THE HISTOLOGY OF ARRESTED DEVELOPMENT IN EMBRYO SACS AND EMBRYOS OF TWO UNCULTIVATED PEANUT SPECIES *ARACHIS SPP.*

By

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ABSTRACT

Some disease resistant wild species of Arachis (peanuts) have a low seed yield although they flower and produce fertile pollen. Probable causes of the reduced seed-yield may include inadequate self-pollination, pollen-stigma incompatibility, egg sterility, syngamy failure, or embryo abortion. These phenomena were investigated in a study which is part of a peanut breeding program designed to incorporate disease resistance from wild species of Arachis into cultivated types of peanuts. Plants investigated were pollinated by hand, flowers collected 1-7 days after pollination, and the styles and ovaries dissected from the flowers. Styles were observed for pollen tube growth using fluorescent microscopy, and sectioned ovaries of killed and fixed material were studied to determine if embryos were present.

It was found that the two rhizomatous species studied had low seed yield which may be caused by two different types of incompatibility. Arachis hagenbeckii Harms (PI 276233) appeared to have post-fertilization embryo abortion and either sterile egg cells or failure in fertilization. Arachis sp. (PI 262286) appeared to have incomplete pollen tube growth with only a few pollen tubes reaching the ovule, and then only triple fusion of the polar nuclei took place. However, these observations are inconclusive because of the relative small amount of materials examined.

Chapter 1

INTRODUCTION

The genus Arachis comprises 30 to 50 species (Gregory et. al., 1973) with A. hypogaea, the cultivated peanut, being the best known. Presently, considerable effort is being expended to develop improved varieties. This effort involves the transfer of disease resistant genes from wild taxa of peanuts to cultivated peanuts (Norden, 1973). Hybrids are being developed as a means to transfer the disease resistant genes. Unfortunately, low fertility of the wild Arachis species hinders the development of hybrids. At Oklahoma State University a peanut breeding program was initiated to combine the desirable traits of selected wild taxa with those of the cultivated peanut. A part of that investigation, a study of the factors of fertility in two wild taxa possessing desirable pest resistant traits, was undertaken. The objectives were to determine the reasons for the arrested development of the embryo sacs and embryos in the hope that further research will find the means to overcome these barriers so that successful hybrids can be achieved.

Two rhizomatous wild peanuts, A. hagenbeckii (PI 276233) and Arachis sp. (PI 262286), are resistant to early leafspot Cercospora arachidicola and northern rootknot nematode Meloidogyne arenaria respectively. Unfortunately, they reproduce asexually by rhizomes and have low seed yield. These two species have been observed to

have profuse flowering but fail to set seed in either the field or in greenhouses at Stillwater, Oklahoma. Pollen of these wild peanuts is considered functional because it has germinated in artificial culture (Banks, unpublished) and affected fertilization in certain crosses involving other species of Arachis (Gregory and Gregory 1979).

Although little is known about embryology of the wild species, considerable embryological research has been conducted on A. hypogaea (Smith 1956a, 1956b). Its fertilization and early development have been investigated. Smith (1954) found that A. hypogaea L. (cultivated peanut) plants usually flower profusely. However, less than 15 percent of the fruits and seed survive to maturity even though flowers contain fertile pollen and fertile, receptive pistils (Smith 1956b). Approximately two-fifths of the A. hypogaea flowers failed to begin fruit development. Self pollination appears to be the rule although some cross pollination by bees occurs in the field (Culp et al., 1968).

Self-pollination occurs slightly before petal expansion, about sunrise on clear summer days, but varies somewhat with weather conditions. Twelve hours or so after pollination the pollen tube grows down through the long style, enters the embryo sac and releases its two sperm nuclei which begin the triple fusion process. The stigma is receptive somewhat before and at the time that the anthers dehisce (Smith, 1956a). The embryo sac of Arachis is of the polygonum type with an 8-nucleate 7-celled embryo sac. Usually the synergids of the embryo sac are short-lived structures which degenerate and disappear prior to or soon after fertilization. Much of the central cell becomes filled with starch grains after the 7-celled embryo sac has

become fully differentiated and before syngamy. In the majority of plants starch grains appear when the embryo sac is mature and there is a maximum number of grains shortly after fertilization. This condition occurs quite often in the Leguminosae. Syngamy and hypostace formation take place after starch grain appearance in Glycine, a related genus (George, George and Herr, 1979).

Assuming that pollen is viable, in the non-seed producing wild species reasons for their poor seed set could include: (1) inadequate self-pollination, (2) female (egg) sterility, (3) presence of pollen-stigma incompability factors, (4) syngamy failure, or (5) post-fertilization failure. Each of these possibilities will be discussed.

Inadequate self pollination could be brought about by mechanical failure in pollen transfer to stigmas. Anther dehiscence might not occur when the stigmatic crest was receptive. The failure of pollen being available when the stigma is receptive may possibly occur in A. hypogaea (Oakes, 1958).

The egg may be either non-functional or may not develop properly.

Improper interaction of haploid pollen tubes and the diploid cells of the stigma and style resulting in arrested pollen tube growth in the pistil may be one pollen-stigma incompatibility factor. This gametophytic incompatibility occurs primarily in plants such as Arachis, which have binucleate pollen (Brewbaker, 1964).

SYNGAMY FAILURE

Failure to set seed could be due to the failure of double fertilization mechanisms which would lead to syngamy failure. To date there is no evidence that either cytological irregularity at meiosis or

failure of syngamy is an important cause of seed failure in A. hypogaea. This may not hold true for other species of Arachis. It has been observed by Smith that double fertilization does not always occur with A. hypogaea or its hybrids (Smith, 1956a). When embryo development occurs but triple fusion fails, the unfertilized polar nuclei remain surrounded by starch grains as on the day of anthesis. When syngamy fails but endosperm develops, the starch is metabolized and disappears as it does following normal fertilization (Smith, 1956a).

POST FERTILIZATION FAILURE

Post fertilization failure could be due to embryo abortion, endosperm abortion, or limited embryo development. Severe delay in embryo and endosperm divisions may be a cause of embryo abortion in A. hypogaea as it may have been in interspecific crosses of Phaseolus. (Rabakoarihanta et al., 1979). Pollination and syngamy do not necessarily ensure fruit development. Fruit and seed failures occur in A. hypogaea at various times during peg development and fruit maturation (Smith, 1956b). Smith (1954) found that in the two varieties he studied 93.3% of the egg cells in all ovules were fertilized but only 53.5% of the potential fruits actually elongated by means of pegs. Under normal conditions of flowering, fruiting and vegetative growth, 30% to 50% of the flowers fail to initiate fruit development. The remaining ovaries remained dormant and failed to develop. The mature fruits harvested represented only about 13.5% of the original flower production (Smith, 1954).

In several plant species, malfunction of the endosperm after triple fertilization has been demonstrated to be the cause of early

embryo abortion and seed failure (Smith, 1956b). Johansen and Smith (1956) showed that the endosperm collapsed, followed by hyperplastic development of the maternal tissues of the seed in crosses of A. hypogaea and A. diogoii. In the instances of early embryo abortion of A. hypogaea cited above (Smith, 1956b), no irregularities in endosperm growth or morphology were detected. Smith (1956b) also reported that the common occurrence of the failure of the apical embryo to mature is not due to failure of the endosperm to maintain the nutritional balance among the tissues of the developing seed as Brink and Cooper (1947) have found in Medicago sativa. Failure occurred because the supply of nutrient materials was not sufficient to maintain both the rapid elongation of the peg and the continued growth of the seed it contained (Smith, 1956b). Murthy et al. (1981), after studying seed failure in A. hagenbeckii and A. glabrata indicated there were atypical embryo sac like structures encountered in A. glabrata. However, in their opinion, the real abnormality was encountered in fertilization. It proceeded very slowly and took place at a very low frequency.

Chapter 2

MATERIALS AND METHODS

Rhizomatous wild peanuts A. hagenbeckii Harms (PI 276233) and Arachis sp. (PI 262286) were selected for this study because of their known resistance to Cercospora leafspot and northern rootknot nematode respectively. Both exhibit profuse flowering but have little or no seed set. The wild Arachis species Arachis hagenbeckii Harms (PI 276233) and Arachis sp. (PI 262286) have not been successfully hybridized with cultivated peanuts (Gregory and Gregory, 1979). They will

hybridize with a third Arachis species Arachis spegazzinii Krap et Greg nom nud (PI 263133). Arachis spegazzinii Krap et Greg nom nud also hybridizes when used as the female with Arachis hypogaea (Gregory and Gregory, 1979) and is referred to as a "bridge species." Arachis hypogaea (Chico), an early maturing Spanish cultivated peanut was used as the cultivated representative and is a very good genotype for use in peanut breeding in Oklahoma. Table I summarizes the crosses studied in this project and is based on the crossibility work of Gregory (Gregory and Gregory, 1979) and Banks (unpublished).

The principal part of this study involved the natural selfing of Arachis hagenbeckii Harms (PI 276233) and Arachis sp. (PI 262286) as indicated by the starred crosses in Table I. The results of the crosses from non-starred combinations are summarized in Chapter 4.

TABLE I

CROSSES STUDIED. SPECIES LISTED ACROSS THE TOP OF THE TABLE ARE POLLEN SOURCES, AND THOSE LISTED DOWN THE LEFT SIDE ARE EGG SOURCES

	Arachis hagenbeckii (PI 276233)	Arachis sp. (PI 262286)	Arachis spegazzinii Krap et Greg nom nud (PI 263133)	Arachis hypogaea (Chico)
Arachis hagenbeckii Harms (PI 276233)	Self (Poor sd production)*	Poor	Inadequately tested	0
Arachis sp. (PI 262286)	Unknown	Self (Poor sd production)*	Inadequately tested	0
Arachis spegazzinii Krap et Greg nom nud (PI 263133)	†	†	Self (good sd production)	†
Arachis hypogaea (Chico)	0	0	0	Self (good sd production)

*Natural selfs
†Hybrids achieved
0 Hybrids attempted but not achieved
sd = Seed

Two pots each of Arachis hagenbeckii Harms (PI 276233), Arachis sp. (PI 262286), Arachis spegazzinii Krap et Greg nom nud (PI 263133) and Arachis hypogaea (Chico) were placed in a walk-in type growth chamber with a 28C, 12 hour photoperiod (6:00 a.m. - 6:00 p.m.) and a 22C 12 hour day regime designed to insure consistent flowering (Banks, 1976). Flowers were pollinated by hand to insure that pollen reached the stigma, labeled with tags and collected one to seven days later. The whole flowers were fixed in FPA (Johansen, 1940). The stigmas, styles and ovaries were dissected and Martin's (1959) fluorescence technique was used to determine if pollen tube germination had occurred. Styles, in dilute aniline blue, were observed under fluorescence microscopy (Ramming et al., 1973). Observations were made using a Schott BG-12 exciter filter and either a Schott OG-1 or OG-9 barrier filter. Illumination was by means of an HBO 200 watt mercury arc light source. In this technique, pollen tubes exhibit a yellowish fluorescence (Fig. 2).

Ovaries of those flowers not used in the pollen tube study were fixed in FPA, dehydrated, and embedded in paraffin (Johansen, 1940). The ovaries were sectioned at 12 micrometers, stained with Johansen's safranin and fast green procedure (Johansen, 1940) and examined microscopically. Photomicrographs (Fig. 1) were made of those ovule sections containing identifiable structures. With the use of photomicrographs it was possible to determine if an ovule contained an egg (Fig. 3), an embryo (Fig. 4), endosperm and starch (Fig. 5) or other structures. The presence and condition of the egg apparatus and sequence of fertilization and embryo formation were determined using these preparations.

Fig. 1. Two embryos in a sectioned peanut ovule. *Arachis sp.* (PI 262286) X *Arachis spegazzinii* Krap et Greg *nom nud*

Fig. 2. Pollen tube growth in a peanut style - whole amount section seen by fluorescence staining. *Arachis hypogaea* (Chico)

Fig. 3. Light micrograph of an egg in a *Arachis sp.* (PI 262286) self.

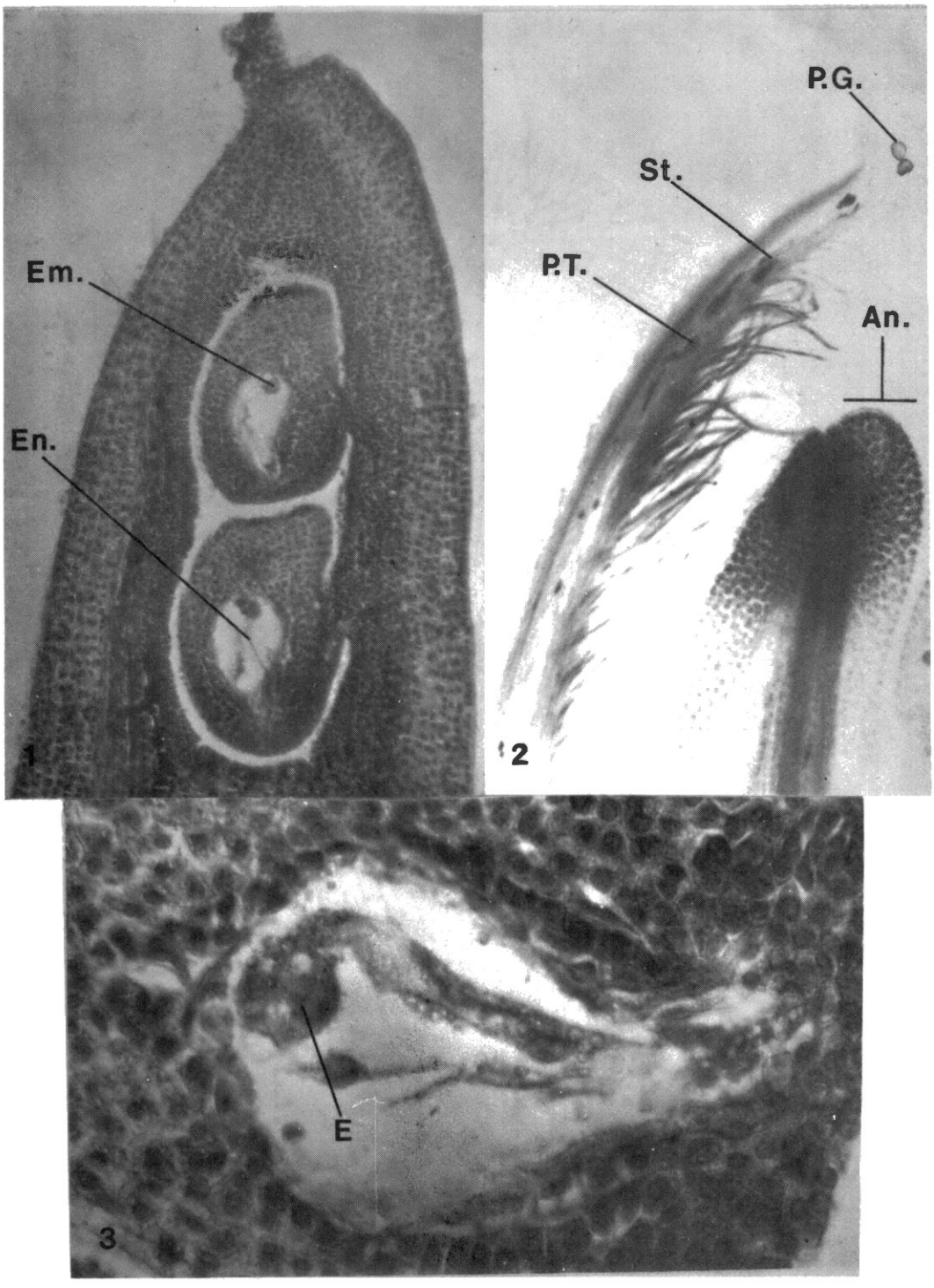
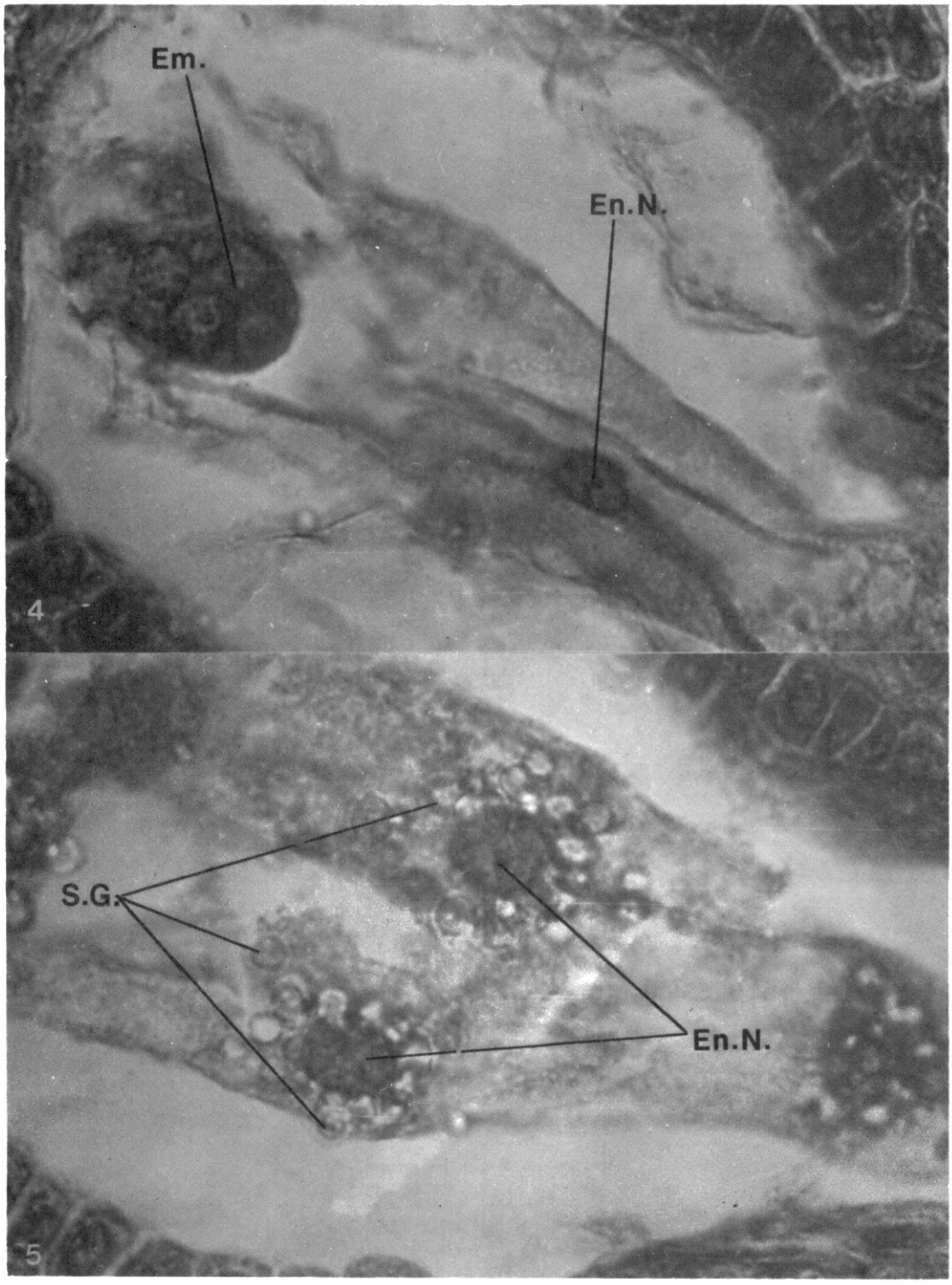

Em.
En.
1
P.G.
St.
P.T.
An.
2
E
3

Fig. 4. Light micrograph of an embryo and endosperm in an Arachis hypogaea (Chico) X Arachis Krap et Greg nom nud cross.

Fig. 5. Light micrograph of endosperm nuclei and starch grains of a Arachis hypogaea (Chico) X Arachis spegazzinii Krap et Greg nom nud cross.


Em.
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S.G.
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Chapter 3

RESULTS AND DISCUSSION OF *ARACHIS HAGENBECKII* HARMS (PI 276233) AND *ARACHIS SP.* (PI 262286)

The results of this chapter and Chapter 4 are summarized in Table II. All 26 of the styles of *Arachis hagenbeckii* Harms (PI 276233) studied showed pollen tube growth well into the style proper. This suggests stylar incompatibility is not the cause of low seed set in *Arachis hagenbeckii* Harms (PI 276233). However, since only five styles of *Arachis sp.* (PI 262286) showed pollen tube growth, stylar incompatibility cannot be ruled out. Each flower of both wild species examined contained two ovules per ovary. There were ten ovules of the total seventeen flowers, or thirty-four ovules, that had observable ovules which had either eggs or embryos. Some difficulty in interpretation of this material was experienced due to the small embryo size and poorly prepared slides.

Thirteen ovules of *Arachis hagenbeckii* Harms (PI 276233) contained conspicuous starch grains without developing endosperm, which suggests that triple fusion had failed to take place. The relatively low number of eggs (seven and fourteen) and embryos (three and zero) for *Arachis hagenbeckii* Harms (PI 276233) and *Arachis sp.* (PI 262286) respectively suggest partial sterility as well.

Endosperm development was noted in five of the ovules of *Arachis hagenbeckii* Harms (PI 276233) and nine ovules in *Arachis sp.* (PI 262286).

These observations suggest that pollen tubes germinate relatively well in both of the rhizomatous species studied but successful fertilization is less common. When fertilizations occur embryo abortions apparently take place resulting in low seed set.

Of the twenty-six observable ovules prepared from the thirty-six flowers of Arachis sp. (PI 262286) material not one contained a distinguishable embryo. Fourteen of the Arachis sp. (PI 262286) showed eggs while the rest were not identifiable as containing eggs. Endosperm was present in only nine of the ovules of Arachis sp. (PI 262286). Since endosperm can be present without developing embryos, triple fusion can occasionally take place without fertilization of the egg.

Twelve of the ovules of Arachis sp. (PI 262286) self had starch grains present. Two of these ovules had both endosperm and starch which might be expected since starch is consumed in endosperm formation.

It is apparent that fertilization of the polar nuclei can occur on occasion in Arachis sp. (PI 262286) but that fertilization of the egg must be rare if it occurs at all. Of the 11 styles of Arachis sp. (PI 262286) checked for pollen tube germination five of the styles showed pollen tube growth. It is possible that pollen tubes reach the ovules in about half the styles of Arachis sp. (PI 262286) and that when this occurs only fertilization of the polar nuclei takes place.

The results of this study indicate that low seed yield in the two rhizomatous species may be caused by different types of self incompatibility. Arachis hagenbeckii Harm (PI 276233) apparently permits pollen tube germination in its stigmas and at least partially successful triple fusion. Post fertilization embryo abortion apparently takes place in the ovules which are successfully fertilized and either

syngamy failure or egg sterility accounts for the unfertilized ovules.

Arachis sp. (PI 262286) apparently permits only partially successful pollen tube growth and of those pollen tubes that reach the ovule only triple fusion is afforded with syngamy occurring only rarely.

Much more study is needed to confirm these conclusions and to determine the methods for overcoming the incompatibility problems suggested.

Chapter 4

RESULTS AND DISCUSSION OF ATTEMPTED HYBRIDS AND SELFS OF SURVEY MATERIALS

The content of this chapter is summarized in Table II. Of the three Arachis hagenbeckii Harms (PI 276233) X Arachis sp. (PI 262286) cross ovules studied two had embryos: one with endosperm, one without endosperm, and one identifiable embryo structure but with endosperm. Of the nine styles observed for pollen tube germination, eight had pollen tube growth and one did not. Since some embryos were present and pollen tube growth took place most of the time it is assumed that the poor seed set is due to post-embryo abortion.

In the two attempts of Arachis hagenbeckii Harms (PI 276233) X Arachis spegazzinii Krap et Greg nom nud (PI 263133) only one ovule was studied. This ovule contained an egg and starch. Of the sixteen styles checked for pollen tube growth seven had pollen tube germination while nine showed no growth. It is possible that fertilization occurred only about 50% of the time. More material should be sectioned

and studied to determine the cause of incompatibility.

In the Arachis hagenbeckii Harms (PI 276233) X Arachis hypogaea (Chico) cross twelve of the twenty sectioned ovules were interpretable. Of these twelve ovules eleven had eggs but none had embryos. Pollen tube growth was not traced, in this cross twelve of the ovules contained starch grains. This finding suggests that fertilization did not take place in this cross. A study of the pollen tube germination in this cross should be undertaken to determine if pollen tube germination is taking place.

The only study done on the Arachis sp. (PI 262286) X Arachis hagenbeckii Harms (PI 276233) cross was to determine that of the six styles observed for pollen tube growth three showed pollen tube germination.

Of the six observed ovules of the Arachis sp. (PI 262286) X Arachis spegazzinii Krap et Greg nom nud (PI 263133) species four contained eggs and none of them contained embryos. It was not determined what percentage of the pollen grains germinated.

In the Arachis sp. (PI 262286) X Arachis hypogaea (Chico) cross only one of the eighteen ovules sectioned contained any interpretable structure. This structure was an egg. Pollen tube development was not traced in this cross.

Of the four observed ovules of the Arachis spegazzinii Krap et Greg nom nud (PI 263133) X Arachis hagenbeckii Harms (PI 276233) cross two had embryos, and one had an egg cell. One of the ovules with an embryo contained endosperm, but starch grains were not found in any of the ovules. All three of the styles observed indicated pollen tube into the style. This cross has been found to produce viable seed as would be expected by the successful pollen tube germination embryo development.

It has been reported that the Arachis spegazzinii Krap et Greg nom nud (PI 263133) X Arachis sp. (PI 262286) cross produces viable seed. This cross was not studied in this project except for a pollen tube germination study. Of the six styles observed every onehad successful pollen tube germination.

Of the eight observed ovules in the Arachis spegazzinii Krap et Greg nom nud (PI 263133) self it was found that all had embryos and every one of the five styles observed for pollen tube germination showed successful pollen tube germination. This was expected because the Bridge species usually produce viable seed in nature.

Arachis spegazzinii Krap et Greg nom nud (PI 263133) X Arachis hypogaea (Chico) is a cross yielding a high percentage of seed, but of the four observed ovules two contained eggs and two contained no apparent internal structure. Pollen tube development was not studied for this cross.

Of the sixteen observed ovules of the Arachis hypogaea (Chico) X Arachis hagenbeckii Harms (PI 276233), thirteen had eggs and none of them had embryos. The styles of this cross were not studied. It could be assumed that either the pollen tubes did not grow or incompatibility inhibited fertilization at some stage of development.

The Arachis hypogaea (Chico) X Arachis spegazzinii Krap et Greg nom nud (PI 263133) cross produced twenty-four observed ovules of which seventeen had embryos, three had eggs, three showed unidentifiable structures and one had no distinguishable structures. This cross has been shown in the past to produce no viable seed and it is assumed that post-embryo failure is involved.

In the Arachis hypogaea (Chico) self there were four observed

ovules. One had an embryo, two had eggs and one contained an unidentified structure. The three styles observed had well developed pollen tubes. This cross is known to result in viable seed. The two unfertilized eggs were not expected since not all flowers of cultivated plants produce seed.

From this study it is determined that sexual incompatibility in Arachis species may be caused by a number of different factors. This study is considered as extremely preliminary and therefore inconclusive. Much more work involving larger numbers of selfed and crossed materials where critical assessments of the structures can be made will be necessary to properly clarify the reasons for poor seed production in these and other wild species of Arachis.

Table II shown on the following pages represents a summary of some of the work attempted. The hand pollinated crosses and selfs are listed across the top while along the side is listed the various categories of observation.

TABLE II

DISPOSITION OF SECTIONED OVULES WITH RESPECT TO ANATOMICAL FEATURES NOTED

	A X A	A X B	A X Bridge	A X Cultivated	B X A	B X B	B X Bridge	B X Cultivated	Bridge X A	Bridge X B	Bridge X Bridge	Bridge X Cultivated	Cultivated X A	Cultivated X B	Cultivated X Bridge	Cultivated X Cultivated
Total number of flowers sectioned	17	2	1	10	0	36	10	9	4	0	6	3	12	0	14	2
Number of observable ovules	18 52%	3 75%	1 50%	12 60%	-	26 36%	6 30%	1 5%	4 50%	-	8 66%	4 66%	16 69%	-	24 88%	4 100%
Number of obscure ovules	16 47%	1 25%	1 50%	8 40%	-	46 63%	14 70%	17 94%	4 50%	-	4 33%	2 33%	7 30%	-	3 11%	0 0%
Number of observable ovules with embryos	3 16%	2 66%	0 0%	0 0%	-	0 0%	1 16%	0 0%	2 50%	-	8 100%	0 0%	0 0%	-	17 70%	1 25%
Number of observable ovules with eggs	7 38%	0 0%	1 100%	11 91%	-	14 53%	4 66%	1 100%	1 25%	-	0 0%	2 50%	13 81%	-	3 12%	2 50%
Number of observable ovules structures not identified	6 33%	0 0%	0 0%	1 8%	-	7 26%	1 16%	0 0%	1 25%	-	0 0%	0 0%	3 18%	-	3 12%	1 25%
Number of observable ovules with no observable structures	2 11%	1 33%	0 0%	0 0%	-	5 19%	0 0%	0 0%	0 0%	-	0 0%	2 50%	0 0%	-	1 4%	0 0%

A = Arachis hagenbeckii Harms (PI 276233)
B = Arachis sp. (PI 262286)

Bridge = Arachis spegazinii Krap et Greg nom nud
Cultivated = Arachis hypogaea (Chico)

TABLE II (Continued)

	A X A	A X B	A X Bridge	A X Cultivated	B X A	B X B	B X Bridge	B X Cultivated	Bridge X A	Bridge X B	Bridge X Bridge	Bridge X Cultivated	Cultivated X A	Cultivated X B	Cultivated X Bridge	Cultivated X Cultivated
Number of observable ovules with starch and no endosperm	13 72%	0 0%	1 100%	12 100%	-	10 38%	1 16%	0 0%	0 0%	-	1 12%	0 0%	14 87%	-	7 29%	1 25%
Number of observable ovules with starch	17 94%	0 0%	1 100%	12 100%	-	12 46%	3 50%	0 0%	0 0%	-	2 25%	0 0%	16 100%	0	19 79%	2 50%
Number of observable ovules with endosperm and no starch	1 5%	2 66%	0 0%	0 0%	-	7 26%	1 16%	0 0%	1 25%	-	2 25%	2 50%	0 0%	-	5 20%	0 0%
Number of observable ovules with endosperm	5 27%	2 66%	0 0%	0 0%	-	9 34%	3 50%	0 0%	1 25%	-	3 37%	2 33%	2 12%	-	17 70%	1 25%
Number of observable ovules with both starch and endosperm	4 22%	0 0%	0 0%	0 0%	-	2 7%	2 33%	0 0%	0 0%	-	1 12%	0 0%	2 12%	-	12 50%	1 25%
Number of styles checked for pollen tube growth	26	9	16	0 0%	6	11	3	0 0%	3	6	5	0 0%	0 0%	0 0%	0 0%	3
Number of styles with pollen tube germination	26 100%	8 88%	7 43%	-	3 50%	5 45%	2 66%	-	3 100%	6 100%	5 100%	-	-	-	-	3 100%

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VITA 2

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Thesis: THE HISTOLOGY OF ARRESTED DEVELOPMENT IN EMBRYO SACS AND EMBRYOS OF TWO WILD PEANUT ARACHIS SPECIES AND SELECTED HYBRIDS

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Education: Graduated from Edmond High School, Edmond, Oklahoma, in 1968; received the Bachelor of Science degree from Central State University, Edmond, Oklahoma, in May, 1972; completed the requirements for the Master of Science degree at Oklahoma State University, December, 1982.

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