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RESEARCH ARTICLE

Karyotypic studies in wild germplasm of *Arachis* (Leguminosae)

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Abstract The karyomorphology for eight diploid species of *Arachis* belonging to three sections has been described for the first time, Sect. *Extranervosae*: *A. macedoi* (2n = 20m) and *A. retusa* (2n = 14m + 6sm); Sect. *Heteranthae*: *A. sylvestris* (2n = 16m + 4sm); Sect. *Procumbentes*: *A. chiquitana* (2n = 18m + 2sm); Sect. *Arachis*: *A. cruziana* (2n = 18m + 2sm), *A. herzogii* (2n = 18m + 2sm), *A. simpsonii* (2n = 20m) and *A. williamsii* (2n = 20m). A pair of satellited chromosomes was observed in all species. A chromosomes were found in *A. chiquitana*, *A. herzogii* and *A. simpsonii*. Karyotypic differences between sections were observed, but not enough to establish a characteristic karyotype pattern for each section. However, the species may be differentiated by the presence of A chromosomes, the type and position of satellites, and the karyotype formulae. These results are discussed with regard to karyotype evolution in *Arachis* to contribute to understanding the role of chromosome changes in the evolution of the genus.

Keywords *Arachis* · Chromosomes · Karyotype evolution · Wild peanut

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Introduction

Arachis (Leguminosae) is an indigenous and economically important genus of South America. It comprises 80 annual and perennial species (Krapovickas and Gregory 1994; Valls and Simpson 2005), classified into nine sections based on morphological and chromosomal features, cross compatibility and hybrid fertility (Krapovickas and Gregory 1994).

Peanut (*A. hypogaea* L.) is one of the most important sources of dietary protein in the world. However, considering their productivity, this crop remains underexploited because of its susceptibility to pests and diseases. The major constraint to the genetic improvement of peanut is the narrow genetic base of the extant crop. Wild *Arachis* species, by contrast, are diverse and have the genetic variability and agronomically useful characters needed to improve the peanut (Holbrook and Stalker 2003) and constitute valuable

resources for the genetic upgrading of peanut. In this sense, information on the cytogenetics and phylogenetic relationships among wild species and between these species and the peanut is critical to the rational development of breeding programs and complete utilization of the wild materials.

Chromosomal data have played a pivotal role in accelerating crop improvement (Jauhar 2006) and our comprehension of the phylogenetic relationships between wild and cultivated species (Cao 2003). On the other hand, cytogenetic studies are also relevant in the study of plant evolution and diversification (Stebbins 1971). Moreover, the analysis of karyotype characteristics has contributed valuable data for inferring evolutionary trends within particular plant groups and analyzing traits such as changes in chromosome numbers (Mercado-Ruaro and Delgado-Salinas 1998), in karyotype symmetry, and in chromosome length (Poggio et al. 2007).

Karyotype analysis in *Arachis* began with studies by Husted (1933), who described the SAT and A chromosomes (i.e. pair of chromosomes that is conspicuously smaller than any of the others of the complement and present a differential condensation pattern) morphology for *A. hypogaea*. After these pioneer reports, several authors described karyotypes from different species, although most of them included few entities and generally those involved in the origin of the peanut (section *Arachis*) (Smartt et al. 1978; Stalker 1991). The most comprehensive work on cytogenetics in *Arachis* was carried out by Fernández and Krapovickas (1994), who analysed species belonging to different sections. They proposed a general trend of karyotype evolution on the basis of the SAT morphology and the presence of A chromosomes. They found A chromosomes only in species of section *Arachis* and described different types of SAT chromosomes. Until that time, all species had been found to have a base chromosome number of $x = 10$. However, three species of section *Arachis* (Lavia 1996, 1998; Peñaloza et al. 1996) and recently one entity of section *Erectoides* with $x = 9$ were found (Peñaloza and Valls 2005). Karyotypes of species of section *Trierectoides*, *Erectoides* and *Procumbentes*, of all varieties of the *A. hypogaea* and seven accessions of *A. stenosperma* Krapov. et W.C. Gregory were further described in order to characterize the *Arachis* germplasm (Lavia 2001; Lavia and Fernández 2004; Custodio et al. 2005). In view of all available information in the literature, the chromosome numbers of 95% of the entities are known; however, only 27% of the *Arachis* species have been karyotyped, and trends in chromosome evolution within the genus have not been well established yet.

To increase the knowledge of this genus, we have been characterizing the chromosomes of wild species of *Arachis*. As part of this study, in this paper we present our analysis of the karyotypes of eight diploid taxa of *Arachis* from Brazil and Bolivia. These data, together with karyotypes previously published, are jointly analysed to investigate chromosomal traits in relation to the infrageneric classification and to infer a possible direction in the chromosomal evolution of the genus.

Materials and methods

The studied material is presented in Table 1. Voucher specimens were deposited in the herbaria of the Instituto de Botánica del Nordeste (CTES), Corrientes, Argentina and of the Centro Nacional do Recursos Genéticos e Biotenología (CEN), Brasilia, Brazil.

Seeds for this study were provided by the Instituto Nacional de Tecnología Agropecuaria-Manfredi (Córdoba, Argentina) and the Texas Experiment Agricultural Station (Stephenville, Texas, USA). Mitotic preparations were obtained from root tips of germinating seeds. After a pretreatment of 3 h in 0.002 M 8-hydroxyquinoline solution at room temperature, the material was fixed in acetic acid-ethanol (1:3), stained following Feulgen's technique, and then squashed in a drop of 2% acetic orcein.

For the numerical characterization of the karyotypes, the following parameters were calculated: total chromosome length (TCL), mean length of the chromosomes (ML), mean centromeric index (CI), arm ratio [$AR = \Sigma(b/B)/n$] (where b and B are the mean length of short and long arms of each pair homologues, respectively, n is the number of homologues), intrachromosomal asymmetry index (A_1) and interchromosomal asymmetry index (A_2) (Romero Zarco 1986). Satellite (SAT) chromosomes were classified according to Fernández and Krapovickas (1994) and Lavia (2000).

Chromosome morphology was determined using the centromeric index (short arm × 100/total length). Accordingly, chromosomes were classified as

Table 1 Locality, collector and voucher number of the studied species of *Arachis*

Species	Collector and locality
Section *Extranervosae* Krapov. et W.C. Gregory	
A. macedoi Krapov. et W.C. Gregory	VR 7533. Brasil, MG, Capinopolis, Faz. Santa Terezinha
A. retusa Krapov., W.C. Gregory et Valls	VSW 9950. Brasil, GO, Monte Alegre, 590 m
Section *Heteranthae* Krapov. et W.C. Gregory	
A. sylvestris (A. Chev.) A. Chev.	VVeSv 8520. Brasil, PI, Mun. Oeiras, 35 km NE de Cristino Castro, BR-135
	VRSv 10891. Brasil, BA, Pindobaçu
Section *Procumbentes* Krapov. et W.C. Gregory	
A. chiquitana Krapov., W.C. Gregory et C. E. Simpson	KSSc 36027. Bolivia, Santa Cruz, Prov. Chiquitos, 30 km NE de San José de Chiquitos, 60°47′ W, 17°47′ S
Section *Arachis*	
A. cruziana Krapov., W.C. Gregory et C.E. Simpson	KSSc 36024. Bolivia, Santa Cruz, Prov. Chiquitos, 2 km W de San José, 60°47′ W, 17°47′ S
A. herzogii Krapov., W.C. Gregory et C.E. Simpson	KSSc 36030. Bolivia, Santa Cruz, Prov. Chiquitos, 18 km N de San José 60°47′ W, 17°47′ S
A. simpsonii Krapov. et W.C. Gregory	VSPmSv 13732. Brasil, MT, Porto Esperidião
A. williamsii Krapov. et W.C. Gregory	WiCl 1118. Bolivia, Beni, Trinidad, Universidad Técnica del Beni

Abbreviations of collectors: Cl, D. Claure; K, A. Krapovickas; Pm, R.N. Pittman; R, V.R. Rao; S, C.E. Simpson; Sc, A. Schinini; Sv, G.P. Silva; V, J.F.M. Valls; W, W.L. Werneck; Wi, D.E. Williams

metacentric (m) = 50–37.5 and submetacentric (sm) = 37.5–25. Idiograms were constructed by organizing the chromosomes into groups according to their centromeric index (m, sm), ordering them by decreasing length within each category, and finally numbering them consecutively using the same scheme.

Parameter means were compared by one-way ANOVA after Bartlett's test of homogeneity (InfoStat 2008). Also, Tukey's test was used to examine karyotype similarity among the species. A data matrix of 18 operational taxonomic units (OTUs) × 11 karyotype variables was constructed using the data obtained in this paper and those obtained from Lavia (1998, 2001). The NTSYS-PC program developed by Rohlf (1994) was used to standardize the data matrix, to calculate the average taxonomic distance, and to generate a phenogram. Clustering was performed using the unweighted pair-group method (UPGMA). The cophenetic correlation was 0.82, indicating a good fit between the cophenetic value matrix and the mean taxonomic distance matrix.

Results

Mitotic chromosomes and idiograms are shown in Figs. 1 and 2. Karyotype formula and the parameters analysed for the species in this work and previous reports (Lavia 1998, 2001) are summarised in Table 2.

The mean chromosome length differed significantly among the species (Table 2). The chromosomes of all species are small according to the Lima de Faria classification (1980), with a mean chromosome length of 1.75 μm, with a range from 1.41 μm in *A. retusa* to 2.11 μm in *A. williamsii*.

Different types of SAT chromosomes were found (Table 2). Although the satellite was borne by different chromosome pairs, they always were located at the distal end of the long arm, except in one accession of *A. sylvestris* that was on the short arm (VRSv 10891, Fig. 2d). In accessions of *A. sylvestris* the SAT chromosomes were type 10 and corresponded to pair 1, whilst in *A. retusa* and *A. macedoi* they were type 3A and corresponded to pair 3 (Figs. 2a, b). *Arachis chiquitana* presented SAT chromosomes type 3B for pair 4 (Fig. 2i). On the other hand, *A. cruziana* and *A. simpsonii* have type 3A for pair 1 and 6, respectively (Figs. 2e, h), *A. herzogii* has type 5 for pair sm 10 (Fig. 2g), and, finally *A. williamsii* has type 6 for pair 5 (Fig. 2f).

A chromosomes were not found in sections *Extranervosae* and *Heteranthae* (Fig. 2), but were present in *A. chiquitana* belonging to section

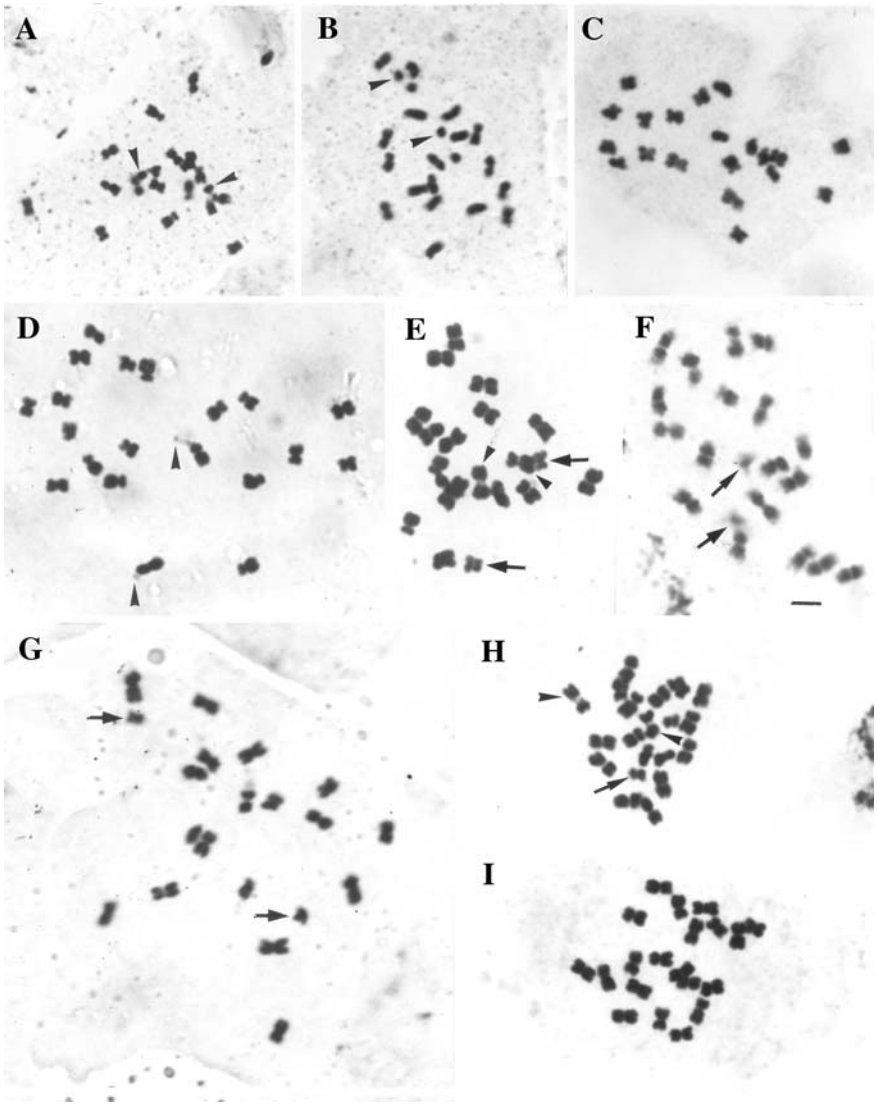

Fig. 1 Mitotic chromosomes of *Arachis* species. **a** *A. macedoi*; **b** *A. retusa*; **c** and **d** *A. sylvestris*; **e** and **f** *A. chiquitana*; **g** *A. herzogii*; **h** *A. simpsonii*; **i** *A. williamsii*. Arrowheads show satellites, arrows show A chromosomes. Bar = 2 μm

Procumbentes and in *A. herzogii* and *A. simpsonii* in section *Arachis* (Figs. 1e–h and 2g–i).

The karyotypes of *A. macedoi*, *A. retusa*, *A. sylvestris*, *A. cruziana*, *A. chiquitana*, *A. herzogii* and *A. simpsonii* are reported for the first time here, whilst the karyotype of *A. williamsii* was presented previously in Seijo et al. (2004). As a whole, the karyotypes were symmetrical, and the karyotype formula was fairly constant. *Arachis macedoi*, *A. williamsii* and *A. simpsonii* have exclusively m pairs, while the other species have karyotypes composed of m and sm chromosomes.

Intrachromosomal (A_1) and interchromosomal (A_2) asymmetry indices were plotted in a scatter diagram (Fig. 3) and were compared with those of other species of the genus previously reported (Lavia 1998, 2001). Values obtained for A_1 were similar for all species (range 0.14–0.28), but the A_2 was more variable (range 0.05–0.35).

Discussion

General karyotype characteristics

In general, *Arachis* chromosomes are small (Fernández and Krapovickas 1994; Lavia 2001), as was the case for all species studied here. Species of section

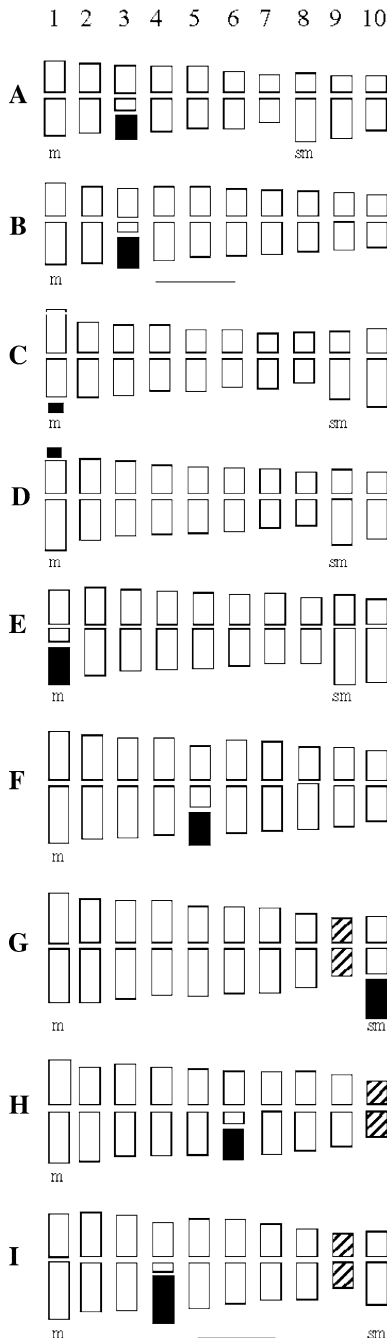

Fig. 2 Idiograms of *Arachis* species. Section *Extranervosae*: **a** *A. retusa*; **b** *A. macedoi*. Section *Heteranthae*: **c** *A. sylvestris* 8520; **d** *A. sylvestris* 10891. Section *Arachis*: **e** *A. cruziana*; **f** *A. williamsii*; **g** *A. herzogii*; **h** *A. simpsonii*. Section *Procumbentes*: **i** *A. chiquitana*. Bar = 2 μm

Extranervosae and *Heteranthae* have the shortest chromosomes, whilst the species of section *Arachis* have the longest ones. Moreover, the entities of this genus are characterized by gradual differences in chromosome size, as shown by the idiograms in Fig. 2.

According to the karyotype data previously published for *Arachis* (Fernández and Krapovickas 1994; Lavia 2000), SAT chromosomes type 10 is exclusive to section *Heteranthae*, while type 9 is exclusive to section *Procumbentes* (Fernández and Krapovickas 1994; Lavia 1996, 2001; Peñaloza and Valls 2005; present study). In the results analysed in the present report, however, *A. chiquitana* and *A. vallsii* Krapov. et W.C. Gregory in section *Procumbentes* have the 3B type. On the contrary, species in section *Arachis* have the greatest variability with SAT chromosomes type 3A, 5, 6, 7 and 8 (Fernández and Krapovickas 1994; Lavia 1996, 1998, 2000, 2001; Peñaloza and Valls 2005; present study). On this basis, it has been suggested that section *Arachis* is in dynamic process of chromosome evolution (Fernández and Krapovickas 1994).

With respect to the structural evolution of SAT chromosomes in the genus, Fernández and Krapovickas (1994) have suggested that either type 2 or 3 is the most primitive and that the other SAT chromosomes are derived types originating from inversions and translocations. However, when the different sizes and morphologies of these chromosomes are considered, deletions or additions of genetic material could have been involved in the variability of SAT chromosomes.

The A chromosomes have been found only in species of section *Arachis*, and the species with this chromosome have been included within the A genome group. An association between species with the A genome and life cycle was observed; that is, all perennial species have A genome, whilst annuals (except *A. duranensis* Krapov. et W.C. Gregory, *A. trinitensis* Krapov. et W.C. Gregory and *A. schininii* Krapov., Valls et C.E. Simpson) do not (Lavia et al. 2008). Moreover, the crossability among perennial species is higher than among annuals (Krapovickas and Gregory 1994); probably because the species that including in the A genome group are more homogeneus genomically than the species of the non A genome group.

The karyotypes presented in this work consist mainly of m and sm chromosomes, like those generally reported for the genus (Fernández and Krapovickas 1994; Lavia 1998, 2001). Subtelocentric

Table 2 Karyotype parameters for *Arachis* species

Section and species	Karyotype	Genome	SAT	TCL	ML	Size range	CI	A_1	A_2	AR	CL
Section *Trierectoides*											
A. tuberosa[1]	20m	E_1E_1	8	48.88	2.44	1.69–3.31	46.24	0.14	0.13	1.17	P
Section *Erectoides*											
A. douradiana[1]	18m + 2sm	–	2	59.29	2.96	1.87–4.93	43.96	0.20	0.21	1.31	P
Sect. *Procumbentes*											
A. appressipila[1]	14m + 6sm	E_2E_2	9	47.53	2.38	1.88–2.93	42.73	0.24	0.13	1.38	P
A. chiquitana[3]	18m + 2sm	AA	3B	41.16	2.06[a]	1.13–2.75	44.25	0.19	0.20	1.29	P
A. subcoriacea[1]	18m + 2sm	–	9	46.07	2.30	1.54–3.09	42.93	0.23	0.05	1.38	P
A. vallsii[1]	18m + 2sm	–	3B	35.39	1.77	1.25–2.42	44.61	0.18	0.32	1.26	P
Section *Extranervosae*											
A. macedoi[3]	20m	ExEx	3	30.56	1.52[c]	1.06–2.46	44.35	0.19	0.27	1.27	P
A. retusa[3]	14m + 6sm	ExEx	3	28.24	1.41[c]	0.91–1.83	40.76	0.28	0.35	1.54	P
Section *Heteranthae*											
A. sylvestris											
10891[3]	16m + 4sm	AmAm	10	32.08	1.60[c]	1.18–2.31	43.62	0.22	0.20	1.31	A
8520[3]	16m + 4sm	AmAm	10	30.70	1.53[c]	1.06–2.57	42.37	0.25	0.23	1.40	A
Section *Arachis*											
A. cruziana[3]	18m + 2sm	BB	3	37.10	1.85[b]	1.32–2.57	42.05	0.25	0.08	1.43	A
A. decora[4]	16m + 2sm	–	3	33.66	1.87	1.37–2.39	45.41	0.22	0.16	1.32	A
A. herzogii[3]	18m + 2sm	AA	5	40.72	2.04[a]	1.06–2.75	43.79	0.20	0.13	1.34	P
A. palustris[2]	16m + 2sm	–	3	33.23	1.85	1.35–2.35	43.64	0.22	0.17	1.31	A
A. praecox[2]	16m + 2sm	–	3	35.28	1.96	1.65–2.25	43.47	0.23	0.12	1.32	A
A. simpsonii[3]	20m	AA	3	38.05	1.90[a]	1.06–2.57	45.41	0.16	0.17	1.22	P
A. stenosperma[4]	18m + 2sm	AA	3	44.6	2.23		44.60	0.19	0.20		P
A. williamsii[3]	20m	BB	6	42.15	2.11[a]	1.39–2.90	44.49	0.20	0.13	1.26	A
F (ANOVA)					80.34		3.85	3.39		4.43	
P					<0.05		<0.05	<0.05		<0.05	

Karyotype, SAT chromosome type (SAT), total chromosome length (TCL) in μm, mean length by chromosome (ML) in μm, size range, centromeric index (CI), intrachromosomic asymmetry index (A_1), interchromosomic asymmetry index (A_2), arm ratio (AR) cycle life (CL), perennial (P) and annual (A)

Note: Values in column ML followed by the same letter are not significantly different

[1] Lavia (2001)

[2] Lavia (1998)

[3] Present work

[4] Lavia (unpublished)

chromosomes are unusual in *Arachis*; however, *A. batizocoi* Krapov. et W.C. Gregory has only one pair (Fernández and Krapovickas 1994) and *A. glandulifera* Stalker has six (Fernández and Krapovickas 1994) or four pairs (Robledo and Seijo 2008). According to the karyotypes of the species studied here and those of Fernández and Krapovickas (1994) and Lavia (1998, 2001), the karyotype formula within the genus and within the sections is variable and includes 20m, 18m + 2sm, 16m + 4sm, 14m + 6sm, 12m + 8sm, 12m + 6sm + 2st and 8m + 12st; the most frequent are 16m + 4sm, 18m + 2sm and 20m. The greatest variability was observed within section *Arachis*, with five different types of formula.

The centromeric index indicates that all karyotypes are moderately symmetric and fall into category 1A of the Stebbins's asymmetry classification

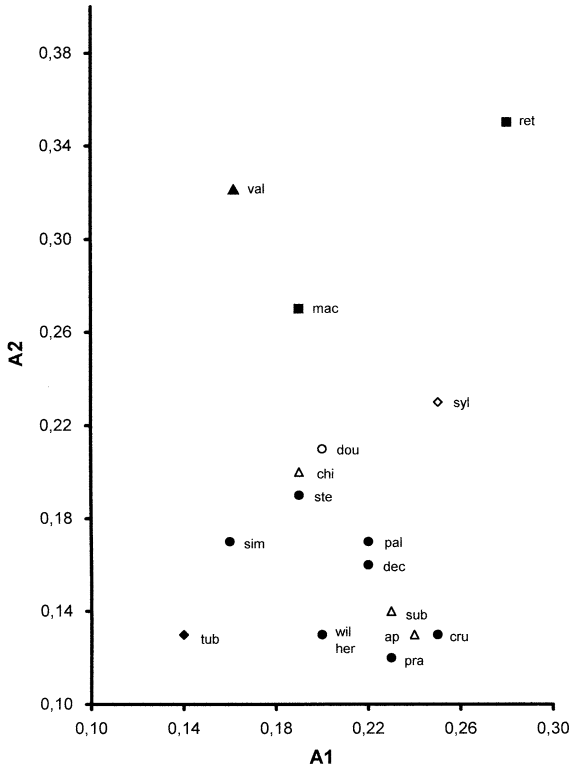

Fig. 3 Scatter diagram showing the relation between intrachromosomal (A_1) and interchromosomal (A_2) asymmetry indices for *Arachis* species. Values of A_1 and A_2 are summarized in Table 2. Symbols: ◆ section *Trierectoides*, ○ section *Erectoides*, ■ section *Extranervosae*, ◇ section *Heteranthae*, △ section *Procumbentes*, ● section *Arachis*. Abbreviations: app = *A. appressipila*, chi = *A. chiquitana*, cru = *A. cruziana*, dec = *A. decora*, dou = *A. douradiana*, her = *A. herzogii*, mac = *A. macedoi*, pal = *A. palustris*, pra = *A. praecox*, ret = *A. retusa*, sim = *A. simpsonii*, ste = *A. stenosperma*, sub = *A. subcoriacea*, syl = *A. sylvestris*, tub = *A. tuberosa*, val = *A. vallsii*, wil = *A. williamsii*

(Stebbins 1971). Values obtained for A_1 indicate little variation among the chromosome arms in the different taxa; however, data for the A_2 indices show differences among the size of the different chromosomes in each taxa (Table 2). Figure 4 shows that the karyotype of *A. retusa* (ret) is the least symmetrical with higher variation in length among its chromosomes, whereas *A. tuberosa* Bong. ex Benth. (tub) is the most symmetrical.

The phenogram in Fig. 4, that includes present and previous data (Lavia 1998, 2001), shows the following clusters: (1) *A. douradiana* Krapov. et W.C. Gregory (section *Erectoides*), characterised by the longest chromosomes (2.96 µm). (2) *A. macedoi*, *A. retusa*

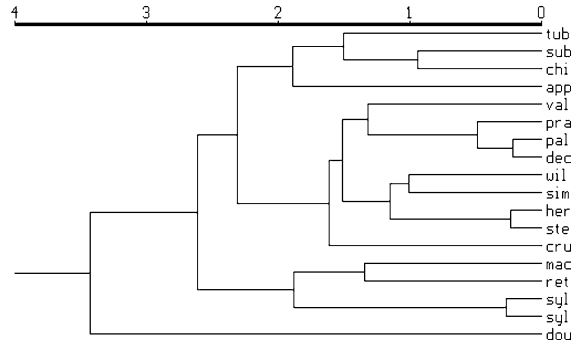

Fig. 4 Phenogram of unweighted-pair group method (UPGMA) derived from average taxonomic distance for *Arachis* species. Cophenetic correlation coefficient (*r*) 0.82. Abbreviations: app = *A. appressipila*, chi = *A. chiquitana*, cru = *A. cruziana*, dec = *A. decora*, dou = *A. douradiana*, her = *A. herzogii*, mac = *A. macedoi*, pal = *A. palustris*, pra = *A. praecox*, ret = *A. retusa*, sim = *A. simpsonii*, ste = *A. stenosperma*, sub = *A. subcoriacea*, syl = *A. sylvestris*, tub = *A. tuberosa*, val = *A. vallsii*, wil = *A. williamsii*

(section *Extranervosae*) and *A. sylvestris* (section *Heteranthae*) with a mean chromosome length of 1.51 µm, separated from species of the other sections because of their smallest chromosomes. Within this cluster, the species of each section separated into two subgroups: section *Extranervosae* and section *Heteranthae*. (3) All species of section *Arachis* and *A. vallsii* (section *Procumbentes*), mean chromosome length of 1.93 µm. In this group, the species of *Arachis* section with $x = 9$ (*A. praecox* Krapov., W.C. Gregory et Valls, *A. palustris* Krapov., W.C. Gregory et Valls and *A. decora* Krapov., W.C. Gregory et Valls) form a subgroup, and the entities with basic chromosome number $x = 10$ (*A. stenosperma*, *A. williamsii*, *A. herzogii* and *A. simpsonii*) constitute another subgroup, except for *A. cruziana* Krapov., W.C. Gregory et C.E. Simpson (section *Arachis* with $x = 10$), which is by itself and characterized by the presence of 4 sm chromosomes. (4) *A. tuberosa* (section *Trierectoides*) and *A. subcoriacea* Krapov. et W.C. Gregory, *A. chiquitana* and *A. appressipila* Krapov. et W.C. Gregory (section *Procumbentes*), mean chromosome length of 2.29 µm. The entities that compose the groups and subgroups mainly belong to the same sections; therefore, their grouping by chromosomal features agrees with the actual taxonomic subgeneric classification.

Consequently, in a detailed analysis of karyotypes, we did not find any chromosome features that were diagnostic for the sections, but a combination of

features—chromosome mean length, presence of A chromosomes, type of SAT chromosomes, karyotype formula and the asymmetry indices—allows us to differentiate the species. Hence, the bulk of the chromosome features are useful in karyologically distinguishing the species in the genus *Arachis*.

Reassessment of *A. chiquitana* and *A. vallsii*

Species included in section *Procumbentes* are characterized by a mean chromosome size ~2.30 μm, SAT chromosomes type 9 and lack of A chromosomes (Fernández and Krapovickas 1994; Peñaloza and Valls 2005; Lavia 2000). However, two exceptional cases were found.

Arachis chiquitana, included in section *Procumbentes*, presents A chromosomes and SAT chromosome type 3B, which are not characteristic of section *Procumbentes*. The presence of A chromosomes is exclusive of section *Arachis*. Additionally, *A. chiquitana* grouped with *A. duranensis*, *A. kuhlmannii* Krapov. et W.C. Gregory and *A. stenosperma* (all species from section *Arachis* with A chromosomes) in a phenogram based on reproductive and vegetative traits (Stalker et al. 1991). Therefore, the position of *A. chiquitana* within section *Procumbentes* must be revised, and we suggest it be placed in section *Arachis*.

On the other hand, *A. vallsii*, included in section *Procumbentes*, presents SAT chromosome type 3B and a mean chromosome length of 1.77 μm. Considering that both chromosomal characters are not characteristic of section *Procumbentes* and that this species differs morphologically from the other species of this section, *A. vallsii* should be removed from section *Procumbentes* (Lavia 2001); moreover, taking on account morphological and chromosomal features we propose including it in section *Arachis*.

Chromosome evolution

Eukaryotic chromosomes can vary in size, shape, number and redundancy. These features are subject to evolutionary changes and thus may vary between and even within individual organisms (Schubert 2007). In certain taxonomic groups is a natural tendency to produce changes in chromosomal complement by reiteration of the same cytogenetical mechanism; this process is karyotypic orthoselection (White 1965).

Arachis species have been shown to be diverse by morphological analysis (Krapovickas and Gregory 1994) and molecular and biochemical markers (Stalker et al. 1994; Moretzsohn et al. 2004). Concerning chromosome numbers, *Arachis* has two basic chromosome numbers, $x = 9$ and $x = 10$, with somatic numbers of $2n = 18$ (5% of species), $2n = 20$ (89% of species) and $2n = 40$ (6% of species). The existence of some species with $2n = 40$ and $2n = 18$ shows that polyploidy and aneuploidy or dysploidy have been involved in the evolution of *Arachis* species, but these numerical changes have not played a very important role in the diversification of the genus.

The size of the chromosome is also a feature subject to evolutionary change. This variation could originate by reciprocal translocation, loss of dispensable parts, insertion, and sequence amplification (Schubert 2007). The direction of chromosome evolution could be toward an increase (Brandham and Doherty 1998) or decrease in chromosome size (Martel et al. 2004). The size of the chromosomes varies more than twofold in *Arachis* species, between 3.09 μm in *A. major* (Fernández and Krapovickas 1994), and 1.41–1.60 μm in species of section *Extranervosae* and *Heteranthae* (present work). In *Arachis*, the short chromosomes should be a more ancestral character than longer ones because (1) *Extranervosae* and *Heteranthae* are considered among the oldest sections (Krapovickas and Gregory 1994), and their species have the smallest chromosomes, and (2) *Arachis* and *Procumbentes* sections are considered among the most advance and their species have larger chromosomes (Lavia 1998, 2001; present work). This suggestion is congruent with the proposal that species with a lower DNA content are more primitive (Singh et al. 1996; Lavia and Fernández 2008). An exception to this general trend is section *Trierectoides*, which in spite of being considered as the most ancestral by exomorphology (Krapovickas and Gregory 1994), has high values for chromosome length.

The *Arachis* karyotypes are moderately symmetrical and consist almost exclusively of m and sm chromosomes (Fernández and Krapovickas 1994; Lavia 1998, 2001, present study). The interspecific differences in chromosomes in the genus would not be attributable, for the most part, to large translocations because previous meiotic studies reported

normal bivalent configurations without multivalent ones in most interspecific hybrids (Fernández and Lavia unpublished; Tallury et al. 2005), except for quadrivalents reported in hybrids between *A. duranensis* and *A. villosa* Benth., *A. correntina* (Burkart) Krapov. et W.C. Gregory, *A. diogoi* Hoehne, *A. stenosperma* and *A. cardenasii* Krapov. et W.C. Gregory (Singh and Moss 1984). These species, however, all belong to section *Arachis*, and homologous chromosomes could have associated to form the quadrivalents. Therefore, the accumulation of smaller chromosome rearrangements derived from cryptic structural changes may be the reason for much of the variation in chromosome types and asymmetry in the genus *Arachis*.

One of the chromosomal parameters most used to unravel evolution in plants is chromosomal symmetry. Symmetrical karyotypes are widely accepted to be more primitive than asymmetrical ones, when the comparison is between the same groups of vascular plants (Stebbins 1971); but this rule is not constant because reverse cases have been observed (Jones 1970). In *Arachis*, an association between asymmetry and advanced taxa was not detected nor an association between asymmetry and shorter total genome length as found in *Lathyrus* in section *Notholathyrus* (Seijo and Fernández 2003). On the contrary, the most asymmetrical karyotype (*A. retusa*) (Fig. 3) has the shortest total chromosome length, and a group with more symmetrical karyotypes ($A_1 = 0.16$–0.25, $A_2 = 0.12$–0.21), constituted by species of sections *Arachis* and *Procumbentes*, sections considered among the most advanced, have intermediate chromosome length. Therefore, the possible evolutionary trend in *Arachis* would be, from species with smallest chromosomes and more asymmetrical karyotypes in sections considered more ancestral, to species with longer chromosomes and more symmetrical karyotypes. Therefore, increases in chromosome length are accompanied by increases in karyotype symmetry. The extra DNA probably was unequally distributed in the chromosome arms of the complement, giving rise to species with larger chromosomes and more symmetrical karyotypes in the advanced sections.

In conclusion, during the diversification of the genus *Arachis*, some changes in chromosome numbers have occurred ($2n = 18$, 20, 40) and the chromosome length would have increased but without great changes in chromosome morphology. Therefore, despite the increase in chromosome size, the *Arachis* genus has maintained karyotype uniformity (all species present almost exclusively m and sm chromosomes) because the addition of DNA in the chromosomes could have been nonrandom via the process of karyotypic orthoselection.

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References

Brandham PE, Doherty MJ (1998) Genome size variation in the Aloaceae, an angiosperm family displaying karyotypic orthoselection. Ann Bot (Lond) 82:67–73. doi:10.1006/anbo.1998.0742

Cao W (2003) Cytogenetic and molecular genetic evidence on evolution of genus *Triticum*. In: Sharma AK, Sharma A (eds) Plant genome. Biodiversity and evolution. vol 1A: Phanerogam—Angiosperm. Science Publishers, Enfield (NH), USA, pp 223–247

Custodio AR, Peñaloza APS, Valls JFM (2005) Further cytogenetic information on *Arachis stenosperma* (Leguminosae). Cytologia (Tokyo) 70:331–335. doi:10.1508/cytologia.70.331

Fernández A, Krapovickas A (1994) Cromosomas y evolución en *Arachis* (Leguminosae). Bonplandia 8:187–220

Holbrook CC, Stalker HT (2003) Peanut breeding and genetic resources. In: Janick J (ed) Plant breeding reviews, vol 22. Wiley, Hoboken, pp 297–356

Husted L (1933) Cytological studies on the peanut, *Arachis*. I. Chromosome number and morphology. Cytologia (Tokyo) 5:109–117

InfoStat (2008) InfoStat versión 2008. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina

Jauhar PP (2006) Modern biotechnology as an integral supplement to conventional plant breeding: the prospects and challenges. Crop Sci 46:1841–1859. doi:10.2135/cropsci2005.07-0223

Jones K (1970) Chromosome changes in plant evolution. Taxon 19:172–179. doi:10.2307/1217950

Krapovickas A, Gregory WC (1994) Taxonomía del género *Arachis* (Leguminosae). Bonplandia 8:1–186

Lavia GI (1996) Estudios cromosómicos en *Arachis* (Leguminosae). Bonplandia 9:111–120

Lavia GI (1998) Karyotypes of *Arachis palustris* and *A. praecox* (section *Arachis*), two species with basic chromosome number $x = 9$. Cytologia (Tokyo) 63:177–181

Lavia GI (2000) Chromosome studies in wild *Arachis* (Leguminosae). Caryologia 53:277–281

Lavia GI (2001) Chromosomal characterization of germplasm of wild species of *Arachis* L. belonging to sections *Trierectoides*, *Erectoides* and *Procumbentes*. Caryologia 54:115–119

Lavia GI, Fernández A (2004) Karyotypic studies in *Arachis hypogaea* L. varieties. Caryologia 57:353–359

Lavia GI, Fernández A (2008) Genome size in wild and cultivated peanut germplasm. Plant Syst Evol 272:1–10. doi:10.1007/s00606-007-0632-0

Lavia GI, Fernández A, Seijo JG (2008) Cytogenetic and molecular evidences on the evolutionary relationships among *Arachis* species. In: Sharma AK, Sharma A (eds) Plant genome. Biodiversity and evolution. vol 1E: Phanerogam—Angiosperm. Science Publishers, Calcutta, Kolkata, India, pp 101–134

Lima de Faría A (1980) Classification of genes, rearrangements and chromosomes according to the field. Hereditas 93:1–46

Martel E, Poncet V, Lamy F, Siljak-Yakovlev S, Lejeune B, Sarr A (2004) Chromosome evolution of *Pennisetum* species (Poaceae): implications of ITS phylogeny. Plant Syst Evol 249:139–149. doi:10.1007/s00606-004-0191-6

Mercado-Ruaro P, Delgado-Salinas A (1998) Karyotypic studies on species of *Phaseolus* (Fabaceae: Phaseolinae). Am J Bot 85:1–9. doi:10.2307/2446547

Moretzsohn MC, Hopkins MS, Mitchell SE, Kresovich S, Valls JFM, Ferreira ME (2004) Genetic diversity of peanut (*Arachis hypogaea* L.) and its wild relatives based on the analysis of hypervariable regions of the genome. BMC Plant Biol 4:11. http://www.biomedcentral.com/1471-2229/4/11. doi:10.1186/1471-2229-4-11

Peñaloza APS, Valls JFM (2005) Chromosome number and satellited chromosome morphology of eleven species of *Arachis* (Leguminosae). Bonplandia 15:65–72

Peñaloza APS, Pozzobon MT, Valls JFM (1996) Cytogenetic findings in wild species of *Arachis* (Leguminosae). Proceedings of the 42nd Congresso Nacional de Genética, Caxambu, Brasil 42:42

Poggio L, González G, Naranjo CA (2007) Chromosome studies in *Hippeastrum* (Amaryllidaceae): variation in genome size. Bot J Linn Soc 155:171–178. doi:10.1111/j.1095-8339.2007.00645.x

Robledo G, Seijo GJ (2008) Characterization of *Arachis* D genome by FISH chromosome markers and total genome DNA hybridization. Genet Mol Biol 31:717–724

Rohlf FJ (1994) NTSYS-pc. Numerical taxonomy and multivariate analysis system, version 1.8. Exeter Software, New York, USA

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

Schubert I (2007) Chromosome evolution. Curr Opin Plant Biol 10:109–115. doi:10.1016/j.pbi.2007.01.001

Seijo JG, Fernández A (2003) Karyotype analysis and chromosome evolution in South American species of *Lathyrus* (Leguminosae). Am J Bot 90:980–987. doi:10.3732/ajb.90.7.980

Seijo JG, Lavia GI, Fernández A, Krapovickas A, Ducasse D, Moscone EA (2004) Physical mapping of 5S and 18S-25S rRNA genes evidences that *Arachis duranensis* and *A. ipaensis* are the wild diploid species involved in the origin of *A. hypogaea* (Leguminosae). Am J Bot 91:2293–2303. doi:10.3732/ajb.91.9.1294

Singh AK, Moss JP (1984) Utilization of wild relative in genetic improvement of *Arachis hypogaea* L. Theor Appl Genet 68:355–364. doi:10.1007/BF00267889

Singh KP, Raina SN, Singh AK (1996) Variation in chromosomal DNA associated with the evolution of *Arachis* species. Genome 39:890–897. doi:10.1139/g96-112

Smartt J, Gregory WC, Gregory MP (1978) The genomes of *Arachis hypogaea*. 1. Cytogenetic studies of putative genome donors. Euphytica 27:665–675. doi:10.1007/BF00023701

Stalker HT (1991) A new species in section *Arachis* of peanuts with a D genome. Am J Bot 78:630–637. doi:10.2307/2445084

Stalker HT, Dhesi JS, Parry DC, Hahn JH (1991) Cytological and interfertility relationships of *Arachis* section *Arachis*. Am J Bot 78:238–246. doi:10.2307/2445247

Stalker HT, Philips TD, Murphy JP, Jones TM (1994) Variation of isozyme patterns among *Arachis* species. Theor Appl Genet 87:746–755. doi:10.1007/BF00222901

Stebbins GL (1971) Chromosomal evolution in higher plants. Edward Arnold, London

Tallury SP, Hilu KW, Milla SR, Friend SA, Alsaghir M, Stalker HT, Quandt D (2005) Genomic affinities in *Arachis* section *Arachis* (Fabaceae): molecular and cytogenetic evidence. Theor Appl Genet 111:1229–1237. doi:10.1007/s00122-005-0017-0

Valls JFM, Simpson CE (2005) New species of *Arachis* (Leguminosae) from Brazil, Paraguay and Bolivia. Bonplandia 14:35–64

White MJD (1965) Principles of karyotype evolution in animals. In: Genetics Today. Proceedings of the XI international congress of genetics, The Hague, 1963, 391–397