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Chromosome Loss and Meiotic Behaviour in Interspecific Hybrids in the Genus *Arachis* L. and their Implications in Breeding for Disease Resistance

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With 6 figures and 7 tables

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The cultivated peanut,
and is cultivated in most tropical and subtropical areas of the world. Between thirty and fifty other species of the genus are recognised, most of which are diploid (2n=20), and native to South America (Gregory et al. 1973). During the past fifteen years many of these species have been collected and studied. Some of these wild species are resistant to important diseases, *A. cardenasii* for example is immune to leafspot caused by *Cercospora personata* (Abdou et al. 1974, Sharief 1972). Interspecific hybridisation, colchicine treatment of the triploid, and backcrossing the resultant hexaploid to the tetraploid species, is one method that is being used to introduce resistance into this cultivated legume (Moss and Spielman 1976, Spielman and Moss 1976).

Husted (1933) observed that one pair of "A" chromosomes is distinctly smaller than the remainder of the complement of *A. hypogaea*. Smartt et al. (1978) have since shown that a smaller pair of chromosomes is present in all the diploid species of the section *Arachis* except *A. batizocoi*.

Aneuploidy in the genus *Arachis* was first reported in a single plant of *A. rasteiro* (Husted 1936). He also reported an aneuploid in *A. hypogaea* where one plant had 41 chromosomes plus a small fragment. There were no phenotypic differences between the aneuploid and the other two plants grown from the same batch of seed. However, very few pods were produced on the aneuploid and none of these reached more than 1.0 cm in length at maturity. Kumar and D'Cruz (1957) produced an aneuploid hybrid with 2n=41 from backcrossing the incompletely sterile triploid hybrid, *A. hypogaea* × *A. villosa*, to the cultivated parent. The progeny resembled *A. villosa* in its spreading habit, was an annual like *A. hypogaea* but, unlike the triploid, was fertile.

The seeds produced from this aneuploid were noticeably smaller than either of the original parents, but no chromosome numbers or meiotic studies of the progeny were reported, so it is not known if the aneuploid condition was inherited.

The chromosome behaviour and some morphological characters of *A. hypogaea*, *A. villosa*, the triploid hybrid between these two species, one hexaploid from the hybrid and its six progeny, have been studied (D'Cruz and Upadhyaya 1962). The hexaploids were found to be more robust with larger flowers and darker green leaves; they showed a range of fertility and also a range of chromosome numbers. The C_1 hexaploid had a chromosome complement of 2n = 60, four of the C_2 generation also had 2n = 60, whilst one plant had a chromosome complement of 2n = 58 and the other 2n = 62. The two aneuploids produced pollen grains of which approximately 50% were filled, but the seed set was low. No information was given on the meiotic behaviour or whether the chromosome number of these two plants was stable in further generations.

Smartt and Gregory (1967) were also able to produce triploid interspecific hybrids. Some of these hybrids e.g. *A. hypogaea* × *A. villosa* and *A. hypogaea* × *A. villosa correntina* were not completely sterile and produced progeny with chromosome numbers in the pentaploid-hexaploid range, whilst *A. hypogaea* × *A. cardenasii* produced progeny which had chromosome complements that approximated to the tetraploid, pentaploid and hexaploid level. Colchicine treatment of the triploid, *A. hypogaea* × *A. cardenasii*, produced hexaploids (2n=60) which were morphologically identical to those progeny where the chromosome complement had doubled spontaneously.

An investigation of the chromosome numbers of some F_6 progeny of *A. hypogaea* × *A. cardenasii* hexaploid hybrids produced by J. Smartt in 1963 revealed that as many as twenty chromosomes had been lost from these plants whilst left to self in the field (Spielman 1976). It was feared that the twenty *A. cardenasii* chromosomes were spontaneously eliminated from the allohexaploid hybrid. The selective shedding of chromosomes from interspecific hybrids has been reported in other species. Subrahmanyan and Kasha (1973) first described the elimination of *Hordeum bulbosum* chromosomes from hybrids between the closely related species *H. bulbosum* and *H. vulgare*. Barclay (1975) crossed *Triticum aestivum* with *H. bulbosum* and produced polyhaploid wheat plants as the barley chromosomes were eliminated from the hybrid. Proposed causes of this phenomenon which does not normally occur when the same genomes are present are genic disharmony between parental genomes, differences in cell cycle times and genic factors controlling chromosome stability.

The implications of selective chromosome loss from interspecific hybrids for the plant breeder attempting to introduce useful genes from wild *Arachis* species into the cultivated peanut appeared serious. Thus an investigation of meiosis in hexaploid interspecific hybrids was undertaken to see if any irregularities in chromosome behaviour might be responsible for chromosome elimination. This paper also reports the occurrence of fertile aneuploids in *A. hypogaea* and in *A. hypogaea* × *A. cardenasii* hybrids following the loss of chromosomes from the hexaploid hybrids. In conjunction with this a morphological

investigation of F_8 *A. hypogaea* × *A. cardenasii* hybrids was carried out to detect the presence of recombinant genotypes which would indicate that a useful hybrid genotype could be maintained despite chromosome loss from the polyploid interspecific hybrid.

Materials and Methods

The material considered in this paper comprises plants from the following sources:

(a) Five varieties of *A. hypogaea* grown at Reading.
(b) Members of the F_6 generation of a cross between *A. hypogaea* and *A. cardenasii* obtained from North Carolina, U.S.A.
(c) Members of the F_8 generation of the above cross, but obtained from Texas, U.S.A. (where the later generations of the North Carolina stock had also been grown).
(d) The F_2 allohexaploid progeny of crosses between 3 *A. hypogaea* varieties and *A. cardenasii* produced and grown under glass at Reading.

(a) Five varieties of *A. hypogaea* grown at Reading

Plants of five varieties of *A. hypogaea* were grown in the glasshouse at Reading and allowed to self. The pods were harvested, dried, shelled and the smaller seeds selected for study.

(b) Members of the F_6 generation of a cross between *A. hypogaea* and *A. cardenasii* obtained from North Carolina, U.S.A.

A. hypogaea (U.S.P.I. 261942 or 261943) was crossed by SMARTT (1964) with *A. cardenasii* (U.S.P.I. 262141). The interspecific F_1 hybrids were sterile and had chromosome numbers of $2n = 30$. Subsequent colchicine treatment produced three hexaploids ($2n = 60$) from one of the hybrids and these were allowed to self. Cuttings from two F_3 seedlings (from one of the F_2 hexaploids) were planted in the field in North Carolina and kept as two separate lots, as were the F_4 and F_5 generations. A single pod was harvested from each of the F_5 plants in 1973 and sent to Reading in 1974, by courtesy of Professor W. C. GREGORY. From this seed, 25 double-seeded pods from each lot were selected at random, shelled, labelled and one of the seeds grown in a glasshouse at Reading, for mitotic studies. These plants were subsequently crossed with *A. hypogaea* and *A. cardenasii* (as the male parents) and as a result four F_1 hybrids were obtained which were grown at Reading and their chromosome numbers determined.

(c) Members of the F_8 generation of the above cross, but obtained from Texas, U.S.A. (where the later generations of the North Carolina stock had also been grown)

In 1977 a number of the pods harvested from the F_7 generation of SMARTT's hybrids grown in Texas were sent to Reading and the progeny of six randomly selected plants were grown in the glasshouse and the somatic chromosome numbers of the plants determined. The resultant twenty F_8 plants were scored for floral and vegetative characters to determine whether recombination and segregation were taking place. Flower characters were recorded daily throughout the flowering period and vegetative characters scored four and seven weeks after planting. Mitotic preparations were made of young root tips collected from seedlings in the morning and pretreated in a saturated solution of para-dichlorobenzene for six hours. They were then transferred to a fixative of 3 absolute alcohol : 1 glacial acetic acid and placed in a refrigerator overnight. Hydrolysis in 1 N hydrochloric acid for 15 minutes at 60 °C was followed by staining Feulgen reagent for two hours. When the tips were stained the root caps were carefully removed and the tips of individual roots were squashed in propionic orcein for microscopic examination.

(d) The F_2 allohexaploid progeny of crosses between 3 *A. hypogaea* varieties and *A. cardenasii* produced and grown under glass at Reading

Flower buds were collected from F_2 hexaploid hybrids of three genotypes: *A. hypogaea* 'F 439.2' × *A. cardenasii*, *A. hypogaea* 'F 452.4' × *A. cardenasii* and *A. hypogaea* 'Samaru 38' × *A. cardenasii*. The buds were collected before midday and were less than 1.5 cm in length when removed from the enclosing bracts.

The buds were fixed in a 3 : 4 : 1 absolute alcohol, chloroform, glacial acetic acid fixative which had previously been saturated with ferric acetate and kept at 14—17 °C for 24 hours

(RAMAN and KESAVAN 1963). Fixed buds were stored in 70 % alcohol at 4 °C until required. The anthers were dissected out of the fixed buds and tapped out in 1 % acetocarmine solution. Gentle tapping and warming resulted in good spreading and differential staining of the cytoplasm and chromosomes in the microsporocytes. Thirty eight pollen mother cells (PMC's) in diakinesis were examined and the chromosome associations recorded.

Results

(a) Five varieties of *A. hypogaea* grown at Reading

The small seeds of the *A. hypogaea* varieties germinated normally and produced plants which were fertile with no obvious morphological differences in leaf shape, habit, flower colour or size from those grown from larger seeds. Cytological examination showed that some of the plants were aneuploids with 42 or 44 chromosomes (*Table 1*). It was also noted that those plants with the diploid number of 40 had four chromosomes which were smaller than the remainder of the complement. Similarly there were six and eight smaller chromosomes in the plants with 42 and 44 chromosomes respectively (*Fig. 1*).

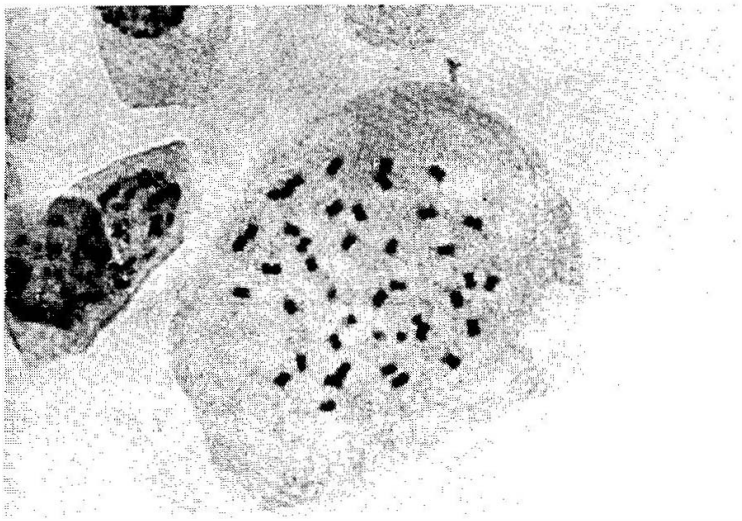

Fig. 1 Somatic metaphase root tip chromosomes of *A. hypogaea* aneuploid (2n = 42) showing six smaller chromosomes (× 3200)

(b) Members of the F_6 generation of a cross between *A. hypogaea* and *A. cardenasii* obtained from North Carolina, U.S.A.

Forty one of the supposed hexaploid seeds survived to maturity. Among these plants there were three different chromosome numbers of 2n=40, (*Fig. 2*)

Tab. 1 Chromosome numbers of plants of *A. hypogaea* grown from small seeds

A. hypogaea varieties	Chromosome number (2n)		
	40	42	44
'G 153'	6	1	0
'F 439.2'	2	2	0
'F 452.4'	1	1	0
'Samuru 61'	3	4	1
Unnamed	1	3	1
Total	13	11	2

Tab. 2 Chromosome numbers of hexaploid F_6 seed of the hybrid *A. hypogaea* × *A. cardenasii*

Seed characters		Chromosome number (2n)		
		40	42	44
Testa colour	brown	16	11	3
	brown/purple	3	0	1
	red or purple	3	3	1
Seed size	large	7	4	3
	medium	9	7	2
	small	6	3	0

42 (*Fig. 3*) and 44; the chromosome numbers of two plants were never established; it is possible that there was variation within these plants. The distribution of chromosome numbers with testa colour and seed size is summarised in *Table 2*. All the plants were fertile and flowered profusely. Some had long trailing laterals or pods with long constrictions (characters associated with the male parent), or purple testas (as in the *A. hypogaea* parent) or very thick dark green leaves, a condition often associated with an increase in ploidy level (RAMAN and MANIMEKALAI 1975).

Fig. 2 Somatic metaphase root tip chromosomes of F_6 *A. hypogaea* × *A. cardenasii* (2n = 40) showing four smaller chromosomes (× 3200)

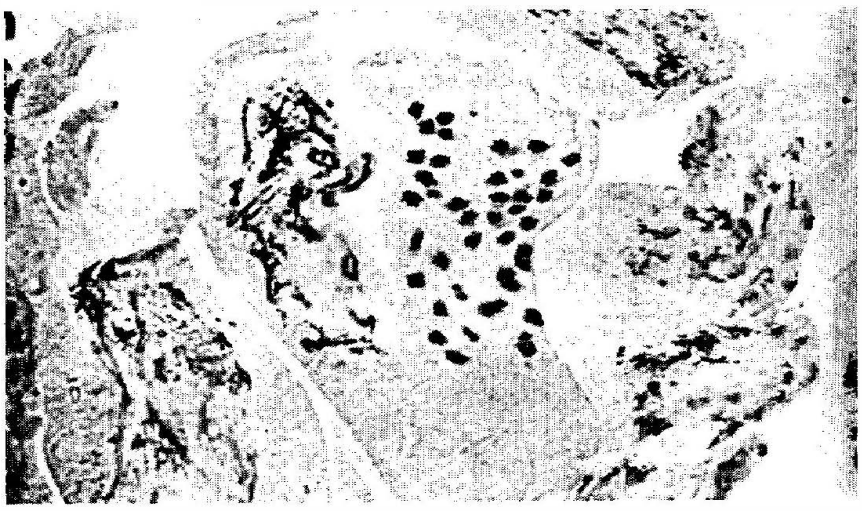

Fig. 3 Somatic metaphase root tip chromosomes of F_6 *A. hypogaea* × *A. cardenasii* (2n = 42) showing six smaller chromosomes (× 3200)

Tab. 3 Chromosome numbers of progeny from crosses of F_6 seed

Parents ♀	Parents ♂	Progeny No.	Progeny 2n	Progeny Seed colour
F_6 2n = 44 light brown seed	*A. hypogaea* 'F 452.4' 2n = 40 brown seed	1	40	brown
F_6 2n = 42 purple seed	*A. cardenasii* 2n = 20 brown seed	1	32	purple
F_6 2n = 44 brown seed	*A. cardenasii* 2n = 20 brown seed	2	34	purple

Backcrosses of the "reduced hexaploids" to the cultivated *A. hypogaea* and to *A. cardenasii* were successful. In three progeny from the backcrosses to *A. cardenasii* the number of extra chromosomes in the progeny was the same as in the female parent (*Table 3*). All the plants which had been grown of the F_7 and F_8 generations had chromosome numbers near the tetraploid level. These plants were the result of selfing by hand pollination of F_6 and F_7 plants respectively (*Table 4*). The F_8 seed of plant number 88/5 (2n=42) was from the F_7 plant which had a chromosome number of 2n=40.

(c) Members of the F_8 generation of the above cross, but obtained from Texas U.S.A. (where the later generations of the North Carolina stock had also been grown).

The chromosome numbers of eleven of the twenty F_8 plants were determined; at least one of the progeny of each F_7 plant was scored. The 2n numbers were found to be 40 in each case.

Observations on the colours of the standards showed two groups, one like *A. cardenasii* (301-126, 301-131 and 301-170y) and the other with the dark orange standard and yellow centre found in many *A. hypogaea* cultivars. All the F_8 plants exhibited the standard crescent common in *A. hypogaea* which is absent in *A. cardenasii*. Only two families (301-92y and 300-178) had floral measurements comparable with that of *A. cardenasii*. The progeny of 300-178

Tab. 4 Chromosome numbers of some F_6 plants and their F_7 and F_8

Plant no.	Chromosome numbers (2n) F_6	F_7	F_8
87/3	44 (1)*)	44 (6)	—
87/10	42 (1)	42 (7)	—
87/15	42 (1)	44 (1)	—
88/5	40 (1)	40 (1)	42 (5)
		42 (2)	—
88/20	40 (1)	40 (2)	—
		42 (3)	—

*) Figures in brackets are numbers of plants examined.

Tab. 5 Chromosome associations in PMC's at diakinesis of *A. hypogaea* 'F 439.2' × *A. cardenasii* hexaploids

2n	No. of PMC's	Chromosome association					
		I	II	III	IV	VI	VIII
58	1	26	9	—	2	1	—
64	1	15	21	1	1	—	—
64	1	20	20	—	1	—	—
64	1	22	21	—	—	—	—
64	1	24	20	—	—	—	—
64	1	28	16	—	1	—	—
64	1	28	18	—	—	—	—
64	1	36	14	—	—	—	—
64	1	36	14	—	—	—	—
64	1	37	12	1	—	—	—
64	1	38	13	—	—	—	—
64	1	41	6	1	—	—	1
Mean associations cell^{-1}		29.25	15.33	0.25	0.417	0.083	0.083

was unique for it had the long hypanthium as well as heavy pubescence, both characters associated with *A. cardenasii.* The progeny of 300-178 and 301-126 had purple stems whilst segregation for this trait had occurred in the progeny of 301-131 as green and purple stemmed sister plants were observed.

(d) The F_2 allohexaploid progeny of crosses between 3 *A. hypogaea* varieties and *A. cardenasii* produced and grown under glass at Reading.

A. hypogaea 'F 439.2' × *A. cardenasii* (*Table 5*)

The somatic chromosome number of this hybrid was 64 except in one bud which showed 2n = 58. Normally the chromosomes associated to form bivalents or remained unpaired at diakinesis. Occasional trivalents and quadrivalents were observed as well as a hexavalent and octavalent. The PMC's of this hybrid fell into two groups with respect to the number of univalents formed; a "high pairing group" with 15—28 and a "low pairing group" with 36—41 unpaired chromosomes respectively. A "t" test significant at the 0.1% level verified that the two groups were not part of the same population.

A. hypogaea 'Samru 38' × *A. cardenasii* (*Table 6*)

The chromosome number of the buds was 64 with three exceptions where 60, 66 and 68 chromosomes were present. Ten PMC's of this hybrid were studied and no chromosome associations higher than quadrivalents were observed. Single quadrivalents were found in five PMC's (mean number cell^{-1} 0.56). One PMC exhibited two trivalents (mean number cell^{-1} 0.22), the remainder showed bivalents and univalents ranging in number from 22—28 (mean number cell^{-1} 24.1) and 8—20 (mean number cell^{-1} 13.11) respectively. The hypothesis that two pairing populations existed in the PMC's of this hybrid was supported by a "t" test which showed that the difference between

the group with 8—14 univalents and that with 20 univalents was significant at the 0.1% level.

A. hypogaea 'F 452.4' × *A. cardenasii* (*Table 7*)

Seven of the sixteen PMC's contained four or more quadrivalents (mean number cell^{-1} 2.88) and two PMC's had a single hexavalent (mean number cell^{-1} 0.13). *Figures 5* and *6* show PMC's with five quadrivalents. Four trivalents were observed (mean number cell^{-1} 0.25) of which two occurred in one PMC. The range of bivalents was 12—32 (mean number cell^{-1} 21.63) whilst as many as thirty two univalents were observed in one PMC (mean number cell^{-1} 5.44). The chromosome numbers in the PMC's of this genotype showed more variation than those found in the others.

One PMC was found with a two armed dicentric half chromatid bridge and an acentric fragment at anaphase 1 of meiosis (*Fig. 4*).

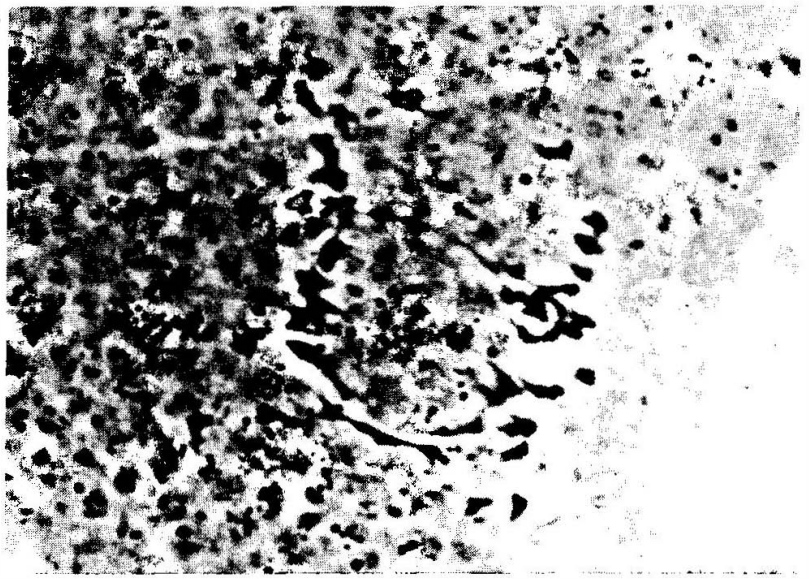

Fig. 4 Pollen mother cell of *A. hypogaea* 'F 452.4' × *A. cardenasii hexaploid* hybrid showing acentric bridge and fragment at anaphase I of meiosis (× 51 200)

Discussion

The presence of aneuploids in the species *A. hypogaea* would be expected because univalents, bivalents, trivalents and quadrivalents have been observed at meiosis in PMC's (D'Cruz and Upadhyaya 1962). The aneuploid condition could arise from an irregular distribution of chromosomes e.g. non-disjunction or 3-1 disjunction of a quadrivalent at anaphase I or the irregular distribution of univalents and trivalents. However, the frequency of univalents and trivalents at meiosis in PMC's is low (0.025 cell^{-1}, each, as reported by D'Cruz and Upadhyaya 1962) and therefore the frequency of uneven numbered aneuploids from this source would also be expected to be low. This is confirmed by these results which also indicate that quadrivalents either separate 2-2 or 0-4. Whether aneuploidy involves only one homoeologous group of chromosomes which behave in this manner, or the whole complement needs further study. These results and the morphological similarity of the aneuploids indicates the former. The small size of the seeds which produce

aneuploid plants is probably a contributory factor as to why few aneuploids of *A. hypogaea* have been reported. The smaller seeds do not always germinate, nor do they always produce aneuploids, as shown in *Table 1*, and the seedlings are sometimes less vigorous (SIVASUBRAMANIAN and RAMAKRISHNAN 1974) and therefore may not survive in competition with more vigorous euploids. Also, the small seeds may be lost at harvest, especially if seed is graded before sowing. The aneuploid nature of the F_6 plants which were originally hexaploids is more complex. It is known that the hexaploid level was reached in the F_2 generation (SMARTT 1964, GREGORY, pers. comm.), and so chromosome loss took place in the intermediate generations. This could have occurred by several methods.

One method is by cross-pollination with other plants, probably tetraploid *A. hypogaea*, when the plants were grown in the field. Although the number of bees found at Raleigh, North Carolina are probably insufficient to effect the necessary number of cross pollinations, this is a possible cause of the reduction in chromosome number.

From the chromosome counts obtained of the few F_7 and F_8 plants which have been grown it would appear that chromosome loss has ceased and the numbers have stabilised near to but not below the tetraploid level (*Table 4*). It is interesting to note the number of chromosomes in the progeny of the backcrosses (*Table 3*). If meiosis was regular the progeny of the F_6 ($2n=44$) × *A. hypogaea* ($2n=40$) cross would be expected to have 42 chromosomes. However, the single progeny examined had $2n=40$. This could have resulted from non-disjunction as a few quadrivalents were observed in the F_6 plants as well as in *A. hypogaea*. From the results of the backcrosses to the diploid parent, where extra chromosomes also appeared in the progeny, non-disjunction probably occurred in the F_6 plants.

The multivalents observed at diakinesis, examples of which are shown in *Figures 5* and *6*, could be the result of autosyndetic pairing between *A. hypogaea* chromosomes or heterogenic associations between *A. hypogaea* and *A. cardenasii* chromosomes. HUSTED (1933, 1936) was the first to observe multivalents in PMC's of *A. hypogaea* and it has since been recognised that

Tab. 6 Chromosome associations in PMC's at diakinesis of *A. hypogaea* 'Samaru 38' × *A. cardenasii* hexaploids

2n	No. of PMC's	Chromosome association			
		I	II	III	IV
60	1	10	25	—	—
64	1	8	28	—	—
64	1	10	27	—	—
64	1	12	24	—	1
64	2	12	26	—	—
64	1	14	23	—	1
64	1	20	20	—	1
66	1	12	22	2	1
68	1	20	22	—	1
Mean associations cell^{-1}		13.11	24.10	0.22	0.56

all tetraploid members of the genus can form one to three quadrivalents (RAMAN and KESAVAN 1963). If the quadrivalents observed in the allohexaploid hybrids reported here were due only to autosyndesis, one would expect their frequency to be similar to that found in *A. hypogaea*. The results show that this is not the case, the frequency of multivalents in the hexaploid hybrids and in *A. hypogaea* 'F 452.4' × *A. cardenasii* (*Table 7*) in particular, is higher than in *A. hypogaea*.

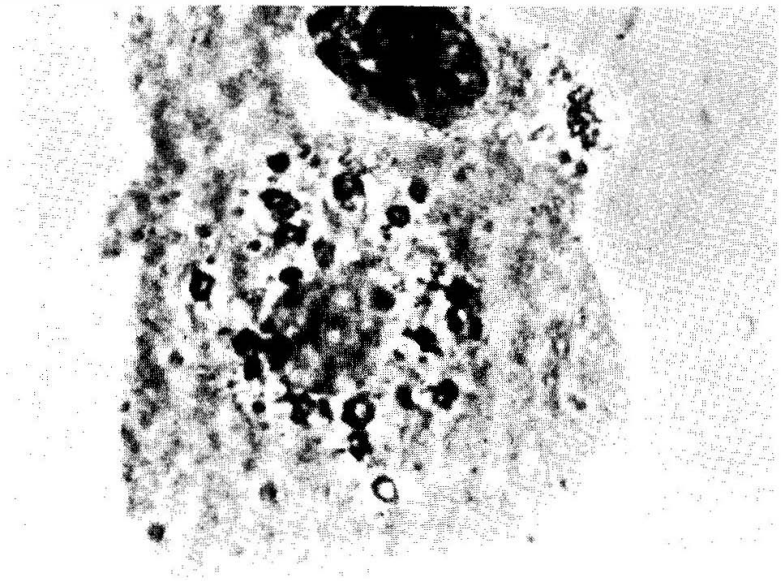

Fig. 5 Pollen mother cell of *A. hypogaea* 'F 452.4' × *A. cardenasii* hexaploid hybrid (2n = 62) showing 5 IV, 20 II and 2 I at diakinesis (× 51 200)

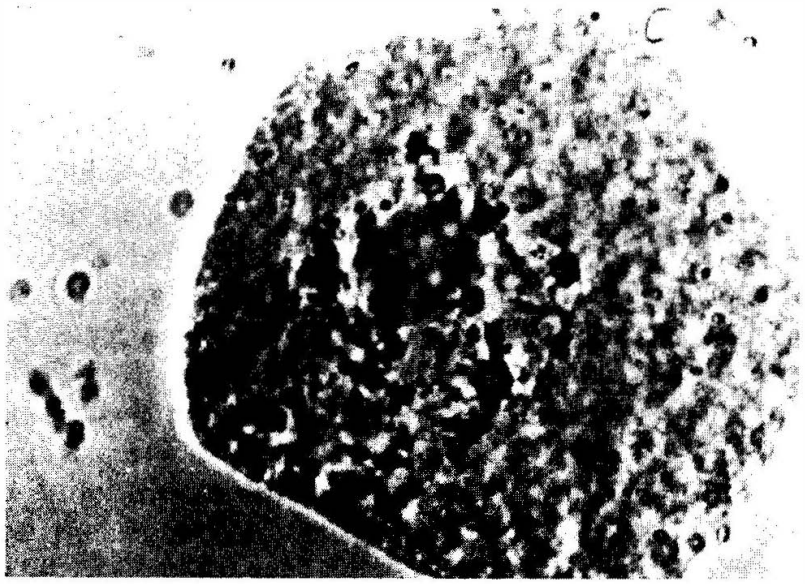

Fig. 6 Pollen mother cell of *A. hypogaea* 'F 452.4' × *A. cardenasii* hexaploid hybrid (2n = 64) showing 5 IV, 22 II at diakinesis (× 51 200)

It is possible that the presence of the *A. cardenasii* genome could induce unusually high pairing to occur between the *A. hypogaea* chromosomes, but the heterogenetic association of *A. hypogaea* and *A. cardenasii* chromosomes is a more likely cause.

The frequency of multivalents, including hexavalents and octavalents, and the segregation occurring in the progenies of hexaploids, indicates that

Tab. 7 Chromosome associations in PMC's at diakinesis of *A. hypogaea* 'F 452.4' × *A. cardenasii* hexaploids

2n	No. of PMC's	Chromosome association				
		I	II	III	IV	VI
56	1	6	18	2	2	—
58	1	—	21	—	4	—
60	1	4	26	—	1	—
60	1	32	12	—	1	—
61	1	6	22	1	2	—
62	1	—	29	—	1	—
62	1	—	18	—	5	1
62	1	1	19	1	5	—
62	1	2	20	—	5	—
62	1	6	16	—	6	—
62	1	10	24	—	1	—
64	1	—	32	—	—	—
64	1	—	22	—	5	—
64	1	4	17	—	5	1
64	1	4	26	—	2	—
64	1	12	24	—	1	—
Mean associations cell^{-1}		5.44	21.63	0.25	2.88	0.13

pairing is occurring between *A. hypogaea* and *A. cardenasii* chromosomes. It is not sufficient to indicate that these two species have a genome in common. In the *A. hypogaea* 'F 439.2' × *A. cardenasii* (*Table 5*) hybrids the mean number of unpaired chromosomes was 29.25 cell^{-1}, whereas it was 13.11 and 5.44 cell^{-1} in the *A. hypogaca* 'Samaru 38' × *A. cardenasii* (*Table 6*) and *A. hypogaea* 'F 452.4' × *A. cardenasii* (*Table 7*) allohexaploid hybrids respectively. Pairing thus appears to occur most readily between the genomes in the genetic and cytoplasmic — nuclear environment of 'F 452.4' × *A. cardenasii* and least readily in that of 'F 439.2' × *A. cardenasii*. It is possible that the three distinct *A. hypogaea* genotypes show differing degrees of structural difference from the *A. cardenasii* chromosomes. The identity of the unpaired chromosomes has not been determined. Failure to pair may occur because the chromosomes show cryptic structural differences as mentioned above, and the remaining mixture of *A. hypogaea* and *A. cardenasii* chromosomes are too dissimilar to form associations. This is a possible explanation where univalents are present in small numbers (as in most *A. hypogaea* 'F 452.4' × *A. cardenasii* PMC's), but where larger numbers of univalents are involved one would expect sufficient homologous or homoeologous chromosomes to be present for a greater level of pairing to occur than was observed. Alternatively, genetic factors, possibly within the *A. cardenasii* genome may act to prevent chromosome pairing. We have no knowledge of variation within *A. cardenasii* in this respect and a study of large populations of hybrids is needed.

The high numbers of univalents found in *A. hypogaea* 'F 439.2' × *A. cardenasii* and *A. hypogaea* 'Samaru 38' × *A. cardenasii* allohexaploids are inconsistent with the findings of previous investigators. The results for the

A. hypogaea 'F 452.4' × *A. cardenasii* hybrids however are comparable with those found D'CRUZ and CHAKRAVARTY (1961) and D'CRUZ and UPADHYAYA (1962) for an allohexaploid F_2 plant from an *A. hypogaea* × *A. villosa* cross and its progeny. Although little is known about the cytology of *A. hypogaea*, it is difficult to accept that genotypic differences between three *A. hypogaea* cultivars from the same subspecies are responsible for this discrepancy in meiotic behaviour and further investigation into this phenomenon is required.

The variation in chromosome number within a genotype is linked to the number of multivalents formed at diakinesis. *A. hypogaea* 'F 439.2' × *A. cardenasii* and *A. hypogaea* 'Samaru 38' × *A. cardenasii* allohexaploids consequently showed little deviation from a full complement of 64 chromosomes, whereas *A. hypogaea* 'F 452.4' × *A. cardenasii* hybrids had somatic numbers of 56, 58, 60, 61, 62 and 64. KUMAR et al. (1957) found similar variation in the chromosome numbers of the progeny of an allohexaploid hybrid between *A. hypogaea* and *A. villosa*. This type of variation is caused by the production of unbalanced karyotypes through unequal divisions in PMC's with univalents and multivalents in the preceding generation. Heterogenetic association may not be restricted to multivalent formation in hybrids, but may also be involved in bivalent formation as suggested by RAMAN and MANIMEKALAI (1975). Pairing of this type would be expected to give rise to karyological instability or segregation in successive generations.

The two armed dicentric half-chromatid bridge and acentric fragment observed could have been caused either by crossing over between inverted segments on homologous chromosomes or by spontaneous chromosome breakage and fusion. Spontaneous breakage and fusion is a more common cause (HAGA 1953) and may be of two types (LEWIS and JOHN 1966). One of these, producing a pseudo-bridge as the result of exchange, rather than union, between non-sister chromatids and the implications of exchange, particularly between *A. hypogaea* and *A. cardenasii* chromosomes are important in the field of peanut breeding. KUMAR et al. (1957) also observed chromatin bridges in allohexaploid *A. hypogaea* × *A. villosa* hybrids.

Chromosome loss had obviously occurred in the allohexaploid *A. hypogaea* × *A. cardenasii* hybrids produced by Smartt, to give near tetraploid F_6 plants. However, several F_8 plants exhibited *A. cardenasii* type floral and vegetative morphology. The expression of these characters indicates either that not all *A. cardenasii* chromosomes are lost and that one or more remain in the chromosome complement at the tetraploid level, or that exchange of genetic material between *A. hypogaea* and *A. cardenasii* chromosomes had taken place. If the loss of chromosomes from hexaploid hybrids which had been described is found to be a common occurrence, the implications for the peanut breeder are serious. The breeder's attempts to transfer genetic material between species will be futile unless recombination occurs, or there is sufficient homoeology between certain *A. hypogaea* and *A. cardenasii* chromosomes to permit the replacement of specific *A. hypogaea* chromosomes by *A. cardenasii* chromosomes and for this hybrid complement to be perpetuated. The results show that *A. cardenasii* genes are present in the F_8 generation of an *A. hypogaea* × *A. cardenasii* cross despite chromosome loss and that recombination has taken place.

Evidence is provided not only by the *A. cardenasii* characters appearing in the F_8, but also by the cytological data. The high frequencies of multivalents observed in the PMC's of the allohexaploid hybrids, particularly the *A. hypogaea* 'F 452.4' × *A. cardenasii* genotypes are only explained satisfactorily by the heterogenetic association of *A. hypogaea* and *A. cardenasii* chromosomes.

RAMAN (1973) suggests that the frequency of quadrivalents in an allopolyploid hybrid is higher than in the parent, the increase resulting from chromosomal exchanges which have occurred through allosyndetic pairing. Where heterogenetic pairing is taking place, the possibility of gene exchange and recombination exists and tetraploids derived from hexaploids where heterogenetic pairing has occurred are worthy of attention by the plant breeder. Disease resistant plants with good yield potential have been selected from such material at ICRISAT. The observed bridge and fragment could have arisen through a "U" type exchange involving non-sister chromatids in a bivalent; and if, as suggested by RAMAN and MANIMEKALAI (1975), heterogenetic pairing is involved in bivalent formation, the possibility exists that segments of *A. hypogaea* and *A. cardenasii* chromosomes could be exchanged by this means.

As mentioned above, the mechanism leading to chromosome loss is probably heterogenetic pairing giving rise to gametes with unbalanced karyotypes, the resultant plants stabilising near the tetraploid level. In addition, where pairing is restricted and large numbers of univalents are present, the possibility of unequal segregation and univalent loss is increased.

A PMC which produces thirty two bivalents or one quadrivalent and twenty nine bivalents at meiosis as in *A. hypogaea* 'F 452.4' × *A. cardenasii* can go on initiating gametes with balanced karyotypes. However, a PMC showing one quadrivalent, twelve bivalents and thirty two univalents will not have the same capacity to produce gametes with a perfectly regular chromosome number, and thus the tendency for chromosome loss to occur is greater. Competition between gametes of balanced and unbalanced karyotypes may have the effect of retarding the rate of chromosome loss, so that it is less rapid than might be expected.

Summary

The association of small seeds and aneuploidy in the tetraploid species *A. hypogaea* was investigated and from the results obtained, together with other reports, it may be concluded that fertile aneuploids were formed due to abnormal pairing and non-disjunction of the chromosomes at meiosis. Chromosome loss from hexaploid hybrids of *A. hypogaea* × *A. cardenasii* has been reported. Plants which were hexaploid in the F_2 generation had chromosome numbers near the tetraploid level in the F_6 generation. Meiotic behaviour in hexaploid *A. hypogaea* × *A. cardenasii* hybrids was investigated. The multivalents observed at diakinesis in the PMC's of hexaploid *A. hypogaea* × *A. cardenasii* hybrids were produced by the heterogenetic association of *A. hypogaea* and *A. cardenasii* chromosomes. This allosyndetic pairing could lead to the production of unbalanced gametes and the consequent karyological in-

stability which was observed in the *A. hypogaea* 'F 452.4' × *A. cardenasii* hybrids. This instability in chromosome number may result in chromosome loss and it is proposed that chromosome loss in the studied hybrids occurred by this method. Evidence supporting the occurrence of heterogenetic pairing was provided by a morphological study of twenty tetraploid F_8 progeny of an *A. hypogaea* × *A. cardenasii* cross. Despite the loss of up to twenty chromosomes in these plants, *A. cardenasii* characters were still present and therefore either one or more entire *A. cardenasii* chromosomes were present in the genome, or genetic material from *A. cardenasii* chromosomes had been incorporated into the *A. hypogaea* complement. The implications of recombination are important for the plant breeder in the face of chromosome loss from polyploid interspecific hybrids.

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Zusammenfassung

Chromosomenverlust und meiotisches Verhalten in Artbastarden des Genus *Arachis* L. und ihre Folgen für die Zucht krankheitswiderständiger Pflanzen

Das Auftreten von kleinen Körnern und der Aneuploidie in tetraploiden Arten von *A. hypogaea* wurde untersucht. Die Ergebnisse, sowie andere Mitteilungen, lassen erkennen, daß fruchtbare Aneuploide durch abnormale Paarung und Nichttrennung der Chromosomen während der Meiose entstehen. Über Chromosomenverlust in hexaploiden Bastarden von *A. hypogaea* × *A. cardenasii* ist schon berichtet worden; die in der F_2 hexaploiden Pflanzen hatten in der F_6-Generation Chromosomenzahlen, die der tetraploiden Stufe nahekamen.

Das meiotische Verhalten in den hexaploiden *A. hypogaea* × *A. cardenasii*-Bastarden wurde untersucht. Multivalente Assoziationen, die in der Diakinese in den PMC beobachtet wurden, sind durch heterogenetische Assoziation der Chromosomen entstanden. Diese allosyndetische Paarung führt zur Entstehung unbalancierter Gameten sowie zu karyotypischer Instabilität, die in *A. hypogaea* 'F. 452.4' × *A. cardenasii*-Bastarden beobachtet wurde. Diese Instabilität in der Chromosomenzahl kann Chromosomenverlust zur Folge haben; es wird vermutet, daß der Chromosomenverlust in den untersuchten Bastarden auf diesem Wege entstanden ist. Ein Beweis für das Vorkommen von heterogenetischen Paarungen wurde durch morphologische Untersuchung von 20 tetraploiden F_8-Pflanzen aus der Kreuzung *A. hypogaea* × *A. cardenasii* erhalten. Trotz des Verlustes von bis zu 20 Chromosomen, waren die *A. cardenasii*-Merkmale bei diesen Pflanzen immer noch vorhanden. Es wird deshalb angenommen, daß entweder ein oder mehrere vollständige *A. cardenasii*-Chromosomen in dem Genom anwesend gewesen sind, oder daß genetisches Material aus *A. cardenasii* wurde in *A. hypogaea*-Chromosomen aufgenommen wurde. Angesichts des Chromosomenverlustes in polyploiden Artbastarden sind die Folgen der Rekombination für den Züchter von großer Bedeutung.

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