Assessment of Genetic Diversity and Sectional Boundaries in Tetraploid Peanuts (*Arachis*)

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ABSTRACT

Tetraploid populations of peanuts were examined using randomly amplified polymorphic DNA (RAPD) methods. Sectional alignments within the genus are supported in the section *Arachis* but not in *Rhizomatosae*. Because of low genetic diversity and similarity between it and its closest wild relative, *A. monticola*, previous hypotheses of a single origin in the domesticated peanut are supported. Four populations of *A. glabrata* showed high levels of genetic diversity and are genetically different from *A. pseudovillosa* even though they occur in the same section. These data provide the first evidence of high genetic diversity within wild, perennial, tetraploid peanuts, and for possible multiple origins of tetraploids in the section *Rhizomatosae*.

Key Words: RAPD, section *Arachis*, section *Rhizomatosae*.

Arachis L., the genus that includes the cultivated peanut, has about 69 species occurring in South America east of the Andes, south of the Amazon, north of La Plata, and from northwest Argentina to northeast Brazil. The species have been assigned to nine sections on the basis of cross-incompatability and exo-morphologic character clustering (Krapovickas and Gregory, 1994). Section *Rhizomatosae* contains one diploid species (*A. burkartii* Handro) and two tetraploid species [*A. glabrata* Benth. and *A. pseudovillosa* (Chodat & Hassl.) Krapov. & W.C. Gregory]. The largest section, *Arachis*, contains many diploid species and two tetraploids (*A. monticola* Krapov. & Rigoni and *A. hypogaea* L., the cultivated peanut) (Fernandez and Krapovickas, 1994; Krapovickas and Gregory, 1994). Evidence from meiotic chromosome associations (Seetharam *et al*., 1973; Stalker, 1985), karyotypes (Stalker and Dalmacio, 1986), isozymes (Lu and Pickersgill, 1993; Stalker *et al*., 1994), and DNA restriction fragment length polymorphisms (RFLP) (Halward *et al*., 1991, 1992; Kochert *et al*., 1991, 1996) indicate that the cultivated peanut is an allotetraploid. Evidence from nuclear cDNA and chloroplast DNA RFLP data (Kochert *et al*., 1996) as well as sequence data (Jung *et al*., 2003) support *A. duranensis* Krapov. & W.C. Gregory and *A. ipaensis* Krapov. & W.C. Gregory as the species most similar to progenitors that hybridized producing *A. hypogaea*. RFLP data (Kochert *et al*., 1996) suggests that *A. monticola* is likely the existing taxon that most resembles the original allotetraploid that resulted from hybridization between the aforementioned species. However, sequence data used to generate phylogenetic trees (Jung *et al*., 2003) and cytogenetic evidence (Stalker and Dalmacio, 1986) suggest that *A. monticola* is not a progenitor of the cultivated peanut. While a great deal has been elucidated in regard to the domesticated peanut and its wild relatives, little is known about the origins of the other tetraploids in the genus, other than that they likely evolved independently of the domesticated peanut and its wild relatives (Smartt and Stalker, 1982; Halward *et al*., 1991, 1992).

RAPD methods have previously been used in genetic studies of peanuts and were used to examine several wild tetraploid species in the genus. Halward *et al*. (1992) examined two peanut cultivars; 25 unadapted germplasm lines of *A. hypogaea*, the putative, allotetraploid progenitor of the cultivated peanut (*A. monticola*); a tetraploid from section *Rhizomatosae* (*A. glabrata*); and 29 diploid species using 10 random primers. No variation was found in *A. hypogaea* cultivars or germplasm, but variation was identified among the *Arachis* species. They concluded that the tetraploids in section *Arachis* and *Rhizomatosae* were the result of polyploidy evolving twice within the genus (Halward *et al*., 1992). Intraspecific variation was lowest in *A. hypogaea* and *A. monticola* and the two species clustered together in dendograms based on RAPD data (Hilu and Stalker, 1995). *Arachis glabrata* was genetically distinct when compared to the *A. hypogaea-A.monticola* complex (Halward *et al*., 1992). In addition, a diploid *Rhizomatosae* species (*A. burkartii*) was shown to be genetically different from tetraploid taxa of the section (Gimenes *et al*., 2002).

Because hypotheses regarding the origin of the domesticated peanut have been established (Ko-

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Table 1. Germplasm collections of *Arachis* used in this investigation from Simpson and Higgins (1984).

	Species/variety	Plant introduction no.	Country	State, nearest town, or route
1	*A. glabrata* var. *glabrata*	262801	Argentina	Corrientes, Loreto
2	*A. glabrata* var. *glabrata*	338303	Brazil	Matogrosso, Ponta Pora
3	*A. glabrata* var. *glabrata*	276202	Brazil	Minis Garais, Capinopolis
4	*A. glabrata* var. *glabrata*	262827	Paraguay	On Ruta 2, Asuncion to Hernandarias
5	*A. hypogaea* subsp. *hypogaea*	475984	Bolivia	Santa Cruz, Natividad
6	*A. hypogaea* subsp. *hypogaea*	476069	Brazil	Parana, CENARGEN, Brasilia
7	*A. hypogaea* subsp. *fastigiata*	476166	Peru	San Martin, Tarapoto
8	*A. monticola*	468196	Argentina	Jujuy, Yala
9	*A. monticola*	468199	Argentina	Jujuy, Yala
10	*A. pseudovillosa*	276217	Brazil	Finca elvira; 43 km by road; NW of Ponta Porá, Mato Grosso, Brazil

chert *et al*., 1996) and taxonomic boundaries have been defined for sections and species within the genus (Krapovickas and Gregory, 1994), RAPD markers would be useful in examining these hypotheses as well as other tetraploids in the group in regard to their genetic diversity and sectional affinities. Specifically, the objectives of this investigation were to examine the two sections that contain tetraploid species (*Arachis* and *Rhizomatosae*), assess the levels of genetic diversity in *A. hypogaea*, *A. monticola*, and *A. glabrata*, as well as evaluate the position of *A. pseudovillosa* within the genus.

Materials and Methods

Plant Materials. Seeds from 10 collections (Table 1) were obtained from Dr. Charles Simpson at the Texas Agric. Exp. Sta., Stephenville, TX. Plants were grown in the Dept. of Biological Sciences Greenhouse at Tarleton State Univ., Stephenville,TX. Young leaf material from these plants was used for DNA isolation.

DNA Isolation. Total genomic DNA used as templates was isolated using the DNeasy Plant Maxi Kit (Qiagen, Valencia, CA). Nucleic acid purity and approximate concentration was determined using an ultraviolet spectrophotometer.

RAPD Protocols. The RAPD procedure followed that of Williams *et al*. (1990) with some modifications (Hilu and Stalker, 1995). Ten samples of tetraploid peanut species (Table 1) were run with 40 10-base oligonucleotide primers (kits A and B from Operon Technologies, Inc., Almeda, CA). Only clear, well-separated amplification products were scored, and each of these was tested several times for repeatability. Of the 40 10-base oligonucleotide primers initially evaluated, 16 were polymorphic and utilized for analyses (primers OPA 1, 3, 4, 7, 8–11, 13, 14, 18–20 and OPB 10, 12, 17).

Each reaction mixture for PCR amplification totaled 25 μL and contained 2.5 μL of 20× reaction buffer, 2 mM $MgCl_2$; 1 mM each of dATP, dCTP, dGTP, and dTTP; 5 to 6 pM of single PCR primer; 0.9 unit of *Taq* polymerase (Qiagen); and approximately 25 ng of genomic DNA template. All reaction mixtures were prepared as master mixes for each primer to minimize measurement errors. Amplification was carried out in a Techne Genius thermal cycler using one initial denaturation cycle at 95 C for 5 min followed by 75 cycles at 95 C for 10 sec, 36 C for 10 sec, and 72 C for 2 min. On completion of the cycles, samples were refrigerated at 4 C before electrophoresis. Amplification products were separated by electrophoresis in a 1.5% agarose gel. About 20 μL of sample was loaded in the gel and electrophoresed in Tris-acetate-EDTA (TAE) buffer at 100 millivolts for 4 to 5 hr. A 1 kb DNA marker (Fisher Scientific) was used as a standard. DNA in gels was stained with ethidium bromide and photographed under ultraviolet light.

Data Analysis. Polymorphic bands were scored as either present or absent and analyzed using Numerical Taxonomy and Systematics Personal Computer (NTSYS-pc) software (Rohlf, 1990) using the unweighted pair group method using arithmetic averages (UPGMA) analysis based on Dice's similarity between populations, given by $2a/(2a + b)$, where a is the number of positive matches (shared bands) and b is the number of mismatches. Similarity values obtained from the Dice algorithm were utilized to group accessions via the UPGMA method to produce a single unrooted phenogram. The cophenetic coefficients for the clusters and the correlation between these coefficients and the similarity matrix were computed (Sneath and Sokal, 1973). The Dice coefficient was used to calculate similarities on the basis of shared presence of a DNA band and excluded shared absence of

Table 2. Dice's similarity between accessions of *Arachis*.[a]

	1	2	3	4	5	6	7	8	9	10
1	1.0	.636	.667	.686	.391	.409	.406	.406	.427	.376
2		1.0	.650	.650	.455	.458	.469	.469	.434	.301
3			1.0	.643	.434	.423	.434	.434	.426	.342
4				1.0	.443	.460	.457	.457	.420	.315
5					1.0	.982	.988	.989	.952	.382
6						1.0.	.994	.994	.958	.385
7							1.0	1.0	.952	.382
8								1.0	.952	.382
9									1.0	.403
10										1.0

[a]Numbers on the top and side of the table correspond to Table 1.

fragments from the analysis (Sneath and Sokal, 1973).

Results

A total of 16 reliable RAPD primers were scored from the 40 primers that were used in the investigation, and 156 loci were identified for a mean of 9.75 loci per primer. Ninety-two percent of the loci were polymorphic. Forty-three RAPD markers were observed exclusively in *A. glabrata*, none in *A. hypogaea*, one in *A. monticola*, and 15 in *A. pseudovillosa*.

The five accessions of *A. hypogaea* and *A. monticola* had genetic similarity values that ranged from 0.952 to 1.000 (Table 2), whereas four accessions of *A. glabrata* had values that ranged from 0.683 to 0.686 (Table 2). *Arachis pseudovillosa* had a genetic similarity value that was far from either of the aforementioned species with a similarity value of 0.382 to 0.403 to the *A. hypogaea* and *A. monticola* group and 0.301 to 0.376 to the *A. glabrata* group (Table 2).

Clusters formed from UPGMA analysis indicate that accessions of *A. glabrata* are more genetically diverse than accession in the *A. hypogaea-A. monticola* complex (Fig. 1). Within this complex, *A. hypogaea* subsp. *fastigiata* (population 7) is identical to an accession of *A. monticola* (accession 8 in Fig. 1). *Arachis pseudovillosa* is distinct from the other groups, but clusters slightly closer to the *A. hypogaea-A. monticola* complex (Fig. 1).

Discussion

The tetraploids in section *Arachis* cluster together and are genetically very similar (Table 1; Fig. 1). This is concordant with previous investigations using isozymes (Lu and Pickersgill, 1993; Stalker *et al*., 1994), RAPD data (Hilu and Stalker, 1995), and RFLP data (Halward *et al*., 1991, 1992; Kochert *et al*., 1991). The two species included in this investigation from the section *Rhizomatosae* do not cluster together (Fig. 1) and are genetically diverse (Table 1). The section *Rhizomatosae* has been described as problematic and needs further investigation (Smartt and Stalker, 1982). Our data supports the hypothesis that the tetraploid species in section *Rhizomatosae* arose from a polyploidization event that was independent of that which formed the *A. hypogaea-A. monticola* complex (Smartt and Stalker, 1982; Halward *et al*., 1991). Because *A. glabrata* and *A. pseudovillosa* are genetically divergent, this investigation also provides support for multiple origins of the tetraploids *A. pseudovillosa* and *A. glabrata* in the section *Rhizomatosae*. Perenniality and asexual reproduction by rhizomes could allow for more recovered examples of chromosome doubling (Grant, 1971) in different geographic regions and involving more progenitor populations. Multiple origins of taxa in the section *Rhizomatosae*, as opposed to the probable single origin of tetraploids in the section *Arachis* (Kochert *et al*., 1996), could account for the differences in genetic diversity observed in

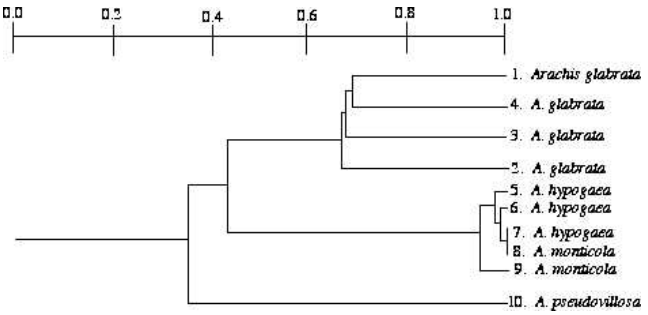

Fig. 1. Grouping of tetraploid species of *Arachis* based on the unweighted pair group method.

tetraploids within the two sections. The diversity of four populations of *A. glabrata* indicates that this tetraploid species may have evolved several times at different locations, or more likely, because of its very broad distribution in diverse habitats, selection resulted in genetic diversity within the species.

Our limited RAPD data supports the hypothesis that *A. monticola* and *A. hypogaea* are genetically similar, with *A. monticola* possibly being the direct, ancestral species of the cultivated peanut (Hilu and Stalker, 1995). This hypothesis has also been supported by isozyme (Lu and Pickersgill, 1993; Stalker *et al*., 1994), RAPD (Hilu and Stalker, 1995), and RFLP data (Halward *et al*., 1991, 1992; Kochert *et al*., 1991). Genetic analysis of the RAPD data in this study indicates that *A. monticola* is genetically more similar to *A. hypogaea* subsp. *fastigiata* than *A. hypogaea* subsp. *hypogaea*. Previous researchers using isozymes (Lu and Pickersgill, 1993; Stalker *et al*., 1994) supported this pattern, but RFLP variation indicated that *A. monticola* is more closely related to *A. hypogaea* subsp. *hypogaea* (Paik-Ro *et al*., 1992). Even though the relationship of *A. monticola* to a particular subspecies of *A. hypogaea* remains unresolved, our limited data and that from isozyme (Lu and Pickersgill, 1993; Stalker *et al*., 1994), RAPD (Hilu and Stalker, 1995), and RFLP data (Halward *et al*., 1991, 1992; Kochert *et al*., 1991) indicate that there is very little interspecific genetic diversity within the *A. hypogaea*-*A. monticola* complex or intraspecific genetic diversity in *A. hypogaea*. However, He and Prakash (1997) detected low levels of DNA polymorphism using DNA amplification fingerprinting and amplified fragment length polymorphism approaches; but they also concluded that the level of genetic variation in cultivated peanut is still low compared to most other cultivated crops.

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Literature Cited

Fernandez, A., and A. Krapovickas. 1994. Cromosomas y evolución en *Arachis* (Leguminosae). Bonplandia 8:187-220.

Gimenes, M.A., C.R. Lopes, and J.F.M. Valls. 2002. Genetic relationships among *Arachis* species based on AFLP. Genet. and Mol. Biol. 25:349-353.

Grant, V. 1971. Plant Speciation. Columbia Univ. Press, New York.

Halward, T.M., H.T. Stalker, E.A. LaRue, and G. Kochert. 1991. Genetic variation detectable with molecular markers among unadapted germ-plasm resources of cultivated peanut and related wild species. Genome 34:1013-1020.

Halward, T., T. Stalker, E. LaRue, and G. Kochert. 1992. Use of single-primer DNA amplifications in genetic studies of peanut (*Arachis hypogaea* L.). Plant Mol. Biol. 18:315-325.

He, G., and C.S. Prakash. 1997. Identification of polymorphic DNA markers in cultivated peanut (*Arachis hypogaea* L.). Euphytica 97:143-149.

Hilu, K.W., and H.T. Stalker. 1995. Genetic relationships between peanut and wild species of *Arachis* sect. *Arachis* (Fabaceae): Evidence from RAPDs. Plant Syst. Evol. 198:167-178.

Jung, S., P.L. Tate, R. Horn, G. Kochert, K. Moore, and A.G. Abbott. 2003. The phylogenetic relationship of possible progenitors of the cultivated peanut. J. Hered. 94:334-340.

Kochert, G., T. Halward, W.D. Branch, and C.E. Simpson. 1991. RFLP variability in peanut (*Arachis hypogaea* L.) cultivars and wild species. Theor. Appl. Genet. 81:565-570.

Kochert, G., H.T. Stalker, M. Gimenes, L. Galgaro, C.R. Lopes, and K. Moore. 1996. RFLP and cytogenetic evidence on the origin and evolution of allotetraploid domesticated peanut, *Arachis hypogaea* (Leguminosae). Amer. J. Bot. 83:1282-1291.

Krapovickas, A., and W.C. Gregory. 1994. Taxonomia del genero *Arachis* (Leguminosae). Bonplandia 8:1-186.

Lu, J., and B. Pickersgill. 1993. Isozyme variation and species relationships in peanut and its wild relatives (*Arachis* L. Leguminosae). Theor. Appl. Genet. 85:550-560.

Paik-Ro, O.G., R.L. Smith, and D.A. Knauft. 1992. Restriction fragment length polymorphism evaluation of six peanut species within the *Arachis* section. Theor. Appl. Genet. 84:201-208.

Rohlf, F.J. 1990. Numerical Taxonomy and Multivariate Analysis System, Ver. 1.80. Exeter Software, New York.

Seetharam, A., K.M.D. Nayar, R. Sreekantaradhya, and D.K.T. Achar. 1973. Cytological studies on the interspecific hybrid of *Arachis hypogaea* × *Arachis duranensis*. Cytologia 38:277-280.

Simpson, C.E., and D.L. Higgins. 1984. Catalog of *Arachis* germplasm collections in South America, 1976–1983. Texas Agric. Exp. Sta., The Texas A&M Univ. System, College Station, TX.

Smartt, J., and H.T. Stalker. 1982. Speciation and cytogenetics in *Arachis*, pp. 21-49. *In* H.E. Pattee and C.T. Young (eds.) Peanut Science and Technology. Amer. Peanut Res. Educ. Soc., Yoakum, TX.

Sneath, P.H., and R.R. Sokal. 1973. Numerical Taxonomy. W. H. Freeman, San Francisco.

Stalker, H.T. 1985. Cytotaxonomy of *Arachis*, pp. 65-79. *In* J.P. Moss (ed.) Proc. Intl. Workshop on the Cytogenetics of *Arachis*, Hyderabad, India. 31 Oct.–2 Nov. ICRISAT, Patancheru, India.

Stalker, H.T., and R.D. Dalmacio. 1986. Karyotype analysis and relationships among varieties of *Arachis hypogaea*. Caryologia 51:617-629.

Stalker, H.T., T.D. Phillips, J.P. Murphy, and T.M. Jones. 1994. Variation of isozyme patterns among *Arachis* species. Theor. Appl. Genet. 87:746-755.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafaliski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18:6531-6535.