Comparative Studies of Seed Proteins and Enzymes of Species and Collections of **Arachis** by Gel Electrophoresis

John P. Cherry[1]
Southern Regional Research Center[2]
New Orleans, Louisiana 70179

ABSTRACT

Proteins and six enzymes (esterase, peroxidase, catalase, leucine aminopeptidase, alcohol dehydrogenase, achromatic oxidase) in individual seeds from 36 species and collections of **Arachis** were characterized by using polyacrylamide and starch gel electrophoretic techniques. These data were used to evaluate the biosystematics of **Arachis.** Support was found for the thesis that each wild relative of the cultivated peanut, **Arachis hypogaea** L., has been properly classified into coherent groups of species under the following taxonomic sections: Axonomorphae, Erectoides, Caulorhizae, Rhizomatosae, Extranervosae, Pseudoaxonomorphae and Triseminalae. For example, the mobility of most bands in the gel patterns of proteins and enzymes of **A. hypogaea** coincided very closely with those of the wild species, **A. monticola** PI 219824, supporting studies which have shown that these two species are closely related and should be classified together. The Similarity Index method, based upon the percent of protein and enzyme bands with similar and dissimilar mobilities compared among gels of seeds of species and collections, produced s-values that further supported the taxonomy of this genus as it is known today.

Additional keywords: Wild species, Cultivars, Chemotaxonomy, Similarity Index method.

Substantial evidence suggests that the genetic base or gene pool of the cultivated peanut, *Arachis hypogaea,* does not have the reserve germ plasm needed to resist many of the new agricultural problems brought on by pollution, dwindling water supplies, and the necessity for biological control methods against insects and plant pathogens (Hammons, 1972, 1973; Cherry *et al.,* 1971, 1974; Cherry and Ory, 1973a, b; Cherry, 1974; Simpson and Haney, 1973; Miller, 1973). Fortunately, there are about 50-70 wild species of *Arachis* containing untapped genetic resources for future breeding programs with *A. hypogaea* (Gregory *et al.,* 1973).

Only a partially complete taxonomic evaluation of *Arachis* has been published, thus, much work remains in this area (Gregory *et al.* 1973; Leppik, 1971; Smartt and Gregory, 1967; Raman, 1973; Krapovickas, 1969). A joint effort in different disciplines is needed to simultaneously evaluate the genetic and biochemical potentials of these wild species for improving the commercial varieties of *A. hypogaea.*

Gel electrophoretic techniques have been used to study the biosystematics of different genera (Sheen, 1972; Cherry *et al.,* 1970, 1971, 1972; Cherry and Ory, 1973a, b; Cherry, 1974; Mitra *et al.,* 1970; Siddiq *et al.,* 1972). Utilizing these biochemical tools, this study characterized and compared the storage proteins and six enzymes from extracts of individual seeds of 36 species and collections of *Arachis* in order to initiate the chemotaxonomy of this genus. Proteins and the enzymes esterase, peroxidase, catalase, leucine aminopeptidase, alcohol dehydrogenase and achromatic oxidase were chosen because well-established staining techniques are available for their detection in electrophoretic gels (Brewbaker *et al.,* 1968; Scandalios, 1969); all have been reported for peanuts (Cherry *et al.,* 1971, 1973; Cherry and Ory 1973a, b; Cherry, 1974).

Materials and Methods

The 35 collections of wild species (originally from South America) and 15 cultivars of **A. hypogaea** (United States) from which seeds were available are listed in Table 1. See Gregory **et al.** (1973) for a detailed description of the collection sites. Preliminary studies suggested that there are species among the undescribed collections labeled as **Arachis** species (**A.** sp.); however, there is not yet sufficient information for peanut taxonomists to assign specific names. Four to 17 seeds (random samples of field grown and/or greenhouse plots) from each wild species or collection, 84 seeds (4 seeds from each of 21 plants) of Virginia 56R grown in Louisiana and 12 seeds (random samples of field-grown plots) of each of the other cultivars were individually examined for protein and enzyme composition. For further details of completed research on **A. hypogaea,** see Cherry **et al.** (1971), Cherry and Ory (1973a, b) and Cherry (1974).

The testa was removed and each seed ground individually with a mortar and pestle in pH 7.9 phosphate buffer, I = 0.01, and centrifuged at 39,000 x g for 30 min. The partially purified extract from each seed was submitted to starch slab gel electrophoresis (Brewbaker **et al.,** 1968) to fractionate leucine aminopeptidase, catalase, alcohol dehydrogenase and achromatic oxidase into distinct bands which were visualized by procedures of Brewbaker **et al.** (1968) and of Scandalios (1969). Achromatic oxidase and alcohol dehydrogenase were identified on the same starch slab as clear areas and dark blue bands, respectively, on a light blue background. The remaining portion of each seed extract was examined for proteins, esterase and peroxidase by polyacrylamide disc gel electrophoresis (Cherry **et al.,** 1970, 1972; Cherry and Ory 1973a, b). Thus, protein and six enzymes were examined in an extract of a single seed. The exact position of each band within the patterns was confirmed by examining 1:1 mixtures of extracts from different species or collections representing each of the taxonomic sections of **Arachis.**

Results and Discussion

The first classification of species of the genus *Arachis* as collections into natural groups (sections and series) based on plant morphology, geographical area where the samples were collected, species cross-compatibility and hybrid fertility among species was recently published by Gregory

[1]National Research Council Postdoctoral Research Associate. Present address: Department of Food Science, University of Georgia Experiment Station, Experiment, Georgia 30212.

[2]One of the facilities of the Southern Region, Agricultural Research Service, U.S. Department of Agriculture.

et al. (1973). A number of undescribed collections (*A.* sp.) are listed in sections of Axonomorphae, Erectoides, Rhizomatosae and Pseudoaxonomorphae because they lack formal descriptions (Table 1). A chemotaxonomic evaluation of *Arachis* follows.

PROTEINS AND ENZYMES OF ARACHIS

SECTION AXONOMORPHAE

Species of Axonomorphae are naturally distributed in the central area of South America (Gregory *et al.*, 1973) and samples of this section used in this study were collected in northern Argentina and western Paraguay (Table 1). Studies to determine cross-compatibility among plants of *Arachis* demonstrated that *A. monticola* PI 219824 and *A. hypogaea* of series Amphiploides, section Axonomorphae, are closely related. Moreover, these species have formed hybrids of variable fertility with species of series Annuae and Perennes in this section (Smartt and Gregory, 1967; Raman, 1973). Polyacrylamide gel electrophoresis of seed extracts from Axonomorphae showed that the species and collections of this section contained large amounts of protein polymorphism (Fig. 1). In

Table 1. List of species and collections of Arachis L. (n = 10) studied and arranged according to Gregory et al. (1973).

	Species	Collection Number[1]	P.I. Number[2]	Location	(Collectors)[3]
Section I.	Axonomorphae				
Series:	Annuae (2n)				
	A. duranensis	7988	219823	Argentina	(K)
	A. sp.	10038	263133	Argentina	(GKP)
Series:	Perennes (2n)				
	A. correntina	7830	262137	Argentina	(K)
	A. correntina	7897	262134	Argentina	(K)
	A. correntina	9530	262808	Argentina	(GKP)
	A. chacoense	10602	276235	Paraguay	(GKP)
	A. villosa	22585	298636	Argentina	(B)
Series:	Amphiploides (4n)				
	A. monticola	7264	219824	Argentina	(K)
	A. hypogaea[4]	-	-	United States	
	subspecies hypogaea variety hypogaea				
	subspecies fastigiata variety vulgaris				
Section II.	Erectoides				
Series	Tetrafoliolatae (2n)				
	A. paraguariensis	9646	262842	Brazil	(GKP)
	A. benthamii	9764	262859	Brazil	(GKP)
	A. sp.	9835	262308	Brazil	(GKP)
	A. sp.	9841	262278	Brazil	(GKP)
	A. sp.	9990	261877	Brazil	(GKP)
	A. sp.	10002	262140	Brazil	(GKP)
	A. sp.	10543	276209	Brazil	(GK)
	A. sp.	10573	276225	Paraguay	(GK)
	A. sp.	10576	276228	Paraguay	(GK)
	A. sp.	10580	276229	Paraguay	(GK)
	A. paraguariensis	11462	331188	Paraguay	(KCF)
	A. paraguariensis	11488	331187	Paraguay	(KC)
Series:	Procumbensae (2n)				
	A. rigonii	10034	262142	Bolivia	(GKP)
Section III.	Caulorhizae (2n)				
	A. pintoi	12787	338447	Brazil	(GK)
Section IV.	Rhizomatosae				
Series:	Eurhizomatosae (4n)				
	A. sp.	9553	262801	Argentina	(GKP)
	A. sp.	9570	262817	Paraguay	(GKP)
	A. sp.	9667	262848	Brazil	(GKP)
	A. sp.	9815	262794	Brazil	(GKP)
	A. glabrata	9830	262797	Brazil	(GKP)
	A. sp.	9882	262286	Brazil	(GKP)
	A. sp.	9935	262301	Brazil	(GKP)
	A. sp.	10105	276200	Brazil	(GKP)
	A. sp.	10596	276233	Paraguay	(GK)
Section V.	Extranervosae (2n)				
	A. villosulicarpa	14445	336985	Brazil	(KHE)
Section VI.	Pseudoaxonomorphae (2n)				
	A. sp.	12943	338452	Brazil	(GK)
	A. sp.	12946	338453	Brazil	(GK)
Section VII.	Triseminalae (2n)				
	A. pusilla	12922	338449	Brazil	(GK)

[1]From Gregory et al. (1973).

[2]Plant Introduction (P.I.) numbers from Gregory et al. (1973).

[3]Collectors listed by Gregory et al. (1973).

[4]Peanut cultivars examined: var. hypogaea, Virginia botanical (a) runner market type: Early Runner, Florunner, Virginia Bunch 67, and (b) Virginia market type; Virginia 56R, Virginia 61R, Florigiant, NC 17, NC 5, NC 2; var. vulgaris, Spanish botanical type: Tifspan, Starr, Argentine, Spancross, Comet, Spanhoma.

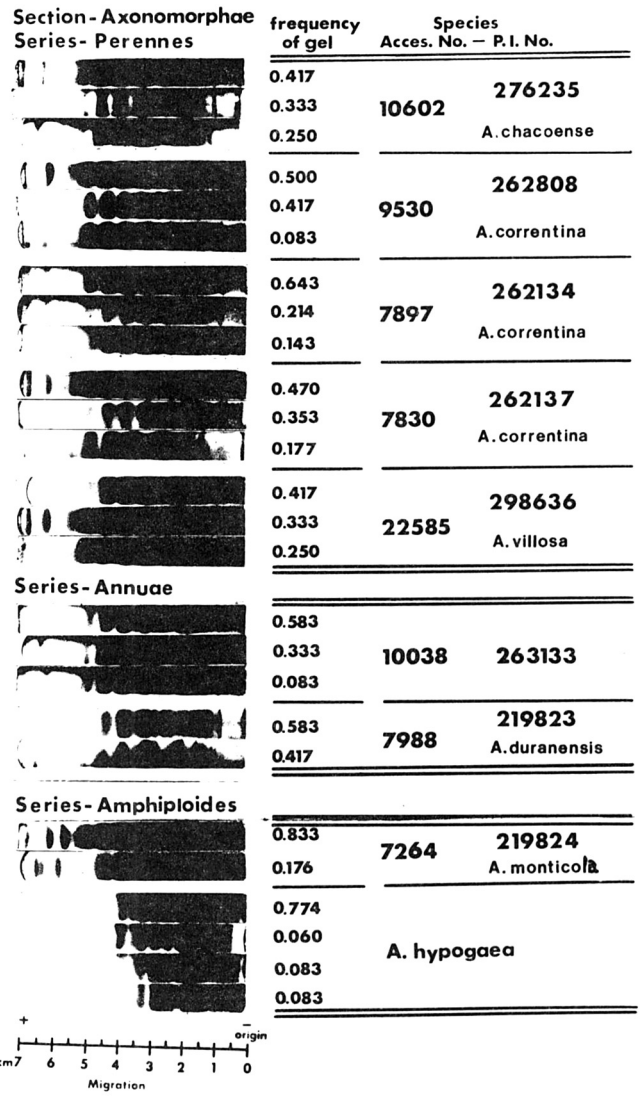

Fig. 1. Polyacrylamide disc gel electrophoretic patterns of proteins of individual seeds from proposed species of section Axonomorphae. Gel frequencies showing intraspecific polymorphism, accession numbers (Acces. No.) and plant introduction numbers (P. I. No.) or names (where assigned) for each species are presented.

general, gels showed a group of major (dark staining) bands in region 1.0-5.0 cm. Patterns differing from these gels contained quantitative or qualitative banding variations in region 1.0-2.5 and 4.0-5.5 cm. Because patterns of both high- and low-frequency gels were similar among most of the species and collections of this section, it was difficult to use protein patterns alone to differentiate the series Perennes, Annuae and Amphiploides. Seed samples of *A. correntina* PI 262134, 262137, 262808 collected at different locations in Argentina, showed similar patterns.

On the other hand, species and collections within each of these series showed esterase patterns which were more similar to one another than to members of the other groups in this section (Fig. 2). *Arachis duranensis* PI 219823 and *A.* sp. PI 263133 of Annuae contained three major bands in

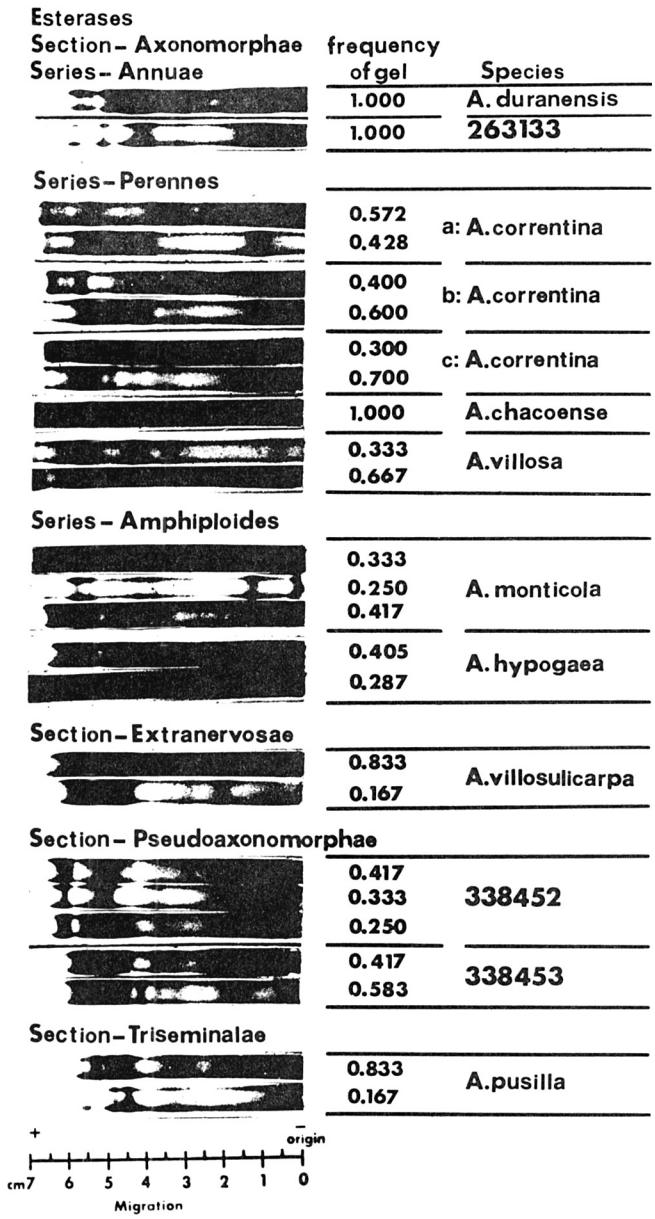

Fig. 2. Polyacrylamide disc gel electrophoretic patterns of esterases of individual seeds from sections Axonomorphae, Extranervosae, Pseudoaxonomorphae and Triseminalae. Gel frequencies showing intraspecific polymorphism and plant introduction numbers or names (where assigned) for species are presented.

region 3.5-5.5 cm and the species of Perennes had a major band in region 5.5 cm; these bands were not clearly shown in ether Annuae or Amphiploides. Many esterase bands were common to all of these species confirming their classification into this section. For example, two major bands in region 5.0-6.0 cm were clearly shown in all of the patterns of *A. monticola* PI 219824 and *A. hypogaea* and in a number of gels of species in Perennes. In general, most of the patterns of *A. villosa* PI 298636 and *A. correntina* PI 262134, 262137, 262808 coincided more closely with those of Amphiploides than with those of Annuae. This observation supported breeding studies suggesting that *A. villosa* PI 298636 appeared to be a close relative

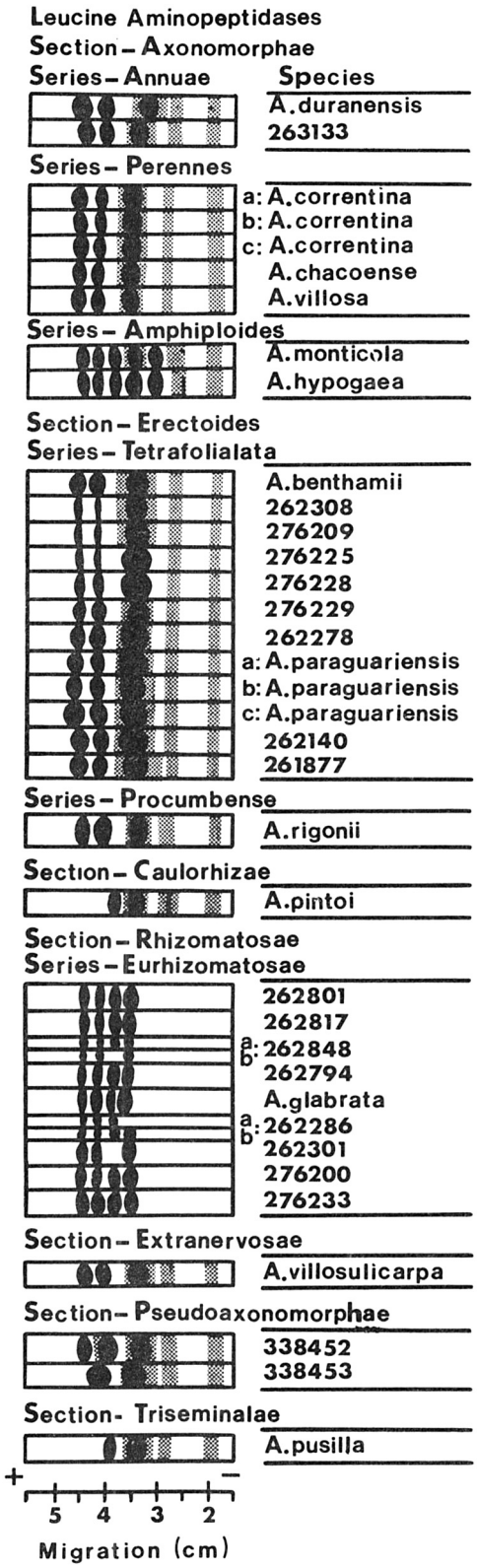

Fig. 3. Starch slab gel electrophoretic patterns of leucine aminopeptidases of seeds from the seven sections of Arachis. The plant introduction numbers of three collections (a, b, c) of A. correntina PI 262134, 262137, 262808 and A. paraguariensis PI 262842, 331187, 331188 are listed in Figs. 1 and 6. Collections PI 262848 and PI 262286 of Rhizomatosae showed one variable pattern (b).

of cultivated *A. hypogaea* (Raman, 1973). Zymograms occurring most frequently among the three collections of *A. correntina* PI 262134, 262137, 262808 were very similar.

The seeds of Axonomorphae showed little or no intraspecific polymorphism in the patterns of leucine aminopeptidase, catalase, alcohol dehydrogenase and achromatic oxidase (Figs. 3, 4). Leucine aminopeptidase patterns distinguished the species of Amphiploides from those of Perennes and Annuae. *Arachis villosa* PI 298636 could be distinguished from the other plants of this section by comparing catalase zymograms. No differences among species and collections of this section were noted for alcohol dehydrogenase. Achromatic oxidase distinguished Perennes from the other series of this section and its pattern for *A. chacoense* PI 276235 was unique. For peroxidase, most of the variability among species and collections of Axonomorphae was in region 2.0-4.0 cm (Fig. 5). *Arachis hypogaea* contained more peroxidase activity than the other species of Axonomorphae.

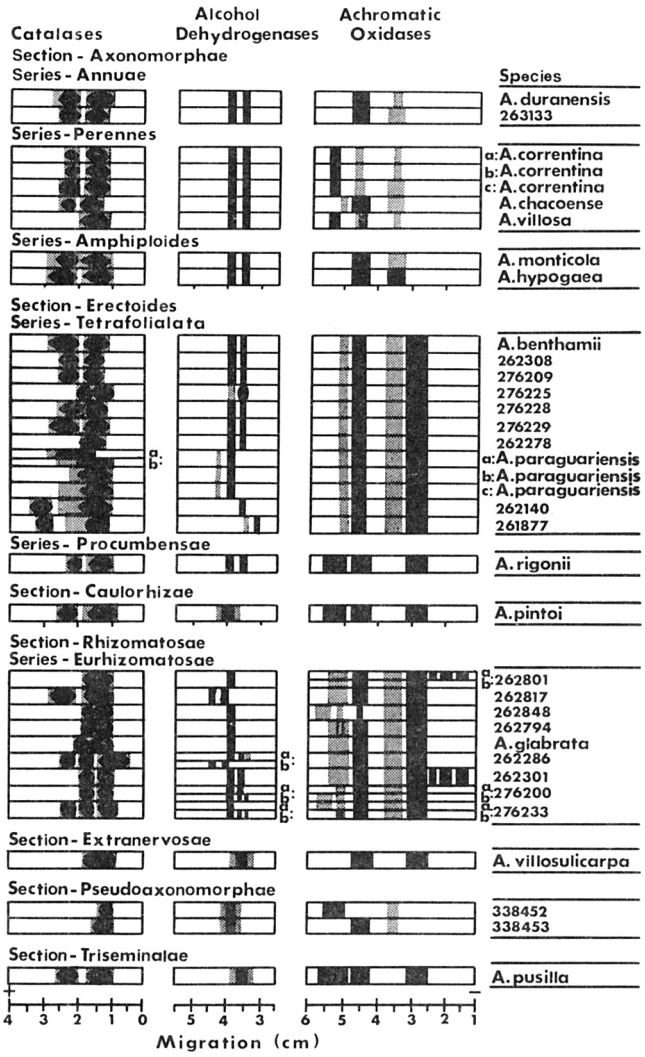

Fig. 4. Starch slab gel electrophoretic patterns of catalases, alcohol dehydrogenases and achromatic oxidases from the sections of Arachis. The plant introduction numbers of the three collections (a, b, c) of A. correntina PI 262134, 262137, 262808 and A. paraguariensis PI 262842, 331187, 331188 are listed in Figs. 1 and 6. Variable patterns (b) are noted for collections of Erectoides and Rhizomatosae.

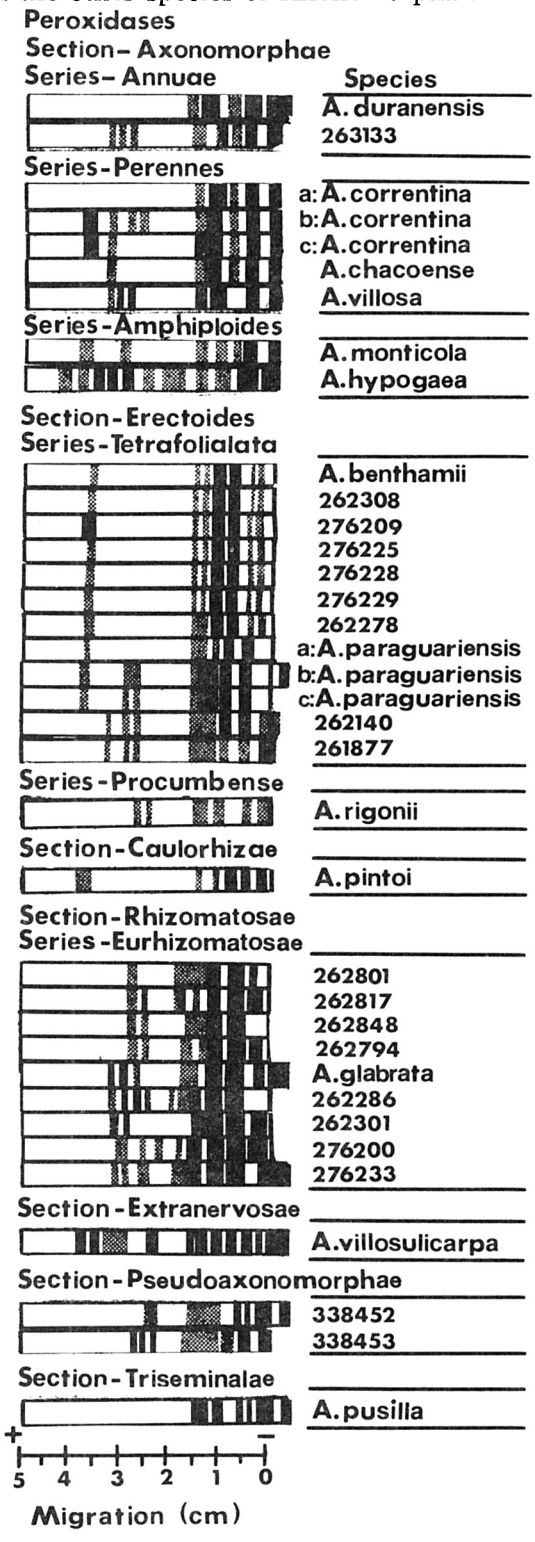

Fig. 5. Polyacrylamide disc gel electrophoretic patterns of peroxidases of the sections of Arachis. Plant introduction numbers or names (where assigned) of species are presented. The plant introduction numbers of three collections (a, b, c) of A. correntina PI 262134, 262137, 262808 and A. paraguariensis PI 262842, 331187, 331188 are listed in Figs. 1 and 6.

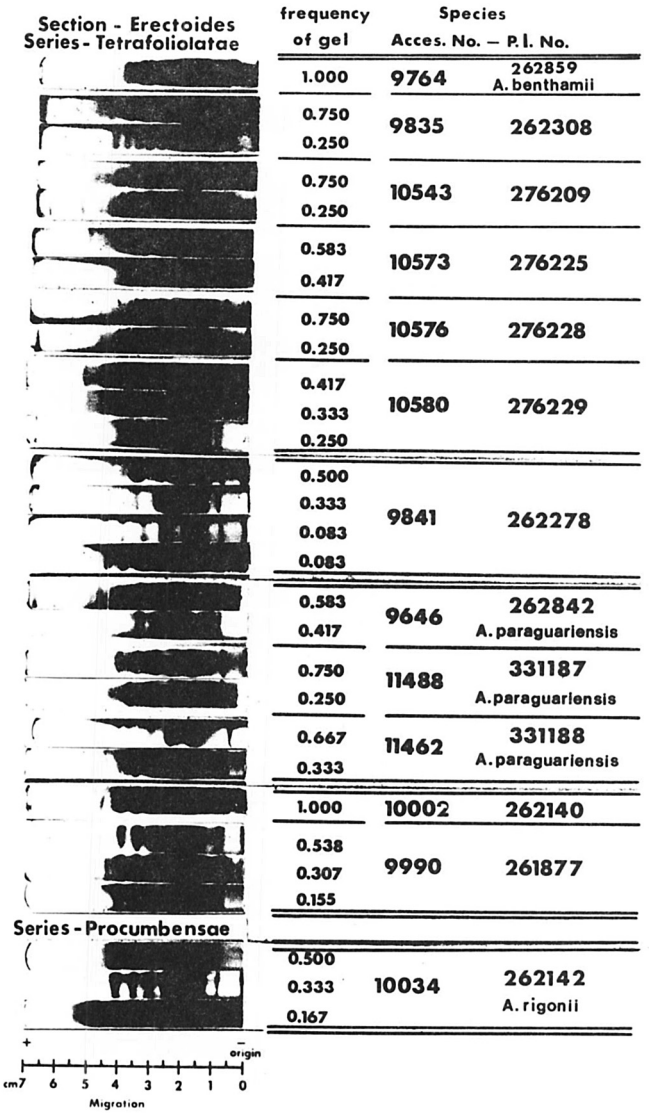

Fig. 6. Polyacrylamide disc gel electrophoretic patterns of proteins of Erectoides. Gel frequencies, accession numbers (Acces. No.) and plant introduction numbers (P. I. No.) or names (where assigned) for each species are presented.

SECTION ERECTOIDES

The species and collections of Tetrafoliolatae and Procumbensae of Erectoides were gathered in Paraguay and south central Brazil (Table 1). Seeds of this section contained lower quantities of protein in region 3.0-5.0 cm than those of Axonomorphae (*cf.* Figs. 1, 6). However, protein band similarities could be noted in the patterns of *A. rigonii* PI 262142 and *A.* sp. PI 262140 and PI 261877 of Erectoides and the species of Axonomorphae. Examination of protein patterns of seeds of Erectoides showed that the gel patterns occurring in high frequency for *A. benthamii* PI 262859, and *A.* sp. PI 262308, PI 276209, PI 276225, PI 276229, PI 262140, and PI 261877 from Tetrafoliolatae contained more protein in region 1.5-3.0 cm than those of the other species or collections of this section, including *A. rigonii* PI 262142 and A. sp. PI 276228, PI 262278, PI 262842, PI 331187, and PI 331188. Moreover, the gel patterns of *A.* sp. PI 276228 and two of the samples collected for *A.paraguariensis* (PI 262842, PI 331187) contained very little protein in region 1.5-3.0 cm. Intraspecific polymorphism for the species and collections of this section was mainly among the proteins in regions 1.0-3.0 (quantitative variations) and 3.0-5.0 cm (qualitative). For *A. paraguariensis,* PI 262842, 331187, 331188, the gels occurring in high frequency in collections PI 331187 and PI 331188 were more similar to one another than to those of PI 262842.

The esterase patterns of species from Tetrafoliolata could be grouped as follows: (1) *A. benthamii* PI 262859 and *A.* sp. PI 262308, PI 276209, PI 276225, PI 276228, PI 276229, and PI 262278; (2) the three collections of *A. paraguariensis* PI 262842, 331187, 331188 and (3) *A.* sp. PI 262140 and PI 261877 (Fig. 7). The patterns of group (1) contained a major (dark staining) esterase in region 6.0 cm which was not consistent in the other spe-

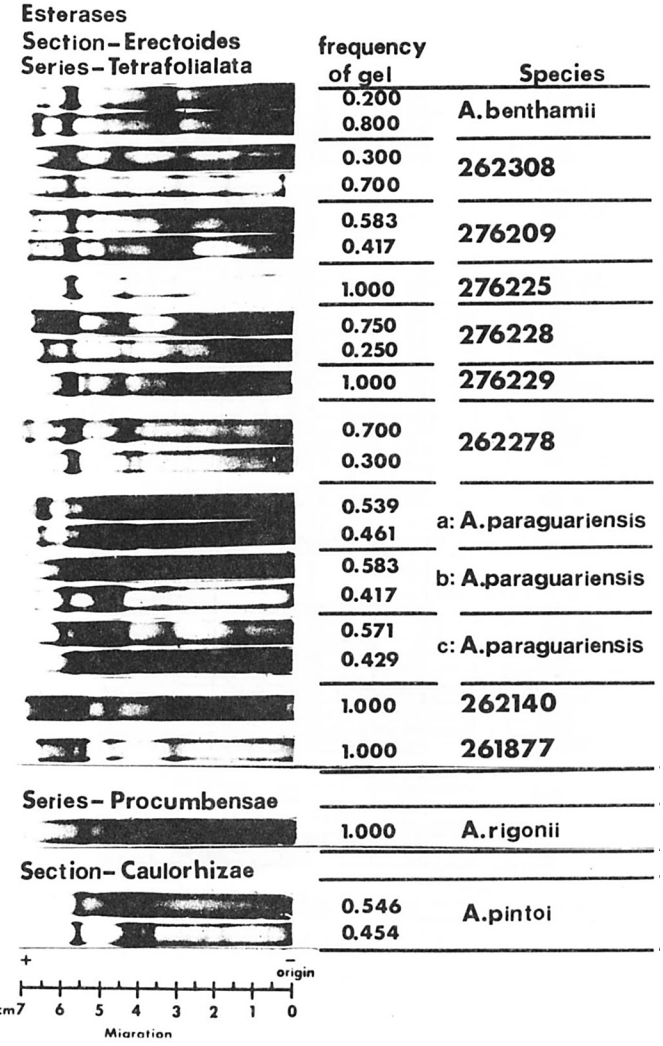

Fig. 7. Polyacrylamide disc gel electrophoretic patterns of esterases of Erectoides and Caulorhizae. Gel frequencies and plant introduction numbers or names (where assigned) for species are presented.

cies and collections of Erectoides. A large amount of esterase activity in region 4.0-5.0 cm was typical of the gels of *A. paraguariensis* PI 262842, 331187, 331188. Moreover, as with proteins, the esterase patterns of PI 331187 (b) and PI 331188 (c) were very similar. The remaining two species or group (3) contained esterases in region 5.5-7.0 cm which were not consistently found in the other patterns of this section.

The esterase patterns of *A. rigonii* PI 262142 differed from those of Tetrafoliolatae, supporting the placement of this species into a separate series, Procumbensae (Fig. 7). However, some of the esterases of *A. rigonii* PI 262142 were observed in *A.* sp. PI 262140 and PI 261877 of Tetrafoliolatae. The esterases in the gels from *A. rigonii* PI 262142 and *A. pintoi* PI 338447 of section Caulorhizae showed many similarities (Fig. 7). These latter two species showed similar patterns for catalase and achromatic oxidase (Fig. 4) confirming the preliminary report that Caulorhizae may be closely related to Erectoides (Gregory *et al.*, 1973).

The patterns for peroxidase and alcohol dehydrogenase separated the species and collections of Erectoides into the same groups distinguished by the esterases (Figs. 4, 5). The species and collections of Tetrafoliolatae could not be distinguished from each other by their leucine aminopeptidase and achromatic oxidase patterns (Figs. 3, 4). Achromatic oxidase did distinguish between seeds of Procumbensae and Tetrafoliolatae (Fig. 4).

SECTION RHIZOMATOSAE

Samples of Rhizomatosae were collected in southwestern Brazil and Paraguay (Table 1). The protein patterns of these seeds compared more closely to those of Erectoides than Axonomorphae (*cf.* Figs. 1, 6, 8). Much intra- and/or interspecific polymorphism was noted for the proteins and enzymes of these seeds making it difficult to determine the chemotaxonomy of this section (Figs. 3-5, 8-9). Moreover, specific esterase bands in region 4.0-6.5 cm were infrequent in one species and frequent in another whereas the reverse was true with other patterns (Fig. 9). For example *A.* sp. PI 262801 had a pattern occurring with a frequency of 0.600 while *A.* sp. PI 262817 had a similar gel with a frequency of 0.400. The relationship was similar between gels of *A.* sp. PI 276286 and PI 276233 (*cf.* gels with high and low frequencies of 0.800 and 0.286 in these two species, respectively). In *A.* sp. PI 262848, a gel with a low frequency of 0.200 occurred in all of the patterns for *A.* sp. PI 262794. Esterase bands in other gels were present in two different patterns. The most frequent gel in *A.* sp. PI 262848 showed a combination of

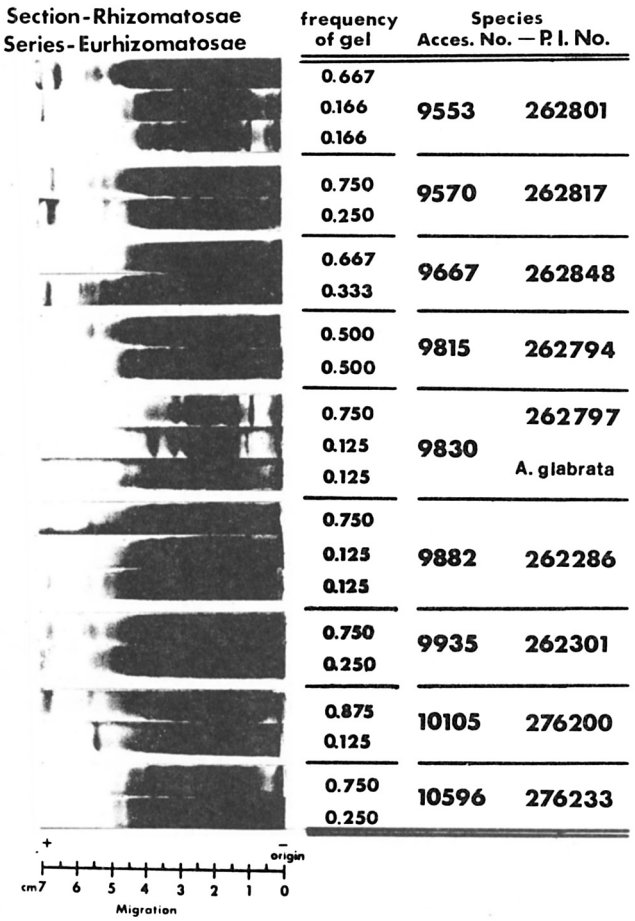

Fig. 8. Polyacrylamide disc gel electrophoretic patterns of proteins of Rhizomatosae. Gel frequencies, accession numbers (Acces. No.) and plant intorduction numbers (P. I. No.) or names (where assigned) for each species are presented.

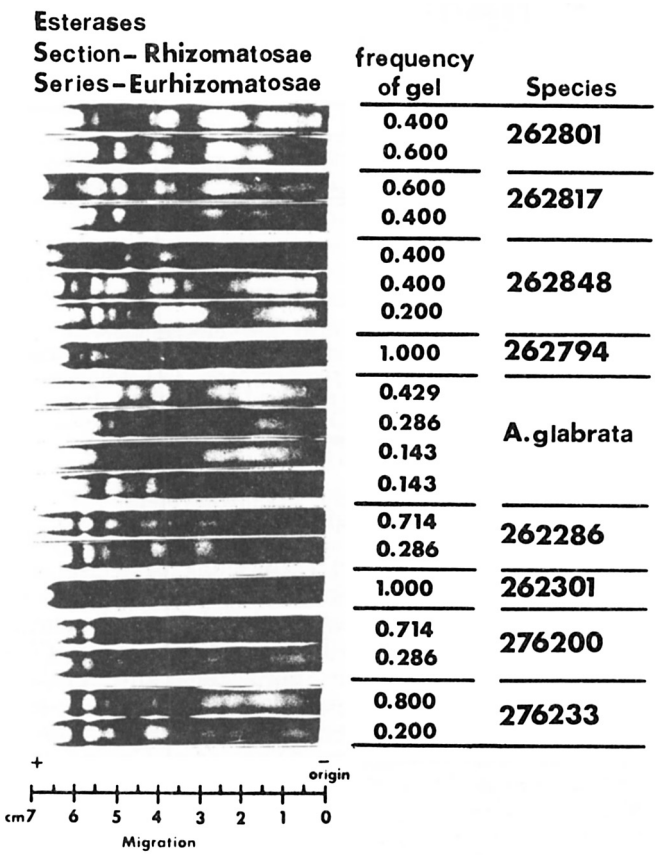

Fig. 9. Polyacrylamide disc gel electrophoretic patterns of esterases of Rhizomatosae. Gel frequencies and plant introduction numbers or names (where assigned) for species are presented.

the esterases found in *A.* sp. PI 262801 and PI 262817. Similar combinations were observed among the gels of *A.* sp. PI 276200, PI 276233, and PI 262301.

Plants of Eurhizomatosae have a chromosome number of 4n (Gregory *et al.*, 1973). Since these plants are polyploids, the extra genetic material may be responsible for the increase in molecular polymorphism shown by their protein and enzyme contents as was discussed for similar observations in the genus *Gossypium* (*Cherry* et al., 1970, 1972). However, qualitative and semiquantitative protein differences on electrophoretic gels have been shown to exist among seeds of vaious cultivated peanut types of *A. hypogaea* grown in different geographical locations (Cherry *et al.*, 1971; Ory and Cherry, 1972; Cherry, 1974). Therefore, it should be noted that possibly some of the variability in proteins and enzymes of wild species and collections of Arachis examined in ths study are due to environmental as well as genetic effects.

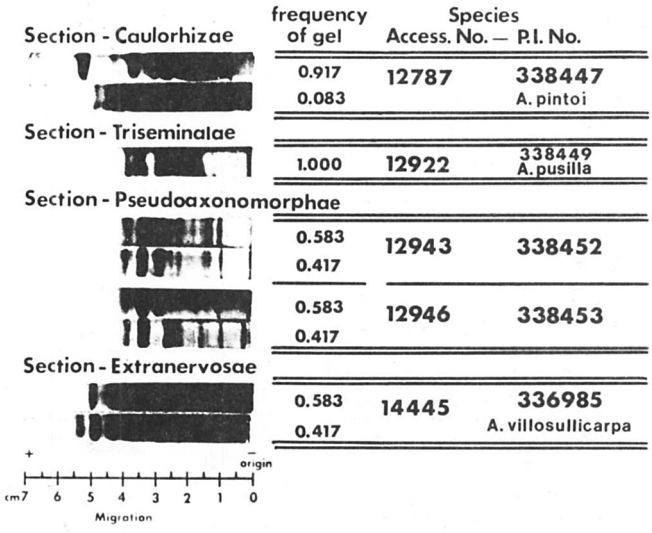

Fig. 10. Polyacrylamide disc gel electrophoretic patterns of proteins of Caulorhizae, Triseminalae, Pseudoaxonomorphae and Extranervosae. Gel frequencies, accession numbers (Acces. No.) and plant introduction numbers (P. I. No.) or names (where assigned) for each species are presented.

SECTIONS CAULORHIZAE, EXTRANERVOSAE, PSEUDOAXONOMORPHAE AND TRISEMINALAE

Available species and collections of these sections were collected in eastern (Caulorhizae), south-central (Extranervosae) and northeastern (Pseudoaxonomorphae, Triseminalae) Brazil (Table 1). Proteins, esterase, peroxidase and catalase distinguished the seeds of specimens of these sections while similar zymograms of leucine aminopeptidase were noted among species of Caulorhizae and Triseminalae and Extranervosae and Pseudoaxonomorphae, respectively (Figs. 2-6, 10). Caulorhizae and Pseudoaxonomorphae and Extranervosae and Triseminalae, respectively, exhibited similar patterns for alcohol dehydrogenase while Caulorhizae and Triseminalae produced identical gels of achromatic oxidase. The two collections of Pseudoaxonomorphae differed in their patterns of esterase, peroxidase, leucine aminopeptidase and achromatic oxidase activities, but showed similar gels for proteins, catalase and alcohol dehydrogenase.

SIMILARITY INDEX VALUES AMONG ARACHIS SPECIES

Proteins and enzyme bands within electrophoretic gels may be assumed to represent a random sample of gene loci of species within a genus (Sheen, 1972). The Similarity Index method (Sokal and Sneath, 1963) was shown by Sheen (1972) to be useful for evaluating genetic similarities or genomic homologies among species when based on their contents of similar and dissimilar isozymes in gel patterns. For our estimations, similarities and dissimilarities were a measure (cm) of the regions of overlap and nonoverlap, respectively, of bands in gels compared among species and/or collections. The positions of bands in the gels were determined by measuring the distances of their fronts and trailing edges from the origins. The Similarity Index value (s-value) for any two species or collections or both was an estimate of the percent of total band overlap in their gel patterns (Sheen, 1972). When an overlap region in the gel patterns involved major and minor bands, the estimate of similarity was arbitrarily weighed by a factor of 0.7. The s-values among species of *Arachis* were determined by combining the comparisons of the proteins and six enzymes (Table 2).

Within *Arachis* the s-values ranged from 19 (least related species or collections) to 86% (closely related). S-values were highest among seeds within species and/or collections within a series or section than s-values of seeds from other groups. For example, in series Annuae and Amphiploides of Axonomorphae, the s-value was 57% for *A. duranensis* PI 219823 and A. sp. PI 263133 and it was 59% for *A. hypogaea* and *A. monticola* PI 219824. Comparisons among these series showed that the s-value was 39% for *A. duranensis* PI 219823, *A. monticola* PI 219824, 33% for *A. duranensis* PI 219823, *A. hypogaea,* 39% for *A.* sp. PI 263133, *A. monticola* PI 219824 and 40% for *A.* sp. PI 263133, *A. hypogaea.* Within series Perennes of this section, *A. correntina* PI 262134, 262137, 262808, *A. chacoense* PI 276235 and *A. villosa* PI 298636 were within 42 to 45%, but comparisons among Annuae and Perennes were 32 to 40%, Annuae and Amphiploides, 33 to 40%, and Perennes and Amphiploides, 30 to 38%. The three collections of *A. correntina* PI 262134, 262137, 262808 of Perennes had s-values of 50, 52 and 57%, and the three collections of *A. paraguariensis* PI 262842, 331187, 331188 of series Tetrafoliolatae, section Erectoides, had s-values of 70, 74 and 86%. The range of s-values among Axonomorphae - Erectoides pairs was 26 to 31%.

Preliminary genetic studies (unpublished data) and present results observed on most of the enzyme patterns (Figs. 3-5, 7) suggested that the species and collections of series Tetrafoliolatae or Erectoides may be subdivided as follows: (1) *A. benthamii* PI 262859, and *A.* sp. PI 262308, PI

Table 2. Combined Protein and Enzyme Similarity Index Values among Arachis Species.

	Species	P.I. Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1	A. duranensis	219823																																				
2	A. sp.	263133	57																																			
3	A. correntina	262137	38	34																																		
4	A. correntina	262134	36	40	52																																	
5	A. correntina	262808	36	40	50	57																																
6	A. chacoense	276235	36	36	44	42	43																															
7	A. villosa	298636	32	36	45	43	42	42																														
8	A. monticola	219824	39	39	36	33	38	34	32																													
9	A. hypogaea	-	33	40	32	32	34	30	31	59																												
10	A. paraguariensis	262842	29	29	27	29	26	32	30	28	28																											
11	A. benthamii	262859	32	34	31	33	32	34	32	33	35	45																										
12	A. sp.	262308	32	33	27	29	29	33	26	30	30	40	55																									
13	A. sp.	262278	31	31	29	28	27	28	27	28	29	41	45	43																								
14	A. sp.	261877	29	30	27	27	27	28	29	30	33	39	37	36	41																							
15	A. sp.	262140	31	29	26	26	28	28	31	31	32	39	39	38	41	70																						
16	A. sp.	276209	28	29	29	28	33	32	29	28	28	38	56	54	48	34	37																					
17	A. sp.	276225	29	28	25	27	27	27	27	26	28	38	47	49	43	35	38	44																				
18	A. sp.	276228	30	31	27	26	30	33	28	31	31	39	50	46	49	37	42	49	48																			
19	A. sp.	276229	34	33	25	29	30	30	29	31	33	39	52	50	46	40	42	50	48	56																		
20	A. paraguariensis	331188	32	30	26	31	29	29	31	30	28	70	43	38	37	42	44	39	38	39	42																	
21	A. paraguariensis	331187	31	30	26	31	28	30	31	29	28	74	43	38	37	42	41	38	38	37	40	86																
22	A. rigonii	262142	38	32	27	31	28	30	30	30	28	34	33	33	34	36	37	31	32	34	35	35	34															
23	A. pintoi	338447	31	31	32	30	32	30	30	31	27	32	33	33	29	33	34	33	32	32	32	33	33	38														
24	A. sp.	262801	24	25	25	25	25	25	29	27	27	29	27	23	23	25	28	24	24	24	26	28	29	27	28													
25	A. sp.	262817	26	28	25	27	31	27	25	31	33	30	35	32	27	31	33	31	29	31	31	32	33	29	34	50												
26	A. sp.	262848	25	23	25	24	25	23	24	25	25	30	28	25	26	26	28	25	28	25	25	31	32	24	27	47	50											
27	A. sp.	262794	23	22	25	22	24	25	25	27	26	28	27	27	28	29	32	27	31	27	26	28	29	26	30	46	55	52										
28	A. glabrata	262797	21	22	22	25	23	22	27	27	26	32	30	28	26	31	33	27	31	26	28	30	32	27	31	44	49	44	58									
29	A. sp.	262286	23	25	24	28	25	25	25	27	28	30	30	27	27	29	28	28	28	27	26	29	31	26	27	38	48	45	47	45								
30	A. sp.	262301	22	22	24	24	26	23	27	28	26	23	25	24	24	26	27	22	28	22	23	25	28	25	27	48	41	38	44	42	41							
31	A. sp.	276200	24	25	24	23	24	24	28	27	27	27	31	27	30	29	32	28	32	26	27	29	31	30	28	37	47	44	51	46	45	47						
32	A. sp.	276233	25	25	25	28	24	26	27	27	28	28	29	26	27	31	34	29	29	29	29	31	30	30	30	37	47	41	45	45	51	38	56					
33	A. villosulicarpa	336985	27	28	27	28	27	30	33	28	27	26	27	25	23	29	32	26	27	24	26	29	30	29	26	26	21	24	24	25	26	27	23	25				
34	A. sp.	338452	27	24	20	19	22	23	23	26	24	26	22	22	23	23	24	19	20	21	22	24	25	28	26	22	22	23	23	24	19	20	20	20	25			
35	A. sp.	338453	29	28	24	25	27	25	26	27	26	31	28	25	27	27	28	23	26	23	24	29	29	29	28	27	25	26	25	28	21	23	22	23	28	57		
36	A. pusilla	338449	29	30	27	29	30	31	30	29	29	26	31	29	23	29	32	27	23	26	27	29	28	31	32	25	26	20	22	25	23	24	25	27	42	28	30	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36

276209, PI 276225, PI 276228, and PI 276229; (2) *A.* sp. PI 262278; (3) the three collections of *A. paraguariensis* (PI 262842, PI 331187, PI 331188); and (4) *A.* sp. PI 261877 and PI 262140. The s-values among plants within groups (1), (3) and (4) averaged 51, 77 and 70%, respectively, whereas comparisons among these groups ranged from 39 to 46%. Further, as similarly suggested by their esterase patterns, the s-values indicated that *A.* sp. PI 262278 of group (2) could be classified with (1). Low s-values averaging 34% among plants of Tetrafoliolatae and Procumbensae warranted this sub-classification within Erectoides. The observation that protein patterns of *A. rigonii* PI 262142 were similar to those of some species in Axonomorphae (e.g., *A. duranensis* PI 219823) and Caulorhizae (*A. pintoi* PI 338447) was verified by relatively high s-values, averaging 38%, and suggested that there may be some genetic homology among these sections.

The protein and enzyme patterns within Rhizomatosae did not clearly distinguish the species of this section into a sub-classification. Among *A. glabrata* PI 262797 and *A.* sp. PI 262801, PI 262817, and PI 262848, s-values were relatively high (44 to 58%). Other s-values suggested that the following species may be closely related: (1) *A.* sp. PI 262801 and PI 262301, (2) *A.* sp. PI 262817 and PI 262286 and *A. glabrata* PI 262797, (3) *A.* sp. PI 262848 and PI 262794, (4) *A.* sp. PI 262794 and PI 276200 and *A.* glabrata PI 262797, and (5) *A.* sp. PI 262286 and PI 276233; the latter collection also compared closely with PI 276200.

An s-value of 57% for the two collections of Pseudoaxonomorphae warranted their classification into this section. Comparisons of these two collections with other seeds in the genus ranged between 19 to 31%. Most of the s-values among *A. villosulicarpa* PI 336985 and *A.* pusilla PI 338449 and other species and collections of *Arachis* ranged between 21 to 31% giving support to the suggestion that these plants should be placed in sections Extranervosae and Triseminalae, respectively. However, an s-value of 42% for these two species indicated that they may be closely related.

The importance of taxonomic studies has been stressed (Frankel and Bennett, 1970). Wild species contain new sources of germ plasm which can be used to decrease "genetic erosion" (Miller, 1973) by increasing the variability of the genetic base in cultivated varieties. Once the cross-compatibility of species within a genus is understood, studies can begin to determine "bridge species" for the introduction of new genetic information into cultivated varieties (Simpson and Haney, 1973). Separation of proteins and enzymes of seeds into discrete bands with distinct physicochemical characteristics on polyacrylamide and starch electrophoretic gels, and evaluating these data by the Similarity Index method, confirmed the taxonomic arrangement of *Arachis* species in present sections. The data in this presentation should promote more detailed biochemical assays at the species level

and provide corresponding genetic patterns to help use these materials in improving commercial peanuts during future breeding programs.

Acknowledgments

I thank Drs. W. C. Gregory and M. P. Gregory for the samples of seeds of wild species and collections of **Arachis** which they had collected from the original sources; Dr. A. D. French for preparation of the computer program used to obtain the s-values among species, Mr. J. Bergquist for photographing the electrophoretic gels and Drs. W. C. Gregory, R. L. Ory, R. O. Hammons, and C. T. Young for suggestions and review of the manuscript. Appreciation is also expressed to the Southern Regional Research Center for financial support during July 1-31, 1973 to complete this work.

Literature Cited

1. Brewbaker, J. L., M. D. Upadhya, Y. Makinen, and T. MacDonald. 1968. Isozyme polymorphism in flowering plants. III. Gel electrophoretic methods and applications. Physiol. Plant. 21:930-940.
2. Cherry, J. P., F. R. H. Katterman, and J. E. Endrizzi. 1970. Comparative studies of seed proteins of species of **Gossypium** by gel electrophoresis. Evolution 24:431-447.
3. Cherry, J. P., N. J. Neucere, and R. L. Ory. 1971. Comparison of proteins of peanuts grown in different areas. I. Disc electrophoretic analysis of qualitative and quantitative variations. J. Amer. Peanut Res. & Ed. Assoc. 3:63-74.
4. Cherry, J. P., F. R. H. Katterman, and J. E. Endrizzi. 1972. Seed esterases, leucine aminopeptidases and catalases of species of the genus **Gossypium.** Theoret. Appl. Genet. 42:218-226.
5. Cherry, J. P. and R. L. Ory. 1973a. Electrophoretic characterization of six selected enzymes of peanut cultivars. Phytochem. 12:283-289.
6. Cherry, J. P. and R. L. Ory. 1973b. Characterization of isoperoxidase activity in **Arachis hypogaea** cultivars. Phytochem. 12:1581-1583.
7. Cherry, J. P., J. M. Dechary, and R. L. Ory. 1973. Gel electrophoretic analysis of peanut proteins and enzymes. I. Characterization of DEAE-cellulose separated fractions. J. Agr. Food Chem. 21:652-655.
8. Cherry, J. P., R. Y. Mayne, and R. L. Ory. 1974. Proteins and enzymes from **Arachis hypogaea** L. grown in different areas. IX. Electrophoretically detected changes in 15 peanut cultivars after **Aspergillus parasiticus** contamination. Physiol. Pl. Path. 4:425-434.
9. Cherry, J. P. 1974. Electrophoretic observations on protein changes and variability during development of the peanut. J. Agr. Food Chem. 22:723-724.
10. Frankel, O. H. and E. Bennett. 1970. Genetic resources in plants — their exploration and conservation. F. A. Davis Co., Philadelphia, Pa.
11. Gregory, W. C., M. P. Gregory, A. Krapovickas, B. W. Smith, and J. A. Yarbrough. 1973. Structure and genetic resources of peanuts, pp. 47-133. In: Peanuts — culture and uses. Published by the Amer. Peanut Res. and Ed. Assoc., Inc., Stillwater, Oklahoma.
12. Hammons, R. O. 1972. Peanuts. Chapter 13, pp. 217-223, 253. In: Genetic Vulnerability of Major Crop Plants. Nat. Acad. Sci. Nat. Res. Council, Agric. Board, Washington, D. C.
13. Hammons, R. O. 1973. Early history and origin of the peanut, pp. 17-45. In: Peanuts — culture and uses. Published by the Amer. Peanut Res. and Ed. Assoc., Inc., Inc., Stillwater, Oklahoma.
14. Krapovickas, A. 1969. The origin, variability and spread of the groundnut (**Arachis hypogaea**). In: P. J. Ucko and G. W. Dimbleby (Eds.), The domestication and exploitation of plants and animals. Aldine Publishing Co., Chicago, Ill.
15. Leppik, E. E. 1971. Assumed gene centers of peanuts and soybeans. Economic Bot. 25:188-194.
16. Miller, J. 1973. Genetic erosion: Crop plants threatened by government neglect. Science 182:1231-1233.
17. Mitra, R. D., D. R. Jagannath, and C. R. Bhatia. 1970. Disc electrophoresis of analogous enzymes in **Hordeum.** Phytochem. 9:1843-1850.
18. Raman, V. S. 1973. Genome relationships in **Arachis.** Oleagineux 28:137-140.
19. Scandalios, J. G. 1969. Genetic control of multiple molecular forms of enzymes in plant: A review. Biochem. Genet. 3:37-79.
20. Sheen, S. J. 1972. Isozymic evidence bearing on the origin of **Nicotiana tabacum L.** Evolution 26:143-154.
21. Simpson, C. E. and R. L. Haney. 1973. Wild species help breeders develop better peanuts for the future. Texas Agric. Prog. 19:10-11.
22. Siddiq, E. A., Y. S. Nerkar, and S. L. Mehta. 1972. Intra and inter subspecific variation in soluble proteins in **Oryzae sativa L.** Theoret. Appl. Genet. 42:-351-356.
23. Smartt, J. and W. C. Gregory. 1967. Interspecific cross-compatibility between the cultivated peanut **Arachis hypogaea L.** and other members of the genus **Arachis.** Oleagineux 22:455-459.
24. Sokal, R. R. and P. H. Sneath. 1963. Principles of numerical taxonomy. W H. Freeman and Co., San Francisco, Calif.