

Chromosome Pairing in F₁ Hybrid *Arachis hypogaea* L. × *A. monticola* Krap. et Rig.

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Summary. Chromosome pairing at pachytene in the F₁ hybrid *Arachis hypogaea* × *A. monticola* was studied. Pairing was remarkably regular and segment by segment except for some minor differences. Chromosomes were identified individually at pachytene. The idiograms of *A. hypogaea* and *A. monticola* were identical. Meiosis was regular and fertility was high in the hybrid indicating that the taxa concerned were very closely related.

Key words: *Arachis hypogaea* – *A. monticola* – F₁ hybrid – Pachytene pairing – Chromosome identification – Nucleolar budding

Introduction

Studies on chromosome pairing at the pachytene stage of meiosis help in identifying and describing the individual chromosomes and those on species hybrids give precise information in elucidating the chromosome relationships between the species concerned. However, difficulties encountered in obtaining well-stained preparations with a good spread of chromosomes at the pachytene stage coupled with a comparatively higher chromosome number have been eluding workers interested in the cytogenetics of the genus *Arachis*.

Most of the work on the chromosomes of the groundnut has been done at the somatic metaphase, which has only resulted in karyotyping them in a routine fashion (Husted 1931, 1933, 1936; Babu 1955; D'Cruz and Tankasale 1961; Raman 1976; Singh et al. 1980; Stalker 1980). Since chromosomes at this stage were very small (0.3 to 3 μm), it was not possible to identify them with ordinary light microscopic studies. However, chromosomes at the pachytene stage of meiosis, in their greatly extended state, offer a number of criteria for their individual identification. Earlier studies in this direction by Murty et al. (1981a) have succeeded in identifying the individual chromosomes in two varieties of groundnut, *Arachis hypogaea* L. and have presented a key for their easy identification. Kirti

et al. (1981) have extended these studies to the diploid species of the section *Arachis*.

The groundnut, *A. hypogaea* is a tetraploid (2n=40) and belongs to the section *Arachis* (Gregory and Gregory 1976). *Arachis monticola* is the only other tetraploid species (2n=40) in the section which contains several diploid species (2n=20). The study of pairing relationships of the 2 tetraploid taxa could throw some light on the origin of the cultivated groundnut. *A. hypogaea* and *A. monticola* intercross freely (Smartt and Gregory 1967; Gregory and Gregory 1976; Smartt et al. 1978; Moss 1980).

In the present study *A. hypogaea* and *A. monticola* were crossed to obtain F₁ hybrids and chromosome pairing at pachytene of these hybrids was studied to elucidate the relationship between the two species and confirm our observations and identification of chromosomes of *A. hypogaea* as reported in an earlier study (Murty et al. 1981a). Chromosome relationship of the two species *A. hypogaea* and *A. monticola* are discussed below.

Materials and Methods

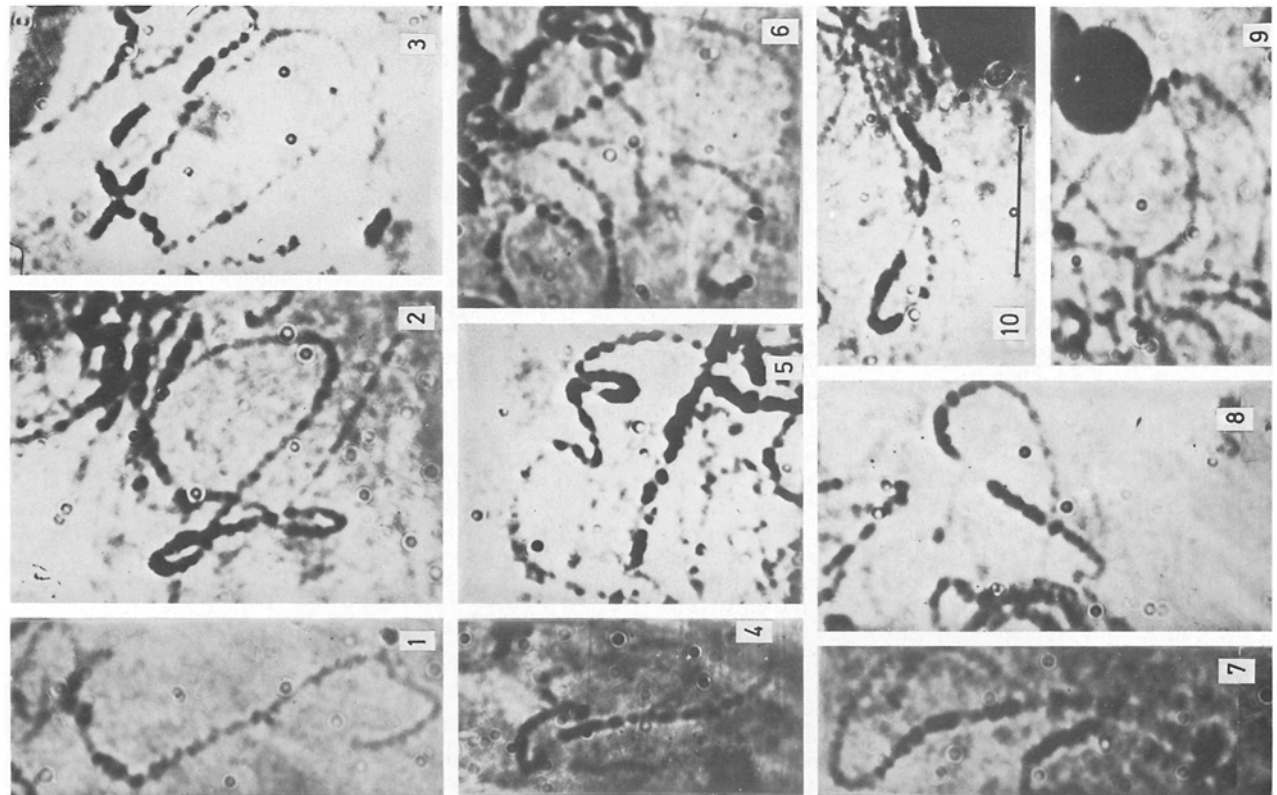
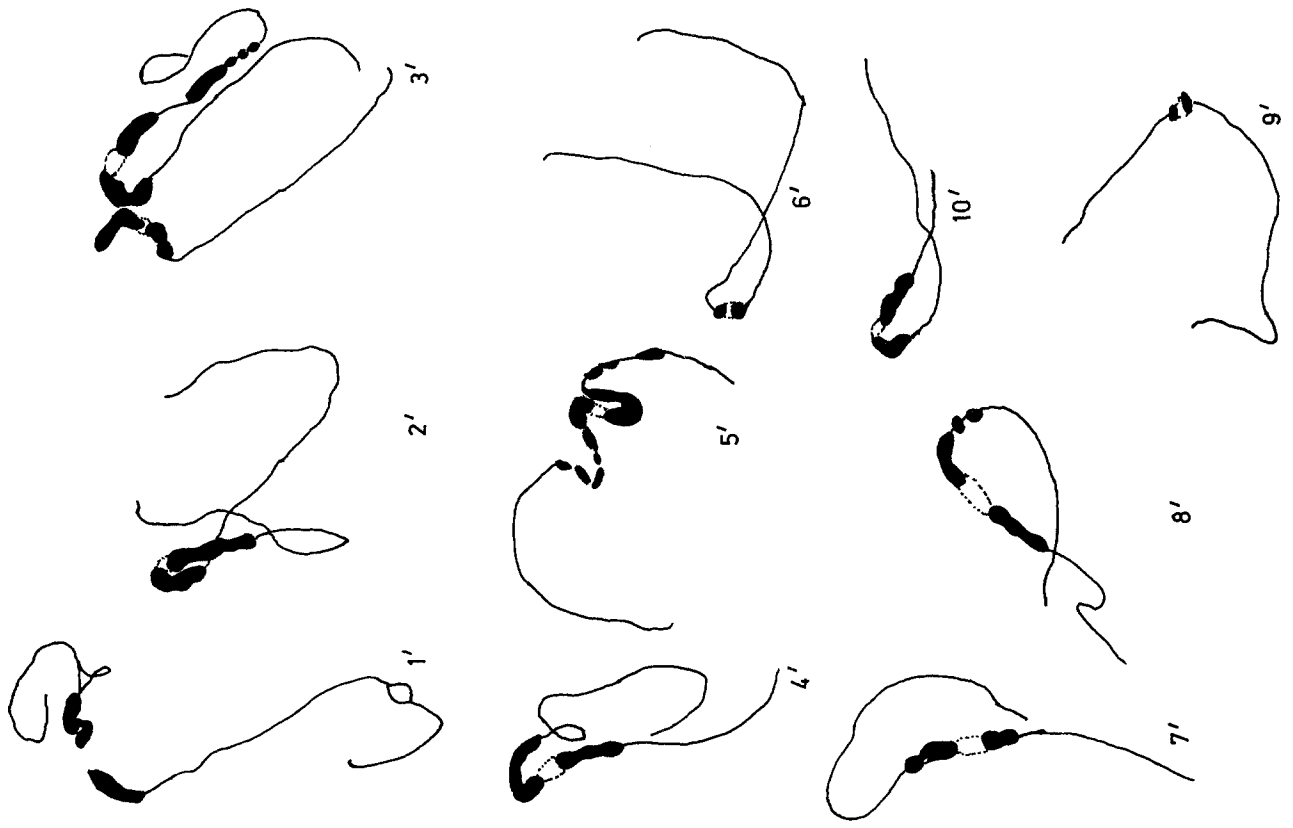
The seed material of *A. monticola* Krap. et Rig. was obtained from Prof. V. S. Raman of Coimbatore. A Spanish variety 'TMV-2' of the groundnut, *A. hypogaea* L. was the other parent used in the study.

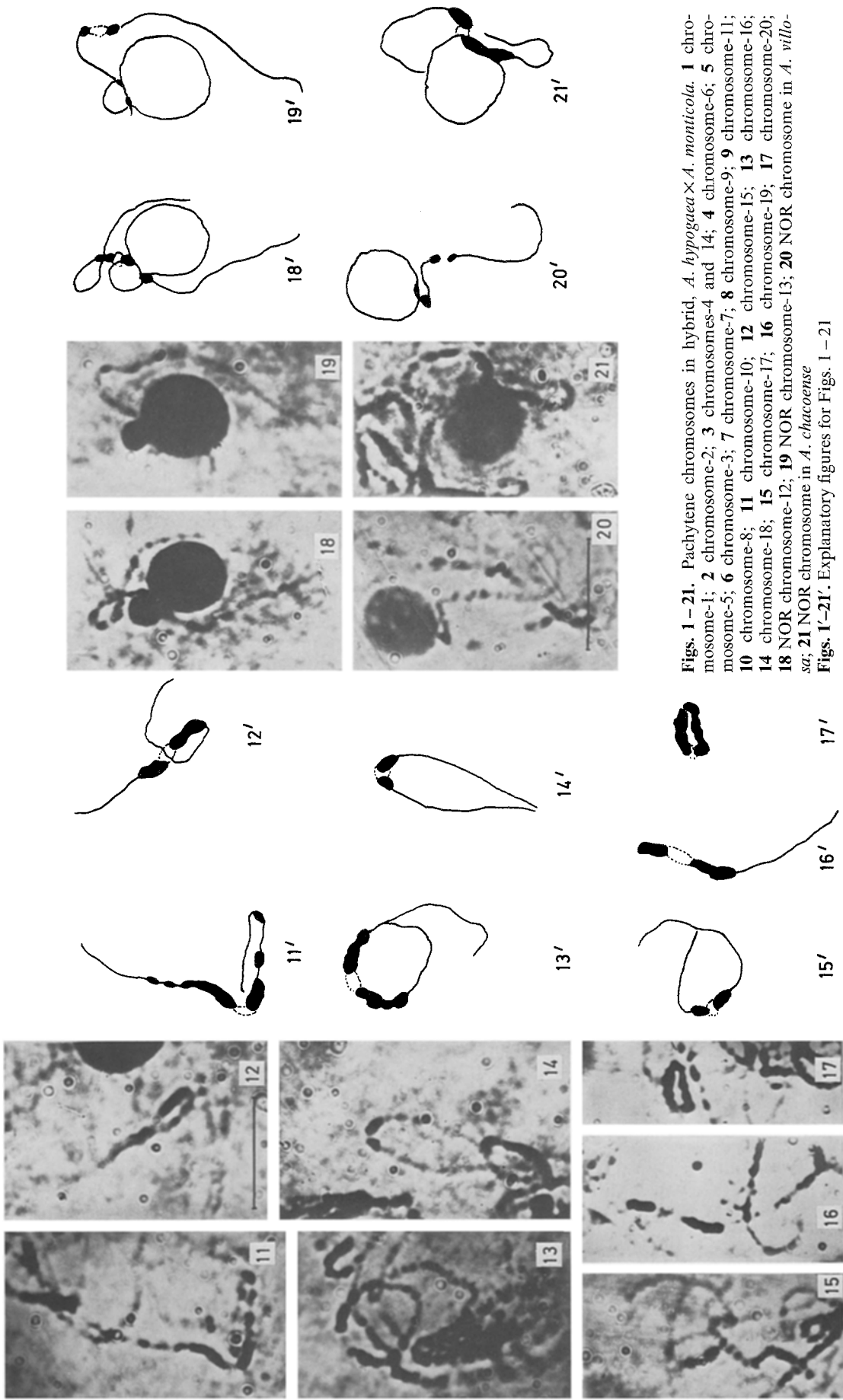
F₁ hybrids were obtained in the field according to the method of crossing outlined by Murty et al. (1981b). Out of the 32 seedlings obtained, 22 (69.1%) were F₁ hybrids.

A Standard propionic carmine schedule was found to give a satisfactory staining of pachytene chromosomes. Measurements of chromosomes were taken from camera lucida drawings. Even though the spread of the chromosomes appeared to be fairly good, only one or two chromosomes per PMC could be followed from end to end as "throw-offs".

Results and Discussion

Pachytene chromosomes were of the differentiated type, differentiation being into eu- and heterochromatic regions. Chromosomes could be easily identified on the basis of 1) total length, 2) arm ratio, 3) extent of hetero-





Figs. 1 - 21. Pachytene chromosomes in hybrid, *A. hypogaea* × *A. monticola*. **1** chromosome-1; **2** chromosome-2; **3** chromosomes-4 and 14; **4** chromosome-6; **5** chromosome-5; **6** chromosome-3; **7** chromosome-7; **8** chromosome-9; **9** chromosome-11; **10** chromosome-8; **11** chromosome-10; **12** chromosome-15; **13** chromosome-16; **14** chromosome-18; **15** chromosome-17; **16** chromosome-19; **17** chromosome-20; **18** NOR chromosome-12; **19** NOR chromosome-13; **20** NOR chromosome in *A. villosa*; **21** NOR chromosome in *A. chacoense*
Figs. 1'-21'. Explanatory figures for Figs. 1 - 21

chromatic segments into arms and 4) the nucleolus attachment. Eight chromosome pairs can be readily identified by simple observation. They are:

1) The smallest of the complement, the completely heterochromatic A-chromosome pair (Fig. 17). This has been designated as chromosome no. 20.

2) Two 'eu-chromosome' pairs, which have very small blocks of heterochromatin or large chromomeres on either side of the centromeres. One of them was longer, having an almost median centromere, and the second one was shorter with a submedian centromere (Figs. 6, 9). These have been designated as chromosomes 3 and 11 respectively.

3) Two chromosome pairs, with the short arm fully comprised of heterochromatin. These also can be divided into long and short ones (Figs. 3, 16). These have been designated as chromosomes 14 and 19, respectively.

4) Two nucleolus organizer chromosome pairs: one of them having a median centromere and a secondary constriction just near the centromere which was the site of nucleolus attachment (Fig. 18). The second pair had a submedian centromere and the nucleolus attachment was at the end of the short arm (Fig. 19). The former nucleolus organizer pair resembled that of *A. chacoense*

(Fig. 21) and *A. batizocoi*, and the latter that of *A. villosa* (Fig. 20). They have been designated as chromosomes 12 and 13 respectively.

5) One long median chromosome pair, having two large distinct heterochromatic segments in the short arm (Fig. 3). This has been designated chromosome 4.

These are the chromosome pairs that can be readily recognized. The rest of the pairs can be identified on the basis of length: long ($> 45 \mu\text{m}$), medium ($30 \mu\text{m}$ to $44 \mu\text{m}$) and short ($< 30 \mu\text{m}$). These can be further classified on the basis of position of the centromere (i.e. arm ratio) as median (A.R. > 0.75) and submedian (A.R. < 0.74). There are 6 classes and each class is comprised of 2 chromosome pairs. Further, intra-class distinction can be made on the basis of total length and extent of the heterochromatin. (Table 1). On the basis of the above criteria, all 20 chromosome pairs can be identified individually and numbered in the order of decreasing lengths – the longest in the complement occupying the first position. The criterion for chromosome numbering cannot simply be total length. Specific markers should also be used in chromosome identification. Since the length of the chromosomes is greatly dependent upon the degree of contraction (Darlington 1937), the position of a chromosome pair in one particu-

Table 1. Data on pachytene chromosomes of the hybrid, *A. hypogaea* × *A. monticola* (in microns)

Sl. no. assigned by Murty et al. (1981)	Chromosome number	Total length	Short arm		Long arm		A/R short arm/long arm)
			Length	Heterochromatic Segment	Length	Heterochromatic Segment	
1.	long submedian	58.8 ± 2.9	20.1 ± 1.2	5.5 ± 0.7	36.4 ± 2.0	4.1 ± 0.8	0.55
2.	long median	53.4 ± 1.4	23.3 ± 0.7	5.6 ± 0.5	28.3 ± 0.9	6.2 ± 0.5	0.83
3.	long 'Eu-chromosome'	43.6 ± 3.3	19.2 ± 1.7	–	23.5 ± 1.9	–	0.82
4.	Chromosome with short arm having 2 heterochromatic blocks	54.2 ± 5.3	25.6 ± 1.9	3.2 ± 0.6/ 2.8 ± 0.5	27.7 ± 3.3	3.9 ± 0.3	0.92
5.	long median	47.9 ± 1.1	20.9 ± 1.1	3.4 ± 0.4	25.4 ± 0.8	3.5 ± 0.3	0.82
6.	long submedian	48.6 ± 0.8	16.5 ± 0.6	4.3 ± 0.6	30.8 ± 1.0	4.9 ± 0.6	0.54
7.	medium submedian	41.5 ± 0.5	14.8 ± 0.4	5.3 ± 0.6	25.0 ± 0.4	4.8 ± 0.5	0.59
8.	medium submedian	36.4 ± 0.8	12.2 ± 0.7	4.0 ± 0.6	22.6 ± 0.7	4.4 ± 0.3	0.54
9.	medium median	40.8 ± 0.5	17.5 ± 0.5	4.4 ± 0.4	20.6 ± 0.3	4.6 ± 0.4	0.85
10.	medium median	31.8 ± 0.6	14.8 ± 0.3	4.8 ± 0.4	16.4 ± 0.5	3.8 ± 0.3	0.88
11.	short 'Eu-chromosome'	35.5 ± 1.0	11.4 ± 0.3	–	23.3 ± 1.1	–	0.49
12.	nucleolus organizer	36.8 ± 1.6	15.3 ± 2.5	–	19.8 ± 2.0	–	0.77
13.	nucleolus organizer	26.6 ± 7.1	7.0 ± 3.8	–	18.8 ± 3.8	–	0.37
14.	chromosome-short arm fully heterochromatic	29.5 ± 0.6	4.6 ± 0.7	4.6 ± 0.7	24.3 ± 1.4	4.3 ± 0.6	0.19
15.	short submedian	28.3 ± 1.2	9.2 ± 0.8	3.2 ± 0.5	17.5 ± 0.8	3.8 ± 0.7	0.52
16.	short median	26.0 ± 0.9	11.7 ± 0.6	3.6 ± 0.5	13.2 ± 0.5	3.7 ± 0.6	0.88
17.	short submedian	20.8 ± 0.8	6.8 ± 1.0	1.0	13.0 ± 0.4	1.1	0.53
18.	short median	25.4 ± 4.9	11.3 ± 2.0	1.3	13.4 ± 1.5	1.3	0.84
19.	chromosome with short arm fully heterochromatic	19.2 ± 1.4	4.1 ± 0.7	4.1 ± 0.7	13.3 ± 1.9	4.1 ± 0.6	0.30
20.	A-chromosome	13.0 ± 0.5	4.6 ± 1.2	4.6 ± 1.2	6.5 ± 1.2	6.5 ± 1.2	0.71

lar study could be different from the position assigned to it in another study. However, since major criteria for identifying the individual chromosomes of the groundnut had already been established, the numbering of chromosomes designed by Murty et al. (1981) was taken as the standard for *A. hypogaea*.

Studies on chromosome pairing at pachytene in the F₁ *A. hypogaea* × *A. monticola* have revealed the following facts. The pairing of chromosomes was remarkably regular, generally with bivalent pairing. Pairing was segment by segment except for some difference in chromosome pair 1. In this chromosome the length of the short arms was slightly different, probably due to some duplication/deletion. In this region, there was a folding-back of the extra segment (Fig. 1). Otherwise, the rest of the pairing generally adjusted. The pairing was normal in the rest of the chromosome complement. This indicates that the idiogram that has been proposed for *A. hypogaea* by Murty et al. (1981) is equally applicable for *A. monticola*. Thus, the genomes of *A. hypogaea* and *A. monticola* are very similar.

The general chromosome configuration at diakinesis and metaphase I ranged from 16-ring bivalents + 4-rod bivalents to 20-ring bivalents. Higher chromosome associations (one per PMC) were observed occasionally. These were rings or chains of four chromosomes. However, these associations were not observed in pachytene.

Chromosome segregation at anaphase I and II was normal. This was followed by full pollen fertility as in the respective parents. Thus, very good chromosome pairing followed by good recombination potential and high pollen fertility indicates that the species are very closely related, even to the extent of rating them as conspecific. *A. monticola* can even be taken as *A. hypogaea* var. 'monticola'. Gregory and Gregory (1976) have pointed out that it is the current wild descendant of the amphidiploid species ancestral to the cultigen.

The observation of two nucleolus organizer chromosome pairs in the hybrid deserves some attention. *A. hypogaea* (Spanish variety 'TMV-2'), analyzed in an earlier study (Murty et al. 1981), shows the occurrence of only one nucleolus organizer chromosome pair, which closely resembles the one observed in diploid species *A. chacoense* and *A. batizocoi*. However, another chromosome pair (no. 13) in that study had a morphology similar to the second nucleolar bivalent observed in the present study. This pair is very similar to the one in the diploid *A. villosa*. However, variety 'Virginia M-13' of *A. hypogaea* had occasionally 2 nucleolus organizer pairs attached to the nucleolus. In the present study the occurrence of 2 pairs associated with nucleolar activity has been unambiguously established. However, the 2 pairs never seemed to have functioned together in the formation of the nucleolus. Therefore, as far as was observed, only one nucleolus organizer functions in any

PMC and there seems to be no dominant-recessive relationship in the formation of the nucleolus: the formation of the nucleolus by either of the 2 NORs being random. However, in the parent *A. hypogaea* ('Spanish TMV-2'), the median nucleolus organizer resembling that of *A. chacoense* and *A. batizocoi* assumes the function; the activity of the second NOR being suppressed (Murty et al. 1981). The shape of the nucleolus in the diploid species, *A. chacoense*, *A. batizocoi* and *A. villosa* was always either round or spherical (Figs. 20, 21) without any budding. A similar situation occurred with respect to the triploid hybrids of *A. hypogaea* × diploid *A. chacoense*. In the tetraploid hybrid of the present study as well as in *A. hypogaea* (Murty et al. 1981) and *A. glabrata* (2n=40) (Unpubl. data), the nucleolar budding was a general phenomenon. This phenomenon of nucleolar budding can somehow be related to the higher ploidy status of the individual. The site of the nucleolus attachment is the region where the bud joins the main body of the nucleolus (Fig. 18, 19). Both *A. hypogaea* ('Spanish TMV-2') and *A. monticola* contributed 2 nucleolus organizing chromosomes each to the hybrid resulting in two homologous pairs. Based on observations of chromosomes at pachytene, it is possible that *A. villosa*, or a form closely similar to it, could be one of the progenitors of the present day cultivated groundnut and may have donated the A genome. *A. batizocoi* was supposed to have donated the B-genome. Earlier workers have supposed that *A. cardenasii* donated the A-genome. However, more information in the chromosomes pairing at pachytene in various interspecific hybrids involving the groundnut and the diploid species is essential before coming to any conclusion on the origin of the groundnut. However, since *A. monticola* forms a fertile hybrid with *A. hypogaea* and chromosome pairing is normal in the hybrid, it is quite possible that the wild *A. monticola* is the immediate ancestor of *A. hypogaea*.

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