Theor. Appl. Genet. 61, 305–314 (1982)

Utilization of Wild Relatives in Genetic Improvement of *Arachis hypogaea* L.

Part 2: Chromosome Complements of Species in Section *Arachis* *

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Summary. The chromosome complements of 12 taxa in section *Arachis* were karyotypically and meiotically analysed. In taxa with 2n = 20 the arm ratio of the respective pair of chromosomes was taken as an independent quantitative character and statistically analysed by Mahalanobis D^2. Two clusters were formed, one represented solely by *A. batizocoi* and the other consisting of the remaining 11 taxa. This grouping was confirmed by canonical analysis. In the larger group of species, *A villosa* and *A. correntina* were closely related karyotypically and on D^2 distance, while *A. cardenasii* forms a distinct subgroup. *A. cardenasii* lacks the short "A" chromosome recorded in other species of this group, and *A. batizocoi* is no longer the only species to have a pair of chromosomes with a secondary constriction. The taxa with 2n = 40, *A. monticola* and *A. hypogaea,* are karyotypically very similar, though there is a difference in the number of chromosome pairs with a secondary constriction. On the basis of karyomorphological affinity, especially in relation to marker chromosomes, *A. cardenasii* is probably one of the ancestors of the tetraploid species studied.

Key words: *Arachis* – Karyomorphology – D^2 analysis and genetic distance – Evolution – Crop improvement

Introduction

The genus *Arachis* has received less attention from cytogeneticists than many other crop genera. This is partly because *Arachis* chromosomes are technically difficult to observe and also because there has been no concerted effort to isolate and utilize aneuploids or haploids, as in wheat or potatoes, or to utilize wild species in the improvement of the crop, as in wheat or tobacco. This paper reports the results of cytological analysis of species being used in a crop improvement programme at ICRISAT.

Ghimpu (1930) and Kawakami (1930) were the first to determine the chromosome number of 2n = 40 for *A. hypogaea.* Later, Husted (1933, 1936) identified a pair of small chromosomes, and a pair of chromosomes with a secondary constriction and a satellite, which were designated the "A" and "B" chromosomes, respectively. Since then there have been several other reports, either confirming the earlier studies, or reporting chromosome counts for different species of the genus (Mendes 1947; Krapovickas and Rigoni 1951; Babu 1955; Raman 1958, 1959; D'Cruz and Tankasale 1961).

The taxonomy of *Arachis* has been studied by Gregory et al. (1973, 1980). The genus was divided into seven sections, based on morphological similarities, cross-compatibility and fertility of hybrids. *A. hypogaea* is classified in section *Arachis,* which presently consists of one other tetraploid species with 2n = 40 and eight diploids with 2n = 20.

Smartt (1964) observed that several diploid species of section *Arachis* had "A" chromosomes, and Smartt et al. (1978) reported that *A. batizocoi* alone had "B" chromosomes. Based on these observations as well as those on pollen fertility of several F_1 hybrids of these species, it was tentatively proposed that *A. batizocoi* and *A. cardenasii* contributed "B" and "A" chromosomes respectively to *A. hypogaea.* Earlier, Gregory and Gregory (1976) had suggested *A. hypogaea* to be a possible tetraploid of an intrasectional hybrid between *A. cardenasii* and *A. duranensis.* These hypotheses are yet to be verified.

In this study the chromosomes of 12 taxa in the section *Arachis,* including *A. hypogaea,* have been studied in detail, and their full somatic complements have been analysed by D^2 statistics and canonical analysis. This will add to the knowledge of species relationships within the section and the evolution of *A.*

* Approved as ICRISAT Journal Article No. 169 and released for publication

0040-5752/82/0061/0305/$ 2.00

hypogaea, and ensure the most efficient use of wild species in the improvement of *A. hypogaea.*

Materials and Methods

The taxa studied in the present investigation and their sources are listed in Table 1. The accessions used were received from collections maintained and classified on the basis of their morphological affinities. We have followed the classification of Krapovickas and Gregory (Gregory et al. 1973); collections 410 and 10038 represent two different species tentatively named *A. stenosperma* and *A. spegazzini* (Gregory Pers. Comm.). In *Arachis* species 10038, plants with large leaflets and small leaflets were recorded by Gregory and maintained separately (Pers. Comm.). At ICRISAT the populations from these accessions showed no variability in morphology or chromosome number, other than indicated by the standard deviations quoted in this paper.

The cytological technique of Singh et al. (1980) was used for the study of somatic complements. For meiotic analysis flower buds were first fixed in Carnoy fluid II for 24 h, then in Carnoy fluid I for 24 h and squashed in 1% acetocarmine.

Detailed observations and photomicrography were done on temporary preparations. Arm lengths were measured from ten cells in each taxon, and arm ratios were calculated. Chromosomes were paired according to their structural homology, based on arm length and ratio, and were ranked in decreasing order of length. Duncan's Multiple Range test was used to distinguish groups of chromosomes based on length, within each species.

The observations for 20 chromosome species were statistically analysed by Mahalanobis D^2. The arm ratio of each pair of chromosomes in every species was taken as an independent quantitative character. The calculations of D^2 values involves the steps discussed by Murty and Arunachalam (1967), where D^2 between i-th and j-th species for K characters were obtained by the equation

$$D_{ij}^2 = \sum_{t=1}^{k} (Y_i t - Y_j t)^2$$

The K components D^2 for each combination were ranked in descending order of magnitude.

Treating D^2 as the generalised statistical distance, all species were grouped into clusters using the Tocher method (Rao 1952). The criterion used in clustering was that the distance between any two species in the same cluster was less than the intercluster distance. The permissible increase of D^2 within a cluster was the maximum D^2 value shown by a species to its nearest species (Singh and Chaudhary 1977). Species were also grouped by canonical analysis to verify the clusters derived by D^2 statistics (Peter et al. 1977).

For karyotypic formulae, chromosomes were classified on the basis of arm ratio (Stebbins 1958):

Type of chromosome	Abbreviation	Arm ratio
Metacentric	M	1.0–1.09
Sub-metacentric	Sm	1.1–2.0
Sub-telocentric	St	2.01 or more
(a) Small satellite	Sat (S)	
(b) Large satellite	Sat (L)	

Results

Species with 2n = 20

A. villosa and *A. correntina* have very similar karyotypes (Figs. 1, 2); the genetic distance based on arm ratio is 0.05 (Fig. 3). Chromosomes 1 and 2 have a similar arm ratio but differ in length. Chromosomes 3 to 5 form a group which cannot be distinguished by length in *A. correntina,* but one, designated 3 by us, has the nucleolar organizer, and the other two can be distinguished by arm ratio. Chromosomes 6 and 7 are similar to each other. There are differences between the species in chromosomes 8 and 9, but both have a short median 10th chromosome (Fig. 2, Table 2).

Table 1. Taxa investigated

Name of species	PI No.	Coll. No.	Source
A. villosa Benth	not available	not available	TNAU, Coimbatore, India
A. correntina (Burk) Krap. et Greg. nom. nud.	262809	9530	NCSU, USA
A. chacoense Krap. et Greg. nom. nud.	276235	10602	NCSU, USA
A. species	338280	410	
A. species	263133/331189	10038 LL	NCSU, USA
A. species	263133/331189	10038 SL	NCSU, USA
A. duranensis Krap. et Greg. nom. nud.	219823	7988	NCSU, USA
A. cardenasii Krap. et Greg. nom. nud.	262141	10017	NCSU, USA
A. batizocoi Krap. et Greg.	338312	9484	NCSU, USA
A. monticola Krap. et Rig.	219824, 263393, 298364, 298365, 331338-39	7264 (104, 105)	NCSU, USA
A. hypogaea L. ssp. *hypogaea* Krap. et Rig. (cv. 'Robut 33-1')		–	RORS, Kadiri, India
A. hypogaea L. ssp. *fastigiata* Waldron (cv. 'TMV-2')		–	OES, Tindivanam, India

TNAU = Tamil Nadu Agricultural University; NCSU = North Carolina State University; RORS = Regional Oilseeds Research Station; OES = Oilseeds Experimental Station

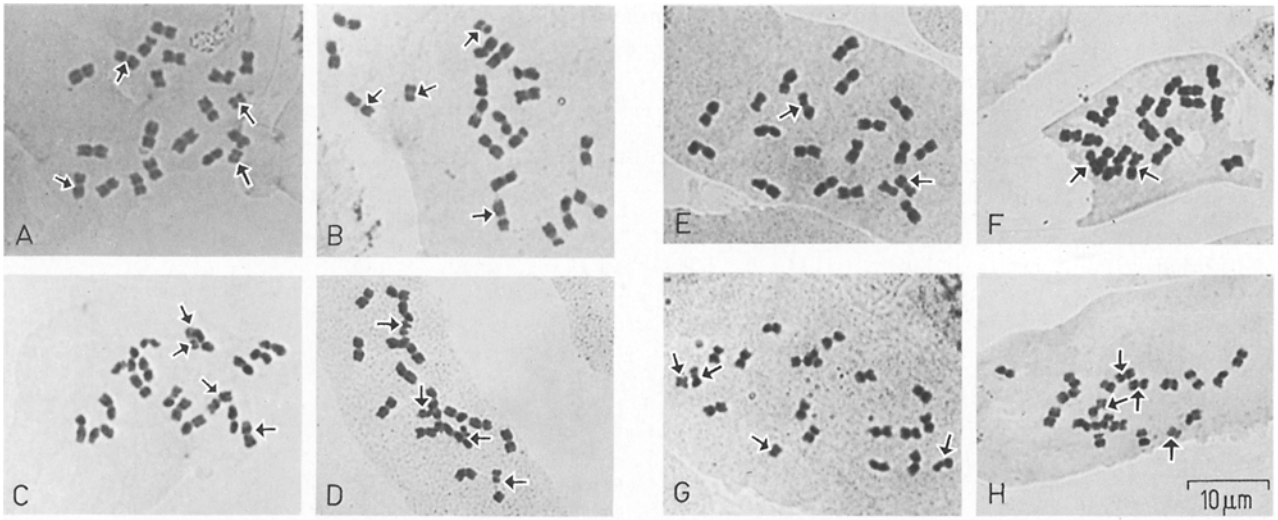

Fig. 1 A–H. Somatic Cells at metaphase. **A** *A. villosa*; **B** *A. correntina*; **C** *A. chacoense*; **D** *A.* species HLK-410; **E** *A. cardenasii*; **F** *A. batizocoi*; **G** *A. duranensis*; **H** *A.* species 10038 LL. Arrows indicate small chromosomes and chromosomes with secondary constriction

Fig. 2. Karyograms of diploid *Arachis* species. Chromosomes with secondary constriction are circled.
* Difference in length between adjacent chromosome pairs statistically significant at 5%.
** Difference in length between adjacent chromosome pairs statistically significant at 1%.

Table 2. Means and standard errors of arm ratios of respective pairs of chromosomes in diploid species of the section *Arachis*

Name of Species	Chromosome pairs									
	I	II	III	IV	V	VI	VII	VIII	IX	X
A. villosa	1.06 ± 0.035	1.16 ± 0.080	1.11 ± 0.058	1.31 ± 0.051	1.08 ± 0.066	1.09 ± 0.061	1.09 ± 0.096	1.80 ± 0.218	1.13 ± 0.063	1.00 ± 0.00
A. correntina	1.08 ± 0.039	1.06 ± 0.066	1.11 ± 0.057	1.34 ± 0.094	1.11 ± 0.078	1.09 ± 0.038	1.18 ± 0.100	1.99 ± 0.210	1.23 ± 0.137	1.02 ± 0.033
A. chacoense	1.17 ± 0.056	1.10 ± 0.046	1.39 ± 0.090	1.27 ± 0.079	1.07 ± 0.045	1.13 ± 0.081	1.09 ± 0.048	1.35 ± 0.132	1.59 ± 0.127	1.06 ± 0.050
A. species HLK-410	1.13 ± 0.054	1.05 ± 0.047	1.06 ± 0.026	1.21 ± 0.096	1.06 ± 0.038	1.21 ± 0.045	1.14 ± 0.060	1.28 ± 0.081	1.42 ± 0.122	1.30 ± 0.155
A. species 10038 (LL)	1.08 ± 0.055	1.06 ± 0.040	1.26 ± 0.082	1.27 ± 0.010	1.05 ± 0.034	1.04 ± 0.033	1.10 ± 0.054	1.14 ± 0.089	1.66 ± 0.094	1.13 ± 0.078
A. species 10038 (SL)	1.10 ± 0.040	1.13 ± 0.090	1.40 ± 0.076	1.22 ± 0.010	1.11 ± 0.069	1.08 ± 0.032	1.28 ± 0.174	1.16 ± 0.080	1.70 ± 0.189	1.14 ± 0.108
A. duranensis	1.10 ± 0.039	1.08 ± 0.031	1.03 ± 0.073	1.04 ± 0.053	1.18 ± 0.071	1.08 ± 0.042	1.47 ± 0.215	1.15 ± 0.059	1.73 ± 0.013	1.05 ± 0.051
A. cardenasii	1.25 ± 0.019	1.10 ± 0.081	1.48 ± 0.095	1.26 ± 0.096	1.22 ± 0.114	1.32 ± 0.076	1.21 ± 0.119	1.52 ± 0.178	1.32 ± 0.180	1.49 ± 0.122
A. batizocoi	2.02 ± 0.252	1.11 ± 0.045	1.20 ± 0.110	1.51 ± 0.120	1.24 ± 0.149	1.36 ± 0.125	1.55 ± 0.130	1.03 ± 0.035	1.54 ± 0.030	1.28 ± 0.104

The next group of 5 species shows wider diversity. *A. chacoense,* and both the forms of *Arachis* species 10038, have the satellite on chromosome 3. The satellite chromosome in *A. duranensis* is chromosome 2, which is not significantly longer than chromosome 3. The satellite in *Arachis* species HLK 410 is on chromosome 5, which is shorter than chromosome 3; however, there is no significant difference in length between chromosomes 3 and 4 or between 4, 5 and 6. There is little difference between these species with respect to chromosome length and arm ratio of the other chromosomes; chromosomes 1 and 2 can be grouped together. Chromosome 10 is distinct in all these species. Chromosome 9 is also distinct in length and/or arm ratio and is nearly subtelocentric in many of the species (Figs. 1, 2).

A. cardenasii differs from the above species, in that chromosome 10 is longer and the satellite is on chromosome 9 (Figs. 1, 2). The arm ratios of other chromosome pairs are also different from the preceding species

Table 3. Details of genetic complements of species of section *Arachis*

Name of species	n	2n	Range of L/S arm ratio	Mean L/S arm ratio	Karyotypic formula
A. villosa	10	20	1.00 – 1.80	1.19	5M + 4Sm + 1SmSat(S)
A. correntina	10	20	1.02 – 1.99	1.22	4M + 5Sm + 1SmSat(S)
A. chacoense	10	20	1.07 – 1.59	1.22	3M + 6Sm + 1SmSat(S)
A. species HLK-410	10	20	1.05 – 1.30	1.20	3M + 6Sm + 1MSat(L)
A. species 10038 LL	10	20	1.06 – 1.66	1.18	4M + 5Sm + 1SmSat(S)
A. species 10038 SL	10	20	1.08 – 1.70	1.23	1M + 8Sm + 1SmSat(S)
A. duranensis	10	20	1.04 – 1.73	1.22	5M + 4Sm + 1MSat(L)
A. cardenasii	10	20	1.10 – 1.52	1.32	1M + 8Sm + 1SmSat(L)
A. batizocoi	10	20	1.03 – 2.02	1.39	1M + 7Sm + 1SmSat(L) + 1St
A. monticola	20	40	1.05 – 1.38	1.22	5M + 13Sm + 1SmSat(S) + 1SmSat(L)
A. hypogaea ssp. *hypogaea*	20	40	1.05 – 1.47	1.20	8M + 11Sm + 1SmSat(L)
A. hypogaea ssp. *fastigiata*	20	40	1.04 – 1.40	1.18	5M + 14Sm + 1SmSat(L)

Table 4. D^2 values arranged in increasing order of magnitude among diploid species of section *Arachis*

A. villosa (1)	*A. correntina* (2)	*A. chacoense* (3)	*A.* species HLK-410 (4)	*A.* species 10038 (LL) (5)	*A.* species 10038 (SL) (6)	*A. duranensis* (7)	*A. cardenasii* (8)	*A. batizocoi* (9)
0.050 (2)	0.050 (1)	0.066 (6)	0.184 (5)	0.070 (6)	0.066 (3)	0.258 (5)	0.379 (3)	0.805 (4)
0.223 (3)	0.273 (3)	0.70 (5)	0.243 (6)	0.070 (3)	0.070 (5)	0.352 (3)	0.415 (6)	0.842 (8)
0.287 (4)	0.376 (4)	0.223 (1)	0.269 (3)	0.184 (4)	0.243 (4)	0.347 (4)	0.443 (4)	0.853 (3)
0.293 (5)	0.390 (5)	0.269 (4)	0.287 (1)	0.258 (7)	0.357 (1)	0.374 (6)	0.551 (2)	0.862 (6)
0.357 (6)	0.431 (6)	0.273 (2)	0.347 (7)	0.293 (1)	0.374 (7)	0.472 (1)	0.571 (5)	0.945 (5)
0.472 (7)	0.551 (8)	0.352 (7)	0.376 (2)	0.390 (2)	0.415 (8)	0.609 (2)	0.594 (1)	1.149 (7)
0.594 (8)	0.609 (7)	0.379 (8)	0.443 (8)	0.571 (8)	0.431 (2)	0.883 (8)	0.842 (9)	1.233 (1)
1.233 (9)	1.244 (9)	0.853 (9)	0.805 (9)	0.945 (9)	0.862 (9)	1.149 (9)	0.883 (7)	1.244 (2)

Table 5. Canonical vectors

Vector	Chromosome pairs									
	I	II	III	IV	V	VI	VII	VIII	IX	X
1.	0.5450	−0.0017	0.0706	0.0917	0.0856	0.1874	0.2456	−0.6705	0.330	0.1624
2.	0.6152	0.0266	−0.0461	0.1978	0.1032	0.1116	0.2869	0.4666	−0.4850	0.1456
3.	−0.1268	0.0168	0.7331	−0.2078	0.0418	−0.1389	0.0465	0.0238	−0.1042	0.6069
4.	0.5454	−0.0018	0.0694	0.0920	0.0857	0.1879	0.2455	−0.6704	0.3329	0.1617

The sum of all canonical roots = 2.8909. $\lambda_1 = 1.4937$; $\lambda_2 = 0.7598$; $\lambda_3 = 0.2834$; $\lambda_4 = 0.2149$; Contribution of $\lambda_1 = 51.6712\%$; $\lambda_2 = 26.2840\%$; $\lambda_3 = 9.8020\%$; $\lambda_4 = 7.4342\%$

(Table 2). This is reflected in the D^2 values between this species and other preceding species which are always greater than the average D^2 for the cluster (Fig. 3).

A. batizocoi is distinct from all the preceding species, sufficiently so to be separated into another cluster (Fig. 3). It has chromosome 10 longer than in any other species and also differs in the arm ratio of respective pairs of chromosomes, as well as in the range and mean of the arm ratio of the total complement (Tables 2, 3).

The analysis of D^2 values among different diploid species of section *Arachis* (Table 4), and grouping by the Tocher method, shows that there are only 2 clusters among the species studied (Fig. 3).

The canonical analysis showed that the first two canonical roots accounted for 78% of the total variation. The magnitude of the vectors indicates the relative importance of the individual characters in the differentiation of the taxa. This shows that the majority of the variation between the wild diploid taxa studied can be attributed to chromosome pairs 1, 7, 8 and 9 (Table 5), though all species fall in one region of the Z_1, Z_2 graph (Fig. 4). All these species showed regular bivalent formation. Observations on meiotic plates revealed that the "A" chromosomes, wherever present, did not stain as intensely as the rest of the chromosomes; but there was no other indication of the presence of heterochromatin with the staining techniques used.

Species with 2n = 40

In *A. monticola* and the *A. hypogaea* cultivars studied, both the "A" and "B" marker chromosomes were present (Figs. 5, 6). In *A. monticola*, two pairs of chromosomes have secondary constrictions, the 2nd with a small satellite and the 8th with a comparatively large satellite. In *A. hypogaea* the 8th pair of chromosomes has a large satellite. Both these species are similar karyomorphologically, with small differences in

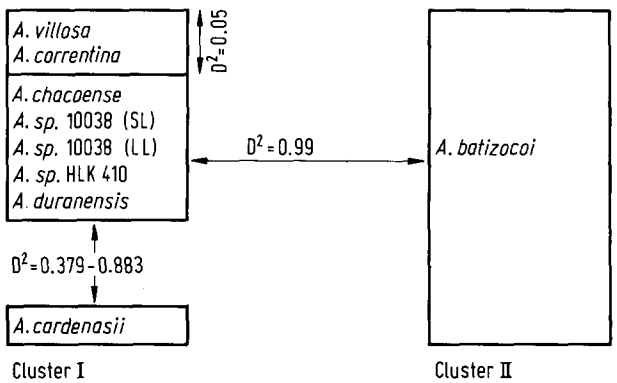

Fig. 3. Cluster diagram. D^2 distances among species of section *Arachis*. Average intracluster $D^2 = 0.335$

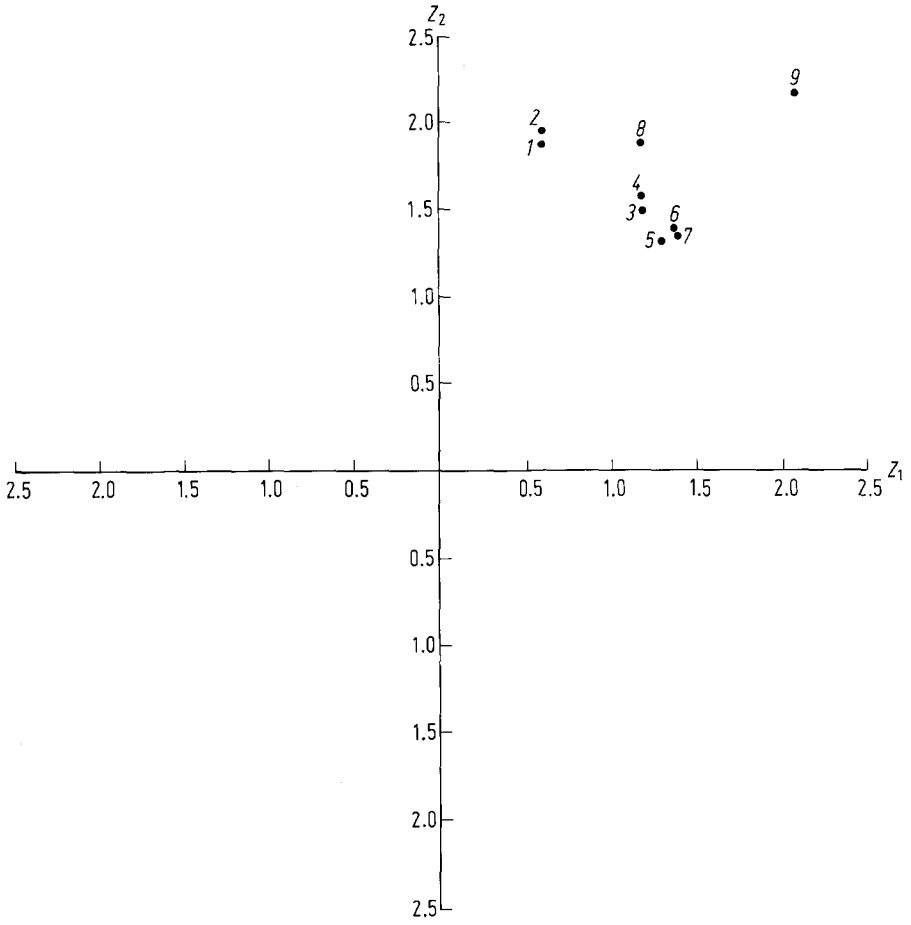

Fig. 4. Z_1 and Z_2 graph. *(1) A. villosa; (2) A. correntina; (3) A. chacoense; (4) A. species* HLK-410; *(5) A.* species 10038 (LL); *(6) A.* species 10038 (SL); *(7) A. duranensis; (8) A. cardenasii; (9) A. batizocoi*

Table 6. Means and standard errors of arm ratios of respective pairs of chromosomes in tetraploid species of the section *Arachis*

Name of species	Chromosomes pairs									
	I	II	III	IV	V	VI	VII	VIII	IX	X
A. monticola	1.27 ± 0.084	1.18 ± 0.088	1.20 ± 0.087	1.34 ± 0.088	1.19 ± 0.111	1.08 ± 0.057	1.18 ± 0.050	1.26 ± 0.077	1.05 ± 0.043	1.23 ± 0.107
A. hypogaea ssp. *hypogaea*	1.28 ± 0.041	1.12 ± 0.059	1.21 ± 0.088	1.06 ± 0.037	1.09 ± 0.065	1.21 ± 0.060	1.12 ± 0.066	1.16 ± 0.072	1.09 ± 0.070	1.23 ± 0.088
A. hypogaea ssp. *fastigiata*	1.35 ± 0.104	1.14 ± 0.057	1.16 ± 0.077	1.05 ± 0.026	1.16 ± 0.075	1.31 ± 0.068	1.18 ± 0.079	1.09 ± 0.070	1.26 ± 0.086	1.29 ± 0.082
Name of species	Chromosome pairs									
	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX
A. monticola	1.09 ± 0.058	1.32 ± 0.147	1.07 ± 0.059	1.13 ± 0.090	1.09 ± 0.078	1.39 ± 0.129	1.15 ± 0.093	1.63 ± 0.164	1.34 ± 0.141	1.10 ± 0.069
A. hypogaea ssp. *hypogaea*	1.08 ± 0.027	1.34 ± 0.038	1.07 ± 0.040	1.31 ± 0.112	1.08 ± 0.058	1.11 ± 0.072	1.07 ± 0.057	1.43 ± 0.164	1.47 ± 0.111	1.04 ± 0.038
A. hypogaea ssp. *fastigiata*	1.06 ± 0.040	1.23 ± 0.092	1.11 ± 0.071	1.09 ± 0.063	1.15 ± 0.063	1.24 ± 0.005	1.11 ± 0.059	1.40 ± 0.096	1.14 ± 0.092	1.05 ± 0.056

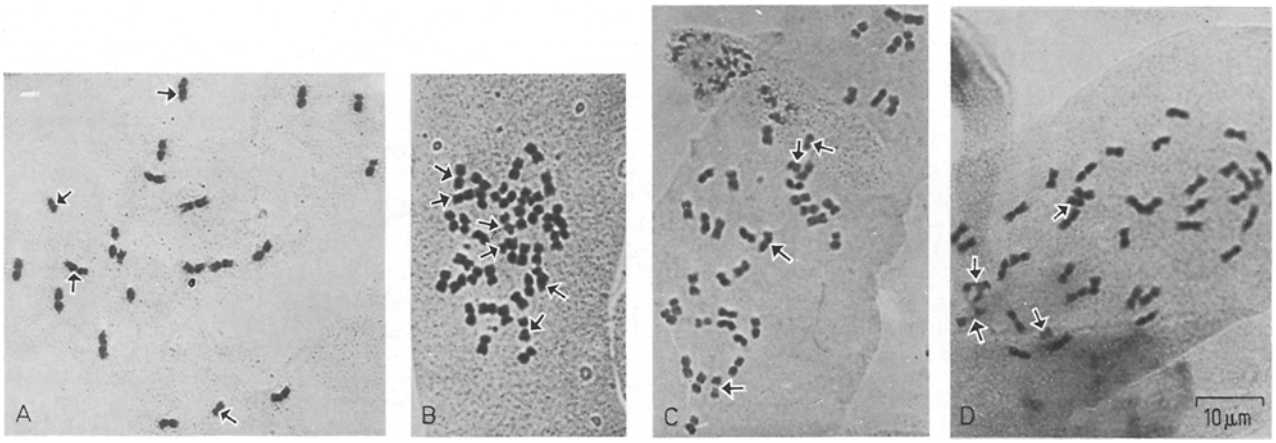

Fig. 5. A–D. Somatic Cells at metaphase. **A** *A.* species 10038 SL; **B** *A. monticola*; **C** *A. hypogaea hypogaea* (Robut 33-1); **D** *A. hypogaea fastigiata* (TMV 2). Arrows indicate small chromosomes and chromosomes with secondary constriction

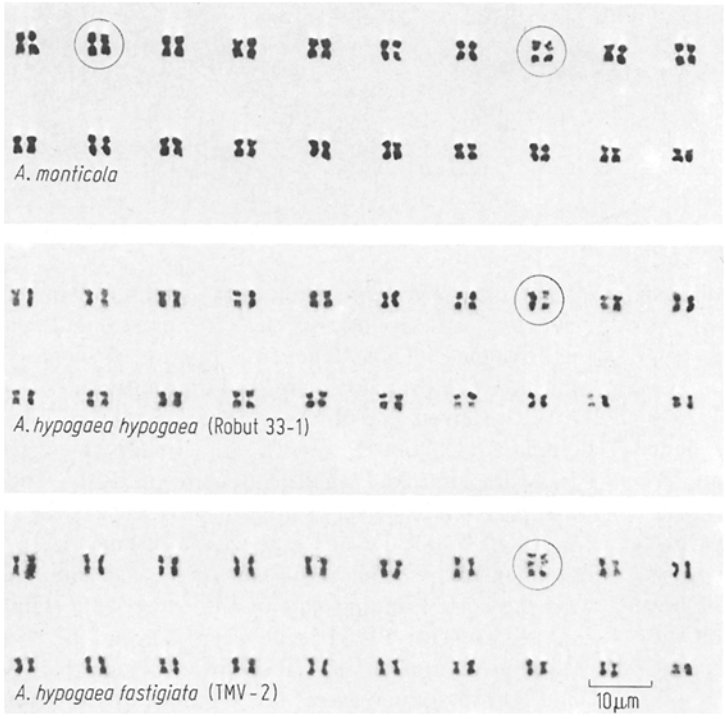

Fig. 6. Karyograms of tetraploid *Arachis* species. Chromosomes with secondary constriction are circled

metacentric and submetacentric chromosomes and mean arm ratios (Tables 3, 6). In *A. monticola* and *A. hypogaea* 6% and 8% of the cells, respectively, show either one quadrivalent or one trivalent.

Discussion

Base Number

Members of the section *Arachis* fall into two groups. One has 40 chromosomes and is represented only by *A. monticola* and the cultivated *A. hypogaea*. The other group has 20 chromosomes and is represented by species listed in Table 1. A survey of cytological studies in the genus *Arachis* shows that taxa with $2n=20$ predominate. Thus the basic chromosome number can be assumed to be 10; $2n=20$ is the commonest number. This is corroborated by regular bivalent formation in 20 chromosome species and the absence of any species with 30 chromosomes. *A. monticola* and *A. hypogaea* are therefore tetraploids which have attained diplontic behaviour, though they sometimes show sec-

Table 7. Chromosomal association and chiasma frequency in species of section *Arachis*

Name of species	Bivalent					Chiasmata					Remarks
	Ring		Rod		Total	No. Chiasma/Cell		No. Term. Chiasma/Cell		Termina-lization coefficient	
	Range	Mean	Range	Mean		Range	Mean	Range	Mean		
A. villosa	8 – 10	9.10	0 – 2	0.60	10	18 – 21	20.43	16 – 20	19.86	0.97	IV in 4% of cells
A. correntina	8 – 10	9.44	0 – 2	0.56	10	18 – 21	20.28	17 – 20	19.44	0.95	
A. chacoense	9 – 10	9.64	0 – 1	0.36	10	19 – 21	20.52	18 – 20	20.04	0.97	
A. species HLK 410	9 – 10	9.66	0 – 1	0.44	10	19 – 21	19.50	19 – 20	19.20	0.98	
A. species 10038 LL	8 – 10	9.60	0 – 2	0.40	10	18 – 20	20.10	18 – 20	19.66	0.97	
A. species 10038 SL	7 – 10	9.40	0 – 3	0.04	10	17 – 21	19.60	15 – 20	19.25	0.97	IV in 5% of cells
A. duranensis	9 – 10	9.76	0 – 1	0.024	10	19 – 23	21.24	19 – 21	20.18	0.95	
A. cardenasii	8 – 10	9.50	0 – 2	0.48	10	18 – 20	19.50	18 – 20	19.40	0.99	
A. batizocoi	8 – 10	9.50	0 – 2	0.50	10	18 – 22	19.83	18 – 20	19.36	0.97	
A. monticola	16 – 20	18.28	0 – 4	1.40	20	35 – 40	38.12	34 – 40	37.68	0.98	III or IV in 6% of cells
A. hypogaea ssp. *hypogaea*	15 – 20	19.18	0 – 4	1.72	20	38 – 40	36.52	38 – 40	36.34	0.99	III or IV in 8% of cells
A. hypogaea ssp. *fastigiata*	18 – 20	19.19	0 – 2	0.81	20	38 – 40	39.18	38 – 40	39.00	0.99	

ondary associations in the form of quadrivalents and trivalents (Table 7).

Diploid Species

Our investigations on diploid species agree in general with earlier reports on the distribution of "A" and "B" chromosomes (Smartt loc. cit.). However, we also observed differences between species in the smallest pair of chromosomes and in the number and type of chromosomes with a secondary constriction. Thus the pair of chromosomes with a secondary constriction was not a feature of *A. batizocoi* alone, as previously reported (Smartt et al. 1978).

On the basis of chromosome length, the range and mean of arm ratios (Table 2), karyotypic formulae (Table 3) and genetic distance calculated by D^2 statistics (Table 4), *A. batizocoi* stands distinct from the rest of the diploid species investigated (Fig. 3).

A. villosa and *A. correntina* are very similar karyomorphologically and there is a very low genetic distance between them (0.05) compared to the average distance among the species of this cluster (0.335). These two species are also very similar morphologically. Stalker and Wynne (1979) observed predominantly bivalent formation at meiosis and good pollen fertility in an *A. villosa* × *A. correntina* hybrid. Thus all the available evidence presently justifies the use of *A. villosa* ssp. *correntina* Burk. rather than treating *A. correntina* as a separate species. The presence of some quadrivalents (Table 7) in meiosis in *A. villosa* is indicative of a probable reciprocal translocation.

We received two forms of *Arachis* species 10038, designated LL and SL by W. C. Gregory to signify types with large and small leaflets, respectively. These were expected to be very similar in karyotype. This was confirmed by the D^2 analysis, with a distance of 0.07, but when each chromosome pair of SL was compared with the corresponding pair of LL, there were significant differences ($P = 0.05$) between LL and SL with respect to chromosome 3 and chromosome 7; all the other chromosomes were not significantly different. These two forms of *Arachis* species 10038 are similar to *A. chacoense* cytologically, but the two taxa are morphologically distinct, and belong to the series *Annuae* and *Perennes* of section *Arachis;* the former was collected in Argentina, the latter in Paraguay.

A. cardenasii differs from the other diploid species in karyomorphology, karyotypic formula and range and means of arm ratios, though the distance by the method of D^2 analysis is not sufficient to justify a third group. Values of D^2 between *A. cardenasii* and other members of the group, from 0.379 to 0.883, are all greater than the average intracluster distance of 0.335, indicating that *A. cardenasii* can be considered as a subgroup within the larger cluster.

The remaining taxa, *Arachis* species HLK 410 and *A. duranensis* which are not very different from each other cytologically, were collected from Parana, Brazil and Salta, Argentina and may be of common origin or represent one large interfertile group though geographically isolated.

The grouping was verified by canonical analysis. When Z_1 and Z_2 values were plotted, all the investigated species fall in one region, indicating that all these species have not diverged greatly (Fig. 4).

Evolution of Diploid Species

Taxa with a symmetrical karyotype (i.e., maximum number of metacentric chromosomes) are thought to be more primitive than those with asymmetrical karyotype (Stebbins 1958). An arm ratio equal or close to 1.0 reflects a primitive nature. On this criterion, *A. villosa* and *A. correntina* have to be considered as the most primitive among the species investigated. The other species are more advanced. *A. cardenasii* and *A. batizocoi,* with mean arm ratios of 1.316 and 1.392 respectively, are the most advanced of the diploid species investigated (Table 3). *A. batizocoi* can also be considered as the most advanced because of the occurrence of a pair of chromosomes with subterminal centromere (Table 2), which might have arisen by deletion, unequal translocation or pericentric inversion.

The changes that have taken place in the evolution of the "A" genome species have been within chromosomes 1 and 3 to 9, and only *A. cardenasii* shows a difference with regard to chromosome 10. The nucleolar organiser chromosome has been involved in these changes in *Arachis* species HLK 410 and *A. cardenasii.*

All the diploid species studied form bivalents at meiosis in almost all cells. They have similar mean chiasma frequencies (19.5–20.52) and similar terminalization coefficients (0.95–0.98) (Table 7). In all interspecific hybrids of these species formation of some bivalents (our unpublished data) indicate that they have originated from a common ancestor, and that the genomes have not diverged sufficiently to prevent recombination between species.

Tetraploid Species

The pair of chromosomes in *A. monticola* with a small satellite is lacking in both the cultivars studied which belong to two botanical subspecies of *A. hypogaea.* Otherwise the two tetraploid species are very similar to each other karyomorphologically and in means and range of arm ratios. There is a very high compatibility between the two species and pollen fertility is high in F_1 hybrids (our unpublished data), thus indicating that they are phylogenetically very close, as also suggested by Smartt (1964).

Meiotic analysis in these taxa showed regular bivalent formation in a majority of the cells, suggesting their allopolyploid nature. The secondary association observed in a few cells, in the form of a trivalent plus a univalent or a quadrivalent (Table 7) may be indicative of their possible segmental allopolyploid nature.

Evolution of the Tetraploid Species

The karyotype differences between the two tetraploid species studied could have arisen at the tetraploid level, or the species could have evolved separately. *A. cardenasii* has been suggested as the donor of the "A" chromosomes, and therefore, of the "A" genome, on morphological and phytogeographical considerations (Smartt et al. 1978). The presence of a previously unnoticed satellite chromosome comparable to that in the tetraploids, and the larger size of chromosome 10 in *A. cardenasii* indicate that this species could be one of the ancestors of the tetraploids.

A. batizocoi is no longer the only known diploid species in the genus *Arachis* having a pair of chromosomes with a secondary constriction, though its karyotype is still markedly different from the other species studied. The other species have previously been recognized by the presence of a pair of short "A" chromosomes, though the shortest chromosome in *A. cardenasii* is longer than that in the rest of this group. Although some of these species can be distinguished karyomorphologically, *A. batizocoi* is the only one with a karyotype distinct enough to be separated as a different cluster on the basis of D^2 statistics and cluster analysis. *A. monticola* and *A. hypogaea* have two pairs and one pair of chromosomes with secondary constriction, respectively. Karyological affinity of chromosomes with secondary constriction between *A. cardenasii* and *A. monticola* and *A. hypogaea* suggest *A. cardenasii* as an ancestor of the tetraploid species.

Acknowledgement

We thank Mr. R. W. Gibbons, Program Leader, Groundnut Improvement Program for his valuable suggestions during the preparation of the manuscript and those who provided the seed of *Arachis* species, especially Prof. W. C. Gregory.

Literature

Babu, C.N. (1955): Cytogenetical investigations on groundnuts. 1: The somatic chromosomes. Indian J. Agric. Sci. **25,** 41–46

D'Cruz, R.; Tankasale, M.P. (1961): A note on the chromosome complement of four groundnut varieties. Indian Oilseeds J. **5,** 58–59

Ghimpu, V. (1930): Recherches cytologiques sur les genres: *Hordeum, Acacia, Medicago, Vitis* et *Quercus.* Arch. d'Anat. Microsc. **26,** 136–234

Gregory, W.C.; Gregory, M.P.; Krapovickas, A.; Smith, B.W.; Yarbrough, J.A. (1973): Structure and genetic resources of peanuts. In: Peanuts-Culture and Uses, pp. 47–133. Stillwater Oklahoma (USA): Oklahoma State University

Gregory, W.C.; Gregory, M.P. (1976): Groundnuts. Evolution of Crop Plants (ed. Simmonds, N.W.), pp. 151–154. London: Longmans

Gregory, W.C.; Krapovickas, A.; Gregory, M.P. (1980): Structure, Variation, Evolution, and Classification in *Arachis.* In: Advances in Legume Science (eds. Summerfield, R.J.; Bunting, A.H.), pp. 469–481. Kew (England): Publ. Royal Botanic Garden

Husted, L. (1933): Cytological studies of the peanut *Arachis* I. Chromosome number and morphology. Cytologia **5,** 109–117

Husted, L. (1936): Cytological studies of the peanut *Arachis* II. Chromosome number, morphology and behaviour and their application to the origin of cultivated forms. Cytologia **7,** 396–423

Kawakami, J. (1930): Chromosome numbers in the Leguminosae. Bot. Mag. **44,** 319–328

Krapovickas, A.; Rigoni, U.A. (1951): Estudios citologicos en el genera *Arachis* Rev. Invest. Agric. (B. A.) **5,** 289–294

Mendes, A.J.T. (1947): Estudios citologicos no genero *Arachis.* Bragantia **7,** 257–267

Murty, B.R.; Arunachalam, V. (1967): Computer programme for some problems in Biometrical Genetics. 1: Use of Mahalonobis D^2 in classifactory problems. Indian J. Genet. **27,** 60–69

Peter, K.V.; Jain, R.C.; Raju, R.P.; Srivastva, U.K. (1977): Statistics and Computer Programme in Biometric Research. G. B. Pant University of Agriculture and Technology: Exp. Stn. Tech. Bull. **104,** 71–75. Pantnagar (India)

Raman, V.S. (1958): Studies in the genus *Arachis* II. Chromosome numbers of certain species of *Arachis.* Indian Oilseeds J. **2,** 72–73

Raman, V.S. (1959): Studies in the genus *Arachis* III. A natural interspecific hybrid. Indian Oilseeds J. **3,** 226–228

Rao, C.R. (1952): Advanced Statistical Methods in Biometric Research. New York: John Wiley and Sons

Singh, A.K.; Moss, J.P.; Sastri, D.C. (1980): Utilization of wild relatives in genetic improvement of *Arachis hypogaea* I. Techniques. In: Proc. Seminar on Current Methodological Approaches in Cytogenetics (eds. Roy, R.P.; Sinha, U.). Delhi: Hindasia Publ. (in press)

Singh, R.K.; Chaudhary, B.O. (1977): Biometrical Methods in Quantitative Genetic Analysis. New Delhi: Kalyani Publ.

Smartt, J. (1964): Cross-compatibility relationships between the cultivated peanut *Arachis hypogaea* L. and other species of the genus *Arachis.* Ph. D. Thesis, N. C. State University, No. 65, 8968. University Microfilms Int., Ann. Arbor. MI

Smartt, J.; Gregory, W.C.; Gregory, M.P. (1978): The genome of *Arachis hypogaea* L. Cytogenetic studies of putative genome donors. Euphytica **27,** 665–675

Stalker, H.T.; Wynne, J.C. (1979): Cytology of interspecific hybrids in section *Arachis* of peanuts. Peanut Sci. **6,** 110–114

Stebbins, G.L. (1958): Longevity, habitat and release of genetic variability in the higher plants. Cold. Spring Harbor Symp. Quant. Biol. **23,** 365–378

Received June 30, 1981
Communicated by J. MacKey

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