Karyotype of *Zea luxurians* and *Z. mays* subsp. *mays* using FISH/DAPI, and analysis of meiotic behavior of hybrids[1]

Graciela E. González and Lidia Poggio

Genome Downloaded from www.nrcresearchpress.com by Universitat de Valencia on 11/17/11
For personal use only.

Abstract: The karyotypes of *Zea luxurians* and a race of maize from northwestern Argentina are described and compared using 4′,6-diamidino-2-phenylindole (DAPI) banding and fluorescent in situ hybridization (FISH) to localize the 180 bp knobs. The meiotic behavior of the F_1 artificial hybrids *Z. luxurians* × maize is also analyzed to determine the genomic relationships between both species. Neocentromere activity at knobs in the meiosis of the hybrids is particularly discussed. The meiotic behavior and the high pollen sterility of the hybrid revealed genetical and (or) chromosomal divergences, leading to postzygotic reproductive isolation among their parents. Here, we propose that maize shows lower genomic affinity to *Z. luxurians* than to other species of the genus with $2n = 20$.

Key words: Zea, hybrid, karyotypes, neocentromeres, 4′,6-diamidino-2-phenylindole (DAPI), fluorescent in situ hybridization (FISH).

Résumé : Les caryotypes du *Zea luxurians* et d'une race de maïs provenant du nord-ouest de l'Argentine sont décrits et comparés en faisant appel aux bandes DAPI (4′,6-diamidino-2-phénylindole) et à l'hybridation in situ en fluorescence (FISH) pour localiser les protubérances à répétitions de 180 pb. Le comportement méiotique a également été examiné chez les hybrides F1 artificiels *Zea luxurians* x maïs pour étudier les relations génomiques entre les deux espèces. Les auteurs discutent particulièrement d'une activité néocentromérique à l'endroit des protubérances lors de la méiose chez les hybrides. Le comportement méiotique et la grande stérilité pollinique chez l'hybride indiquent des divergences génétiques ou chromosomiques, menant ainsi à un isolement reproductif des parents, lequel serait dû à des barrières post-zygotiques. Les auteurs proposent que le maïs présente une moindre affinité génomique avec le Zea *luxurians* qu'avec les autres espèces au sein de ce genre qui possèdent 2n = 20 chromosomes.

Mots-clés : Zea, hybride, caryotype, néocentromères, 4′,6-diamidino-2-phénylindole (DAPI), l'hybridation in situ en fluorescence (FISH).

[Traduit par la Rédaction]

Introduction

The genus *Zea* (Poaceae, Maydeae) is classified into the *Luxuriantes* section and the *Zea* section (Doebley 1990). The *Luxuriantes* section (Doebley and Iltis) includes the perennials *Zea diploperennis* Iltis (Doebley and Guzman) and *Z. perennis* (Reeves and Mangelsdorf), and the annuals *Z. luxurians* (Durieu and Ascherson) (Bird) and *Z. nicaragüensis* (Iltis and Benz 2000). The *Zea* section consists only of the annual *Z. mays* L. with the following four subspecies: *Z. mays* subsp. *mays* (maize), *Z. mays* subsp. *mexicana*, *Z. mays* subsp. *parviglumis*, and *Z. mays* subsp. *huehuetenanguensis* (Doebley 1990). All species have $2n = 20$, except *Z. perennis* with $2n = 40$.

Crosses between different taxa of *Zea* have been performed to assess the genomic affinity of the parental species by meiotic pairing analysis of the hybrids (Naranjo et al. 1990, 1994; Poggio et al. 1990, 1999, 2000*a*, 2000*b*, 2005; González et al. 2004, 2006). These authors provided cytogenetic evidence for the cryptic polyploid nature of the genus, and concluded that maize and its wild relatives are allotetraploids with a basic chromosome number of five ($x = 5$). In fact, genetic mapping provided compelling evidence that maize is a segmental allopolyploid, having undergone extensive chromosomal rearrangement (Moore et al. 1995; Gaut and Doebley 1997; Soltis and Soltis 1999).

The in situ hybridization (ISH) techniques have frequently been applied to analyze the genomic affinities among *Zea* species (Poggio et al. 1999, 2000*a*, 2000*b*, 2005; Takahashi et al. 1999; González et al. 2004, 2006). On the other hand, the relationships among species of the *Luxuriantes* section and subspecies of the *Zea* section have been investigated by

Received 29 May 2010. Accepted 1 September 2010. Published on the NRC Research Press Web site at genome.nrc.ca on 14 December 2010.

Corresponding Editor: M. Puertas.

G.E. González[2] and L. Poggio. Department of Ecology, Genetics and Evolution (FCEN, UBA), University of Buenos Aires, Buenos Aires, Argentina.

[1]In memoriam of Dr. Carlos A. Naranjo.

[2]Corresponding author (e-mail: gegonzalez@ege.fcen.uba.ar).

RIGHTSLINK

Genome Downloaded from www.nrcresearchpress.com by Universitat de Valencia on 11/17/11
For personal use only.

analyzing the meiotic behavior of their hybrids, and GISH (genomic in situ hybridization) and FISH (fluorescent in situ hybridization) in species and hybrids (Poggio et al. 1999, 2000*a*, 2000*b*, 2005; González 2004; González et al. 2004, 2006). However, the relationship between *Z. luxurians* and maize, which differ in DNA content, has not been sufficiently explored using cytogenetic methods. *Z. luxurians* shows the highest DNA content among *Zea* species with $2n = 20$ chromosomes (2C = 8.83 pg), whereas in maize it ranges between 2C = 5.86 and 6.79 pg (Laurie and Bennett 1985; Tito et al. 1991; Poggio et al. 1998; Rosato et al. 1998). In the genus *Zea*, variation in DNA content has been proposed to be mainly due to differences in the amount of heterochromatin, which is mainly located in distal heterochromatic blocks called knobs (Laurie and Bennett 1985; Poggio et al. 1998). Knobs occur in all *Zea* species with $2n = 20$, varying in size and number across maize races and their wild relatives (Kato 1976; McClintock et al. 1981; González 2004; Poggio et al. 2005). The knobs correspond to C- and 4′,6-diamidino-2-phenylindole (DAPI)-positive bands on mitotic metaphase chromosomes, are located subterminally in maize and terminally in *Z. luxurians* (Tito et al. 1991; Poggio et al. 1998, 2005), and consist mainly in a "tandem" repeat of a 180 bp sequence (Peacock et al. 1981). The analysis of the C-banding pattern revealed that maize has shorter chromosomes with smaller and fewer knobs compared with *Z. luxurians* (Tito et al. 1991; Ellneskog-Staam et al. 2007). In *Z. luxurians*, a positive correlation between high DNA content and high number and size of knobs was found (Tito et al. 1991). On the other hand, in the genus *Zea* no correlation was found between chromosome length and percentage of knob heterochromatin (Ellneskog-Staam et al. 2007).

In this study the karyotypes of *Z. luxurians* and a race of maize from northwestern Argentina are described and compared using DAPI banding and FISH, to localize the 180 bp knob sequence. The meiotic behavior of the artificial F_1 *Z. luxurians* × maize hybrids is analyzed to determine the genomic relationship between parental species. Neocentromere activity at knobs in the meiosis of the hybrids is particularly discussed.

Materials and methods

Plant material

The materials used in this study were the Guatemalan *Z. luxurians* '9478' provided by the International maize and wheat improvement center (CIMMYT) and the Argentine maize race Amarillo Chico (VAV 6451) from the Vavilov Laboratory, University of Buenos Aires (UBA). They were cultivated in the greenhouse of the Faculty of Agronomy, UBA.

Karyotyping

DAPI banding was performed according to Summer (1990). At least 10 cells of each species were analyzed. Chromosomal parameters were measured using the freeware program MicroMeasure 3.3 (http://www.colostate.edu/depts/biology/micromeasure). The relative chromosome length, arm ratio, and centromeric indexes were calculated to determine the karyotypes. The total chromosome volume (TCV) was estimated using the formula $TCV = (\pi \times r^2 \times TCL) \times 2$ (r, average chromatid radius; TCL, total chromosome length). The chromosomes were ordered from the largest to the smallest, as usual for maize, and chromosome morphology was described according to Levan et al. (1964), which classify chromosomes as metacentric (m), sub-metacentric (sm), sub-telocentric (st), and telocentric (t). To estimate the karyotype asymmetry, two numerical parameters were used, following Romero Zarco (1986): A1 (intrachromosomal asymmetry index = 1 – (short arm/long arm)/n) and A2 (interchromosomal asymmetry index = standard deviation (S)/mean chromosome length (X)). Both indexes are independent of the number and size of the chromosomes.

Meiotic analysis

Interspecific crossings between *Z. luxurians* (female) and maize (male) were carried out in the greenhouse to obtain the F_1 hybrid plants ($2n = 20$). About 100 plants of *Z. luxurians* were hand-pollinated with a bulk of pollen from 10 maize plants. Only 2 of 20 F_1 hybrid seeds giving rise to adult plants.

Young panicles from *Z. mays* subsp. *mays*, *Z. luxurians*, and their F_1 hybrids were fixed in a 3:1 solution of absolute ethanol – acetic acid and squashed in 2% acetic haematoxylin. The pairing configurations were determined at diakinesis – metaphase I. Only plates showing well-spread cells were scored.

Normal (stained) and aborted (unstained) pollen grains were distinguished using Alexander's stain (Alexander 1969).

Fluorescence in situ hybridization (FISH)

The 180 bp knob sequence in maize was obtained from GenBank (http://www.ncbi.nlm.nih.gov/). The sequence was isolated and amplified from total genomic DNA of maize by PCR methods. The primers were designed using the Primer3 program (version 0.6) provided by the Whitehead Institute for Biomedical Research & Howard Hughes Medical Institute, USA (http://frodo.wi.mit.edu/primer3/primer3_code.html). This probe was biotin-labeled with the Nick translation kit (Boehringer Mannheim, Germany), following the manufacturer's procedures.

Fixed root tips of *Z. luxurians* and maize were treated with an enzyme solution (2% cellulase Onozuka R10 and 20% pectinase), and squashed in a drop of 45% acetic acid. Slides with well-spread metaphases were selected by phase-contrast light microscopy. The in situ hybridization technique was performed as described by Cuadrado and Jouvé (1995), with minor modifications.

The DAPI and FISH slides were observed with a Zeiss AxioPhot epifluorescence microscope (Carl Zeiss, Germany), and microphotographs were taken, in black and white, with a Leica CCD digital camera.

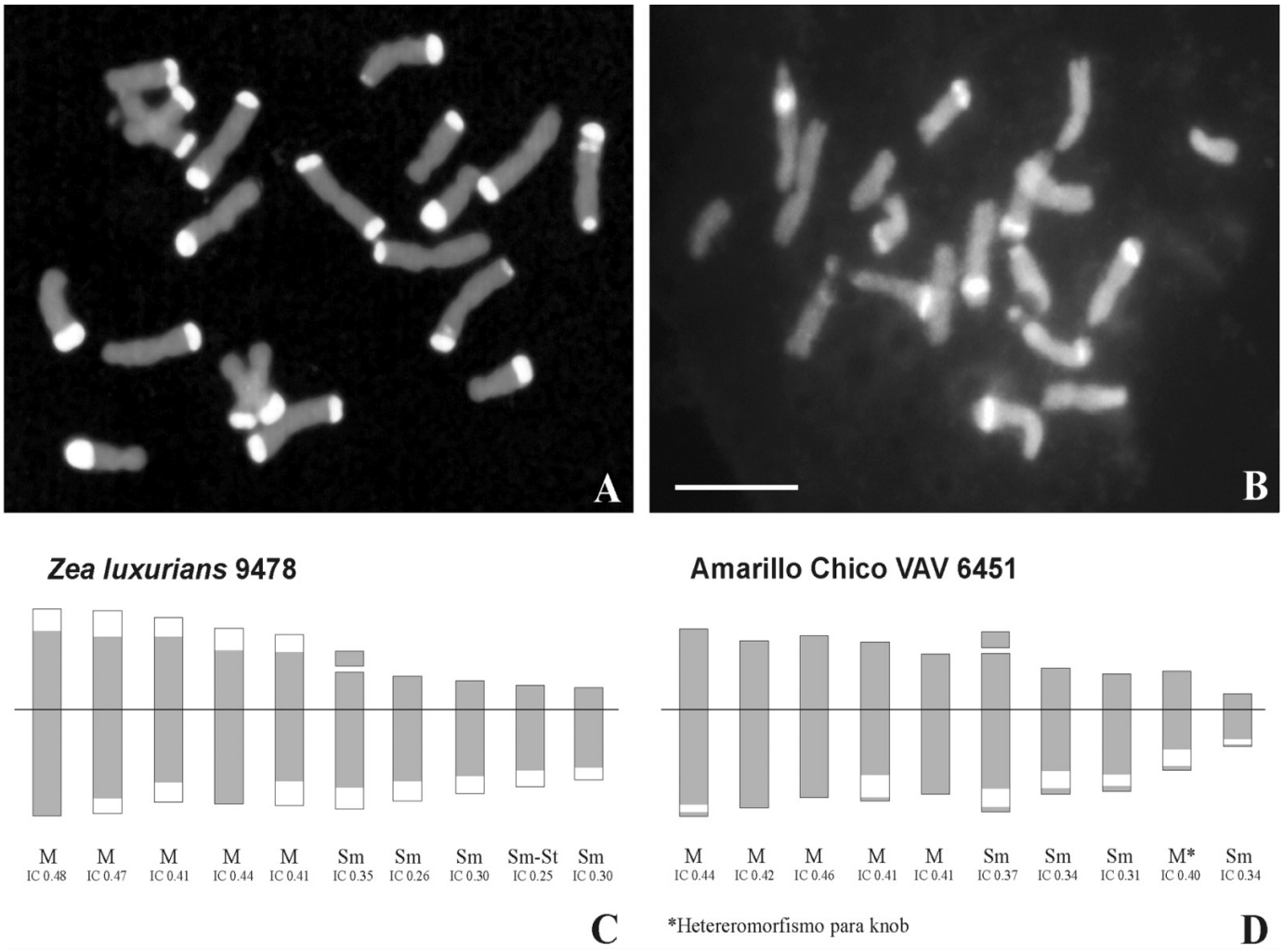
Results

Karyotypes

The karyotype formula was 5 m + 4 sm + 1 sm-st for *Z. luxurians* and 6 m + 4 sm for maize (race Amarillo Chico) (Fig. 1). The asymmetry indexes were A1 = 0.39

RIGHTSLINK

Fig. 1. (A and B) 4′,6-Diamidino-2-phenylindole (DAPI) banding and fluorescent in situ hybridization (FISH) using 180 pb knob sequence as probe on mitotic metaphase of *Zea luxurians* (A) and maize race Amarillo Chico (B), the last one with one chromosome out of plate. DAPI-positive bands and FISH-positive signals are coincident. (C and D) Idiograms of *Z. luxurians* (C) and maize race Amarillo Chico (D), the position of DAPI-positive bands and hybridization signals are indicated in white bars. M, metacentric; Sm, sub-metacentric; IC, centromeric index. Scale bar = 10 μm.



and A2 = 0.15 for *Z. luxurians* and A1 = 0.36 and A2 = 0.27 for maize.

DAPI banding and FISH using the 180 bp knob repeat as a probe were performed on mitotic metaphase chromosomes of maize and *Z. luxurians*. The 180 bp knob sequence showed positive hybridization signals on all heterochromatic DAPI-positive bands (Figs. 1A, 1B). These results were used to analyze the karyotypes of both species and to construct their idiograms (Figs. 1C, 1D).

The mean TCL was 173 μm for *Z. luxurians* and 135 μm for maize, being the *Z. luxurians* karyotype 28.6% larger than that of maize. It is interesting to point out that the karyotype of *Z. luxurians* was larger than that of the Amarillo Chico maize population (168 μm vs. 124 μm, respectively) regardless of the heterochromatic blocks. On the other hand, the average TCV of *Z. luxurians* was about 28% higher than that of maize.

The percentage of knob heterochromatin, which was calculated as a percentage of TCL, was 21% for *Z. luxurians* and 7.6% for maize. The DAPI-positive heterochromatic bands of *Z. luxurians* were larger than those of maize (Fig. 1). Knob size ranged between 2.74 μm and 0.8 μm (mean: 1.75 μm) in *Z. luxurians*, and between 1.62 μm and 0.54 μm (mean: 1.04 μm) in maize. Therefore, the knobs of *Z. luxurians* were approximately 40% larger than those of maize.

Meiotic analysis

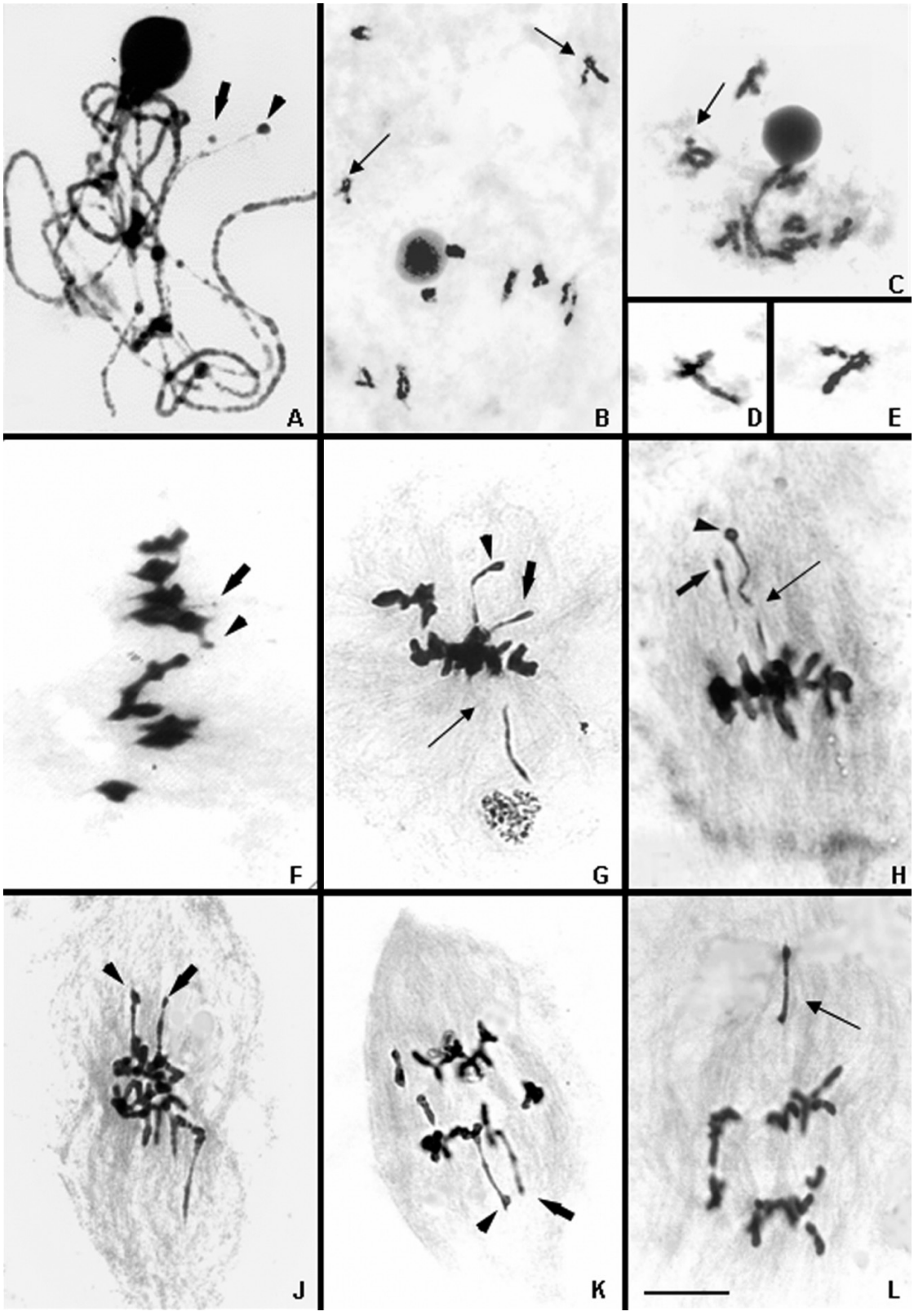
The meiotyc pairing of *Z. luxurians* and maize was regular, showing 10 homomorphic bivalents (II) in all the analyzed cells (at least 50 cells per species).

In the F_1 hybrids *Z. luxurians* × maize ($2n = 20$), loss of pairing between telomeric regions heterozygous for knobs was observed at pachytene (Fig. 2A). The analysis of 90 meiocytes in young anthers revealed that the most frequent meiotic configuration at diakinesis and metaphase I was 10 II (97% of the cells studied) (Fig. 2B), while the rest of the cells showed 9 II + 2 I. The mean number of ring bivalents per cell was 5.78, ranging between 5 and 7. Most of the bivalents were heteromorphic (Figs. 2B–2E).

Genome Downloaded from www.nrcresearchpress.com by Universitat de Valencia on 11/17/11
For personal use only.

RIGHTSLINK

Fig. 2. Meiotic behaviour of F_1 hybrid *Zea luxurians* × maize ($2n = 20$). (A) Pachytene. Arrows show loss of pairing between telomere regions heterozygous for knob sizes. (B and C) Ten heteromorphic bivalents in diakinesis, arrows show two of them. (D and E) Diakinesis detailed heteromorphic bivalents. (F) Metaphase I. (G and H) Metaphase II. (J–L) Anaphase II. In F–K, neocentromeric activity of the small (arrows) and big (arrowhead) knobs are shown. In G, H, and L, zones of chromosomal stretching and breakage are indicated with a slim arrow. In L, the slim arrow indicates a chromosome displaying neocentromeric activity in the different sized knobs of both chromatids. Scale bar = 10 μm.



Genome Downloaded from www.nrcresearchpress.com by Universitat de Valencia on 11/17/11
For personal use only.

RIGHTSLINK

Neocentromere activity at knobs was detected at metaphase I (Fig. 2F) and clearly observed at metaphase II (Figs. 2G, 2H) and anaphase II (Figs. 2J–2L). Although the neocentromere activity was observed in most of the chromosomes, seven of them showed very conspicuous neocentromeres in all cells studied (91 cells at metaphase II and 43 cells at anaphase II). Stretching and breakage of some arms were observed in chromosomes with neocentromere activity (Figs. 2G–2K). In Fig. 2L, the arrow indicates a chromosome displaying neocentromeric activity in the different sized knobs of both chromatids.

The pollen grains produced by the F_1 hybrid *Z. luxurians* × maize plants were highly sterile (97%), as indicated by Alexander's staining.

Discussion

The karyotypes of *Z. luxurians* and the maize population native to northwestern Argentina (race Amarillo Chico) were described by DAPI banding and FISH using the 180 bp knob sequence as a probe. It was observed that in both species the DAPI-positive bands co-localized with the 180 bp knob sequence. In different lines and races of maize from northwestern Argentina, Tito et al. (1991) and Poggio et al. (1998) found that the DNA content increases with increasing number and size of heterochromatic knobs, as evidenced by C and DAPI banding. We also found that the heterochromatic bands of *Z. luxurians* are larger than those of maize. This is in accordance with the fact that the DNA content of *Z. luxurians* is higher than that of lines and races of maize (Poggio et al. 1998; Rosato et al. 1998). As expected, *Z. luxurians* present a higher TCL and TCV in relation to maize. It is interesting to point out that when the knobs were excluded from the calculation, TCL of *Z. luxurians* was also higher than maize. On this basis, the variation in TCL between the studied species is probably due not only to differences in number and size of knob but also to differences in the amount of interspersed DNA, such as retrotransposon families (Meyers et al. 2001). These differences may also account for the dissimilar karyotypic formulae and related parameters of the studied species.

The karyograms constructed in this work are not strictly comparable to those obtained by Ellneskog-Staam et al. (2007), because of differences in the criteria used for chromosome ordering. The A1 and A2 asymmetry indexes are related to the number, size, and localization of the knobs on different chromosome arms. *Zea luxurians* showed higher intrachromosomal asymmetry (A1) but lower interchromosomal asymmetry (A2) than maize. This could be due to differences in the size and the distribution of the knobs on the chromosomal arms.

The study of meiotic pairing of artificial hybrids has proven to be a very useful tool for analyzing relationships among species of the *Luxuriantes* section and subspecies of the *Zea* section (Poggio et al. 1999, 2005; González 2004; González et al. 2004, 2006). The genomic affinity between *Z. luxurians* and maize through the meiotic behavior of their hybrid had not been carefully performed, and the results reported herein provide new insights on the relationships between maize and its wild relatives.

Despite the very large difference in chromosome size between *Z. luxurians* and maize, the fidelity of pairing, excepting knob zones, is quite high in the F_1 hybrids (Fig. 2A). They showed 10 heteromorphic bivalents, that is, recombinant chromosomes with chromatids of different size. In these heteromorphic bivalents, one chromatid of each chromosome had a large knob from *Z. luxurians* while the other chromatid had a small knob from maize or none, depending on the chromosome involved (Figs. 2B–2E).

Neocentromere activity in plants is an example of noncentromeric chromosome movement that is meiosis limited (Dawe and Hiatt 2004) and can be very conspicuous, often stretching chromosome arms the entire length of the spindle (Dawe 2009). In the hybrids, all the studied cells showed neocentromere activity at the heterochromatic knobs at metaphase–anaphase I and metaphase–anaphase II.

In the F_1 hybrid of *Z. luxurians* × maize, the larger knobs (from *Z. luxurians*) were closer to the poles than the smaller ones (from maize) at anaphase I and II (Figs. 2G–2H). The migration occurred towards the pole to which the larger knob is directed. This is in agreement with Yu et al. (1997), who found that neocentromeres move much faster on the spindle than true centromeres and, in maize, the speed of this movement would be correlated with knob size. Moreover, stretching and occasionally breakage of some arms were observed in chromosomes with neocentromere activity. This has been previously reported for maize by Rhoades and Vilkomerson (1942). Neocentromere activity in maize is related to genes present in the abnormal chromosome 10 (Ab-10) (Yu et al. 1997; Hiatt et al. 2002). However, the Ab-10 was undetected in more than 10 parental maize plants with neocentromere activity studied herein. It should be noted that the Ab-10 chromosome is unusual in maize races from South America (McClintock et al. 1981; Poggio et al. 1998; Rosato et al. 1998). Nevertheless, the Ab-10 chromosome could have occurred at low frequency in the male parent, because a bulk of pollen was used for inbreeding. On the other hand, it is important to point out that the neocentromeres may become activated in the absence of the Ab-10 knob (Rhoades and Dempsey 1986; Dawe 2009). The presence of neocentromeres when the Ab-10 is absent may also be caused by stress resulting from hybridization between distant taxa, a phenomenon called "genomic shock" by McClintock (1984). In fact, in other taxa, neocentromeres were only observed after interspecific cross (Walters 1952; Hayman 1955; Jones 1969; Manzanero et al. 2000; Carvalho et al. 2008).

Previous GISH experiments on maize chromosomes using total genomic DNA of *Z. luxurians* as a probe showed low-intensity hybridization signals in comparison with those observed using probes from other species of *Zea* with $2n = 20$ (Poggio et al. 2000*a*, 2000*b*; González 2004). The meiotic behavior and the high pollen sterility of the hybrid reveal genetical and (or) chromosomal divergences leading to postzygotic reproductive isolation. These findings let us to propose that maize shows lower genomic affinity to *Z. luxurians* than to other species of the genus with $2n = 20$.

Acknowledgements

This research was supported by grants from the Agencia Nacional de Promoción Científica y Técnica (FONCYT)

Genome Downloaded from www.nrcresearchpress.com by Universitat de Valencia on 11/17/11
For personal use only.

RIGHTSLINK

PICT-14119, the University of Buenos Aires (X-178), and CONICET (PIP-0342). The authors are members of the National Council of Scientific Research of Argentina (CONICET).

References

Alexander, M.P. 1969. Differential staining of aborted and nonaborted pollen. Stain Technol. **44**(3): 117–122. PMID:4181665.

Carvalho, A., Guedes-Pinto, H., Heslop-Harrison, J.S., and Lima-Brito, J. 2008. Wheat neocentromeres found in F_1 Triticale × Tritordeum hybrids (AABBRHch) after 5-azacytidine treatment. Plant Mol. Biol. Rep. **26**(1): 46–52. doi:10.1007/s11105-008-0021-z.

Cuadrado, A., and Jouvé, N. 1995. Fluorescent in situ hybridization and C-banding analyses of highly repetitive DNA sequences in the heterochromatin of rye (*Secale montanum* Guss.) and wheat incorporating *S. montanum* chromosome segments . Genome, **38**(4): 795–802. doi:10.1139/g95-101. PMID:7672610.

Dawe, R.K. 2009. Maize centromeres and knob (neocentromeres). *In* Handbook of maize genetics and genomics. *Edited by* J.L. Bennetzen and S. Hake. Springer, New York. pp. 239–250.

Dawe, R.K., and Hiatt, E.N. 2004. Plant neocentromeres: fast, focused, and driven. Chromosome Res. **12**(6): 655–669. doi:10.1023/B:CHRO.0000036607.74671.db. PMID:15289670.

Doebley, J.F. 1990. Molecular systematics of *Zea* (Gramineae). Maydica, **35**: 143–150.

Ellneskog-Staam, P., Henry Loaisiga, C., and Merker, A. 2007. Chromosome C-banding of the teosinte *Zea nicaraguensis* and comparison to other *Zea* species. Hereditas, **144**(3): 96–101. doi:10.1111/j.2007.0018-0661.01989.x. PMID:17663701.

Gaut, B.S., and Doebley, J.F. 1997. DNA sequence evidence for the segmental allotetraploid origin of maize. Proc. Natl. Acad. Sci. U.S.A. **94**(13): 6809–6814. doi:10.1073/pnas.94.13.6809. PMID:11038553.

González, G.E. 2004. Afinidades genómicas y mapeo cromosómico en maíz y especies relacionadas, a través de estudios de citogenética clásica y de hibridación in situ. Ph.D. thesis, Depto. de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

González, G.E., Confalonieri, V., Comas, C., Naranjo, C.A., and Poggio, L. 2004. GISHGenomic in situ hybridization reveals cryptic genetic differences between maize and its putative wild progenitor *Zea mays* subsp. *parviglumis*. Genome, **47**(5): 947–953. doi:10.1139/g04-038.

González, G.E., Comas, C., Confalonieri, V., Naranjo, C.A., and Poggio, L. 2006. Genomic affinities between maize and *Zea perennis* using classical and molecular cytogenetic methods (GISH-FISH). Chromosome Res. **14**(6): 629–635. doi:10.1007/s10577-006-1072-3. PMID:16964569.

Hayman, D.L. 1955. Centromeric behavior of the univalents in two *Phalaris* hybrids. Aust. J. Biol. Sci. **8**: 241–253.

Hiatt, E.N., Kentner, E.K., and Dawe, R.K. 2002. Independently regulated neocentromere activity of two classes of tandem repeat arrays. Plant Cell, **14**(2): 407–420. doi:10.1105/tpc.010373. PMID:11884683.

Iltis, H.H., and Benz, B.F. 2000. *Zea nicaraguensis* (Poaceae), a new teosinte from Pacific Coastal Nicaragua. Novon, **10**(4): 382–390. doi:10.2307/3392992.

Jones, G.H. 1969. Further correlations between chiasmata and U-type exchanges in rye meiosis. Chromosoma, **26**(1): 105–118. doi:10.1007/BF00319501.

Kato, Y.T. 1976. Cytological studies of maize (*Zea mays* L.) and teosinte (*Zea mexicana* Schrader Kuntze) in relation to their origin and evolution. Mass. Agric. Exp. Stn. Bull. **635**: 1–185.

Laurie, D.A., and Bennett, M.D. 1985. Nuclear DNA content in the genera *Zea* and *Sorghum*. Intergeneric, interespecific and intraspecific variation. Heredity, **55**(3): 307–313. doi:10.1038/hdy.1985.112.

Levan, A., Fredga, K., and Sandberg, A. 1964. Nomenclature for centromeric position of chromosomes. Hereditas, **52**(2): 201–220. doi:10.1111/j.1601-5223.1964.tb01953.x.

Manzanero, S., Puertas, M.J., Jiménez, G., and Vega, J.M. 2000. Neocentric activity of rye 5RL chromosome in wheat. Chromosome Res. **8**(6): 543–554. doi:10.1023/A:1009275807397. PMID:11032323.

McClintock, B. 1984. The significance of responses of the genome to challenge. Science, **226**(4676): 792–801. doi:10.1126/science.15739260. PMID:15739260.

McClintock, B., Kato, Y.T., and Bluemenshein, A. 1981. Chromosome constitution of races of maize: its significance in the interpretation of relationships between races and varieties in the Americas. Colegio de Postgraduados, Chapingo, México.

Meyers, B.C., Tingey, S.V., and Morgante, M. 2001. Abundance, distribution, and transcriptional activity of repetitive elements in the maize genome. Genome Res. **11**(10): 1660–1676. doi:10.1101/gr.188201. PMID:11591643.

Moore, G., Devos, K.M., Wang, Z., and Gale, M.D. 1995. Cereal genome evolution. Grasses, line up and form a circle. Curr. Biol. **5**(7): 737–739. doi:10.1016/S0960-9822(95)00148-5. PMID:7583118.

Naranjo, C.A., Molina, M.C., and Poggio, L. 1990. Evidencias de un número básico x = 5 en el género *Zea* y su importancia en estudios del origen del maíz. Acad. Nac. Cs. Ex. Fís. Nat., Buenos Aires **5**: 43–53.

Naranjo, C.A., Poggio, L., Molina, M.C., and Bernatené, E. 1994. Increase in multivalent frequency in F_1 hybrids of *Zea diploperennis* × *Zea perennis* by colchicine treatment. Hereditas, **120**(3): 241–244. doi:10.1111/j.1601-5223.1994.00241.x.

Peacock, W.J., Dennis, E.S., Rhoades, M.M., and Pryor, A.J. 1981. Highly repeated DNA sequence limited to knob heterochromatin in maize. Proc. Natl. Acad. Sci. U.S.A. **78**(7): 4490–4494. doi:10.1073/pnas.78.7.4490. PMID:16593063.

Poggio, L., Molina, M.C., and Naranjo, C.A. 1990. Cytogenetic studies in the genus *Zea*. 2. Colchicine induced multivalents. Theor. Appl. Genet. **79**: 461–464.

Poggio, L., Rosato, M., Chiavarino, M., and Naranjo, C.A. 1998. Genome size and environmental correlations in maize. Ann. Bot. (Lond.), **82**: 107–115. doi:10.1006/anbo.1998.0757.

Poggio, L., Confalonieri, V., Comas, C., González, G.E., and Naranjo, C.A. 1999. Genomic affinities of *Zea luxurians*, *Z. diploperennis*, and *Z. perennis*: meiotic behavior of their F_1 hybrids and genomic in situ hybridization (GISH). Genome, **42**: 993–1000. doi:10.1139/gen-42-5-993.

Poggio, L., Confalonieri, V., Comas, C., González, G.E., and Naranjo, C.A. 2000*a*. Evolutionary relationships in the genus *Zea*: analysis of repetitive sequences used as cytological FISH and GISH markers. Genet. Mol. Biol. **23**(4): 1021–1027. doi:10.1590/S1415-47572000000400048.

Poggio, L., Confalonieri, V., González, G.E., Comas, C., and Naranjo, C.A. 2000*b*. Aportes de la citogenética molecular al análisis de divergencias genómicas crípticas en el género *Zea* (Poaceae). Bol. Soc. Argent. Bot. **35**: 297–304.

Poggio, L., González, G.E., Confalonieri, V., Comas, C., and Naranjo, C.A. 2005. The genome organization and diversification of maize and its allied species revisited: evidences from classical and FISH-GISH cytogenetic analysis. Cytogenet. Genome Res. **109**(1–3): 259–267. doi:10.1159/000082408. PMID:15753585.

Rhoades, M., and Dempsey, E. 1986. Evidence that the K10 knob

Genome Downloaded from www.nrcresearchpress.com by Universitat de Valencia on 11/17/11
For personal use only.

RIGHTSLINK

is not responsible for preferential segregation and neocentromere activity. Maize Genet. Coop. News Lett., **60**: 26–27.

Rhoades, M.M., and Vilkomerson, H. 1942. On the anaphase movement of chromosomes. Proc. Natl. Acad. Sci. U.S.A. **28**(10): 433–436. doi:10.1073/pnas.28.10.433. PMID:16588574.

Romero Zarco, C. 1986. A new method for estimating karyotype asymetry. Taxon, **35**(3): 526–530. doi:10.2307/1221906.

Rosato, M., Chiavarino, A.M., Naranjo, C.A., Cámara-Hernández, J., and Poggio, L. 1998. Genome size and numerical polymorphism for B- chromosome races of maize (*Zea mays* ssp. *mays*, Poaceae). Am. J. Bot. **85**(2): 168–174. doi:10.2307/2446305.

Soltis, D.E., and Soltis, P.S. 1999. Polyploidy: recurrent formation and genome evolution. Trends Ecol. Evol. **14**(9): 348–352. doi:10.1016/S0169-5347(99)01638-9.

Summer, A.T. 1990. Chromosome banding. Unwin Hyman, London. pp. 434.

Takahashi, C., Marshall, J.A., Bennett, M.D., and Leitch, I.J. 1999. Genomic relationships between maize and its wild relatives. Genome, **42**(6): 1201–1207. doi:10.1139/gen-42-6-1201. PMID: 10659788.

Tito, C., Poggio, L., and Naranjo, C.A. 1991. Cytogenetics studies in the genus *Zea*: DNA content and heterochromatin in species and hybrids. Theor. Appl. Genet. **83**: 58–64.

Walters, M.S. 1952. Atypical chromosome movements in meiotic anaphase of *Bromus pitensis* × *Bromus marginatus*. Am. J. Bot. **39**(9): 619–625. doi:10.2307/2438366.

Yu, H.G., Hiatt, E.N., Chan, A., Sweeney, M., and Dawe, R.K. 1997. Neocentromere-mediated chromosome movement in maize. J. Cell Biol. **139**(4): 831–840. doi:10.1083/jcb.139.4.831. PMID:9362502.

Genome Downloaded from www.nrcresearchpress.com by Universitat de Valencia on 11/17/11
For personal use only.

RIGHTSLINK