

GENOME SIZE IN THREE SPECIES OF *Glandularia* AND THEIR HYBRIDS TAMAÑO DEL GENOMA EN TRES ESPECIES DE *Glandularia* Y SUS HÍBRIDOSFerrari M.R.¹, Greizerstein E.J.^{2,3}, Poggio L.^{4*}

ABSTRACT

In this work the relationship between genome size of *Glandularia* species and the meiotic configurations found in their hybrids are discussed. *Glandularia incisa* (Hook.) Tronc., growing in two localities of Corrientes and Córdoba provinces, Argentina, with different ecological conditions, showed inter-population variability of the 2C-value. The DNA content found in the Corrientes locality (2.41 pg) was higher than that obtained in the Córdoba locality (2.09 pg) which has more stressful environmental conditions than the former. These values are statistically different from those that were found in *Glandularia pulchella* (Sweet) Tronc. from Corrientes (1.43 pg) and in *Glandularia perakii* Cov. et Schn from Córdoba (1.47 pg). The DNA content of the diploid F₁ hybrids, *G. pulchella* × *G. incisa* and *G. perakii* × *G. incisa*, differed statistically from the DNA content of the parental species, being intermediate between them. Differences in the frequency of pairing of homoeologous chromosomes were observed in the hybrids; these differences cannot be explained by differences in genome size since hybrids with similar DNA content differ significantly in their meiotic behavior. On the other hand, the differences in the DNA content between the parental species justify the presence of a high frequency of heteromorphic open and closed bivalents and univalents with different size in the hybrids.

Key words: Intra-specific DNA content variability, homoeologous pairing, heteromorphic bivalents.

RESUMEN

En el presente trabajo se discute la relación entre el tamaño del genoma en especies de *Glandularia* y las configuraciones meióticas encontradas en sus híbridos. El valor 2C mostró variabilidad interpoblacional en muestras de *Glandularia incisa* (Hook.) Tronc. coleccionadas en dos localidades con diferentes condiciones ecológicas (provincias de Corrientes y Córdoba, Argentina). El contenido de ADN encontrado en Corrientes (2,41 pg) fue mayor que el obtenido en Córdoba (2,09 pg) donde se registran condiciones ambientales más estresantes. Estos valores son estadísticamente diferentes de los determinados en *Glandularia pulchella* (Sweet) Tronc. de Corrientes (1.43 pg) y en *Glandularia perakii* Cov. et Schn de Córdoba (1.47 pg). El contenido de ADN de los híbridos diploides F₁, *G. pulchella* × *G. incisa* y *G. perakii* × *G. incisa*, difirió estadísticamente del contenido de ADN registrado en las especies parentales siendo intermedio entre ellas. Las diferencias observadas en la frecuencia de apareamiento de cromosomas homeólogos no pueden explicarse por diferencias en el tamaño del genoma, ya que híbridos con un contenido de ADN similar difieren significativamente en su comportamiento meiótico. Sin embargo, la diferencia en el contenido de ADN entre las especies parentales explica la presencia de una alta frecuencia de bivalentes heteromórficos tanto abiertos como cerrados y univalentes con diferentes tamaños.

Palabras clave: Variabilidad intra-específica del contenido de ADN, apareamiento homoeólogo, bivalentes heteromórficos.

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INTRODUCTION

Genome size varies among species and its diversification accompanies the evolution of many groups of plants (Bennett and Leitch, 2005; Leitch *et al.*, 2005; Gregory *et al.*, 2007; Leitch and Leitch, 2013; Poggio *et al.*, 2014). Significant variation in DNA content was found among species of the same genera, among populations of one species or even among individuals belonging to the same population or cultivar (Cavallini and Natalli, 1991; Grehilhuber and Leitch, 2013; Realini *et al.*, 2016).

Variation in genome size arises by increase and/or decrease of DNA content. The increase arises predominantly through polyploidy and amplification of non-coding repetitive DNA heterochromatin and retrotransposons. Moreover, recombination-based processes are mechanisms involved in decrease in genome size or genome downsizing (Soltis *et al.*, 2003; Bennetzen *et al.*, 2005; Grover and Wendel, 2010; Hidalgo *et al.*, 2017). Besides, numerical polymorphism of B-chromosomes can modify the size of the genome (Kalendar *et al.*, 2000; Gregory, 2004; Bennet and Leitch, 2005; Grehilhuber and Leitch, 2013; Fourastie *et al.*, 2018).

Several studies reported some relationships between the DNA content and phenotypic characteristics such as cell size, duration of the cell cycle, growth rate, leaf expansion, flowering time, weediness, invasiveness, seed weight, and minimum generation time (Grime and Mowforth, 1982; Bennett, 1987; Ohri and Pistrick, 2001; Beaulieu *et al.*, 2007; Greihuber and Leitch, 2013; Leitch and Leitch, 2013; Fourastie *et al.*, 2018).

Genome size was also associated with ecological parameters (temperature, precipitation and length of the growing season) and geographical parameters (altitude and latitude) (Greihuber and Leitch, 2013; Fourastie *et al.*, 2018). These correlations suggest a biological role for genome size or “nucleotype”, term coined to describe the condition of the nucleus that affects the phenotype independently of the informational content of the DNA (Bennett, 1971; 1972).

Glandularia J.F. Gmel is a genus of the Verbenaceae family composed of ca. 100 species with a North-South American disjoint distribution (O’Leary and Peralta, 2007; Peralta and Múlgura, 2011). Many of these species, their hybrids and polyploids have great ornamental potential due to their colourful flowers, long flowering period and low water requirements (Imhof *et al.*, 2013; González Roca *et al.*, 2015). The chromosome numbers of numerous species have been studied, and it was found that the South American species are mostly diploid ($2n=2x=10$) whereas the North American species are hexaploid ($2n=6x=30$) or tetraploid ($2n=4x=20$) (Schnack and Covas, 1945; Solbrig *et al.*, 1968; UMBER, 1979; Poggio *et al.*, 1993; 2016; Turner and Powell, 2005).

Schnack and Solbrig (1953) and Solbrig *et al.* (1968) carried out an extensive hybridization program between

South American species of the genus *Glandularia* and recently, many artificial hybrids were obtained with ornamental purposes (Imhof, 2014).

Poggio *et al.* (2016) analysed *G. pulchella* × *G. incisa* F₁ natural hybrids observing variability in the frequency of bivalents and univalents. They also reported the presence of heteromorphic bivalents and differences in the size of univalents.

In the present work, DNA content and its variations are reported for the first time in *G. pulchella*, *G. incisa*, *G. perakii* and their F₁ hybrids, *G. pulchella* × *G. incisa* and *G. perakii* × *G. incisa*, species and their hybrids have the same chromosome number ($2n=10$) (Poggio *et al.* 2011; Poggio *et al.*, 2016). Moreover, the effect of DNA content of the parental species on meiotic pairing of homoeologous chromosome in the natural hybrids is discussed. These studies could shed light on the biological importance of variation in genome size and processes of hybrid speciation.

MATERIALS AND METHODS

Glandularia incisa, *G. pulchella* and their natural hybrids (HA1, HA2, HA3, and HA4): Argentina, province of Corrientes, Dpto. Capital, Aeropuerto Cambá Punta.

G. incisa, *G. perakii* and their natural hybrids (HB1): Argentina, province of Córdoba, Embalse Río Tercero.

Taxonomic identification of the species and natural hybrids was made according to morphological criteria described by Poggio *et al.* (1993).

Herbarium materials were deposited in Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina.

The distance between Corrientes Dpto. Capital and Embalse Río Tercero is 726 Km. Geographical and ecological differences between the two localities are presented in Table 1 (Cabrera, 1976).

Meiotic studies

These studies were done in immature flowers fixed in 3:1 (ethanol: acetic acid). The anthers were squashed in 2% acetic haematoxylin as stain and 1% ferric citrate as mordant (Nuñez, 1968). In the present paper at least 50 Metaphase I were studied in the parental species and in *G. perakii* × *G. incisa* natural hybrid (HB1). The meiotic determinations made in *G. pulchella* × *G. incisa* were taken from Poggio *et al.* (2016). Hybrids HA1, HA2, HA3 and HA4 in the present paper correspond, respectively to the hybrids named H5, H8, H9, H4 by Poggio *et al.* (2016).

Feulgen staining and cytophotometry

DNA content was measured in meiotic cells stained with the Feulgen Reaction. Immature flowers were fixed

in 3:1 (ethanol: acetic acid). The staining method was performed as described in Naranjo *et al.* (1998) and the measurements of DNA content were done in telophase I nuclei (2C). The amount of Feulgen staining per nucleus, expressed in arbitrary units, was measured at a wavelength of 570 nm using the scanning method in a Zeiss Universal Micro spectrophotometer (UMSP 30). The DNA content expressed in picograms was calculated using *Allium cepa* Ailsa Craig as a standard (2C-DNA=33.55 pg; Bennett and Smith, 1976).

Statistical analysis

Differences in DNA content were tested through analysis of variance (ANOVA) and multiple contrasts were performed with the LSD Fisher method (Fisher, 1932). These statistical analyses were considered significant if P values were <0.05 and were performed using the Infostat program, FCA, National University of Córdoba (Di Rienzo *et al.*, 2015).

Table 1. Ecological conditions in Corrientes Dpto. Capital, Province of Corrientes, Argentina and in Embalse Río Tercero Province of Córdoba, Argentina.

	Localities	
	Corrientes Dpto. Capital	Embalse Río Tercero
Phytogeographic Regions	Provincia Chaqueña	Provincia del Espinal
Latitude	27° 47' S	38° 18' S
Altitude	63 m.a.s.l.	387 m.a.s.l.
Average precipitation/year	1249 mm	783 mm
Average Maximal temperature (January)	33.5° C	30.4° C
Average Minimal temperature (July)	11° C	2.8° C
Soil characteristics	Clay soils with high moisture	Stony soils with low humidity

RESULTS

The 2C-DNA content of *G. pulchella* and *G. incisa* and their hybrids collected in the Province of Corrientes showed significant differences ($F_{6,76}=20.83$; $P<0.0001$).

Contrasts performed with the LSD Fisher method are presented in Table 2.

Table 2. Nuclear DNA content in *G. pulchella*, *G. incisa*, and *G. pulchella* x *G. incisa*, collected in Province of Corrientes, Argentina. Means with the same letters are not significantly different ($P<0.05$). Pu= *G. pulchella*, In= *G. incisa* and HA1 - HA4= *G. pulchella* x *G. incisa*.

Material	2C-DNA (pg) mean ± SE	Number of cells studied	Mean value (pg) mean ± SE
<i>G. pulchella</i>			
Pu1	1.45 ± 0.03 ^{AB}	25	1.43 ± 0.06
Pu2	1.52 ± 0.02 ^A	25	
Pu3	1.31 ± 0.03 ^B	17	
<i>G. incisa</i>			
In1	2.34 ± 0.12 ^E	25	2.41 ± 0.10
In3	2.29 ± 0.05 ^E	25	
In2	2.30 ± 0.06 ^E	25	
In4	2.71 ± 0.05 ^F	25	
Hybrid			
HA1	1.84 ± 0.05 ^C	36	1.87 ± 0.06
HA2	1.78 ± 0.05 ^C	25	
HA3	1.81 ± 0.13 ^C	25	
HA4	2.04 ± 0.03 ^D	33	

The 2C-DNA content of *G. perakii*, and *G. incisa*, and their hybrids collected in the Province of Córdoba showed significant differences ($F_{9,127}=44.85$; $P<0.0001$). Contrasts performed with the LSD Fisher method are presented in Table 3. Numerical polymorphism for B chromosomes was detected in *G. incisa*, *G. perakii* and hybrids collected in the Córdoba locality. DNA content was determined only in plants without B chromosomes for a better comparison with plants from other localities.

Intra-population significant differences ($P<0.001$) were detected in the three species and in their hybrids studied in the present work. Inter-population significant differences ($P<0.001$) were detected between *G. incisa* collected in the provinces of Corrientes and Córdoba. In Figure 1, a graphic representation is

presented of 2C-DNA content of the parental species and their hybrids in collections made in the provinces of Corrientes (A) and Córdoba (B). The DNA content of the hybrids, *G. pulchella* × *G. incisa* and *G. perakii* × *G. incisa* was intermediate between the parental species (Table 1, Table 2 and Figure 1). DNA content of HA1, HA2, and HA3, did not show significant differences among them, but they differed from that of HA4.

The three parental species presented five homomorphic bivalents in all Metaphase I (more than n=50 cells were studied in each species) (Figure 2 a and b). The hybrids had univalents of different size and up to five heteromorphic bivalents (Figure 2 c-f).

The mean frequency and standard deviation of bivalents in the natural hybrids *G. pulchella* × *G. incisa* was HA1: 4.98±0.16, HA2: 5.00±0.00, HA3: 2.80±1.21 and HA4: 3.50±0.72. HA1 and HA2 did not present significant differences between them (Poggio *et al.*, 1993).

Table 3. Nuclear DNA content in *G. perakii*, *G. incisa* and *G. perakii* × *G. incisa* collected in Province of Córdoba, Argentina. Means with the same letters are not significantly different ($P \leq 0.05$). Pe = *G. perakii*, In = *G. incisa* and HB1 = *G. perakii* × *G. incisa*.

Material	2C-DNA (pg) mean ± SE	Number of cells studied	Mean value (pg) mean ± SE
<i>G. perakii</i>			
(Pe1)	1.39±0.03 ^A	25	
(Pe2)	1.36±0.05 ^A	20	
(Pe3)	1.55±0.07 ^B	17	1.47±0.06
(Pe4)	1.57±0.05 ^B	16	
<i>G. incisa</i>			
In5	2.04±0.08 ^C	20	
In6	2.24±0.04 ^D	20	
In7	2.08±0.04 ^E	20	2.09±0.10
In8	2.00±0.05 ^E	20	
Hybrid			
HB1	1.76±0.04 ^F	25	1.76±0.06

DISCUSSION

Significant differences in the DNA content of *G. incisa* with *G. pulchella* and *G. perakii* were found. The mean DNA 2C-values and the variation ranges were determined in *G. incisa* 2.25 pg (2.00 pg - 2.71 pg), *G. pulchella* 1.43 pg (1.31 pg - 1.52 pg) and *G. perakii* 1.47 pg (1.36 pg - 1.57 pg). According to the Kew DNA C-values, these are the first reports for genus *Glandularia*. These values are between the minimum value (*Tectona grandis* 0.96 pg) and the maximum value (*Lantana camara* 5.50 pg) determined in five species belonging to different genera of the Verbenaceae family (RBG Kew DNA C-values, 2017).

Inter-population variability of the 2C-value was observed in *G. incisa* growing in two localities with different geographical and ecological conditions (Corrientes and Córdoba Provinces). The values of DNA content obtained in both localities differed significantly and the 2C values determined in the Corrientes population were 15% greater than that reported in the Córdoba population. The population of *G. incisa* with the smaller genome was found in drier and more stressful conditions. These results are in agreement with the reported in different studies, suggesting that variation in DNA amount has adaptive significance related to environmental, climatic and phenological parameters such as temperature, precipitation, length of growing season and type of soil (Bennett, 1987, reviewed in Grehilhuber and Leitch, 2013). Another important difference between the two populations is that *G. incisa* growing in Córdoba presented polymorphism for B chromosomes (0-6 B's) whereas B chromosomes were not detected in plants growing in Corrientes. However, this fact does not affect the comparisons performed because measurements in the Córdoba population were carried out in cells without B chromosomes.

In addition to the interpopulation variation reported in this paper, the three species showed intrapopulation variability. The origin of this variation in the nuclear DNA content would be the result of a fraction potentially unstable such as transposable elements subject to environmental and/or genetical events that induce deletion and amplification of sequences (Grover and Wendel, 2010).

It is usually expected that the DNA content of interspecific hybrids were in an intermediate range between the respective parental species, although different authors have found, in some cases, that hybrids have more or less DNA content than their parents. This would be indicating the occurrence of genetic and epigenetic changes that were reported in newly-formed hybrids in several groups of plants (Rayburn *et al.*, 1993; Grattapaglia and Bradshaw, 1994; Ma and Gustafson, 2005).

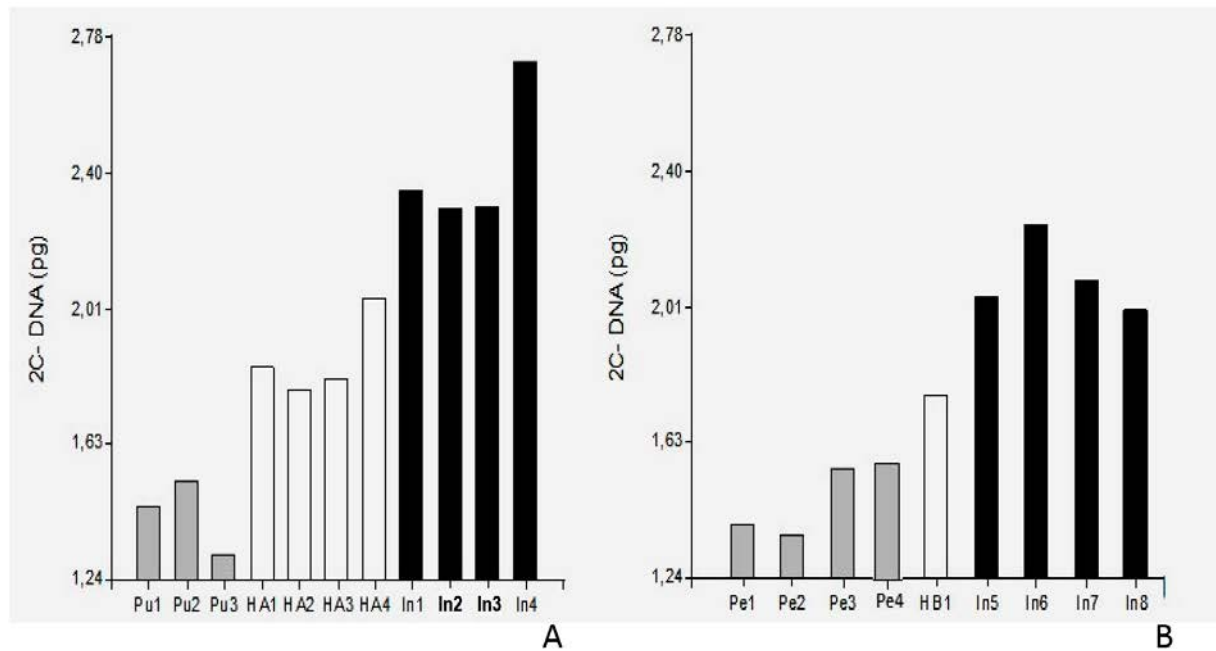


Figure 1. Graphic comparison of 2C-DNA content in *G. pulchella*, *G. perakii*, *G. incisa* and the hybrids *G. pulchella* × *G. incisa* and *G. perakii* × *G. incisa*.

A) Materials collected in the Province of Corrientes: *G. pulchella* (Pu, grey); *G. incisa* (In, black), hybrids (HA, white).

B) Materials collected in the Province of Córdoba. *G. perakii* (Pe, grey); *G. incisa* (In, black); hybrid (HB, white). Different numbers indicate different individuals in each taxa.

The natural F_1 hybrids *G. pulchella* × *G. incisa* and *G. perakii* × *G. incisa*, analyzed in the populations of Corrientes and Córdoba, have intermediate 2C-DNA values between the parental values and differed statistically from them.

Differences in DNA content are usually correlated with karyotype parameters and can affect the entire chromosome complement or they may be restricted to a subset of chromosomes. So far, the *G. incisa*, *G. pulchella* and *G. perakii* karyotypes have been analysed (data not published) and all of them show metacentric chromosomes, being larger the chromosomes of *G. incisa*. This leads us to suggest that changes in DNA content occurred in the whole-chromosome complement adding or losing equal DNA amounts to both arms or in the pericentromeric region, maintaining their metacentric morphology (in preparation).

Solbrig *et al.* (1968) made artificial crosses between species of *Glandularia* and observed homoeologous pairing in F_1 hybrids and suppression of homoeologous pairing in the allotetraploids. Poggio *et al.* (2016) reported that the F_1 hybrids *G. pulchella* × *G. incisa* had variability in homoeologous pairing forming from one to

five heteromorphyc bivalents and univalents of different size. These authors explained the homoeologous pairing in the F_1 hybrids by suggesting the presence of a pairing regulator gene/s that precluded homoeologous pairing when homologous genomes are in two doses in the polyploids, and display incomplete penetrance when homologous genomes are in one dose in the diploids.

The differences in DNA content found in the parental genomes could explain the presence of heteromorphyc bivalents in the F_1 hybrids. However, the genome size cannot explain the differences observed in homoeologous pairing since hybrids with similar DNA content differed significantly in their meiotic behavior.

The differences in the frequency of pairing of homoeologous chromosomes that were observed in the hybrids cannot be explained by the genome, size of the parental species, since hybrids with similar DNA content differed significantly in their meiotic behavior. On the other hand, the differences in the DNA content between the parental species could justify the presence of a high frequency of heteromorphyc open and closed bivalents as well as univalents with different size in the hybrids.

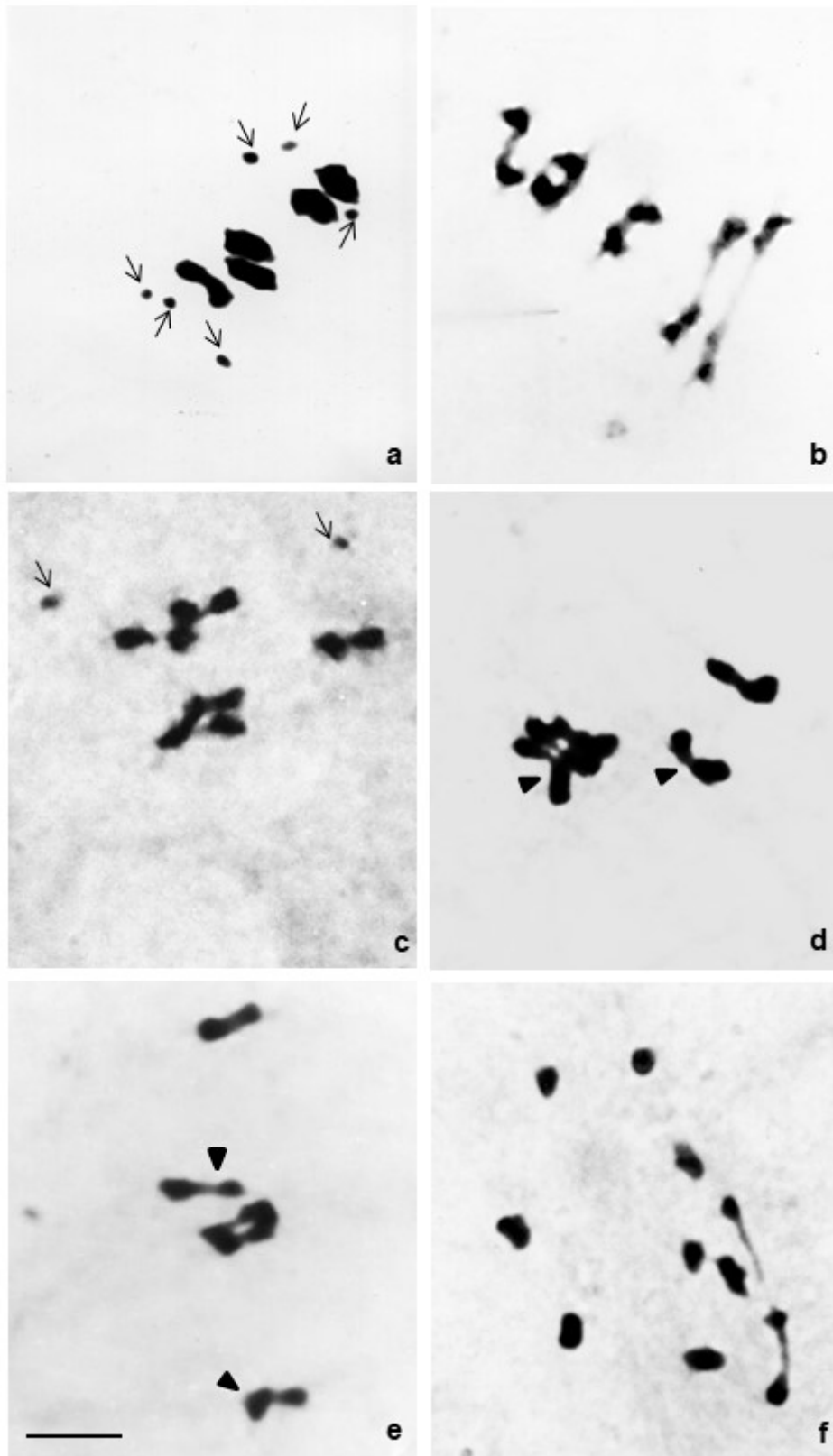


Figure 2. Metaphase I in *Glandularia* species and F₁ hybrids.

a) *G. incisa* (collected in Córdoba) with five homomorphic bivalents and six B chromosomes.

b) *G. pulchella* with five homomorphic bivalents.

c-f) hybrids with univalents and heteromorphic bivalents. c) *G. perakii* x *G. incisa* with two B

chromosomes and five heteromorphic bivalents. d) *G. perakii* x *G. incisa* with five heteromorphic

bivalents. e) *G. pulchella* x *G. incisa* with five heteromorphic bivalents. f) *G. pulchella* x *G. incisa* with one

heteromorphic bivalent and eight univalents. Arrows show B chromosomes and arrow heads indicate

some notorious heteromorphic bivalents. Bars 10 µm.

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