CP 205

Breeding Strategies for Utilization of Wild Species of *Arachis* in Groundnut Improvement

1557

J.P. Moss[1]

Abstract

Utilization of wild species entails bringing genomes together and producing progenies which can be subjected to selection. The degree of similarity between species and between genomes dictates the techniques to be used. Studies on cytology, genetics, morphology, and evolution lead to a knowledge of species relationships, ploidy levels, and the taxonomic limits to hybridization. Closely-related species at the same ploidy level present few problems, but genes controlling chromosome pairing, and variation in number and position of chiasmata, may reduce or enhance the production of desirable segregants.

Differences in ploidy level between wild and cultivated species present problems in interspecific breeding, but ploidy manipulation has been successfully used, not only to achieve fertility in sterile hybrids, but also to adjust ploidy levels prior to hybridization. Induction of haploidy also has a role to play.

Aneuploidy, and substitution and addition lines can be useful tools in the identification and manipulation either of chromosomes carrying the genes that are to be transferred, or of genes controlling pairing or crossability that can be used to facilitate gene transfer.

Using these techniques, a wide range of interspecific Arachis *hybrids have been produced at ICRISAT, and derivatives with disease resistance and good yield have been introduced into the trials of the All India Coordinated Project for Oilseeds.*

Résumé

Stratégies de sélection pour l'utilisation des espèces sauvages. L'utilisation des espèces sauvages implique de rassembler des génomes et de produire des descendances pouvant être soumises à une sélection. Le degré de similitude entre les espèces et entre les génomes impose les techniques à utiliser. Les études de cytologie, génétique, morphologie et évolution, conduisent à une connaissance des relations des espèces, des niveaux de ploidie, et des limites taxonomiques à l'hybridation. Les espèces voisines de même niveau de ploidie posent peu de problèmes, mais les gènes contrôlant l'appariement des chromosomes et les différences de nombre et de position des chiasmas peuvent réduire ou accroître la production des ségrégants intéressants.

Les différences du niveau de ploidie entre les espèces sauvages et cultivées posent des problèmes pour la sélection interspécifique, mais la manipulation de la ploidie s'est révélée être un instrument puissant, utilisé non seulement pour rendre fertiles les hybrides stériles, mais également pour ajuster les niveaux de ploidie avant l'hybridation. L'induction de l'haploidie a également un rôle à jouer.

L'aneuploïdie, et les lignées de substitution et d'addition, peuvent se révéler très utiles pour l'identification et la manipulation soit des chromosomes portant les gènes à transférer, soit des gènes contrôlant l'appariement ou l'aptitude au croisement, qui peuvent être utilisés pour permettre le transfert de gènes.

Grâce à ces techniques, une gamme assez large d'hybrides interspécifiques d' Arachis *ont été produits à l'ICRISAT. Les dérivés de ceux-ci présentant les caractères de résistance aux maladies et de bon rendement font l'objet actuellement des essais de l'All India Coordinated Project for Oilseeds (Projet Coordonné Indien sur les Oléagineux).*

1. Principal Cytogeneticist, Groundnut Improvement Program, ICRISAT, Patancheru P.O., A.P. 502324, India

International Crops Research Institute for the Semi-Arid Tropics. 1985. Proceedings of an International Workshop on Cytogenetics of *Arachis*, 31 Oct - 2 Nov 1983, ICRISAT Center, India. Patancheru, A.P. 502 324, India: ICRISAT.

Introduction

Wild relatives of crop plants contain many characters, which may be valuable to the breeder in crop improvement. Foremost among these are disease and pest resistance, but wild species have also been shown to contribute other desirable characters, such as increased yield (Takeda and Frey 1976). The wild species of *Arachis* have been of interest primarily as a source of disease and pest resistance (Subrahmanyam et al. 1985; Amin 1985). The pioneering work on crossability (Gregory and Gregory 1979) made the utilization of wild *Arachis* species an attainable goal, and the early screening for disease resistance (Abdou et al. 1974; Sharief 1972) indicated which species were to be used.

When the desired character is not available in the cultivated germplasm, wild species and induced mutations present alternative sources of variability to the plant breeder. In the cultivated groundnut mutagens have produced some interesting novel plant forms but few, if any, widely adopted new cultivars have been released for commercial production (Gregory 1966; Anon 1967; Patil 1977). The use of tissue culture for the release of somaclonal variation (Larkin and Scowcroft 1981) or for selection of cells in culture has yet to be attempted in *Arachis*.

Wild species can be of value even when good resistance is available in the cultivated germplasm, as they may have different resistance genes which can be utilized to give more stable resistance (Singh et al. 1985). The choice of cultivated parent is important, and there are many genotypes of cultivated germplasm from which to choose. Although there is no evidence of crossability genes in *Arachis* as effective as those in wheat, cultivars differ in their crossability with wild species.

This paper discusses the stategies used to obtain interspecific derivatives which can be used in crop improvement programs.

Wild Species

In any genus, the wild species available to the plant breeder consist of a number of accessions, whose correct taxonomy may or may not be known. Accessions of the same species may differ from one another, and all accessions should be screened for desirable characters. Where more than one species or accession is resistant to a pathogen, the type of resistance may differ, and it may be possible to transfer different components of resistance (Subrahmanyam et al. 1983). The wild species with the desired character may be very similar to, and freely crossable with, the cultivated species, and the progeny of the interspecific hybrids may be fully fertile. Hammons (1970) crossed *A hypogaea* with *A monticola* and developed 'Spancross' without the application of special cytogenetic techniques. This successful production of a cultivar indicated that pairing and recombination between wild and cultivated chromosomes had occurred.

A number of intrasectional hybrids have been produced within section *Arachis* (Raman and Kesavan 1962; Gregory and Gregory 1979; Moss et al. 1981). However, only a few of these had been used in attempts to transfer characters from wild species into the cultivated groundnut (Moss and Spielman 1976; Stalker et al. 1979; Moss 1980) until ICRISAT groundnut cytogeneticists began an extensive program of interspecific hybridization (ICRISAT 198.). Despite many attempts, very few hybrids have been produced between *A hypogaea* and species of section *Rhizomatosae* (Gregory and Gregory 1979; Moss and Sastri 1982) and none of these have been used in a breeding program. Attempts to tap this valuable source of resistance genes by bridge crossing, using a third species compatible with both parents, have not been fruitful (Stalker 1981).

There are seven sections with a wide range of morphology and two ploidy levels in the genus. Some intersectional hybrids have been produced, but some sections are isolated, and all sections other than *Arachis* are isolated from *A hypogaea*. There are, therefore, two major priorities in gene transfer from wild *Arachis* species. One is to transfer resistance to the two major leaf spot diseases, *Cercospora arachidicola* Hori and *Cercosporidium personatum* Berk et Curt., from compatible wild species in section *Arachis* (Abdou et al. 1974; Subrahmanyam et al. 1985). The second is to tap the virus resistance of section *Rhizomatosae* (Subrahmanyam et al. 1985, in press). The former involves ploidy manipulations among hybrids that are comparatively easy to obtain. The latter involves a major input to overcome barriers to hybridization between sections *Arachis* and *Rhizomatosae*, with the possibility that the techniques developed will be of value in producing other intersectional hybrids.

The production of an interspecific hybrid brings genomes together in the cytoplasm of one of the species. Often that is a considerable achievement,

but the desired end product is usually the cytoplasm and genome of the cultivated species with only a small part of the wild species genes. It is often more difficult to produce the agronomically-acceptable line than to produce the original interspecific hybrid. Production of interspecific derivatives involves the disciplines of cytogenetics and plant breeding. Where resistance genes are to be transferred, the assistance of pathologists and entomologists will be needed. The success of utilization of wild species of *Arachis* at ICRISAT owes much to the strong interdisciplinary cooperation in the Groundnut Improvement Program.

Intrasectional Hybrids

Intrasectional hybrids have been produced in most sections (Gregory and Gregory 1979) but their possible use in the improvement of *A. hypogaea* has not been explored. The possibility of using induced auto- or allotetraploids from intrasectional hybrids has not been researched. Emphasis has been placed on hybrids within section *Arachis* to utilize resistance to leaf spots and rust (*Puccinia arachidis* Speg.) diseases because of the crossability of most section *Arachis* species with *Arachis hypogaea*.

The cultivated groundnut is a successful allotetraploid, probably originating from two section *Arachis* diploid species. There is some controversy as to the donor of the A genome but *A. batizocoi* which is always cited as the B genome donor (Smartt et al. 1978) is the only known B genome species. Little is known of the genomes in other sections, as detailed karyomorphological studies have only been completed in section *Arachis* (Stalker and Dalmacio 1981; Singh and Moss 1982) and few intersectional hybrids have been produced or studied (Stalker 1981).

There are several routes to transfer genes from a diploid wild species to a cultivated tetraploid. Amphiploidy and autotetraploidy have been used extensively at ICRISAT (Singh 1985). Haploidy could be a valuable tool, not only to transfer genes from wild species, but also in breeding programs using cultivated germplasm. Haploidy cannot be induced in *Arachis* at present, though some progress has been made in anther culture techniques (Mroginski and Fernandes 1979, 1980). Aneuploidy has been reported in the genus *Arachis* (Spielman et al. 1979), and aneuploids could be used in future to transfer whole chromosomes by substitution or addition. Aneuploidy is also useful in the manipulation of genes controlling chromosome pairing or crossability.

All available means of interspecific gene transfer by sexual hybridization involve the production of a hybrid between the wild species and a cultivar or germplasm line of the cultivated species. This may be the product of the first hybridization in the crossing program, or the cultivated species may not be used as a parent until after a series of crosses and/or ploidy manipulations. The primary wild x cultivated hybrids may be sterile, or only partially fertile, (e.g., most triploids), or may be fully fertile and vigorous, (e.g., some amphiploid x *A. hypogaea* hybrids). Some of the latter hybrids must be repeatedly backcrossed to *A. hypogaea* to produce genetically - and cytologically-stable tetraploid lines compatible with *A. hypogaea*, and with the desired genes from the wild species. The ideal introgressed line will have a genetic and cytoplasmic background in which the desired gene can be expressed. Such lines must also have suitable agronomic characters, such as yield, duration, and plant habit, that make them more useful to conventional plant breeders as sources of resistance. When backcrossing wild species hybrids to the cultivated species, the parental cultivar is not always used as recurrent parent. The introduction of another cultivar can bring in other useful characters, as well as inducing variation in the genetic background for the best expression of the desired gene. It is the best use of resources for the cytogeneticist to produce *A. hypogaea*-like derivatives that can be used by many breeders, rather than for individual breeders to use the primary hybrids. The breeder can then cross these lines to locally adapted cultivars without undesirable wild species characters occurring in the progenies.

The fertility, genomic constitution, and degree of recombination of the hybrids produced after the first crossing of the wild and cultivated species are the major factors that determine the time taken to achieve productive *A. hypogaea*-like derivatives of value to the breeder as sources of wild species genes. However, a knowledge of the genomes of the species does not imply that one can successfully transfer genes. Often that knowledge comes from the pairing behaviour at meiosis in hybrids. Thus, the attempts to transfer genes, and the cytogenetic analysis of species and interspecific hybrids have been a concurrent process at ICRISAT. Where cytogenetic studies have provided a sound base for planning future strategies for inter-

specific gene transfer (ICRISAT 1981; Singh and Moss 1982, 1984). The primary hybrid provides the first opportunity for wild and cultivated chromosomes to pair and recombine, and the progeny produced provides the first opportunity to select recombinants. This generation is the equivalent of an F_2 in a conventional breeding program, and for successful gene transfer this F_2 population must satisfy a number of requirements:

Size. In practice, the population should be as large as can be handled, to increase the opportunity of selecting fertile, productive plants which have the desired character combinations. Vegetative propagation of the primary wild x cultivated hybrid may be necessary to produce large segregating generations.

Range of genetic and cytoplasmic backgrounds. The cytogeneticist must produce more than one primary hybrid, produce reciprocals, use different accessions of wild species, and use different genotypes of the cultivated parent as these may differ in genes controlling pairing (Singh and Moss 1984). In one population, Company et al. (1982) reported that disease resistance was closely linked with undesirable pod shape, but disease-resistant lines with good agronomic characters,including pod shape have been selected from another population (Moss 1984).

Effective screening. Facilities to screen large populations are essential. The frequency of desirable segregants may be low; for example, the number of disease-resistant plants may be adequate, but many of these may be sterile or may have undesirable wild species characters (ICRISAT 1980; Company et al. 1982)

Working with large populations obviates the need to count chromosomes or check meiosis in all plants. Chromosome counts and meiosis checks are only made on key hybrids and plants with abnormal characters or reduced fertility. A number of plants from each selection are checked for chromosome number and regular meiosis (Table 1)

Table 1. Program for development of wild species derivatives[1].

Season	Activity	Selection criteria
1979 rainy	Seed increase, preliminary observations	Disease resistance, Yield, Season length, Chromosome number, Growth habit
1979-80 postrainy	Seed increase	Yield, Uniformity, Season length
1980 rainy	Progeny rows of selected plants	Disease resistance, Yield, Pod characters, Uniformity, Cytological stability
1980-81 postrainy	Seed increase	Yield, Uniformity
1981 rainy	Selection of lines	Disease resistance, Yield
1981-82 postrainy	Seed increase	Yield, Uniformity
1982 rainy	a Line selection	Disease resistance, Yield, Haulm yield, Oil yield
	b Seed increase for conservation	
	c Testing at different locations	
1982-83 postrainy	Seed increase	Yield

1 Lines with sufficient seed tested in randomized block or triple lattice designs

Results

A number of lines with resistance to late leaf spot and/or rust have been selected. The material which is most advanced was received as progenies of interspecific hybrids among the germplasm transferred to ICRISAT during 1978 and 1979. The species hybrids from which selections were made are listed in Table 2. Whilst this germplasm was being grown in the ICRISAT Post-Entry Quarantine Isolation Area, it was observed that these progenies were still segregating and that there were some desirable plants. A program of single plant selection and progeny row testing was started. Disease resistance was assessed in the rainy season when natural levels of inoculum were high, and the postrainy season was used for generation advance and assessment of yield, crop duration, uniformity,

and other agronomic characters (Table 1). Early generation yield selections were based on number of pods per plant, and in later generations on pod weight per plot. Selections were grown in replicated trials as soon as possible, usually in the second or third generation after single-plant selections were made (Plate 4a). Trials were also grown at two sites in Tamil Nadu, South India, where there was a heavy incidence of leaf spot but little rust infection and so minimal interaction between pathogens.

A number of interspecific derivatives have been entered in varietal trials of the All India Coordinated Research Project on Oilseeds (AICORPO). One derivative, ICGS 50, [*A hypogaea* × *A cardenasii* (115 M), Selection H3/4E] was entered in the initial evaluation trial for virginia bunch types, in 1983 rainy season. It was retained in Zone IV (Southeastern) and Zone V (Peninsular) for further testing in the 1984 rainy season.

Two derivatives were entered in the foliar disease resistant varieties trial in 1983. Twenty entries and 3 controls were successfully grown at 6 locations. The entries were ICG FDRS 17 [*A hypogaea* × *A cardenasii* (9B) Selection 1/IV/3/11] and ICG FDRS 18 [*A hypogaea* × *A. cardenasii* (115 M) Selection H3/4L B4]. At Aliyarnagar, both entries were the most resistant to rust and late leaf spot. At Dharwad, ICG FDRS 18 was resistant to early and late leaf spots. At ICRISAT, both entries had higher pod yield than all three controls. ICG FDRS 17 yielded 3535 kg/ha, ICG FDRS 18, 3360 and the controls 2250, 1843, and 1715 kg/ha. ICG FDRS 18 was one of three entries with the highest shelling percentage (64%), and also had the highest hundred kernel weight of 37 g. ICG FDRS 17 was the most resistant to late leaf spot. At Jalgoam, ICG FDRS 18 recorded the highest pod yield, 2055 kg/ha. At Kadiri, ICG FDRS 17 and 18 were resistant to late leaf spot, and at Vriddhachalam FDRS 17 was the most resistant to late leaf spot and rust (AICORPO 1984).

These and other selections from wild species derivatives also have good haulm yield, producing up to 6500 kg/ha of field-dried hay at harvest (Moss 1984). Many tetraploid wild species derivatives with disease resistance and good agronomic potential have been distributed to breeders.

Table 2. Pedigrees of hybrids from which wild species derivatives with resistance to disease, and with good agronomic characters have been selected.

Pedigree	Interspecific hybrid
82[1] × 34 - 9B	*A hypogaea* × *A cardenasii*
82 × 34 - 115M	*A hypogaea* × *A cardenasii*
82 × 19	*A hypogaea* × *A batizocoi*
82 × 19 × 8	*A hypogaea* × (*A batizocoi* × *A spegazzinii*)
HP12 - 8B	(*A batizocoi* × *A hypogaea* × *A spegazzinii*)

1 82, 34, 19, 8, are Gregory's parental numbers. (Gregory and Gregory 1979) HP12 designates a hybrid combination, and 9B, 115M and 8B are the original hybrid numbers.

Intersectional Hybrids

Very few intersectional hybrids have been produced (Gregory and Gregory 1979). Of the 42 possible intersectional combinations, including reciprocals, only eight have been produced, six of these involve section *Erectoides*. Although there has been some interest in bridge crosses, attempting to use the crossability of species of section *Erectoides* with species of other sections to transfer genes to *A. hypogaea*, there has been no success.

The major problem in intersectional crossing has been that few pegs are produced, and most hybrid pegs stop growing before they reach the soil. Pods are occasionally formed, but they contain very small seeds. Embryo rescue by tissue culture has been suggested, but has not been successful enough to be used as a routine technique. Ovule culture has been attempted as a means of rescuing embryos which are too small to excise and culture (Martin 1970).

Therefore emphasis has recently been placed on this technique at ICRISAT (Nalini and Sastri 1985). We all await with interest the production of a range of hybrids which can be studied in detail to further understand the genomic constitution of the genus, and to see whether whole genomes, or only parts of genomes are being transferred, and whether genes from other sections can be used in genetic improvement of *A. hypogaea*.

Protoplast culture in *Arachis* is a recent development, some success in culture, and in regeneration of callus, has been achieved (Oelck et al. 1982; Rugman and Cocking 1985). Hopefully the use of protoplasts to transfer whole or parts, of genomes will become a reality in legumes in the near future.

References

Abdou, Y.A-M., Gregory, W.C., and Cooper, W.E. 1974. Sources and nature of resistance to *Cercospora arachidicola* Hori and *Cercosporidium personatum* (Berk et Curtis) Deighton in *Arachis* species. Peanut Science 1(1):6-11.

AICORPO (All India Coordinated Research Project on Oilseeds). 1984. Groundnut Annual Progress Report 1983-84. Rajendranagar, Hyderabad, A.P. 500 030, India. Indian Council of Agricultural Research, Directorate of Oilseeds Research.

Amin, P.W. 1985. Resistance of wild species of groundnut to insect and mite pests. Pages 57-60 *in* Proceedings of an International Workshop on Cytogenetics of *Arachis*, 31 Oct - 2 Nov 1983, ICRISAT Center, India. Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Anon. 1967. List of mutant varieties. Mutation Breeding Newsletter 7:12-13.

Company, M., Stalker, H.T., and Wynne, J.C. 1982. Cytology and leafspot resistance in *Arachis hypogaea* x wild species hybrids. Euphytica 31:885-893.

Gregory, M.P., and Gregory, W.C. 1979. Exotic germplasm of *Arachis* L. interspecific hybrids. Journal of Heredity 70: 185-193.

Gregory, W.C. 1966. Mutation breeding. Pages 189-218 *in* Plant breeding, a symposium. (Frey, K.J., ed.) Ames, Iowa:Iowa State University Press.

Hammons, R.O. 1970. Registration of Spancross peanuts. Crop Science 10:459.

ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). 1980. Cytogenetics. Pages 133-141 *in* Annual Report 1979-80. Patancheru, A.P., 502 324, India: ICRISAT.

ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). 1981. Utilization of wild species. Pages 198-213 *in* Annual Report 1980. Patancheru, A.P. 502 324, India: ICRISAT.

Larkin, P.J., and Scowcroft, W.R. 1981. Somaclonal variation - a novel source of variability from cell cultures for plant improvement. Theoretical and Applied Genetics 60: 197-214.

Martin, J-P. 1970. Culture in vitro d'ovules d'arachide. Oléagineux 25: 155-156.

Moss, J.P. 1980. Wild species in the improvement of groundnuts. Pages 525-535 *in* Advances in legume science: proceedings of the International Legume Conference, 24-29 July 1978, Kew, Surrey, UK (Summerfield, R.J., and Bunting, A.H., eds.). Vol.2. Kew, Surrey, UK: Royal Botanic Gardens.

Moss, J.P. (In press.) Wild species in crop improvement *in* Proceedings of the Inter Center Seminar on IARC's and Biotechnology, 23-27 Apr 1984, International Rice Research Institute, Los Banos, Laguna, Phillipines.

Moss, J.P., and Spielman, I.V. 1976. Interspecific hybridisation in *Arachis*. Proceedings of the American Peanut Research and Education Association, Inc. (APREA) 8(1):88 (Abstract).

Moss, J.P., and Sastri, D.C. 1982. Wide hybridization in *Arachis*: problems and prospects. Page 4 *in* Abstracts of proceedings of the International Symposium on New Genetical Approaches to Crop Improvement, 6-10 Feb 1982, Karachi, Pakistan. Karachi, Pakistan: Pakistan Atomic Energy Agricultural Research Center.

Moss, J.P., Spielman, I.V., Burge, A.P., Singh, A.K., and Gibbons, R.W. 1981. Utilization of wild *Arachis* species as a source of *Cercospora* leafspot resistance in groundnut breeding. Pages 673-677 *in* Perspectives in cytology and genetics (Manna, G.K., and Sinha, U., eds.) New Delhi, India: Hindasia.

Mroginski, L.A. and Fernandez, A. 1979. [The culture *in vitro* of anthers of groundnut species (Leguminosae).] Cultivo *in vitro* de anteras de especies de *Arachis (Leguminosae)* (In Es., Summaries in En., Fr.) Oléagineux 34(5):243-248.

Mroginski, L.A. and Fernandez, A. 1980. [Obtained plantlets by *in vitro* culture of anthers of wild species of *Arachis* (Leguminosae).] Obtención de plántulas por cultivo *in vitro* de anteras de especies silvestres de *Arachis* (Leguminosae) (In Es., summaries in En., Fr.) Oléagineux 35(2): 89-92.

Nalini Mallikarjuna and Sastri, D.C. 1985. In vitro culture of ovules and embryos from some incompatible interspecific crosses in the genus *Arachis* L. Pages 93-99 *in* Proceedings of an International Workshop on Cytogenetics of *Arachis*, 31 Oct - 2 Nov 1983, ICRISAT Center, India. Patancheru A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Oelck, M.M., Bapat, V.A., and Schieder, O. 1982. Protoplast culture of three legumes: *Arachis hypogaea*, *Melilotus officinalis* and *Trifolium resupinatum*. Zeitschrift für Pflanzenphysiologie 106:173-177.

Patil, S.H. 1977. Radiation induced mutants for improving groundnut production. Indian Farming 26 (10):19-22.

Raman, V.S. and Kesavan, P.C. 1962. Studies on a diploid interspecific hybrid in *Arachis*. Nucleus 5:123-126.

Rugman, Emma E. and Cocking, E.C. 1985. The development of somatic hybridisation techniques for groundnut improvement. Pages 167-174 *in* Proceedings of an International Workshop on Cytogenetics of *Arachis*,

31 Oct - 2 Nov 1983, ICRISAT Center, India Patancheru, A.P. 502324, India: International Crops Research Institute for the Semi-Arid Tropics

Sharief, Y. 1972. The inheritance of cercospora leafspot resistance in *Arachis* species. Ph.D. thesis, North Carolina State University, USA.

Singh, A.K. 1985. Genetic introgression from compatible wild species into cultivated groundnut. Pages 107-117 *in* Proceedings of an International Workshop on Cytogenetics of *Arachis*, 31 Oct - 2 Nov 1983, ICRISAT Center, India Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Singh, A.K., and Moss, J.P. 1982. Utilization of wild relatives in genetic improvement of *Arachis hypogaea* L. Part 2. Chromosome complement of species in section *Arachis*. Theoretical and Applied Genetics 61: 305-314.

Singh, A.K., and Moss, J.P. 1984. Utilization of wild relatives in genetic improvement of *Arachis hypogaea* L. V. Genome analysis in section *Arachis* and its implications in gene transfer. Theoretical and Applied Genetics 68: 1-10.

Singh, A.K., Subrahmanyam, P., and Moss, J.P. 1984. The dominant nature of resistance to *Puccinia arachidis* in wild *Arachis* species. Oleagineux 39: 535-537.

Smartt, J., Gregory, W.C., and Gregory, M.P. 1978a. The genomes of *Arachis hypogaea* L. 1. Cytogenetic studies of putative genome donors. Euphytica 27: 665-675.

Spielman, I.V., Burge, A.P., and Moss, J.P. 1979. Chromosome loss and meiotic behaviour in interspecific hybrids in the genus *Arachis* L. and their implications in breeding for disease resistance. Zeitschrift für Pflanzenzuchtung 83: 236-250.

Stalker, H.T. 1981. Hybrids in the genus *Arachis* between sections *Erectoides* and *Arachis*. Crop Science 21: 359-362.

Stalker, H.T., and Dalmacio, R.D. 1981. Chromosomes of *Arachis* species, section *Arachis*. Journal of Heredity 72: 403-408.

Stalker, H.T., Wynne, J.C., and Company, M. 1979. Variation in progenies of an *Arachis hypogaea* x diploid wild species hybrid. Euphytica 28: 675-684.

Subrahmanyam, P., McDonald, D., Gibbons, R.W., and Subba Rao, P.V. 1983. Components of resistance to *Puccinia arachidis* in peanuts. American Phytopathology 73(2): 253-256.

Subrahmanyam, P., Ghanekar, A.M., Nolt, B.L., Reddy, D.V.R., and McDonald, D. 1985. Resistance to groundnut diseases in wild *Arachis* species. Pages 49-55 *in* Proceedings of an International Workshop on Cytogenetics of *Arachis*, 31 Oct - 2 Nov 1983, ICRISAT Center, India Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Subrahmanyam, P., Moss, J.P., McDonald, D., Subba Rao, P.V., and Rao, V.R. (In press.) Resistance to *Cercosporidium personatum* leafspot in wild *Arachis* species. Plant Disease.

Takeda, K. and Frey, K.J. 1976. Contributions of vegetative growth rate and harvest index to grain yield of progenies from *Avena sativa* x *A. sterilis* crosses. Crop Science 16: 817-821.

Taxonomy and Means of Utilization — Discussion

Rees:
I am struck by the lack of references to single gene "markers" or to linkage data, either from the cultivated or wild species. Are such data difficult to obtain? They might well be useful to mark and follow the transmission of chromosomes in hybrids, hybrid derivatives, and in aneuploids?

Moss:
There is very little information on gene markers in *Arachis*, and very few linkage groups are known (see Wynne, J.C., and Coffelt, T.A. 1982. Genetics of *Arachis hypogaea* L. Pages 50-94 *in* Peanut science and technology (Pattee, H.E., and Young, C.T., eds.). Yoakum, Texas, USA: American Peanut Research and Education Society, Inc.)

Appa Rao:
Is there any information on the assignment of linkage groups to the chromosomes?

Stalker:
Only a few linkage groups are known, I recollect three being reported. As aneuploids are being developed the association of genes to specific chromosomes will be possible. Some work is in progress at several universities to characterize linkage groups, but the work is long term and information is slow to accumulate. Presently no genes have been associated with any particular chromosome.

Rees:
I think it may well be a difficult task to construct linkage maps in *Arachis* species. The reason is that the chiasmata at meiosis are highly localised towards the distal regions of the chromosomes. Unless master genes are located within these distal segments they cannot, by normal means, be mapped. Genes in interstitial segments will remain inseparable by crossing over.

Stalker:
Only three linkage groups have been described in *Arachis* and the majority of genetic work associated with morphological variants has been with seed coat color. Approximately 20 other genes have been identified. As a result of the few genetic studies, as compared to maize or tomato, linkage of genes with specific wild species traits are not known, and extensive work will be required before these association can be made.

Singh:
Dr. Murty, your inference, based on pachytene analysis, of putting *A. batizocoi* out of section *Arachis* or assigning it a different status within the section does not conform with other results. In biosystematic studies the conclusions are made based on collective evidence from different sources, such as morphology, geographical distribution, mitotic karyotype affinities, crossability, and pairing of chromosomes in F_1 hybrids, nearly all of which indicate that *A. batizocoi* is in section *Arachis*.

Murty:
F_1 hybrids of *A. hypogaea* with A genome species have higher pollen fertilities than those between *A. hypogaea* x *A. batizocoi*. All section *Arachis* hybrids have reasonable levels of fertility except those involving *A. batizocoi*. *A. batizocoi* has entirely different leaf anatomy from other section *Arachis* species. Amphidiploids among A genome species look more like *A. monticola* or *A. hypogaea*, but this is not so for those between A genome species and *A. batizocoi*.

Stalker:
Why do you consider *A. batizocoi* as belonging to a different section from *Arachis*?

Murty:
We do not consider *A. batizocoi* as belonging to another section. However, we do consider *A. batizocoi* as very diferent from other section *Arachis* species for the following reasons: it forms mostly sterile hybrids with other section *Arachis* species including the cultivated groundnut and it does not have the completely heterochromatic chromosome (A chromosome) characteristic of all other section *Arachis* species.

We do not at this stage recommend that *A. batizocoi* be separated from section *Arachis* but we emphasize its dissimilarity with the other section *Arachis* species.

N.C.Subrahmanyam:
How many genotypes from the species did you use for the measurements, how many cells were scored for each chromosome measurement and what is the coefficient of variation for each chromosome?

Murty:
One genotype of each species was used, and more than 30 pollen mother cells were scored. Coefficients of variation were very low.

Stalker:
How do you account for the fact that the somatic chromosome data of Stalker and Dalmacio, or Singh and Moss (which are very similar) is so greatly different from your data on meiotic chromosomes? This is especially evident for chromosomes with the nucleolar organizer, which is most easily observed?

Murty:
Differential condensation of eu- and heterochromatin during mitosis and meiosis, and also the pretreatment agents used by mitotic cytologists, may perhaps be responsible for mitotic karyotypes differing from pachytene karyotypes.

Sastri:
Based on pachytene analysis of representatives of different sections, will it be possible to predict the cytological performance of intersection hybrids?

Murty:
It is possible. It is likely that essentially true pairing resulting in chiasma formation and exchange of chromosome segments may occur to a certain extent in intersectional hybrids, since the basic karyotype in several sections appears to be the same. There should be homoeologies in the case of the six differentiated chromosomes.

Moss:
How much do your results correlate with mitotic results? ICRISAT's and NCSU's results on mitosis agree in broad principle, variations in numbering being consistent with stastitical variation due to fixing, staining, etc. Can you identify which meiotic chromosomes in your study correspond to the accepted numbering of the karyotype?

Murty:
The classification of chromosomes at pachytene in *Arachis* species has been based to a large extent on morphology. Slight length and arm ratio differences were shown to be adjusted at pairing in hybrids as has been found in other species. That the morphological criteria are accurate was confirmed by observing chromosome pairing in interspecific hybrids such as *A. hypogaea* x *A. monticola*, *A. hypogaea* x *A villosa* and *A hypogaea* x *A chacoense*.

Singh:
In our study of somatic complements *A glabrata* does not have the distinct A chromosome. However, it was shown to have a similar type of secondary construction to that present in *A batizocoi*, which has also been found to cross with *A glabrata* by Gregory and Gregory.

Murty:
The A chromosome was distinguished, by size only, by Husted and subsequent workers as a very small chromosome. At pachytene, we characterized it as the completely heterochromatic chromosome. The nucleolar organizers in *A glabrata* are of two types: metacentric and submetacentric. *A batizocoi* has centric nucleolar organizers.

Amin:
A monticola is very similar to *A hypogaea* and they also produce fertile hybrids. The pest reaction of *A monticola* and *A hypogaea* to a large number of sucking and defoliating insects is similar. Based on the available evidence, can we really call them separate species?

Stalker:
A hypogaea and *A monticola* are the same biological species, but different morphological species. Whether *A monticola* should be made a subspecies of *A hypogaea* is a taxonomic question whose answer depends on whether you are a "lumper" or "splitter". Because they are morphologically different, and the literature is full of references where they are two species, *A hypogaea* and *A monticola*, I believe the taxa should be referred to as two species.

Murty:
If you assume *A hypogaea* or *A monticola* to have been derived from an A genome species x *A batizocoi*, don't you also expect that *A. hypogaea* x A genome hybrids should have similar cytology and fertility to *A. hypogaea* x *A batizocoi* hybrids?

Stalker:
With the present accumulation of information, I believe *A. hypogaea* and *A. monticola* were derived sometime in ancient history, from a hybrid between an A genome and a B genome species. However, generalizations concerning meiotic behaviour of

triploid, interspecific hybrids should not be made using the presently-available cytological reports. To do a proper job, one should hybridize a common *A. hypogaea* with all the section *Arachis* species and then observe meiosis under uniform environments. The question of triploid fertility has to do with the frequency of unreduced gametes in specific hybrid combinations, not the closeness or relationship of the two species which were hybridized.

Rees:
Atreya has provided sophisticated information about the composition of the nuclear DNA in *Arachis* species. I would have thought that estimates of nuclear DNA amounts in themselves would be useful. Are there such estimates?

Atreya:
Yes. Resslar et al, 1981. [American Journal of Botany 68(2): 149-153] reported DNA amounts, and according to them the values are such that they cannot be used as a character in determining the ancestry of the cultivated groundnut.

Sastri:
Can we use haploid tissue for these studies? Resslar showed differences between the two subspecies of *A. hypogaea*.

Atreya:
Theoretically this is a good suggestion, but the amount of haploid tissue that is required to isolate pure DNA is impractical, especially when genomes of different species are to be analysed. Ressler's work is quantitative, it shows the amount of DNA per cell in different species in section *Arachis*. Our work is in analysing genomes (DNA) in terms of the DNA quality, so the work cannot be compared, each aspect gives different information. However, DNA content per cell together with qualitative analysis of DNA in different species, would help in establishing the extent of genome relationships or divergence between species.

Rees:
Peroxidase may be a 'model enzyme', but whatever the advantages or disadvantages of peroxidases as isozymes for distinguishing between varieties or species, I am sure that the comparisons would be that much more effective if five or six different groups of isozymes were used.

P.Subrahmanyam:
Utilization of high-yielding, rust-resistant *A. hypogaea* genotypes in backcrossing with hexaploids or pentaploids to produce near tetraploids may be useful in accumulation of rust-resistant genes from both wild species and cultivated groundnut. What are the problems involved in utilizing rust-resistant *A. hypogaea* in backcrossing?

Moss:
The combination of genes from wild species and from *A. hypogaea* may give more stable resistance, because there is evidence that they are different genes. However, it is difficult to detect the presence of both genes. At the time of backcrossing hexaploids, when we are losing chromosomes, is not the best time to introduce the other gene. This should be done by the breeder at the tetraploid level. The genes can then be detected either by their phenotype, if they affect different components of resistance, or by genotype, by crossing with a susceptible tester variety, and examining F_1 populations. In fact we have used NC 107090 in some of our backcrosses, but will not test for the presence of both genes until the tetraploid level.

N.C.Subrahmanyam:
On the problem of achieving recombination between chromosomes from wild relatives and those of the cultivated species it may be worthwhile using autotetraploids of wild diploid relatives which show useful characters, crossing them with the cultivated species, and then looking for recombinants.

Moss:
This is already being done (ICRISAT Annual Report 1983, pp 212-214) and is a valuable method.

Rao:
When we harvest groundnuts we observe many immature pods. Can we reduce the length of the flowering period to get more filling (i.e., make groundnut determinate with synchronous maturity)? Are there any such types in wild germplasm? Can we transfer the trait to cultivated species?

Moss:
We found a line with the opposite effect in one of our populations, it produced so many pegs that few pods matured. We have also found many other variants, but not the one you describe. The physiologists are now looking more closely at our lines and we hope something will materialize.