INDUCED CHROMOSOMAL CHANGES IN *LINUM*

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1. Introduction

Nuclear changes associated with environmentally induced heritable variation in flax plants of the variety Stormont Cirrus have been reported earlier (Evans, Durrant and Rees, 1966; Evans, 1968). Plants of the large stable genotroph (*L*), produced by giving high nitrogen and high temperature treatment to seedlings of the commercial variety Stormont Cirrus, were found to contain 16 per cent. more nuclear DNA than plants of the small stable genotroph (*S*) produced by giving high phosphate treatment under similar conditions. It was inferred that the difference in nuclear DNA was most probably located in the chromosomes but there was no definite proof of this. It is impracticable to carry out karyotype analysis of the somatic chromosomes because they are very small and do not take up stain easily. However, examination and analysis of metaphase 1 of meiosis can be satisfactorily carried out and used to ascertain whether any structural differences exist between chromosomes of the *L* and *S* genotroph—such as would be expected if the variation in DNA is located in the chromosomes.

The method adopted was to produce autotetraploids of *L* and *S* plants and of their hybrids and subsequently to determine the pairing behaviour of chromosomes in the " parent " and " hybrid " tetraploids. The argument is that if the chromosomes of *L* and *S* are structurally different the pairing in their tetraploid hybrid will tend towards bivalent formation rather than quadrivalents. A comparison of the relative frequencies of bivalents and quadrivalents in the " parent " and " hybrid " tetraploids serves consequently as a measure of any structural divergence between *L* and *S* chromosomes. The analysis is essentially of the type described by Darlington (1937).

2. Materials and methods

Seeds of *L* and *S* genotrophs of Stormont Cirrus were obtained from stocks grown under uniform conditions for several generations following " conditioning ". The $L \times S$ and $S \times L$ were obtained from crosses carried out under field conditions the previous year. Although flax is an inbreeder the *L* and *S* seed used were also obtained from crosses between plants within the respective genotroph. In this way any effects on plant development that might be attributable to seed damage due to the emasculation of the flowers are common to both " hybrids " and " parents ".

Induction of polyploidy. The technique adopted for the induction of tetraploidy was both simple and effective. Seeds were sown on damp filter paper in petri dishes and incubated at 25° C. for two days, by which time the radicles were about a centimetre long. These seedlings were then placed in 0·1 per cent. aqueous colchicine solution for two hours at room

temperature followed by a thorough washing in distilled water before planting in John Innes No. 1 compost in shallow boxes in a warm greenhouse.

Cytological techniques. Flower buds were fixed in Carnoy's fixative to which a few drops of Ferric chloride solution had been added as a mordant. Squash preparations were made in the usual way using aceto-carmine stain. Chiasma frequency and the distribution of the various chromosome configurations found at M_1 of meiosis were scored in 10 pollen mother cells per plant.

3. Results

(a) *Chromosome pairing in* L *and* S *and their hybrids*

The mean frequency per *p.m.c.* of the different types of chromosome configurations for each plant of the two autotetraploid genotrophs and of the two tetraploid crosses is given in table 1. No data for trivalent frequency

Table 1

Mean frequency per p.m.c. *of the different types of chromosome configuration together with mean chiasma frequency for each plant of the two autotetraploid genotrophs and the reciprocal crosses between them*

	Plants	1	2	3	4	5	6	Mean
	Chiasmata	57·3	57·9	57·3	56·9	58·2	58·8	57·7
$L \times L$	IV	8·5	9·5	9·9	9·6	9·6	10·0	9·52
	II	12·6	10·3	9·7	9·6	9·6	10·0	10·30
	I	0·5	0·5	0·7	0·6	0·2	0	0·42
	Chiasmata	57·4	—	58·2	55·3	56·1	56·9	56·8
$S \times S$	IV	8·9	—	9·7	9·5	9·3	9·7	9·42
	II	11·0	—	10·5	9·9	10·7	9·8	10·38
	I	0·6	—	0·3	1·0	1·1	0·7	0·74
	Chiasmata	56·4	56·4	55·9	54·0	55·2	56·3	55·7
$L \times S$	IV	7·9	7·2	7·1	7·5	6·8	7·5	7·33
	II	13·6	14·9	14·7	13·5	15·2	13·8	14·29
	I	0·6	1·1	0·7	1·8	1·5	0·9	1·10
	Chiasmata	55·8	56·4	55·4	56·9	54·3	56·0	55·8
$S \times L$	IV	5·9	6·8	8·1	7·6	6·7	8·2	7·22
	II	17·6	15·6	12·9	13·9	14·9	13·3	14·70
	I	0·6	1·6	1·5	1·2	1·2	0·6	1·12

are given in view of the very low number of such configurations found in this material. Analyses of variance of the data are given in table 2.

From these analyses it will be seen first that there is no significant difference in mean quadrivalent, bivalent or univalent frequency between the *L* and *S* tetraploid genotrophs. Secondly, there are no significant differences in the frequencies of these configurations between the recriprocal crosses. Thirdly, and in marked contrast, the variation in frequency of all three configurations between the parents and the F_1 cross is highly significant ($P = <0{\cdot}01$). Most important, the mean quadrivalent frequency is higher ($\bar{d} = 2{\cdot}19$) in the parents than in the hybrids and the mean bivalent frequency is lower ($\bar{d} = 4{\cdot}15$). It is appreciated that the frequencies of quadrivalents and bivalents are not independent of one another. That there are highly

significant differences in respect of *both* configurations leaves no doubt, however, of the variation in pairing between " hybrids " and " parents ". The higher quadrivalent and lower bivalent frequencies in *p.m.c.* of the parents as compared with the hybrids is precisely what would be expected as a result of structural divergence affecting the homology of *L* and *S* chromosomes.

TABLE 2

The analyses of variance of the frequency distribution of the different types of chromosome configuration in the autotetraploid L *and* S *genotrophs and the crosses between them*

Configuration	Item	d.f.	Mean square	Variance ratio	P
IV	Total	22			
	Genotroph	3	9·263	26·31	< 0·001
	L−*S*	—	—	$t =$ 0·279	N.S.
	L×*S*−*S*×*L*	—	—	$t =$ 0·322	N.S.
	P−F_1	—	—	$t =$ 8·831	< 0·001
	Error	19	0·352		
II	Total	22			
	Genotroph	3	33·21	24·65	< 0·001
	L−*S*	—	—	$t =$ 0·114	N.S.
	L×*S*−*S*×*L*	—	—	$t =$ 0·612	N.S.
	P−F_1	—	—	$t =$ 8·557	< 0·001
	Error	19	1·347		
I	Total	22			
	Genotroph	3	0·663	4·51	< 0·01
	L−*S*	—	—	$t =$ 1·380	N.S.
	L×*S*−*S*×*L*	—	—	$t =$ 0·091	N.S.
	P−F_1	—	—	$t =$ 3·438	< 0·01
	Error	19	0·147		

While the tendency for higher bivalent frequencies in the hybrid is reasonably interpreted as being due to structural changes in the *Linum* chromosomes there is an alternative possibility that needs to be taken into account. It is that the chiasma frequencies are lower in the " hybrid " than in the " parents ". If this is so there are good reasons for expecting the quadrivalent frequencies in the hybrid to be lower and the bivalent frequencies higher (Durrant, 1960; Hazarika and Rees, 1967; John and Henderson, 1962). The evidence which relates to this alternative possibility is discussed below.

(b) *Chiasma frequency and chromosome pairing*

The mean chiasma frequencies for parent and hybrid genotrophs are given in table 1 and an analysis of variance of the data given in table 3. This analysis does show that although there is no significant difference in chiasma frequency between *L* and *S* genotrophs and also no reciprocal difference in the cross between them, there is nevertheless a significant

decrease (P = <0·001) in mean chiasma frequency in the hybrids as compared with the " parents ".

Whether the drop in hybrid chiasma frequencies is itself a reflection of structural non-homology between parental chromosomes or whether it may be a consequence of genotypic control cannot be determined from the present results. It is, however, possible to determine whether this drop in hybrid chiasma frequencies accounts directly for the variation in pairing configurations between hybrids and parents.

TABLE 3

The analysis of variance of mean plant chiasma frequency in p.m.c.s *of the two autotetraploid genotrophs and the reciprocal cross between them*

Item	d.f.	Mean square	Variance ratio	P
Total	22			
Genotroph	3	5·430	6·418	<0·01
$L-S$	—	—	$t = 1·62$	N.S.
$L\times S-S\times L$	—	—	$t = 0·179$	N.S.
$P-F_1$	—	—	$t = 4·036$	<0·001
Error	19	0·846		

(c) *Chiasma frequencies and the pairing pattern*

Chiasma frequency is known to have a profound effect on the distribution of the various chromosome configuration at meiosis in *p.m.c.*'s of some autotetraploids. For example, Hazarika and Rees (1967) found that in autotetraploid rye an increase in chiasma frequency was accompanied by an increase in quadrivalent frequency and a decrease in bivalent, trivalent and univalent frequency. This agreed with the statistical predictions made by Durrant (1960).

On the face of it, therefore, a correlation between chiasma frequency and the pattern of chromosome pairing provides a ready explanation for the lower quadrivalent and higher bivalent frequency found in the hybrid between the autotetraploid *L* and *S* genotrophs of Stormont Cirrus. This possibility, however, is ruled out by the following evidence. In fig. 1 where bivalent and quadrivalent frequencies are plotted against the chiasma frequencies of plants in all four families it will be seen that there is no indication whatever of a correlation between the chiasma frequency and the frequencies of bivalents and quadrivalents. A regression analysis confirms that there is no significant correlation.

Further evidence for the independence of quadrivalent and bivalent frequency on chiasma frequency in this material is found from an analysis of the results at cell level. Table 4 gives the mean frequency per cell of quadrivalents and bivalents for each chiasma frequency value in the range 52-60 for all four types. This type of treatment of the data can separate the effect of chiasma frequency from any other factor which might affect the degree of chromosome association at metaphase 1 of meiosis. The correlation can be analysed either by a joint regression analysis or by a straightforward analysis of variance. Since no significant regression of chromosome configuration frequency on chiasma frequency was found in the

previous section it was felt that an analysis of variance would be a more appropriate way of handling these particular data. This analysis is given in table 5. The analysis shows that chiasma frequency has no significant

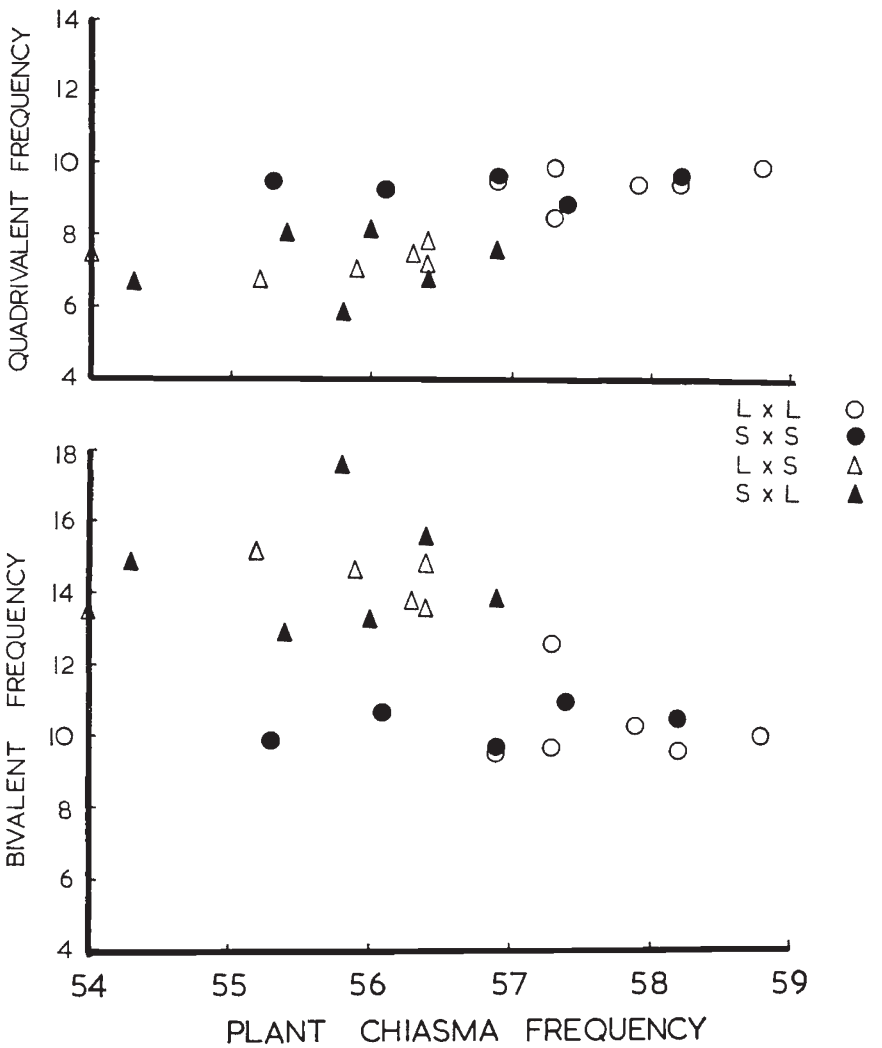

FIG. 1.—The average frequency per *p.m.c.* of quadrivalents and bivalents plotted against mean cell chiasma frequency in plants of the autotetraploid *L* and *S* genotrophs and the reciprocal cross between them.

effect on the frequency of either quadrivalents or bivalents. This is in agreement with the previous analyses on a plant basis. The analysis also confirms the previous conclusion, viz., that the mean cell quadrivalent and bivalent frequencies do not vary significantly between *L* and *S* or between reciprocal crosses but that the difference between " parents " and " hybrids " is highly significant ($P = < 0{\cdot}001$). Above all the analysis establishes that

the higher quadrivalent and lower bivalent frequency per *p.m.c.* of the parents as compared with the crosses is, virtually, independent of the cell chiasma frequency.

TABLE 4

The average frequency of quadrivalents and bivalents in pollen mother cells of specific chiasma frequency in plants of autotetraploid L *and* S *genotroph and the reciprocal cross between them*

Cell-chiasma frequency	IV					II				
	L×*L*	*S*×*S*	*L*×*S*	*S*×*L*	Total	*L*×*L*	*S*×*S*	*L*×*S*	*S*×*L*	Total
53	8·0	8·3	7·3	6·5	30·1	13·0	11·7	13·2	15·4	53·3
54	9·0	9·3	7·4	7·0	32·7	10·0	10·3	13·8	15·0	49·1
55	9·4	8·9	7·3	8·4	34·0	10·8	11·4	14·7	12·4	49·3
56	9·6	9·6	7·3	8·0	34·5	10·4	9·7	13·9	13·2	47·2
57	9·4	9·1	7·1	7·3	32·9	11·1	10·7	14·8	14·8	51·4
58	9·4	10·2	6·8	8·3	34·7	10·6	9·3	16·1	13·3	49·3
59	9·7	8·7	7·7	7·3	33·4	10·3	12·0	14·5	15·0	51·8
60	9·9	9·7	9·0	6·8	35·4	10·3	10·7	12·0	16·5	49·5
Total	74·4	73·8	59·9	59·6		86·5	85·8	113·0	115·7	
	148·2		119·5			172·3		228·6		

TABLE 5

The analysis of variance of average quadrivalent and bivalent frequency in pollen mother cells of specific chiasma frequency in the autotetraploid L *and* S *genotroph and the reciprocal cross between them*

Configuration	Item	d.f.	Mean square	Variance ratio	P
IV	Total	31			
	Chiasma frequency	7	0·673	2·064	N.S.
	Type	3	8·59	26·349	
	L—*S*	1	0·225	—	N.S.
	L×*S*—*S*×*L*	1	0·0056	—	N.S.
	P—F_1	1	25·740	78·966	<0·001
	Error (interaction)	21	0·326		
II	Total	31			
	Chiasma frequency	7	0·923	—	N.S.
	Type	3	33·167	23·708	
	L—*S*	1	0·031	—	N.S.
	L×*S*—*S*×*L*	1	0·423	—	N.S.
	P—F_1	1	99·053	70·803	<0·001
	Error (interaction)	21	1·399		

(d) *The distribution of chiasmata*

Detailed analysis of the distribution pattern of chiasmata between the various configurations at M_1 of meiosis in these autotetraploids provides further information about the consequences of the structural divergence between the *L* and *S* genotrophs inferred above.

The chiasma frequency of quadrivalents. Figure 2 gives the average quadrivalent chiasma frequency per cell for each specific *p.m.c.* chiasma frequency from 52 to 60 for both parents and hybrids. The joint regression analysis

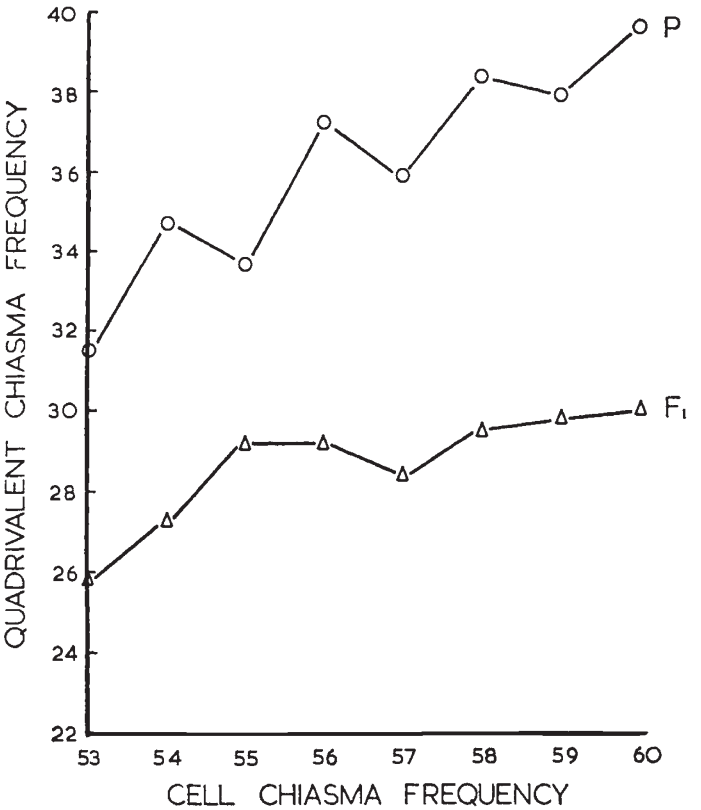

FIG. 2.—The regression of the average cell chiasma frequency of quadrivalents on *p.m.c.* chiasma frequency in the autotetraploid " parents " and " hybrids ".

TABLE 6

Joint regression analysis of the average quadrivalent chiasma frequency in p.m.c *of specific chiasma frequencies in autotetraploid plants of the parent genotrophs and the hybrid between them*

Item	d.f.	Mean square	Variance ratio	P
Joint regression	1	46·406	46·545	< 0·001
Heterogeneity of regression	1	4·905	4·920	< 0·05
Heterogeneity of means	1	221·265	221·931	< 0·001
Error	12	0·997	—	—
Total	15	—	—	—

given in table 6 confirms, as one would indeed expect from the earlier results, that the quadrivalent chiasma frequency is greater in the parents than in the hybrids for comparable *p.m.c.* chiasma frequency. However it is

important to note that the variation in slopes of the regression lines is also significant at the 5 per cent. level. The rate of increase of chiasma frequency in quadrivalents with increasing *p.m.c.* chiasma frequency is higher in the " parents " than in hybrids. The significance of this observation is discussed in a subsequent section.

The chiasma frequency of bivalents. Clearly if the chiasma frequency of quadrivalents increases more rapidly with increasing *p.m.c.* chiasma frequency in the parents, then the increase in bivalent chiasma frequency in the parents must be correspondingly less rapid than in the hybrids. That this is so is shown in fig. 3 where the bivalent chiasma frequency is plotted

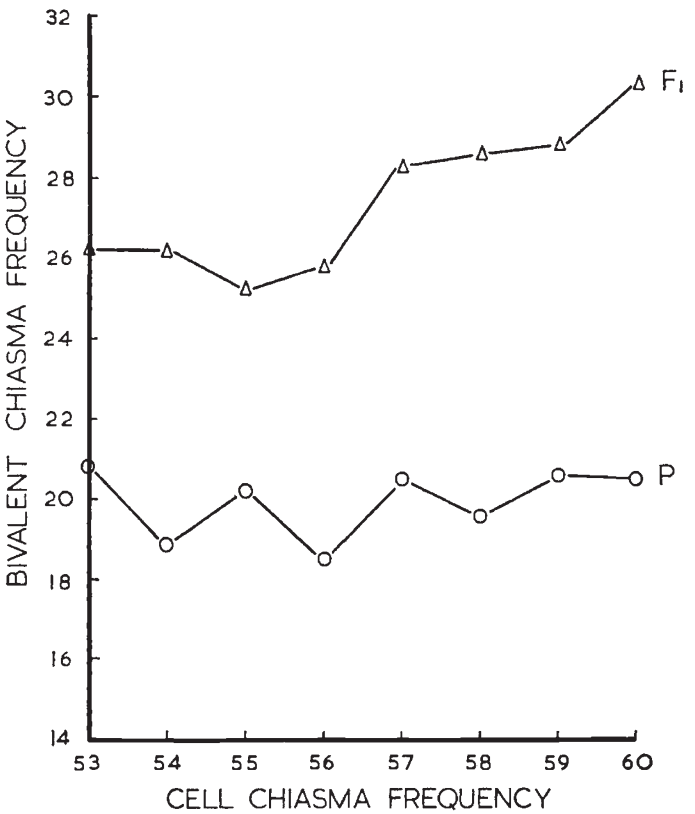

FIG. 3.—The regression of the average chiasma frequency of bivalents on cell chiasma frequency in pollen mother cells of autotetraploid large and small Genotroph (Parents) and the crosses between them.

against *p.m.c.* chiasma frequency. A joint regression analysis given in table 7 shows that the heterogeneity of regression lines is significant at the 5 per cent. level.

These observations show that with increasing *p.m.c.* chiasma frequencies the additional chiasmata in the hybrids tend to be allocated mainly to bivalents rather than to quadrivalents. The indication is, as might be expected from the previous results in section 3 (*c*), that pairing and hence chiasma formation is more effective between pairs of chromosomes, than

between associations of four. As has already been observed, the most probable explanation for this is a structural divergence affecting the homology of the *L* and *S* chromosomes.

TABLE 7

A joint regressino analysis of the average bivalent *chiasma frequency in pollen mother cells of specific chiasma frequency in plants of autotetraploid* L *and* S *genotrophs and the cross between them*

Item	d.f.	Mean square	Variance ratio	P
Joint regression	1	13·721	14·368	< 0·01
Heterogeneity of regression	1	7·956	8·331	< 0·05
Heterogeneity of means	1	228·766	239·545	< 0·001
Error	12	0·955	—	—
Total	15	—	—	—

4. CONCLUSION

The crossing and grafting experiments of Durrant (1962) established that the heritable changes induced in *Linum* were, almost certainly, of nuclear origin. This conclusion was supported by cytochemical investigations which revealed that differences in nuclear dry mass and in nuclear DNA were associated with the induction of heritable change. The present cytological investigation shows that the nuclear changes, in turn, have affected chromosome homology and hence, it must be concluded, the structure and organisation of the chromosomes themselves.

Permanent changes in chromosome structure of two main kinds can be envisaged. The first is longitudinal replication of chromosome regions (*cf.* Rees *et al.*, 1966). The second is a change in *polynemy*, *i.e.* in the degree of strandedness (*cf.* Darlington, 1965). There is a third type of change that may also be worth considering, namely that involving material which is not part of the permanent chromosome " thread " but is supplementary in the same sense that an episome may be supplementary to a bacterial chromosome.

There is, as yet, no evidence that allows one to distinguish between these various possibilities.

5. SUMMARY

1. A method involving the analysis of chromosome " pairing " at meiosis in autotetraploids of two flax genotrophs and their hybrids is described as a means of finding whether any divergence in chromosome structure had taken place.

2. Analysis of M_1 of meiosis showed that there were: (i) no difference in the frequencies of the various chromosome configurations between the two genotrophs or between the reciprocal crosses; (ii) significantly lower quadrivalent and higher bivalent frequencies per *p.m.c.* in the crosses compared with the parent genotrophs.

3. This difference in pairing pattern is independent of chiasma frequency.

4. Lower quadrivalent and higher bivalent frequencies in *p.m.c.*'s of the parents as compared with the hybrids is expected as a result of structural

divergence affecting the homology of chromosomes from the two genotrophs. Two possible kinds of permanent structural changes are envisaged.

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6. References

DARLINGTON, C. D. 1937. *Recent Advances in Cytology*, 2nd Ed. Churchill, London.

DARLINGTON, C. D. 1965. *Cytology*. Churchill, London.

DURRANT, A. 1960. Expected frequencies of chromosome associations in tetraploid with random chiasma formation. *Genetics, 45*, 779-783.

EVANS, G. M., DURRANT, A., AND REES, H. 1966. Associated nuclear changes in the induction of flax genotrophs. *Nature, 212*, 697-699.

EVANS, G. M. 1968. Nuclear changes in flax. *Heredity, 23*, 25-38.

HAZARIKA, M. H., AND REES, H. 1967. Genotypic control of chromosome behaviour in Rye. X. Chromosome pairing and fertility in autotetraploids. *Heredity, 22*, 317-332.

JOHN, B., AND HENDERSON, S. A. 1962. Asynapsis and polyploidy in Schistocerca paranensis. *Chromosoma, 13*, 111-147.

REES, H., CAMERON, F. M., HAZARIKA, M. H., AND JONES, G. H. 1966. Nuclear variation between diploid angiosperms. *Nature, 211*, 828-830.

REES, H., AND HAZARIKA, M. H. 1968. Chromosome evolution in Lathyrus. *Chromosomes Today*, Vol. II (in press).