

Genome sizes in diploid and allopolyploid *Arachis L.* species (section *Arachis*)

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Abstract Species of section *Arachis* with $x = 10$ are important for peanut breeding and have been organized in five different genomes (A, B, D, F and K). The few available estimates of the DNA content are inconsistent and hampered the understanding of the evolutionary trends and in decision making for genomic studies of the group. Considering that, the objectives of this research were to measure the DNA content for all available (26) species and to make evolutionary inferences at the diploid and tetraploid level for section *Arachis*. The 2C values obtained by flow cytometry ranged from 2.55 to 3.22 pg among the diploid species. The annual species belonging to different genomes tend to have different genome sizes. However, the 2C values of the perennial species of the A genome were distributed almost along the whole range of genome sizes here observed. The

distribution of 2C values partially support the genome arrangement proposed for the section. The comparisons of 2C values with karyotype parameters suggests that changes in DNA content have been proportionally distributed among the chromosome arms, and that the heterochromatic fraction was not directly involved in that changes. Within the A genome, the annual species has lower DNA content than the perennial ones, according to the nucleotype hypothesis. However, the lack of significant relationships with geoclimatic variables suggests that there are many intrinsic factors determining particular ecological roles of the DNA content in the different lineages of section *Arachis*. The constancy of the Cx values observed in the polyploids compared to those of the parental species suggests that the allopolyploidization event that originated the cultivated peanut did not induce significant changes in the genome size.

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Abbreviations

DAPI 4',6-diamidino-2-phenylindole (DAPI)
Pg Picograms
Mbp Mega base pairs
GISH Genome *in situ* hybridization

Introduction

An immense amount of effort was invested into the collection and maintenance of *Arachis* germplasm that

is held in few genebanks around the world (Bertioli et al. 2011). Efforts to improve the knowledge of *Arachis* germplasm mainly included morphological and taxonomic studies (Krapovickas and Gregory 1994; Valls and Simpson 2005), cross compatibility assays (Gregory and Gregory 1979; Stalker 1991; Simpson 2001; Tallury et al. 2005), cytogenetic studies and molecular markers characterization. Cytogenetic studies revealed that section *Arachis* is composed of two allotetraploids species ($2n = 4x = 40$, AABB) and 29 diploid species with two basic chromosome numbers (26 with $x = 10$ and three with $x = 9$). Molecular cytogenetic studies arranged the 26 species with $x = 10$ in five different genomes (A, B, D, F, and K; Robledo and Seijo 2010) and three karyotype groups (La Plata River Basin, Chiquitano, and Pantanal, all included in the A genome; Robledo et al. 2009). The three species with $2n = 2x = 18$ were recently assigned to the G genome (Silvestri et al. 2014).

A series of studies using molecular markers that included isozymes and proteins (Singh et al. 1991; Lu and Pickersgill 1993), RFLP (Kochert et al. 1991; Gimenes et al. 2002), RAPD (Halward et al. 1992; Hilu and Stalker 1995; Subramanian et al. 2000), AFLP (He and Prakash 2001; Milla et al. 2005; Tallury et al. 2005) and microsatellites (Moretzsohn et al. 2004; Gimenes et al. 2007; Koppolu et al. 2010) have been conducted to understand the genetic relationships between *Arachis* species, but with more emphasis on section *Arachis*. More recently, chloroplast and nuclear sequence analyses (Grabiele et al. 2012; Moretzsohn et al. 2013) were used to investigate the molecular variation within and among species and the origin of peanut. Characterization of these germplasm, however, has lagged behind in many other aspects, such as reproductive biology, physiology and genome size.

The genome size or “C-value” of an organism makes reference to the nuclear DNA content per haploid complement of chromosomes (Greilhuber et al. 2005). It was observed that this parameter widely varies among angiosperms by more than 2,500 folds (Bennett 1987) and can be correlated with life histories (Bennett 1972; Nandini and Murray 1997), climatic factors and geographical distribution (Bennett 1976; Poggio et al. 1989, 1998; Wakamiya et al. 1993; MacGillivray and Grime 1995; Bottini et al. 2000). Despite being a useful descriptor for

characterization of plant genetic resources (Ozias-Akins and Jarret 1994; Rayburn et al. 1997; Hendrix and Stewart 2005; Loureiro et al. 2007), nuclear DNA contents were studied in a very limited number of *Arachis* species and the available genome size estimations are controversial. Most of these determinations were made by the Feulgen densitometry method (Dhillon et al. 1980; Reslar et al. 1981; Singh et al. 1996; Lavia and Fernández 2008). However, measurements in *A. duranensis* and *A. hypogaea* by flow cytometry evidenced that the data obtained by Feulgen densitometry [except in Dhillon et al. (1980) for *A. hypogaea*] overestimated the genome sizes by two (Temsch and Greilhuber 2000, 2001). Variability in estimated 2C values among those reports brought much confusion for germplasm characterization, comprehensive understanding of the evolutionary trends and in decision making for genomic studies. In this sense, several genome projects in diploid and tetraploid species are being developed (www.peanutbase.org) and the representation of different sequences in the genomes is being investigated in peanut and wild relatives (Nielen et al. 2009, 2011; Bertioli et al. 2013), but without having a precise determination of the DNA content for the species.

Here, an attempt is made to resolve the ambiguities concerning the DNA content of diploid and tetraploid species of section *Arachis* with $x = 10$ measuring the 2C nuclear DNA contents by flow cytometry. The aims of this work were: (1) to determine the 2C nuclear DNA content for all available species; (2) to analyse the interspecific variation of nuclear DNA content with the genome and karyotype constitution of the species; (3) to document the possible changes in the Cx values resulting from hybridization and chromosome duplication that gave rise to peanut; and (4) to assess the significance of genome size variation in a biological and biogeographical context.

Materials and methods

Plant material

Seeds of the species with $x = 10$ of the *Arachis* section were obtained from the peanut germplasm collections maintained at the INTA EEA Manfredi (Córdoba, Argentina), at the Instituto de Botánica del

Nordeste (Corrientes, Argentina) and at the Texas Agriculture Experimental Station (Stephenville, Texas, USA). The materials analysed, accession numbers and their original provenances are detailed in Table 1. Geographical distributions of the accessions studied for each species are shown in Fig. 1. Voucher specimens for these materials are deposited at the herbarium of the Instituto de Botánica del Nordeste (CTES). Since the aim of this study was to survey the interspecific variation of 2C DNA nuclear content, and because it was demonstrated that intra-specific variation of genome size is lower than 6.5 % (Temsh and Greilhuber 2000, 2001) only one accession per species was used for this study. Additionally, in order to compare the genome size with the karyotype parameters of the species, the selected accessions of the 26 species here studied were the same as those for which chromosome data were published (Seijo et al. 2004; Robledo and Seijo 2008, 2010; Robledo et al. 2009).

Determination of chromosome number

In order to confirm the chromosome numbers and banding patterns for each accession analysed, secondary root meristems obtained from germinated seed were pretreated with 0.002 M 8-hydroxyquinoline for 3 h at 25 °C and fixed in 3:1 absolute ethanol : glacial acetic acid (v/v) for at least 24 h at –20 °C. Somatic chromosome spreads were prepared according to Schwarzacher et al. (1980). Root apices were macerated in a digestion solution of 1 % (w/v) cellulose from *Trichoderma viridae* (Onozuka R-10, Serva) plus 10 % (v/v) pectinase solution from *Aspergillus niger* in 40 % glycerol (Sigma-Aldrich) dissolved in 0.01 mol/L citric acid/sodium citrate buffer pH 4.8, at 37 °C for 2 h. Subsequently, the meristematic cells were removed from the root tip and squashed in 45 % acetic acid. After removal of the coverslip with CO₂, the slides were air dried, aged for 1–2 days at room temperature, and then kept at –20 °C until use. Preparations were stained with 2 mg/mL of 4',6-diamidino-2-phenylindole (DAPI) and mounted with Vectashield medium (Vector Laboratories). Chromosomes were viewed and photographed with a Leica DMRX epifluorescence microscope. Digital images were combined and processed using Photoshop v.7.0 (Adobe).

Flow cytometry

Holoploid genome size (as defined by Greilhuber et al. 2005) of *Arachis* species was estimated by flow cytometry using fresh young leaves. Nuclei of *Paspalum intermedium* Munro ex Morong, accession Sch 28857 (diploid, 2C = 1.42 pg; Vaio et al. 2007) were used as internal standard for diploid *Arachis* species, while those of *Paspalum notatum* Chirú biotype (hexaploid, 2C = 3.57 pg; Vaio et al. 2007) were used for tetraploid ones. These standards were selected because they are among the most reliable and always available in the flow cytometer laboratory at IBONE (Corrientes, Argentina). Approximately 15 mg of *Arachis* leaf tissues and 15 mg of the internal standard were finely chopped using a sharp razor blade in presence of 0.5 mL PARTEC absolute buffer (High resolution DNA kit), and then filtered through a 30 µm nylon mesh to remove cellular debris. The nuclei suspension was incubated for 2–5 min at room temperature with 1.5 mL of staining solution containing 1 µg/µL propidium iodide and 5 U/mL of RNase. Immediately, measurements were performed with a PARTEC II flow cytometer (Partec). One to three individuals were measured for every accession with three runs (5,000–10,000 nuclei per analysis) from independent isolations for each individual. The data analysis was performed using the Partec II FloMax software. The absolute value of DNA content of each sample was calculated as described in Doležel and Bartos (2005): DNA content of the sample = $(\bar{X} \text{ peak of sample} \times \text{G1 DNA content (2C) of the standard}) / \bar{X} \text{ G1 peak of the standard}$. Cx values, representing the DNA content of one non-replicated monoploid genome with the chromosome number *x* (Greilhuber et al. 2005), were calculated as the 2C nuclear DNA content divided by ploidy level and expressed in pg (1 pg of DNA = 978 Mbp).

Statistical analysis

All statistical analyses were performed using the InfoStat software version 2013 (Di Rienzo et al. 2013). The mean, standard deviation and the coefficient of variation of 2C value were calculated for each species. One-way analysis of variance (ANOVA) at a significance level of 5 % ($\alpha = 0.05$) was used to evaluate the differences in DNA content between species, and

Table 1 List of *Arachis* species studied with their provenance, collectors and accession number

Taxon	Provenance, collectors and collection number	Code
<i>A. batizocoi</i> Krapov. et W. C. Gregory	Bolivia, Dept. Santa Cruz, Prov. Cordillera, Parapetí. K 9484. (CTES)	1
<i>A. benensis</i> Krapov., W. C. Gregory et C. E. Simpson	Bolivia, Dept. Beni, Prov. Cercado, Trinidad. K, G, S, P, Sc 35005. (CTES)	2
<i>A. cardenasii</i> Krapov. et W. C. Gregory	Bolivia, Dept. Santa Cruz, Prov. Chiquitos, Roboré. K, S, Sc 36015. (CTES)	3
<i>A. correntina</i> (Burkart) Krapov. et W. C. Gregory	Argentina, Prov. Corrientes, Dept. Ituzaingó. K, Ri, P 7897. (CTES)	4
<i>A. cruziana</i> Krapov., W. C. Gregory et C. E. Simpson	Bolivia, Dept. Santa Cruz, Prov. Chiquitos, San José. K, S, Sc 36024. (CTES)	5
<i>A. diogoi</i> Hoehne	Paraguay, Dept. Alto Paraná, Puerto Casado. G, K 10602. (CTES)	6
<i>A. duranensis</i> Krapov. et W. C. Gregory	Argentina, Salta, San Martín, Campo Durán. K 7988. (CTES)	7
<i>A. glandulifera</i> Stalker	Bolivia, Bolivia, Dept. Santa Cruz, Prov. Velasco. San Ignacio Se, Sn 3710 (CTES)	8
<i>A. gregoryi</i> C. E. Simpson, Krapov. et Valls	Brazil, St. Mato Grosso, Mun. Pontes e Lacerda. V, Of, Sv 14753. (CEN)	9
<i>A. helodes</i> Martius ex Krapov. et Rigoni	Brazil, St. Mato Grosso, Cuiabá. K, G 30029. (CTES)	10
<i>A. herzogii</i> Krapov., W. C. Gregory et C. E. Simpson	Bolivia, Dept. Santa Cruz, Prov. Chiquitos, San José de Chiquitos. K, S, Sc 36029. (CTES)	11
<i>A. ipaënsis</i> Krapov. et W. C. Gregory	Bolivia, Dept. Tarija, Prov. Gran Chaco, Ipa. K, G, B, P, Sc, S 30076. (CTES)	12
<i>A. kempff-mercadoid</i> Krapov., W. C. Gregory et C. E. Simpson	Bolivia, Dept. Santa Cruz, Prov. Ñuflo de Chavez, Ascensión de Guarayos. K, G 30090. (CTES)	13
<i>A. krapovickasii</i> C. E. Simpson, D. E. Williams, Valls et I. G. Vargas	Bolivia, Dept. Santa Cruz, Prov. Chiquitos, San José de Chiquitos. Wi, S, Vg 1291. (CTES)	14
<i>A. kuhlmannii</i> Krapov. et W. C. Gregory	Brazil, St. Mato Grosso do Sul, 70 km from Cáceres on road to Cuiabá. K, G 30034. (CTES)	15
<i>A. linearifolia</i> Valls, Krapov. et C. E. Simpson	Brazil, St. Mato Grosso, Santo Antonio de Leverger. V, Po, B (CEN, CTES)	16
<i>A. magna</i> Krapov., W. C. Gregory et C. E. Simpson	Bolivia, Dept. Santa Cruz, Prov. Velasco, San Ignacio. K, G, Sc, S 30097. (CTES)	17
<i>A. schininii</i> Krapov. Valls et C. E. Simpson	Paraguay, Dept. Amambay, Bella Vista. V, S, W 9923. (CTES)	18
<i>A. simpsonii</i> Krapov. et W. C. Gregory	Bolivia, Dept. Santa Cruz, Prov. Sandoval, San Matías. Se, Sn 3722. (CTES)	19
<i>A. stenoperma</i> Krapov. et W. C. Gregory	Brazil, St. Mato Grosso, Barra do Garças. V, S, Sa, Gd, W 7762. (CTES)	20
<i>A. trinitensis</i> Krapov. et W. C. Gregory	Bolivia, Dept. Beni, Prov. Cercado, Trinidad. Wi 1117. (CTES)	21
<i>A. valida</i> Krapov. et W. C. Gregory	Brazil, St. Mato Grosso do Sul, Corumbá, Fazenda Vale do Paraíso. K, G 30011. (CTES)	22
<i>A. villosa</i> Benth	Uruguay. S 862 (CTES)	23
<i>A. williamsii</i> Krapov. et W. C. Gregory	Bolivia, Dept. Beni, Prov. Cercado, Trinidad. Wi 1118. (CTES)	24
<i>A. monticola</i> Krapov. et Rigoni	Argentina, Prov. Jujuy, Dept. Capital, Yala. Se, Sn 2774. (CTES)	25
<i>A. hypogaea</i> L. subsp. <i>hypogaea</i> var. <i>hypogaea</i> (race Guaycurú)	Argentina, CTES. FCA 27	26

B = D. Banks; Bi = L. B. Bianchetti; G = W.C. Gregory; Gd = I. J. de Godoy; K = A. Krapovickas; Of = F. O. Freitas; P = J. Pietrarelli; Po = A. Pott; Ri = V.A. Rigoni; S = C.E. Simpson; Sa = H.T. Stalker; Sc = A. Schinini; Se = J. G. Seijo; Sn = V.G. Solís Neffa; Sv = G. P. Silva; V = J. F. M. Valls; Vg = I. G. Vargas; W = W.L. Werneck; Wi = D.E. Williams; FCA = plant introduction number of Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste, Argentina; Prov. = province; Dept. = department; St. = state

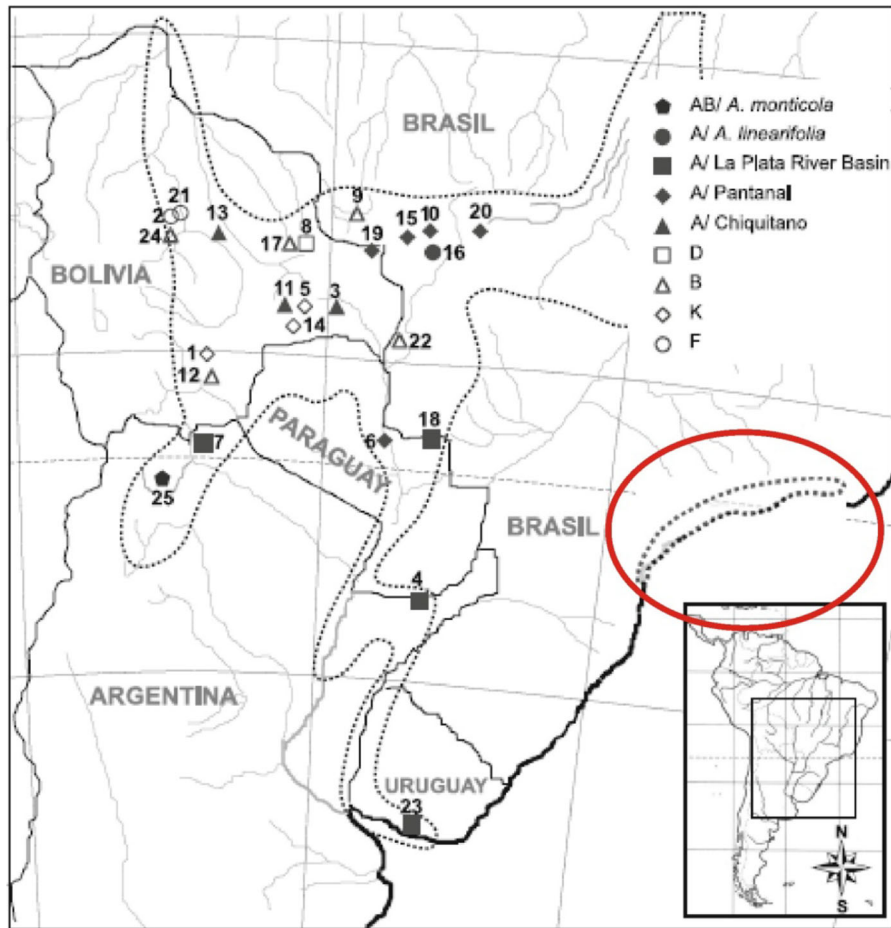


Fig. 1 Geographic distribution of the localities in which the accessions of the wild *Arachis* species analysed in this study were collected. For location details and species code see

Table 1. The *dash-dotted line* denotes the distribution of the *Arachis* section. Each genome and karyotype group (of the A genome) is indicated with different symbol

the Turkey 5 % post hoc test was used to evaluate differences between group of species.

The relationship between DNA content and the karyotype morphology in *Arachis* species was evaluated by comparing the values of DNA content here obtained with the karyotype parameters previously published by our laboratory (TCL: total chromosome length, A_1 : intrachromosomal asymmetry index; A_2 : interchromosomal asymmetry index, HT: total content of DAPI⁺ heterochromatin expressed as percentage of the TCL, KF: karyotype formula). The relationship between DNA content and CMA⁺ heterochromatin was not investigated since in section *Arachis* the latter is restricted to the nucleolar organizer regions. All karyotype measurements used for the correlation analyses correspond to mean values of three to eight

individuals per accession and at least five metaphases by each individual (Seijo et al. 2004; Robledo et al. 2009; Robledo and Seijo 2010).

The biological significance of the DNA content variation was evaluated in relation to the life cycle, the minimum generation time and the distribution range of the studied species. These data were recovered from Krapovickas and Gregory (1994), Valls and Simpson (2005), from personal observations of the authors in wild and cultivated populations, and from herbarium material. The DNA content of the species here studied was also analysed in relation to geographic (latitude, longitude and altitude) and 19 bioclimatic variables (Annual Mean Temperature, Mean Monthly Temperature Range, Isothermality, Temperature Seasonality, Max Temperature of Warmest Month, Min Temperature of Coldest

Month, Temperature Annual Range, Mean Temperature of Wettest Quarter, Mean Temperature of Driest Quarter, Mean Temperature of Warmest Quarter, Mean Temperature of Coldest Quarter, Annual Precipitation, Precipitation of Wettest Month, Precipitation of Driest Month, Precipitation Seasonality, Precipitation of Wettest Quarter, Precipitation of Driest Quarter, Precipitation of Warmest Quarter, Precipitation of Coldest Quarter) to gain insights into the ecological significance of the variation observed. Data for bioclimatic variables were extracted from the WorldClim database (Hijmans et al. 2005).

Results

Genome size variation

DNA contents (2C), coefficients of variation, genome/karyotype groups, ploidy levels, and life cycles for all the *Arachis* species analysed are shown in Table 2. The 2C values among diploid species ranged from 2.55 (*A. duranensis*) to 3.22 pg (*A. magna*), which represents a difference of 1.26 fold. Considering all the diploid species, the genome size varied in a continuous fashion (Fig. 2) and statistical differences were found only for some pairs of species ($F = 12.51$; $p < 0.0001$), regardless of their genome constitution (Table 2).

2C values, genomes and karyotype groups

The range of genome size variation within the B, F and K genome groups was lower than that observed in the A genome group (Table 2; Fig. 2). Comparing exclusively the annual species, *A. duranensis* (A genome) showed the lowest genome size while those of the B genome had the highest DNA content (Fig. 4A). The species of the K genome showed the largest range of variation (from 2.59 pg in *A. cruziana* to 2.83 pg in *A. batizocoi*). The 2C value of the D genome species was in the range of the K genome species, and the genome sizes of these species (D and K genome) were closer to the value of *A. duranensis* (A genome) than to those of the B genome species. The F genome species showed intermediate genome sizes between of the values of the K–D and B genome species (Fig. 4A).

The genome size of the perennial species of the A genome were distributed almost along the whole range

of DNA content of the annual species here studied (Fig. 4B). Those belonging to the Chiquitano group tended to have the lowest 2C values, while those of the La Plata River Basin group the highest ones (Table 2; Fig. 4B). The species of the Pantanal group presented medium genome sizes.

2C values and karyotype parameters

Chromosome numbers of all the materials analysed were confirmed by DAPI staining. All the taxa showed $2n = 20$ chromosomes, excepting *A. hypogaea* and *A. monticola* that had $2n = 40$ (Table 2). The species of the A and K genomes and the A chromosome complements of *A. hypogaea* and *A. monticola* showed conspicuous centromeric bands in all (or in some cases in nine) chromosomes pairs. The unique species of the D genome showed seven chromosomes pairs with centromeric bands, two chromosome pairs with interstitial bands and two without bands. Meanwhile, the species of the F genome showed tiny centromeric bands in six or seven pairs of chromosomes, and those species of the B genome and the B chromosome complements of *A. hypogaea* and *A. monticola* were deprived of DAPI⁺ centromeric heterochromatin. Figure 4 presents metaphase plates of representative species belonging to different genomes and karyotype groups.

The comparison of genome size with the karyotype parameters revealed that the species with longer karyotypes tended to have higher 2C values (although not statistically significant; Table 3). The DNA content was not correlated to the karyotype asymmetry indices (A_1 and A_2), and the karyotype formula remained almost constant in spite of the 0.67 pg of variation. This was particularly evident in the perennial species of the A genome. Small changes in karyotype formula like those observed in *A. batizocoi*, or more largely like in *A. glandulifera*, seems to be not related with changes in genome size. Surprisingly, a negative correlation ($r = -0.66$; $p = 6.3 \times 10^{-4}$) was found between the genome sizes and the DAPI⁺ heterochromatin content (Fig. 5).

2C values and life cycle

The analysis of genome size in species with different life cycles within a single genome was only possible among species of the A genome. Within it, *A.*

Table 2 Nuclear DNA contents (2C), genome constitution, karyotype group and life cycle of diploid and allotetraploid species analysed of section *Arachis*

Species	Code	n	2C value (pg)	% CV	Statistical* grouping	Genome/Karyotype group	Ploidy x = 10	Life cycle group
<i>A. batizocoi</i>	1	3	2.83 ± 0.08	3.65	abcde	K	2x	a
<i>A. benensis</i>	2	3	2.91 ± 0.03	3.25	cdef	F	2x	a
<i>A. cardenasii</i>	3	3	3.01 ± 0.07	4.15	defg	A/Chiquitano	2x	p
<i>A. correntina</i>	4	3	2.85 ± 0.09	3.55	bcde	A/La Plata River Basin	2x	p
<i>A. cruziana</i>	5	3	2.59 ± 0.07	4.33	ab	K	2x	a
<i>A. diogoi</i>	6	3	2.84 ± 0.14	2.86	bcde	A/Pantanal	2x	p
<i>A. duranensis</i>	7	3	2.55 ± 0.07	3.76	a	A/La Plata River Basin	2x	a
<i>A. glandulifera</i>	8	2	2.69 ± 0.18	2.99	abc	D	2x	a
<i>A. gregoryi</i>	9	3	3.17 ± 0.13	3.79	fg	B	2x	a
<i>A. helodes</i>	10	3	2.81 ± 0.02	2.59	abcd	A/Pantanal	2x	p
<i>A. herzogii</i>	11	3	2.95 ± 0.07	4.11	cdefg	A/Chiquitano	2x	p
<i>A. ipaënsis</i>	12	3	3.19 ± 0.08	3.71	fg	B	2x	a
<i>A. kempff-mercadoi</i>	13	3	2.69 ± 0.06	3.37	abc	A/Chiquitano	2x	p
<i>A. krapovickasii</i>	14	3	2.74 ± 0.04	3.99	defg	K	2x	a
<i>A. kuhlmannii</i>	15	3	3.09 ± 0.09	4.11	efg	A/Pantanal	2x	p
<i>A. linearifolia</i>	16	1	3.04 ± 0.11	3.90	defg	A/?	2x	p
<i>A. magna</i>	17	3	3.22 ± 0.06	3.70	g	B	2x	a
<i>A. schinonii</i>	18	3	3.18 ± 0.13	4.11	fg	A/La Plata River Basin	2x	b-p**
<i>A. simpsonii</i>	19	3	3.08 ± 0.08	3.94	defg	A/Pantanal	2x	p
<i>A. stenosperma</i>	20	3	2.96 ± 0.04	3.36	cdefg	A/Pantanal	2x	p
<i>A. trinitensis</i>	21	3	2.84 ± 0.03	4.30	bcde	F	2x	a
<i>A. valida</i>	22	3	3.16 ± 0.02	4.12	fg	B	2x	a
<i>A. villosa</i>	23	3	3.04 ± 0.04	2.94	defg	A/La Plata River Basin	2x	b-p
<i>A. williamsii</i>	24	3	3.20 ± 0.01	3.68	g	B	2x	a
<i>A. monticola</i>	25	3	5.70 ± 0.15	2.76		AB	4x	a
<i>A. hypogaea</i>	26	3	5.60 ± 0.08	2.80		AB	4x	a

pg picograms, n number of individuals analysed, CV coefficient of variation, a annual, p perennial, b-p biennial or perennial, ? not assigned to any karyotype group yet

* Statistical grouping for diploid species. Same letter are not statistically different according to the multiple comparison Tukey test ($p < 0.0001$). Statistical analysis was performed using mean values obtained from three individuals

** *A. schinonii* was considered for this study as biennial or perennial due to the morphology of the roots and to green house observations

duranensis (2C = 2.55 pg) showed significant smaller ($F = 12.51$; $p < 0.0019$) genome size (Table 2) than any of the perennial species (except *A. kempff-mercadoi*). *Arachis schinonii* needs a special consideration since this A genome species was described as annual in its original taxonomic treatment (Valls and Simpson 2005); however, it unexpectedly showed the highest 2C values (3.18 pg) of this genome group. The morphological analyses of the herbarium specimens showed that some plants of this species have tap roots with a crown diameter of up to 0.7 cm, a character which suggests that those plants may have behaved as

perennials (or at least biennials). Considering that, here and for further analyses, *A. schinonii* will be considered as a perennial species, similarly as we treated the biennial-perennial *A. stenosperma* (Table 4).

2C values and geoclimatic variables

The results of the Pearson correlation analyses did not show significant correlation between the DNA content of the diploid *Arachis* species here analysed with any of the geographic and bioclimatic variables considered (Table 5).

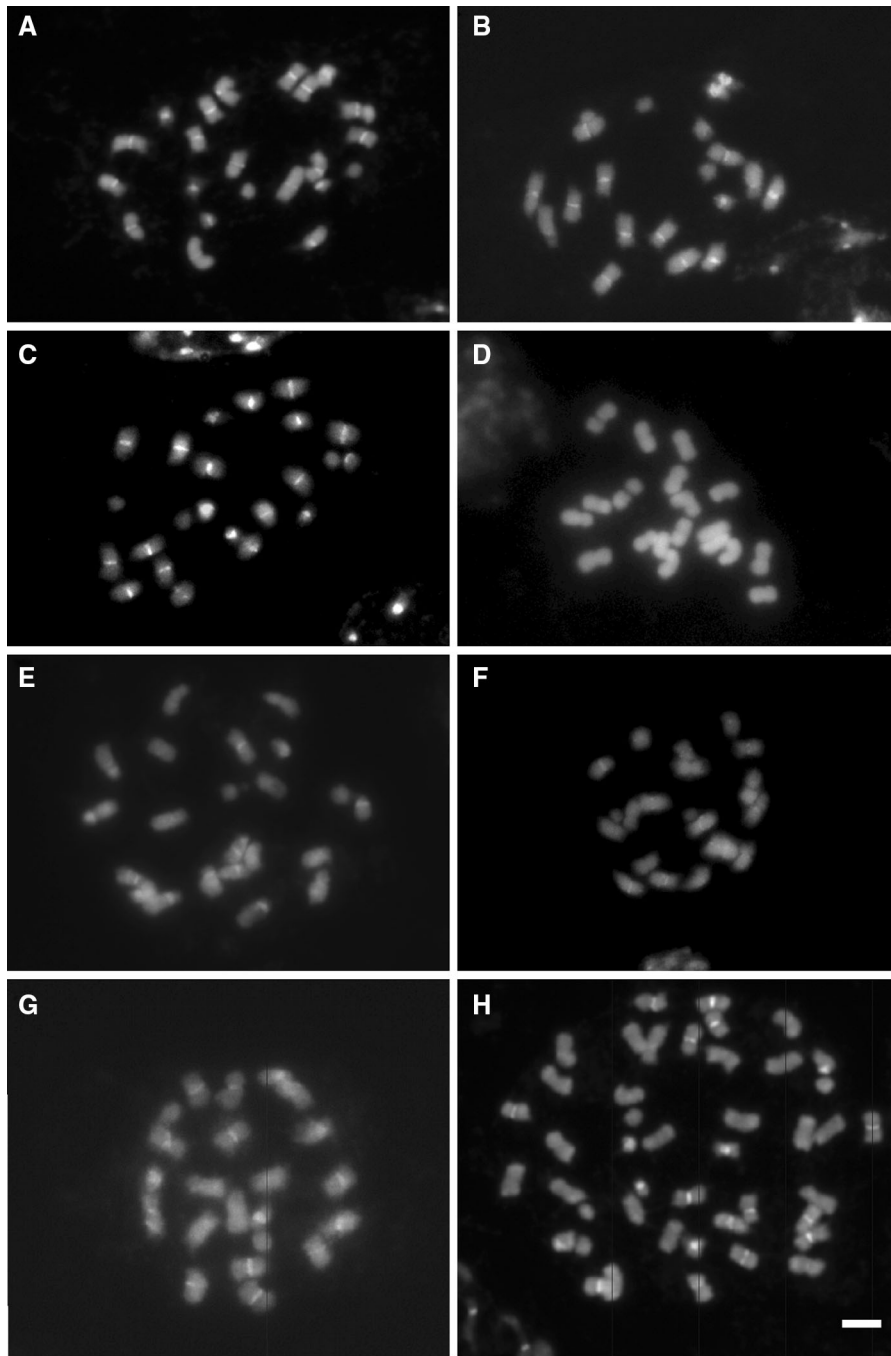


Fig. 2 Representative metaphases of *Arachis* species (section *Arachis*) belonging to different genomes and karyotype groups stained with DAPI. **A** *A. duranensis*, A genome (La Plata River Basin karyotype group); **B** *A. cardenasii*, A genome (Chiquitano

karyotype group); **C** *A. helodes*, A genome (Pantanal karyotype group); **D** *A. ipaënsis*, B genome; **E** *A. glandulifera*, D genome; **F** *A. trinitensis*, F genome; **G** *A. batizocoi*, K genome; and **H** *A. hypogaea*, AB genome. Scale bar 3 μ m

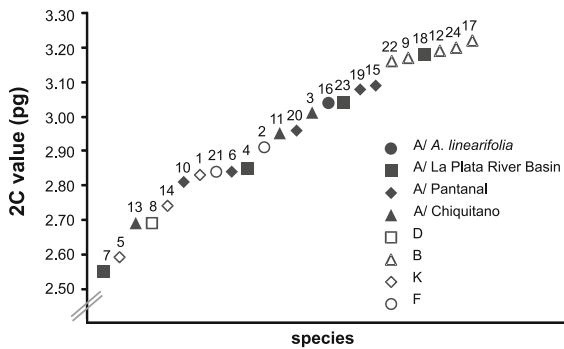


Fig. 3 DNA content (2C) of the species of the Arachis section with $2n = 2x = 20$ analysed in this study. DNA contents were plotted in an increasing order revealing a continuous fashion. Data are the means of three independent measurements. Different genomes and karyotype groups are indicated by different symbols and species are encoded by the numbers used in Table 1

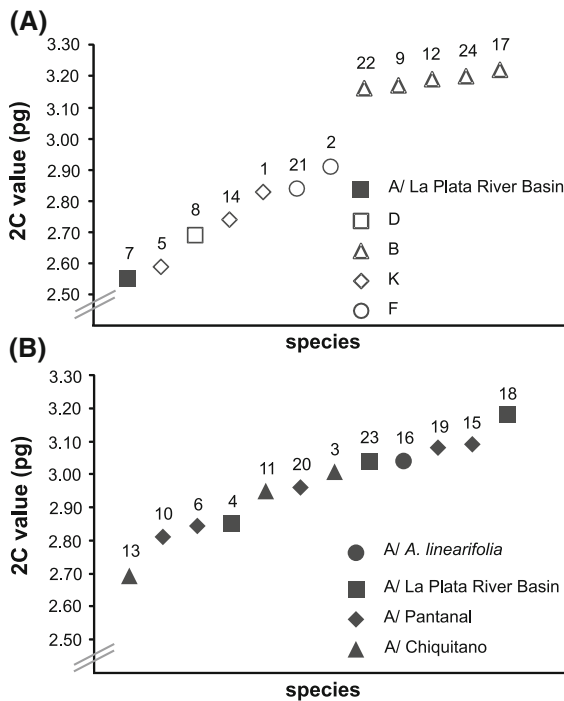


Fig. 4 DNA content (2C) of the species with annual and perennial life cycle of the Arachis section. **A** DNA contents of annual species of five different genomes are represented in an increasing order. **B** DNA contents of the perennial species of the A genome are represented in an increasing order; the karyotype group of each species is also indicated. Species are encoded by the numbers used in Table 1

2C values and allopolyploidization

The genome sizes of both allotetraploid (AABB) species, the wild *A. monticola* ($2C = 5.70$ pg) and the cultivated peanut ($2C = 5.60$), were very similar (Table 2) and not statistically different ($F = 2.65$; $p = 0.178$). Considering that *A. duranensis* ($2C = 2.55$ pg) and *A. ipaënsis* ($2C = 3.19$ pg) are the most probable A and B genome donors of the tetraploids respectively, the expected 2C value for the polyploids was 5.74 pg. The values of genome size here observed for the tetraploids did not show statistical significant deviation from the expected value ($F = 0.87$; $p = 0.4659$). Consistently, Cx values for *A. monticola* (1.394 Mbp) and *A. hypogaea* (1.369 Mbp; Table 3), although lower, were not statistically different from the mean Cx value (1.403 Mbp) expected from values of the parental diploids species.

Discussion

This work reports about the genome size of the diploid and allotetraploid species with $x = 10$ of the Arachis section using flow cytometry. It is expected that the data here obtained will represent a keystone in comparative studies of the genomes of *Arachis* species, since it is the first in which a comprehensive analysis of the genome sizes of most species of the section Arachis was performed using a standardized flow cytometric technique. Moreover, this investigation provides additional insight about the genomic differentiation within the section Arachis.

2C values and genomes

Genome size estimations were in the range of the values published previously for *A. duranensis*, *A. monticola* and *A. hypogaea* using flow cytometry (Temsch and Greilhuber 2000, 2001); however, they were almost the half compared with the data published for the same species using Feulgen densitometry (Ressler et al. 1981; Singh et al. 1996; Lavia and Fernández 2008; Table 4). According to Temsch and Greilhuber (2000, 2001), the discrepancy in DNA content of *Arachis* species using Feulgen densitometry and flow cytometry points to unrecognized technical

Table 3 Cx values, karyotype formulae, total chromosome length, asymmetry indexes and total heterochromatin of the analysed species of *Arachis*

Species	Cx (Mbp)*	KF	TCL (μm)	Asymmetry indexes		TH (%)
				A1	A2	
<i>A. batizocoi</i>	1,384	7 m + 3 sm	36.15	0.29	0.08	11.63
<i>A. benensis</i>	1,423	8 m + 2 sm	25.18	0.24	0.07	7.52
<i>A. cardenasii</i>	1,472	9 m + 1 sm	33.57	0.24	0.16	11.90
<i>A. correntina</i>	1,394	8 m + 2 sm	32.89	0.24	0.16	12.09
<i>A. cruziana</i>	1,267	8 m + 2 sm	28.22	0.26	0.09	12.55
<i>A. diogoi</i>	1,389	9 m + 1 sm	34.36	0.19	0.16	13.44
<i>A. duranensis</i>	1,247	9 m + 1 sm	28.95	0.21	0.15	14.59
<i>A. glandulifera</i>	1,315	8 m + 4 sm + 8 st	38.22	0.49	0.09	13.09
<i>A. gregoryi</i>	1,550	8 m + 2 sm	28.74	0.23	0.10	0.78
<i>A. helodes</i>	1,374	9 m + 1 sm	29.48	0.21	0.16	12.42
<i>A. herzogii</i>	1,443	9 m + 1 sm	37.48	0.21	0.14	12.15
<i>A. ipaënsis</i>	1,560	9 m + 1 sm	29.56	0.20	0.09	0
<i>A. kempff-mercadoi</i>	1,315	9 m + 1 sm	35.53	0.20	0.13	13.85
<i>A. krapovickasii</i>	1,340	6 m + 4 sm	34.79	0.30	0.11	11.36
<i>A. kuhlmannii</i>	1,511	9 m + 1 sm	39.77	0.22	0.17	12.92
<i>A. magna</i>	1,575	9 m + 1 sm	31.04	0.17	0.09	0
<i>A. schininii</i>	1,555	9 m + 1 st	29.13	0.24	0.13	10.28
<i>A. simpsonii</i>	1,506	9 m + 1 sm	28.19	0.21	0.15	14.67
<i>A. stenosperma</i>	1,447	9 m + 1 sm	35.69	0.19	0.15	13.21
<i>A. trinitensis</i>	1,389	10 m	29.02	0.20	0.07	5.89
<i>A. valida</i>	1,545	10 m	32.81	0.21	0.08	1.22
<i>A. villosa</i>	1,487	9 m + 1 sm	38.12	0.20	0.16	11.85
<i>A. williamsii</i>	1,565	10 m	29.39	0.15	0.08	0.71

Chromosome data was compiled from Robledo and Seijo (2008), Robledo et al. (2009) and Robledo and Seijo (2010)

KF karyotype formula, TCL total chromosome length, TH Total heterochromatin measured as percentage of the TCL

* 1 pg DNA = 978 Mbp according to Doležel and Bartos (2005)

measurement problems that cause non-stoichiometric binding of the chromophore, rather than to intraspecific variation. Therefore, for further discussion only the flow cytometric estimations of DNA content published previously (Temsch and Greilhuber 2000, 2001) and those obtained in this study will be considered.

The comparison of the DNA contents of the annual species belonging to different genomes partially supports the genome arrangement of section *Arachis* recently proposed (Robledo and Seijo 2010). The fact that the genome size for the D genome species was in the range of that found for K genome species (*A. batizocoi*, *A. cruziana* and *A. krapovickasii*), and that all of them were more similar to that observed in *A. duranensis* (A genome) is in accordance with the karyotype similarity (mainly the banding pattern) observed by Robledo and Seijo (2010) for these groups. In agreement, the phylogenetic analysis based on the non-transcribed region of the 5S rDNA

(Grabiele et al. 2012) evidenced that the species of the A, K and D genomes are grouped in a single cluster different from other cluster composed of F and B genome species. Similarly, the K and D genomes appeared more closely related to the A genome than to the B genome in a phylogenetic tree based on introns of single-copy gene sequences (Moretzsohn et al. 2013). On the other side, the finding that the genome sizes of the F genome species (*A. benensis* and *A. trinitensis*) and of the K genome species are separated by a gap 0.25 pg or more from those of the B genome sensu stricto species (*A. ipaënsis*, *A. gregoryi*, *A. magna*, *A. valida* and *A. williamsii*) support the segregation of the former (F and K genome) from the B genome sensu lato (Robledo and Seijo 2010).

DNA content and karyotype data

Chromosome numbers were determined for all the accessions used for DNA content estimations. In all

cases, the chromosome number $2n = 20$ or $2n = 40$ and the banding patterns here observed were coincident with the data published before for these *Arachis* species (Fernández and Krapovickas 1994; Lavia 1996; Peñaloza and Valls 2005; Robledo and Seijo 2008; Robledo et al. 2009; Robledo and Seijo 2010).

The observation that the *Arachis* species with longest karyotypes tended to have higher 2C values was an expected result. However, the lack of statistically significant correlation of these parameters in section *Arachis* could be attributed to the facts that DNA content variation may not only change the chromosome length but also the chromosome width, and that the proportion of the changes in each of these parameters may be variable among species (Bennett et al. 1982). The general conservation of the asymmetry indexes and karyotype formula suggest that changes in DNA may have been non-randomly accumulated in the chromosome complements of the species, but rather, that the changes in DNA have been proportionally distributed among each chromosome arm.

Variation in genome size among related homoploid species, as those observed in section *Arachis*, involve quantitative changes mainly in the repetitive fraction of DNA (Schmidt and Heslop-Harrison 1998). The negative correlation found between the genome size and the DAPI⁺ heterochromatin content suggests that the satellite sequences of the heterochromatic bands did not greatly participate in the changes of genome size. By contrast, the comparison of homeologous genomic sequences of *A. duranensis* (A genome) and *A. ipaensis* (B genome) indicated that there are highly conserved microsyntenic regions flanked by repetitive DNA regions, mainly LTR retrotransposons that were completely different in the A and B genomes (Bertioli et al. 2013). Notably, a large proportion of the sequence space is accounted for by relatively few transposons that have been active since the evolutionary divergence of the two genomes (Bertioli et al. 2013). Fluorescent *in situ* hybridization of some LTR retroelements onto mitotic metaphases of A and B genome species demonstrated that the hybridization signals were evenly distributed along most of the chromosomes of the complements (Nielen et al. 2009, 2011). Therefore, the combined results of BAC sequencing and FISH analysis using LTR retrotransposons support the occurrence of proportional changes of DNA content among chromosome arms during

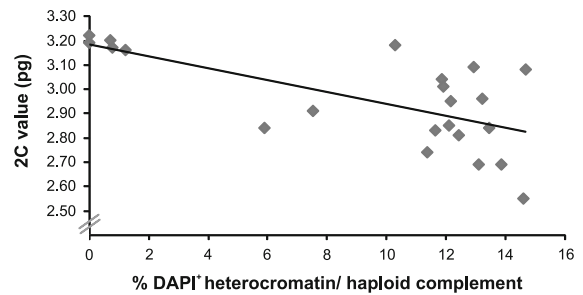


Fig. 5 Dispersion diagram representing the relationships between 2C values with total DAPI⁺ heterochromatin expressed as the percentage of total chromosome length. A trend line was represented in the graph. Each diamond symbol represents one species

genome and species differentiation. The above discussion provides an explanation to the conservation of the karyotype formula in spite of the quantitative changes of DNA content observed in the species of section *Arachis*.

2C values and life cycle

The lower genome size here observed in the annual species *A. duranensis* compared to the perennial species of the A genome (statistically significant with all except with *A. kempff-mercadoi*) is in complete consistence with previous observations in several other leguminous (Raina et al. 1986; Naranjo et al. 1998; Chalup et al. 2014) and non leguminous plants (Sims and Price 1985; Jakob et al. 2004; Albach and Greilhuber 2004; Bancheva and Greilhuber 2006) in which annual species tend to have smaller genomes than perennial ones. According to the nucleotype hypothesis proposed by Bennett (1972), lower DNA contents are associated not only with a shortening of the life cycle but also with a reduction of the minimum generation time (Bennett 1972, 1987). In accordance, the lowest DNA content observed in *A. duranensis* is not only associated with the annual life cycle, but also with a reduction of the whole vegetative and reproductive period when compared to the perennial species of the A genome.

2C values and geoclimatic variables

Correlations of the genome size variation with geographic or bioclimatic variables have been

Table 4 Comparison of the DNA contents so far published for species of section *Arachis* ($x = 10$) with the results obtained in this study

Species (accepted name)	Feulgen's cytophotodensitometry			Flow cytometry					
	Ressler et al. (1981)			Lavia and Fernández (2008)			Temsh and Greilhuber (2000, 2001)		
	Name and accession	2C	Name and accession	2C	Name and accession	2C	Name and accession	2C	
<i>A. duranensis</i>	<i>A. duranensis</i> Krap. et Greg. nom. nud. (K 7988)	4.92	<i>A. duranensis</i> Krap. et Greg. nom. nud. (28 accessions)	5.33–5.91 (mean 5.64)	(20 accessions)	2.54–2.69 (mean 2.63)	(K 7988)	2.55	
	<i>A. spegazzini</i> Greg. et Greg. nom. nud. (GKP 10038)	5.00	<i>A. spegazzini</i> Greg. et Greg. nom. nud.	5.82					
<i>A. herzogii</i>					(KSSc 36030)	4.21	(KSSc 36029)	2.95	
<i>A. stenosperma</i>	<i>A. stenosperma</i> Greg. et Greg. nom. nud. (HLK 408–410)	5.53	<i>A. stenosperma</i> * Greg. et Greg. (<i>A. stenocarpa</i>) nom. nud.	6.10	(VKSSv 9010, VGaSv 12646)	5.74, 5.62	(VSSaGdW 7762)	2.96	
<i>A. cardenasii</i>	<i>A. cardenasii</i> Krap. et Greg. nom. nud. (GKP 10017)	5.68	<i>A. cardenasii</i> * Krap. et Greg.	6.10			(KSSc 36015)	3.01	
<i>A. helodes</i>	<i>A. helodes</i> Mart. ex Krap. et Rig. (GKP 9926)		<i>A. helodes</i> * Krap. et Greg.	6.10			(KG 30029)	2.81	
<i>A. diogoi</i>	<i>A. chacoense</i> Krap. et Greg. nom. nud. (GKP 10062)	5.94	<i>A. chacoense</i> * Krap. et Greg. nom. nud.	6.37			(GK 10602)	2.84	
<i>A. correntina</i>	<i>A. villosa</i> Benth. var. <i>correntina</i> Burk. (GKP 9530–9531)	5.83	<i>A. correntina</i> * Krap. et Greg.	6.42			(KRiP 7897)	2.85	
<i>A. villosa</i>	<i>A. villosa</i> Benth. var. <i>villosa</i> (PI 210554)	5.98	<i>A. villosa</i> * Benth.	6.56			(S 862)	3.04	
<i>A. batizocoi</i>	<i>A. batizocoi</i> Krap. et Greg. nom. nud. (K 9484)	4.96	<i>A. batizocoi</i> * Krap. et Greg.	5.33	(K 9505)	4.97	(K 9484)	2.83	
<i>A. ipaënsis</i>			<i>A. ipaënsis</i> Greg. et Greg. nom. nud.	5.66			(KGBFScS 30076)	3.19	
<i>A. monticola</i>	<i>A. monticola</i> Krap. et Rig. (K 7264)	10.39	<i>A. monticola</i> Krap. et Rig. (3 accessions)	11.28–11.34 (mean 11.31)	(HLP 104)	5.98	SeSn (2774)	5.70	

Table 4 continued

Species (accepted name)	Feulgen's cytophotodensitometry		Singh et al. (1996)		Lavia and Fernández (2008)		Flow cytometry	
	Ressler et al. (1981)		Singh et al. (1996)		Lavia and Fernández (2008)		Temsch and Greilhuber (2000, 2001)	
	Name and accession	2C	Name and accession	2C	Name and accession	2C	Name and accession	2C
<i>A. hypogaea</i>	<i>A. hypogaea</i> L. ssp. <i>hypogaea</i> var. <i>hypogaea</i> (486 GKP Bolivia)	10.36	<i>A. hypogaea hypogaea</i>	11.27	Nigeria*	5.91	FCA 27	5.60
<i>A. hypogaea</i>	<i>A. hypogaea</i> L. ssp. <i>fastigiata</i> Waldron var. <i>fastigiata</i> (GKP 190, Tarapoto, Peru)	11.10	<i>A. hypogaea fastigiata</i>	10.97	USA*	5.95		

Names of the species are provided for each report when they were used as nomen nudum (nom. nud.) or when the accession number was not provided in the original report

* Accession number not provided

demonstrated in several plant groups (Price et al. 1981; Rayburn and Auger 1990; Chalup et al. 2014). Within *Arachis* genus, the genome size variation observed in different accessions of *A. duranensis* [one of the most variable species of the genus and with a widespread distribution (Krapovickas and Gregory 1994; Stalker et al. 1995)] was found to be inversely correlated with altitude (Singh et al. 1996) and with altitude and latitude (Temsch and Greilhuber 2000). The fact that our analyses considering the whole section *Arachis* did not show significant correlation with any of the 18 environmental and the three geographic variables tested could be attributed to the real inexistence of a relationship, to under sampling or to several intrinsic particularities of this taxonomic group that may be blurring the correlation. Among the latter, it should be considered that (1) the total amount of variation in genome size is significant but relatively low while the distribution area of the species of *Arachis* section is very large; (2) the section is composed of at least five lineages with different karyotype structures and different genome components, that may have different genomic constrains for adaptative answers; (3) in general, each of these lineages have different ecological preferences, but, those particular preferences may occur very close at a microgeographic scale; (4) exceptionally, species of different lineages may have very similar ecological preferences. Thus, these intrinsic factors may be determining different genomic answers to similar evolutionary defeats, and at the time, the differential ecological role that the nuclear DNA content may have in the species of section *Arachis*.

2C values and allopolyploidization

The AABB allotetraploids (*A. monticola* and *A. hypogaea*) arose via a single hybridization/polyploidization event between *A. duranensis* (A genome) and *A. ipaensis* (B genome) some 5,000–9,000 years ago (Grabiele et al. 2012). Most allopolyploid species display lower DNA Cx values than the expected from the sum of the parental genomes although in a few number of species the genome size are very close to the sum of their diploid progenitors, like in tobacco (Leitch and Bennett 2004), *Hordeum* (Jakob et al. 2004) and AD *Gossypium* species (Wendel et al. 2002). Our results evidenced that the AABB species of *Arachis* are among the latter group of polyploids, since

Table 5 Correlation analyses of DNA content (2C) with geographic and the main bioclimatic variables for the studied species of section *Arachis*

Species	GS	Latitude	Longitude	Altitude	AMT	MMTR	TS	TAR	MTWaQ	MTCQ	AP	PS	PWeQ	PDQ
<i>A. batizocoi</i>	2.83	-20.08	-63.23	1,143.00	21.05	13.39	303.33	22.00	24.03	16.93	792.00	88.69	444.00	28.00
<i>A. cruziana</i>	2.59	-17.08	-60.75	314.00	24.25	13.28	206.15	20.00	25.92	21.33	1,253.00	63.51	592.00	104.00
<i>A. krapovickasii</i>	3.05	-18.24	-60.85	306.00	24.76	12.90	228.40	19.70	26.87	21.53	878.00	61.17	396.00	68.00
<i>A. benensis</i>	2.91	-14.82	-64.92	155.00	25.94	11.36	159.97	17.20	27.32	23.63	1,882.00	60.34	848.00	145.00
<i>A. trinitensis</i>	2.84	-14.81	-64.90	156.00	25.96	11.37	159.97	17.30	27.33	23.65	1,884.00	60.45	851.00	146.00
<i>A. ipaensis</i>	3.19	-21.00	-63.38	619.00	23.00	13.24	384.01	24.80	27.17	18.00	739.00	87.42	404.00	12.00
<i>A. magna</i>	3.22	-16.37	-60.97	405.00	23.37	14.93	186.91	22.10	25.03	20.78	1,119.00	67.12	530.00	66.00
<i>A. valida</i>	3.16	-19.18	-57.48	192.00	25.37	10.24	224.18	16.20	27.47	22.32	1,124.00	60.59	491.00	94.00
<i>A. gregoryi</i>	3.17	-15.99	-59.56	282.00	24.49	13.12	178.91	19.10	25.92	21.87	1,485.00	69.94	709.00	80.00
<i>A. williamsii</i>	3.20	-14.80	-64.87	158.00	25.91	11.38	159.96	17.20	27.25	23.60	1,886.00	60.58	853.00	146.00
<i>A. duranensis</i>	2.55	-22.25	-63.72	596.00	21.60	12.91	386.03	24.10	25.73	16.40	1,036.00	84.87	530.00	24.00
<i>A. correntina</i>	2.85	-27.63	-56.80	72.00	22.30	11.38	417.41	22.40	27.33	17.25	1,616.00	22.64	476.00	281.00
<i>A. schinii</i>	3.18	-22.38	-56.40	220.00	23.54	11.63	275.62	19.30	26.45	19.93	1,389.00	36.46	496.00	179.00
<i>A. villosa</i>	3.04	-31.02	-57.95	56.00	18.98	12.13	486.88	25.30	25.07	13.25	1,283.00	25.05	401.00	233.00
<i>A. kemppf-mercadoi</i>	2.69	-15.72	-63.08	213.00	25.51	11.92	194.62	17.60	27.23	22.78	1,087.00	63.44	496.00	101.00
<i>A. cardenasii</i>	3.01	-18.35	-59.75	250.00	25.42	13.03	225.47	19.30	27.58	22.37	1,053.00	52.86	425.00	98.00
<i>A. herzogii</i>	2.95	-17.82	-60.75	282.00	24.81	12.76	218.27	19.70	26.82	21.68	1,018.00	65.00	480.00	76.00
<i>A. diogoi</i>	2.84	-22.28	-57.98	78.00	24.97	11.27	316.28	19.70	28.57	20.90	1,213.00	43.91	455.00	132.00
<i>A. helodes</i>	2.81	-16.00	-56.47	142.00	26.00	11.58	159.17	17.80	27.33	23.60	1,237.00	67.48	555.00	54.00
<i>A. kuhlmannii</i>	3.09	-16.13	-57.30	151.00	26.09	12.18	177.26	18.30	27.53	23.45	1,274.00	70.81	605.00	60.00
<i>A. simpsonii</i>	3.08	-18.23	-60.85	305.00	24.78	12.88	225.89	19.60	26.90	21.60	882.00	61.24	398.00	68.00
<i>A. stenoperma</i>	2.96	-15.53	-52.22	328.00	25.17	13.15	140.70	19.20	26.43	23.12	1,569.00	84.42	796.00	24.00
<i>A. linearifolia</i>	3.04	-15.88	-56.03	146.00	26.17	11.56	150.32	18.00	27.37	23.92	1,285.00	70.07	587.00	42.00
<i>A. glandulifera</i>	2.69	-16.17	-62.02	499.00	23.25	13.39	186.68	19.20	24.90	20.55	1,121.00	60.15	496.00	88.00
<i>Arachis</i> species	<i>p</i>	0.95	0.33	0.39	0.52	0.93	0.68	0.77	0.49	0.53	0.84	0.69	0.96	0.93
	<i>r</i>	0.01	0.21	-0.18	0.14	-0.02	-0.09	-0.06	0.15	0.14	0.04	-0.08	0.01	0.02

GS Genome Size, AMT Annual Mean Temperature, MMTR Mean Monthly Temperature Range, TS Temperature Seasonality, TAR Temperature Annual Range, MTWaQ Mean Temperature of Warmest Quarter, MTCQ Mean Temperature of Coldest Quarter, AP Annual Precipitation, PS Precipitation Seasonality, PWeQ Precipitation of Wettest Quarter, PDQ Precipitation of Driest Quarter

the genome sizes estimated for the wild (*A. monticola*, 5.70 pg) and the cultivated (*A. hypogaea*, 5.60 pg) did not show statistically significant departure from the expected 2C value (5.74 pg). The constancy in the Cx values suggests that the hybridization and chromosome doubling events that occurred during the origin of the cultivated peanut did not induce significant changes in genome size.

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