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GENOMIC RELATIONSHIPS BETWEEN THE CULTIVATED PEANUT (*ARACHIS HYPOGAEA*, LEGUMINOSAE) AND ITS CLOSE RELATIVES REVEALED BY DOUBLE GISH[1]

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Arachis hypogaea is a natural, well-established allotetraploid (AABB) with $2n = 40$. However, researchers disagree on the diploid genome donor species and on whether peanut originated by a single or multiple events of polyploidization. Here we provide evidence on the genetic origin of peanut and on the involved wild relatives using double GISH (genomic in situ hybridization). Seven wild diploid species ($2n = 20$), harboring either the A or B genome, were tested. Of all genomic DNA probe combinations assayed, *A. duranensis* (A genome) and *A. ipaensis* (B genome) appeared to be the best candidates for the genome donors because they yielded the most intense and uniform hybridization pattern when tested against the corresponding chromosome subsets of *A. hypogaea*. A similar GISH pattern was observed for all varieties of the cultigen and also for *A. monticola*. These results suggest that all presently known subspecies and varieties of *A. hypogaea* have arisen from a unique allotetraploid plant population, or alternatively, from different allotetraploid populations that originated from the same two diploid species. Furthermore, the bulk of the data demonstrated a close genomic relationship between both tetraploids and strongly supports the hypothesis that *A. monticola* is the immediate wild antecessor of *A. hypogaea*.

Key words: allopolyploidy; *Arachis*; genome constitution; GISH; heterochromatin; peanut origin.

The cultivated peanut (*Arachis hypogaea*) is one of the major oilseed crops of the tropics and subtropics, although it is also cultivated in the warm areas of the temperate regions (Hammons, 1994). Based on morphological features, i.e., the ramification pattern and the presence/absence of flowers on the main axis, two subspecies with partial genetic isolation are recognized (Gregory et al., 1980). The two subspecies are further divided into six botanical varieties based on other morphological traits and growth habit (Table 1) (Krapovickas and Gregory, 1994).

The origin of this cultigen has long interested plant taxonomists, geneticists, and breeders. However, our knowledge about its origin is very limited compared with other major crops. Archeological studies indicate the presence of *A. hypogaea* in the Huarmey Valley, near the Peruvian coast, around 5000 yr BP (Bonavia, 1982). In spite of these findings, the region of peanut domestication is thought to have been far from this valley because no wild species of *Arachis* are presently found in the area. Furthermore, wild *Arachis* species would not be expected to have occurred in the valley spontaneously because the genus is geocarpic and mostly distributed in central Brazil, east Bolivia, Paraguay, and north Argentina below 1200–1500 m a.s.l. Therefore, the Andes mountains would have been an insurmountable barrier, preventing the species from reaching the Pacific coast by natural dispersion. According to the current species distribution and the variability of the landraces, an area comprising south Bolivia to north Argentina was traditionally considered the primary candidate location for the domestication of peanut, as shown in Fig. 1 (Krapovickas and Gregory, 1994). However, pod samples recently found in the Casma Valley on the Pacific coast of Peru date between 3500 and 3800 yr BP (Fig. 1) and strongly resemble those of three different wild species; this finding suggests that ancient people from northwest Peru may have used and even cultivated some 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*.

Peanut is an allotetraploid with $2n = 4x(2A + 2B) = 40$ (Husted, 1936; Stebbins, 1957; Seijo et al., 2004), and most authors support the hypothesis of a single origin for *A. hypogaea* because of the very limited genetic variability among landraces and commercial cultivars (Kochert et al., 1996; Raina et al., 2001; Milla et al., 2005). However, a different origin for each subspecies was advanced based on cross compatibility experiments (Singh and Moss, 1982) and isozyme analyses (Lu and Pickersgill, 1993). In this context, the morphological variability and partial reproductive isolation of the subspecies may support a multiple origin hypothesis for peanut, probably involving different diploid species (Krapovickas and Gregory, 1994). An alternative hypothesis proposes a single origin of the ancestral tetraploid, but because the cultigen was dispersed throughout South America by man, introgression from

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TABLE 1. List of the *Arachis* species and samples studied, their provenance, life cycle, chromosome number, genome formula, and sources of metaphases and DNA for GISH experiments. Species are ordered first by ploidy level and then by genomic constitution.

Taxon	Provenance, collection no.[a]	Life cycle[b]	2*n*	Haploid genome formula	Source of metaphases for GISH experiments	Source of total DNA probes for GISH experiments
A. hypogaea L. subsp. *hypogaea* var. *hypogaea* (race Guaycurú)	Argentina, Corrientes. FCA 27	annual	40	AB	X	
A. hypogaea subsp. *hypogaea* var. *hirsuta* Köhler (race Mitad del Mundo)	Ecuador, San Antonio. B, P, Va 732 (MDI 86–2769)	annual	40	AB	X	
A. hypogaea subsp. *fastigiata* Waldron var. *fastigiata* (race Colorado)	Argentina, Corrientes. FCA 31	annual	40	AB	X	
A. hypogaea subsp. *fastigiata* var. *aequatoriana* Krapov. and W. C. Gregory (race Zaruma)	Ecuador, Quito. B, P, Z 683 (MDI 86–2535)	annual	40	AB	X	
A. hypogaea subsp. *fastigiata* var. *peruviana* Krapov. and W. C. Gregory (race Tingo María)	Perú, Casma. MDI 87–2753 (PI 393641)	annual	40	AB	X	
A. hypogaea subsp. *fastigiata* var. *vulgaris* C. Harz (race Blanco Manfredi)	Argentina. MDI 68–2551	annual	40	AB	X	
A. monticola Krapov. and Rigoni	Argentina, Prov. Jujuy, Dept. Capital, Yala. Se, Sn 2774	annual	40	AB	X	
A. cardenasii Krapov. and W. C. Gregory	Bolivia, Dept. Santa Cruz, Prov. Chiquitos, Roboré. G, K, P 10017 (PI 262141)	perennial	20	A		X
A. correntina (Burkart) Krapov. and W. C. Gregory	Argentina, Prov. Corrientes, Dept. Ituzaingó. K 7897 (PI 331192)	perennial	20	A		X
A. duranensis Krapov. and W. C. Gregory	Argentina, Prov. Salta, Dept. San Martín, Campo Durán. K 7988 (PI 219823)	annual	20	A		X
A. villosa Benth.	Argentina, Prov. Corrientes, Dept. Paso de los Libres, Laguna Mansa. Se, Sn 2866	perennial	20	A		X
A. ipaensis Krapov. and W. C. Gregory	Bolivia, Dept. Tarija, Prov. Gran Chaco, Ipa. K, G, B, P, Sc, S 30076 (PI 468322)	annual	20	B		X
A. williamsii Krapov. and W. C. Gregory	Bolivia, Dept. Beni, Prov. Cercado, Trinidad. W 1118	annual	20	B		X
A. batizocoi Krapov. and W. C. Gregory	Bolivia, Dept. Santa Cruz, Prov. Cordillera, Parapetí. K 9484 (PI 298639)	annual/ biannual	20	B?		X

[a] B = D. Banks, G = W. C. Gregory, K = A. Krapovickas, P = J. Pietrarelli, S = C. E. Simpson, Sc = A. Schinini, Se = J. G. Seijo, Sn = V. G. Solís Neffa, Va = F. Valenzuela, W = D. E. Williams, Z = H. Zurita; FCA = plant introduction number of Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste, Argentina; MDI = plant introduction number of Instituto Nacional de Tecnología Agropecuaria (INTA) Manfredi, Argentina; PI = plant introduction number of United States Department of Agriculture (USDA), USA. Prov. = province, Dept. = department.

[b] a = annual, b = biannual, p = perennial.

sympatric wild *Arachis* species may has led to the present subspecies and varieties. In spite of several attempts to resolve this uncertainty, no data conclusively locate the center(s) of origin or resolve whether the cultigen has single or multiple origins. Moreover, even though researchers generally agree that the wild peanut progenitors come from within section *Arachis*, the identity of these progenitors has long been a matter of speculation. More than eight diploid species having either the A or B genome have been considered as involved in the origin of peanut. Different authors, using morphology and cross compatibility data, have proposed *A. correntina*, *A. duranensis*, *A. cardenasii* (all with A genome), and *A. batizocoi* (B genome?) as probable parents of *A. hypogaea* (Krapovickas, 1973; Gregory and Gregory, 1976; Singh and Smartt, 1998). Classical chromosome analyses suggested that *A. duranensis* and *A. ipaensis* (B genome), or *A. trinitensis* Krapov. and W. C. Gregory (A genome) and *A. williamsii* (B genome), could be the genome donors of the cultigen (Fernández and Krapovickas, 1994; Lavia, 1999). Even though most molecular markers suggested a single origin for domesticated peanut, they revealed different species as ancestors. For instance, from restriction fragment length polymorphism (RFLP) analysis, *A. duranensis* and *A. ipaensis* were proposed as the most likely progenitors of *A. hypogaea* (Kochert et al., 1991, 1996), while from randomly amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) analyses, *A. villosa* and *A. ipaensis* were considered as the best candidates (Raina et al., 2001). On the other hand, PCR amplified fragment length polymorphism (AFLP) data showed that at least three diploid species with the A genome and three with the B genome display small genetic distance when compared with the cultigen (Milla et al., 2005), while microsatellite markers demonstrated that, although *A. duranensis* and *A. ipaensis* are closely related to the cultigen, a group of other species having the A or B genome could be considered possible genome donors (Moretzsohn et al., 2004).

A different approach involving the analysis of the number of ribosomal gene clusters by fluorescent in situ hybridization (FISH) showed that *A. villosa* and *A. ipaensis* are the most probable donors of the A and B genome, respectively (Raina and Mukai, 1999a). Our previous results on chromosome mapping of the rDNA loci by FISH indicated a high degree of homeology between the chromosomes of *A. ipaensis* and those of the B genome in the tetraploid species, *A. hypogaea* and *A. monticola* (Seijo et al., 2004). However, rDNA clusters mapped very similarly in *A. duranensis*, *A. correntina*, and *A. villosa* and also in the A genome of the tetraploids. Therefore, there is no conclusive evidence of which diploid species harboring the A genome could be the actual progenitor of *A. hypogaea*/*A. monticola*. In this context, more data are

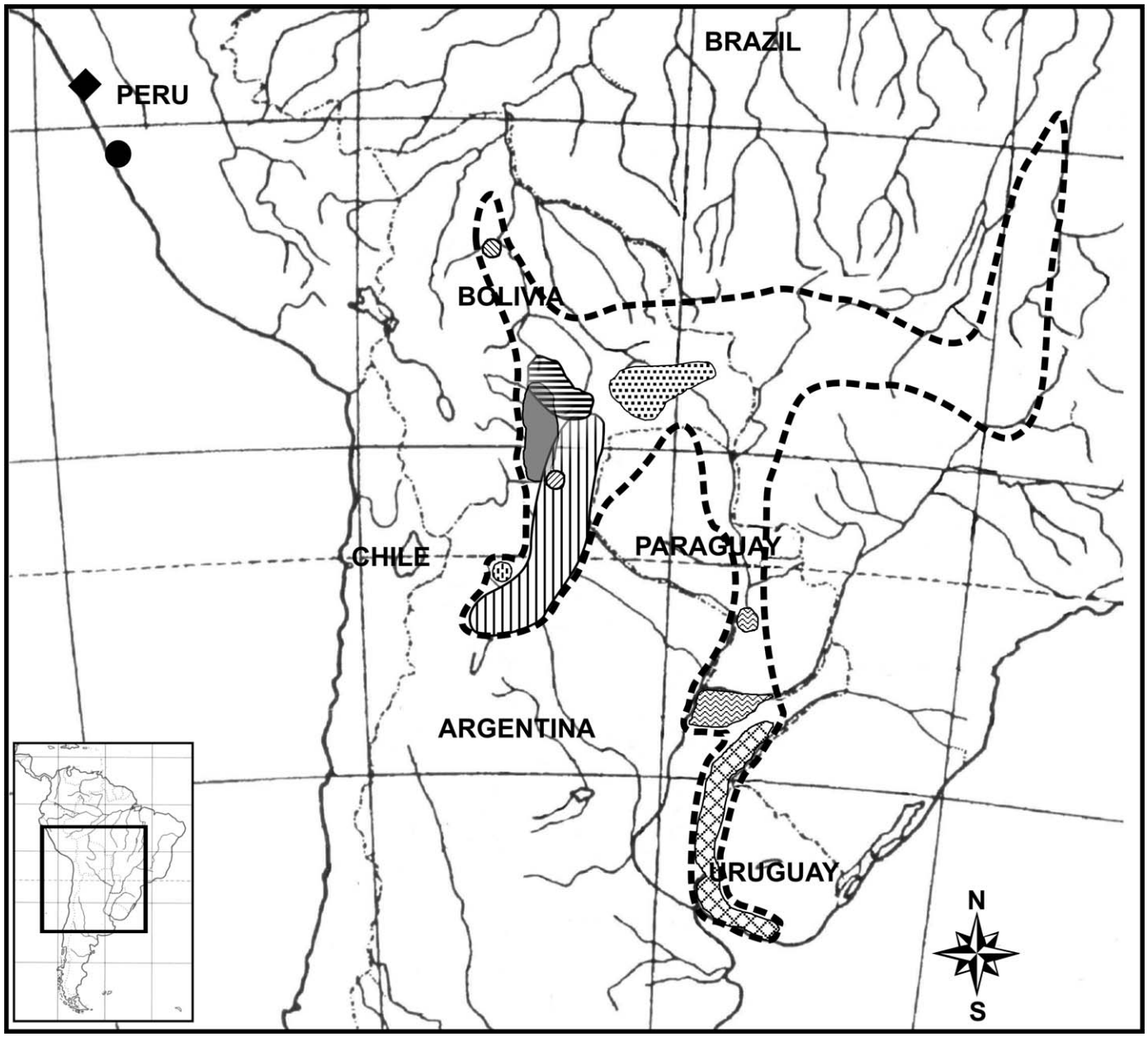

Fig. 1. Geographical distribution of the wild *Arachis* species studied and the major center of variability of *Arachis hypogaea* var. *hypogaea*. = *A. batizocoi*, = *A. cardenasii*, = *A. correntina*, = *A. duranensis*, = *A. hypogaea* var. *hypogaea*, = *A. ipaensis*, = *A. monticola*, = *A. villosa*, = *A. williamsii*. Archeological records in *Arachis*: ◆ = Huarmey Valley, ● = Casma Valley. Dashed line indicates the geographical distribution of the genus.

needed to clarify which diploid species with the A genome have the greatest genetic affinity to the corresponding chromosome subset of the tetraploids.

Genomic in situ hybridization (GISH) has been shown to be very useful in clarifying the genomic constitution of allopolyploid species (e.g., wheat, oat, bananas, and tobacco) and in revealing rearranged chromosome segments in hybrids and genome introgression between species (cf. Kenton et al., 1993; Bennett, 1995; Heslop-Harrison and Schwarzacher, 1996; Mukai, 1996; Leitch and Bennett, 1997; Osuji et al., 1997; Lim et al., 2000; Li et al., 2001; Chase et al., 2003). The first attempt to investigate the genomic composition of *A. hypogaea* by this approach was performed as simple GISH on an unidentified material of the cultigen, using labeled total genomic DNA from a potential diploid ancestor (genomic probe) and an unlabeled (blocking) total genomic DNA ("cold probe") from another diploid species of *Arachis* (Raina and Mukai, 1999b). Double GISH, which uses two differentially labeled genomic DNA probes, offers an advantage over the simple GISH in that the DNA probes of both candidate parents (in approximately the same concentrations) compete to hybridize with homologous sequences without the interference

of highly concentrated "cold probes"; thus, double GISH provides more realistic patterns of genome affinities (Moscone et al., 1996). Another advantage of this technique is that it recognizes different target genomes in a single experiment.

Therefore, in the present work we used genomic DNA probes from the diploid *Arachis* species related to the tetraploid species pair, *A. hypogaea* and *A. monticola*, in double GISH experiments with the somatic chromosomes of the six botanical varieties and two subspecies of the cultigen and of the wild tetraploid species. Our objectives were (1) to find which diploid species participated in the origin of *A. monticola*/*A. hypogaea* by comparing the genomic hybridization pattern of the putative progenitors DNA with the chromosomes of the tetraploids, (2) to determine whether the subspecies or varieties of the cultigen originated by a single or multiple events of hybridization/polyploidization, (3) to clarify the genomic relationship between the wild tetraploid *A. monticola* and the cultivated peanut, and (4) to determine the occurrence of intergenomic structural changes in the allotetraploids after the hybridization/polyploidization.

MATERIALS AND METHODS

Plant material—Samples of varieties and subspecies of *A. hypogaea* and wild species of *Arachis* were obtained from the peanut germplasm collection at the experimental station INTA Manfredi in Córdoba, Argentina, and at the Instituto de Botánica del Nordeste in Corrientes, Argentina. The original provenances, voucher specimens, and life cycle of the accessions studied are listed in Table 1, with species ordered first by ploidy level, then by genomic constitution. The geographic distribution of the wild *Arachis* species, the center of major diversity of *A. hypogaea* subspecies *hypogaea*, and the locations of archeological remains are mapped in Fig. 1.

Chromosome preparations—Plants obtained from seeds were grown in pots. Collected root tips (5–10 mm long) were pretreated with 2 mmol/L 8-hydroxyquinoline for 3 h at room temperature (Fernández and Krapovickas, 1994) and then fixed in 3 : 1 absolute ethanol : glacial acetic acid for a minimum of 12 h at 4°C. Somatic chromosome spreads were prepared according to Schwarzacher et al. (1980). Root tips were macerated in 1% (w/v) cellulose Onozuka R-10 (from *Trichoderma viridae*; Serva, Heidelberg, Germany) plus 10% (v/v) pectinase dissolved in 40% glycerol (from *Aspergillus niger*, Sigma, St. Louis, Missouri, USA) in 0.01 mol/L citric acid/sodium citrate buffer, pH 4.8, at 37°C for 2 h, and then squashed in 45% acetic acid. After removal of the coverslip with CO_2, slides were air dried, aged for 1–2 d at room temperature, and then kept at −20°C until use.

DNA extraction—Total genomic DNA was extracted from young actively growing leaves of all the diploid species assayed using a DNeasy 96 plant kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA concentrations were determined by spectrophotometry and gel electrophoresis.

Probe labeling and fluorescent in situ hybridization—Total DNA from the diploid species used as probes in fluorescent in situ hybridization experiments were labeled with digoxigenin-11-dUTP (Roche, Mannheim, Germany) or biotin-11-dUTP (Sigma) by nick translation.

Pretreatment of preparations, chromosome and probe denaturation, conditions for the in situ hybridization (hybridization mixes contained DNA probes at a concentration of 2.5–3.5 ng/μL), posthybridization washing, blocking, and indirect detection by fluorochrome-conjugated antibodies were performed according to Moscone et al. (1996). The first set of antibodies consisted of mouse anti-biotin (Dakopatts, Dako, Carpinteria, California, USA) and sheep anti-digoxigenin conjugated to fluorescein isothiocyanate (FITC) (Roche) in phosphate-buffered saline (PBS; 0.13 mol/L NaCl, 0.007 mol/L Na_2HPO_4, 0.003 mol/L NaH_2PO_4) and 3% (w/v) bovine serum albumin (BSA). The second set of antibodies consisted of rabbit anti-mouse conjugated to tetramethyl-rodamine isothiocyanate (TRITC) (Dakopatts) and FITC-conjugated rabbit anti-sheep (Dakopatts) in PBS and 3% (w/v) BSA. Preparations were counterstained and mounted with Vectashield medium (Vector Laboratories, Burlingame, California, USA) containing 2 μg/mL 4′-6-diamidino-2-phenylindole (DAPI, Sigma).

The DAPI counterstaining subsequent to GISH resulted in a C banding-like pattern with major heterochromatin bands fluorescing more intensely, thus aiding chromosome identification (cf. Moscone et al., 1999; Seijo et al., 2004).

Each GISH experiment included two total genomic DNA probes differentially labeled (with digoxigenin or biotin), one from a diploid species with the A genome and the other from a diploid species with the B genome. For reducing interpretation errors due to poor labeling, each genomic DNA probe was checked by dot blot and also was hybridized onto the chromosomes of the same species. Probes that produced uniform hybridization patterns and high fluorescent signal intensity in these test experiments were then used to analyze the genomic composition of the tetraploids. Our GISH protocol included a hybridization mixture combined with post-hybridization washes, both steps containing 60% formamide and 2 × SSC (saline sodium citrate buffer) at 37 °C, which resulted in a comparatively high stringency (82–85%).

Fluorescence microscopy and image acquisition—Chromosomes were viewed and photographed with a Leica (Heerbrugg, Switzerland) DMLB fluorescence microscope equipped with a computer-assisted Leica DC 250 digital camera system. Red, green, and blue images were captured in black and white using appropriate filters for TRITC, FITC, and DAPI excitation, respectively. Digital images were pseudocolored and combined using IM 1000 Leica software, then imported into Adobe (San Jose, California, USA) Photoshop, version 7.0, for final processing. Chromosomes were designated following the nomenclature of Seijo et al. (2004).

RESULTS

Double GISH experiments were carried out on metaphase spreads of the tetraploid *Arachis* species pair, i.e., the wild *A. monticola* and the cultigen *A. hypogaea*, to cast light on their amphidiploid origin. We tested all the possible combinations among the seven diploid *Arachis* species ($2n = 2x = 20$) that have been proposed as parents of the cultigen. Under our experimental conditions, a clear discrimination of the chromosome subsets from the corresponding parental genomes in the tetraploids was possible without the need of unlabeled blocking DNA.

Some representative somatic metaphases of the tetraploid species *A. hypogaea* probed with total DNA of various wild diploid species are shown in Fig. 2A–H. From Among all the assayed combinations, the DNA of *A. duranensis* (with A

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Fig. 2. Somatic metaphases of *Arachis hypogaea* (subsp. *hypogaea* var. *hypogaea*, race Guaycurú) after double genomic in situ hybridization (GISH). A 4′-6-diamidino-2-phenylindole (DAPI) counterstain (blue, shown in black and white) after the GISH was used to highlight the heterochromatic bands and to stain euchromatin in A, C, E, and G. Figs. B, D, F, and H show the somatic chromosomes of *A. hypogaea* (same metaphase as in A, C, E, and G, respectively) after GISH with different combinations of total DNA probes from diploid wild *Arachis* species labeled either with green fluorescein isothiocyanate (FITC) or red tetramethyl-rodamine isothiocyanate (TRITC). (B) *A. ipaensis* (red) and *A. duranensis* (green). (D) *A. ipaensis* (red) and *A. villosa* (green). (F) *A. ipaensis* (red) and *A. cardenasii* (green). (H) *A. williamsii* (red) and *A. duranensis* (green). In some metaphases, chromosomes A9 and A10 are indicated. If the secondary constriction of chromosome A10 is extended, the short arm and the proximal segment of the long arm are indicated by an asterisk and the separated satellite is marked by a degree sign. Scale bar = 5 μm.

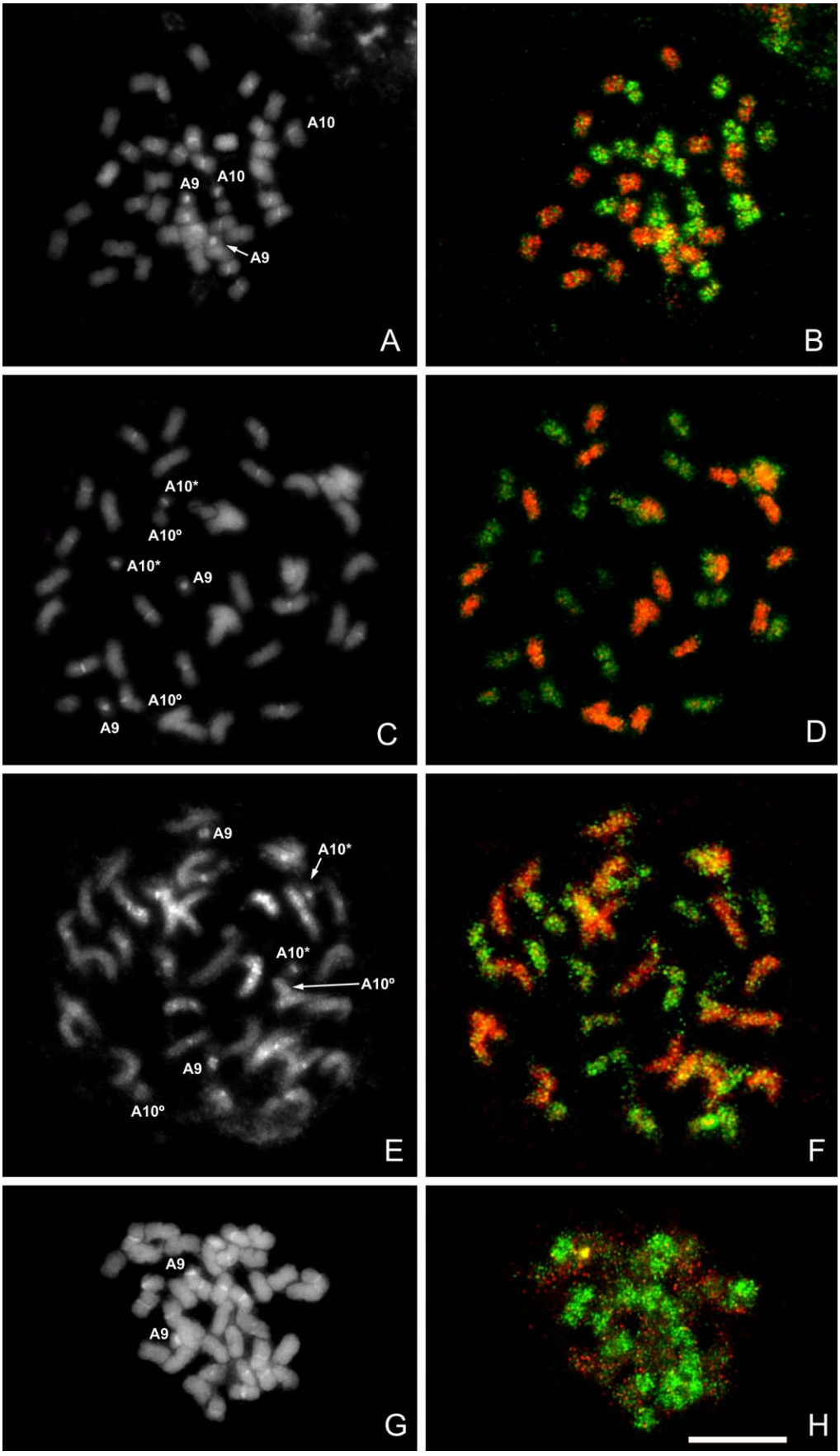
A10
A9
A10
A9
A
B
A10*
A10°
A10*
A9
A10°
A9
C
D
A9
A10*
A10*
A10°
A9
A10°
E
F
A9
A9
G
H

TABLE 2. Summary of GISH experiments with *Arachis* species.

Source of metaphases		Source of probes						
Species	Chromosome subset	*A. duranensis*	*A. villosa*	*A. correntina*	*A. cardenasii*	*A. ipaensis*	*A. williamsii*	*A. batizocoi*
A. hypogaea	A genome (banded) cromosomes	++++	+++	+++	++	−	−	−
	B genome (nonbanded) chromosomes	−	−	−	−	++++	+	+
A. monticola	A genome (banded) cromosomes	++++	+++	+++	++	−	−	−
	B genome (nonbanded) chromosomes	−	−	−	−	++++	+	+

Note: Similar GISH patterns were obtained in all the botanical varieties and subspecies of *A. hypogaea*. Rows are chromosome subsets of somatic metaphase preparations from the respective tetraploid species and columns are probed total genomic DNAs from the respective wild diploid species. Intensity of the hybridization GISH signals (genome painting), i.e., degree of binding of probed DNA of the diploid species to the respective subsets of metaphase chromosomes in the tetraploids, is indicated by an increasing number of +. Lack of or very low hybridization signals are indicated by −.

genome) and *A. ipaensis* (with B genome) yielded the most intense and uniform hybridization pattern onto the respective *A. hypogaea* chromosome subsets (Fig. 2A, B). A similar pattern was observed in all the varieties of the cultigen and also in the wild tetraploid *A. monticola* (data available upon request). With this probe combination, the genomic DNA of *A. duranensis* hybridized preferentially to 20 chromosomes of *A. hypogaea*, all with heterochromatic bands (A genome), while the genomic DNA of *A. ipaensis* hybridized preferentially to the remaining 20 chromosomes, all without heterochromatic bands (B genome). This result is congruent with the karyotype features of the probed diploid species, *A. duranensis* with centromeric bands in all chromosomes and *A. ipaensis* without them (see Seijo et al., 2004).

In *A. hypogaea* and *A. monticola*, the smallest chromosome pair A9 (with a conspicuous heterochromatic band) and the short arm together with the proximal segment of the long arm of the SAT chromosome pair A10 had less intense hybridization signal than did the rest of the A genome chromosomes when probed with total DNA of *A. duranensis*. However, the satellite of pair A10 produced a strong signal, similar to the rest of the chromosomes of the A complement (Fig. 2A–F). This hybridization pattern was equivalent to that observed when total DNA from an A-genome species was probed with its own chromosome complement. The poor DNA hybridization in some regions of the complement may reflect that they posses a lower repetitive DNA content than the regions with strong hybridization.

GISH also demonstrated two other regularities within the A genome. On one hand, centromeric heterochromatic regions had weak hybridization signals, while pericentromeric and interstitial segments had the most intense fluorescence. This pattern may reflect different accessibility of the probes to the homologous sequences along the chromosomes. The proteinase treatment in our FISH protocol was optimum for euchromatic regions but could not be strong enough to expose the DNA of the heterochromatic segments to the probes. However, longer exposure to proteinase, which enhanced the hybridization on the heterochromatic bands, resulted in a major loss of chromosome morphology. On the other hand, the weak fluorescence at the distal chromosome regions could be due to differential chromatin condensation. It should be noted that in the metaphase spreads selected for FISH experiments, the chromosomes usually are not very condensed to facilitate loci mapping. In such conditions, less compact chromatin is expected to occur in the distal chromosome regions yielding lower flourescense intensity in GISH assays.

In all cases, the combined double GISH images (overlapping of single green and red images) showed dispersed yellow spots on the chromosomes of both genomes in the tetraploids, indicating that DNA probes from the diploids hybridize indistinctly to some genomic regions of the tetraploids. Thus, it is most likely that some dispersed repetitive sequences of the tested diploids are present in the chromosomes belonging to both the A and B genome of the tetraploids. Still, the one-colored GISH pattern throughout the entire length of every chromosome in all screened metaphases of the tetraploid species indicates that the repetitive element compositions of the A and B genomes are distinct and that no large intergenomic translocations have occurred after the allopolyploidization event.

Concerning the other diploid species analyzed within the A genome group, hybridization patterns obtained with metaphases of *A. hypogaea*/*A. monticola* and genomic DNA probes from *A. villosa* (Fig. 2C, D), *A. correntina*, and *A. cardenasii* (Fig. 2E, F) were similar to the pattern obtained with the DNA probe from *A. duranensis*. However, probes from *A. villosa*, *A. correntina*, and *A. cardenasii* produced less intense fluorescence, and the hybridization pattern was not uniform (Table 2). On the other hand, the DNA probes from the other species within the B genome group, *A. batizocoi* and *A. williamsii* (Fig. 2G, H), had less homology to the B chromosomes of the cultigen than did *A. ipaensis*, giving weaker hybridization signals or, even, failing to label the corresponding chromosome set of the tetraploid species (Table 2).

DISCUSSION

Origin of the tetraploid Arachis hypogaea/A. monticola species pair and their genome donors—Interspecific hybridization and polyploidization are major forces in plant evolution and have played an important role in the origin of many crops (Hilu, 1993; Singh, 2003). Our results confirm that *A. hypogaea* is allotetraploid; using GISH with genomic DNA of *A. duranensis* (A genome) and of *A. ipaensis* (B genome), we clearly discriminated two sets of 20 chromosomes each. Further, the two chromosome sets in *A. hypogagea* (one with constitutive heterochromatin expressed in centromeric bands and the other one without) agreed with the expected reunion of the chromosome complements of *A. duranensis* (with bands) and of *A. ipaensis* (without bands) in a hybridization/polyploidization event.

That both subspecies and all the botanical varieties of the cultigen had identical patterns of genomic hybridization 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. Alternatively, cultivated peanut could have arisen from different allotetraploid populations, with all the allotetraploid populations originating from the same two diploid species, i.e., there was a recurrent formation with polytopic origin (cf. Brochmann et al., 1992). The common ancestry of all infraspecific taxa of *A. hypogaea* is supported by the fact that they share the same number of 45 S and 5 S rDNA loci, which are mapped in identical chromosome positions (Seijo et al., 2004). Also, the low genetic variability so far detected with molecular markers in the cultivated peanut reflects a large bottleneck in the origin of the cultigen (Halward et al., 1991; Kochert et al., 1996; Herselman, 2003).

However, possible introgression from other diploid species of *Arachis* cannot be fully discarded. Our results suggest that, if introgressions from other wild species have occurred during the history of peanut culture, the mechanism of intergenomic gene transference did not involve large chromosome segments or entire chromosomes because no fragments of alien chromatin in the karyotype of *A. hypogaea* were detected with GISH. Data from RFLP and RAPD marker analyses, which demonstrated that chromosome recombination instead of chromosome substitution was the mechanism of introgression of *A. cardenasii* (A genome) characters into the genome of *A. hypogaea* (Garcia et al., 1995), appears to support our findings.

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 (that is, they are separated by an isthmus), unlike the fruit of any cultivated peanut. GISH results demonstrate a very similar genome composition in *A. monticola* and *A. hypogaea*. In addition, the complements of both tetraploid species seem to have the same rDNA order according to the physical mapping of five 45 S and two 5 S rDNA loci (Seijo et al., 2004). Furthermore, whenever these species were included in molecular marker assays, they always clustered together (Gimenes et al., 2002; Milla et al., 2005). Therefore, the bulk of data indicates 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, we propose the following model for the origin of *A. hypogaea*. A hybridization event followed by chromosome duplication or fusion of unreduced gametes could have given rise to a wild tetraploid with two complements of the A genome and two complements of the B genome. After the origin of this wild allotetraploid (which probably 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 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 proposed model is supported by the present-day geographical distribution, morphological characters, and cross-compatibility of the extant wild diploid species postulated as the peanut progenitors. *Arachis monticola* grows in a restricted area in northwest Argentina, very close to the center of variability of *A. hypogaea* var. *hypogaea* (southeast Bolivia), a taxon that displays a series of characters considered as ancestral for the crop. Based on this distribution, the peanut parents have been postulated to be wild diploid species living in this region (see Fig. 1). Our GISH results have revealed that the diploid species are *A. ipaensis*, which bears the B genome and *A. duranensis*—instead of *A. villosa* as proposed by Raina and Mukai (1999a, b)—which has the A genome. This result is completely congruent with the 45 S and 5 S rDNA loci number and distribution found with FISH mapping (Seijo et al., 2004). Moreover, an amphidiploid from *A. ipaensis* and *A. duranensis* that was recently synthesized artificially (Fávero et al., 2006) is morphologically very similar to *A. monticola*, can hybridize with all varieties of the cultigen and produce fertile offspring. Therefore, the bulk of evidence supports the model advanced here.

Allopolyploids combine and maintain diploid sets of chromosomes from two or more parental species (Leitch and Bennett, 1997). This special type of polyploids is fertile, well adapted, genetically stable, and common in nature; however, genome restructuring usually occurs during the allopolyploid establishment in order to stabilize the newly built complex genome (Soltis and Soltis, 1999). Intergenomic translocations are among the most easily detectable structural chromosome modifications, e.g., in the amphidiploid tobacco (Kenton et al., 1993; Moscone et al., 1996; Lim et al., 2000). Nevertheless, according to our genomic hybridization patterns, no large chromosome rearrangements could be detected between the A and B complements of *A. monticola*/*A. hypogaea*. These findings are in agreement with the conservative positions of the 45 S and 5 S rDNA loci in the A and B complements of the tetraploids compared to those observed in the proposed progenitors, *A. duranensis* and *A. ipaensis*, respectively (Seijo et al., 2004).

Genomic considerations—Classical cytogenetic methods (Fernández and Krapovickas, 1994; Lavia, 1999) combined with crossability assays (Gregory and Gregory, 1979) have established the first genomic analysis between species of section *Arachis*. Species with the small A chromosome were usually considered as bearing the A genome, while species without the A chromosome were commonly referred as having the B genome (non A). Furthermore, a third genome named D has been assigned to only one species, *A. glandulifera* Stalker, which has mostly submetacentric chromosomes (Stalker, 1991). This genomic classification was supported to some extent by additional crossing experiments (Krapovickas and Gregory, 1994; Singh and Smartt, 1998) and also by FISH analysis of the 5S and 45 S rRNA genes (Seijo et al., 2004). However, the distribution of heterochromatic bands in diploid species considered as putative parents of *A. hypogaea*/*A. monticola* demonstrates some discrepancies with the proposed genomic classification. A typical feature of all A genome species is the presence of conspicuous heterochromatic bands at the centromere region of most or all chromosomes, being particularly evident in the A9 chromosome (Seijo et al., 2004). In contrast, except for *A. batizocoi*, the species with the B genome lack those heterochromatic bands. For this reason, we previously postulated that *A. batizocoi* has a different genomic constitution, one that is neither A nor the B (discussed next).

GISH experiments demonstrated a considerable sequences differentiation between the chromosomes of the A and B

genomes in the tetraploids. Also, the DNA hybridization patterns of the extant diploid species with the chromosome subsets of the allotetraploids demonstrate that the A genome species are more similar to each other than those with the B genome. The low affinity between the genomic DNA of *A. batizocoi* and the unbanded genome of the tetraploids was expected from the results obtained by FISH because this diploid species is very distinct from other B genome entities having heterochromatic bands. However, that genomic DNA of *A. williamsii* also had low affinity with the unbanded genome in *A. hypogaea* was unexpected because, like *A. ipaensis*, *A. williamsii* lacks centromeric bands (Seijo et al., 2004) and is considered a close relative of *A. ipaensis*. The present observations agree with our previous findings on rDNA mapping (Seijo et al., 2004) and with RFLP (Gimenes et al., 2002) and AFLP marker analyses (Milla et al., 2005), which revealed a lower genetic distance between species with the A genome than between species with the B. On the basis of the available data, the species with the B genome do not seem to constitute a natural group, and a reappraisal of the genomic constitution within the section *Arachis* seems to be needed.

GISH relies largely on the hybridization of genome-specific repetitive sequences. While gene sequences are often very similar over large taxonomic distances, repetitive DNA motifs vary in both sequence and abundance, even between closely related species. It has been suggested that evolutionary changes in repetitive DNA and its genome and chromosome distribution are related to speciation (Dean and Schmidt, 1995; Heslop-Harrison, 2000; Dechyeva et al., 2003). With our GISH results, we demonstrated a clear differentiation among the diploid species of *Arachis*. The very high cross DNA hybridization within A-genome species indicates that they share a large number of sequences. However, species lacking the A-chromosome pair, broadly referred to as B-genome members, appear more differentiated in repetitive DNA sequences. From these data, we can conclude that divergence in the content of the genomic repetitive element accompanied speciation or has helped to drive speciation in *Arachis*.

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