observed at diakinesis, a close agreement between percentage alternate orientation, percentage pollen stainability and percentage seed set was noticed for the interchanges in the present study (Table 6). The same type of relation between percentage alternate disjunction and percentage hatched eggs was noticed for interchanges in German cockroach (Ross & Cochran, 1975).

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REFERENCES

- BURNHAM, C. R. (1934). Cytogenetic studies of an interchange between chromosomes 8 and 9 in maize. *Genetics* **19**, 420-447.
- BURNHAM, C. R. (1956). Chromosome interchanges in plants. *Botanical Review* **22**, 419-552.
- BURNHAM, C. R. (1962). *Discussions in cytogenetics*. Minneapolis: Burgess Publishing Co.
- COCHRAN, D. G. (1976). Disjunction types and their frequencies in two heterozygous reciprocal translocations of *Blattella germanica* (L.). *Chromosoma* (Berl.) **59**, 129-135.
- COCHRAN, D. G. (1977). Patterns of disjunction frequencies in heterozygous reciprocal translocations from the German Cockroach. *Chromosoma* (Berl.) **62**, 191-198.
- DENNHÖFER, L. (1975). Inherited preferential segregation in translocation heterozygotes of the mosquito, *Culex pipiens* L. *Theoretical and Applied Genetics* **45**, 250-253.
- ENDRIZZI, J. E. (1974). Alternate-1 and alternate-2 disjunction in heterozygous reciprocal translocations. *Genetics* **77**, 55-60.
- HAGBERG, A. (1954). Cytogenetic analysis of erectoides mutations in barley. *Acta Agricultura Scandinavica* **49**, 472-490.
- JOHN, B. & LEWIS, K. R. (1965). *The meiotic system*. Protoplasmatologia VI/FI. Vienna: Springer.
- LACADENA, J. R. & CANDELA, M. (1977). Centromere co-orientation at metaphase I in interchange heterozygotes of rye, *Secale cereale* L. *Chromosoma* (Berl.) **64**, 175-189.
- ROBINSON, A. S. (1976). Progress in the use of chromosomal translocations for the control of insect pests. *Biological Reviews* **51**, 1-24.
- ROSS, M. H. & COCHRAN, D. G. (1975). Two new reciprocal translocations in German cockroach. *Journal of Heredity* **66**, 79-88.
- SAKAI, R. K., BAKER, R. H., MIAN, A. & SAID, S. (1972). Sterility enhancement in the mosquito *Culex tritaeniorhynchus*. *Canadian Journal of Genetics and Cytology* **14**, 215-220.
- SYBENGA, J. (1968). Orientation of interchange multiples in *Secale cereale*. *Heredity* **23**, 73-79.
- SYBENGA, J. (1975). *Meiotic configurations*. Berlin-Heidelberg-New York: Springer.
- THOMPSON, J. B. (1956). Genotypic control of chromosome behaviour in rye. II. Disjunction at meiosis in interchange heterozygotes. *Heredity* **10**, 99-108.