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CAUSES AND CONSEQUENCES OF DNA CONTENT VARIATION IN *ZEA*

CAUSAS Y CONSECUENCIAS DE LA VARIACIÓN DEL CONTENIDO DE ADN EN *ZEA*

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ABSTRACT

Cytogenetic evidence indicates that *Zea*, which comprises maize (*Z. mays* ssp. *mays*) and its wild relatives, is an allopolyploid genus. Our research group has carried out numerous cytogenetic studies on *Zea* species, mainly focused on native Argentinian and Bolivian maize landraces. We found a wide inter- and intraspecific genome size variation in the genus, with mean 2C-values ranging between 4.20 and 11.36 pg. For the maize landraces studied here, it varied between 4.20 and 6.75 pg. The objectives of this work are to analyze the causes of genome size variation and to discuss their adaptive value in *Zea*. This variation is mainly attributed to differences in the heterochromatin located in the knobs and to the amount of interspersed DNA from retrotransposons. Polymorphisms in presence or absence of B-chromosomes (Bs) and the population frequency of Bs are also a source of genome size variation, with doses ranging between one and eight in the landraces analyzed here. Correlation analysis revealed that the percentage of heterochromatin is positively correlated with genome size. In addition, populations cultivated at higher altitudes, which are known to be precocious, have smaller genome sizes than do those growing at lower altitudes. This information, together with the positive correlation observed between the length of the vegetative cycle and the percentage of heterochromatin, led us to propose that it has an adaptive role. On the other hand, the negative relationship found between Bs and heterochromatic knobs allowed us to propose the existence of an intragenomic conflict between these elements. We hypothesize that an optimal nucleotype may have resulted from such intranuclear conflict, where genome adjustments led to a suitable length of the vegetative cycle for maize landraces growing across altitudinal clines.

Key words: B chromosomes, heterochromatin, intragenomic conflict, knobs, maize landraces

RESUMEN

La evidencia citogenética indica que el género *Zea*, el maíz (*Z. mays* ssp. *mays*) y sus parientes silvestres, posee un origen alopoliploide. Nuestro grupo de investigación ha realizado numerosos estudios en especies de *Zea*, principalmente en maíces nativos de Argentina y Bolivia. En este género, hallamos una amplia variación inter e intraespecífica en el tamaño del genoma, con valores 2C medios que oscilan entre 4,20 y 11,36 pg. El valor 2C medio de los maíces nativos estudiados varió entre 4,20 y 6,75 pg. Los objetivos de este trabajo son analizar las causas de la variación del tamaño del genoma en *Zea* y discutir su valor adaptativo. Esta variación se atribuye principalmente a las diferencias en la heterocromatina de los *knobs* y en la cantidad de ADN intercalado de los retrotransposones. Otras fuentes de variación son los polimorfismos para presencia/ausencia de cromosomas B (Bs) y para la frecuencia poblacional de Bs en las razas analizadas, con dosis que oscilan entre uno y ocho Bs. El porcentaje de heterocromatina se correlaciona positivamente con el tamaño del genoma. Las poblaciones cultivadas en altitudes altas, que son precoces, tienen tamaños de genoma más pequeños que las que crecen en bajas altitudes. Esta información, junto con la correlación positiva observada entre la duración del ciclo vegetativo y el porcentaje de heterocromatina, nos llevó a proponer el rol adaptativo de la heterocromatina. Por otro lado, la relación negativa encontrada entre Bs y *knobs* heterocromáticos nos permitió proponer la existencia de un conflicto intragenómico entre estos elementos. Hipotetizamos que de este conflicto intranuclear habría resultado el nucleotipo óptimo, donde ajustes genómicos condujeron a una duración adecuada del ciclo vegetativo en las razas de maíz que crecen a lo largo de clines altitudinales.

Palabras clave: conflicto intragenómico, cromosomas B, heterocromatina, *knobs*, maíz nativo

INTRODUCTION

The genus *Zea* (Poaceae - Maydeae) includes the perennial species *Z. perennis* (Hitch.) (Reeves & Mangelsdorf) and *Z. diploperennis* Iltis (Doebley & Guzman), and the annual species *Z. luxurians* (Durieu & Ascherson) (Bird), *Z. nicaragüensis* (Iltis & Benz) and *Z. mays* L. The latter has four subspecies: *Z. mays* ssp. *mays* (the maize), *Z. mays* ssp. *parviglumis*, *Z. mays* ssp. *huehuetenanguensis* and *Z. mays* ssp. *mexicana* (Doebley, 1990; Iltis and Benz, 2000). All taxa have 2n=20, except for *Z. perennis* with 2n=40.

Cytogenetic evidence indicates that maize and its wild relatives (the teosintes) are cryptic polyploids. In the pioneering studies of Anderson (1945) maize was considered as allotetraploid 2n=4x=20, derived from extinct ancestors with 2n=10. Meiotic studies of intra- and interspecific hybrids confirmed their allopolyploid nature, indicating that maize and its wild relatives are tetraploids, except for the octoploid *Z. perennis*, with x=5 being the basic chromosome number (revisited in Poggio *et al.*, 2005; Poggio and González, 2018). Further molecular studies provided compelling evidence for the allopolyploid nature of maize (Moore *et al.*, 1995; Gaut and Doebley, 1997; White and Doebley, 1998; Swigonova *et al.*, 2004).

Our research group has carried out numerous cytogenetic studies on *Zea* species, mainly focused on native Argentinian and Bolivian maize landraces (Tito *et al.*, 1991; Quintela Fernández *et al.*, 1995; Poggio *et al.*, 1998, 1999a, 1999b, 2000a, 2000b, 2005; Rosato *et al.*, 1998; González *et al.*, 2004, 2006, 2013; González and Poggio, 2011, 2015, 2021; Realini *et al.*, 2016, 2018, 2021; Fourastié *et al.*, 2017; Poggio and González, 2018).

Maize is one of the most important cereal crops worldwide, growing in a broad range of agro-ecological regions. In South America, many landraces are adapted to a great variety of climatic conditions at different growing altitudes (Roberts *et al.*, 1957; Wellhausen *et al.*, 1957; Timothy *et al.*, 1961; Rodríguez *et al.*, 1968; Salhuana and Machado, 1999; Sánchez *et al.*, 2007; Cámara Hernández *et al.*, 2011; Orozco-Ramírez *et al.*, 2016). Cámara Hernández *et al.* (2011) described more than 50 morphological native landraces from Northern Argentina, which are cultivated using ancestral farming practices. Of these, 36 landraces grow from lowlands to highlands in Northwestern Argentina (NWA), while 15 are cultivated at lower altitudes in Northeast Argentina (NEA). They are potential sources of genetic variation, constituting useful reservoirs of tolerance and resistance alleles for biotic and abiotic stresses.

The objectives of this work are to analyze genome size variation in the genus *Zea* based on studies carried out by our research group, to discuss the causes of such variation and to explore the relationship between genome size and cytological, phenological and environmental characteristics.

INTER- AND INTRASPECIFIC VARIATION IN THE GENOME SIZE OF *ZEA*

Genome size plays an important role in the cytogenetic variation of a taxon or group of taxa. This character is measured by microdensitometry with Feulgen's stain or, most often, using flow cytometry (Tito *et al.*, 1991; Dolezel *et al.*, 2007). DNA content is usually expressed in picograms (pg) as the 2C-value, which refers to the amount of genomic DNA in unreplicated somatic cells (Poggio *et al.*, 2010).

In genus *Zea*, the interspecific variation in the mean DNA content has been reported to range between 4.2 and 11.36 pg (Tables 1 and 2) (Laurie and Bennett, 1985; Tito *et al.*, 1991; Fourastié *et al.*, 2017). In maize, intraspecific variability in the DNA content has been recorded in native landraces from the following sites: a) NEA, cultivated in lowlands from 98 to 591 m.a.s.l.; b) NWA, growing along a broad altitudinal cline from 80 to 3900 m.a.s.l.; and c) Bolivia, cultivated from 200 to 3250 m.a.s.l. (Table 2) (Quintela Fernández *et al.*, 1995; Rosato *et al.*, 1998; Realini *et al.*, 2016; Fourastié *et al.*, 2017; González and Poggio, 2021). Table 2 shows the range of mean 2C-values (4.20-6.75 pg) for these landraces. A similar genome size variation has been obtained for other American maize populations growing along altitudinal clines (Laurie and Bennett, 1985; Rayburn and Auger, 1990; Díez *et al.*, 2013; Bilinsky *et al.*, 2018).

Table 1. DNA content in *Zea* species. Data from Tito *et al.* (1991).

Species (somatic chromosome number)	Mean 2C-value (pg) (±SD)
Zea luxurians (2n=20)	8.83 (± 0.08)
Zea diploperennis (2n=20)	6.36 (± 0.06)
Zea perennis (2n=40)	11.36 (± 0.11)
Zea mays ssp. mays (2n=20) C-tester line	5.86 (± 0.05)
Zea mays ssp. mexicana (2n=20)	6.79 (± 0.05)

Table 2. Genome size and B-chromosome variations in maize landraces.

Source of maize landraces	Altitude m.a.s.l. (range)	2C-Value* (range)	Dose of Bs (range)	Frequency of Bs (range)
NWA	80-3900	4.20-6.75 pg	1 - 8	0-100%
Bolivia	200-3250	5.26-6.23 pg	1 - 5	16.6-81.8%
NEA	98-591	4.62-6.29 pg	-------	-------

Ref.: NWA: Northwestern Argentina, NEA: Northeastern Argentina, m.a.s.l.: meters above sea level. *The 2C-value was measured in plants without B chromosomes (A-DNA). Data from: Rosato *et al.*, 1998; Realini *et al.*, 2016; Fourastié *et al.*, 2017; González *et al.*, 2021.

CAUSES OF GENOME SIZE VARIATION

In *Zea*, genome size variation has been mainly attributed to differences in the heterochromatin located in knobs and to interspersed DNA amount (e.g. retrotransposon families), making up over 70% of the nuclear genome (SanMiguel *et al.*, 1998; Meyers *et al.*, 2001; Realini *et al.*, 2016; Fourastié *et al.*, 2017). The presence of accessory chromosomes (B chromosomes; Bs) also contribute to DNA content variation, as discussed later (Rosato *et al.*, 1998; Fourastié *et al.*, 2017; González and Poggio, 2021).

Chromosomes of *Zea* species have blocks of heterochromatin called knobs (Kato, 1976; McClintock, 1978). This heterochromatin is highly condensed and composed of highly repeated DNA sequences (Peacock *et al.*, 1981). Knobs can be visualized in dividing cells as well as in interphase nuclei as chromocenters (Wan and Widholm, 1992). During pachytene of *Zea* species, knobs can be found at 34 different chromosomal positions in the karyotype (Kato, 1976). They are detected using C and DAPI chromosome banding (González *et al.*, 2013). At the molecular level, knobs are composed of two sequence families of 180 base pairs (180-bp) and 350 base pairs (TR-1), and may contain several retrotransposons (Dennis and Peacock, 1984; Ananiev *et al.*, 1998). The sequence families, which are represented by thousands to millions of tandemly arranged copies, conform the different knob types (knobs exclusively containing 180-bp repeats, knobs exclusively containing TR-1 repeats and knobs with different proportion of both sequences) (Ananiev *et al.*, 1998).

As mentioned above, DAPI banding identifies knobs as DAPI-positive bands that are A-T rich (Figure 1 A, B, C, F). However, this technique does not provide information on knob sequences. The fluorescent *in situ* hybridization (FISH) allows the detection and localization of specific sequences on interphase nuclei and metaphase chromosomes (Poggio *et al.*, 1999b; 2005; González *et al.*, 2006; González and Poggio, 2011; 2015). The hybridization of the 180-bp and TR-1 knob sequences on mitotic metaphases of *Zea* is used to determine the sequence composition of each DAPI-positive band (*i.e.* each knob) (Figure 1 D, E) (Albert *et al.*, 2010; González *et al.*, 2013).

Different maize landraces and teosintes show a wide variation in the size, number, chromosome position and sequence composition of heterochromatic knobs (Kato, 1976; McClintock *et al.*, 1981; González *et al.*, 2013), thus serving as valuable cytological markers. For example, they have been used for the cytogenetic characterization of landraces from Northern Argentina (Realini *et al.*, 2016; Fourastié, 2017).

In *Zea*, DNA content is positively correlated with the number and size of knobs, as well as with the percentage of heterochromatin in the karyotype (Laurie and Bennett, 1985; Tito *et al.*, 1991; Poggio *et al.*, 1998; Realini *et al.*, 2016; Fourastié *et al.*, 2017; González and Poggio, 2021). The percentage of heterochromatin in a *Zea* karyotype is calculated by summing all chromosomal portions occupied by the DAPI-positive bands detected in the chromosomal complement. *Z. luxurians* has the highest DNA content of the 2n=20 species within the genus (Table 1), possibly due to the larger number and size of knobs, which are at terminal position on almost all chromosomes (Figure 1 C, D) (González and Poggio, 2011; González *et al.*, 2013). On the contrary, DAPI-banding and FISH experiments did not detect conspicuous knobs in the octoploid *Z. perennis*, showing the lowest DNA content per basic genome (Cx) among *Zea* species (Kato and López, 1989; Tito *et al.*, 1991; González *et al.*, 2013) (Table 1; Figure 1 F). The small quantity of knob sequences in *Z. perennis* was postulated to be a consequence of the genome downsizing occurring during the process of secondary polyploidization in this species (Poggio *et al.*, 2005; González and Poggio, 2015).

In maize landraces, the percentage of heterochromatin

is positively correlated with the genome size (González and Poggio, 2011; Realini *et al.*, 2016; Fourastié *et al.*, 2017), suggesting that the variation in DNA content is mainly due to differences in the size, and to a lesser extent, in the number of knobs. However, some authors provided evidence for the presence of other sources than heterochromatin knobs contributing to genome size (revisited in Realini *et al.*, 2016; 2018). Transposable elements (TEs) play a role in the dynamics of the nuclear genome, either through polymorphic insertions and deletions or by mediating ectopic recombination events leading to structural variation in the genome (SanMiguel and Bennetzen, 1998; Meyers *et al.*, 2001). Recently, Coutinho Silva *et al.* (2020) demonstrated that in maize a higher 2C-value is associated with a more abundant distribution of LTR-retrotransposons in the karyotype, mainly from the *Grande* family. Further studies will enhance the knowledge on the differential composition of retrotransposon families in native maize landraces from Argentina, and its influence on genome size variation.

NEGATIVE CORRELATION BETWEEN DNA CONTENT AND ALTITUDE OF CULTIVATION IN MAIZE LANDRACES

In NWA, Rosato *et al.* (1998) reported a significant negative correlation between DNA content and altitude of cultivation in landraces growing along an altitudinal cline. This was further supported by Fourastié *et al.* (2017) for other NWA populations. Recently, González and Poggio (2021) observed that the Bolivian landraces cultivated at higher altitudes have lower DNA content than those growing at lower altitudes (Figure 2). Negative correlations between genome size and altitude of cultivation were also detected in different altitudinal clines from the American continent (Rayburn and Auger, 1990; Díez *et al.*, 2013; Bilinsky *et al.*, 2018).

HETEROCHROMATIN HAS ADAPTIVE SIGNIFICANCE ALONG AN ALTITUDINAL CLINE

Realini *et al.* (2016) observed a positive correlation between the length of the vegetative cycle and the percentage of heterochromatin in maize landraces from lowlands in NEA. Knob heterochromatin is the latest component to finish DNA replication because increased DNA packaging extends DNA synthesis, leading to a longer cell cycle, which may affect the rate of cell division and plant development (Pryor *et al.*, 1980; Buckler *et al.*, 1999; Greilhuber and Leitch, 2013). On this basis, length of the vegetative cycle was proposed to be optimized through artificial selection for an appropriate

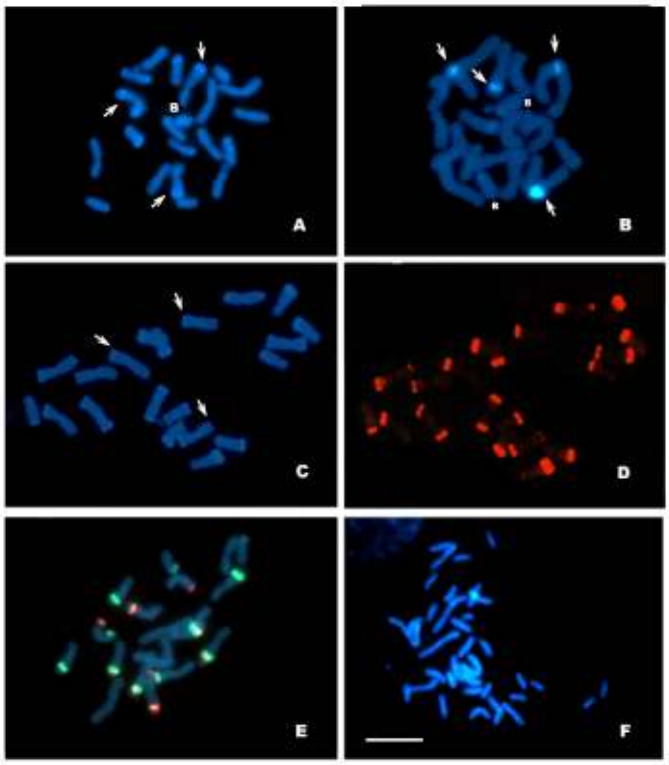

Figure 1. Mitotic metaphases of *Zea*. **A:** DAPI banding on NWA maize Blanco y Ocho Rayas landrace, with 1 B chromosome. **B:** DAPI banding on NWA maize Amarillo Chico landrace, with 2 B chromosomes. **C:** DAPI staining on *Z. luxurians*. **D:** Fluorescent *in situ* hybridization (FISH) on mitotic metaphase of *Z. luxurians* hybridized with 180bp knob sequence detected with Cy3 (red). **E:** FISH on mitotic metaphase of NEA maize Pipoca Colorado landrace simultaneously hybridized with 180bp knob sequence detected with antidigoxigenin-FITC (green) and with TR-1 knob sequence detected with Cy3 (red). **F:** DAPI banding of *Z. perennis*. Ref.: white arrows show some DAPI-positive bands (knobs). Bar=10 µm.

percentage of heterochromatin (Realini *et al.*, 2016; 2021; Bilinsky *et al.*, 2018).

As mentioned above, the percentage of heterochromatin is positively correlated with genome size. In addition, DNA content is higher in inbred lines with long vegetative cycles than in precocious ones, and their F1 hybrids have an intermediate genome size (González and Poggio, in prep.). Jian *et al.* (2017) also observed a high correlation between genome size and flowering time in inbred lines growing under tropical conditions. These results may explain the negative correlation detected between genome size and percentage of heterochromatin with cultivation altitude (Figure 2) (Tito *et al.*, 1991; Poggio *et al.*, 1998; Fourastié *et al.*, 2017; González and Poggio, 2021). Thus, the fact that maize landraces at high altitudes are precocious and show a reduction in heterochromatin percentage most likely represents an adaptation to a shorter growing season typical of highlands, with natural selection acting on the flowering time across altitudinal clines (Bilinsky *et al.*, 2018). This reinforces the hypothesis that the percentage of heterochromatin has adaptive value along altitudinal clines.

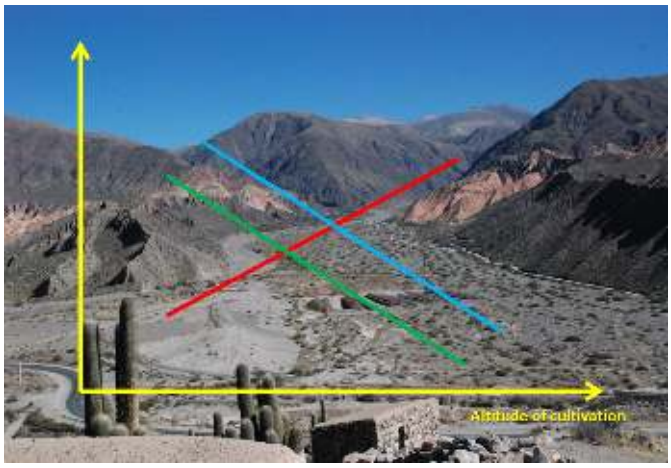

Figure 2. Relationships of DNA content (blue), percentage of heterochromatin (green) and dose and frequency of B chromosomes (red) with cultivation altitude in maize landraces. This graph only represents the trends for the relationships. Photograph taken by Julián Cámara Hernández.

B-CHROMOSOME POLYMORPHISMS AS SOURCES OF GENOME SIZE VARIATION

In *Zea*, B chromosomes (Bs) are also regarded as a source of genome size variation (McClinctock *et al.*, 1981; Kato and López, 1990; Poggio *et al.*, 1998; Rosato *et al.*, 1998; Cheng and Lin, 2003; Lamb *et al.*, 2007; Rosado *et al.*, 2009). Supernumerary Bs are dispensable chromosomes lacking homology with any member of the normal complement, the A-chromosome (A) set. These accessory chromosomes represent a specific type of selfish genetic element with mechanisms of drive which allow them to increase their transmission rates through different processes of non-mendelian inheritance (Jones and Houben, 2003; Houben *et al.*, 2014; Blavet *et al.*, 2021). Although Bs follow their own species-specific evolutionary pathways, it is widely accepted that they are derived from their respective A complement (revisited in Houben *et al.*, 2014).

Polymorphisms for presence/absence and different doses of Bs have been reported in annual *Zea* species (Longley, 1937; Ting, 1976; Kato, personal communication). Polymorphisms are well documented in maize, particularly for landraces from NWA and Bolivia (McClinctock *et al.*, 1981; Quintela Fernández *et al.*, 1995; Rosato *et al.*, 1998; Fourastié *et al.*, 2017; González and Poggio, 2021). In these landraces, a wide variation of population frequency of Bs was observed. The doses range between one and eight Bs per plant, with two Bs per plant being the most frequently reported dose (Table 2). Fourastié *et al.* (2017) proposed that the frequency of Bs depends not only on the cultivation altitude, but also on the genotypical and nucleotypical backgrounds of the landraces.

In NWA and Bolivian landraces, which grow along a wide altitudinal cline, the mean number and frequency of Bs are significantly and negatively correlated with the 2C-value of the A-chromosome complement (A-DNA) and positively correlated with altitude of cultivation (Figure 2) (Quintela Fernández *et al.*, 1995; Poggio *et al.*, 1998; Rosato *et al.*, 1998; Fourastié *et al.*, 2017; González and Poggio, 2021). These authors also found a significant negative correlation between the mean number of Bs and the percentage of knob heterochromatin. Moreover, they hypothesized that Bs are maintained at higher frequencies in populations with low percentage of heterochromatin to preserve an optimal nucleotype (*sensu* Bennett, 1972). Such term defines the conditions of the nucleus affecting cell and developmental parameters such as cell volume, nuclear volume, chromosome size, mitotic cycle time, duration of meiosis and minimum generation time (Bennett, 1987; Poggio *et al.*, 1998). The negative association observed between the frequency of Bs and the percentage of heterochromatin suggests that there is a maximum limit on the mass of nuclear DNA that allows the optimum nucleotype (Rosato *et al.*, 1998; Fourastié *et al.*, 2017). Based on the analysis of many landraces from NWA and Bolivia, González and Poggio (2021) proposed that the optimal nucleotype is the result of an intragenomic conflict between Bs and heterochromatin knobs, where genome adjustment may lead to an appropriate length of the vegetative cycle for maize landraces growing across altitudinal clines.

A better understanding of the causes accounting for genome size variation and their adaptive significance in maize landraces is essential for the development of successful breeding and conservation programs.

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