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Cytogenetic studies in the genus *Zea*

2. Colchicine-induced multivalents

L. Poggio [1,2], M.C. Molina [1] and C.A. Naranjo [1]

Consejo Nacional de Investigaciones Cientificas y Técnicas (CONICET), Argentina
[1] Instituto Fitotécnica de Santa Catalina, C.C.4, Llavallol, 1836 Buenos Aires, Argentina
[2] Department of Biological Sciences, University of Buenos Aires, Argentina

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Summary. Premeiotic colchicine treatment brings about the production of one to five quadrivalents in *Zea mays* ssp. *mays* (maize, 2n = 20) and an increase in the number of quadrivalents from five to ten in *Zea perennis* (2n = 40). The results confirm the allotetraploid nature of maize and suggest that the species possesses two homoeologous genomes (A2A2 B2B2) that fail to pair, probably due to the presence of Ph-like genes. Moreover, the autoallooctoploid nature of *Zea perennis*, with a genome formula A′1A′1 A″1A″1 C1C1 C2C2, is supported by the present results.

Key words: *Zea* – *Zea mays* ssp. *mays* – *Z. perennis* – Cytogenetics – Colchicine-induced multivalents

Introduction

Colchicine is commonly used for induction of polyploidy. However, it has been demonstrated that premeiotic colchicine (0.5%–1%) treatments reduce the pairing of homologues, inducing partial asynapsis (Driscoll and Barber 1967; Dover and Riley 1973), although they do not affect intrachromosomal pairing in isochromosomes (Driscoll and Darvey 1970). If colchicine is applied before the last premeiotic mitosis, then cells with double the normal number of chromosomes show bivalent formation exclusively (Driscoll and Barber 1967; Feldman and Avivi 1988). Moreover, colchicine in low concentrations (0.5×10^{-4} *M*), does not cause asynopsis, and induces homoeologous pairing (multivalents and heteromorphic bivalents) and interlocking of bivalents at first meiotic metaphase (Driscoll and Barber 1967; Feldman and Avivi 1988). According to Feldman and Avivi (1988), colchicine does not suppress pairing and crossing-over, but does interfere with the nonrandom chromosomal arrangement at interphase, a presynaptic event that is necessary for the regularity of meiotic pairing. Most of the studies mentioned above were done in wheat (*Triticum aestivum*), where the *Ph1* gene, located on the long arm of the *5B* chromosome, is mainly responsible for the cytological diploid-like behavior in meiosis (Feldman and Avivi 1988).

Jackson (1982) advanced a model to explain pairing and chiasma failure in homologous or homoeologous genomes. His model assumes that a genome has a genetically controlled specific site of attachment to the nuclear membrane. This premeiotic genome placement would be caused by Ph-like genes. Jackson and Murray (1983) demonstrated that diluted solutions of colchicine (0.5×10^{-4} *M*) applied at the onset of meiosis can disrupt this genetically controlled position, promoting intergenomal pairing and revealing cryptic genome homologies. Using this technique, they induced quadrivalent formation in *Helianthus,* and interpreted it as evidence of ancient polyploidy. Murray et al. (1983), using the same treatment in other species, observed an increase of the quadrivalent frequency in most of the cases, probably due to a disruption of the bivalent promoting mechanism.

Zea is an important genus of the tribe Maydeae, for which new cytological evidence that five is the original basic chromosome number was given by Molina and Naranjo (1987) and Naranjo et al. (1989). These authors postulate that maize and its related wild species (2n = 20) are cryptic tetraploids that originated by allopolyploidy from different diploid species (2n = 10), probably extinct nowadays. In the present paper, the effect of premeiotic treatment with diluted solutions of colchicine on the meiotic behavior of *Z. mays* ssp. *mays* (2n = 20) and *Z. perennis* (2n = 40) was studied. The aim of this experi-

ment was to provide new evidence for the amphiploid origin of the above mentioned species and for their genome constitution as proposed by Molina and Naranjo (1987) and Naranjo et al. (1989).

Materials and methods

The origin of the materials studied is as follows: *Zea mays* ssp. *mays*, maize "Colorado Klein"; *Z. perennis*, Mexico, Jalisco, Ciudad Guzmán, Leg. Dra. Prywer, cultivated at the "Instituto Fitotécnico de Santa Catalina" since 1962.

The colchicine treatment was done according to Jackson and Murray (1983) with minor modifications. The stems of five plants were cut under a diluted solution of colchicine (0.5×10^{-4} *M*) and maintained therein for 12 h (keeping the submerged portion of the stem in absolute darkness). Before fixation, the stems were placed for 24 h in tap water. Control material of *Z. mays* ssp. *mays* was fixed without colchicine treatment. Treated and untreated materials were fixed in 3:1 (absolute alcohol: acetic acid) solution. Anthers were squashed in 2% acetic haematoxylin (Núñez 1968). The pairing configurations were determined at diakinesis-metaphase I. Only cells with well-spread plates were scored.

Results and discussion

In *Zea mays* ssp *mays* (2n = 20), untreated material shows only bivalents (Fig. 1 a), while *Z. perennis* (2n = 40) shows 5 IV + 10 II in most of the cells, and a maximum of 6 IV (+8 II) (Molina and Naranjo 1987). In the treated materials of *Z. mays* ssp. *mays*, 1–5 IV were found in 61% of the cells and an increase of up to 10 IV was found in *Z. perennis* (Table 1; Fig. 1). Differences between the number of quadrivalents in treated and untreated material of *Z. perennis* were subjected to a test of homogeneity and were found highly significant ($p = 4.486 \times 10^{-7}$).

The analysis of chromosome pairing in our treated material reveals some interesting features with regard to its genome organization. In *Z. mays* ssp. *mays*, associations of four chromosomes, which were no doubt quadrivalents (Fig. 1 b and c), were observed. On the other hand, other associations were difficult to interpret in a similar manner (Fig. 1 d), probably due to a considerable contraction of the chromosomes by the colchicine, which makes it difficult to determine whether or not all of the chromosomes are connected by chiasmata. There is also the possibility of interlocked bivalents or of secondary association in pairs rather than at random. Secondary association occurs between genetically equivalent bivalents (homoeologous) (Kempanna and Riley 1964; Riley 1960). In untreated *Z. mays* ssp. *mays* and other 2n = 20 taxa such as *Z. mays* ssp. *mexicana* and *Z. diploperennis* (Molina and Naranjo 1987; Naranjo et al. 1989), bivalents were seen in proximity (Fig. 1 a) but never in close contact, as was found in treated cells (Fig. 1 d).

From meiotic configurations in several species and also in intra- and interspecific hybrids of *Zea*, Molina and Naranjo (1987) and Naranjo et al. (1989) collected evidence supporting a tetraploid condition for the taxa with 2n = 20. They stated that the meiotic configurations in *Z. mays* ssp. *mays* are those expected of a typical allotetraploid, and they proposed the genome formula A2A2 B2B2. The associations now found in treated material, i.e., quadrivalents and interlocked or strongly secondarily associated bivalents, suggest the existence of homoeology between genomes A2 and B2. This allows us to hypothesize that the diploid-ancestors of *Z. mays* ssp. *mays* were more related to each other than was expected from the alloploid-like pairing behavior found in the latter. This pairing pattern probably is genetically determined, as it is seen in the genetic system most studied, i.e., the diploidizing genes of *Triticum aestivum*, mainly the *Ph1* gene. In the presence of *Ph* no homoeologous pairing occurs between the three genomes of wheat, but intergenomic pairing does occur in its absence. Feldman and Avivi (1988) discussed several results of the effect of different doses of *Ph1* on chromosomal pairing. These authors have presented evidence that some of these effects can be phenocopied by premeiotic treatment with colchicine, since that gene affects microtubule response to several anti-microtubule agents.

The great similarity between maize and wheat with regard to the effects of premeiotic treatment with colchicine permits the inference to be drawn that Ph-like genes are also present in maize. The results obtained in the present work allow the 2n = 20 taxa of *Zea* to be considered as allotetraploids possessing two homoeologous genomes (A2A2 B2B2) that fail to pair, probably due to the presence of *Ph*-like genes.

Table 1. Frequency of quadrivalents per cell in untreated (U.T.) and treated (T.) materials

Species	IV/cell	0	1	2	3	4	5	6	7	8	9	10	No. cells
Z. mays ssp. *mays*	U.T.	200	–	–	–	–	–	–	–	–	–	–	200
	T.	85	49	26	18	16	23	–	–	–	–	–	217
Z. perennis	U.T.	–	3	6	10	32	76	7	–	–	–	–	134
	T.	–	–	–	6	18	33	39	35	12	3	1	149

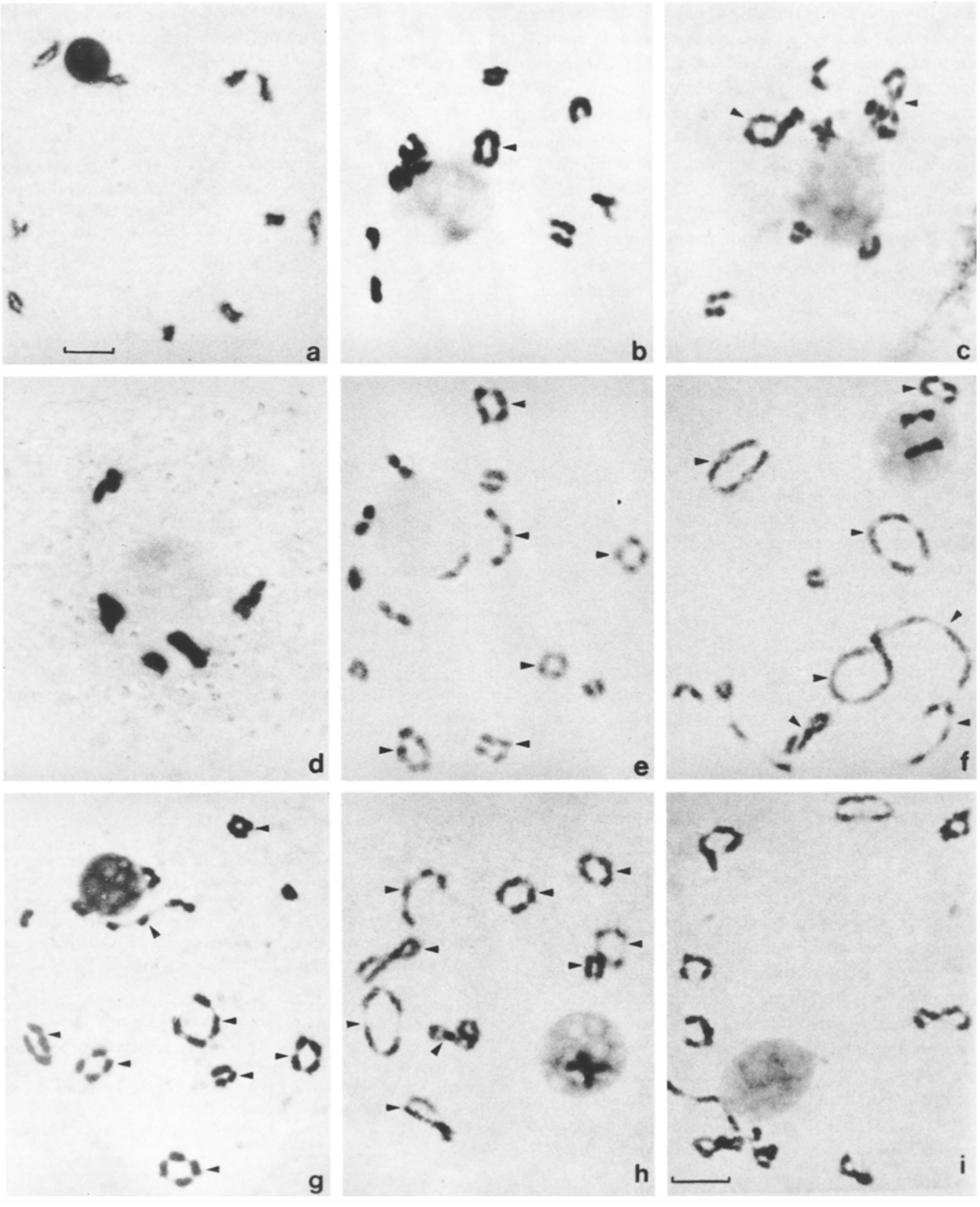

Fig. 1 a–i. Meiotic chromosomes. **a–d** *Z. mays* ssp. *mays* (2n = 20). **a** Diakinesis with ten bivalents in untreated material, showing secondary association. **b–d** Colchicine-treated materials. **b** Diakinesis with one quadrivalent (IV) + eight bivalents (II). **c** Diakinesis with 2IV + 6II. **d** Prometaphase I with 5IV. **e–i** Diakinesis of colchicine-treated *Z. perennis* (2n = 40). **e** 6IV + 8II. **f** 7IV + 6II. **g** 8IV + 4II. **h** 9IV + 2II. **i** 10 IV. In cells with bivalents, *arrows* show the quadrivalents. *Bars* represent 10 μm; **b–i** all with the same enlargement

Since chromosome pairing of homoeologous genomes can fail because they are too distant for synaptic initiation, the terms "typical" and "segmental allopolyploid" (according to Stebbins 1946) should be used with the proviso that they are not indicating a measure of chromosome homology (Jackson 1984). As Stebbins (1971) has stated, the presence or absence of multivalent configurations in a natural polyploid may provide some indication as to whether or not it is of hybrid origin, but by itself this criterion is by no means decisive. The attempt to classify polyploids into clearly defined categories is meaningless, and emphasis must be placed upon their origin and evolution.

Feldman and Avivi (1988) published results supporting the hypothesis that the *Ph1* gene controls the association of chromosomes by affecting chromosome sets rather than individual chromosomes. In 2n = 20 species of *Zea*, a double spindle with five bivalents each is frequently observed at late metaphase I (Naranjo et al. 1989). In treated cells at late metaphase I having two spindles (with five bivalents each), the latter are seen more separate than in untreated material. Apparently, colchicine disrupts the position of whole chromosomal sets, either approximating them (IV formation) or separating them (more distance between groups of five bivalents). The nature and origin of the double spindle, the existence of Ph-like genes in maize and wild related species, and the action of colchicine on the chromosome sets and on the spindle formation is being studied at present in our laboratory.

From the meiotic pairing found in species and hybrids of *Zea, Z. perennis* was considered as an autoallooctoploid with the genome formula A′1A′1 A″1A″1 C1C1 C2C2 (Molina and Naranjo 1987). The presence of a higher frequency of quadrivalents and the absence of octovalents and hexavalents in treated materials of this species indicate a high affinity within each of genomes A and C and no noticeable homoeology between them. However, the possibility cannot be ruled out that this species requires a different colchicine concentration to disrupt the position of its genomes, or possesses Ph-like genes with a particular mode of action, or that its genomes A and C have a strong preferential affinity.

The results lend support to Jackson's (1982) point of view. He stated that colchicine may be a valuable tool for evolutionists and plant breeders. Besides the evolutionary considerations stated above, the possible existence of Ph-like genes in maize and the possibility of obtaining intergenomal pairing producing new recombinations would open new perspectives in breeding programs.

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