

## Meiotic Behaviour in Wild Diploid *Arachis* (Leguminosae) Species

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**Summary** Meiotic studies of *A. chiquitana*, *A. matiensis*, *A. subcoriacea* (Sect. *Procumbentes*), *A. triseminata* (Sect. *Triseminatae*), *A. benensis*, *A. diogoi*, *A. herzogii* and *A. kempff-mercadoi* (Sect. *Arachis*) are reported for the first time. Meiotic behaviour was essentially regular in most of the species, with 10 II at diakinesis-metaphase I, except in *A. benensis* which presented 8 II+1 IV in 2% of the pollen mother cells. Most of the bivalents were of the ring type, and only one or two bivalents were usually of the rod type. Chiasmata were always located in distal to terminal position even at diplotene and diakinesis. Normal segregation of bivalents is in accordance with the recorded high meiotic indexes and pollen stainability. Only one pair of chromosomes was easily recognized, that being the very small “A” pair in the species with A genome of *Arachis* section. This small pair can be used at meiosis to distinguish between the A and B genomes species and may be a useful marker to detect allopolyploids involving both genomes.

**Key words** *Arachis*, Meiotic behaviour, Wild diploid.

*Arachis* is an indigenous genus of South America, and consists of about 80 species. Krapovickas and Gregory (1994) have recognized 9 sections based on morphological characters, cross compatibility and fertility of the hybrids. The tetraploid cultigen *A. hypogaea* ( $2n=40$ ) is the most important species of the genus and belongs to section *Arachis*. In this section, there are another tetraploid species, *A. monticola*, and more than 27 diploid species. Many of these diploid species were found to be resistant to the primary diseases and pests of *A. hypogaea* and are being tested in crosses for introduction of resistance genes. Species of other sections have also been tested in crossing programs, however, the hybrids are rarely produced and they usually present a high degree of sterility.

Regarding gene introduction, the best results were obtained by chromosome doubling of wild species and hybrids before crossing with *A. hypogaea* (Simpson 1991, 2001). By this method reproductive barriers between wild diploid species and the cultivated peanut, due to different ploidy level, can be overcome. As in many instances, the main goal of breeding programs involves the transference of desirable agronomic characters that are present in different wild species to the cultigen. Thus, breeders have been advocated to artificial hybridization of these sources of germplasm.

At this respect, very few (less than 24%) species of *Arachis* have been studied meiotically, and this kind of information is still lacking for several sections. In this sense, as a part of a large investigation on the genome affinities between *Arachis* species the aim of this report was to describe comparatively the meiotic behaviour of 8 wild diploid species of *Arachis* that are cultivated at the Texas Agricultural Experiment Station, USA (TAES) and Instituto de Botánica del Nordeste, Argentina

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(IBONE) with the hope to provide valuable information for the genetic improving programs.

### Materials and methods

The analyzed materials were collected from plants growing under greenhouse conditions at TAES and from plants growing in the experimental field at the Instituto de Botánica del Nordeste (CTES). Vouchers of original collections are kept at CTES herbarium. The localities of collection and collectors are shown in Table 1.

Meiotic study was performed on inflorescences fixed in ethanol:lactic acid 5:1 for 24 h (Fernández 1973), transferred to 70% ethanol and kept refrigerated until use. Pollen mother cells (PMCs) were stained in a drop of 3% acetic orcein. Representative permanent slides were made using Euparal as mounting medium. Photomicrographs were taken on a Zeiss microscope using Kodalith film.

Meiotic index was calculated by the ratio normal sporads/total of sporads analyzed. Pollen viability was estimated by the acetocarmine-glycerin technique. For each species 500 pollen grains were scored, considering viable grains those with uniformly dark stained cytoplasm.

### Results and discussion

Meiotic behavior of *A. benensis*, *A. chiquitana*, *A. diogoi*, *A. herzogii*, *A. kempff-mercadoi*, *A. matiensis*, *A. subcoriacea* and *A. triseminatae* (Figs. 1, 2) is reported for the first time, all examined species were diploid  $2n=20$ . Chromosome numbers of the analyzed species are in accordance with previous reports based on somatic chromosome counts (Fernández and Krapovickas 1994, Lavia 1996, 2000). In section *Arachis* 2 basic chromosome numbers have been reported,  $x=10$  and  $x=9$  (Lavia 1996, 1998), however, all the species studied here belong to the group of  $x=10$ , which is by far the most widespread number among the species of the genus.

All the studied species presented, in general terms, a regular meiotic behavior with 10 II at diakinesis-metaphase I, except *A. benensis* which presented 8 II+1 IV in 2% of the pollen mother cells (Table 2). At metaphase I, most of the bivalents were of the ring type and only one or two bivalents per PMC were usually of the rod type (Table 2). Chiasmata were always located in distal to terminal position even at diplotene and diakinesis. The tetravalent observed in *A. benensis* probably arose as a consequence of a reciprocal chromosome interchange in heterozygosity. This phenomenon was also observed in other wild *Arachis* species (Singh and Moss 1982, Lavia *et al.* 2001), and

Table 1. Studied material. Collectors and procedence

Species	Collectors and procedence
Section <i>Procumbentes</i>	
<i>A. chiquitana</i> Krapov., W. C. Gregory & C. E. Simpson	KSSc 36027. BOLIVIA. Santa Cruz. Prov. Chiquitos.
<i>A. matiensis</i> Krapov., W. C. Gregory & C. E. Simpson	VSGr 6366. BRASIL. MG. 66 Km de porto Esperidiao.
<i>A. subcoriacea</i> Krapov. & W. C. Gregory	VKSSv 8922. BRASIL. MG. 75 Km E de Cáceres.
Section <i>Arachis</i>	
<i>A. benensis</i> Krapov., W. C. Gregory & C. E. Simpson	KGSPSc 35005. BOLIVIA. Beni. Trinidad.
<i>A. diogoi</i> Hoehne	VPoBi 9147. BRASIL. MS. Corumbá.
<i>A. herzogii</i> Krapov., W. C. Gregory & C. E. Simpson	KSSc 36030. BOLIVIA. Santa Cruz. Prov. Chiquitos.
<i>A. kempff-mercadoi</i> Krapov., W. C. Gregory & C. E. Simpson	KGSSc 30088. BOLIVIA. Santa Cruz. 3 Km arroyo San Joaquín.
Section <i>Triseminatae</i>	
<i>A. triseminata</i> Krapov. & W. C. Gregory	GK 12922. Brasil, BA, 15 km W de Joazeiro.

Collectors: Bi: L. B. Bianchetti, G: W. C. Gregory, Gr: A. Gripe, K: A. Krapovickas, P: J. R. Pietrarelli, Po: A. Pott, S: C. E. Simpson, Sc: A. Schinini, Sv: G. P. Silva, V: J. F. M. Valls.

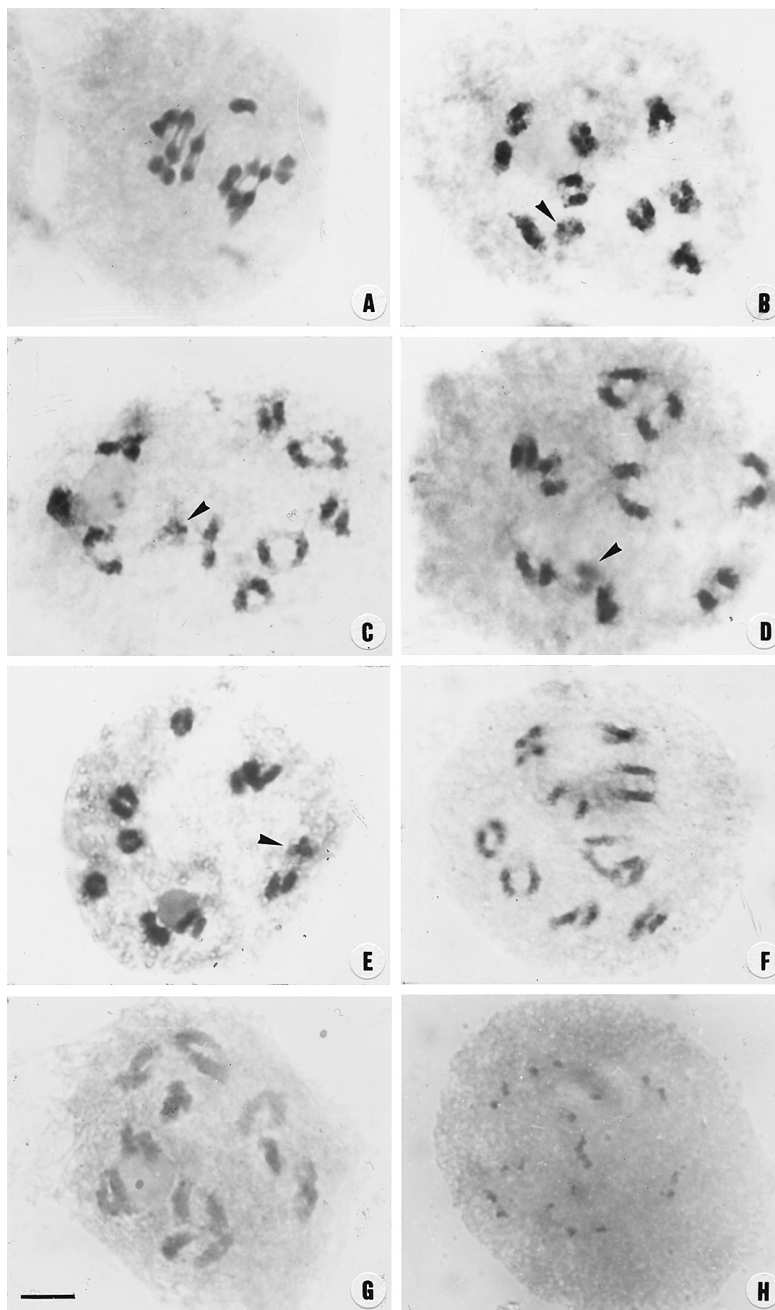


Fig. 1. Meiotic chromosomes A) *A. benensis*, B) *A. chiquitana*, C) *A. diogeni*, D) *A. herzogii*, E) *A. kempff-mercadoi*, F) *A. matiensis*, G) *A. subcoriacea*, H) *A. triseminata*. Arrowheads show the “A” chromosomes. Bar=5  $\mu$ m.

suggests that reciprocal translocations may be one of the mechanisms involved in structural changes of chromosomes and karyotype differentiation among diploids.

Segregation of bivalents was normal, except in *A. herzogii*, which presented 1 bridge in 11% of the studied cells in telophase I, and in *A. subcoriacea* which showed 1 bridge in 1% and 2 bridges in 4% of the analyzed PMCs (Table 3). Additionally, in *A. matiensis* 1 out of plate bivalent

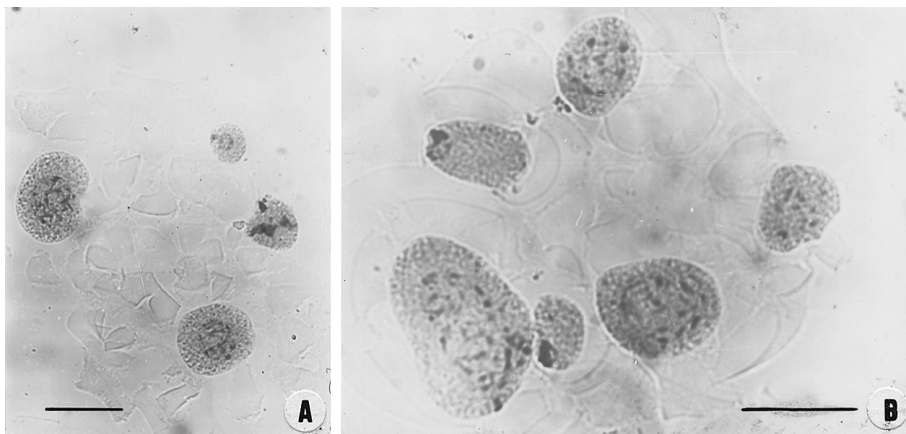


Fig. 2. Abnormal sporads in *A. matiensis*. A) sporad having two big microspores and two smaller ones; B) sporad having more than the four normal microspores. Bar=50  $\mu$ m.

Table 2. Meiotic associations in diakinesis

Species	Meiotic associations			
	<i>n</i>	10 II R	9 II R+1 II A	8 II R+1 IV
<i>A. benensis</i>	60	59	0	1
<i>A. chiquitana</i>	10	8	2	0
<i>A. diogoi</i>	10	2	8	0
<i>A. herzogii</i>	10	2	8	0
<i>A. kempff-mercadoi</i>	15	15	0	0
<i>A. matiensis</i>	10	6	4	0
<i>A. subcoriacea</i>	9	0	9	0
<i>A. triseminata</i>	10	8	2	0

*n*=number of PMCs. R=ring, A=rod.

Table 3. Meiotic behaviour in some wild diploid *Arachis* species

Species	Metaphase I		Telophase I			Metaphase II	Telophase II
	Normal	1 II out of the plate	Normal	1 bridge	2 bridges	Normal	Normal
<i>A. benensis</i>	0	0	11	0	0	0	5
<i>A. diogoi</i>	0	0	0	0	0	0	56
<i>A. herzogii</i>	0	0	50	5	0	50	0
<i>A. kempff-mercadoi</i>	0	0	0	0	0	0	20
<i>A. matiensis</i>	34	5	80	0	0	0	0
<i>A. subcoriacea</i>	0	0	100	1	4	0	0

was observed in metaphase I (14.7%) that segregated separately of the remaining bivalents. Since this bivalent segregates asynchronously, homologous chromosomes would not reach the pole and therefore, they may originate micronuclei in telophase II. These micronuclei ultimately could lead to the formation of micromicrospores as those observed in the tetrad stage (Fig. 2A). This phenomenon is very similar to those observed in other legume groups, such as *Lathyrus* (Seijo 2002), in

Table 4. Analysis of pollen grains in some wild diploid *Arachis* species

Species	No. Pollen grains	Coloured %		Uncoloured %	
		Normal	Macrograin	Micrograin	Normal shaped
<i>A. diogoi</i>	509	99.00	0	0	1.00
<i>A. chiquitana</i>	489	98.80	0	0	1.20
<i>A. herzogii</i>	522	98.28	0.38	0	1.34
<i>A. kempff-mercadoi</i>	500	100.00	0	0	0
<i>A. matiensis</i>	506	96.25	2.00	1.75	0
<i>A. subcoriacea</i>	476	94.50	1.50	0.60	3.40
<i>A. triseminata</i>	341	93.50	1.51	0	4.99

which the high production of micromicrospores was attributed, at least in part, to asynchronous segregation.

The meiotic index, estimated through sporads analysis, was 100% in *A. diogoi*, *A. herzogii*, *A. Kempff-Mercadoi* and *A. triseminata*. In *A. matiensis* this index was lower due to the presence of micromicrospores and microspores larger than the normal ones (Fig. 2). All species presented high pollen viability, which range between 93.5 and 100%. Uncolored pollen grains, macrograins and micrograins (Table 4) caused the reduction of viability, even though each of these categories contributed differentially in the species analyzed. In general, the pollen viability was in accordance with that expected from the observed meiotic behavior. Pollen grains with different volumes, but with similar morphology, were observed in *A. subcoriacea*, *A. triseminata* and *A. herzogii*. The causes of pollen size variation remain unknown since no abnormality in meiosis was observed in these species.

At diplotene and diakinesis one small bivalent was clearly distinguished in *A. herzogii*, *A. kempff-mercadoi*, *A. diogoi* and *A. chiquitana*. This bivalent was usually of the ring type, with a conspicuous heterochromatic pericentromeric region, but with diffuse and poorly stained distal regions. This behavior is in correspondence with that described for the "A" chromosome pair in somatic cells (Fernández and Krapovickas 1994, Lavia 1996, 2000). The "A" chromosome pair was so far only detected in nearly half of the species that belong to section *Arachis*. The presence of these particular pair in *A. chiquitana*, currently included in section *Procumbentes*, needs particular considerations. This finding, together with the fact that none of the species belonging to section *Procumbentes* (*A. kreschmeri*, *A. lignosa*, *A. matiensis*, *A. rigonii*, *A. subcoriacea*, *A. vallsii*) have the "A" chromosome pair (Fernández and Krapovickas 1994, Lavia 2000), may support the relocation of this taxon into section *Arachis* as was suggested by Lavia (2000).

The results we obtained adds to the few data so far known on the meiotic behavior of *Arachis* species and provides some particular chromosome characteristics for the identification of different sections of *Arachis*, as well as some probable mechanisms of chromosome evolution of these groups of species.

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## References

- Fernández, A. 1973. El ácido láctico como fijador cromosómico. *Bol. Soc. Argent. Bot.* **15**: 287–290.
- and Krapovickas, A. 1994. Cromosomas y evolución en *Arachis* (Leguminosae). *Bonplandia* **8**: 187–220.
- Krapovickas, A. and Gregory, W. C. 1994. Taxonomía del género *Arachis* (Leguminosae). *Bonplandia* **8**: 1–186.
- Lavia, G. I. 1996. Estudios cromosómicos en *Arachis* (Leguminosae). *Bonplandia* **9**: 111–120.
- 1998. Karyotypes of *Arachis palustris* and *A. praecox* (section *Arachis*), two species with basic chromosome number  $x=9$ . *Cytologia* **63**: 177–181.
- 2000. Chromosome studies in wild *Arachis* (Leguminosae). *Caryologia* **53**: 277–281.
- , Fernández, A., Simpson, C. and Seijo, G. 2001. Meiotic analysis in wild diploid *Arachis* species. *Cytologia* **66**: 293–298.
- Seijo, J. G. 2002. Estudios citogenéticos en especies Sudamericanas del género *Lathyrus*, Sección *Notolathyrus* (Leguminosae). Ph. D. Thesis, Universidad Nacional de Córdoba, Argentina.
- Simpson, C. E. 1991. Pathways for introgression of pest resistance into *Arachis hypogaea* L. *Peanut Sci.* **18**: 22–26.
- 2001. Use of wild *Arachis* species/Introgression of genes into *A. hypogaea*. *Peanut Sci.* **28**: 114–116.
- Singh, A. K. and Moss, J. P. 1982. Utilization of wild relative in genetic improvement of *Arachis hypogaea* L. *Theor. Appl. Genet.* **61**: 305–314.
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