Relationships between Frequency, Localization and Errors in Chiasma Formation in Desynaptic Rye

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Abstract. Four inbred lines of rye (*Secale cereale*) and the F_1 and F_2 from the cross between two of them have been studied. The results indicate that the genotypes used show variation in chiasma frequency, chiasma error frequency and chiasma localization. Significant correlations between these characters have been found: as chiasma frequency decreases both chiasma error frequency and distal localization increase. These correlations lead us to the assumption that these anomalies are in fact secondary effects of the failure of some preconditions for exchange. It is suggested that one such exchange precondition may be effective pairing.

Introduction

Mutations, genetic unbalance and environmental variation are phenomena leading to the appearance of a series of meiotic anomalies. Some of these anomalies concern different aspects of chiasma formation: chiasma frequency, chiasma distribution and chiasma errors which can be detected by the presence of bridges and fragments at anaphase I.

There is a considerable list of cases in which a reduction in chiasma frequency appearing simultaneously with anaphase I bridges and fragments has been reported; some of them are: *Alopecurus myosuroides* (Johnsson, 1944), *Secale cereale* (Müntzing and Akdik, 1948; Rees, 1955; Jones, 1968), *Scilla sibirica* (Rees, 1952), *Bromus sp.* (Walters, 1952), *Paris verticillata* (Haga, 1953), *Paeonia californica* (Walters, 1956), *Allium cepa* (Koul, 1962), *Elymus farctus* (Heneen, 1963), *Podophyllum peltatum* (Newman, 1967), *Tulipa sp.* (Couzin and Fox, 1973; Southern, 1967a), *Pisum sativum* (Klein and Baquar, 1972). On the other hand, it has been demonstrated that frequency and distribution of chiasmata are inter-related events (Henderson, 1962; John and Henderson, 1962; Southern, 1967b; Carpenter and Sandler, 1974).

The aim of this work was to investigate if there is any genetical reason for the simultaneous appearance of these anomalies. With this in mind, four rye

inbred lines showing different degrees of desynapsis, different frequency of anaphase I bridges and fragments and variation in chiasma distribution, as well as the F_1 and F_2 of a cross between two of these lines were analysed.

Material and Methods

Four inbred lines of rye, *Secale cereale L.* (V, P, M, and A), the F_1 and F_2 of the cross between the lines P and A formed the material for this study. All lines were obtained by self pollination during 14 to 20 generations (V, I_{20}; P, I_{14}; M, I_{15}; A, I_{14}) at the Experimental Station of Aula Dei (C.S.I.C., Zaragoza, Spain).

An open pollinated line of *Secale cereale L.* (cv. Ailés), growing in the same conditions, was used as a control.

All observations were made in Feulgen stained squash preparations of pollen mother cells, following fixation in acetic-alcohol 1:3. Preparations were made permanent by inclusion in sandeural.

The number of bivalent arms per cell associated at metaphase I was taken as an estimate of the number of chiasmata.

Anaphase I fragment sizes were determined by photographic means, as used in previous investigations of this kind (Rees and Thompson, 1955; Jones, 1968).

Results

Metaphase I

Table 1 shows the mean number of ring bivalents, open bivalents, univalents and bound arms per cell observed at metaphase I. Univalents at metaphase I in these lines result from desynapsis (Giraldez and Lacadena, 1976).

Table 1. Mean numbers of ring bivalents per cell (R), open bivalents per cell (O), univalents per cell (U), and bound arms (chiasmata) per cell (B), at metaphase I of the four inbred lines, the F_1 and F_2 of the hybrid between the two inbred lines A and P, and the open pollinated line (cv. Ailés) used as a control

	R	O	U	B	Number of cells	Number of plants
Line V	4.94 ±0.19	1.84 ±0.16	0.43 ±0.08	11.73 ±0.23	600	6
Line P	3.83 ±0.13	2.65 ±0.05	1.01 ±0.18	10.32 ±0.23	600	5
Line M	3.45 ±0.34	2.95 ±0.28	1.19 ±0.15	9.85 ±0.40	400	4
Line A	2.80 ±0.08	3.31 ±0.05	1.75 ±0.08	8.92 ±0.12	2600	10
$F_1(A \times P)$	5.80 ±0.09	1.09 ±0.06	0.19 ±0.06	12.71 ±0.12	500	4
$F_2(A \times P)$	4.80 ±0.19	2.03 ±0.16	0.32 ±0.09	11.64 ±0.23	800	8
Control (cv. Ailés)	6.34 ±0.05	0.65 ±0.05	–	13.34 ±0.05	200	2

In order to demonstrate its random nature, the distribution of chiasmata per cell at metaphase I in rye has been compared with a Poisson series (Sybenga, 1960; Jones, 1967). In some cases, specially when univalents were frequent, the differences between observed and expected values were significant. In our opinion the reason for this lack of fit might be due to the non-binomial nature of the distribution of chiasmata per cell. In our case each pair of homologues can have three different configurations at metaphase I: ring bivalent, open bivalent or univalent pair.

Even when the average chiasma frequency is the same for all seven bivalents, the probability of forming ring bivalents, open bivalents or univalent pairs may not be identical for all chromosomes, as it is dependent on the relative length of the two arms of the chromosomes. At mitosis the length ratio long arm/short arm varies between approximately 1 and almost 2 for rye chromosomes. A simple test made clear that in the range of chiasma frequencies present in this material, the relative frequencies of the different configurations is only slightly affected by such differences in arm length ratio.

Another test on the chromosome pairs and cells in respect to configuration and chiasma frequencies is the comparison of the distribution of rings, open bivalents and univalent pairs per cell with a binomial series. Inbred lines V, P, M and A were tested. The fit was quite good except for two individual plants of P and one of A, which deviated considerably from random. The reason could not be traced. Details about the behaviour of individual plants are available from the authors.

Now, assuming that all seven pairs of homologs have similar chiasma frequencies, a cell containing r ring bivalents, o open bivalents and u univalent pairs will appear with a probability of:

$$\frac{7!}{r! \times o! \times u!} R^r \times O^o \times U^u.$$

This, of course, is the general term of the development of the trinome: $(R+O+U)^7$, in which R, O and U have the same values as indicated above.

Now, the probability for a cell to have B bound arms equals the sum of the terms of this trinomial distribution in which:

$$2r + o = B.$$

For instance, there are two possible ways for a cell to have twelve bound arms, one of them is having six ring bivalents and one univalent pair $\left(\text{probability: } \frac{7!}{6!0!1!} R^6 O^0 U^1 \right)$ the other way is to have five ring bivalents and two open bivalents $\left(\text{probability: } \frac{7!}{5!2!0!} R^5 O^2 U^0 \right)$. The probability for a cell to have twelve bound arms will be the sum of these two probabilities.

Table 2 shows the distribution of bound arms per cell in the four inbred lines as compared to expected distributions calculated as indicated above. Only one

Table 2. Distributions of numbers of chiasmata (bound arms) per cell at metaphase I in the four inbred lines, compared to the corresponding expected distributions (see text). Asterisk indicates significant differences between observed and expected distributions. Last value for each expected distribution stands for the sum of this and the lower classes

| Number of χta per cell	Line							
	V		P		M		A	
	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.
14	65	52.68	10	9.40	5	2.84	12	4.62
13	127	137.77	31	44.96	17	16.99	39	37.83
12	166	169.19	99	100.53	53	46.98	155	142.40
11	132	130.77	164	139.31	66	79.65	340	326.94
10	73	70.72	140	133.43	103	92.45	476	511.03
9	20	28.29	88	93.88	71	77.71	578	575.76
8	9	8.63	36	49.79	45	48.80	466	481.74
7	4	2.04	17	20.26	25	23.24	290	304.22
6	3	0.34	13	6.35	10	8.42	150	145.68
5	1	0.13	2	1.53	4	2.24	71	52.64
4	–		–	0.36	1	0.40	20	14.13
3	–		–		–	0.28	3	3.06
χ^2		9.30		19.33*		6.74		15.44
d.f.		5		7		6		8
χ^2 het[a]		6.82		23.53		7.41		39.56
d.f.		16		17		13		49

[a]   Heterogeneity χ^2

plant of line P showed a significant difference between observed and expected values. It is one of the plants in which also at the configuration distributions deviated from binomial, as indicated above. This is the reason for the deviation indicated for the total for this line. Detailed information about the behaviour of individual plants is available from the authors.

The conclusion that all seven pairs of homologs have similar chiasma frequencies, is justified.

Anaphase I

Bridges and fragments were observed in all plants studied, both in those belonging to pure inbred lines and F_1 or F_2 generations. Even in those plants in which it was not possible to observe anaphase I, cells in subsequent stages (telophase I, diads, or second division) were found in which anaphase I bridges persisted (Fig. 1). Most anaphase I bridges were formed between the two chromosomes of a bivalent (Fig. 1a). In some cases, however, bridges were formed between both chromatids of a univalent dividing equationally (Fig. 1b).

Table 3 shows the mean number of anaphase I bridges per cell in the four inbred lines and the F_1 and F_2 studied. In 500 cells of the open pollinated line used as a control neither bridges nor fragments were found.
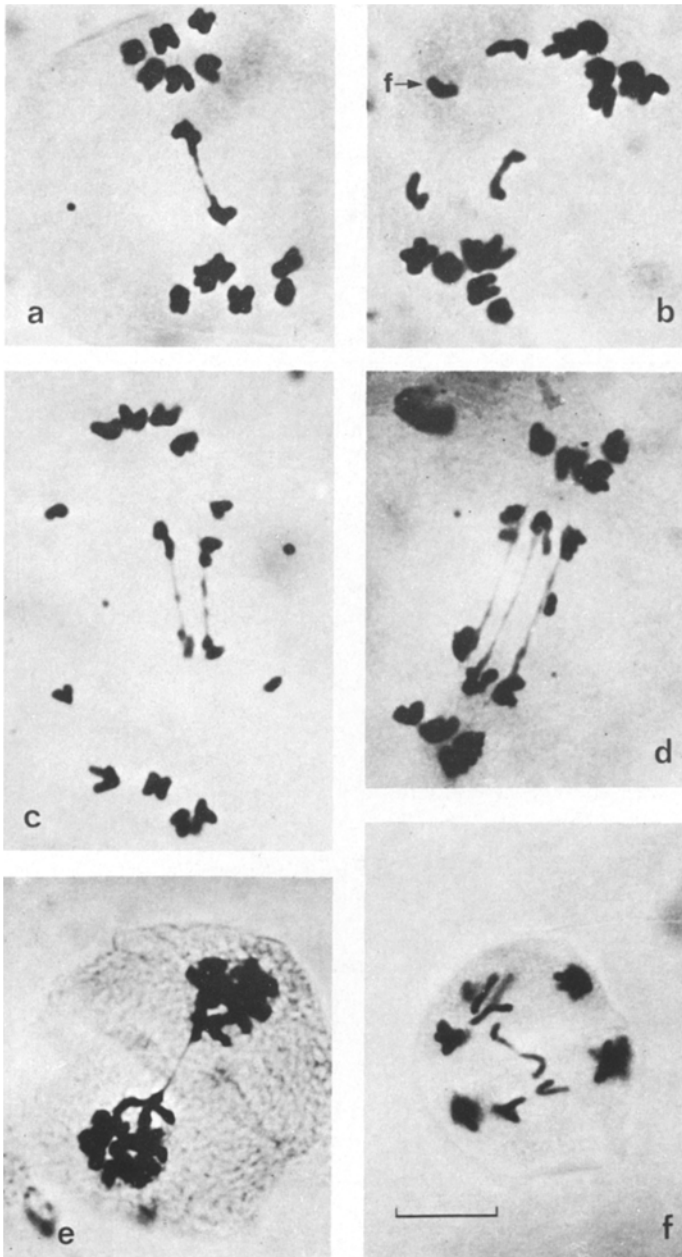
Fig. 1a–f. a Anaphase bridge and fragment formed by a bivalent. **b** Anaphase bridge and fragment formed by both chromatids of a univalent dividing equationally. **c** Two bridges and two fragments at anaphase I. Note the presence of two univalents dividing equationally in the same cell. **d** Three bridges and three fragments at anaphase I. **e** Dyads showing a persistent anaphasic bridge. **f** Anaphase II in which a first division bridge persists

Table 3. Mean numbers of anaphase I bridges per cell in the four inbred lines, the F_1 and F_2 of the cross between the two inbred lines A and P

	Line				$F_1(A \times P)$	$F_2(A \times P)$	Control (cv. Ailés)
	V	P	M	A			
Mean number of bridges	0.25 ±0.04	0.37 ±0.03	0.34 ±0.09	0.44 ±0.02	0.06 ±0.03	0.11 ±0.02	0.00
Number of cells	400	500	200	1400	300	800	500
Number of plants	4	5	2	6	3	8	5

In order to ascertain if all seven bivalents contribute to bridge formation in the same way, the distribution of bridges per cell in each plant was compared with the binomial series:

$$[P+(1-P)]^7$$

in which P is the average probability for a bivalent to form a bridge:

$$P = \frac{\text{Total number of bridges}}{7 \times \text{total number of A I cells}}$$

Table 4 shows the comparison between observed and expected values for each line. As can be seen there is a good fit in all cases. These results are in

Table 4. Distributions of numbers of anaphase I bridges per cell in the four inbred lines compared to the correspondent binomial distributions

Bridges per cell		Line			
		V	P	M	A
0	obs.	314	344	148	886
	exp.	309.30	344.06	143.38	875.22
1	obs.	72	132	41	405
	exp.	81.02	132.11	48.86	425.22
2	obs.	13	22	9	97
	exp.	9.09	21.73	7.13	88.53
3	obs.	1	2	2	11
	exp.	0.56	1.98	0.57	10.24
4	obs.	—	—	—	1
	exp.	0.02	0.10	0.03	0.71
d.f.		1	1	1	2
χ^2		3.0036	0.0013	2.7661	1.9965
		n.s.	n.s.	n.s.	n.s.

Table 5. Mean sizes of anaphase I fragments of inbred lines A and P, and their F_1 and F_2

	Line A	Line P	$F_1(A \times P)$	$F_2(A \times P)$
Mean fragment size (μm^2)	0.761	0.871	1.431	0.982
	±0.025	±0.035	±0.082	±0.042
Number of fragments	322	216	142	377
Number of plants	3	3	3	8
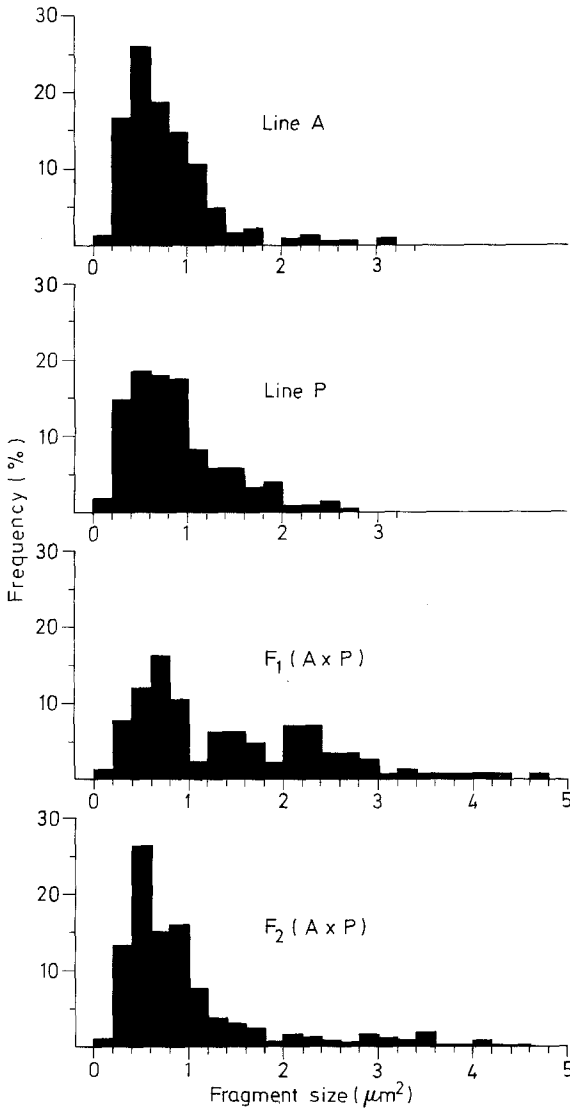
Fig. 2. Fragment size distributions of the inbred lines A and P and their F_1 and F_2
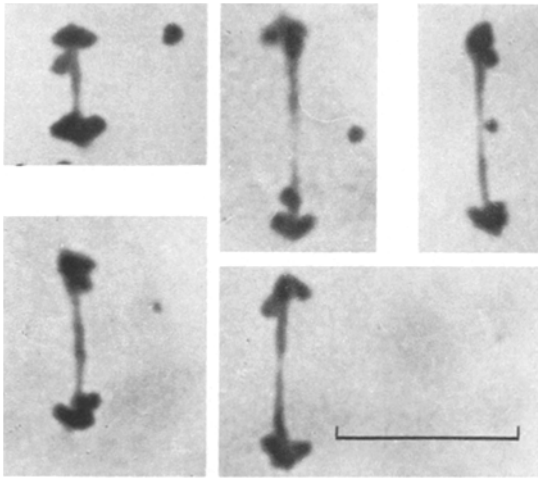
Fig. 3. Some of the bridges and fragments found in a plant (P6) of the inbred line P photographed at the same magnification. Note the variation in fragment size within the same plant

agreement with the assumption that all seven bivalents have the same probability of forming a bridge.

Anaphase I fragments were measured in inbred lines A and P and in their F_1 and F_2.

Table 5 shows the mean sizes of the anaphase I fragments for each plant analysed. The fragment size distributions for each genotype are represented in histogram form in Figure 2. Figure 3 shows, as an example, the variation in fragment size that could be observed within the same plant.

From these results, two interesting observations can be deduced. First, there is an excess of fragments with small size in all cases. Second, there is a considerable variation in fragment size, both within plants and between genotypes.

As will be discussed later, anaphase I bridges and fragments seem to be produced by erroneous chiasmata (U-type exchanges) as those described in another investigations of this type on inbred rye (Rees and Thompson, 1955; Jones, 1968, 1969). Then, fragment size can be taken as an estimation of the length from telomere to the chromosome arm point in which U-type exchange occurred, and the between lines variation in fragment size distribution found in this work could be attributed to the existence of differences in within bivalent U-type exchange distribution.

If this is true, we have different genotypes of rye showing variation both in chiasma frequency, in U-type exchange frequency and in U-type exchange distribution within bivalents.

Figure 4 shows the relationship between the mean number of bound arms per cell at metaphase I and the mean number of bridges per cell at anaphase I for each plant. The regression of mean number of bridges per cell on mean number of bound arms per cell is negative, small, and highly significant ($b = -0.0966$; $t = 8.8458$; $p < 0.001$).
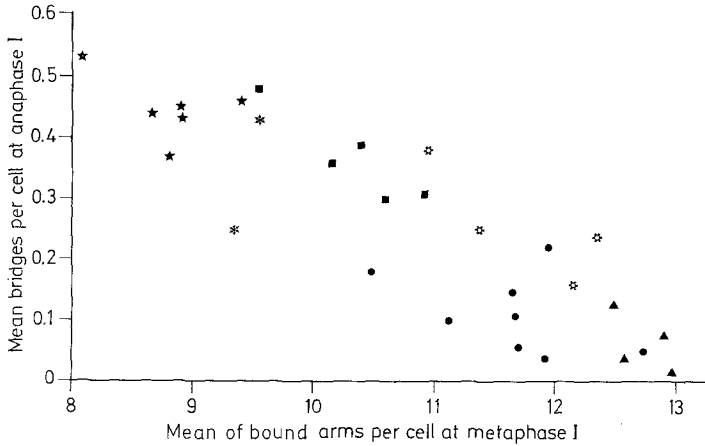
Fig. 4. The mean number of anaphase I bridges per cell plotted against the mean number of chiasmata (bound arms) per cell at metaphase I in each plant. ⋆ Inbred line A; ■ inbred line P; ✩ inbred line V; ✳ inbred line M; ▲ $F_1(A \times P)$; ● $F_2(A \times P)$
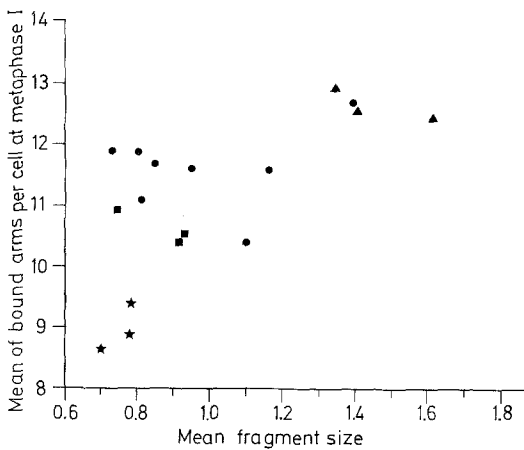

Fig. 5. The mean chiasmata (bound arms) per cell at metaphase I plotted against the mean fragment size in each plant. ⋆ Inbred line A; ■ inbred line P; ▲ $F_1(A \times P)$; ● $F_2(A \times P)$

Figure 5 shows the relationship between the mean number of bound arms per cell at metaphase I and the mean size of fragments at anaphase I for each plant. The regression of bound arms per cell on fragment size is positive, large, and highly significant ($b = 3.1503$; $t = 3.5296$; $p < 0.01$).

Discussion

Anaphase I bridges and fragments have been attributed on a number of occasions to breakage and reunion or U-type exchange (Haga, 1952; Rees, 1952; Rees and Thompson, 1955; Lewis and John, 1966; Jones, 1968, 1969; Couzin

and Fox, 1973). In most of these cases a close relationship between breakage events and crossing-over has been found, leading to the hypothesis that U-type exchanges result from errors in crossing-over.

In this work there are some findings that agree with this hypothesis:

(i) All bivalents have similar chiasma frequencies. If anaphase I bridges are produced by crossing-over errors a similar probability of forming a bridge is expected for all bivalents. The results confirm this expectation.

(ii) Chiasmata in rye are mostly distally localized. As in other studies in rye (Rees and Thompson, 1955; Jones, 1968) an excess of small size fragments have been found in all cases, indicating that most U-type exchanges occur near the telomere.

(iii) In desynaptic rye, it is supposed that two univalents appear when no chiasma is formed between two homologous chromosomes. Then, bridges formed between both chromatids of a univalent dividing equationally (Fig. 1 b) would indicate the occurrence of a breakage and reunion or a U-type error in a sister-chromatid exchange. This same kind of anomaly has been found in other studies in similar material (Jones, 1968; Jones and Brumpton, 1971).

(iv) In desynapsis chiasma formation seems to be affected. This can produce two alternative effects, namely, loss of chiasmata and erroneous chiasmata. This assumption about the effect of desynapsis agrees with the fact that in most cases in which univalents at metaphase I have been observed (desynapsis), a simultaneous appearance of anaphase I bridges and fragments has been found (Johnsson, 1944; Müntzing and Akdik, 1948; Rees, 1952, 1955; Walters, 1952; Haga, 1953; Walters, 1956; Koul, 1962; Heneen, 1963; Newman, 1967; Southern, 1967a; Jones, 1968; Klein and Baquar, 1972; Couzin and Fox, 1973).

If this is true, a correlation between chiasma frequency and bridge frequency can be expected, i.e. the higher the degree of desynapsis, the lower the chiasma frequency at metaphase I and the higher the frequency of anaphase I bridges. The negative correlation between chiasma frequency and anaphase I bridge frequency found in this work (see Fig. 4) agrees with this hypothesis.

It can then be concluded that U-type exchanges are due to erroneous chiasmata.

Fragment size distribution can be taken as an estimate of chiasma error distribution within bivalents. Now, if any chiasma has the same probability of being erroneous, the fragment size distribution can be taken as an estimation of the actual chiasma distribution within bivalents (Jones, 1968). Under this assumption the regression of mean number of chiasmata per cell on mean fragment size found in this work (see Fig. 5) indicates that frequency and distribution of chiasmata are interrelated events, i.e., as the chiasma frequency decreases the distal localization increases.

Desynapsis is an abnormal process leading to the appearance of univalents at metaphase I. As was shown by Giraldez and Lacadena (1976) in this same material, univalents seem to increase in number during metaphase I until the beginning of anaphase I. It was also shown that the higher the degree of desynapsis the earlier the univalents begin to form.

The material studied in the present work shows variation in four different ways: chiasma frequency, chiasma errors, chiasma positions and univalent

formation time. Moreover, all these characters are correlated: as chiasma frequency decreases, chiasma errors increase, chiasma positions tends to be more distal and univalents form earlier.

Mutants affecting the frequency and distribution of crossing-over and simultaneously increasing non-disjunction have been found in *Drosophila* (Carpenter and Sandler, 1974). In that case the genetical reason for the simultaneous appearance of all these abnormalities was claimed to be the failure in some exchange preconditions. The correlations between the different meiotic abnormalities found here suggest that they might be produced by a single controlling system. Probably an exchange precondition is altered, causing as a secondary effect all the abnormalities described.

In spite of the fact that under the light microscope pairing at the end of zygotene seems as a rule to be complete, both in the inbred lines and in the open pollinated line incomplete effective pairing cannot be discarded as the altered exchange precondition since the alteration in pairing effectivity can consist not only in the completion of pairing but in its synchronization with the crossing-over process.

There is good evidence supporting that in maize pairing proceeds from the telomeres to the centromeric zone of chromosomes (Burnham et al., 1972). If pairing in rye proceeds in the same way and if effective pairing is altered in our material it can be expected that the greater the alteration the smaller the chromosomal zone effectively paired. Moreover, the reduction of effective pairing could be larger in the last chromosomal zone to be paired. This would lead to a situation in which with an increase of the alteration effective pairing would be restricted to a shorter chromosome zone near the telomeres, and the fewer chiasmata formed would be located more distally, and the effect of terminalization that leads to univalent formation would be higher.

It is somewhat simplistic to ascribe all effects observed to a limitation in effective pairing. For instance it is improbable that the exchange error itself, leading to U-type exchange, is caused by ineffective pairing.

Probably both are parallel effects of inbreeding, but need not necessarily be causally related.

The variation in several aspects of meiotic chromosome behaviour between inbred lines of rye has been taken as evidence for a direct polygenic control of these processes (Rees, 1955). The results of this work point to the possibility that the alteration in these processes is a secondary effect of a decrease in efficiency of some preconditions necessary for the normal development of meiosis.

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