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Classical and Molecular Cytogenetics in *Arachis*

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ABSTRACT

The progress that has been achieved in understanding the chromosome complements of peanut and its related wild species is reviewed here. Chromosome markers developed by using fluorescent *in situ* hybridization (FISH) with rDNA probes and fluorescent banding allowed the construction of the first chromosome map in peanut and in some diploid taxa. They also revealed the existence of different karyotype structures within the section *Arachis*. Each group of species having different karyotype structure was defined as having different genomes. FISH analysis together with genomic *in situ* hybridization allowed the differentiation of chromosomes from *A. duranensis* and *A. ipaënsis,* the two species involved in the origin of cultivated peanut. The results obtained by these techniques also suggest that the wild tetraploid *A. monticola* is the direct ancestor of peanut and that the Northwest of Argentina and South of Bolivia are the regions from which the AABB tetraploids of section *Arachis* have arisen.

Keywords: Chromosome numbers, karyotypes, FISH markers, genetic and geographic origin, genomes

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3.1 Introduction

Arachis hypogaea (v.n. "peanut", "groundnut") is a cultigen that has become the third most important grain legume crop of the world (Duke 1981) because of its multiple use as human food, vegetable oil, feedstock and ground cover (Wynne and Halward 1989). This species is adapted to a wide ecological range of tropical and subtropical regions and is cultivated under diverse agricultural production systems in Asia, Africa and the Americas (Holbrook and Isleib 2001).

On the bases of morphological features, crossing experiments and seed protein electrophoretic profiles, Krapovickas and Gregory (1994) recognized two subspecies within the cultigen, *hypogaea* and *fastigiata*. Additionally, six botanical varieties have been described, two of them within subsp. *hypogaea* (*hypogaea* and *hirsuta*) and four within subsp. *fastigiata* (*fastigiata*, *aequatoriana*, *peruviana*, and *vulgaris*). Moreover, there are numerous landraces within varieties that are very diverse in their vegetative and reproductive traits (cf. Krapovickas and Rigoni 1960; Grosso et al. 1994; Krapovickas and Gregory 1994; Krapovickas et al. 2009).

Peanut genetic resources are available in the form of naturally evolved landraces of cultivated peanut in the various centers of diversity, breeding lines or materials developed in different peanut producing areas, and also the numerous wild *Arachis* species. In spite of the morphological variability, 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 for the rational development of breeding programs and complete utilization of the wild materials.

The genus *Arachis* is a native of South America and naturally distributed over a large area that extends from the eastern foothills of the Andes Mountains in Bolivia and northern Argentina to the Atlantic coast in Brazil and from the southern limit of the Amazonian rainforest towards the northern coast of La Plata River in Uruguay. The species grow spontaneously from sea level on the Atlantic coast in Brazil and Uruguay to around 1,450 m a.s.l. in the Andes Mountains of Northwest Argentina (Krapovickas and Gregory 1994; Valls and Simpson 2005). Along this area wild *Arachis* species are adapted to a wide variety of habitats. Their ecological preferences range from the xerophytic forests to temporarily flooded areas, and from temperate grasslands to open patches of the subtropical rainforest. Soil preferences are also diverse, ranging from rock outcrops, layers of laterite pebble, heavy soils, poorly drained areas to well drained sandy soils. In spite of the ample

range of ecological preferences displayed by the wild species, the genus as a whole is mainly associated with the savannah-like Cerrado biogeographical region as defined by Cabrera and Willink (1973).

The genus consists of a diverse group of 80 autogamous (some probably with variable percentages of allogamy) and geocarpic taxa (Krapovickas and Gregory 1994; Valls and Simpson 2005). According to morphology, cross-compatibility, viability of the hybrids, geographic distribution and cytogenetics, they have been taxonomically arranged in nine sections —Trierectoides, Erectoides, Procumbentes, Rhizomatosae, Heteranthae, Caulorrhizae, Extranervosae, Triseminatae and Arachis (Krapovickas and Gregory 1994). Trierectoides is considered to be the most ancestral section since the species included in it have tuberous hypocotyls or roots, trifoliated leaves and vaginated stipules, resembling those characters present in the genus *Stylosanthes*. On the other hand, since section Arachis has species with annual and perennial life cycles, with different basic chromosome numbers, ploidy levels and karyotype structures it is considered as the most diverse and derived. Between these two sections, species that belong to sections Erectoides and Procumbentes seem to be the most related to those of section Arachis. Some of the members of sections Rhizomatosae, Heteranthae and Caulorrhizae may produce hybrids with the most derived sections, but others show a strong genetic isolation. Sections Extranervosae and Triseminatae are the most isolated, and their evolutionary position still has to be determined (Krapovickas and Gregory 1994).

Among all the sections, Arachis has received particular attention because it contains the cultivated peanut and its putative wild progenitors. In accordance with its status as the most evolutionarily derived section, geographically it is the most widely distributed. It extends in an east–west direction between the Chapada dos Parecis in the central west of Mato Grosso State (Brazil) and the northern edge of the Chacoan region. From this latitudinal central axis, in the east, the species extend towards the northeast along the Tocantins River (central Brazil) and southward along the Paraguay–Paraná and Uruguay River Basins (Paraguay, Argentina and Uruguay) reaching the northern shore of La Plata River. In the west, they are found towards the northwest along the Mamoré and Guaporé Rivers in north Bolivia and towards the southwest along the Parapetí, Pilcomayo, Bermejo, San Francisco and Juramento River Basins in southern Bolivia and northern Argentina (Krapovickas and Gregory 1994).

Cytogenetics has played a main role among the biosystematic studies carried out in the genus. Many classical cytological studies have been performed in *Arachis*, which delivered important information about the complexity of the peanut genome. The chromosome numbers and the karyotype features of the species as well as the study of the meiotic divisions of interspecific hybrids have provided irreplaceable information to establish

the relationships among species and the taxonomic sections. More recently, chromosome studies using fluorescent banding and *in situ* hybridization of DNA have been important to reveal the genetic origin of the cultigen, to provide a more realistic genome arrangement of the section Arachis and to understand the chromosome structure and genome evolution within the genus.

3.2 Chromosome Numbers

Classical cytogenetic studies began as early as the 1930s decade when the chromosome number of $2n = 40$ (Fig. 3-1a) for the cultigen *A. hypogaea* was determined (Husted 1933, 1936). After these pioneer data, several authors have reported the chromosome numbers for different species, although most of them included few entities and generally those that were considered most related to peanut (Gregory 1946; Mendes 1947; Smartt et al. 1978; Singh and Moss 1982; Smartt and Stalker 1982; Stalker 1991). High-quality chromosome plates from mitotic divisions, however, were obtained only
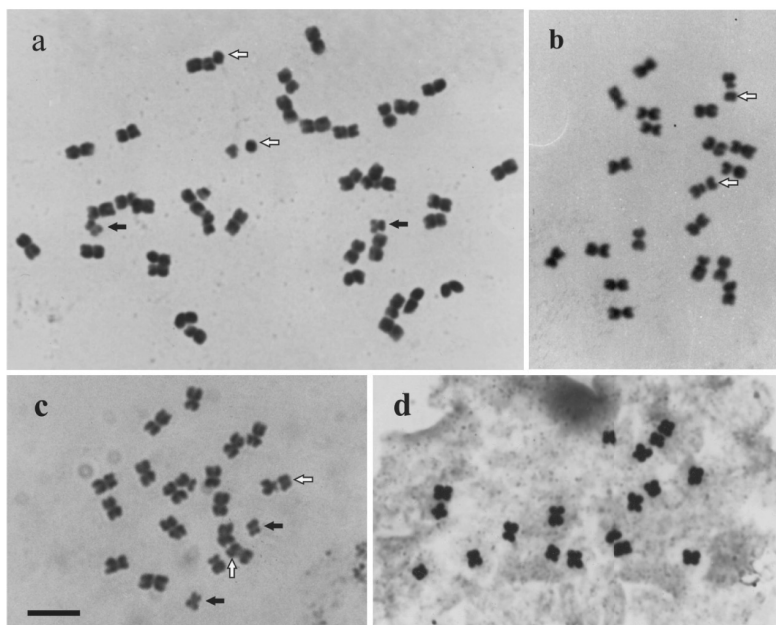

Figure 3-1 Mitotic metaphases of *Arachis* species stained with the Feulgen's technique. **(a)** *Arachis hypogaea* ($2n = 40$) showing A chromosomes and SAT chromosomes type 3. **(b)** *A. ipaënsis* ($2n = 20$) without "A" chromosomes. **(c)** *A. duranensis* ($2n = 20$) showing A chromosomes and SAT chromosomes type 6. **(d)** *A. praecox* with $2n = 18$ chromosomes. Black arrows indicate A chromosomes and white arrows the satellites of SAT chromosomes. Scale bar = 5 μm for all the pictures.

after Fernández and Krapovickas (1994). These authors carried out the most comprehensive work on cytogenetics by classical techniques in *Arachis* analyzing 41 species belonging to eight out of the nine recognized sections. Further contributions made in the last two decades elevated the knowledge of chromosome numbers to 95% of the species of the genus (Lavia 1996, 1998, 2000, 2001; Peñaloza et al. 1997; Lavia and Fernández 2004; Custodio et al. 2005; Peñaloza and Valls 2005). All these studies have revealed that the genus is dibasic, with a great predominance of the species with $x = 10$ (Fig. 3-1b and c) and only four species with $x = 9$ (Fig. 3-1d) (Lavia 1996, 1998; Peñaloza and Valls 1997; Peñaloza and Valls 2005). Three of the latter belong to section Arachis while the remaining species belongs to section Erectoides. They have also revealed that most of the species are diploid and few are polyploids with $2n = 4x = 40$. The latter are restricted to section Arachis, with *A. hypogaea* and *A. monticola*, and to sect. Rhizomatosae with *A. glabrata*, *A. pseudovillosa* and *A. nitida* (Gregory et al. 1973; Fernández and Krapovickas 1994; Peñaloza and Valls 2005). *Arachis pintoi* (section Caulorrhizae) is the only known species of the genus with diploid and triploid cytotypes (Lavia et al. 2011).

3.3 Karyotypes

Chromosome identification in peanut began with Husted (1933), who distinguished two pairs of chromosomes: one of them that borne a secondary constriction (SAT chromosome) and the other that has around half the size of the other chromosomes of the complement (A chromosome) (Fig. 3-1a). Based on these observations and in the meiotic behavior it was established that *A. hypogaea* is an allotetraploid species with two A and two B sets of chromosomes (Smartt et al. 1978). Several investigations carried out on different peanut varieties (D'Cruz and Tankasale 1961; Singh and Moss 1982; Stalker and Dalmacio 1986; Fernández and Krapovickas 1994; Lavia and Fernández 2004) have shown that the karyotypes are highly symmetric with a predominance of *metacentric* chromosomes. The most common karyotype formula found is 38*m* + 2*sm*, although in some landraces of the *fastigiata* and *hypogaea* botanical varieties the formula 36*m* + 4*sm* has been reported (Fernández and Krapovickas 1994; Lavia and Fenández 2004). The average size of chromosomes is 1.88 µm ranging from 0.92 to 2.80 µm and it is impossible to distinguish the A genome chromosomes from those of the B genome. Only one pair of chromosomes with satellites is usually distinguished in all the varieties, although the morphology of these pairs varies among the landraces (Lavia and Fernández 2004). In spite of these minor differences, the karyotypes of the varieties and subspecies are very similar. Even the karyotype described for *A. monticola*, which is a wild tetraploid

closely related to peanut, is indistinguishable from that of the cultigen (Fernández and Krapovickas 2004).

The chromosomes of diploid wild *Arachis* species are also relatively small and their size ranges from 1.11 to 4 µm. The karyotypes are completely or mainly composed of metacentric chromosomes. One or two pairs (up to six only in *A. glandulifera*) of submetacentric or subtelocentric chromosomes can be also present in the karyotypes of some species. In general, the karyotypes are moderately symmetric and fall into the 1A category of the Stebbins's asymmetry classification (Stebbins 1971). The chromosomes within a particular karyotype are very similar to each other and only two types of them are clearly distinguished. One of these types corresponds to the SAT chromosomes, which bear a conspicuous secondary constriction and a satellite ~~of different size~~ (Fig. 3-1b). The other type corresponds to small A chromosomes, which are present only in a group of species (Fig. 3-1c). Almost all species have only one pair of secondary constrictions localized on the long arms of ~~m or sm~~ metacentric or submetacentric chromosomes, which delimits satellites of different sizes. Eleven types of SAT chromosomes have been identified in *Arachis* on the basis of the relative size of the satellites and the proximal segment of the chromosome arm. The variability found in the morphology of these chromosomes has been used to develop a scheme of species arrangement (Fernández and Krapovickas 1994) and was useful to delimit the taxonomic sections.

All the species show allocyclic condensation of the chromosomes. Chromatin condensation begins in the centromeric region and extends toward the telomeres. This phenomenon is particularly observed in A chromosomes, which usually has its distal region uncondensed until late metaphase (Fernández and Krapovickas 1994). In general, secondary constrictions are extended at prophase and early metaphase, and the satellites remain far from the corresponding proximal segments of the chromosome arms; a fact that has frequently conducted to errors in chromosome counts. In late metaphase the chromosomes are uniformly stained along their length and secondary constrictions are very short.

3.4 Chromosome Banding

The first attempt to provide additional chromosome markers involved the application of Giemsa C-banding in a few set of species (Cai et al. 1987). This analysis has revealed large centromeric bands in all the chromosomes of diploid species of the ~~A and non-A genome of~~ section Arachis, of the peanut, and of *A. rigonii* of section Procumbentes. Several interstitial and terminal bands have been also identified in different chromosomes of the species included in that analysis. Giemsa C-banding was later applied to a few other species belonging to different sections (Pierozzi et al. 2001)

also revealing a high predominance of pericentromeric bands. In general, C-banding was helpful to identify unequivocally 3–4 chromosome pairs in addition to the A and the SAT chromosomes.

Counterstaining with DAPI after FISH treatment produced a banding pattern that reveal differences in chromosome structure among different groups of species (Raina and Mukai 1999; Seijo et al. 2004). As opposed to the C-banding, DAPI staining after FISH has revealed that in the tetraploid species, *A. hypogaea* (Fig. 3-2f) and *A. monticola*, half of the chromosomes—those belonging to the A genome—have centromeric C-DAPI$^+$ bands, while the remainder (corresponding to the B genome) have a uniform staining. All *A. hypogaea* varieties and *A. monticola* have a similar distribution and amount of C-DAPI$^+$ heterochromatin, which accounts for about 7% of the total karyotype length (Seijo et al. 2004).

Among the wild species, those included in section Arachis and Heteranthae have been the most comprehensively studied by fluorochrome banding. The heterochromatin patterns revealed the existence of different karyotypes among the species included in section Arachis. All the A genome species have a similar pattern of heterochromatic bands (Fig. 3-2d) with a total amount of heterochromatin that range from 10.28 to 14.67% of the karyotype length. Within this group, nine species have conspicuous C-DAPI$^+$ centromeric bands in all the chromosome pairs, while the others have conspicuous bands in all the chromosomes except in one or two pairs (A7 and A4) with small and faint bands or without them (Robledo et al. 2009).

By contrast, the non-A genome species showed three different patterns of heterochromatic bands (Robledo and Seijo 2010). The group composed of *A. batizocoi* (Fig. 3-2c), *A. cruziana*, and *A. krapovickasii* is characterized by having karyotypes with conspicuous centromeric bands in nine chromosome pairs and a total amount of centromeric heterochromatin that varies from 11.36 to 12.55% of the karyotype length. The karyotypes of *A. trinitensis* (Fig. 3-2a) and *A. benensis* characteristically have small and faint centromeric bands in only seven or eight chromosome pairs and the total amount of heterochromatin per complement varies from 5.89 to 7.52%. The remaining non-A genome species have karyotypes completely devoid of centromeric C-DAPI$^+$ bands. However, while *A. ipaënsis* (Fig. 3-2e) and *A. magna* are completely devoid of detectable heterochromatin, *A. gregoryi*, *A. valida*, and *A. williamsii* have one small interstitial or distal band in the short arms of pair 3. *Arachis glandulifera* (D genome) is very particular in the banding pattern since it shows large centromeric blocks in the four subtelocentric chromosomes, small centromeric blocks in the other three chromosomes, and two interstitial blocks of medium size (Fig. 3-2b). This pattern revealed that its karyotype structure differs from any other *Arachis* species (Robledo and Seijo 2008).
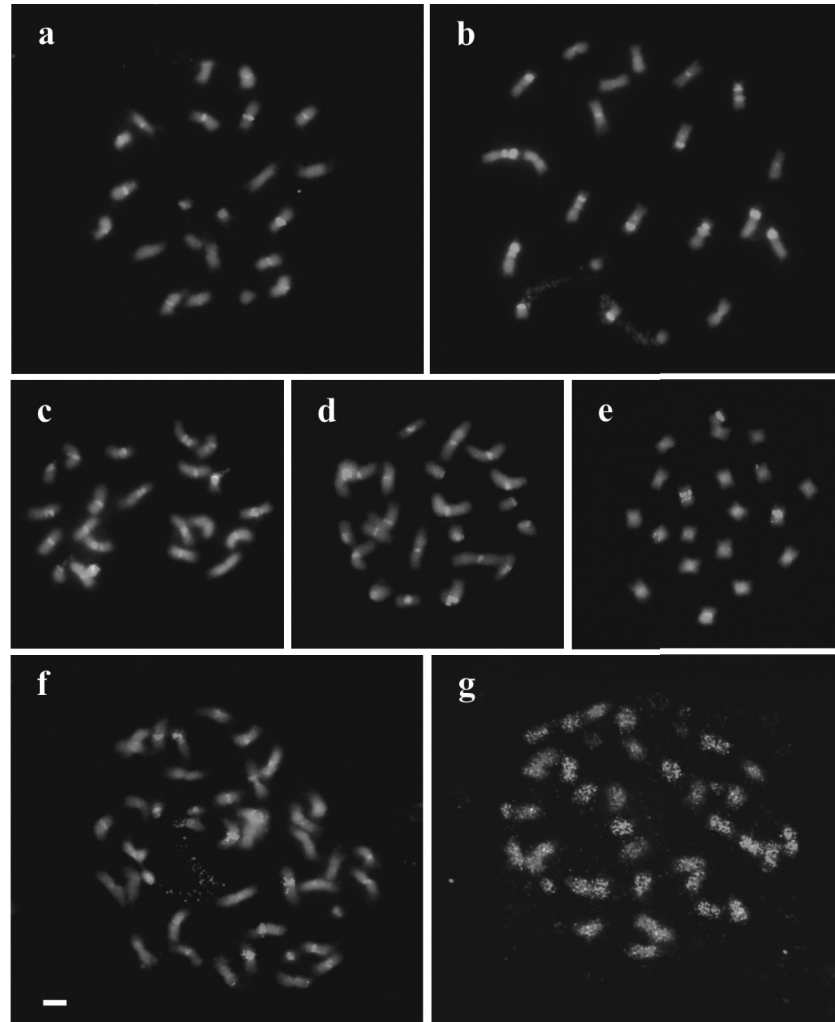
[Margin annotation: All the species with A chromosomes]

[Margin annotation: the species without A chromosomes]

Figure 3-2 Somatic metaphases of *Arachis* species following double fluorescent *in situ* hybridization (a–f), showing yellow-green FITC signals from the 5S rDNA probe and red TRITC signals from the 18S-25S rDNA probe. DAPI counterstaining (light blue) subsequent to the FISH procedure was used to highlight the heterochromatin bands and to stain euchromatin. **(a)** *A. trinitensis* (F genome). **(b)** *A. glanduifera* (D genome). **(c)** *A. batizocoi* (K genome). **(d)** *A. duranensis* (A genome). **(e)** *A. ipaënsis* (B *s.s.* genome). **(f)** *A. hypogaea* (2*n* = 40). **(g)** Somatic metaphase of *Arachis hypogaea* after double genomic *in situ* hybridization (GISH) using total DNA probes of *A. ipaënsis* (red) and of *A. duranensis* (green). Scale bar = 3 μm for all the pictures.

Color image of this figure appears in the color plate section at the end of the book.

Concerning the base composition of the heterochromatic bands found in the species of section Arachis, direct CMA/DAPI staining has demonstrated that all the centromeric ones are composed of AT-rich sequences. By contrast, ~~the~~ bands associated to the rDNA clusters, mainly those located at the secondary constrictions, are CMA_3^+ bands and thus composed of GC rich sequences. (Seijo et al. unpubli.).

As opposed to section Arachis, karyomorphological evaluation of five species of section Heteranthae (Silva et al. 2010) revealed that the pattern of GC rich heterochromatin is diverse and different from those described for section Arachis. There are species with CMA_3^+ centromeric bands in all the chromosomes and others completely deprived of such bands. $DAPI^+$ bands were only found in *A. pusilla* flanking some of the centromeric CMA_3^+ bands.

According to the available data, the analysis of the heterochromatin amount, composition and distribution by fluorochromes constitute one of techniques that provided many useful chromosome markers for the construction of karyotypes and the identification of chromosome homeologies among species. In addition, the variation in the patterns of heterochromatin that has been reported for species within and among sections reflects that it constitutes one of the most dynamic genomic fractions in the evolution of karyotypes within *Arachis*.

3.5 DNA Content

The nuclear DNA content has an important function in the evolution and adaptation of plants (Bennett 1982; Price 1976, 1988) and its comparison among related taxa contributed to clarify phylogenetic relationships and to establish evolutionary trends in several groups of organism. The 2C nuclear DNA amounts have been reported for around 40 *Arachis* species belonging to eight out of the nine taxonomic sections (Resslar et al. 1981; Singh et al. 1996; Temsch and Greilhuber 2000, 2001; Lavia and Fernández 2008). However, there are incongruences between the absolute DNA content values obtained by different authors. Most of the microdensitometric studies found that mean 2C DNA amounts varied from 10.26 to 11.82 pg among accessions of *A. hypogaea* ($2n = 4x = 40$) and from approximately 3 to 7 pg in diploid ($2n = 2x = 20$) species of the genus (Resslar et al. 1981; Singh et al. 1996; Lavia and Fernández 2008). By contrast, flow cytometric measurements, corroborated with microdensitometric analysis, indicated that *A. hypogaea* has a mean 2C = 5.914 pg and *A. monticola* a mean 2C = 5.979 pg (Temsch and Greilhuber 2000). Similar analyzes determined that *A. duranensis* has a mean 2C value of 2.63 pg that represents about half of the values obtained

in other reports (Temsch and Greilhuber 2001). Discrepancies in the DNA content have been attributed by Temsch and Greilhuber (2001) to technical measurement problems that may have remained unrecognized in the other studies; ~~however,~~ thus more research is needed to precisely determine the absolute DNA content of *Arachis* species.

Another point of debate concerns the existence of variation in DNA content among populations of any particular species. Intraspecific variation among different landraces of *A. hypogaea* has been cited by Singh et al. (1996) and by Lavia and Fernández (2008). According to these authors, the accessions belonging to *A. hypogaea* ssp. *hypogaea* (mean value 11.27 pg) with a longer life cycle have significantly larger mean DNA content than the accessions of *A. hypogaea* ssp. *fastigiata* (mean value 10.97 pg) with a shorter life cycle. However, Temsh and Greilhuber (2000) were not able to find that variation in DNA content.

By contrast, significant variation in the 2C amount of DNA has been reported among populations of *A. duranensis*, in spite of the differences in absolute values observed by different authors (Singh et al. 1996; Temsh and Greilhuber 2001). There is a negative correlation of genome size with latitude and altitude above sea level of the collection sites, and it has been postulated that this variation may be due to selection for favorable genome sizes in particular environmental conditions (Singh et al. 1996).

An overview of the published data showed that, in general, the diploid perennial species of section Arachis have about 12% more DNA than the annual species (Singh et al. 1996). Concerning the sections, the species within Procumbentes, Caulorrhizae, Rhizomatosae and Arachis sections have higher values of DNA content than those included in Erectoides, Extranervosae, Triseminatae and Heteranthae sections (Singh et al. 1996; Lavia and Fernández 2008). There is no available data for section Trierectoides. Comparisons of DNA content within section Arachis revealed a great variation in DNA content among the species, in part due to the presence of species with different basic chromosome numbers and levels of ploidy. Considering the Cx values, the genome size per haploid complement is smaller in polyploids than in the parental diploids, suggesting that a postpolyploidization reduction of DNA content has occurred (Lavia and Fernández 2008). Taking in account the whole data for the genus, it has been suggested that there is an apparent tendency to increase the genome size during the evolution of *Arachis* (Lavia and Fernández 2008), as it is the general tendency in angiosperms (Leitch et al. 1998; Soltis et al. 2004). However, the values published for section Arachis suggest that the evolution of the genome size may have been dynamics and complex, involving different cycles of expansions and reductions within particular lineages of species.

3.6 Molecular Cytogenetics

Modern cytogenetic tools, such as fluorescence *in situ* hybridization (FISH) or genomic *in situ* hybridization (GISH) techniques have helped to resolve the chromosome evolution history and enhanced the knowledge on the mechanism that conducted to the genome differentiation in *Arachis*. Localization of the 5S and 18S–26S rDNA genes on the chromosomes by FISH was initially applied to a set of species from different sections of *Arachis* (Raina and Mukai 1999) revealing their usefulness for the characterization of the group. Chromosome mapping of rDNA families by FISH was later used to analyze in detail the karyotypes of all the species included in section Arachis (Seijo et al. 2004; Robledo and Seijo 2008; Robledo et al. 2009; Robledo and Seijo 2010), to propose a new genome arrangement of them and to bring light on the genetic and geographic origin of peanut.

Physical mapping by FISH of the rDNA loci in the six botanical varieties of *A. hypogaea* (Fig. 3-2f) and in *A. monticola* revealed two pairs of 5S and five pairs of 18S–26S rDNA sites. In both species, the 5S loci are proximally located in short arms (pairs A3 and B3), while the 18S–26S rDNA clusters are proximally (pairs A2, A10, B3 and B10) or subterminally placed (B7). One 5S site is syntenic to an 18S–26S site (B3). The high degree of homeology detected between *A. monticola* and *A. hypogaea* strongly shows evidence that they are very closely related taxa. The mapping of the rDNA loci, together with the heterochromatin analysis, provided the first chromosome map for peanut, which by anchoring other different sequence base marker, will be useful to generate an integrated map for the AABB tetraploids of *Arachis*.

Concerning diploid species, those with A chromosomes have only one pair of interstitial (or rarely proximal) 5S rDNA loci located on the A3 pair (Fig. 3-2d). However, the number, size and chromosomal localization of the 18S–26S rDNA loci are variable among the species (Robledo et al. 2009). The number of these gene clusters ranges from two to four pairs (Fig. 3-2d). In general, the largest loci are located in A10 pair, those of intermediate size are borne by the A2 pair, while the smallest and faintest signals (in the cases that the species have more than two loci) were detected in the A7 and A4 chromosomes.

In spite of the general homogeneous structure of the karyotypes, according to the pattern of rDNA loci and heterochromatic bands, the species with A chromosomes have been arranged into three subgroups (Robledo et al. 2009). The first one is defined by having one or two chromosome pairs without or with small heterochromatic bands, and by three to four pairs of 18S–26S rDNA sites. This subgroup includes *A. cardenasii*, *A. herzogii* and *A. kempff-mercadoi*. The remaining species, which characteristically have heterochromatic bands in all the chromosomes, have been arranged in two different subgroups according to the number of 18S–26S rDNA sites.

One of them, includes the species with five or six sites (*A. diogoi*, *A. helodes*, *A. kuhlmannii*, *A. simpsonii* and *A. stenosperma*), and the other, is composed of the taxa with two pairs of sites (*A. correntina*, *A. duranensis*, *A. schininii* and *A. villosa*).

Arachis glandulifera ~~(D genome)~~ has only one pair of 5S rDNA loci subterminally located and five pairs of 18–26S rDNA loci (Fig. 3-2b). These markers, together with the pattern of heterochromatic bands, were sufficient to identify precisely all the chromosome pairs of the karyotype and to the construction of the first wholly-resolved idiogram for an *Arachis* species (Robledo and Seijo 2008).

FISH analysis within ~~the non-A genome species with 2*n* = 20~~ **the species without A chromosomes** revealed that all ~~the species~~ **of them** have one pair of 5S rDNA loci localized in proximal (or rarely interstitial) position on the metacentric pair 3. *Arachis cruziana*, *A. batizocoi* (Fig. 3-2c), and *A. krapovickasii* have two additional pairs of loci. The number of 18S–26S rDNA loci ranges from ~~two~~ **one** in *A. gregoryi* and *A. trinitensis* (Fig. 3-2a) to four pairs in *A. magna* **and** *A. valida*. Most of them are located in proximal or interstitial position, but few are distally or subterminally located.

3.7 **Genome Organization** of Section Arachis

The first genome constitution established within the genus *Arachis* was the AABB for *A. hypogaea* (Smartt et al. 1978). On the basis of the crossing experiments, chromosome features of the karyotypes **and** chromosome pairing in interspecific hybrids, diploid species within the section *Arachis* have been traditionally arranged into three different genome groups. The species characterized by symmetric karyotypes and the small A pair of chromosomes were included within the A genome (Smartt et al. 1978; Fernández and Krapovickas 1994). The species with symmetric karyotypes but without A chromosomes have been considered members of the non-A or B genome (Smartt et al. 1978; Smartt and Stalker 1982; Fernández and Krapovickas 1994), while the only species with an asymmetric karyotype (*A. glandulifera*) has been considered to have the D genome (Stalker 1991).

Molecular cytogenetics revealed a high degree of homogeneity among the karyotypes of the species classically included within the A genome. However, considering the statement that closeness of taxa is usually correlated with the similarity of their heterochromatin and rDNA FISH patterns (Hizume et al. 2002; Liu et al. 2003), the variation in number and positions of C-DAPI$^+$ bands and ~~major~~ 18S–26S rDNA sites among species was used to establish three subgroups of karyotype homeologies. Interestingly, the species within each subgroup tend to be more closely distributed geographically than those belonging to different subgroups; therefore each subgroup was named using a geographical reference

(Robledo et al. 2009). The first one, named Chiquitano, comprised the species (*A. cardenasii*, *A. chiquitana*, *A. herzogii*, and *A. kempff-mercadoi*) that grow in the southern and western portion of the Chiquitanía biogeographic region in Santa Cruz Department of Bolivia. The second subgroup, called Pantanal, includes the species (*A. diogoi*, *A. kuhlmannii*, *A. helodes*, *A. simpsonii* and *A. stenospema*) distributed in the Pantanal biogeographic region, in western Brazil, northern Paraguay and eastern Bolivia. The third subgroup, called La Plata River Basin, corresponds to the species (*A. duranensis*, *A. schininii*, *A. correntina* and *A. villosa*) distributed along the La Plata River Basin (except for the region that comprises the upper stream of the Paraguay River in the Pantanal). Within this scheme, the A genome of *A. hypogaea* falls into the subgroup of La Plata River Basin.

The particular karyotype features of *A. glandulifera*, i.e., assymetric karyotype, the unique pattern of heterochromatin distribution and the 5 pairs of 18–26 rDNA loci, justify the maintenance of this species as having the D genome (Robledo and Seijo 2008).

The other species with $2n = 20$ included within the non-A genome have been arranged in three different karyotype groups according to the number, size and distribution of the rDNA sites and the features of the heterochromatic bands. Moreover, each group of species having different karyotype structure has been further segregated into different genomes (Robledo and Seijo 2010). One of the karyotype groups includes *A. batizocoi*, *A. cruziana*, and *A. krapovickasii* which have conspicuous heterochromatic bands in nine chromosome pairs and three pairs of 5S rDNA loci. This group has been named K genome. The second one, named F genome, is integrated by *A. benensis* and *A. trinitensis* that have karyotypes with small and faint heterochromatic bands in seven or eight chromosome pairs and only two 5S rDNA loci. The last group is composed of all the species without pericentromeric heterochromatic bands in their karyotypes, namely, *A. ipaënsis* (Fig. 3-2e), *A. gregoryi*, *A. magna, A. valida* and *A. williamsii*. It has been proposed that this group should retain the B genome *sensu stricto* designation (Robledo and Seijo 2010). This proposal was based on the fact that the B genome has been originally assigned to one of the chromosome complements of the cultigen (Smartt et al. 1978). Thus, the wild donor of this genome, *A. ipaënsis* (see below), and all the relatives that share the same karyotype structure should belong to the same genome.

In accordance with the particular geographic distribution described for each karyotype subgroup of the A genome species (Robledo et al. 2009), the species included within each of these new genome groups also showed a strong tendency to be co-distributed, mainly those of the F and K genomes. The K genome species are distributed in the northern and northwestern edges of the Chaco Boreal region, whereas the species having the F genomes are restricted to the savannas around Trinidad city, Beni

Cerrado

department in Bolivia. The B genome species are distributed over a large area of semi-deciduous forests and savannas of the ~~cerrado~~ associated with the chiquitano highlands and peripheral mountain ranges of the western portion of the Brazilian Precambrian Shield. The only exception is *A. ipaënsis*, from which the only known population grows at the top of the sand banks of streams in an ecotone between the ~~tucumano-oranence~~ deciduous forest and the chacoan xerophytic forest (Robledo and Seijo 2010).

Tucumano-Oranense

Diploid species with $2n = 18$ of section Arachis are less characterized, but their heterochromatin patterns and rDNA loci features revealed that they have a different genome constitution from those observed in the species with $2n = 20$. Therefore, it was proposed that they should be included in a new genome, named G genome (Silvestri et al. unpubli.).

The new genome arrangement is supported by a set of different kind of data. For *Arachis*, records of pollen stainability lower than 25% in F_1 hybrids are widely accepted as indicative of intergenomic crosses. In all the hybrids obtained from crosses between species that have different karyotype structures (different genomes after Robledo and Seijo 2010) the pollen viability of the F_1 are below 25% (Smartt et al. 1978; Stalker 1991; Krapovickas and Gregory 1994; Tallury et al. 2005; Burrow et al. 2009), thus supporting the arrangements of the groups with different karyotypes as different genomes. Bivalent formation at meiotic metaphases of hybrids obtained by crosses between species with the same genome is usually higher than 9.5 out of the 10 expected. However, in intergenomic hybrids of *Arachis* species, the number of bivalents formed is lower than 7.5 (Stalker et al. 1991; Tallury et al. 2005). The number of bivalents formed in F_1 obtained by crosses between species with large heterochromatic bands (*A. batizocoi*) and those without them (*A. ipaënsis* and *A. williamsii*) is in the range expected for intergenomic hybrids, also supporting the new genome arrangement.

Molecular analyzes performed in section Arachis have usually included incomplete sets of non-A genome species (Milla et al. 2005; Tallury et al. 2005; Burow et al. 2009). However, in all of them, the group of species without bands (i.e., the B genome *s.s.* after Robledo and Seijo 2010) always formed a cluster separated from that formed by *A. batizocoi*, *A. cruziana*, and *A. krapovickasii* (K genome) (Bechara et al. 2010). Similarly, whenever *A. benensis* and *A. trinitensis* (F genome) were included in those analyzes, they were always grouped together and in a separated cluster (Milla et al. 2005). Therefore, the clustering of species observed in those dendrograms highly supports the arrangement of non-A genome species in three different genomes (B, F and K genome) as proposed by Robledo and Seijo (2010).

3.8 Genetic and Geographical Origin of Peanut to

The origin of peanut has long interested plant taxonomists, geneticists and breeders. However, the knowledge about its origin is still limited compared with other major crops. Concerning the genetic origin of peanut, more than eight wild diploid species having either the A or non-A genomes have been considered as involved in the origin of peanut. Since the early 50s, when the first hybrid between *A. hypogaea* and the diploid *A. correntina* (A genome) was obtained (Krapovickas and Rigoni 1954), several other diploid species, ~~with either the A or the non-A genome,~~ have produced hybrids with *A. hypogaea* and, thus, been proposed as putative progenitors of the tetraploids (Krapovickas and Rigoni 1957; Raman 1960; Smartt and Gregory 1967; Krapovickas 1973; Stalker and Wynne 1979; Singh and Moss 1984). Different authors, using morphology and cross compatibility data, have further proposed *A. correntina*, *A. duranensis*, *A. cardenasii* (all with A genome), and *A. batizocoi* (K genome, ~~after Robledo and Seijo 2010~~) as probable parents of *A. hypogaea* (Krapovickas 1973; Gregory and Gregory 1976; Singh and Smartt 1998). Classical chromosome analyzes suggested that *A. duranensis* and *A. ipaënsis* (B genome) (Fernández and Krapovickas 1994), or *A. trinitensis* (F genome ~~after Robledo and Seijo 2010~~) and *A. williamsii* (B genome), could also be the genome donors of the cultigen (Lavia 1996). Assays on molecular markers have also revealed different species as probable ancestors of peanut. For instance, from Restriction Fragment Length Polymorphism (RFLP) analysis, *A. duranensis* and *A. ipaënsis* were proposed as the most likely progenitors of *A. hypogaea* (Kochert et al. 1991, 1996; Burrow et al. 2009), while from Randomly Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeats (ISSR) analyzes, *A. villosa* (A genome) and *A. ipaënsis* were considered as the best candidates (Raina et al. 2001). On the other hand, Amplified Fragment Length Polymorphism (AFLP) data showed that at least three diploid species with the A genome and three with the non-A genome display small genetic distance when compared with the cultigen (Milla et al. 2005), while microsatellite markers demonstrated that, although *A. duranensis* and *A. ipaënsis* are closely related to the cultigen, a group of other species having the A or non-A genome have small genetic distance with peanut (Moretzsohn et al. 2004; Koppolu et al. 2010). Similarly, ITS analysis revealed that a set of species having the A or B genome *s.s.* could have participated in the origin of the tetraploids (Bechara et al. 2010).

The molecular cytogenetic approach involving the analysis of the number (Raina and Mukai 1999) and the mapping (Seijo et al. 2004; Robledo et al. 2009) of ribosomal gene clusters by FISH and heterochromatin distribution showed that the species included in the La Plata River Basin group of the A genome are the most related to the A genome of the

tetraploids. Concerning the donors of the B genome of peanut, detailed karyotype analysis supports *A. ipaënsis* from the B genome *s.s.* group as the most probable B genome donor because (1) its chromosomes are completely deprived of heterochromatic bands (Raina and Mukai 1999; Seijo et al. 2004) and (2) its pattern of rDNA loci is the most similar (among the wild diploids) to that observed in the complement without bands of the cultigen (Seijo et al. 2004; Robledo and Seijo 2010).

Double GISH experiments on metaphase spreads of the wild *A. monticola* and the cultigen *A. hypogaea* (Fig. 3-2g), showed that from all the possible combinations among the diploid *Arachis* species ($2n = 2x = 20$) that have been proposed as parents of the cultigen, the DNA of *A. duranensis* and *A. ipaënsis* yielded the most intense and uniform hybridization pattern onto the respective *A. hypogaea* chromosome subsets (Seijo et al. 2007). A similar pattern was observed in all the varieties of the cultigen and also in the wild tetraploid *A. monticola*, which was interpreted as an evidence of a single origin of these taxa.

Long-standing ideas about the center of origin of the cultivated peanut, which were based on the morphological variability of the landraces and biogeography (Gregory et al. 1980; Krapovickas and Gregory 1994), place the origin of the domesticated peanut in northern Argentina and southern Bolivia, in a transition area between the Tucumano-Boliviano forest and the Chaco lowlands. The distributions of the most accepted putative A and B genome donors for *A. hypogaea* and the location of *A. monticola* in this area provided additional support for this hypothesis. However, archeological studies indicate the presence of *A. hypogaea* in the Huarmey Valley, near the Peruvian coast, around 5000 yr BP (Bonavia 1982). Also pod samples that strongly resemble those of wild species have been found in the Casma Valley on the Pacific coast of Perú that date between 3500 and 3800 yr BP and suggests that ancient people from northwest Perú may have used and even cultivated some wild *Arachis* species (Simpson and Faries 2001). More recently, radiocarbon-dated macrobotanical remains dating around 7840 yr BP, that appear to correspond morphologically to a wild *Arachis* species or to peanut fruits in early stages of domestication, were recovered from sealed house floors and hearths in buried preceramic sites in a tropical dry forest of the Ñanchoc Valley, on the lower western slopes of the Andes in northern Perú (Dillehay et al. 2007). However, since there is no evidence to indicate that the Ñanchoc Valley was a domestication center for any major economic plants, the early adoption of peanut, and other crops in these valley, suggests that *Arachis* species must have been cultivated elsewhere earlier than 8000 yr BP. In spite of these findings, the region of spontaneous origin of peanut is thought to have been far from this valley because no wild species of *Arachis* are presently found in the area. Wild *Arachis* species would not be expected to have occurred in those valleys spontaneously

because the genus originated in the Amambay ranges and nowadays it is mostly distributed in Central-East Brazil, East Bolivia, Paraguay, and North Argentina below 1,200–1,500 m a.s.l. Due to the geocarpic fruits, the Andes mountains would have been an insurmountable barrier, preventing the species from reaching the Pacific coast by natural dispersion. An alternative hypothesis contemplates that peanut may have originated in the gardens of ancient people from South America, who may have used and cultivated wild *Arachis* species (Simpson and Faries 2001). In this scenario, the gardens of those people may have also served as a possible site for the origin of *A. hypogaea*.

The fact that both subspecies and all the botanical varieties of the cultigen had identical patterns of genomic hybridization (GISH) suggests that the same wild species participated in their origin. This statement implies that all presently known varieties and subspecies of peanut arose from a single, unique allotetraploid plant population, i.e., they have a common origin. The common ancestry of all infraspecific taxa of *A. hypogaea* is supported by the low genetic variability so far detected with most molecular markers in the cultivated peanut (Halward et al. 1991; Kochert et al. 1996; Herselman 2003). Although possible introgression from other diploid species of *Arachis* cannot be fully discarded, the fact that fragments of alien chromatin could not be identified in the karyotype of *A. hypogaea* by GISH suggests that if introgressions occurred during the history of peanut culture, the mechanism of gene transference may have not involved large chromosome segments or entire chromosomes (Seijo et al. 2007).

It has been postulated that diploid ancestors of *A. hypogaea* could first have given origin to a wild allotetraploid plant (Krapovickas and Gregory 1994). The unique extant wild tetraploid species so far known within section Arachis is *A. monticola*, which has several morphological traits similar to *A. hypogaea*. It should be noted that the wild condition of *A. monticola* is based mainly on its fruit structure (wherein each seed has its own shell separated by an isthmus) and on its ability to persist as natural populations, unlike the cultivated peanut. FISH and GISH results (Seijo et al. 2004, 2007), as well as molecular marker data (Gimenes et al. 2002; Milla et al. 2005), revealed a very close genomic relationship between both tetraploid species and strongly supports the hypothesis that *A. monticola* is the immediate wild antecessor of *A. hypogaea*. Accordingly, it was proposed that the origin of *A. hypogaea* occurred by an initial hybridization event followed by chromosome duplication or fusion of unreduced gametes that have given rise to a wild tetraploid with two sets of the A genome chromosomes and two sets of the B genome. It has been recently published that sexual polyploidization may have been the mechanism by which the tetraploid *A. glabrata* and the triploid cytotype of *A. pintoi* have arisen (Ortíz et al. 2011; Lavia et al. 2011). The production of unreduced gametes was reported

for few diploid species in section Arachis, but they are common in hybrids (Chatuverdi et al. 1990). These data supports the hypothesis advanced by Seijo et al. (2007) which considers that the AABB tetraploid would have arisen by means of bilateral sexual polyploidization of AB diploid hybrids.

After the origin of the wild allotetraploid, probably *A. monticola* (which possibly had larger seeds than any of the progenitors as a result of the *gigas* effect in polyploids), *A. hypogaea* arose by domestication. High selective pressure in different agroecological environments may have led to the origin of the different subspecies and varieties of the cultigen. Therefore, morphological variability would mainly result from artificial selection, as it is the case in most domesticated crops, rather than from the participation of several species in the origin of *A. hypogaea*.

The artificial resynthesis of an amphidiploid from *A. ipaënsis* and *A. duranensis* (Fávero et al. 2006), that is morphologically very similar to *A. monticola* and that can hybridize with all varieties of the cultigen producing fertile offspring, supports the model advanced.

3.9 Chromosome Evolution in Section Arachis

The analyzes of chromosome numbers have revealed the existence of two basic chromosome numbers and two ploidy levels within the section. Since $x = 10$ is the most widespread basic chromosome number within the genus and since it is present in all the sections considered as ancestral, the $x = 9$ should be considered as a derived character. Aneuploidy and disploidy have been proposed as the mechanisms responsible for the reduction of the chromosome number in *Arachis* (Lavia et al. 2008). The former implies the loss of chromosomes, and consequently of all the genetic information contained by them. On the contrary, the latter involves the distribution of the chromatin of one or more chromosomes in some or all the other chromosomes of the complement by successive unidirectional translocations. In spite of the many cytogenetic studies performed in the genus, evidences to support any of these mechanism as the responsible for the origin of the $x = 9$ are still needed.

The two spontaneous polyploids (*A. monticola* and *A. hypogaea*) of section Arachis are allopolyploids. This type of polyploids combines and maintains diploid sets of chromosomes from two or more parental species (Leitch and Bennett 1997). However, genome restructuring usually occurs during their establishment in order to stabilize the newly built complex genome (Soltis and Soltis 1999; Kenton et al. 1993; Lim et al. 2000). In opposition to this general rule, the GISH patterns observed in the AABB tetraploids of *Arachis* did not reveal large structural rearrangements of chromosomes between the A and B chromosome sets of *A. monticola/A. hypogaea* (Seijo et

al. 2007). These findings are in agreement with the conservative localizations of the ~~45S and 5S~~ 5S and 18S -26S rDNA loci in the A and B complements of the tetraploids compared to those localizations observed in their wild diploid progenitors, *A. duranensis* and *A. ipaënsis*, respectively (Seijo et al. 2004). Moreover, the analysis of few dispersed (Nielen et al. 2010, 2011) and tandemly repetitive (Robledo et al. ~~unpubl.~~ unpubli.) ~~elements~~ sequences showed that in the AABB tetraploids those genomic elements are in almost the same amount than that expected from the addition of the amounts present in their diploid progenitors.

Concerning the structural changes of chromosomes at diploid level, since ~~the karyotype formulae are~~ a karyotype formula is conserved among most of the species, it has been postulated that these types of rearrangements may have not played an important role during the evolution of the section Arachis (Lavia et al. 2009). The exception to this general statement is *A. glandulifera* because it has been reported that its asymmetrical karyotype formula arose by the occurrence of several translocations (Stalker 1991). In spite of the stasis found in the karyotype ~~formulae~~ formula of this group of species, chromosome markers revealed by banding and FISH showed that changes in the amount and distribution of AT rich heterochromatin are one of the most relevant factors that determined the differentiation of the A, B, D, F and K genomes (Raina and Mukai 1999; Seijo et al. 2004; Robledo and Seijo 2010).

GISH on AABB species demonstrated a clear differential hybridization pattern between the chromosomes of the A genome and those of the B genome. Since this technique relies largely on the hybridization of genome-specific repetitive sequences, it was advanced that divergence in the content of the genomic repetitive elements accompanied speciation and has driven genome differentiation in *Arachis* (Seijo et al. 2007). A recent analysis on one of the members of the Ty3-*gypsy* elements present in the *Arachis* genomes, named FIDEL, demonstrated that it is dispersely distributed and that it is more frequent in the A- than in the B-genome, with copy numbers of about 3,000 in *A. duranensis* and 820 in *A. ipaënsis* per haploid genome (Nielen et al. 2010). This was the first evidence of uneven distribution of a dispersed repetitive element among different genomes of *Arachis*. In conclusion, the investigations carried out on dispersed elements and tandemly repetitive sequences of the centromeric heterochomatin strongly support that the changes in the repetitive fraction have played a key role in the chromosomal and genome evolution of *Arachis*.

Acknowledgements

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