Genomic affinities revealed by GISH suggests intergenomic restructuring between parental genomes of the paleopolyploid genus *Zea*

Journal:	<i>Genome</i>
Manuscript ID	gen-2015-0081.R2
Manuscript Type:	Article
Date Submitted by the Author:	25-Aug-2015
Complete List of Authors:	González, Graciela; Instituto de Ecología, Genética y Evolución de Buenos Aires (IEGEBA-CONICET), Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires Poggio, Lidia; Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires
Keyword:	GISH, Intergenomic restructuring, Maize, <i>Zea</i> , Teosintes

SCHOLARONE™
Manuscripts

**Genomic affinities revealed by GISH suggests intergenomic restructuring between
parental genomes of the paleopolyploid genus *Zea***

Graciela Esther González* and Lidia Poggio

Instituto de Ecología, Genética y Evolución (IEGEBA, Consejo Nacional de Investigaciones Científicas y Técnicas - CONICET) - Laboratorio de Citogenética y Evolución (LaCyE), Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina.

Running title: Genomic affinities and intergenomic restructuring in *Zea*.

Author for Correspondence: Graciela Esther González, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Int. Güiraldes N° 2620, Pabellón II, Lab. 110, 4° Piso (1428), Ciudad Autónoma de Buenos Aires, Argentina. Tel.: +54-11-4576-3300 ext. 218. e-mail address: gegonzalez@ege.fcen.uba.ar

Abstract

The present work compares the molecular affinities revealed by GISH with the analysis of meiotic pairing in intra e interspecific hybrids between *Zea* species, obtained in previous works. The joint analysis of these data provided evidence about the evolutionary relationships among the species from the paleopolyploid genus *Zea* (maize and teosintes). GISH and meiotic pairing of intraspecific hybrids revealed high genomic affinity between maize (*Zea mays* ssp. *mays*) and both *Zea mays* ssp. *parviglumis* and *Zea mays* ssp. *mexicana*. On the other hand, when *Zea mays* ssp. *huehuetenanguensis* DNA was probed on maize chromosomes, a lower affinity was detected, and the pattern of hybridization suggested intergenomical restructuring between the parental genomes of maize. When DNA from *Zea luxurians* was used as probe, homogeneous hybridization signals were observed through all maize chromosomes. Lower genomic affinity was observed when DNA from *Zea diploperennis* was probed on maize chromosomes, especially at knob regions. Maize chromosomes hybridized with *Zea perennis* DNA showed hybridization signals on four chromosome pairs: two chromosome pairs presented hybridization signal in only one chromosomal arm, whereas four chromosome pairs did not show any hybridization. These results are in agreement with previous GISH studies that enabled to recognize the genomic source of the chromosomes involved in the meiotic configurations of the *Zea perennis* x maize hybrids. These findings allow postulating that maize has a parental genome not shared with *Zea perennis*, and the existence of intergenomic restructuring between the parental genomes of maize. Moreover, the absence of hybridization signals in all maize knobs indicate that these heterochromatic regions were lost during the *Zea perennis* genome evolution.

Keywords: GISH, Intergenomic restructuring, Maize, Teosintes, *Zea*

Introduction

The meiotic association of homologous or homoeologous chromosomes reveals the relative affinity between genomes in hybrids and polyploid species and detects chromosomal rearrangements acting as reproductive isolation mechanisms (Sybenga 1975). Crosses between different species of *Zea* have been performed to assess the genomic affinities of these species by meiotic pairing analysis of their hybrids. These studies provided cytogenetic evidences to reveal the cryptic polyploid nature of the genus (paleopolyploidy) and concluded that maize and its wild relatives, with $2n=20$ chromosomes, are allotetraploids with a basic chromosome number of five ($x=5$). On the other hand, *Z. perennis* ($2n=40$) is, in all probability, an amphioctoploid (Naranjo *et al.* 1990, 1994; Poggio *et al.* 1990, 1999a, 2000a, 2000b, 2005; González *et al.* 2004, 2006, 2011). One of the most convincing evidence supporting the amphiploid hypothesis was obtained through treatments with dilute concentrations of colchicine (Naranjo *et al.* 1990, 1994; Poggio *et al.* 1990, 2000b, 2005). These treatments, which promote intergenomic pairing in allopolyploids (Poggio *et al.*, 1990), joint to the analysis of the meiotic behavior of species and hybrids, supported the polyploid condition of the genus *Zea* and the existence of two parental homoeologous genomes, arbitrarily named A and B. On these bases, the genomic formulae for all *Zea* species were proposed, being $AxAxBxBx$ for $2n=20$ species, and $ApApApApBp_1Bp_1Bp_2Bp_2$ for *Z. perennis* ($2n=40$) (Naranjo *et al.* 1990, 1994; Poggio *et al.* 1990, 2000b, 2005). Molecular analysis 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; White and Doebley 1998; Swigonova *et al.* 2004; Wei *et al.* 2007; Schanable *et al.* 2011).

The genomic *in situ* hybridization (GISH) is a valuable tool for discriminating between closely related genomes in plants, because the chromosomes and/or genomes may be distinguished on the basis of divergent dispersed repetitive sequences (Bennett 1995; Chester *et al.* 2010; Heslop-Harrison and Schwarzacher 2011). In *Zea*, GISH provided evidence about the evolutionary relationships among maize and some of its allied species (Poggio *et al.* 1999, 2000a, 2000b, 2005; González *et al.* 2004, 2006).

In this work, the results of comparative GISH experiments, using the wild related *Zea* species (teosintes) genomic DNA as probes on maize chromosomes, are discussed and compared with the genomic affinities revealed by meiotic pairing of intra and interspecific hybrids. These data will provide new clues about the evolutionary relationships among maize and its allied species, revealing cryptic genomic divergences between these closely related taxa.

Materials and Methods

Plant materials

The plants of *Zea mays* ssp. *mays* were legated by Vavilov Lab., Facultad de Agronomía de la Universidad de Buenos Aires (FA-UBA): Race Amarillo Chico (accession VAV 6451) from Santa Victoria, Salta, Argentina; Race Amarillo Grande (VAV 6669) from Manuel Belgrano, Jujuy, Argentina and Race Blanco y Ocho Rayas (VAV 6483) from Trancas, Tucumán, Argentina. The teosintes come from México and Guatemala: *Zea mays* ssp. *parviglumis* (Valle del Río Balsas, Guerrero, México, Leg. by CIMMYT), *Zea mays* ssp. *mexicana* (Chalco-Amecameca, Mesa Central, México, Leg. by Dr. A. T. Kato Yamakake from Colegio de Postgraduados, Montecillo, México), *Zea mays* ssp. *huehuetenanguensis* (Huehuetenango, Guatemala, Leg. by Dra. C. Prywed from Colegio de Postgraduados, Montecillo, México), *Zea luxurians*

(Guatemala, Leg. by Dra. C. Prywed), *Zea diploperennis* (San Miguel, Ciudad Guzmán, Jalisco, México, Leg. by Dra. C. Prywed), *Zea perennis* (Piedra Ancha, San Gabriel, Jalisco, México, Leg. by Dra. C. Prywed), All materials were cultivated and are maintained in the greenhouse of FA-UBA.

Cytological preparations

Metaphase chromosome preparations from *Zea mays* ssp. *mays* were obtained from root tips. Seeds were placed in Petri dishes on wet filter paper. Root tips were pre-treated in 0.02 M 8-hydroxyquinoline (Merck) for 3 hours at room temperature and fixed in 3:1 ethylic alcohol – acetic acid for 24 – 48 hours. Fixed root tips were washed in 0.01 M citric acid – sodium citrate, pH 4.6 buffer to remove fixative, and then were transferred to an enzyme solution containing 2% of cellulase Onozuka R10 (Merck) and 20% liquid pectinase (SIGMA). After washing with the above buffer solution, root tips were squashed onto slides in a drop of 45% acetic acid. Preparations showing well-spread metaphase cells were selected by phase-contrast light microscopy. After removal coverslips by freezing, the slides were air-dried to be used for *in situ* hybridization procedures.

DNA probes

Total genomic DNA was isolated from adult leaves from the teosintes with Wizard Genomic DNA Purification Kit (Promega), following manufacturer's procedures.

DNA was labelled with Dig High Prime kit (Boehringer Mannheim) and Biotin Nick Translation kit (Boehringer Mannheim), following manufacturer's procedures.

Genomic *in situ* hybridization-GISH

GISH technique was carried out according to González *et al.* (2004). Slide preparations were incubated in 100 µg/ml of RNase in 2xSSC (Saline-Sodium Citrate) for 1 h at 37 °C in a humidified chamber and washed three times in 2xSSC for 5 min each at room temperature. The slides were post-fixed in freshly prepared 4% (w/v) paraformaldehyde in distilled water for 10 min and then were washed in 2xSSC for 15 min at room temperature. The preparations were dehydrated in a graded ethanol series and air-dried. The hybridization mixture consisted of 50% (w/v) deionised formamide, 10% (w/v) dextran sulphate, 0.1% (w/v) SDS and 0.3 mg/ml of salmon sperm in 2xSSC, then adding 100 ng of the labelled probe to 30 µl of hybridization mixture for each slide. The hybridization mixture was denatured for 15 min at 75 °C, loaded onto the slides and covered with a plastic coverslip. The slides were placed on a thermocycler at 75 °C for 7 min, 45 °C for 10 min and 38 °C for 10 min. Then the slides were incubated overnight at 37 °C. Following hybridization, coverslips were carefully floated off by placing the slides in 2xSSC at 42 °C for 3 min each and then given an astringent wash in 20% formamide in 0.1xSSC at 42 °C for 10 min. After that, the slides were washed in 0.1xSSC at 42 °C for 5 min; 2xSSC at 42 °C for 5 min, then the slides were transferred to detection buffer (4xSSC, 0.2% (v/v) Tween 20) at 42 °C for 5 min and 1 h at room temperature in the same buffer.

To detect digoxigenin-labelled probes, slides were treated with sheep antidigoxigenin-FITC (Boehringer Mannheim), while for biotin-labelled probes, the slides were treated with Streptavidine-CY3 conjugated (SIGMA). The slides were incubated in a 1:40 solution of the corresponding antibody in detection buffer containing 2.5% Bovine Serum Albumine (SIGMA), for 1 h at 37 °C and washed 3 times in detection buffer for 10 min each at room temperature. Slides were

counterstained with 1 µg/ml of 4',6-diamino-2-phenylindole (DAPI) in 4xSSC/Tween buffer, for 25 min at room temperature and then mounted in anti-fade solution (Vector Lab.). Slides were examined with a Carl Zeiss Axiophot epifluorescence microscope with appropriate Carl Zeiss filters coupled with a Leica DC 250 digital camera and with an image analyzer Leica IM 1000.

Results

In GISH experiments total genomic DNA of *Z. m. ssp. mexicana* (Fig. 1 A and B) and *Z. m. ssp. parviglumis* (Fig. 1 C and D) were labelled and used as probes on mitotic metaphases of the maize accessions studied. With both probes, strong signals of hybridization were observed along all maize chromosomes, but being weak or absent in the pericentromeric region and in the region proximal to nucleolar organizer (NOR) located in the short arm of the chromosome pair 6. Moreover, strong fluorescent signals on the DAPI-positive bands (heterochromatic knob regions) and B chromosomes were detected (Fig. 1 A and B).

When genomic DNA of *Z. m. ssp. huehuetenanguensis* was probed on maize chromosomes, many chromosome regions were unlabelled. Three chromosomal pairs displayed one chromosome arm highly labelled and the other one unlabelled or weakly hybridized. The chromosome pair with NOR showed very weak hybridization signals except in the knob region. The rest of the chromosomes displayed a high level of hybridization except in the pericentromeric regions. The knobs and B chromosomes also showed a high level of hybridization (Fig. 1 E-F).

Genomic DNA from *Z. luxurians* showed uniform hybridization signals over all maize chromosomes, being higher on the knob regions (Fig. 1 G and H). In contrast,

probing with genomic DNA from *Z. diploperennis* hybridization signals were observed over all maize chromosomes, although they were weaker than the observed in the species already described. Besides, *ca.* 10 maize chromosomes showed very weak hybridization signal. The knob regions presented the same level of hybridization than that observed along the chromosome arms (Fig. 1 I and J).

Probing several accessions of maize with genomic DNA from *Z. perennis*, high level of hybridization in 4 chromosome pairs and absence of hybridization on other 4 chromosome pairs were revealed. The rest of the chromosomes (2 pairs) presented hybridization signals in a large portion of one of the chromosome arms. The knob regions lacked hybridization signals (Fig. 1 K and L).

The B chromosomes of maize showed high hybridization signal with all teosinte probes (Fig. 1 A, B, E and F).

Discussion

For a long time maize has been considered to be a diploid species, however noteworthy data indicate that it is a cryptic or diploidyzed polyploid. Several studies evaluated the genome affinity based on the estimation of the chromosome meiotic association of species and hybrids of genus *Zea*. These studies provided cytogenetic evidence confirming the cryptic polyploid nature of the genus and established that all *Zea* species have a basic chromosome number of five ($x=5$), that maize and its wild relatives ($2n=20$) are tetraploids, while *Z. perennis* ($2n=40$) is an allooctoploid (Naranjo *et al.* 1990, 1994; Poggio *et al.* 1990, 1999a, 1999b, 2000a, 2000b, 2005; González *et al.* 2004, 2006, 2011). These hypotheses were further confirmed by molecular studies

(Moore *et al.* 1995; White and Doebley 1998; Soltis and Soltis 1999; Schanable *et al.* 2011; Swigonova *et al.* 2004; Wei *et al.* 2007).

In the present work, the genomic affinities revealed by GISH experiments, using teosinte probes on maize chromosomes, are analyzed and compared with those detected through meiotic analysis in previous works.

The hybrids between maize and the two subspecies of the same taxonomical Section *Zea* (Doebley 1990), *Z. m. ssp. parviglumis* and *Z. m. ssp. mexicana*, have regular meiosis, with ten bivalents and high pollen viability (revisited in Poggio *et al.* 2005). When maize chromosomes were hybridized with labelled genomic DNA from *Z. m. ssp. parviglumis* and *Z. m. ssp. mexicana*, high hybridization signals throughout all chromosomes were observed. Similar results were reported by Takahashi *et al.* (1999). It is interesting to point out that, in the present work, bright hybridization signals were detected on the subtelomeric regions on some maize chromosomes, which correspond to the heterochromatic maize knobs (Poggio *et al.* 1999a; González *et al.* 2004, 2011, 2013). However, the pericentromeric regions and the NOR region presented faint hybridization signals with *Z. m. parviglumis* and *Z. m. mexicana* probes. This could be due to higher divergence in these regions, which consist mostly in repetitive sequences (Heslop-Harrison and Schwarzacher 2011). The high genomic affinities, revealed by GISH, between maize and *Z. m. ssp. parviglumis* and *Z. m. ssp. mexicana* are in agreement with the meiotic pairing of their hybrids. All these results indicate that the three subspecies are very close, with little divergences among its genomes.

When maize chromosomes were hybridized with labelled genomic DNA from *Z. m. ssp. huehuetenanguensis* (Section *Zea*), it was observed that, in addition to the absence of hybridization signals in the centromeric and NOR regions, entire

chromosome arms or part of them were unlabelled. Taking into account the genomic formulae proposed for these taxa, AABB (Naranjo *et al.* 1990), this result let to postulate that one of the maize parental genomes (A or B) presents an important divergence with *Z. m. ssp. huehuetenanguensis* genome. Moreover, the pattern of hybridization observed could be explained by the occurrence of intergenomic recombination between A and B maize parental genomes. This phenomenon is frequent in polyploids, indicating that parental genomes may have undergone some rearrangements following hybridization and whole genome duplication. Intergenomic restructuration was also reported in *Avena* (Hayasaki *et al.* 2000), *Poa jemtlandica* (Brysting *et al.* 2000) and the first generation of newly synthesized *Brassica* allopolyploids (Ma and Gustafson 2005), among others (Soltis *et al.* 2014a, b).

The meiotic analysis of the hybrids between maize and two teosinte species from Section Luxuriantes (Doebley 1990), *Z. luxurians* and *Z. diploperennis*, showed 10 bivalents with regular meiotic pairing. However, both hybrids differ in pollen fertility and seed viability (Naranjo *et al.* 1990; González 2004; González and Poggio 2011). González and Poggio (2011) analyzed the meiotic behavior of F1 artificial hybrids *Z. luxurians* x maize to determine the genomic relationships between both parental species. These hybrids presented 10 heteromorphic bivalents and several meiotic abnormalities that explained their high pollen sterility, which determine the postzygotic isolation between parental species. When labelled DNA from *Z. luxurians* was probed on maize chromosomes, homogeneous hybridization signals were observed through all chromosomes. However, the hybridization signals were weaker than those observed with *Z. m. ssp. parviglumis* and *Z. m. ssp. mexicana* probes, except in the knob regions, where high hybridization signals were seen. This indicates a high homology between knob sequences of maize and *Z. luxurians*, which is greater than that detected in the rest

of the chromosomes. Even lower genomic affinity was observed when total DNA of the perennial teosinte *Z. diploperennis* was probed on maize chromosomes. In this experiment, all maize chromosomes showed hybridization signals, although variation in the intensity of the signals among chromosomes was found, because *ca.* 10 chromosomes showed lower intensity of hybridization. According to the hypothesis proposed by Naranjo *et al.* (1990), these results would suggest that the allotetraploids maize and *Z. diploperennis* differ in one parental genome (A or B), or that these genomes experimented divergence and/or fractionation bias during the evolutionary process. On the other hand, these divergences are not reflected in the meiotic behavior of the F1 hybrids *Z. diploperennis* x maize, which presented regular meiotic chromosome pairing and high pollen viability (Naranjo *et al.* 1990; Poggio *et al.* 2005). In relation to the knobs, *Z. diploperennis* probe showed low hybridization signals on these heterochromatic regions of maize chromosomes. This result was previously observed by Albert *et al.* (2010) and could be explained by the existence of divergences in the sequence composition of knobs of maize and *Z. diploperennis*.

Zea perennis is a perennial autoalloctoploid species ($2n=40$) from the Section Luxuriantes, with a genomic formulae $ApAp\ Ap'Ap'\ Bp_1Bp_1\ Bp_2Bp_2$ (Doebley 1990; Naranjo *et al.* 1990). The meiotic analysis of the hybrids *Z. perennis* x maize ($2n=30$), whose genomic formulae is $ApAp'\ Am\ Bp_1Bp_2\ Bm$, revealed that $5III+5II+5I$ was the most frequent chromosome configuration at metaphase I (Poggio *et al.* 2005; González *et al.* 2006). GISH experiments enabled to recognize the genomic source of each chromosome involved in the meiotic configurations of these hybrids. These results revealed that, in these hybrids, five maize chromosomes have pairing affinities with *Z. perennis* chromosomes, jointly constituting the trivalents (III), while the other five maize chromosomes remain as univalents (González *et al.* 2006). In the present work,

an interesting result was obtained when *Z. perennis* genomic DNA was probed onto the maize chromosomes. It was observed a high level of hybridization on 4 chromosome pairs and absence of hybridization on other 4 chromosome pairs, while the remaining 2 chromosomes pairs presented hybridization signal in only one of their chromosomal arms. The entire chromosomes and the chromosome arms with hybridization signals could represent one of the parental genomes of maize with high sequence homology with *Z. perennis* genomes. They would correspond to the “pan handle” of the trivalents of the hybrids *Z. perennis* x maize described by González *et al.* (2006). Besides, the chromosomes and chromosomal arms without hybridization signals, could belong to the parental maize genome with low sequence homology with *Z. perennis* genomes and are, in all probability, the chromosomes that remain as univalents in the hybrids (González *et al.* 2006). Then, the analysis of the meiotic behaviour of the hybrids and the molecular affinities detected by GISH support the hypothesis that maize has a parental genome not shared with *Z. perennis*. Moreover, the two chromosome pairs showing hybridization signals in one arm reveal the existence of intergenomic restructuring between the maize parental genomes A and B. These intergenomic rearrangements would have occurred during the evolution and/or domestication of maize. Genomic restructuring following allopolyploidization was reported on several genus (revisited in Soltis *et al.* 2014a, b).

Another interesting result that emerges when *Z. perennis* genomic DNA is used as a probe on maize chromosomes is the absence of hybridization signals on all the maize heterochromatic knobs. Also, the lack of hybridization signal on knobs was observed when genomic DNA from *Z. perennis* was hybridized onto the other teosintes (Poggio *et al.* 1999a, 2005). These results would indicate that the knob sequences were lost during the *Z. perennis* genome evolution. In relation to this, it is interesting to point

out that the *Z. perennis* DNA content *per* basic genome is smaller than in other *Zea* species with $2n=20$ (Tito *et al.* 1991). It has been frequently documented that the major trend in vascular plants is a decrease in the genome size *per* haploid genome when a polyploidization event occurs (Leitch and Bennett 2004; Leitch and Leitch 2013). This genome downsizing, which could be involved in the genetic and cytogenetic diploidization of polyploids, consists in non-random deleting of coding and non-coding sequences, changes in retroelements, chromosome reorganization, loss of chromosomes or entire genomes (Ma and Gustafson 2006; Jones and Langdom 2013; Leitch and Leitch 2013; Poggio *et al.* 2014).

The present work provides new clues for understanding the genome organization and diversification of *Zea* species and the origin of domesticated maize.

Acknowledgements

Authors gratefully acknowledge grants from the Agencia Nacional de Promoción Científica y Técnica, University of Buenos Aires and the National Council of Scientific Research of Argentina (CONICET). The authors specially acknowledge the valuable suggestions from the Associate Editor and the two anonymous reviewers, which have improved the manuscript.

References

Albert, P.S., Gao, Z., Danilova, T.V., and Birchler, J.A. 2010. Diversity of chromosomal karyotypes in maize and its relatives. *Cytogenetic and Genome Research* 129:6-16.

- Bennett, M.D. 1995. The development and use of genomic *in situ* hybridization (GISH) as a new tool in plant biosystematics. In: Brandham PE, Bennett MD, eds. Kew Chromosome Conference IV. Kew, 167-183.
- Brysting, A.K., Holst-Jensen, A., and Leitch, I.J. 2000. Genomic origin and organization of the hybrid *Poa jemtlandica* (Poaceae) verified by genomic *in situ* hybridization and chloroplast DNA sequences. *Annals of Botany* 85:439-445.
- Chester, M., Leitch, A., Soltis, P.S., and Soltis, D.E. 2010. Review of the application of modern cytogenetic methods (FISH/GISH) to the study of reticulation (polyploidy/hybridization). *Genes* 1:166-192.
- Doebley, J.F. 1990. Molecular systematics of *Zea* (Gramineae). *Maydica* 35:143-150.
- Gaut, B.S., and Doebley, J.F. 1997. DNA sequence evidence for the segmental allotetraploid origin of maize. *Proceedings of the National Academy of Sciences of the United States of America* 94:6809-6814.
- 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*. PhD Thesis, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.
- 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 (GISH-FISH). *Chromosome Research* 14:629-635.
- González, G.E., Confalonieri, V., Comas, C., Naranjo, C.A., and Poggio, L. 2004. GISH Genomic *in situ* hybridization reveals cryptic genetic differences between maize and its putative wild progenitor *Zea mays* subsp. *parviglumis*. *Genome* 47:947-953.

- González, G.E., Fourastié, M.F., and Poggio, L. 2013. Número y composición de secuencias de los *knobs* (DAPI-FISH) y su utilidad en la caracterización de accesiones de maíz y teocintle. *Revista Fitotecnia Mexicana* 36:127-135.
- González, G.E., and Poggio, L. 2011. Karyotype of *Zea luxurians* and *Z. mays* subsp. *mays* using FISH/DAPI and analysis of meiotic behavior of hybrids. *Genome* 54:26-32.
- Hayasaki, M., Morikawa, T., and Tarumoto, I. 2000. Intergenomic translocations of polyploid oats (genus *Avena*) revealed by genomic *in situ* hybridization. *Genes and Genetic Systems* 75:167–171.
- Heslop-Harrison, J.S., and Schwarzacher, T. 2011. Organization of the plant genome in chromosomes. *The Plant Journal* 66:18-33.
- Jones, R.N., and Langdom, T. 2013. The plant nucleus at war and peace: genome organization in the interphase nucleus. In: Leitch IJ, Greilhuber J, Dolezel J, Wendel JF, eds. *Plant Genome Diversity*, Vol. 2. Springer, 13-31.
- Leitch, I.J., and Bennett, M.D. 2004. Genome downsizing in polyploid plants. *Biological Journal of the Linnean Society* 82:651–663.
- Leitch, I.J., and Leitch, A.R. 2013. Genome size diversity and evolution in land plants. In: Leitch IJ, Greilhuber J, Dolezel J, Wendel JF, eds. *Plant Genome Diversity*, Vol. 2. Springer, 307-322.
- Ma, X.F., and Gustafson, J.P. 2006. Timing and rate of genome variation in *Triticale* following allopolyploidization. *Genome* 49:950–958.
- Moore, G., Devos, K.M., Wang, Z., and Gale, M.D. 1995. Cereal genome evolution. Grasses, line up and form a circle. *Current Biology* 5:737-739.

- 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. Academia Nacional de Ciencias Exactas, Físicas y Naturales de Buenos Aires 5:43-53.
- Naranjo, C.A., Poggio, L., Molina, M.C., and Bernatené, E. 1994. Increase in multivalent frequency in F1 hybrids of *Zea diploperennis* x *Zea perennis* by colchicine treatment. Hereditas 120:241-244.
- Poggio, L., Confalonieri, V., Comas, C., Cuadrado, A., Jouvé, N., and Naranjo, C.A. 1999a. Genomic *in situ* hybridization (GISH) of *Tripsacum dactyloides* and *Zea mays* ssp. *mays* with B-chromosomes. Genome 42:687-691.
- Poggio, L., Confalonieri, V., Comas, C., González, G.E., and Naranjo, C.A. 1999b. Genomic affinities among *Zea luxurians*, *Zea perennis* and *Zea diploperennis*: meiotic behaviour in the F1 and genomic *in situ* hybridization (GISH). Genome 42:993-1000.
- Poggio, L., Confalonieri, V., Comas, C., González, G.E., and Naranjo, C.A. 2000a. Evolutionary relationships in the genus *Zea*: analysis of repetitive sequences used as cytological FISH marker. Genetic and Molecular Biology 23:1021-1027.
- Poggio, L., Confalonieri, V., Comas, C., González, G.E., and Naranjo, C.A. 2000b. Aportes de la citogenética molecular al análisis de divergencias genómicas crípticas en el género *Zea* (Poaceae). Boletín de la Sociedad Argentina de Botánica 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 cytogenetics analysis. Cytogenetic and Genome Research 109:259-267.
- Poggio, L., Molina, M.C., and Naranjo, C.A. 1990. Cytogenetic studies in the genus *Zea*. 2. Colchicine induced multivalents. Theoretical and Applied Genetics 79:461-464.

- Poggio, L., Realini, M.F., Fourastié, M.F., García, A.M., and González, G.E. 2014. Genome downsizing and karyotype constancy in diploid and polyploid congeners: a model of genome size variation. *AoB PLANTS* 6: plu029; doi:10.1093/aobpla/plu029.
- Schnable, J.C., Springer, N.M., and Freeling, M. 2011. Differentiation of the maize subgenomes by genome dominance and both ancient and ongoing gene loss. *Proceedings of the National Academy of Sciences of the United States of America* 108:4069-74.
- Soltis, D.E., and Soltis, P.S. 1999. Polyploidy: recurrent formation and genome evolution. *TREE* 14:48-352.
- Soltis, D.E., Visger, C.J., and Soltis, P.S. 2014a. The polyploidy revolution then...and now: Stebbins revisited. *American Journal of Botany* 101:1057–1078.
- Soltis, P.S., Liu, X., Marchant, D.B., Visger, C.J., and Soltis, D.E. 2014b. Polyploidy and novelty: Gottlieb's legacy. *Philosophical Transactions of the Royal Society of London B* 369: 20130351.
- Swigonova, Z., Lai, J., Ma, J., Ramakrishna, W., Llaca, V., Bennetzen, J.L., Messing, J. 2004. On the tetraploid origin of the maize genome. *Comparative and Functional Genomics* 5:281–284.
- Sybenga, J. 1975. *Meiotic configurations*. Berlin, Heidelberg, New York: Springer-Verlag.
- Takahashi, C., Marshall, J.A., Bennett, M.D., and Leitch, I.J. 1999. Genomic relationships in maize and its wild relatives. *Genome* 42:1201-1207.
- Tito, C., Poggio, L., and Naranjo, C.A. 1991. Cytogenetic studies in the genus *Zea*: DNA content and heterochromatin in species and hybrids. *Theoretical and Applied Genetics* 83:58-64.

Wei, F., Coe, E., Nelson, W., *et al.* 2007. Physical and genetic structure of the maize genome reflects its complex evolutionary history. PLOS Genetics doi: 10.1371/journal.pgen.0030123.

White, S., and Doebley, J. 1998. Of genes and genomes and the origin of maize. Trends in Genetics 14:327-332.

Draft

Fig. 1: GISH on mitotic metaphase chromosomes of *Zea mays* ssp. *mays* probed with:

A: *Z. m. ssp. mexicana* DNA (detected with red-Cy3). Inner square: detail of one chromosome pair (from another metaphase plate) lacking hybridization signal on the pericentromeric region. **C:** *Z. m. ssp. parviglumis* DNA (detected with red-Cy3). Inner square: detail of chromosome pair six (from another metaphase plate) without hybridization signal on the short arm. **E:** *Z. m. ssp. huehuetenanguensis* DNA (detected with yellow-green FITC). **G:** *Z. luxurians* DNA (detected with yellow-green FITC). **I:** *Z. diploperennis* DNA (detected with yellow-green FITC). **K:** *Z. perennis* DNA (detected with yellow-green FITC). **B, D, F, H, J and L:** DAPI counterstaining. White arrows indicate several knob regions, yellow arrows point the short arm of chromosome 6, arrowhead point chromosomes with hybridization signal on only one arm, and green arrows point to lack of hybridization signals on pericentromeric regions (A-F) or onto entire chromosomes (I-L). B: B chromosomes. Bar: 10µm.

Unable to Convert Image

The dimensions of this image (in pixels) are too large to be converted. For this image to convert, the total number of pixels (height x width) must be less than 40,000,000 (40 megapixels).

Draft