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Restriction fragment length polymorphism evaluation of six peanut species within the *Arachis* section *

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Summary. Restriction fragment length polymorphisms (RFLP) were assessed among accessions within six peanut species of the *Arachis* section: tetraploid cultivated species, *A. hypogaea*; tetraploid wild species, *A. monticola*; and four diploid wild species, *A. batizocoi, A. cardenasii, A. duranensis* and *A. glandulifera*. While the two tetraploid species did not show polymorphism with 16 *Pst*I-generated random genomic probes, two of seven seed cDNA probes detected polymorphisms. The RFLP variation detected by two seed cDNA probes appeared to be related to structural changes occurring within tetraploid species. The botanical var. 'fastigiata' (valencia market type) of *A. hypogaea* subspecies *fastigiata* was shown to be the most variable. *Arachis monticola* was found to be more closely related to *A. hypogaea* subspecies *hypogaea* than to subspecies *fastigiata*. Diploid species *A. cardenasii, A. duranensis*, and *A. glandulifera* showed considerable intraspecific genetic diversity, but *A. batizocoi* showed little polymorphism. The genetic distance between the cultivated peanut and wild diploid species was found to be closest for *A. duranensis*.

Key words: Restriction fragment length polymorphism – Tetraploid cultivated peanut – *Arachis* species – Genetic diversity – Genetic distance

Introduction

Peanuts are an important crop throughout the world. While they are used as a source of high quality vegetable oil in many countries, in the United States peanuts are an important food source and consumed as roasted nuts, peanut butter, or as a confectionery product.

The cultivated peanut, *Arachis hypogaea* L., is believed to have originated in the northwestern Argentina-southern Bolivia region of South America (Hammons 1982). Peanuts were dispersed during the sixteenth century to Europe, Africa, and Asia by European explorers.

Arachis hypogaea is a tetraploid, self-pollinating species with $2n = 40$ chromosomes that consists of two subspecies, *hypogaea* and *fastigiata*. Subspecies *hypogaea* is further divided into two botanical varieties: 'hypogaea', which includes runner and virginia U.S. market types, and 'hirsuta', or peruvian market type. Subspecies *fastigiata* is also divided into two varieties: 'fastigiata', or valenica market type, and 'vulgaris', or spanish market type. *Arachis hypogaea* belongs to section *Arachis*, one of seven sections within genus *Arachis* (Gregory et al. 1973). In the *Arachis* section *A. monticola* is the only wild tetraploid species with $2n = 40$ chromosomes, while the remaining species are diploids with $2n = 20$ chromosomes. *Arachis hypogaea* is cross compatible with *A. monticola*, and the F_1 hybrids are fertile. *Arachis* wild diploid species can be hybridized with cultivated peanut, but infertile triploids are mostly produced. On the basis of cytological evidence diploid species in the *Arachis* section have been grouped into different genomic clusters: the 'A' genomic group, represented by most of the diploid species; the 'B' genomic group, represented by *A. batizocoi* (Singh and Moss 1982); and a further 'D' genomic group, represented by *A. glandulifera* (Stalker and Moss 1987).

Stable bivalent pairing with occasional multivalent formation and the presence of two pairs of morphologically distinguishable chromosomes in the *A. hypogaea* genome (Husted 1936) have suggested it to be of allopolyploid origin. On the basis of cytological evidence, *A. batizocoi* ('B' genome species) is considered to be one

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of the diploid progenitors of cultivated peanut (Smartt et al. 1978; Singh and Moss 1984; Singh 1986). Several 'A' genome species have been postulated to be the other diploid progenitor of *A. hypogaea* (Seetharam et al. 1973; Smartt et al. 1978).

Genetic analysis of interspecific hybrids has been used in the past to understand the genetic relatedness among peanut species. Analysis of restriction fragment length polymorphisms can provide additional information to improve our understanding of these relationships. On the basis of RFLP band sharing between cultivated peanut and 14 wild *Arachis* species, *A. ipaensis*, *A. duranensis*, and *A. spegazzinii* were considered to be the most closely related to the diploid progenitor species of allotetraploid cultivated peanut (Kochert et al. 1991).

In the study reported here random genomic probes and seed cDNA probes were used for RFLP analysis on a number of accessions within six species of the *Arachis* section; the cultivated tetraploid species, *A. hypogaea*; the wild tetraploid species, *A. monticola*; and four wild diploid species, *A. batizocoi, A. cardenasii, A. duranensis* and *A. glandulifera*. The objective of this work was to gain information about the genetic variation and genomic origin of cultivated peanut by evaluating the average genetic diversity within species and the genetic distance among species of *Arachis*.

Materials and methods

Plant materials

Fourteen accessions of *A. hypogaea*, 2 accessions of an advanced generation *A. hypogaea* subsp. *fastigiata* × *A. cardenasii* interspecific hybrid with 2n = 40, seven accessions of *A. monticola*, four accessions of *A. batizocoi*, four accessions of *A. cardenasii*, five accessions of *A. duranensis*, and four accessions of *A. glandulifera* were used for RFLP analysis (Table 1). *A. hypogaea* accessions were chosen to represent as diverse a group as possible of all three commercial botanical varieties of plants. In the case of the wild species, as many of the available seed-propagated accessions as possible were used. Plants were grown in the greenhouse or in the field during April through May. Based on preliminary DNA hybridization patterns showing the homogeneity of individual plants within *A. glandulifera* accession PI468342, unopened leaves from several young plants were pooled for each accession of cultivated or wild species, then immediately frozen in liquid N_2 and stored at −70 °C.

Probes

A total of 23 random genomic and seed cDNA probes were used. Sixteen genomic probes ranging from 0.6 to 2 kb in size were selected at random from an *Arachis hypogaea Pst*I-generated genomic library in PUC 18. The genomic library was constructed in our laboratory from the spanish type, high oleic acid peanut line FL 435. In addition, seven seed cDNA probes ranging from 0.6 to 2 kb in size were used. The *Arachis hypogaea* seed cDNA library in lambda gt11 was provided by Dr. A. G. Abbott, Clemson University and was made from maturity stage II seeds of spanish type, high oleic acid peanut line FL 435. The isolation of genomic clones followed the alkali-SDS lysis method described by Ish-Horowicz and Burke (1981), and the isolation of seed cDNA clones was done by PCR amplification of individual plaques (Herman et al. 1990).

Plant DNA isolation

Total DNA was isolated from peanut leaf tissue by using two methods. For the greenhouse plants, the DNA was extracted according to the procedure of Saghai-Maroof et al. (1984) with the following modifications: DNA was extracted in 2× CTAB extraction buffer, followed by two chloroform/isoamyl alcohol (24:1) extractions and precipitation with CTAB precipitation buffer. For field-grown plants, the nuclei were extracted prior to DNA extraction, based on the procedure of Bernatzky and Tanksley (1986). Young leaves (3–5 g) were ground to a fine powder in liquid N_2. That powder was rapidly mixed into a

Table 1. Peanut accessions used for RFLP analysis

Species Subspecies	– market types (variety)	Genome-Ploidy	Accessions
A. hypogaea		AB-2n = 4x = 40	
hypogaea	– Runner US market type (hypogaea)		Southern Runner, Okrun, F79308-1, 22-714
	– Virginia US market type (hypogaea)		F89109, F1036, F89-2513, PI362130
fastigiata	– Valencia type (fastigiata)		F89501, F89509, F89512
	– Spanish type (vulgaris)		F89506, F89508, F435, F79-1339
A. hypogaea × *A. cardenasii*		AB-2n = 4x = 40	NC104, NC303
A. monticola Krap. et Rig.		AB-2n = 4x = 40	263393, 219824, 405933, 468196, 467260, 467261, 468199
A. batizocoi Krap. et Greg.		B -2n = 2x = 20	PI298639, PI468327, PI468328, PI468326
A. cardenasii Krap. et Greg.		A -2n = 2x = 20	PI262141, PI475999, PI476011, PI476014
A. duranensis Krap. et Greg.		A -2n = 2x = 20	PI219823, PI468200, PI468201, PI475844, PI475846
A. glandulifera Stalker		D -2n = 2x = 20	PI468336, PI468341, PI468342, PI468343

nuclei extraction buffer, filtered through one layer of miracloth, and centrifuged at 100 *g*. After nuclei lysis in a buffer containing 2% CTAB, the DNA was extracted by incubating the buffer solution at 60 °C for 15 min, purified one time by extraction with chloroform/isoamyl alcohol (24:1) and precipitated with ice-cold isopropanol. DNA precipitates were resuspended in TE at 60 °C and centrifuged to remove insoluble materials. The supernatant was further precipitated with two volumes of ice-cold ethanol. After drying, the DNA was resuspended in TE and stored at −20 °C. The quantity and quality of the nucleic acids were determined by absorbance measured at 260 nm and 280 nm.

Restriction enzyme digestion, blotting, and hybridization

Fifteen micrograms of total nucleic acids were digested with restriction enzymes using 1.3 U/μg nucleic acid. Six-base restriction endonucleases (*Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I) and four-base endonucleases (*Rsa*I and *Hae*III) were used for digesting the DNA. Restriction enzyme-digested DNA was electrophoresed on a 0.8% agarose gel at 2 V/cm overnight, Southern blotted onto a Hybond N membrane (Amersham), and UV crosslinked for 2 min at 300 nm. Fifty nanograms of probe DNA was labeled with alpha-P^{32} dCTP by random primer extension (Feinberg and Vogelstein 1983). Pre-hybridization and hybridization were done at 65 °C with 7% SDS and denatured salmon sperm DNA (Church and Gilbert 1984). Washing was done three times for 10 min each at 65 °C with 2 × SSC, then 1 × SSC, and finally with 0.5 × SSC (all washes included 0.1% SDS). Hybridized blots were autoradiographed using X-ray film (Kodak XAR-5) and two intensifying screens at −70 °C for 3–7 days.

RFLP data analysis

Restriction fragment sizes were estimated by the computer program of Schaffer and Sederoff (1981) using fragments of *Hin*dIII-digested bacteriophage lambda and *Hae*III-digested phi X174 as fragment size standards.

RFLP data were analyzed using the computer program of Swofford and Selander (1981). The average genetic diversity within and between species was determined from the frequencies of restriction fragment sizes following Nei's methods calculating the unbiased estimates of average heterozygosity (gene diversity) and genetic distance (Nei 1987). The cluster analysis based on Nei's distance coefficients used the unweighted pair-group method (Sneath and Sokal 1973).

Results

Sixteen random *Pst*I-generated genomic probes and seven seed cDNA probes were hybridized to accessions of tetraploid species: 3–7 accessions of *A. monticola*, 7–16 accessions of *A. hypogaea*, and 1–2 accessions of

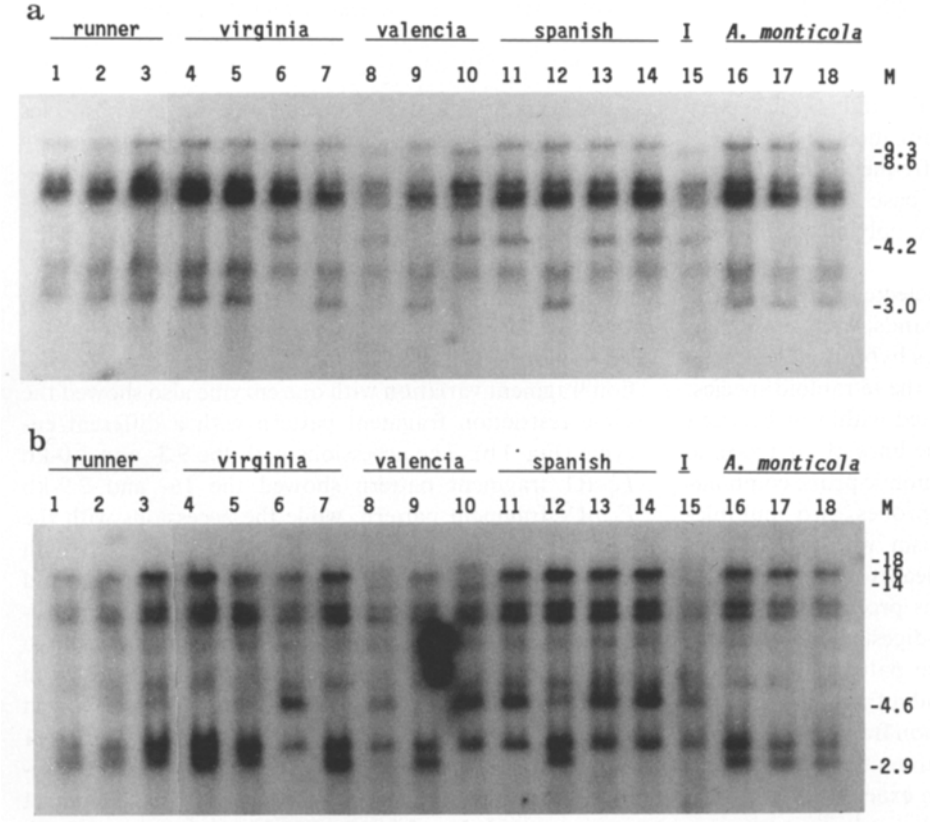


Fig. 1 a, b. RFLP patterns detected by C03 among the accessions of tetraploid species. **a** *Eco*RI digestion: **b** *Eco*RV digestion. *Lanes 1–7 A. hypogaea* subsp. *hypogaea* (*1–3* runner type, *4–7* virginia type), *lanes 8–14 A. hypogaea* subsp. *fastigiata* (*8–10* valencia type, *11–14* spanish type), *lane 15* interspecific origin (NC104), *lanes 16–18 A. monticola*, *M* marker

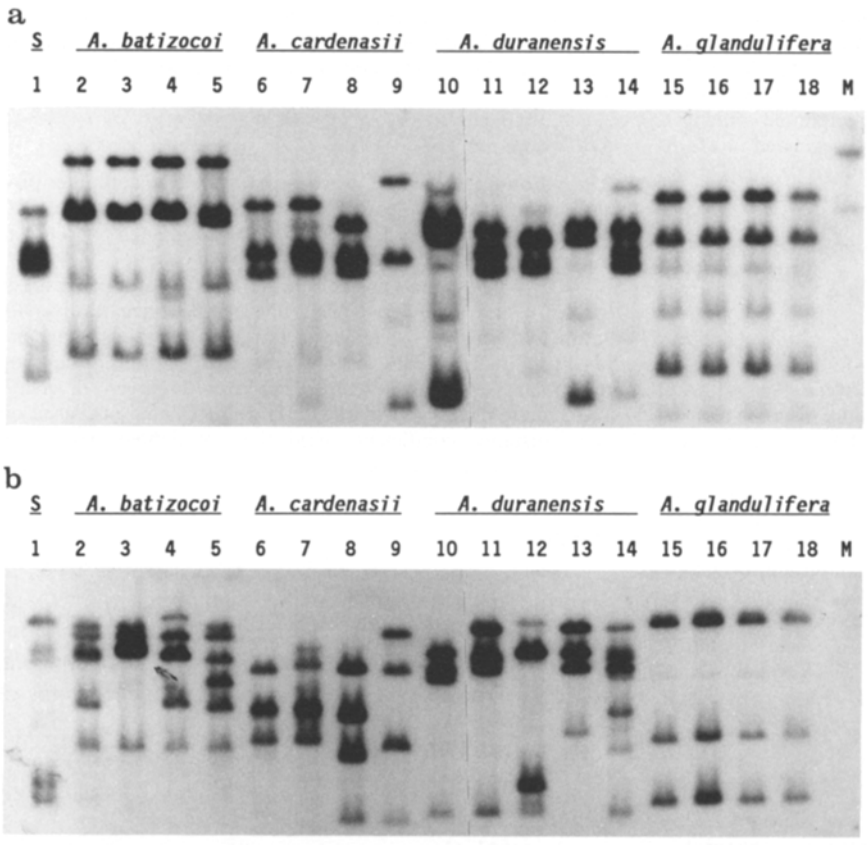


Fig. 2 a, b. RFLP patterns detected by C03 among cultivated peanut and four diploid species. **a** *Eco*RI digestion; **b** *Eco*RV digestion. *Lane 1 A. hypogaea* (southern runner), *lanes 2–5 A. batizocoi, lanes 6–9 A. cardenasii, lanes 10–14 A. duranensis, lanes 15–18 A. glandulifera, M* marker

A. hypogaea × *A. cardenasii*. *Eco*RI and *Eco*RV were used as restriction endonucleases for most of the RFLP work because *Hin*dIII and *Rsa*I frequently showed incomplete digestion and the four-base recognition enzyme, *Hae*III, failed to show more polymorphism than the six-base recognition enzymes.

Most genomic probes (14/16) detected the RFLP pattern of a few restriction fragment bands, while more than half (4/7) of the seed cDNA probes hybridized to multiple bands among the accessions of the tetraploid species. No polymorphism could be detected within or between *A. hypogaea*, *A. monticola*, and the lines of interspecific origin with the 32 endonuclease-genomic probe combinations. Of the seven seed cDNA probes, two multiple-band probes showed polymorphism within tetraploid species regardless of the endonucleases used. Figure 1 a shows three hybridization patterns produced by multiple-band probe CO3 and *Eco*R1-digested DNA within the tetraploid species. These three patterns involved a fragment of either 9.3 or 8.6 kb and a fragment of either 4.2 or 3.0 kb. Although the restriction fragment variation shown by probe CO3 did not differentiate the species, subspecies, or variety classification exactly, several fragment differences were noticed within subspecies or species. While the 3.0-kb fragment was present in the majority of subspecies *hypogaea* and *A. monticola* accessions, the 4.2-kb fragment was more common in subspecies *fastigiata* accessions. The 8.6-kb fragment hybridized by CO3 was unique to the valencia market type of subspecies *fastigiata* and NC104 of the interspecific hybrid. The 8.6-kb band of NC104 was not unexpected because the female parent of the interspecific hybrid was the valencia market type of the *A. hypogaea* subspecies. When a different restriction enzyme was used with the same probe, the group of accessions which showed the same restriction fragment variation with one enzyme also showed the same restriction fragment pattern with a different enzyme (Fig. 1 b). The accessions with the 9.3- and 3.0-kb *Eco*R1 fragment pattern showed the 16- and 2.9-kb *Eco*RV fragment pattern, while the accessions with the 9.3- and 4.2-kb *Eco*R1 fragment pattern and those with the 8.6- and 4.2-kb *Eco*R1 pattern revealed the 16- and 4.6-kb, and 18-, 14- and 4.6-kb *Eco*RV patterns, respectively. Probe C34 and *Eco*RI-digested DNA showed two hybridization patterns within the tetraploid species in which either a 4.5-, or a 4.0-kb fragment was involved (data not shown). The accessions with a 4.5-kb *Eco*R1 restriction fragment pattern showed a 17-kb *Eco*RV fragment pattern,while those with a 4.0-kb *Eco*R1 fragment pattern revealed a 15-kb *Eco*RV fragment pattern. Both the 4.0-kb *Eco*R1 and 15-kb *Eco*RV fragments were unique to subspecies *fastigiata*. The RFLP data shown

Table 2. Restriction fragments lengths detected by nine probes in five *Arachis* section species

Species accessions	Probe								
	G12-V	G20-I	G22-I	G23-V	G25-V	G30-I	C14-I	C21-I	C23-I
A. hypogaea									
Southern runner	5.6/3.2	3.6/2.3	3.8/6.4	12.0/18.1	5.6/4.5	10.2/23.4	2.5/2.8	4.1/7.6	16.0/10.7
A. batizocoi									
PI298639	5.6	3.0	8.9	15.1	5.6	17.5	2.0	4.4	9.5
PI468327	5.6	3.0	8.9	15.1	5.6	12.5	2.0	4.4	9.5
PI468328	5.6	3.0	8.9	15.1	5.6	12.5	2.0	4.4	9.5
PI468326	5.6	3.0	8.9	15.1	5.6	12.5	2.0	4.4	9.5
A. cardenasii									
PI262141	5.6/12.2	3.6	3.8	12.0	5.6/9.8	28.4	2.5	23.0	21.5
PI475999	5.6/12.2	3.6	3.8	12.0	5.6/9.8	28.4	2.3	11.0	17.5
PI476011	5.6	3.6	3.8	12.0	9.8	28.4	2.3	11.0	21.5/10.7
PI476014	12.2	10.6	8.9	12.0	6.2	28.4	2.2	11.0	10.7
A. duranensis									
PI219823	5.6	3.6/2.3	4.1	12.0	5.6	28.4	2.5	4.1	16.0
PI468200	5.6	3.6	3.8	8.5/4.1	5.6	28.4	2.6	4.1	16.0
PI468201	5.6/3.2	3.6/2.3	3.8	12.0/18.1[a]	5.6/4.5[a]	28.4/23.4[a]	2.3/2.8[a]	4.1/7.6[a]	9.0
PI475844	5.6	3.6	4.1	12.0	5.6	28.4	2.3	4.1	16.0
PI475846	5.6	3.6	4.1	8.5/4.1	5.6	10.2	2.5	4.1/4.6[a]	16.0
A. glandulifera									
PI468336	5.6/4.4	3.0	13.9	15.1	17.0	19.8/12.5[a]	3.0	10.3	14.4
PI468341	5.6/2.4	3.0/1.8	8.9	15.1	20.8	19.8	3.0	10.3	14.4
PI468342	5.6/2.4	3.0	9.4	15.1	20.8	19.8	3.0	10.3	14.4
PI468343	5.6/2.4	3.0	10.2	15.1	20.8	19.8	3.0	10.3	14.4

-I and -V designates endonuclease *Eco*RI and *Eco*RV

[a] Indicates a lightly hybridized band. The fragment sizes in kb represent +/− 150 bp

by two cDNA probes suggested that the valencia market type of subspecies *fastigiata* was the most variable and that *A. monticola* was more closely related to subspecies *hypogaea* than to subspecies *fastigiata*. Because the use of different enzymes with the same probes did not change the hybridization pattern variation in the group of accessions, the polymorphism shown by two cDNA probes within both tetraploid species was most likely related to structural changes rather than restriction site gain/loss.

The assessment of RFLPs was further expanded to the wild diploid species. The two multiple-band seed cDNA probes, CO3 and C34, that had shown polymorphism within the tetraploid species also hybridized to multiple bands in the diploid species and displayed highly polymorphic restriction fragment patterns among the accessions within *A. cardenasii* and *A. duranensis* regardless of the endonucleases used (Fig. 2). Because it was difficult to analyze the genetic diversity within or between species with the multiple restriction fragment bands, seven of the previous 16 genomic probes and three seed cDNA probes that had shown two bands on the tetraploids were hybridized to DNA of four accessions of *A. batizocoi*, four accessions of *A. cardenasii*, five accessions of *A. duranensis* and four accessions of *A. glan-*

Table 3. Estimates of Nei's unbiased average gene diversity within species

Species	Mean sample size per locus	Percentage of loci polymorphic[a]	Mean genetic diversity
A. hypogaea	8.3	0.0	0.000
A. batizocoi	4.0	10.0	0.043
A. cardenasii	4.0	80.0	0.461
A. duranensis	5.0	80.0	0.316
A. glandulifera	4.0	50.0	0.239

[a] Percentage of loci polymorphic indicates the percentage of probes showing polymorphism within a species

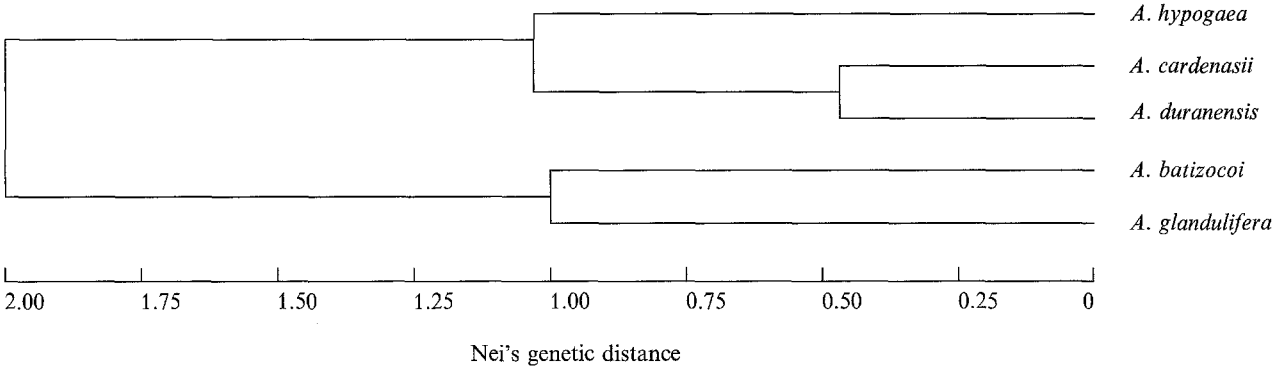
Table 4. Estimates of Nei's unbiased genetic distance coefficients among five *Arachis* section species

Species	*A. batizocoi*	*A. cardenasii*	*A. duranensis*	*A. glandulifera*
A. hypogaea	1.875	1.211	0.832	2.859
A. batizocoi		1.566	1.061	1.005
A. cardenasii			0.469	2.432
A. duranensis				2.082

dulifera. All three seed cDNA probes and six of the seven genomic probes showed hybridization pattern variation among the diploid species.

The average gene diversity within species and the genetic distance among five species, *A. hypogaea, A. batizocoi, A. cardenasii, A. duranensis,* and *A. glandulifera,* were determined from the data shown in Table 2. Since the second enzyme was not likely to be independent of the first enzyme, the data was based on one enzyme and nine probes that hybridized to one band in diploid species and two bands in tetraploid species. However, some *A. duranensis* accessions showed lightly hybridized bands which were visible when DNA hybridization was repeated, suggesting heterogeneity within accessions. Those lightly hybridized fragments were not included in the analysis because of the low frequency of their occurrence (Fig. 3). Table 3 shows Nei's unbiased average gene diversity within five *Arachis* section species. *Arachis hypogaea* showed no gene diversity within species with the nine few-band probes. *Arachis batizocoi* showed little diversity within species, and some of the restriction fragment sizes were unique when compared to the other species (Table 2). These findings corroborate previous cytological findings about the genetic relationship between *A. batizocoi* and other *Arachis* section diploid species (Singh 1986; Stalker and Wynne 1979). *Arachis cardenasii* and *A. duranensis* showed considerable gene diversity within species, suggesting genetic diversification among the 'A' genome species. *Arachis glandulifera* was relatively diverse among accessions, but it showed unique restriction fragment sizes and more bands were noticed with probe G12 (Table 2). Table 4 shows Nei's unbiased genetic distance coefficients among *A. hypogaea* and four diploid species, and Table 5 shows a cluster analysis based on those coefficients. The distance analysis agreed with a genomic classification determined by conventional studies, except for *A. hypogaea* (See Table 1 for the genomic classification of species). The two 'A' genome species, *Arachis cardenasii* and *A. duranensis* were closely related with the smallest distance coefficient of 0.469. *Arachis batizocoi* and *A. glandulifera* were more distantly related, and had a coefficient of 1.005. The distance coefficients support that *A. batizocoi* and *A. glandulifera* may represent two independent genomes. The proposed 'AB' genome amphidiploid *A. hypogaea* was the most closely related to *A. duranensis* with a distance coefficient of 0.832. The cluster analysis using unweighted pair-group method clustered *A. hypogaea* with two 'A' genome spe-

Table 5. Unweighted pair group clustering of five *Arachis* section species



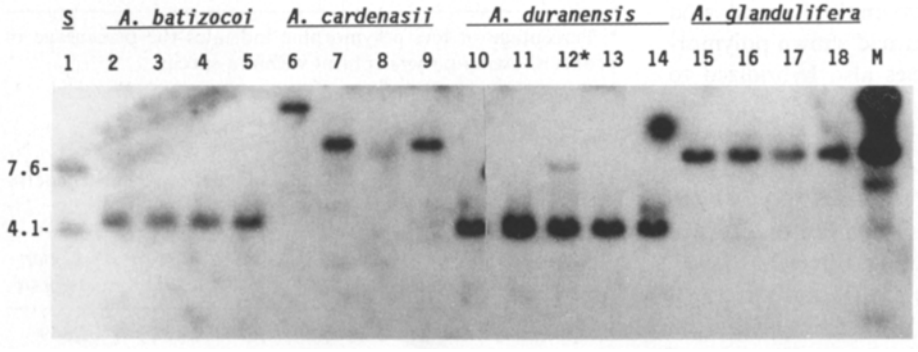


Fig. 3. RFLP patterns detected by probe C21 and *Eco*R1 among cultivated peanut and four diploid species. *Lane 1 A. hypogaea* (southern runner), *lanes 2–5 A. batizocoi, lanes 6–9 A. cardenasii, lanes 10–14 A. duranensis, lanes 15–18 A. glandulifera, M* marker. * Indicates *A. duranensis* accession PI468201

cies, *A. cardenasii* and *A. duranensis* (Table 5), suggesting its close relatedness to 'A' genome species.

Discussion

Whereas outcrossing crop species have generally shown abundant restriction fragment polymorphism, restriction fragment diversity within self-pollinating species such as wheat, tomato, and soybean (Sharp et al. 1989; Helentjaris et al. 1985; Keim et al. 1989) has been much smaller. Cultivated peanut, a self-pollinating species, follows that same general pattern in that no polymorphism was found with 30 probe-enzyme combinations when *Pst*I-generated genomic probes were used. Since polymorphism is necessary for RFLP mapping and for the use of those markers for genetics and breeding, it would be ideal if restriction fragment polymorphism were present between the two completely cross-compatible species, *A. hypogaea* and *A. monticola*. Unfortunately, both our data and a previous RFLP study on peanut (Kochert et al. 1991) indicate that there is little variation between these two species. The lack of polymorphism within and between tetraploid species suggests that the origin of tetraploid species is narrow based, possibly from a single hybrid plant that doubled its chromosome number following interspecific hybridization, similar to what has been suggested for wheat (Sharp et al. 1989).

Structural changes within the tetraploid species were shown by two seed cDNA probes that hybridized to multiple bands and showed highly polymorphic restriction fragment patterns within 'A' genome species *A. cardenasii* and *A. duranensis*. Recent molecular studies of genome organization in eukaryotes indicate that many genes do not exist as single copies in the genome, but rather as clusters (Nei 1987). If the multiple bands detected by the seed cDNA probes indicate multiple copies of the genes, the increased polymorphism in multiple loci regions may be due to inexact homeologous pairings, which result in deletion and insertion events (Ohno 1970). In peanut, four of the seven seed cDNA probes hybridized to multiple bands and were more effective in detecting polymorphism within tetraploid species than the *Pst*I-generated random genomic probes. Many of the genes expressed in the seed may be related to storage oil, storage protein, and carbohydrate synthesis, which may be encoded by multiple gene families. The storage proteins in seeds have been known to be coded by multigene families (Higgins 1984).

It has been suggested that cultivated peanut evolved from 'A' and 'B' genome species with sufficient diversity to produce bivalent pairing in the tetraploid. The genetic distance analysis showed that the 'A' genome species, *A. duranensis* is the most closely related to *A. hypogaea*, suggesting that species as a likely 'A' genome donor of cultivated peanut. *Arachis batizocoi* is the only 'B' genome species known. However, the lack of restriction fragment homology and the large distance coefficient shown between *A. batizocoi* and *A. hypogaea* do not lend much support for that species as a 'B' genome donor of *A. hypogaea*. It is possible that the 'B' genome donor of cultivated peanut has not been found yet, is extinct, or that *A. hypogaea* originated from 'A' × 'A' hybridization.

On the basis of data showing the close relatedness of 'A' genome species to cultivated peanut and the large genetic diversity within 'A' genome species, we cannot exclude the possibility that the cultivated peanut evolved from the closely related two 'A' genome species. Because the lack of polymorphism within tetraploid species indicates that the genomic changes sufficient to cause diploid pairing did not occur after the initial tetraploid was formed, another mechanism would be necessary to produce diploid pairing in cultivated peanut if cultivated peanut originated from two closely related species. In hexaploid wheat there are one or more genes on chromosome 5B that prevent homeologous pairing between very closely related progenitors, thus making it possible to have bivalent pairing with high fertility and stable inheritance (Riley et al. 1960).

An interesting result was noticed in one *A. duranensis* accession, PI468201, which showed unique polymorphic bands similar in size to those of *A. hypogaea*, although sometimes lightly hybridized. These unique bands of the *A. duranensis* accession enabled *A. duranensis* to reconstitute the restriction fragment patterns of *A. hypogaea* within the species over 11 of a total of 13 probes tested (Table 2, example in Fig. 3). It is not clear yet whether the light hybridization of the bands was due to their being present in only one of several pooled plants of the *A. duranensis* accession, PI468201. The seed propagation of plants may have generated genetic diversity within accessions. Individual plants from *A. duranensis* accession PI468201 and more *Arachis* diploid species need to be screened in order to determine whether a plant with the *A. hypogaea*-like pattern can be found.

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