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Variation of isozyme patterns among *Arachis* species

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Abstract. The genus *Arachis* contains a large number of species and undescribed taxa with patterns of genetic variation that are little understood. The objectives of this investigation were to estimate genetic diversity among species of *Arachis* by utilizing electrophoretic techniques and to establish the potential for use of isozymes as markers for germplasm introgression. One-hundred-and-thirteen accessions representing six of the seven sections of the genus were analyzed for isozyme variation of 17 enzymes. Section *Rhizomatosae* species were not included because they produce very few seeds. Seeds were macerated and the crude extract was used for starch-gel electrophoretic analyses. Although the cultivated species has few polymorphic isozymes, the diploid species are highly variable and two-to-six bands were observed for each isozyme among accessions. Because of the large number of isozyme differences between *A. hypogaea* and *A. batizocoi* (the presumed donor of the B genome), this species can no longer be considered as a progenitor of the cultivated peanut. Seed-to-seed polymorphisms within many accessions were also observed which indicate that germplasm should be maintained as bulk seed lots, representative of many individuals, or as lines from individual plants from original field collections. The area of greatest interspecific genetic diversity was in Mato Grosso, Brazil; however, the probability of finding unique alleles from those observed in *A. hypogaea* was greatest in north, north-central, south and southeast Brazil. The large number of polymorphic loci should be useful as genetic markers for interspecific hybridization studies.

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Key words: Peanut – Speciation – *Arachis hypogaea* – Groundnut

Introduction

The genus *Arachis* comprises a large number of spcies distributed throughout South America. Five previous taxonomic works (Chevalier 1933, 1934, 1936; Hoehne 1940; Hermann 1954) are outdated because of the large number of new accessions obtained during the past 30 to 40 years (see Gregory et al. 1973; Simpson and Higgins 1984). Subsequent to earlier works, 23 species have been validly described and at least 12 additional unofficial names have been commonly used (Resslar 1980; Stalker 1991). At least 70 species are currently believed to exist in the genus (Valls et al. 1985; Krapovickas 1990). Based on morphological comparisons, geographical distributions, cross-compatibility relationships, and hybrid fertility, Gregory et al. (1973) divided the genus into seven sections that are useful to delineate cross-compatibility groups and to initially separate taxa.

Most *Arachis* species are diploids ($2n = 2x = 20$), but tetraploids ($2n = 4x = 40$) are found in the sections *Rhizomatosae* and *Arachis*. The cultivated peanut (*Arachis hypogaea* L.) and its putative wild progenitor (*A. monticola* Krap. et Rig.) are 40-chromosome species in the section *Arachis*. Based on cluster analyses of morphological traits, Stalker (1990) showed relationships among 73 accessions in this section and estimated that 20 to 25 species will eventually be described from the existing collections. The taxonomic treatments of the section *Arachis* and the genus as a whole remain greatly deficient.

A limited number of cytogenetic studies characterizing species relationships in *Arachis* have been reported. Most species in the section *Arachis* have an A genome and interspecific hybrids within this section have a high degree of chromosome homology; *A. batizocoi* Krap. et Greg. (B genome) and *A. glandulifera* Stalker (D genome) are the two exceptions (Stalker and Moss 1987; Stalker 1991). Chromosome homologies have also been noted between species of the sections *Erectoides* and *Arachis* (Stalker 1981) and *Erectoides* and *Rhizomatosae* (Stalker 1985), but little is known about cytological relationships among most species in the genus.

Several types of chemical analyses have been reported to give preliminary indications of systematic relationships in *Arachis*. For *A. hypogaea*, Tombs (1963) showed variation in seed storage proteins between cultivars, but diversity is insufficient to be useful for cultivar identification (Bianchi-Hall et al. 1991). Ory and Cherry (1972) established several enzyme systems and reported differences between healthy and damaged peanut seeds; but Grieshammer and Wynne (1990b) found that only 3 (glutamate oxaloacetate transaminase, isocitrate dehydrogenase, and phosphohexose isomerase) out of 25 isozymes showed polymorphisms among 68 *A. hypogaea* genotypes. Furthermore, each of these polymorphic isozymes had only two forms. They concluded that isozyme analysis is of little use for molecular characterization of the cultivated peanut. Lacks and Stalker (1993) analyzed a large number of *A. hypogaea* lines from the South American centers of origin and found very little variation in the cultivated species. Cherry (1975) analyzed crude protein extracts and six enzymes of 36 *Arachis* accessions. Based on similarity values of banding pattern, he concluded that species could be grouped into the seven sections outlined by Gregory et al. (1973). Klozova et al. (1983) also observed seed protein patterns by polyacrylamide-gel electrophoretic techniques and found similar patterns of variability.

Stalker et al. (1989) analyzed 80 accessions of *Arachis* for fatty-acid profiles and reported conservation of oleic/linoleic ratios (which they believed to be important for conditioning survival in arid regions) in the sections *Extranervosae* and *Trisiminalae*. They concluded that the *Extranervosae* species may be the most ancient in the genus. Kochert et al. (1991) and Paik-Ro et al. (1992) observed little differentiation among cultivated lines for restriction fragment length polymorphisms (RFLPs), but diploid species in the section *Arachis* were highly variable. Halward et al. (1992) also found that analysis by single-primer DNA amplification (RAPDs) grouped accessions in this section according to published species descriptions and, for unnamed taxa, into similar groups as the morphological clusters reprted by Stalker (1990).

Although cross-compatibility, as well as cytological and biochemical analysis data have begun to answer questions about species relationships in *Arachis*, few estimates of genetic divergence in the genus are currently available. The objectives of this investigation were (1) to establish the potential for isozymes as markers for introgression and germplasm enhancement and (2) to analyze patterns of variation associated with geographical distribution and species relationships.

Materials and methods

Seeds of 109 accessions of diploid *Arachis* species, three tetraploid accessions of *A. monticola* (7264, 30062, 30063), and one *A. hypogaea* cultivar (NC 4), were obtained from the 1989 seed nursery at the Sandhills Research Station, N.C. (see Table 1). One representative *A. hypogaea* line was used for comparison based on previous studies showing little isozyme variation in the species (Grieshammer and Wynne 1990a; Lacks and Stalker 1993). The accessions represent six of the seven *Arachis* sections as outlined by Gregory et al. (1973). Species in the section *Rhizomatosae* were not included because they produce very few seeds under cultivated conditions. Procedures for enzyme extractions, starch-gel preparation, electrophoresis, and enzyme staining followed those published by Grieshammer and Wynne (1990 a). Mature undamaged seeds were imbibed for 24 h at room temperature. The seed coat was removed and seeds were macerated at 4 °C; the tissues analyzed included cotyledons and embryos. Five individual seeds from each accession were initially analyzed for each enzyme but, when seed-to-seed variation was observed, an additional 15 to 30 seeds were scored for the specific variable enzymes.

Isozyme banding patterns were scored for 17 enzyme systems (see Table 2) and data recorded for the presence or absence of the respective bands. Bands were numbered consecutively from the cathode end of the gel for each enzyme. Principal component analysis (SAS 1982), based on the covariance matrix of band presence or absence, was used to separate the 113 accessions. A second principal component analysis, again using the covariance matrix of band presence or absence, was restricted to the 83 accessions of the section *Arachis*. Hedrick's probability of genotypic identity (Hedrick 1971) was then used to estimate genetic similarity among species accessions.

Results

An extensive amount of isozyme polymorphism was observed among the *Arachis* accessions (Tables 1–3; Fig. 1). Although only two different bands were evident for IDH and TPI (see Table 2 for these and subsequent abbreviations), six bands were observed for MNR and PHI (Table 2). The banding patterns for TPI and SAD were difficult to score because they appeared as small or lightly-stained clusters of bands, so scoring was done on the basis of band clusters. A total of 66 different bands or band clusters were observed for the 17 enzymes (Table 2). However, all combinations of two or more band pairs for each enzyme were not observed. For example, more than one band per presumably inbred

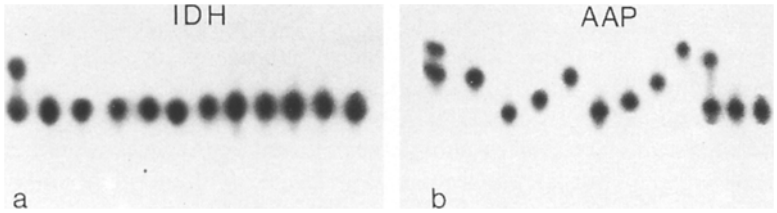

Fig. 1. Representative variation of *Arachis* species for IDH (**a**) and for AAP (**b**). From left to right in both **a** and **b** are *A. hypogaea* cv NC 4, *A. stenosperma* 410, *A. batizocoi* 30081, *A.* sp. 30109, *A. stenosperma* 7762, *A. batizocoi* 9484, *A.* sp. 36027, *A.* sp. 36031, *A. helodes* 30030, *A. glandulifera* 30091, *A. batizocoi* 30079, and *A.* sp. 30093

Table 1. *Arachis* species analyzed for isozyme polymorphism

Species	No. accessions		No. polymorphic enzymes	Avg. no. isozyme bands/acc.	Avg. no. different bands from NC 4
	Total	Polymorphic			
Section *Arachis*					
A. hypogaea	1	0	0	34.0	–
A. monticola	3	3	3 (0–1)[a]	31.7 (30–33)	4.7 (3–6)
A. batizocoi	6	3	2 (0–1)	25.3 (25–26)	20.5 (20–22)
A. cardenasii	8	6	5 (0–3)	26.0 (24–27)	12.3 (11–14)
A. chacoense	1	1	1	27.0	13.0
A. correntina	8	7	8 (0–5)	25.9 (21–28)	11.8 (10–13)
A. diogoi	2	2	4 (0–2)	23.5 (21–26)	15.0 (13–17)
A. duranensis	16	13	11 (0–3)	27.8 (26–31)	11.3 (7–19)
A. glandulifera	4	3	2 (0–1)	28.3 (26–33)	9.4 (7–12)
A. helodes	3	2	4 (0–3)	28.0 (25–30)	11.7 (9–15)
A. ipaensis	1	0	0	23.0	13.0
A. spegazzinii	3	2	2 (0–2)	26.3 (25–28)	10.3 (9–12)
A. stenosperma	4	3	2 (0–2)	22.0 (21–23)	12.3 (11–13)
A. villosa	2	2	6 (2–5)	28.5 (26–31)	16.0 (9–23)
A. spp. (approximately 10)	21	16	10 (0–3)	26.9 (23–33)	13.0 (9–17)
	83	63			
Section *Ambinervosae*					
A. sp. (1)	2	1	2 (0–2)	28.5 (28–29)	20.0 (17–23)
Section *Caulorhizae*					
A. pintoi	1	1	3	29.0	28.0
Section *Erectoides*					
A. paraguariensis	4	0	0	26.8 (26–28)	14.5 (11–20)
A. rigonii	1	1	1	28.0	12.0
A. spp. (4–7)	16	13	11 (0–4)	28.0 (24–35)	13.4 (5–21)
	21	14			
Section *Extranervosae*					
A. macedoi	3	1	2 (0–2)	24.7 (23–28)	20.7 (19–22)
A. villosulicarpa	1	1	1	25.0	17.0
	4	2			
Section *Triseminalae*					
A. pusilla	2	2	6 (2–4)	28.5 (26–31)	17.5 (15–20)

[a] Numbers in parenthesis represent ranges

genotype was never observed for GA3PD or GALDH, and at most two bands per inbred genotype were observed for AAP, ALD, IDH, SOD, TPI, ADH, LAP-F, LAP-S and PGM. *Arachis hypogaea* cv NC 4 had 34 of the possible 66 bands; three collections of *A. monticola* averaged 31.7 bands (range = 30–33), and the diploid species averaged 26.5 bands with a range of 21 to 35. Large differences were not observed in the total numbers of isozyme bands among species belonging to the different sections (Table 1).

Seed-to-seed expression of isozyme patterns was often different among individual seeds within acces-

Table 2. Isozymes analyze, the corresponding number of unique patterns (bands) observed, and the number of accessions with seed-to-seed polymorphisms

Enzyme	E. C. no.	Code	Number		Number polymorphic accessions
			Unique bands	Banding patterns	
Acid phosphatase	3.1.3.2	ACP	3	7	18
Alanine aminopeptidase	3.4.11.1	AAP	4	7	11
Alcohol dehydrogenase	1.1.1.1	ADH	4	4	5
Aldolase	4.1.2.13	ALD	4	6	3
Galactose dehydrogenase	1.1.1.48	GALDH	3	4	6
Glutamate oxalocetate transaminase	2.6.1.1	GOT	5	6	8
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	GA3PD	4	5	3
Isocitrate dehydrogenase	1.1.1.42	IDH	2	3	0
Leucine aminopeptidase (fast band)	3.4.11.1	LAP-F	4	6	3
Leucine aminopeptidase (slow band)		LAP-S	4	4	6
Malate dehydrogenase	1.1.1.37	MDH	4	16	23
Menadione reductase	1.6.99.2	MNR	6	6	13
Phosphoglucomutase	2.7.5.1	PGM	4	4	6
Phosphohexose isomerase	5.3.1.9	PHI	6	8	23
Shikimate dehydrogenase	1.1.1.25	SAD	3[a]	4	3
Superoxide dismutase	1.15.1.1	SOD	4	5	13
Triose phosphate isomerase	5.3.1.1	TPI	2[a]	3	2
			66	98	

[a] Cluster of two to three small bands versus one darkly-staining band

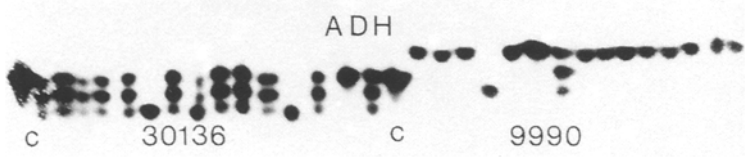

Fig. 2. Seed-to-seed variation in ADH for two section *Erectoides* species accessions of *Arachis*. Cultivar NC 4 is denoted as '*c*'

sions. Thirty-three accessions had monomorphic banding patterns for all 17 enzymes, 34 had one variant, 25 had two variants, 15 had three variants, and the remaining accessions had three-to-five band differences among seeds (Table 1, Fig. 2). The variation within accessions was spread across all sections and not concentrated in any group. Further, accessions which were introduced from South America during the 1960s had approximately the same numbers of polymorphisms as ones introduced during the 1980s, so outcrossing, possibly due to high bee populations in field nurseries, is not believed to be a major factor accounting for the observations. Representative isozyme profiles for many named *Arachis* species are presented in Table 3.

An estimate of genetic diversity in *Arachis* can be illustrated by mapping the number of different isozyme patterns into geographical areas (Fig. 3). Sixty of sixty-six enzyme bands were found in the Mato Grosso state of central Brazil, whereas only 24 were in the Araguaia River Basin in north-central Brazil (which may have been a true function of only one accession represented in the study from this area). The apparent center of diversity for *Arachis* is the state of Mato Grosso, Brazil, which is northwest of the proposed center of origin in the "planaltine ellipse" described by Gregory et al. (1980).

A principal component analysis of the 113 accessions was completed based on the presence or absence of a band within an accession. The analysis grouped most accessions according to sectional classifications (Fig. 4). However, a large amount of variation existed among members of the section *Erectoides* and species did not form a singular group. Even distinct accessions of the same species from the section *Erectoides* (e.g., *A. paraguariensis* Chod. et Hassl.) sometimes did not cluster together. *Arachis rigonii* Krap. et Greg., a mem-

Table 3. Representative isozyme banding patterns for *Arachis* species

Identification	Acc.	ACP	AAP	ADH	ALD	GALDH	GOT	GA3PD	IDH	LAP-F	LAP-S	MDH	MNR	PGM	PHI	SAD
		1 2 3	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3	1 2 3 4 5	1 2 3 4	1 2	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4 5 6	1 2 3 4	1 2 3 4 5 6	1 2
Section *Arachis*																
A. hypogaea	NC4	+ + +	+ − + −	− + − −	− + − −	− + −	− + + + +	− + − −	+ +	− + + −	+ + − −	− − + +	+ + + + + −	− − + −	− + + + − −	− +
A. monticola	30062	+ − −	+ − + −	− + − −	− + − −	− + −	− + + + +	− + − −	− +	− + + −	+ + − −	− − + +	+ + + + + −	− − + −	− + + + − −	− +
A. batizocoi	9484	+ + +	− − − +	+ − − −	+ − − −	+ − −	− − + + +	+ − − −	− +	− + + −	+ + − −	− − − +	+ + + + + −	− − + −	− − + − − −	− +
A. cardenasii	10017	− − +	− − + −	− + − −	− + − −	− + −	− − + + +	− + − −	− +	− + + −	− + − −	+ − − −	+ + + + + −	− − + −	− − + − − −	− +
A. chacoense	10602	− + +	− − + −	− + − −	− + − −	− + −	− − + + +	− + − −	− +	− + + −	− + − −	− − + −	+ + + + + −	− − + −	− + + + − −	− +
A. correntina	9530	− + +	− − + −	− + − −	− + − −	− + −	− − + + +	− + − −	− +	− + + −	+ − − −	− − − −	− − − + + −	− − + −	− + − − − −	− +
A. correntina	9548	+ + +	− − + −	− + − −	− + − −	− + −	+ + + + +	− + − −	− +	− + + −	− + − −	+ − − −	+ + + + + +	− − + −	− + + + − −	− +
A. diogoi	30001	+ + −	+ − + +	− + − −	− + − −	− + −	− − + + +	− + − −	− +	− + + −	− + − −	− + + +	− − − + + −	− − + −	− + − − − −	− +
A. duranensis	7988	− + +	+ − − −	− + − −	− + − −	− + −	− + + + +	− + − −	− +	− + + −	− + − −	− + + +	+ + + + + −	− + − −	− + − + − −	− +
A. duranensis	30070	+ + +	− − + −	− + − −	− + − −	− + −	− + + + +	− + − −	− +	− + + −	+ + − −	− + + +	+ + + + + −	− + + −	− + − − − −	− +
A. duranensis	30074	− − −	− − + −	− + − −	− + − −	− + −	− + + + +	− + − −	− +	− + + −	− + − −	− + + +	+ + + + + −	− − + −	− + − − − −	− −
A. glandulifera	30091	+ + −	− − − +	− + − −	− + − −	− + −	− + + + +	− + − −	− +	− + + −	− − + −	− − − +	+ + + + + −	− − + −	− − − + − −	− +
A. helodes	30029	+ + +	− − + −	− + − −	− + − −	− + −	− − + + +	− + − −	− +	− + + −	− − + −	− + − −	+ + + + + −	− − + −	− + + + − −	− +
A. ipaensis	30076	+ + −	− − + −	− + − −	− + − −	− + −	− − + + +	− + − −	− +	− + + −	− − + −	− − − −	+ + + + + −	− − + −	− + + + − −	− −
A. spegazzinii	10038 ll	+ + +	+ − − −	− + − −	− + − −	− + −	− − + + +	− + − −	− +	− + + −	+ − − −	− − − −	+ + + + + −	− − + −	− − − + − −	− +
A. stenosperma	410	− + +	− − + −	− + − −	− + − −	− + −	− − + + +	− + − −	− +	− + + −	− + − −	− − + −	− − + + − −	− − + −	− + − − − −	− +
A. villosa	22585	+ + +	− − + −	− + − −	− + − −	− + −	− − + + +	− + − −	− +	− + + −	− + − −	− − − −	− − − + + −	− − − −	− + + + − −	− +
Section *Ambinervosae*																
A. sp.	12943	− + +	− − + −	+ + − −	− − + −	− − +	− − + + +	− − + −	− +	− + + −	+ − − −	− + + +	+ + + + + +	− − + −	− + − − − −	− +
Section *Caulorhizae*																
A. pintoi	12787	− + +	− − + −	− − + −	− − + −	− − +	− + + + +	− − + −	+ −	+ + + −	− + − −	+ + − −	+ + + + + +	− + − −	+ − − − − −	− −
Section *Erectoides*																
A. paraguariensis	11462	+ + −	− − + −	+ − + −	− − − +	− − +	− − + + +	− − − +	− +	− − + +	− + − −	− + − −	+ + + + + −	− − + −	− − + − − −	− +
A. rigonii	10034	− + +	− − + −	− + − −	− + − −	− + −	− + + + +	− + − −	− +	− + + −	− + − −	− + + −	+ + + + + −	− − + +	− − − − − −	− +
Section *Extranervosae*																
A. macedoi	10127	− + +	+ − − −	− + − −	− + − −	− + −	− + + + +	− + − −	− +	+ + − −	+ − − −	− − + +	− − − − − +	− − − +	+ − − − − −	− −
A. villosulicarpa	− −	+ + −	− + − −	− + − −	− + − −	− + −	− + + + +	− + − −	− +	− + + −	− + − −	− − + −	− + − − − −	− − − +	+ + − − − −	− +
Section *Triseminalae*																
A. pusilla	12881	− + +	+ − − −	− + − −	− + − −	− + −	− + + + +	− + − −	− +	− + − −	+ − − −	− + + −	− − − + + −	− + − −	+ + + − − −	− +

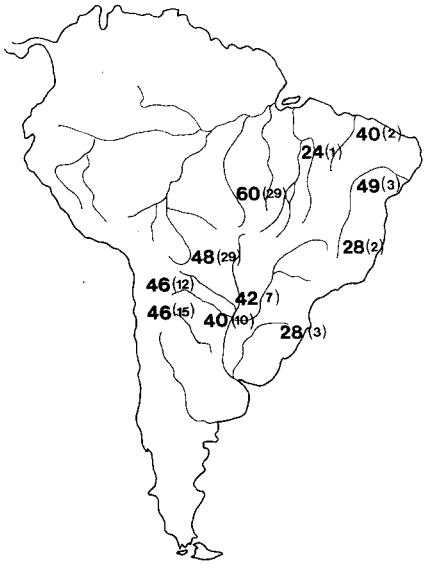

Fig. 3. Number of unique isozyme patterns found in different regions in South America (out of a total of 66 observed) and the number of *Arachis* accessions (*in parentheses*) which were analyzed from each area

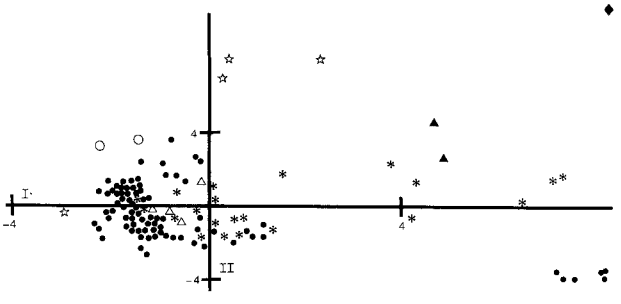

Fig. 4. Projection of 113 accessions of *Arachis* onto the first two principal components. Members of sections are designated as follows: ●, diploid *Arachis* species; △, tetraploid *Arachis* species; ▲, *Ambinervosae*; ◆, *Caulorhizae*; ✲ *Erectoides*; ☆, *Extranervosae*; and ○, *Triseminalae*

ber of the series *Procumbensae* (which is morphologically intermediate between the sections *Arachis* and *Erectoides*) grouped in the midst of the section *Arachis* species, most closely with the *A. duranensis* Krap. et Greg. *nom. nud.* accession 30065. *Arachis pintoi* Krap. et Greg. *nom. nud.*, a species in the section *Caulorhizae* had the highest positive values for both principal components 1 and 2 and was the most distant from all other members of the genus examined (Fig. 4). The section *Extranervosae* accessions of *A. macedoi* Krap. et Greg. *nom. nud.* clustered in the same general area, with low positive values for principal component 1 and high positive values for component 2; whereas, *A. villosulicarpa* Hoehne plotted nearer the section *Arachis taxa* than to *A. macedoi* (Fig. 4).

Most accessions of the section *Arachis* formed a diverse cluster with negative principal component 1 values (Fig. 4). *Arachis ipaensis* Greg. et Greg. *nom. nud.* (A genome) and *A. glandulifera* (D genome) had slightly positive principal component values, whereas *A. batizocoi* was distantly removed from the section *Arachis* as well as other examined members of the genus. The enzymes with the heaviest weighting for the observed separation along principal component axes 1 and 2 included AAP, GALDH, ALD, PHI, and TPI. Members of the sections *Caulorhizae* and *Ambinervosae*, plus one accession in the section *Erectoides* (*A. paraguariensis* 11462), all had unique patterns for ADH and GA3PD different from other species. *Caulorhizae* and *Ambinervosae* taxa also had the same unique patterns for ALD and GALDH; *Extranervosae*, *Caulorhizae*, and *Trisiminalae* species had the same characteristic PHI bands (Table 3).

Because the cultivated peanut belongs to the section *Arachis*, a second principal component analysis was completed using only the 83 accessions in this group. The tetraploid members of the section (*A. hypogaea* and *A. monticola*) grouped closely together, with *A. hypogaea* having higher positive principal component 2 values (Fig. 5). The four *A. glandulifera*, four *A. stenosperma* Greg. et Greg. *nom. nud.*, eight *A. cardenasii* Krap. et Greg. *nom. nud.*, two *A. diogoi* Hoehne, three *A. spegazzinii* Greg. et Greg. *nom. nud.*, and six *A. batizocoi* accessions all formed separate clusters, as would have been expected based on published or proposed species designations. *Arachis duranensis* accessions were more variable, and at least two distinct groups of taxa were observed. The first

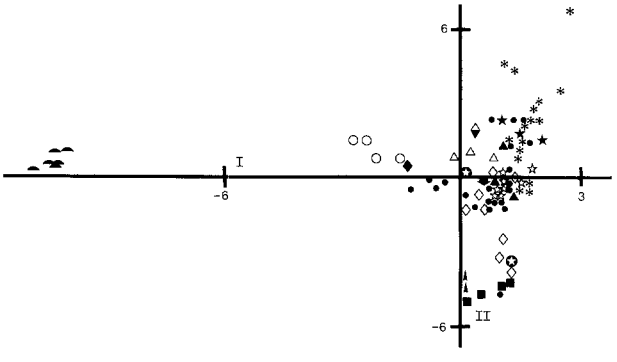

Fig. 5. Projection of 83 section *Arachis* accessions onto the first two principal components. Spacies are designated as follows (numbers of accessions are shown in parentheses): ☁, *A. batizocoi* (6); ☆, *A. cardenasii* (8); ⬇, *A. chacoense* (1); ◇, *A. correntina* (8); ▲, *A. diogoi* (2); ✲, *A. duranensis* (16); ○, *A. glandulifera* (4); ▲, *A. helodes* (3); ⬙, *A. hypogaea* (1); ◆, *A. ipaensis* (1); △, *A. monticola* (3); ★, *A. spegazzinii* (3); ■, *A. stenosperma* (4); ✪, *A. villosa* (2); and ●, species names unknown (21)

Table 4. Hedrick's probability of finding a wild species genotype with unique alleles different from *A. hypogaea* in different regions of South America

Region	%	Region	%
North-central Brazil	24.2	Central Paraguay	4.5
Southeast Brazil	21.2	North Argentina	4.5
South Brazil	19.7	South Bolivia	1.5
Northeast Brazil	16.7	East Bolivia	1.5
East Brazil	4.5	Central Brazil	1.5
East Argentina	4.5		

group, which contained most accessions of *A. duranensis*, had positive values for both principal components 1 and 2 (accession GKBSPSc 30071 had very high positive values for both components and appears to be an outlier). A second group – including accessions 30074, 30075 and 30077 – had negative values for principal component 2. Variation among accessions of *A. duranensis* was observed for banding patterns of ACP, AAP, LAP-S, PGM and PHI. The three *A. spegazzinii* accessions were closely associated (mixed) with the species *A. duranensis*, and they may not represent a unique species, even though leaf morphology is different from that of the *A. duranensis* accessions. Several members of *A. duranensis* also appeared to be closely related to *A. monticola*.

The *A. correntina* (Burk.) Krap. et Greg. *nom. nud.* accessions also formed two, and possibly three, clusters. Unexpectedly, the two accessions of *A. villosa* Burk. plotted far apart and the three accessions of *A. helodes* Martius ex Krap. et Rig. did not group together.

In addition to named species, 23 accessions in the section *Arachis* are unnamed and may represent ten or more unique taxa. Accessions GKSSc 30093, GK 30011, GKBSPSc 30068, and KSSc 36027 had negative principal components 1 and 2 and each most likely represents an individual species. Ten of these twenty-three accessions which are morphologically similar to *A. cardenasii* had positive principal component values, but negative values for the second component, including accessions GK 30006, GK 30017, GK 30035, GK 30038, GKBSPScZ 30085, GKSSc 30088, GKSSc 30089, GKSPScGb 35001, KSBScC 36005, and VSGr 6326 (orange-flower type). GK 30008 (another of the unnamed accessions) grouped with the *A. stenosperma* cluster which had high negative principal component 2 values (Fig. 5). Seven additional unnamed accessions had positive values for components 1 and 2 [GKSSc 30092, KSSc 36036, KSSc 36008, KSSc 36029, VKRSv 6536, and VSGr 6326 (yellow-flower type)].

Arachis batizocoi accessions clustered tightly, but they were isolated from other species of the section *Arachis*. The isozymes most important for this separation included GALDH, ALD, GA3PD, ADH and LAP-S. Several notable similarities were observed among the species *A. batizocoi*, *A glandulifera*, and *A. ipaensis*, including the presence of two lightly-staining fast-migrating bands for TPI, and accessions of all three species had only the fastest migrating enzyme band for MDH. *Arachis batizocoi* and *A. glandulifera* also shared a unique LAP-S band.

To estimate genetic differences among species, Hedrick's probability (Hedrick 1971) was calculated to suggest the areas of South America in which germplasm with unique alleles different from the cultivated peanut could be collected. Species in the state of Mato Grosso, Brazil, and in southern and eastern Bolivia were the most similar to *A. hypogaea* (Table 4). North-central, northeastern, southeastern, and southern Brazil are the areas where the greatest numbers of alleles unique from *A. hypogaea* are most often found (Table 4).

Discussion

Most *Arachis* species are self-pollinating diploids which grow in isoloated populations in South America. Because of the low frequency of polymorphic loci found in *A. hypogaea* (Grieshammer and Wynne 1990 a) and the expected homozygosity of plants within an accession, the variability observed within and, to a lesser degree, among accessions of the same species was unexpected. This variation indicates that either (1) diversity was present at the original collection sites in South America and the variation is being maintained in plant collections or (2) unplanned outcrossing in maintenance nurseries has occurred. The seeds used in this study were harvested from a nursery where a significant amount of within-population crossing occurs due to a large number of bees at the experiment station; but, because plots were isolated, no crossing between accessions is thought to have occurred. Since these nursery populations originated either from cuttings or from small numbers of few seeds, allelic frequencies would most likely be different from those present at the original collection sites in South America. Thus, our analyses were conducted on the presence or absence of banding patterns rather than on the frequencies at which specific bands were observed.

The clusters formed from isozyme analyses of the 113 accessions generally corresponded with the sectional designations outlined by Gregory et al. (1973). Further, when section *Arachis* taxa were analyzed separately, groups generally formed as would have been expected based on morphological species descriptions. Because several species are morphologically variable (e.g., *A. duranensis*, *A. correntina*, *A. car-*

denasii), and many taxa in *Arachis* are as yet unnamed, a comparison of isozyme analyses with the morphological groups reported by Stalker (1990) is informative. In general, both morphological and isozyme data clustered accessions into similar species groups or species-species associations. For example, *A. ipaensis* was closely associated with *A. glandulifera*, *A. spegazzinii* was close to *A. duranensis*, and several of the *A. correntina* accessions were relatively close to *A. stenosperma* (Fig. 5). Within species, neither the *A. correntina* nor the *A. helodes* accessions produced defined morphological or isozyme groups. *Arachis duranensis* showed more isozyme than morphological diversity, but the reverse was found for accessions of *A. cardenasii*. Also, *A. chacoense* Krap. et Greg. *nom. nud.* was associated morphologically with *A. diogoi*, but isozyme patterns showed a closer similarity to *A. helodes* and *A. cardenasii*. Although accession 7762 was collected in central Brazil whereas the *A. stenosperma* accessions 408–410 were found in the southern coastal area, they are very similar morphologically (Stalker 1990), have homologies based on PCR analysis (Halward et al. 1992), and their isozyme patterns are also similar. Thus, accession 7762 should be considered as a member of the species *A. stenosperma*. In general, both validly described and unnamed species grouped as expected based on field observations, and the isozyme data will be useful for delineating species in a formal taxonomic treatment of the genus. However, combinations of isozyme patterns will be necessary to distinguish *Arachis* species.

One major difference between morphological (Stalker 1990) and isozyme analyses was the positioning of *A. batizocoi* in relation to other species of the section *Arachis*. Morphologically, *A. batizocoi* forms a group somewhat intermediate between *A. monticola* and *A. duranensis*; but, in the isozyme analysis, it is very distantly removed from all other species. Halward et al. (1992) and Paik-Ro et al. (1992) also found the same trend on the basis of RAPD and RFLP analyses, respectively. Because *A. batizocoi* has a B genome (Smartt et al. 1978), and is karyotypically unique from other species (Stalker and Dalmacio 1981; Singh and Moss 1982), it was expected to be somewhat distant to other members of the section, but not as far removed as the isozyme data indicated. Further, because *A. glandulifera* has a D genome, it was also expected to form a very distinctive group, but it did not separate far from the other section *Arachis* species and was very similar to the A genome species *A. ipaensis* (Fig. 5).

Gregory and Gregory (1979) hypothesized that species in the sections *Erectoides*, *Extranervosae* and *Rhizomatosae* (series *Prorhizomatosae*) represent the most ancient taxa in the genus. Because *Erectoides* species still maintain cross-compatibility with other sections, they should also have genetic homologies, here represented by isozyme banding patterns. The isozyme analyses showed that *Erectoides* accessions represented the most variable section of the six analyzed. Further, this variation was distributed along a major portion of the first principal component axis, leading to loose associations with several other major groups. Differences between *A. hypogaea* and species accessions of the section *Erectoides* ranged between five and 21, while taxa in other sections ranged between 15 and 28. These data fit expectations that members of the section *Erectoides* are closely related to the section *Arachis* and follow cross-compatibility relationships outlined by Gregory and Gregory (1979). For example, these authors reported that taxa of the sections *Arachis* and *Erectoides* will hybridize, whereas the section *Arachis* species are reproductively isolated from members of other sections. The species in which there is discordance between isozyme data and general patterns of cross-compatibility in the genus is *A. batizocoi*.

Variation was observed among the tetraploid accessions of *A. hypogaea* and *A. monticola* in section *Arachis* for the four enzymes ACP, IDH, MDH, and PHI. Grieshammer and Wynne (1990 a) and Lacks and Stalker (1993) also found variation in *A. hypogaea* for IDH and PHI. The variants in this study included the loss of one and two bands, respectively, in *A. monticola* accessions 30063 and 30062 for ACP; *A. hypogaea* had two IDH bands whereas all three *A. monticola* accessions had only one band; accession 30063 had one different MDH band from other *A. monticola* accessions or *A. hypogaea* and it also lacked the fastest migrating MDH band; and all *A. monticola* accessions had three PHI bands whereas NC 4 had one band. The PHI pattern in *A. monticola* corresponded to the spanish and valencia types of *A. hypogaea* subspecies *fastigiata* (Grieshammer and Wynne 1990 a); and this very limited isozyme data places *A. monticola* closer to subspecies *fastigiata* than to subspecies *hypogaea*, as previously suggested by karyotypic analyses (Stalker and Dalmacio 1986).

The number of isozyme band similarities and differences between *Arachis* diploid species and the cultivated peanut is informative for showing general genetic relationships. A-genome species in the section *Arachis* averaged 10.3 to 15.0 differences from *A. hypogaea*, the B-genome species *A. batizocoi* averaged 20.5 and the D-genome species *A. glandulifera* averaged 9.4 bands different from *A. hypogaea* (Table 1). The conclusion from these data is that the A and D genome species are genetically more closely related to *A. hypogaea* than is *A. batizocoi* even though *A. glandulifera* is not believed to have been involved in the evolution of the cultivated peanut (Stalker 1991). *Arachis batizocoi* has been widely considered as a progenitor species of *A. hypogaea* and the donor of the B genome (Smartt et al. 1978; Smartt and Stalker 1982). A comparison of the 39 bands

present across the 17 enzymes in the two species showed 23 similarities, but also 20 differences (Table 3). Because diploid progenitor species are not expected to have a large number of bands which are not also found in the tetraploid, a critical comparison is the presence of isozyme bands in *A. batizocoi* which are not present in *A. hypogaea*. *Arachis batizocoi* accessions had seven bands not present in cv NC 4, which is more than in all but one accession in the other 20 or more diploid species in the section *Arachis*. Although only one accession was used in this study, NC 4 is believed to be representative of *A. hypogaea* based on other studies by Grieshammer and Wynne (1990 a) and Lacks and Stalker (1993). These extra bands included at least one band in each of the enzymes AAP, ALD, ADH, PHI, GALDH, GA3PD, and LAP-S. Thus, *A. batizocoi* is not a likely progenitor of the cultivated species. This conclusion was also supported by Halward et al. (1992) based on RAPDs and by Kochert et al. (1991) and Paik-Ro et al. (1992) based on RFLP analyses. If *A. batizocoi* is not a progenitor of *A. hypogaea*, then the cultivated peanut cannot have both A and B genomes as researchers have assumed since the mid-1970s, or else some other (perhaps extinct) B-genome species gave rise to the cultivated peanut. An alternative evolutionary pathway is hybridization of two A-genome species, chromosome doubling to the tetraploid level, subsequent diploidization of chromosomes so that few multivalents are observed during meiosis, and one of the two uniquely small chromosome pairs (commonly found in A-genome species) becoming larger through a translocation event. This hypothesis also seems plausible because a higher probability exists for a semifertile hybrid (A-A genome versus A-B genome sterile cross) going through a chromosome doubling event (Harlan and deWet 1975); and because translocations occur at a relatively high frequency in *Arachis* (Stalker and Dalmacio 1986; Stalker 1991; Stalker et al. 1991). Uniformity among *A. hypogaea* genotypes for isozymes (Grieshammer and Wynne 1990a; Lacks and Stalker 1993) and restriction fragment length polymorphisms (Halward et al. 1991) indicates that *A. hypogaea* evolved as a single evolutionary event. Unfortunately, similarities and differences between diploid *Arachis* species and the cultivated peanut have excluded, but not conclusively indicated, other possible progenitors.

This investigation has provided insight into procedures or collecting and preserving the genetic resources of *Arachis*. The areas where variation unique from *A. hypogaea* can most frequently be found is in accessions from the north, east, and south coastal regions of Brazil. These areas also correspond to the locations of species in sections distantly related, and impossible to cross, to the cultivated peanut (e.g., *Caulorhizae*, *Triseminalae*, and *Ambinervosae*). Thus, much of the unique variation may not be present in species which are easily accessible for germplasm improvement. Because polymorphic loci within accessions were observed, natural populations in South America cannot be assumed to be highly inbred or homozygous. The ideal situation would be to collect seeds from many different plants and keep lines separate, but this is impractical. An alternative is to collect seeds from many plants and then harvest large bulked seed populations in maintenance nurseries before distribution. However, a common practice in *Arachis* has been to grow and distribute seeds or cuttings from single plants, which has probably resulted in significant narrowing of the potential germplasm base. Larger seed increases and then bulking seed prior to initial seed distribution would better insure maintenance of genetic diversity in the genus.

Many isozyme banding variants exist in the diploid species of *Arachis* as compared to *A. hypogaea*. Because many of these differences are in the form of additional bands in diploids, as compared to tetraploids, many interspecific hybrids should be easily identified based on electrophoretic profiles. Estimates of germplasm introgression into advanced generation hybrids should also be possible. The genetics of most of the enzyme variation must still be determined, but the variation reported in this study should hold potential for use in future crossing and germplasm utilization programs.

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