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# Cytogenetic studies in the genus *Zea*

## 1. Evidence for five as the basic chromosome number

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**Summary.** New cytological evidence supporting $x = 5$ as the basic chromosome number of the genus *Zea* has been obtained as a consequence of our analysis of the meiotic configurations of *Zea mays* ssp. *mays*, *Z. diploperennis*, *Z. perennis* and of four $F_1$ artificial interspecific hybrids. *Z. mays* ssp. *mays* ($2n = 20$) presents regular meiosis with 10 bivalents (II) and is considered here as a typical allotetraploid ($A_2A_2B_2B_2$). In *Z. diploperennis* ($2n = 20$) 10II are formed in the majority of the cells, but the formation of 1III + 8II + 1I or 1III + 7II + 3I in 4% of the cells would indicate its segmental allotetraploid nature ($A_1A_1B_1B_1$). *Z. perennis* ($2n = 40$) had 5IV + 10II in 55% of the cells and would be considered as an auto-allooctoploid ($A_1'A_1'A_1''A_1''C_1C_1C_2C_2$). *Z. diploperennis* × *Z. mays* ssp. *mays* ($2n = 20$) presents 10II in ca. 70% of the cells and no multivalents are formed. In the two $2n = 30$ hybrids (*Z. mays* ssp. *mays* × *Z. perennis* and *Z. diploperennis* × *Z. perennis*) the most frequent meiotic configuration was 5III + 5II + 5I and in $2n = 40$ hybrid (*Z. diploperennis* × *Z. perennis*) was 5IV + 10II. Moreover, secondary association was observed in the three above-mentioned tetraploid taxa ($2n = 20$) where one to five groups of two bivalents each at diakinesis-metaphase I was formed showing the affinities between homoeologous genomes. The results, as a whole, can be interpreed by assuming a basic $x = 5$ in this polyploid complex. The main previous contributions that support this working hypothesis are reviewed and its phylogenetic implications studied are discussed.

**Key words:** *Zea* species and hybrids – Basic number determination – Meiotic configuration – Secondary association – Phylogeny of *Zea*

## Introduction

*Zea* is an important genus of the Tribe Maydeae and according to Doebley and Iltis (1980) and Iltis and Doebley (1980) is composed of two sections: sect. Luxuriantes Doebley & Iltis, including the perennials *Z. diploperennis* Iltis, Doebley & Guzman and *Z. perennis* (Hitch.) Reeves & Mangelsdorf and the annual *Z. luxurians* (Durieu & Ascherson) Bird., and sect. Zea consisting of only one species, annual *Z. mays* L. which may be divided into three subspecies *Z. mays* ssp. *parviglumis* Iltis & Doebley, *Z. mays* ssp. *mexicana* (Schrader) Iltis & Doebley and maize, *Z. mays* ssp. *mays* Iltis & Doebley. All the above-mentioned species have $2n = 20$ except *Z. perennis* which has $2n = 40$.

Darlington (1956) pointed out that the inference of a basic number in a polyploid series is obviously an important step in fitting chromosome numbers to an evolutionary hypothesis. Sometimes the diploid members of the series have disappeared from the genus. At the same time most of this "polyploid" species actually show a diploid-like chromosome behaviour. According to Darlington "this is the situation in *Zea*. The ten chromosomes of the haploid set in *Zea mays* show no internal relations, no evidence of an earlier doubling. *Zea mays* is what we call and rightly call for all practical purposes, a diploid species. But relatives, both in *Coix* and *Sorghum*, have the haploid number of 5. There is a strong suggestion, therefore, that 5 is the ancestral basic number. This number, although of no practical interest for its future hybridization, is the key to the past history, the phylogeny, of *Zea*".

There are genetical, cytological and biochemical studies in which evidence supporting the tetraploid nature of maize are presented (Rhoades 1951; Ghatnekar 1965; Vijendra Das 1970; Gottlieb 1982; Bennett 1983, 1984). The study of the occurrence of secondary association and the detailed study of meiotic configurations in species and interspecific hybrids would permit Darlington's hypothesis (x = 5) to be tested and confirm the evidence presented in the reports mentioned above.

Darlington (1928) defined secondary pairing or secondary association of bivalents as "the occurrence of bivalents in pairs of groups rather than at random at first metaphase of meiosis in a number of polyploid plant species". Darlington (1928) and Lawrence (1931) ascribed secondary association to the specific affinity between chromosomes. Evidence in favour of the occurrence of secondary association determined by genetical equivalence was obtained when the secondary association was demonstrated in species whose polyploid origin is known. Thus, Riley (1960) found in *Triticum aestivum* (6x) secondary association between bivalents of genetically equivalent chromosomes (homoeologous) and Kempanna and Riley (1964) demonstrated that the phenomenon of secondary association in *Triticum aestivum* is dependent upon the genetic relationships of the associated bivalents. Finally, Lacadena and Puertas (1969) in a quantitative analysis, have statistically demonstrated the secondary association in *Aegilops triaristata* (6x).

Part of our results on the meiotic configuration studies of *Zea* species and hybrids was previously reported (Molina 1978, 1982, 1983, 1985; Molina and Naranjo 1986; Molina and Rosales 1983). In this paper these results as a whole are presented and discussed with the aim of demonstrating that they constitute evidence of x = 5 in the genus *Zea*. Moreover, the occurrence of secondary association in species and hybrids is reported.

## Materials and methods

The origin of the species studied is as follows: *Zea mays* ssp. *mays*; maize "Colorado Klein" and simple hybrid "Asgrow AX 252". *Z. diploperennis*, Mexico, Jalisco, Sierra de Mananthan Occidental, 2 Km E. of Las Joyas, Leg. Rafael Guzman & M.A. de Guzman (No. 1120, Nov. 1980). *Z. perennis*, Mexico, Jalisco, Ciudad Gusmân. Leg. Dra. Prywer, cultivated in "Instituto Fitotécnico de Santa Catalina" since 1962.

To study meiosis the young panicles were fixed in acetic acid: absolute alcohol (1:3) and kept under refrigeration until studied. The anthers were squashed in 2% acetic haematoxylin (Nuñez 1968). The pairing configurations were determined at diakinesis-metaphase I. Only those cells that had well-spread figures were scored.

## Results

The chromosome numbers and the meiotic configurations of three species (*Z. mays* ssp. *mays*, *Z. diploperennis* and *Z. perennis*) and four $F_1$ interspecific hybrids are given in Table 1. The majority of the results could be interpreted only by assuming x = 5 (Molina & Naranjo 1986). In Fig. 1 the most frequent meiotic configurations and hypothetic genomic constitutions of each taxa are presented, and in Fig. 2 the meiotic chromosomes in species and hybrids are illustrated.

In *Z. mays* ssp. *mays* (2n = 20; Table 1, Fig. 2a, b) 10 bivalents are regularly formed. Secondary association was observed and a maximum of five groups of two bivalents each was frequent at diakinesis-metaphase I (Fig. 2a, b). The meiotic configurations are those expected in a typical allotetraploid and the following genome formula is proposed: $A_2A_2B_2B_2$ (Fig. 1).

*Z. diploperennis* (2n = 20; Table 1, Fig. 2c) presents a regular meiosis with the formation of 10 bivalents in

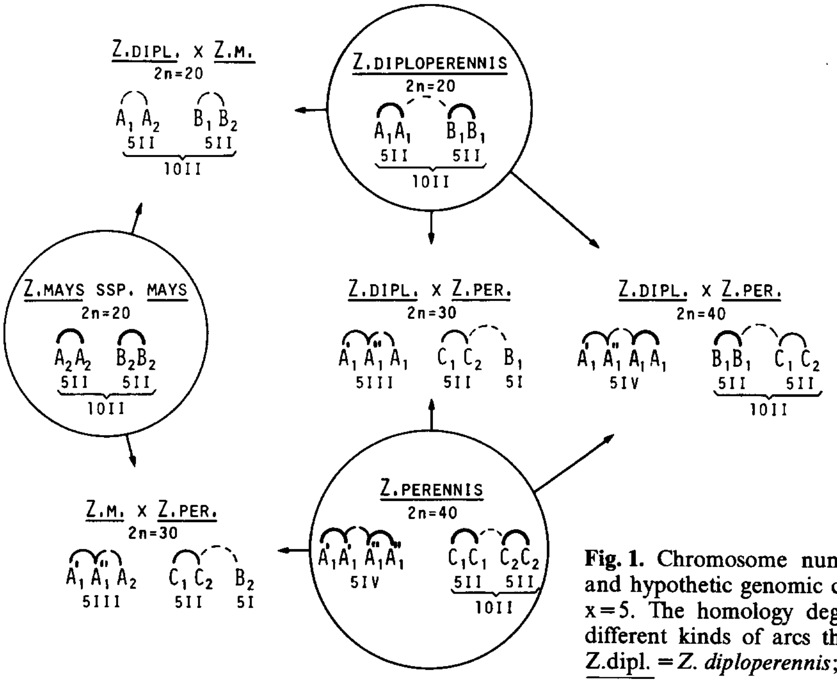

**Fig. 1.** Chromosome numbers, most frequent meiotic configurations and hypothetic genomic constitutions of species and hybrids assuming x = 5. The homology degree between genomes are represented with different kinds of arcs that connect them. Z.m. = *Z. mays* ssp. *mays*; Z.dipl. = *Z. diploperennis*; Z.per. = *Z. perennis*

**Table 1.** Meiotic configuration in species and $F_1$ hybrids studied. (Data partly from Molina 1978, 1982, 1983, 1985; Molina and Naranjo 1986; Molina and Rosales 1983, and original)

| Species or Hybrids | 2n | | diakinesis-metaphase I configuration | | | | % | No. of cells studied |
|---|---|---|---|---|---|---|---|---|
| | | | I | II | III | IV | | |
| *Z. mays* ssp. *mays* | 20 | | – | 10 | – | – | 100 | 200 |
| *Z. diploperennis* | 20 | | – | 10 | – | – | 73 | 100 |
| | | | 2 | 9 | – | – | 23 | |
| | | | 1 | 8 | 1 | – | 3 | |
| | | | 3 | 7 | 1 | – | 1 | |
| | | X̄ | 0.52 | 9.68 | 0.14 | | | |
| *Z. perennis* | 40 | | – | 18 | – | 1 | 1.49 | 134 |
| | | | 1 | 16 | 1 | 1 | 0.75 | |
| | | | – | 16 | – | 2 | 3.73 | |
| | | | 2 | 15 | – | 2 | 0.75 | |
| | | | – | 14 | – | 3 | 6.72 | |
| | | | 2 | 13 | – | 3 | 0.75 | |
| | | | – | 12 | – | 4 | 20.15 | |
| | | | 2 | 11 | – | 4 | 2.98 | |
| | | | 1 | 10 | 1 | 4 | 0.75 | |
| | | | – | 10 | – | 5 | 54.47 | |
| | | | 2 | 9 | – | 5 | 1.49 | |
| | | | 4 | 8 | – | 5 | 0.75 | |
| | | | – | 8 | – | 6 | 5.22 | |
| | | X̄ | 0.164 | 11.02 | 0.014 | 4.44 | | |
| *Z. diploperennis* × *Z. mays* ssp. *mays* | 20 | | – | 10 | – | – | 69.06 | 181 |
| | | | 2 | 9 | – | – | 24.31 | |
| | | | 4 | 8 | – | – | 6.08 | |
| | | | 6 | 7 | – | – | 0.55 | |
| | | X̄ | 0.76 | 9.62 | | | | |
| *Z. mays* ssp. *mays* × *Z. perennis* | 30<br>6 | | 7 | 7 | 3 | – | 2.53 | 79 |
| | | | 6 | 6 | 4 | – | 22.78 | |
| | | | 4 | 7 | 4 | – | 5.06 | |
| | | | 7 | 4 | 5 | – | 1.26 | |
| | | | 5 | 5 | 5 | – | 54.43 | |
| | | | 3 | 6 | 5 | – | 6.32 | |
| | | | 1 | 7 | 5 | – | 1.26 | |
| | | | 4 | 4 | 6 | – | 5.06 | |
| | | | 4 | 3 | 7 | – | 1.26 | |
| | | X̄ | 5.00 | 5.38 | 4.75 | | | |

73% of the cells studied. The formation of 1III + 8II + 1I or 1III + 7II + 3I in 4% of the cells would indicate the existence of some homoeologies between $A_1$ and $B_1$ genomes (Fig. 1) and suggest the probable segmental allotetraploid nature of this taxon. Secondary association was found also in this species and groups of four or five pairs of bivalents are frequent (Fig. 2c).

*Z. perennis* (2n = 40) had 10II + 5IV in 54.47% of the cells studied (Fig. 2d) and the means were II = 11.02, IV = 4.44 (Table 1). These configurations suggest the existence of at least three different genomes ($A_1$, $C_1$, $C_2$; Fig. 1). The formation of 5IV in ca. 55% of the cells would indicate the existence of four highly homologous genomes $A_1$. The cells with less than 5IV (4, 3, 2 or 1IV; Table 1) probably result from lower chiasmata frequency and, in general, 2II are formed by each non occurring IV; in only 1.5% of the cells 1III + 1I was formed. These results suggest high homology by pairs in $A_1$ genomes ($A_1'A_1'$ and $A_1''A_1''$; Fig. 1). Sometimes 6 IV were observed and this could indicate

**Table 1** (continued)

| Species or Hybrids | 2n | diakinesis-metaphase I configuration | | | | % | No. of cells studied |
|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | | |
| *Z. diploperennis* × *Z. perennis* | 30 | 7 | 10 | 1 | – | 0.59 | |
| | | 8 | 8 | 2 | – | 0.59 | |
| | | 7 | 7 | 3 | – | 2.98 | |
| | | 5 | 8 | 3 | – | 3.57 | |
| | | 3 | 9 | 3 | – | 0.59 | |
| | | 12 | 3 | 4 | – | 0.59 | |
| | | 8 | 5 | 4 | – | 1.19 | |
| | | 6 | 6 | 4 | – | 14.28 | |
| | | 4 | 7 | 4 | – | 4.17 | |
| | | 2 | 8 | 4 | – | 1.19 | 168 |
| | | 7 | 4 | 5 | – | 0.59 | |
| | | 5 | 5 | 5 | – | 39.29 | |
| | | 3 | 6 | 5 | – | 4.76 | |
| | | 6 | 3 | 6 | – | 0.59 | |
| | | 4 | 4 | 6 | – | 16.07 | |
| | | 2 | 5 | 6 | – | 4.76 | |
| | | 3 | 3 | 7 | – | 2.98 | |
| | | 2 | 2 | 8 | – | 0.59 | |
| | | – | – | 10 | – | 0.59 | |
| | $\bar{X}$ | 4.73 | 5.25 | 4.91 | | | |
| *Z. diploperennis* × *Z. perennis* | 40 | – | 18 | – | 1 | 2.34 | |
| | | 1 | 14 | 1 | 2 | 0.93 | |
| | | – | 16 | – | 2 | 3.27 | |
| | | 1 | 12 | 1 | 3 | 1.40 | |
| | | 2 | 13 | – | 3 | 2.80 | |
| | | – | 14 | – | 3 | 15.42 | |
| | | 1 | 10 | 1 | 4 | 3.74 | 214 |
| | | 2 | 11 | – | 4 | 3.74 | |
| | | – | 12 | – | 4 | 20.09 | |
| | | 2 | 9 | – | 5 | 2.34 | |
| | | – | 10 | – | 5 | 31.78 | |
| | | – | 8 | – | 6 | 11.22 | |
| | | – | 6 | – | 7 | 0.93 | |
| | $\bar{X}$ | 0.238 | 11.30 | 0.061 | 4.24 | | |

homoeologies between $C_1$ and $C_2$ genomes. On the basis of these results, *Z. perennis* would be considered as an auto-allooctoploid with the genomic formula: $A_1'A_1'A_1''A_1''C_1C_1C_2C_2$ (Fig. 1).

The $F_1$ *Z. diploperennis* × *Z. mays* ssp. *mays* (2n = 20; Table 1, Figs. 1 and 2e) presents the formation of 10II in ca. 70% of the cells studied and the mean of bivalents was 9.62; the rest of the cells presents 2I to 6I ($X = 0.76$, Table 1). Secondary association was observed and a maximum of four groups of two bivalents each at diakinesis-metaphase I was formed (Fig. 2e). These results suggest that there is exclusive allosyndetic pairing ($A_1A_2$ and $B_1B_2$, Fig. 1). The homoeology between $A_1$ and $B_1$ from *Z. diploperennis* is not shown by autosyndetic pairing but is clearly indicated by the secondary association. The high frequency of bivalents formation ($\bar{X} = 9.62$) suggests the existence of homoeologies by pairs $A_1A_2$ and $B_1B_2$ (Fig. 1).

In the $F_1$ *Z. mays* ssp. *mays* × *Z. perennis* (2n = 30; Table 1, Figs. 1 and 2f) 5III + 5II + 5I were formed in ca. 55% of the cells studied (Fig. 2f) and the means were III = 4.75, II = 5.38 and I = 5.00 (Table 1). The 5III could be formed by autosyndetic pairing of $A_1'$ and $A_1''$ genomes from *Z. perennis* and by allosyndetic pairing with the $A_2$ genome from *Z. mays* ssp. *mays* (Fig. 1). A detailed study of trivalents shows that many have a "frying pan" shape and probably the ring part could be formed by the above-mentioned $A_1'A_1''$ pairing (Fig. 2f). The 5II could be formed by autosyndetic pairing between the $C_1$ and $C_2$ homoeologous genomes from *Z. perennis*. Finally the 5I would belong to the $B_2$ genome from *Z. mays* ssp. *mays* (Fig. 1). The same

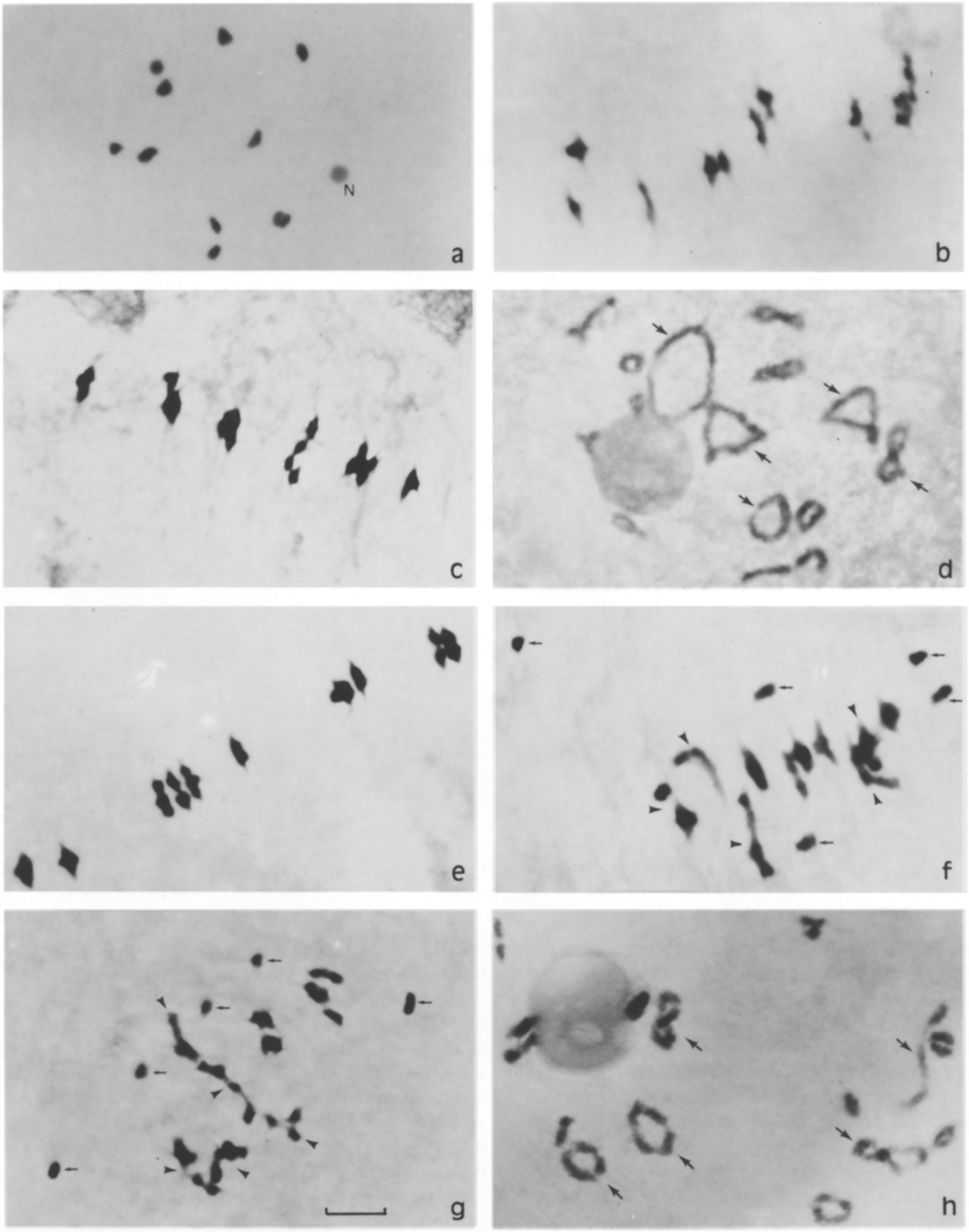

**Fig. 2a–h.** Meiotic chromosomes in species and hybrids. **a–b** *Z. mays* ssp. *mays* (2n = 20) with 10 bivalents; **a** diakinesis; **b** metaphase I; **c** *Z. diploperennis* (2n = 20), metaphase I with 10 bivalents; **d** *Z. perennis* (2n = 40), diakinesis with 5 quadrivalents (➞) + 10 bivalents; **e** *Z. diploperennis* × *Z. mays* ssp. *mays* (2n = 20), metaphase I with 10 bivalents; **f** *Z. mays* ssp. *mays* × *Z. perennis* (2n = 30), metaphase I with 5 trivalents (▸) + 5 bivalents + 5 univalents (→); **g** *Z. diploperennis* × *Z. perennis* (2n ≈ 30), metaphase I with 5 trivalents (▸) + 5 bivalents + 5 univalents (→); **h** *Z. diploperennis* × *Z. perennis* (2n = 40), diakinesis with 5 quadrivalents (➞) + 10 bivalents. Bar represent 10 μm; all with the same enlargement

considerations could be applied in the $F_1$ *Z. diploperennis* × *Z. perennis* (2n = 30; Table 1, Figs. 1 and 2g) interpretations. 5III + 5II + 5I was the configuration present in ca. 40% of the cells studied. In this hybrid the 5III could be formed by autosyndetic pairing of $A_1'$ and $A_1''$ genomes from *Z. perennis* and by allosyndetic pairing with the $A_1$ genome from *Z. diploperennis* showing again the "frying pan" shape of some of the trivalents (Fig. 2g). The 5I could be formed by the $B_1$ genome from *Z. diploperennis.* In both hybrids (2n = 30) the formation of more than 5III would occur due to allosyndetic pairing between $C_1C_2$ with $B_2$ or $B_1$ from *Z. mays* ssp. *mays* or *Z. diploperennis* respectively (Fig. 1). The nonformation of quadrivalents or higher multivalents indicate a) the absence of homoeologies between genomes A and the others ($C_1, C_2, B_1, B_2$) and b) absence of structural heterozygosity between these three species. The occurrence in these two hybrids of fewer than 5III (4 or 3III in 30% of the cells, Table 1) could be attributable to the failure of chiasmata between $A_1'A_1''$ and $A_1$ or $A_2$ genomes. Since the more frequent configuration is 5III + 5II + 5I, no more than 10(III + II) would be expected, because if chiasmata fail in a trivalent one or more bivalents would be added. However, there are some cases where the number of III + II is higher than 10 (i e. 4III + 7II + 4I; Table 1) and a possible explanation is the formation of pseudobivalents between chromosomes from the B genomes.

There is a tendency of A, C and B genomes separation through the trivalents, bivalents and univalents grouped, respectively. In Fig. 2f and g it is possible to see that tendency in the grouped 5III ($A_1'A_1''A_2$ or $A_1'A_1''A_1$), the 5II ($C_1C_2$) and the 5I ($B_2$ or $B_1$). Furthermore, the 5I are grouped, in general, near the bivalents (Fig. 2g).

The $F_1$ *Z. diploperennis* × *Z. perennis* hybrid (2n = 40; Table 1, Figs. 1 and 2h) was probably originated by the fertilization of an unreduced egg cell from *Z. diploperennis* by a normal male gamete from *Z. perennis* (Molina 1985). The formation of bivalents plus quadrivalents was observed in 85% of the cells studied, and 1III + 1I were detected in only 5.07%. The most frequent configuration (5IV + 10II) was observed in ca. 32% of the cells (Fig. 2h). These results suggest high homology between the $A_1'$ and the $A_1''$ genomes, which is also observed in *Z. perennis.*

## Discussion

There have been many previous cytogenetic studies in *Zea* species and hybrids since the earlier contribution of Kuwada (1911). Space does not permit a complete review here, therefore, only the workers which have given information concerning to meiotic configurations principally in hybrids are mentioned and mainly those that furnish evidence of x = 5 will be discussed.

Pasupuleti and Galinat (1982) studied *Z. diploperennis* and found a regular meiosis with 10 bivalents and in *Z. diploperennis* × *Z. mays* $F_1$ hybrids, 10 bivalents were formed in 90% and 9II + 2I or 8II + 4I in 10% of the cells studied. In our study some less regular meioses were found in *Z. diploperennis* and in its hybrids (Table 1).

In relation to *Z. perennis*, Longley (1924) reported 2n = 40 and a regular meiosis with the formation of 20 bivalents for the first time, whereas Longley (1934), Mangelsdorf and Reeves (1939) and Randolph (1955) maintain that it behaves as a "autotetraploid" with only quadrivalent associations. Finally Shaver (1962) in a re-examination of meiotic behaviour of *Z. perennis* founded the mean meiotic configuration of 9.92II + 4.99IV that lies between the above-mentioned extremes. These values are similar to our results (11.02II + 4.44IV; Table 1). According to Pasupuleti and Galinat (1982) a comparative cytological study of the number of knobs and their position of the "diploid" (*Z. diploperennis*) and "tetraploid" perennial teosinte (*Z. perennis*) provides evidence that the latter is an "autotetraploid" derivative of the former. The above-mentioned comparative study was done using *Z. perennis* observations of Longley (1941). Moreover Pasupuleti and Galinar (1982) have suggested that *Z. perennis* should be submerged as a subspecies of *Z. diploperennis.* Assuming an autopolyploid origin of *Z. perennis,* the formation of 10 trivalents were expected in *Z. diploperennis* × *Z. perennis* $F_1$ hybrid. This hypothesis appeared as improbable in view of the meiotic configurations obtained in this hybrid (Molina 1983, 1985 and the present paper). Configurations with 6 to 10III are present in 25.6% and 10III in only 0.59% of the cells studied (means of III = 4.91, II = 5.25, I = 4.73; Table 1). Furthermore, very similar configurations and relative frequency was observed in the hybrid *Z. mays* ssp. *mays* × *Z. perennis* (Table 1). The occurrence in these two 2n = 30 hybrids of similar configurations could be due to a) the A genomes are shared by the three species, b) the B genomes are shared by *Z. diploperennis* and *Z. mays* ssp. *mays* but these are absent in *Z. perennis* and finally c) the C genomes are present only in *Z. perennis* (Fig. 1). The alloploid nature of *Z. perennis* would have been undetected so far because of the similar morphology between *Z. perennis* and *Z. diploperennis.* There are several examples of a cryptic alloploid nature in other genera and a good example in grasses is *Bromus arizonicus* (Stebbins 1971) and in legumes *Lotus corniculatus* (Grant 1965; Grant and Zandstra 1968). On the other hand, these two perennial species (*Z. diploperennis* and *Z. perennis*) have distinct, but similar mitochondrial DNAs (Timothy et al. 1983) and isozyme patterns (Doebley et al. 1984). All this information would support the auto-allooctoploid origin of *Z. perennis* proposed here.

Longley (1924) was the first to report a meiotic study of the $F_1$ hybrid *Z. mays* × *Z. perennis* (2n = 30) and found meiotic configurations that are similar to our results, including 5III + 5II + 5I. The second interesting observation of Longley (1924) was that many of the trivalents have a large part corresponding to a bivalent chromosome and a small loosely attached portion. This observation agrees with the "frying pan" shape of some of the trivalents in this hybrid that are reported in the present paper. Finally, Longley (1924) found the way of possible identification of the univalents present at meiosis in this hybrid, suggested by a study of pollen where the corn involved was the Chinese waxy type. Only 4% of the pollen grains of this hybrid showed *wx* character. On the other hand, there was a very small percentage of meiosis in which there were no laggard chromosomes (univalents). This may indicate that the laggard chromosomes have been contributed to this hybrid by the waxy corn parent (Longley 1924). These results are in total concordance with the genome hypothesis postulated in Fig. 1 where in the *Z. mays* ssp. *mays* x *Z. perennis* hybrid the univalents come from the $B_2$ genome of *Z. mays* ssp. *mays*.

Cytological evidence of internal relations among the 10 chromosomes of the haploid set maize were found by several authors. At the present time there are three types of cytological evidence: a) chromosome pairing in meiosis of haploid maize, b) secondary association and c) three-dimensional distribution of chromosomes in somatic metaphase cells.

a) McClintock (1933) studied prophase pairing in a monoploid maize and reported the occurrence of a high degree of pachytene pairing which looked as intimate as homologous pairing. Chaganti (1965) made a detailed study of various types of chromosome associations in meta-anaphase I in haploid maize and 30% of the cells showed associations implying homology or homoeology. Similar associations have been found by Ting (1966) and recently by the same author (Ting 1985) in haploid maize obtained from anther culture.

These types of pairing are likely to occur where haploids arise from auto-allopolyploid, i.e., in polyhaploids, since they are then likely to have homologous or at least homoeologous members in the haploid complement (John and Lewis 1965). But, what is the nature of pairing in haploid plants of diploid origin? i.e. in haploid rye (John and Lewis 1965; Puertas and Giraldez 1979). Three possible explanations were proposed by Kimber and Riley (1963): 1) pairing may arise from homologies due to the existence of duplicated segments or 2) aneuploid origin of the contemporary chromosome number, or 3) due to an archaic polyploid origin. On the other hand, John and Lewis (1965) proposed that meiotic associations in monoploids may be due to stickness of heterochromatic zones than actual chiasmata. In haploid maize synaptonemal complex has been demonstrated by Ting (1969). Moreover, the occurrence of crossing over in haploid maize was strongly indicated by the study of translocations of haploid origin made by Weber and Alexander (1972).

b) Secondary association was observed in Italian maize (2n = 20) by Ghatnekar (1965). Vijendra Das (1970) found secondary association of bivalents in *Zea mays* (2n = 20) and a maximum of five groups of two bivalents were observed at diakinesis. These indicated that the basic number of *Zea* is five (Vijendra Das 1970). Our observation on secondary association of bivalents in the 2n = 20 taxa (*Z. mays* ssp. *mays*, *Z. diploperennis* and in its $F_1$; Fig. 2a, b, c and e) agree with the results and hypothesis proposed by Vijendra Das (1970).

c) Finally, the 3-D reconstruction study, using electronmicrographs of serial thin sections of somatic metaphase cells, made by Bennett (1983, 1984), showed that the 20 chromosomes of *Zea mays* form four sub-sets of 5 chromosomes rather than two sets of 10. The Bennett model is used for predicting the mean spatial order of chromosomes which are related to the secondary associations of heterologues. As Bennett (1983, 1984) pointed out, his model may have predicted intraspecific homoeology, suggesting that *Zea mays* is a tetraploid with considerable homoeology between chromosomes at corresponding positions in its two natural karyotypes (x = 5) and indicating which chromosomes are homoeologues.

Concentric genome separation is common in hybrids, but side-by-side genome separation appears to be common in established species. This genome separation was observed in somatic metaphase cells in barley, barley x *Secale africanum* hybrid and maize (Bennett 1983). In our case, an interesting genome separation in meiotic metaphase I of the 2n = 30 hybrids was observed. As mentioned previously in results, there is a clear tendency of A, C and B genome separation through the trivalents, bivalents and univalents grouped respectively (Figs. 1 and 2f–g).

Moreover, there is genetic evidence for the amphidiploid origin of maize, Rhoades (1951) in a review on the duplicate genes in maize, pointed out that the architecture of the germ plasm of this species contains many duplicated regions and would represent vestiges reflecting an ancient amphidiploid origin. More recently Gottlieb (1982) proposed that "if many enzymes in a presumed diploid show increased isozyme number, it becomes necessary to re-examine the evidence of diploidy". From this point of view, the numerous duplicated isozymes in maize are intriguing (Gottlieb 1982). This author agrees with Rhoades's hypothesis of the tetraploid nature of maize to explain their numerous duplicated genes.

The tribe Maydeae is composed of seven genera *Zea, Tripsacum, Coix, Trilobachne, Polytoca, Sclerachne* and *Chionachne* (Celarier 1957a). Two basic numbers x = 5(10) and x = 9 are found in Maydeae, which are also present in many genera of the tribe Andropogoneae (Bolkhovsjijh et al. 1969; Celarier 1956, 1957a, b, 1959; Darlington and Wiley 1956). All the genera of Maydeae have species with chromosome number multiples of x = 5 (10, 20, 40) with the exception of *Tripsacum* that have species with 2n = 18, 36, 72 (x = 9). As pointed out for the tribe Andropogoneae by Celarier (1956), the available evidence in Maydeae strongly suggests that x = 5 may have been the original basic number for this tribe, that $x_1 = 10$ was derived from it principally by allopolyploidy and that $x_2 = 9$ is of secondary origin from $x_1 = 10$. At the present time, the Asiatic genus *Coix* is the only one that has diploid species (2n = 10) in the Maydeae. According to Stebbins (1982) *Coix* is certainly derived from the Andropogoneae. A similar situation occurs in Andropogoneae where only two Asiatic or African genera (*Sorghum* and *Elyonurus*) have diploid species (Celarier 1957b).

Stebbins (1982) in a discussion on the original basic number for the Poaceae family pointed out that in the case of Andropogoneae and Maydeae the two alternative hypotheses, aneuploid reduction from x = 12 and secondary polyploid from x = 5, appear to be highly and equally improbable. Acceptance of either requires the assumption that a large number of connecting species have become extinct. The evidence discussed here support the secondary polyploid origin of n = 10 in *Zea*.

Numerous theories and hypotheses on the origin of maize were proposed (Gay 1984). In view of the possible tetraploid nature of maize and other species with 2n = 20, a reconsideration of the "Amphiploid theory" proposed by Anderson (1945) and sustained by Ting (1966) with some modification, seems to be necessary. According to this hypotheses *Z. mays* ssp. *mays* (maize), other wild subspecies (ssp. *mexicana*) could be derived from human selection or both, would be originated by allopolyploidy from different diploid species (2n = 10) probably extinct at the present time. In these diploid species the distinct original genomes (x = 5) that are present in the actual polyploids would be differentiated. A minimum of three distinct original genomes (A, B, and C; Fig. 1) was proposed here to explain the genome constitution and the relationships among the species studied here.

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