MEIOTIC PAIRING IN HAPLOID RYE

by

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INTRODUCTION

Polyhaploids and monoploids are very interesting situations for studying meiotic pairing, especially monoploid forms in which there is no homology with the exception of possible interchromosomal duplications.

Studies on haploid meiosis have shown that in some species bivalents are formed in a very low proportion, while in others meiotic associations of two or more chromosomes were frequently observed (J o h n and L e w i s , 1965).

How can the occurrence of bivalents be explained in monoploids? K i m b e r and R i l e y (1963) proposed three possible explanations. Pairing may arise from homologies due to an archaic polyploid or aneuploid origin of the contemporary chromosome number, or due to the existence of duplicated segments. In addition, J o h n and L e w i s (1965) pointed out that meiotic associations in monoploids may be due to stickiness of heterochromatic zones more that actual chiasmata. These associations give rise to «quasi bivalents» in which metaphasic coorientation can be observed. A fifth explanation could be that pairing could also take place between non-homologous chromosomes (S a d a - s i v a i a h and K a s h a , 1971).

In this work, the meiotic behaviour of a spontaneously arisen haploid of rye was studied.

MATERIAL AND METHODS

The haploid plant studied arose spontaneously within a rye (*Secale cereale* L.) population proceeding from Korea.

The anthers were fixed in acetic alcohol 1:3; hydrolized in HCl 1N for 10 min and stained with fucsin. In order to obtain stained cytoplasm, the cover and the slide were separated and inmersed in a fast-green alcoholic solution. Preparations were then made permanent with Sandeural.

RESULTS

Neither numerical nor structural variations were apparent in mitotic metaphase chromosomes *(figure 1a)*. Moreover, in diploid rye belonging to the same population sample there are no known chromosomal variations. Then, it can be concluded that this haploid plant was karyotipically normal.

The meiotic configurations at metaphase I were scored and the results obtained are shown in *Table 1*. Only nine univalents

Table 1. Chromosome pairing at metaphase 1

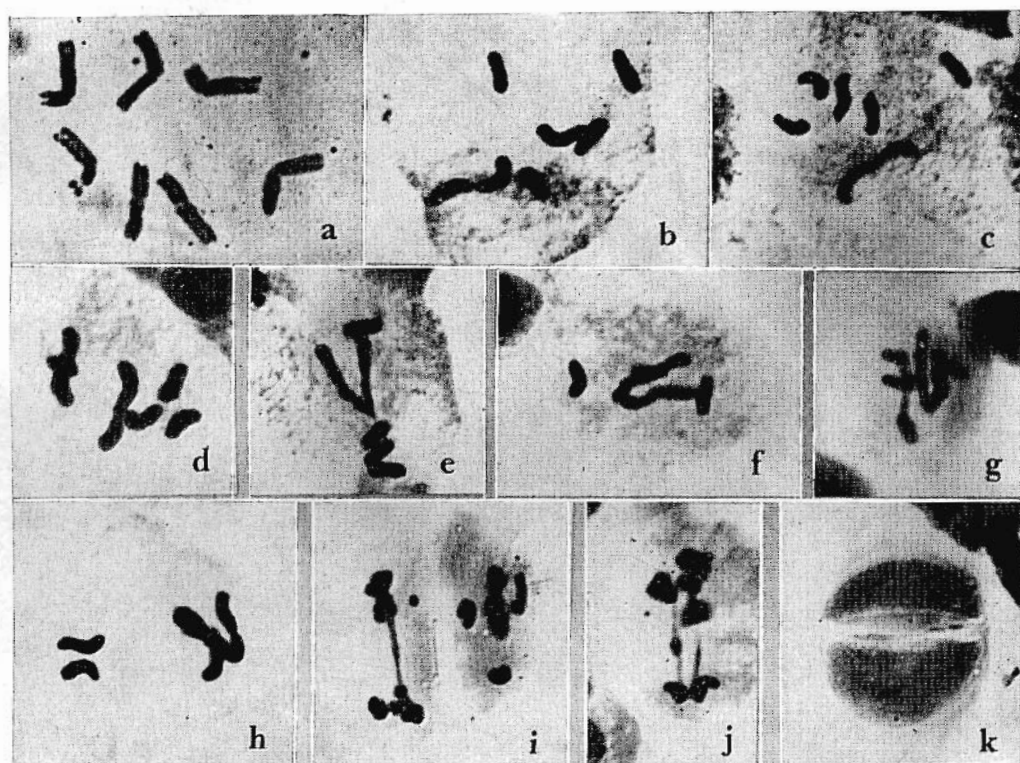| Configurations of PMCs at metaphase I	Frequency	Chiasmata per cell
7^I	268	0
$1^{II}\ 5^I$	128	1
$2^{II}\ 3^I$	34	2
$1^{III}\ 4^I$	7	2
$1^{III}\ 1^{II}\ 2^I$	3	3
$1^{IV}\ 3^I$	2	3
$1^V\ 2^I$	1	4
Total of PMCs	443	

Figure 1. a) Somatic metaphase. b-h) meiotic pairing at metaphase I. b) 7 univalents. c) 1 bivalent and 5 univalents. d) 2 bivalents and 3 univalents. e) 1 trivalent and 4 univalents. f) 1 quadrivalent and 3 univalents. g) 1 trivalent, 1 bivalent and 2 univalents. h) 1 pentavalent and 2 univalents. i) Anaphase II showing 1 bridge and 1 fragment. j) Anaphase I showing 2 bridges and 1 fragment. k) Cytokinesis of an anucleated cell.

showing foldback pairing and two ring shaped bivalents were found. In all other cases univalents were normal, bivalents were rod shaped and multivalents showed a chain configuration. All possible combinations of paired chromosomes up to five were found, but more than five chromosomes were never involved in these associations. In every case a minimum of two univalents per cell was found. Some of the metaphase I cells observed are shown in *figures 1b* to *1h*.

Table 2. Distribution of numbers of chiasmata per cell at metaphase I compared to the correspondent Poisson distribution

	Chiasmata per cell				
	0	1	2	3	4
Observed	268	128	41	5	1
Expected	264.18	136.56	35.30	6.08	0.79
$x^2 = 1\ 6224$			$0\ 5 > p > 0.3$		

The first problem to be analysed is whether a given number of chiasmata per cell occurred preferentially or not. For this, pollen mother cells of this plant were considered as individuals of a population for the character «chiasmata per cell». It was found that the distribution of observed chiasmata per cell fitted a Poisson distribution at the $0.5 > P > 0.3$ level *(Table 2)*.

Anaphase segregation was also studied, the results obtained are shown in *Table 3*. It was observed that within every type of segregation there was a tendency towards a balanced migration of chromosomes; for instance, in class 0 chromosomes dividing equationally, the most frequent class was 4-3, etc.

On the other hand, there was a correspondence between the chromosome arrangements at metaphase I and the observed anaphase segregations; i. e., the most frequent class at metaphase I was 7 univalents *(Table 1)* which corresponds to the most frequent class at anaphase I of 7 chromosomes dividing equationally. It is followed by the class 1 bivalent 5 univalents

corresponding to the segregation 1 chromosome 5 chromatids to each pole, and so on.

An interesting aspect at anaphase I was the presence of both inter and intra chromosomal bridges and fragments; their significance will be discussed later (figure 1i, j).

Table 3. Types of segregations at anaphase I

Chromosomes dividing equationally	Type of reductional segregation	Frequency
0	7 — 0	1
	6 — 1	1
	5 — 2	1
	4 — 3	5
1	6 — 0	—
	5 — 1	—
	4 — 2	1
	3 — 3	1
2	5 — 0	—
	4 — 1	1
	3 — 2	1
3	4 — 0	—
	3 — 1	3
	2 — 2	7
4	3 — 0	1
	2 — 1	3
5	2 — 0	3
	1 — 1	10
6	1 — 0	3
7	0 — 0	19
Total of PMCs		61

A small number of PMCs (about 4 %) showed a chromosome number varying from 21 to 1 and even some of them were apparently anucleated. The integrity of PMCs was accurately established due to the staining with fast-green. In some cases, the anucleated cells underwent cytokynesis at the same time as the normal cells in the anther *(figure 1k)*.

DISCUSSION

In the haploid rye plant studied in this work, chromosome bounds seems to occur at random, as can be deduced from the comparison of the distribution of chromosome bounds per cell with a Poisson distribution. However, this fact does not necessarily mean that all seven chromosomes are involved in pairing with the same probability.

It is remarkable that within a single cell more than 5 chromosomes were never paired; that is, a minimum of two univalents per cell was always observed. In addition, 6 bound chromosome arms are enough to form either 3 rod bivalents, or 1 trivalent plus one bivalent, or one quadrivalent; while at least 8 bound chromosome arms are necessary to form a pentavalent. Therefore, if all chromosome arms were involved in pairing with the same probability it would be expected that the configuration 3^{II} 1^I would appear with higher frequency than the configuration 1^V 2^I and with similar frequency to that of 1^{III} 1^{II} 2^I or 1^{IV} 3^I. However, PMCs with 3 bivalents were never found. These facts could be explained under the assumption that not all seven chromosomes were involved in pairing with the same probability.

Comparing the configurations at metaphase I and the segregations at anaphase I, a correspondence can be observed (see *Tables 1 and 3*). This indicates the existence of a true coorientation of bi or multivalent chromosome associations. In the case that a true metaphase coorientation did not exist, a random segregation at anaphase I would be observed.

J o h n and L e w i s (1965) interpreted all meiotic associations arising at metaphase I of haploid rye as due to stickiness. However we have some findings supporting the existence of

exchanges in the bi or multivalents appearing in the rye haploid studied here.

Bridges and fragments at anaphase I and II have been observed *(figure 1i, j)*. The formation of fragments from persistent heterochromatin fusions, does not seem possible. In the present work bridges were mainly observed with fragments. Therefore, it can be concluded that stickiness was not the only cause of bridge formation and consequently the only cause of chromosome binding at metaphase.

The appearance of bridges and fragments could be explained either by breakages and fusions (L e w i s and J o h n , 1966); erroneous chiasmata (J o n e s , 1968); or by normal exchange in inversely paired chromosomes. This last possibility would occur if regions of ancestral homology (arisen by duplication) were asymetrically situated in two different chromosomes; that is, in an inverted situation in respect to the centromere.

The origin and significance of cells with a variable number of chromosomes remain unknown. Cell fusion, non-disjunction at premeiotic mitosis and chromosome elimination could be possible causes of their presence.

ABSTRACT

The meiotic behaviour of a spontaneously arisen haploid of rye was studied. All the possible combinations of paired chromosomes at metaphase I were found up to five. In any case a minimum of two univalents per cell were observed. The possibility that not all chromosomes were involved in pairing is discussed.

From the observations at anaphase I it was deduced that chromosomes did not segregate equationally or reductionally at random. Comparing the frequency of meiotic configurations at metaphase I with those at anaphase I it was possible to establish a correspondence which is an indication of the existence of actual coorientation.

The relationships between the presence of a high number of bridges and fragments at anaphase I and II with the existence of chromosomal exchanges is discussed.

RESUMEN

Se ha estudiado el comportamiento meiótico de un haploide de centeno aparecido espontáneamente. Se encontraron en todas las combinaciones posibles hasta cinco cromosomas apareados en metafase I. Se observaron en todos los casos un mínimo de dos univalentes por célula. Se discute la posibilidad de que no todos los cromosomas estén implicados en el apareamiento.

De las observaciones en anafase I se dedujo que los cromosomas no segregaban ecuacional o reduccionalmente al azar. Comparando la frecuencia de configuraciones meióticas en metafase I con las de anafase I, fue posible establecer una correspondencia que indica la existencia de coorientación auténtica.

Se discute la relación entre la presencia de un alto número de puentes y fragmentos en anafase I y II, y la existencia de intercambios cromosómicos.

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