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Utilisation of wild relatives in the genetic improvement of *Arachis hypogaea* L.

5. Genome analysis in section *Arachis* and its implications in gene transfer *

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Summary. Cross-compatibility of species in section *Arachis* Krap. et Greg. nom. nud., and chromosome pairing and pollen fertility in their interspecific F_1 hybrids were studied to further understand the phylogenetic relationships among these species. Except those with *A. batizocoi* Krap. et Greg. nom. nud., hybrids between diploid species have near normal bivalent frequency (9.1–9.8) and moderate to high pollen fertility (60–91%). Hybrids between *A. batizocoi* and other species have low bivalent frequency (5.2–6.9) and very low pollen fertility (3–7%). These results confirm the earlier separation of these species into two groups based on karyomorphology and Mahalanobis D^2 calculated on arm ratios. These studies also provide a picture of relative affinities between *A. batizocoi,* the lone member of one cluster, and the other species, and among the rest of the species. They also indicate that the basic chromosome complement in the two groups of species is the same. Chromosome pairing in triploid hybrids, (*A. hypogaea* L. × diploid wild species), suggests that *A. batizocoi* is the closest diploid relative of *A. hypogaea.* It is closer to *A. hypogaea* subspecies *fastigiata* Waldron than to *A. hypogaea* subspecies *hypogaea* Krap. et. Rig. Other diploid species of the section *Arachis* are equidistant from *A. hypogaea,* and have the same genome which has strong homology to one of the genomes of *A. hypogaea.* Based on the present results, the two tetraploid species, *A. monticola* Krap. et Rig. and *A. hypogaea* can be recognised as two forms of the same species. Breeding implications have been discussed in the light of chromosome behaviour observed in hybrids of *A. hypogaea* × diploid species, and on the presumptions that *A. hypogaea* has an AABB genomic constitution, and that among the diploid species, the 'B' genome is present in *A. batizocoi* while the 'A' genome is common to the other diploid species of section *Arachis.*

Key words: *Arachis* – Crossability – Chromosome pairing – Pollen fertility – Genomes – Phylogenetic relationship – Cluster

Introduction

Several workers have reported resistance in many wild *Arachis* L. species to important pathogens and pests of *Arachis hypogaea* L. (Abdou et al. 1974; Subrahmanyam et al. 1980, 1983; Hebert and Stalker 1981). This has given impetus to research on two major aspects – (1) biosystematic analysis to understand phylogenetic relationships between the *Arachis* species and (2) the transfer of useful traits from the wild species into cultivated *A. hypogaea* (Moss 1980).

The genus *Arachis* has been divided into seven sections (Gregory and Gregory 1979). The cultivated species *A. hypogaea* is a tetraploid in section *Arachis* Krap. et. Greg. nom. nud., and the species in this section are cross-compatible and closely related, irrespective of the differences in chromosome number and morphology (Smartt 1964; Gibbons and Turley 1967; Smartt and Gregory 1967; Smartt et al. 1978a; Gregory and Gregory 1979; Singh and Moss 1982). Smartt et al. (1978a) reported that the interspecific hybrids between diploid species with identical karyotypes such as *A. villosa* Benth., *A. correntina* (Burk.) Krap. et Greg. nom. nud. and *duranensis* Krap. et Greg. nom. nud. show moderate to high pollen stainability, and those involving *A. batizocoi* Krap. et Greg. nom. nud. show 0 to a maximum of 2.0% pollen stainability. Singh and Moss (1982) studied the relationships between species of section *Arachis,* including *A. hypogaea,* by the

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analysis of the somatic chromosome complement. Based on karyomorphological affinities and application of Mahalanobis D^2 on arm ratio of chromosome pairs, the diploid species were grouped into two clusters, one comprising only *A. batizocoi* and the other comprising the rest of the species.

The present paper reports cross compatibility between species, and chromosome associations and pollen fertility in F_1 hybrids involving all eight currently available diploid and two tetraploid species of section *Arachis*.

Materials and methods

The identities and sources of the nine wild species used have been listed in a previous communication (Singh and Moss 1982). Among the diploid species are some collections which are yet to be botanically described and named but they have been considered morphologically distinct entities of specific status by Gregory and Krapovickas (personal communication). *A. monticola* Krap. et Rig. used is an accession which does not flower on the main axis. The details of the cultivars used from both subspecies of *A. hypogaea* are given in Table 1.

Table 1. Taxonomic status and sources of cultivars of *A. hypogaea* used in the present study

Name of cultivar	ICG no.[a]	Origin
A. hypogaea L. ssp. *fastigiata* Waldron var. '*fastigiata*' (Valencia)		
1. 'Gangapuri'	2 738	India
A. hypogaea L. ssp. *fastigiata* Waldron var. 'vulgaris' (Spanish)		
2. '99–5'	1 472	Unknown
3. 'Chico'	476	USA
4. 'Tifspan'	3 497	USA
5. '91176'	4 117	India
A. hypogaea L. ssp. *hypogaea* Krap. et Rig. var. 'hypogaea' (Virginia)		
6. 'Robut 33-1'	799	India
7. 'M–13'	156	India
8. 'Makulu Red'	6 391	Zambia

[a] ICRISAT groundnut accession no

All plants were grown and crosses made in a greenhouse at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Center, Patancheru, near Hyderabad, India, during the rainy season of 1979. Some crosses were repeated in subsequent seasons. The diploid species were also crossed with six different cultivars of *A. hypogaea* representing three botanical varieties (Tables 1 and 5). In these crosses *A. hypogaea* was used as a female parent, and no reciprocal crosses were attempted.

For hybridization the flower buds were emasculated between 13.00 and 15.00 h and pollinated the following day between 06.00 and 08.00 h. Relative humidity was maintained at about 60% by running sprinklers for 30 min after emasculation. Only one flower per node was pollinated. Nodes were tagged, and the success of fertilization was checked by recording pegs formed on tagged nodes. For cytological analysis, young flower buds were collected between 06.00–08.00 h and fixed in Carnoy fluid II for 24 h. They were then transferred to Carnoy fluid I containing a drop of 5% ferric chloride solution. The buds were dissected and anthers squashed in 1% acetocarmine. Pollen grains were stained in 1% acetocarmine and stained pollen grains were considered fertile.

Chromosome associations in diploid and triploid hybrids were statistically analysed using one way analysis of variance. Fisher and Behren's *d*-test was used for testing the significance of mean bivalent association between diploid intracluster hybrids of any one species with the hybrids of the remaining species.

Observations

A Diploid species hybrids

Crossability. Of the 56 possible combinations attempted between eight diploid species, 46 combinations produced pods and seeds (Table 2). There were marked differences in reciprocal crossability between pairs of species. *A. chacoense* Krap. et Greg. nom. nud. (PI 276235), as female parent, failed to produce pods with any of the other seven species, and hybrids with *A. cardenasii* Krap. et Greg. nom. nud., as female parent, could not be established, although both were successful as a pollen parent. The crossability between *A. batizocoi* and *A.* species HLK-410 (PI 338280) was

Table 2. Pods per 100 pollinations in crosses between different diploid wild species of section *Arachis*

♀ Parent \ ♂ Parent	*A. villosa*	*A. correntina*	*A. chacoense*	*A.* sp. HLK-410	*A. cardenasii*	*A.* sp. 10038	*A. duranensis*	*A. batizocoi*
A. villosa	–	16.7	15.8	36.7	15.6	0	23.8	15.3
A. correntina	16.2	–	10.5	11.8	7.4	14.9	9.5	14.0
A. chacoense[a]	0	0	–	0	0	0	0	0
A. sp. HLK-410	61.5	37.5	44.5	–	27.1	33.7	35.5	42.3
A. cardenasii	9.3	2.9	9.5	7.5	–	0	1.4	4.8
A. sp. 10038	43.4	55.6	60.4	63.8	17.5	–	53.2	32.8
A. duranensis	58.7	53.7	70.4	71.0	24.7	59.1	–	55.4
A. batizocoi	35.5	30.5	33.5	32.5	32.0	0	30.3	–

[a] Female did not produce peg or pod
0 = not successful

Table 3. Chromosome associations in F_1 hybrids of diploid species of section *Arachis*

Cross	Chromosome associations[f]								Chiasma frequency		Terminalisation of chiasma		Terminalisation coefficient	Pollen stainability %	D^2 distance
	I		II		III		IV								
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean			
Intracluster															
A. correntina × *A. villosa*[a]	0– 2	0.4	9–10	9.8	0	0	0	0	17–21	19.4	17–20	19.2	0.99	83	0.05
A. correntina × *A. chacoense*[a]	0– 4	0.8	8–10	9.6	0	0	0	0	16–20	18.5	16–20	18.5	1.00	75	0.27
A. correntina × *A.* sp. HLK-410[a]	0– 2	0.4	9–10	9.8	0	0	0	0	16–20	18.8	16–20	18.4	0.98	71	0.37
A. correntina × *A. cardenasii*[a]	0– 4	0.7	8–10	9.6	0	0	0–1	0.1	14–20	18.0	14–20	17.9	0.99	77	0.55
A. correntina × *A.* sp. 10038[b]	–	–	–	–	–	–	–	–	–	–	–	–	–	70	0.39
A. correntina × *A. duranensis*[b]	–	–	–	–	–	–	–	–	–	–	–	–	–	73	0.60
A. villosa × *A.* sp. 10038	0– 2	0.3	8–10	9.7	0	0	0–1	0.1	16–20	19.0	16–20	18.7	0.99	71	0.29
A. villosa × *A. duranensis*	0– 2	0.6	8–10	9.5	0	0	0–1	0.1	16–19	17.4	16–20	18.7	0.99	75	0.47
A. villosa × *A. chacoense*	0– 8	1.0	6–10	9.2	0–1	0.08	0–1	0.1	15–20	17.7	17–20	17.4	0.98	71	0.22
A. villosa × *A.* sp. HLK-410[b]	–	–	–	–	–	–	–	–	–	–	–	–	–	71	0.28
A. sp. HLK-410 × *A.*sp. 10038	0– 2	0.1	8–10	9.7	0	0	0–1	0.1	16–20	19.0	16–20	18.9	0.99	80	0.18
A. sp. HLK-410 × *A. chacoense*	0– 4	1.3	8–10	9.4	0	0	0	0	15–20	18.4	15–20	18.4	1.00	91	0.26
A. duranensis × *A. chacoense*	0– 2	0.3	8–10	9.5	0–1	0.03	0–1	0.2	18–22	19.5	17–20	18.8	0.97	60	0.35
A sp. 10038 × *A. chacoense*	0– 2	0.7	8–10	9.4	0–1	0.04	0–1	0.1	15–20	18.8	15–20	18.6	0.99	67	0.07
A. sp. 10038 × *A. duranensis*	0	0	8–10	9.8	0	0	0–1	0.2	17–22	19.6	17–20	19.0	0.96	74	0.07
A. duranensis × *A.* sp. HLK-410	0– 4	0.5	8–10	9.1	0–1	0.04	0–1	0.2	16–20	19.0	16–20	18.4	0.97	70	0.34
A. sp. HLK-410 × *A. cardenasii*[c]	0– 4	0.8	7–10	9.4	0–1	0.03	0–1	0.1	18–22	17.8	14–20	17.7	0.99	70	0.44
A. villosa × *A. cardenasii*[c]	0– 4	1.0	8–10	9.4	0	0	0–1	0.1	14–20	17.6	14–20	17.6	1.00	75	0.59
A. sp. 10038 × *A. cardenasii*[c]	0– 6	1.1	7–10	9.3	0–1	0.1	0	0	15–20	17.9	15–20	16.8	0.94	79	0.57
A. duranensis × *A. cardenasii*[c]	0– 2	0.6	8–10	9.1	0–1	0.1	0–1	0.3	15–21	19.4	14–20	19.3	0.99	81	0.88
Intercluster															
A. batizocoi × *A.* sp. HLK-410[d]															0.80
A. batizocoi × *A. cardenasii*[e]	4– 8	6.0	6– 8	6.9	0–1	0.1	0	0	7–12	10.7	7–12	10.7	1.00	7	0.84
A. batizocoi × *A. chacoense*[e]	4–16	8.2	2– 8	5.2	0–1	0.1	0–1	0.3	4–10	7.6	4–10	7.6	1.00	4	0.85
A. sp. 10038 × *A. batizocoi*[e]	2–10	5.7	4– 9	6.8	0–2	0.2	0	0	9–13	10.7	9–13	10.7	1.00	6	0.94
A. batizocoi × *A. duranensis*[e]	6–12	9.5	4– 7	5.2	0–1	0.04	0	0	2–10	6.0	9–13	6.0	1.00	4	1.14
A. batizocoi × *A. correntina*[e]	4–14	8.9	3– 8	5.4	0–2	0.1	0	0	4–14	9.8	4–12	8.5	0.94	3	1.24
A. batizocoi × *A. villosa*[e]	4–12	7.7	3– 8	5.5	0–2	0.4	0	0	5–12	9.3	5–10	8.3	0.90	4	1.23

[a] Mean bivalent association significantly higher than the rest of intracluster hybrids
[b] Hybrids not analysed
[c] Mean bivalent association significantly lower than the rest of intracluster hybrids
[d] Hybrids died as seedlings
[e] Mean association significantly different from the rest
[f] No. of cells analysed per combination ranged from 20–32

Table 4. Chromosome associations in F_1 reciprocals between diploid species of section *Arachis*

Cross	Chromosome associations								Chiasma frequency		Chiasma terminalisation		Terminalisation coefficient	Pollen stainability %
	I		II		III		IV							
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean		
A. villosa × *A. correntina*	0– 2	0.4	8–10	9.7	0	0	0–1	0.4	17–20	18.3	17–20	18.0	0.99	85
Reciprocal	0– 2	0.4	9–10	9.8	0	0	0	0	17–21	19.4	17–20	19.2	0.99	83
A. villosa × *A. duranensis*	0– 2	0.6	8–10	9.5	0	0	0–1	0.08	16–19	17.4	16–19	17.4	1.00	75
Reciprocal	0– 4	0.7	8–10	9.6	0	0	0	0	16–20	18.8	16–20	18.6	0.99	81
A. correntina × *A.* sp. HLK-410	0– 2	0.4	9–10	9.8	0	0	0	0	16–20	18.8	16–20	18.4	0.98	74
Reciprocal	0– 2	0.2	8–10	9.7	0–1	0.04	0–1	0.08	18–20	19.6	16–20	19.0	0.96	71
A. sp. HLK-410 × *A.* sp. 10038	0– 2	0.1	8–10	9.7	0	0	0–1	0.1	16–20	19.0	16–20	18.9	0.99	80
Reciprocal	0– 2	0.5	6–10	9.2	0–1	0.2	0–1	0.2	17–20	18.6	16–20	18.2	0.97	68
A. batizocoi × *A. villosa*	4–12	7.7	3– 8	5.5	0–2	0.4	0	0	5–12	9.3	5–10	8.3	0.90	4
Reciprocal	4–14	9.8	3– 8	5.1	0	0	0	0	5–12	9.3	5–10	9.3	0.99	4

moderately good (33.5 and 42.3 pods per 100 pollinations), but the hybrids obtained in either direction died as seedlings. Our attempts to sustain these hybrids through different chemical treatments were not successful.

Cytology. The chromosome associations in the diploid species hybrids, except those involving *A. batizocoi,* were near normal (Fig. 1). The mean bivalent associations in these hybrids ranged from 9.1 to 9.8 (Table 3). It was higher in the hybrids of *A. correntina* (9.7) and lower in the hybrids of *A. cardenasii* (9.3) at 5% level of significance than in the hybrids of other species. The number of univalents in these hybrids ranged from 0 to a maximum of eight per pollen mother cell (PMC) with means from 0.1 to 1.3. However, univalent means were not significantly different (Table 3). The chromosome association in hybrids involving *A. batizocoi* was highly irregular (Figs. 2 and 3) although a few PMCs even had nine bivalents (Fig. 4). There were no PMCs without univalents, and means ranged from 5.7 to 9.5. The mean bivalent associations in *A. batizocoi* hybrids ranged from 5.2 to 6.9 and were lower at 1% level of significance than in intracluster hybrids (Table 3). The chromosome associations in some randomly selected reciprocals of these hybrids were analysed and were not significantly different (Table 4). Pollen fertility in the hybrids from the species of the same cluster ranged from 60 to 91%, but it was very low (3% to 7%) in hybrids involving *A. batizocoi* (Table 3).

B Hybrids between diploid and tetraploid species

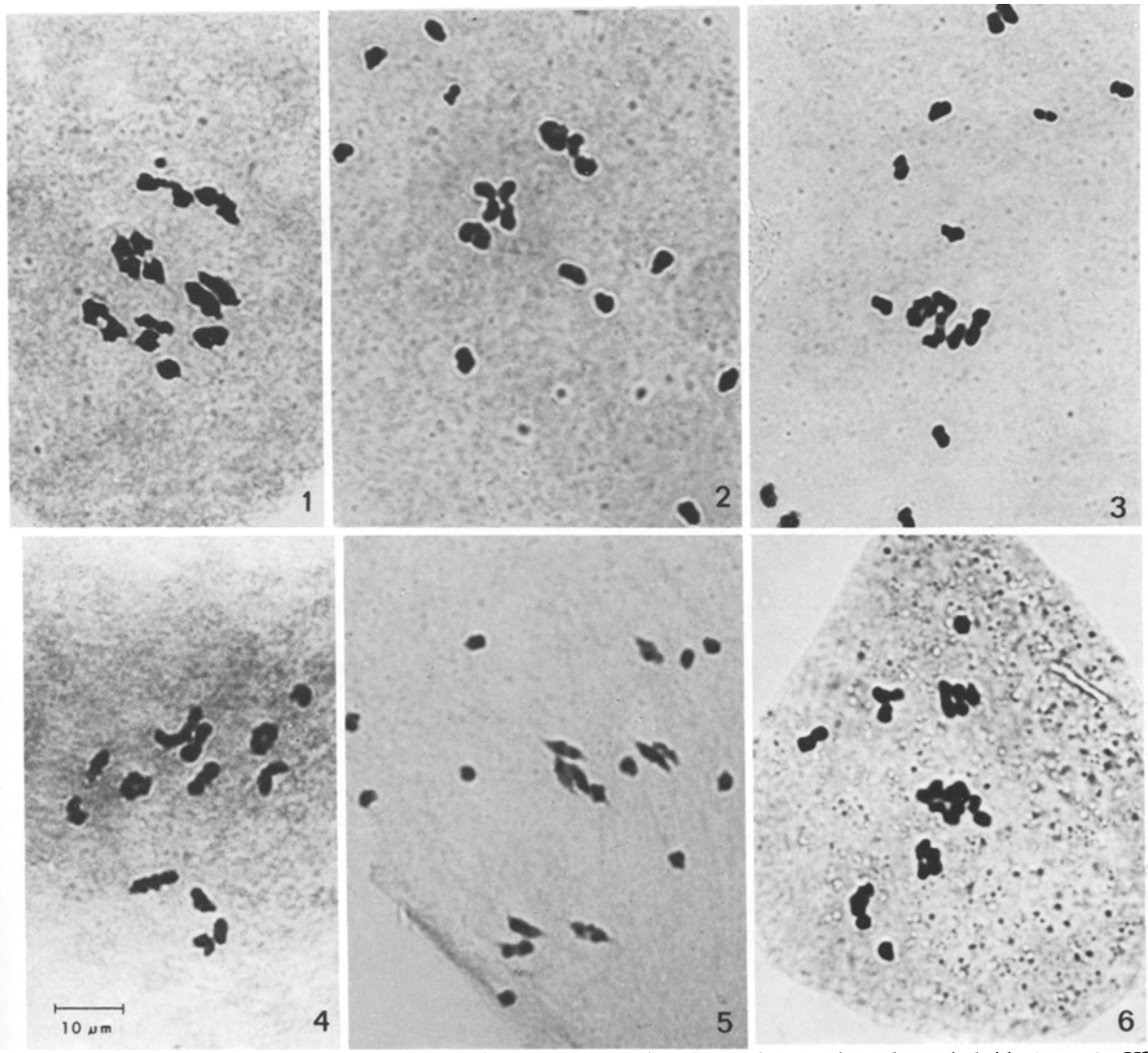
Crossability. Hybrids were obtained from crosses between the eight wild diploid species and one or more cultivars belonging to three botanical varieties of two subspecies of *A. hypogaea* (Table 5). Only one 'Valencia' cultivar was used, and hybridization of this with *A. correntina* and *A. cardenasii* failed. The crosses between both cultivars of the *A. hypogaea* subspecies *hypogaea* and *A. batizocoi* produced mostly immature pods.

Morphology. All established triploid hybrids were vigorous. However, in the case of *A. hypogaea* subspecies *hypogaea* × *A. batizocoi* seedling survival was poor. They had intermediate leaflet size. The yellow flowers of *A. batizocoi, A.* species HLK-410 and *A.* species GKP 10038 (PI 263133) and the perennial habit of species, such as *A. villosa* or *A. chacoense,* were observed in all of their F_1 hybrid progenies.

Cytology. A minimum of one hybrid plant from each of the eight diploid species crossed with the same cultivar of *A. hypogaea* subspecies *hypogaea* ('Robut 33-1') as

Table 5. Pods per 100 pollinations between two subspecies of *A. hypogaea* and wild diploid species

♀ Parent \ ♂ Parent	*A. villosa*	*A. correntina*	*A. chacoense*	*A.* sp. HLK-410	*A. cardenasii*	*A.* sp. 10038	*A. duranensis*	*A. batizocoi*
A. hypogaea hypogaea (Virginia)								
ICG 799	21	27	0	32	0	16	24	15
ICG 6391	0	0	20	14	14	10	21	10
A. hypogaea fastigiata (Spanish)								
ICG 1472	12	11	22	17	5	11	5	13
ICG 3497	15	5	0	0	18	7	12	21
ICG 476	20	17	19	20	23	3	9	0
A. hypogaea fastigiata (Valencia)								
ICG 2738	25	0	8	6	0	20	12	20



Figs. 1–6. Pollen mother cells at metaphase I in interspecific hybrids: **1** 9II + 2I in an intracluster hybrid, *A. species* HLK-410 × *A. chacoense*; **2** 5II + 10I, and **3** 3II + 1III + 1II in an intercluster hybrid, *A. batizocoi* × *A. correntina*; **4** 9II + 2I in an intercluster hybrid, *A. batizocoi* × *A. villosa*; **5** 10II + 10I in a triploid hybrid, *A. hypogaea hypogaea* (4x) × *A. chacoense*; **6** 12II + 1III + 3I in a triploid hybrid *A. hypogaea fastigiata* × *A. batizocoi*

Table 6. Chromosome associations in F_1 hybrids (3x) of *A. hypogaea* (4x) × wild species (2x) of section *Arachis*

Cross	Chromosome associations[b]										Pollen stainability
	I		II		III		IV		V		
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	%
A. hypogaea hypogaea (Robut 33-1)											
A. hypogaea × *A. villosa*	5–14	9.1	5–12	8.6	0–4	1.0	0–2	0.2			19
A. hypogaea × *A. correntina*	6–14	8.3	7–12	9.9	0–2	0.6	0–1	0.1			20
A. hypogaea × *A. chacoense*	7–18	9.7	5–12	8.7	0–3	0.8	0–1	0.1			17
A. hypogaea × *A.* sp. HLK-410	5–14	9.2	7–12	9.6	0–2	0.5	0–1	0.3			13
A. hypogaea × *A. cardenasii*	3–14	8.3	7–12	9.7	0–3	0.5	0–1	0.2			9
A. hypogaea × *A.* sp. 10038	7–14	10.0	6–11	8.0	0–3	1.2	0–1	0.1			11
A. hypogaea × *A. duranensis*	5–12	8.3	8–12	9.4	0–2	1.0	0–1	0.1			18
A. hypogaea × *A. batizocoi*	2– 9	6.2[a]	4–12	8.7	0–5	2.0	0–1	0.1			7
A. hypogaea fastigiata (Tifspan) × *A. batizocoi*	1– 8	4.0[a]	6–12	9.0	0–4	2.1[a]	0–1	0.3	0–1	0.04	10

[a] Mean associations were significantly different from rest
[b] No. of cells analysed per combination ranged from 20–40

Table 7. Crossability and pollen stainability percent of *A. hypogaea* × *A. monticola*

Cross	Crossability (% pods/poll)	Pollen stainability %
A. hypogaea hypogaea (Virginia)		
ICG 156 × *A. monticola*	28	90
Reciprocal	58	90
ICG 6391 × *A. monticola*	27	84
A. hypogaea fastigiata (Spanish)		
ICG 1472 × *A. monticola*	13	90
ICG 4118 × *A. monticola*	17	–

female parent, was analysed cytologically. A hybrid between *A. hypogaea* subspecies *fastigiata* × *A. batizocoi* was also included in the analysis since a differential crossability reaction was observed between the two subspecies of *A. hypogaea* and *A. batizocoi.* All triploid hybrids formed around 10 bivalents and 10 univalents (Fig. 5) except those involving *A. batizocoi,* which showed a lower frequency of univalents at the 5% level of significance, and hybrids of *A. hypogaea* subspecies *fastigiata* × *A. batizocoi,* which showed a higher frequency of trivalents at the 5% level of significance (Fig. 6; Table 6). However, the number of chromosomes associating as bivalents, did not differ significantly between different combinations (Table 6).

Chromosomal irregularities in the form of laggards, bridges, spindle abnormalities and unequal distribution were observed at Anaphase I (A I). At Anaphase II (A II) there were few laggards and no bridges though there were more spindle abnormalities. Pollen stainability ranged from 7% in *A. hypogaea* subspecies *hypogaea* × *A. batizocoi* to 20% in *A. hypogaea* subspecies *hypogaea* × *A. correntina* (Table 6). Stainable pollen grains in these triploids showed much variation in size, except in *A. batizocoi* triploids, where most of them were of uniform size. Despite the low pollen fertility in some of the triploid hybrid plants of *A. hypogaea* subspecies *fastigiata* × *A. batizocoi* a few early flowers produced pegs, while the triploid hybrids of other species produced pegs only a maturity. The pod fertility was very low; a maximum of 30 pods were harvested from a single large old plant of *A. hypogaea* × *A. cardenasii.*

C Tetraploid species

A. monticola crossed more readily with *A. hypogaea* subspecies *hypogaea* than with other botanical varieties. The crossability was higher (58%) in the only reciprocal cross attempted using *A. monticola* as a female parent (Table 7). The chromosome association in the F_1 hybrids was normal in 72% of PMCs at MI culminating into regular distribution of chromosomes in 92% PMCs at AI. The pollen stainability ranged from 84–90% in these hybrids (Table 7).

Discussion

Diploid species

Although several reports are available on hybridization between diploid wild species of the section *Arachis,* they are fragmentary and do not present comprehensive or comparable information on crossability between these species (Raman and Kesavan 1962; Gibbons and Turley 1967; Gregory and Gregory 1979; Stalker and Wynne 1979). The present studies

have provided a comprehensive and comparable picture of crossability under uniform conditions in section *Arachis* and have indicated that crossability could be as low as 17% between the two species *A. villosa* and *A. correntina* which are considered closely related by Burkart (1939) and Singh and Moss (1982), and could be as high as 55% between the distantly related species such as *A. batizocoi* and *A. duranensis* (Singh and Moss 1982). Although crossability has been useful at generic level in grouping the species into seven sections (Gregory and Gregory 1979), it has not corroborated the earlier grouping of diploid species in section *Arachis* and is not useful for tracing intrasectional affinities between species. Crossability between *A. batizocoi* and the other species was near to the mean of intracluster crossability of the respective species as female parent. This suggests that *A. batizocoi,* although genetically distant, is not different from the rest of the species with respect to crossability, and that its geographical isolation to western Brazil along with two other annual species, *A. duranensis* and *A.* species GKP 10038, has not affected its crossing ability with other species of the section. The annual species, including *A.* species HLK 410, were the best female parents (Table 2). The differences in female cross-compatibility are often associated with habit and are explained by faster evolutionary divergence of the annuals as compared to the perennial species (Stebbins 1971). However, the higher female cross-compatibility of annual species in the present investigation may be due to their recent origin. It may also be added that failure of *A. chacoense* to cross with any of other diploid species as a female parent, but its success as a male parent indicates a possible cytoplasmic effect on the crossability of this species. Cytoplasmic influence is also indicated in crosses between *A.* species GKP 10038 and some other species (Table 2).

Karyomorphological similarities and Mahalanobis D^2 calculated on arm ratio of mitotic chromosomes were earlier used for tracing phylogenetic relationships between the species (Singh and Moss 1982). In the present investigation chromosome pairing in F_1 hybrids (which predominantly manifest homology between chromosomes of the two species) was used for tracing affinities and for grouping. This would ascertain whether karyomorphologically identical chromosomes are also genetically identical or not and how they have differentiated. A reduction in bivalent frequency and increase in univalents in a hybrid indicates reduced homology between parental chromosomes, whereas formation of multivalents and a decrease in bivalents indicates repatterning of genetic material and no reduction in homology. However, both affect normal bivalent associations although they have different phylogenetic implications. A high frequency of bivalents in intracluster hybrids (9.1–9.8), such as *A. duranensis* × *A. cardenasii* and *A. correntina* × *A. villosa* suggests strong genomic similarities between these species, whereas the low frequency of bivalents (5.2–6.9) and high frequency of univalents (5.7–9.5) in the hybrids involving *A. batizocoi* suggests the distinct nature of the *A. batizocoi* genome from the other diploid species (Figs. 1–3; Table 3). The pollen fertility in the hybrids, except those of *A. batizocoi,* was moderate to high (60–91%), whereas the hybrids between *A. batizocoi* and the rest of the species showed low pollen fertility (3–7%) (Table 3). These results further strengthen the earlier conclusion of Singh and Moss (1982) on using the D^2 distance for the division of section *Arachis* diploid species into two clusters.

On relative affinities between *A. batizocoi* and species from the other cluster, chromosome associations between *A. batizocoi* and *A.* species HLK-410, the closest intercluster species based on D^2 distance, could not be assessed because of the breakdown of the hybrid seedlings before they flowered. However, the hybrid of *A. batizocoi* with *A. cardenasii,* the next closest species on D^2 distance, had the second lowest univalent frequency (6.0), and highest mean bivalent association (6.9), chiasmata frequency and pollen fertility, strengthening the previous conclusions (Singh and Moss 1982). Interestingly, all three species, *A. batizocoi, A.* species HLK-410, and *A. cardenasii,* have an identical pair of chromosomes with a secondary constriction proximal to the centromere.

Based on the bivalent association (6.8) and the univalent frequency (5.7), the next closest species to *A. batizocoi* is *A.* species GKP 10038. However, it did not cross with *A. batizocoi* as a male parent. Probably these two species have differentiated more through structural changes that have changed the morphology of chromosomes in relation to centromere position, therefore increaing D^2 distance based on arm ratio. But this differentiation is without genetic alteration to decrease chromosome pairing and chiasma frequency per chromosome to the same degree. These changes are probably also associated with cytoplasmic alteration which restricts this combination to one way crossing even though there is comparatively high genomic similarity.

A. duranensis can be considered the next closest to *A. batizocoi* by virtue of an identical pair of chromosomes with secondary constriction and sympatric distribution. However, it is not corroborated by chromosome associations and pollen fertility, which was comparatively lower in their hybrids (Table 3).

Some PMCs in *A. batizocoi* hybrids have six, eight or nine bivalents out of a possible ten (Fig. 4), suggesting that the basic complement in *A. batizocoi* and in other species is the same, but it has differentiated genetically and karyomorphologically mainly through chromosomal changes affecting homology of respective pair of chromosomes, although some PMCs have multivalents.

In the rest of the species hybrids with near normal pairing, when the mean number of bivalents for hybrids involving one species was compared with the mean for all other intracluster hybrids by Fisher and Behren's *d*-test, the hybrids involving *A. cardenasii* had a significantly lower number of bivalents (mean 9.3) than the hybrids of the other species (mean 9.6). This is also supported by low chiasma frequency and a higher terminalisation coefficient in most of its hybrids (Table 3), except *A. duranensis* × *A. cardenasii,* which had higher chiasma frequency, and *A.* species GKP 10038 × *A. cardenasii,* which had lower terminalisation. Thus, these observations are in agreement with earlier observations for a subgroup status of *A. cardenasii* within larger cluster based on D^2 distance (Singh and Moss 1982).

On the other hand, the hybrids between *A. correntina* and other species showed a significantly higher bivalent association (9.7), compared to other species hybrids (9.4), indicating that this species has closer affinities with the rest of the species of the cluster, including *A. cardenasii.* The mean bivalent associations in the hybrids of the rest of the species were not significantly different suggesting that they form a group of very closely related taxa. The higher mean bivalent associations (9.8) and pollen fertility (83%) in the *A. correntina* × *A. villosa* hybrids and identical cytological behaviour of their hybrids with other species suggest a close relationship between *A. correntina* and *A. villosa,* supporting the earlier conclusions on varietal status of these two species (Burkart 1939; Singh and Moss 1982). Similarly, the absence of univalents, the higher mean bivalent association (9.8) and pollen fertility (74%) in hybrids between *A. duranensis* and *A.* species GKP 10038 indicate that they can also be considered as the forms of the same species. This was also evident from their similar morphology, annual habit, sympatric distribution and

D^2 distance. Observation of multivalents in the hybrids of these two species suggests that they have probably differentiated from each other by a reciprocal translocation. Multivalents were also observed in other species hybrids, the higher frequency of quadrivalents in *A. duranensis* hybrids indicates that *A. duranensis* may have differentiated from all the species in the cluster by reciprocal translocations. The mean frequencies in other species hybrids were low and did not indicate a consistent pattern.

Thus the present observations on chromosome association and pollen fertility in conjunction with morphological evidence confirm the earlier grouping of the diploid species of section *Arachis* into two clusters (Singh and Moss 1982). The pairing behaviour and pollen fertility did not differ greatly between the intracluster hybrids but bivalent frequency was significantly lower in the *A. cardenasii* hybrids compared to other species hybrids, suggesting a slightly distant nature of *A. cardenasii* from the rest of the species of the larger cluster. Further, these results indicate that *A. correntina* is the closest to *A. villosa,* confirming the earlier conclusion of Burkart (1937) and Singh and Moss (1982), and that *A. duranensis* is the closest to *A.* species 10038.

Diploid and tetraploid species

Crossability did not indicate any specific relationship between diploid species and any of the subspecies or the botanical varieties of *A. hypogaea,* and crossability probably depends more on the cultivar and species than on subspecies or botanical variety. However, the cross *A. hypogaea* subspecies *hypogaea* × *A. batizocoi* produced mostly immature pods and had a lower seedling survival rate, indicating that *A. batizocoi* is not as closely related to *A. hypogaea* subspecies *hypogaea* as it is to *A. hypogaea* subspecies *fastigiata.*

Chromosome pairing in F1 hybrids between *A. hypogaea* subspecies *hypogaea* and all the diploid species did not differ significantly in relation to bivalent associations, but significantly lower univalent frequencies in the hybrids between two subspecies of *A. hypogaea* and *A. batizocoi,* and a significantly higher trivalent frequency in the hybrid of *A. hypogaea* subspecies *fastigiata* × *A. batizocoi* (Table 6; Fig. 6) suggest that, in phylogenetic terms, *A. batizocoi* is the closest diploid relative in section *Arachis* of *A. hypogaea* and that the *A. batizocoi* genome has greater homology with that of *A. hypogaea* subspecies *fastigiata* than with that of *A. hypogaea* subspecies *hypogaea.* Morphologically too, *A. batizocoi* with its large light green leaves, a limited number of n + 1 branches, and sequential distribution of reproductive branches, is closer to *A. hypogaea* subspecies *fastigiata* than to *A. hypogaea* subspecies *hypogaea.*

In the F1 hybrids between *A. hypogaea* and the rest of the diploid species of section *Arachis,* the chromosomes of diploid species paired with one set of chromosomes of *A. hypogaea* to result in mean bivalent frequencies ranging from 8.0 per PMC in *A. hypogaea* × *A.* species GKP 10038 to 9.9 in *A. hypogaea* × *A. correntina* (Table 5). The other set of *A. hypogaea* chromosome remained unpaired as univalents, or occasionally allosyndetic pairing between genomes leads to the formation of more than ten bivalents, or both autosyndetic and allosyndetic pairing between the chromosomes of the diploid species and *A. hypogaea* lead to the formation of multivalents. The pairing behaviour observed in autotetraploids and amphiploids of these species crossed with *A. hypogaea* confirms these conclusions (unpublished). Chromosome associations in these triploid hybrids were not significantly different enough to indicate any specific relationship between *A. hypogaea* and these species from the larger cluster, and thus they appeared equidistant from *A. hypogaea.* The frequency of chromosome associations in triploid hybrids and amphiploids from the tetraploid to hexaploid (Spielman et al. 1979; Singh and Moss, unpublished) does not indicate either the presence of or the supression of genes controlling pairing. Thus the level of chromosome associations has been considered as an indication of genomic affinities.

Spindle abnormalities observed in triploid hybrids at AI and AII resulted in the formation of restitution nuclei and unreduced gametes; the irregular segregation of chromosomes resulted in the formation of haploid, diploid and hyperdiploid gametes, which were seen as different sized stainable pollen grains. Thus, pollen fertility (stainability) in these triploid hybrids cannot be used as a measure of species relationships, as meiosis is irregular and produces stainable unreduced and haploid to hyperdiploid pollen.

A collective evaluation of data on geographical distribution, morphology, karyomorphology, crossability and chromosome associations in triploid hybrids between *A. hypogaea* and diploid species suggests that *A. batizocoi* is the closest diploid relative of *A. hypogaea* contributing the 'B' genome, while the rest of the diploid species are the close relatives of the 'A' genome contributor to *A. hypogaea*, confirming the amphiploid origin of *A. hypogaea* (Husted 1936; Gregory et al. 1980) like many other tetraploid crop species such as, cotton (Phillips 1976) tobacco (Grestel 1976) and wheat (Mac Key 1975). The morphological similarities, differential success in establishing hybrids between two subspecies of *A. hypogaea* and *A. batizocoi,* the cytological behaviour of their F_1 hybrids and our results on crossing between *A. hypogaea* and amphiploids of *A. batizocoi* (unpublished) indicate that *A. batizocoi* is closest to *A. hypogaea* subspecies *fastigiata* and a probable biphyletic origin for the two subspecies of *A. hypogaea* is more likely than monophyletic. These observations indicate that the 'B' genome of *A. bati-*

zocoi may be a pivotal genome like the "A" genome in tetraploid wheat (Mac Key 1975). The genome is common to the two subspecies of *A. hypogaea* but the cytoplasm may not be, or unlike wheat the other genomes involved are very closely related. The close affinities of other genomes results in fully fertile hybrids between the two subspecies of *A. hypogaea.*

The genus *Arachis* is divided into seven sections, most of the species are diploid (2n = 20), except *A. hypogaea,* its wild form *A. monticola* and *Eurhizomatosae* species. Interestingly the compatibility relationship between tetraploid rhizomatous species and annual species of section *Arachis,* including *A. batizocoi,* and diploid species of *Erectoides* led Gregory and Gregory (1979) to infer another amphiploidization for *Eurhizomatosae* species, involving either *A. batizocoi* or a closely related ancestor with a similar genome. Thus *A. batizocoi* is possibily involved in tetraploidy in the genus, and may have a critical role to play in interspecific gene transfer.

Tetraploid species

Present studies on two tetraploid species, *A. hypogaea* and *A. monticola,* besides confirming the high crossability between the two (Smartt 1964; Singh et al. 1980; Palaniswamy and Raman 1980), further revealed that the accession of *A. monticola* used in the present studies had higher crossability when it was the female parent, and also its crossability was better with *A. hypogaea* subspecies *hypogaea* than with *A. hypogaea* subspecies *fastigiata* var. 'vulgaris'. These results strengthen the suggestion of Krapovickas and Rigoni (1957) that *A. monticola* is a virginia type. The hybrids between the two tetraploid species were vigorous with a dominant runner habit. They showed regular chromosome associations (in 72% of PMCs) and a high pollen fertility (Table 7) suggesting a very close relationship between the two species, to an extent that *A. monticola* can be considered as another subspecies of *A. hypogaea,* but with wild habit, confirming earlier conclusions of Smartt (1964) and Singh and Moss (1982).

Implications for gene transfer

A. hypogaea has been considered to be of amphidiploid origin, with AABB genomic constitution. The morphological similarity between the two (known) marker chromosomes of *A. hypogaea* and those in diploid species led to the attribution of 'B' genome to *A. batizocoi* which contained one marker chromosome, and 'A' genome to an unidentified species of section *Arachis* containing the other marker chromosome (Gregory et al. 1980). The reports of Smartt et al. (1978 a) and Stalker and Wynne (1979) on pollen fertility of diploid species hybrids of section *Arachis* confirmed the existence of two distinct genomes in section *Arachis.* This concept of distinct genomes led Smartt et al. (1978 b) to predict difficulties in incorporating genes from these wild species into *A. hypogaea,* specifically in the light of strong distinction between the two genomes present in wild species and *A. hypogaea.*

However, the present results on chromosome pairing provide evidence that the genomes are not completely unrelated, despite the pollen sterility recorded earlier in the hybrids that involved species with different genomes. The formation of six, eight or nine bivalents in hybrids between *A. batizocoi* (with 'B' genome) and other diploid species (with 'A' genome) and formation of 10–12 bivalents and 1–4 multivalents in triploid hybrids between *A. hypogaea* (with 'AB' genome) and any of the diploid species indicates that intergenomic pairing does occur between the two genomes existing in diploid wild species and those in *A. hypogaea* (Tables 3 and 5). Thus recombination can occur when different genomes from species are brought together afresh by interspecific breeding. Although diploid 'A' genome × 'B' genome hybrids are completely sterile, many triploid and tetraploid hybrids with the AB genome have formed viable seeds.

Thus the observed chromosome behaviour revealed that the degree of intergenomic and intragenomic pairing in interspecific hybrids is adequate to incorporate desired traits from these wild species into the genomes of *A. hypogaea* through meiotic recombination.

The different pathways for gene transfer from wild into cultivated species offer different possibilities for intergenomic pairing. The triploid route, via fertile hexaploids produced by colchicine treatment, should produce satisfactory genetic recombination and desirable segregants through allosyndetic pairing between the two genomes of *A. hypogaea* and that of wild species as was observed by Spielman et al. (1979) in hexaploids. In triploid hybrids 23–29 chromosomes participated in pairing, of which at least 3–6 represent A–B intergenomic pairing in the configuration of trivalents and quadrivalents. When fertile amphiploids, (involving diploid species with A and B genomes) were crossed with *A. hypogaea* intergenomic pairing was also observed in the hybrids (Singh and Moss, unpublished). Triploids are also of value, as they can produce fertile tetraploid progeny (ICRISAT 1982) without induced chromosome doubling as a result of meiotic abnormalities after recombination at metaphase I. However, there can be another method for induction of greater recombination and fertility in the first generation hybrids i.e. crossing of fertile amphiploids of diploid A × B hybrids with *A. hypogaea.* This method has been effective and many hybrids have been produced. The frequency of bivalents and trivalents in these hybrids indicates that besides A–A and B–B intragenomic pairing (upto a maximum of ten bivalents) A–B intergenomic pairing also occurs in bi-

valents in excess of ten, and in trivalents and quadrivalents.

The degree of intergenomic pairing (A–B, A–B–B) observed in tetraploid hybrids between *A. hypogaea* and *A. batizocoi* (4x) (unpublished) and triploid hybrids between *A. hypogaea* and *A. batizocoi* (Table 5) indicates the success of the auto tetraploid route in genetic transfer from this species into *A. hypogaea.* The fertility of the hybrid derivatives between *A. hypogaea* × *A. batizocoi* (4x) and rapid production of stable tetraploids in subsequent generations suggest that intergenomic pairing can produce viable gene combinations, and that the genomes involved are closely related.

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