The Cytology of Caryopsis Development in *Triticum-Agropyron* Amphiploids

by

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Thesis presented to the Faculty of Graduate Studies, McGill University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

1952

TABLE OF CONTENTS

Page

INTRODUCTION 1

MATERIALS AND METHODS 12

RESULTS 18

1. Male and Female Fertility, Spikelet Fertility and Percent Fertilization 18
2. Descriptions of Caryopsis Development in Group 1 Plants 25
3. Appearance and Germinability of Kernels of Group 1 Plants 44
4. Failure during Fertilization 46
5. Comparative Development of the Endosperm and Embryo in Group 2 Plants 55
6. Fertilization Failure in Group 2 Plants 59
7. Chromosome Numbers of Group 2 Plants 69

DISCUSSION 70

1. Female Fertility 70
2. Miscellaneous Abnormalities of the Embryo Sac 71
3. Sterility Caused by Failure during Fertilization 73
4. Sterility Caused by Failure during Development of the Endosperm and Embryo 77
5. Relation of Antipodal Development and Ovule Elongation to Failure during Fertilization 79

SUMMARY 83

PLATES ..... 85 to 90

INDEX TO PLATES ..... 91 to 94

ACKNOWLEDGMENTS 95

REFERENCES 96

I N T R O D U C T I O N

The occurrence of partial sterility in Triticum - Agropyron amphiploids has proved to be a major hindrance to the breeding of hardy perennial wheat and hardy, large-seeded, soil-binding forage crops in Canada and the United States. Following the first successful cross between Triticum and Agropyron credited by Armstrong (1936) to Tzitzin in 1930, extensive hybridization programs to achieve the aforementioned objectives were undertaken in these two countries and in the U.S.S.R. Approximately thirty species of Agropyron were used in crossing with the wheats and in a summary of these, eight were listed by Smith (1942) as having been successfully hybridized. The two species of Agropyron most successfully crossed were Agropyron elongatum (Host) Beauv. and A. intermedium (Host) Beauv. (A. glaucum Desf.) and these have been used exclusively in Canada in crosses with the wheats. Hybrids between the wheats and the eight species of Agropyron were, with the exception of those with A. elongatum, entirely sterile. The latter hybrids showed varying degrees of partial fertility, and from them Armstrong (1945) states it has been possible to develop a number of promising fertile lines.

To produce fertile derivatives of the sterile crosses, somatic doubling was first induced by Canadian workers. Peto (1936) used alternating high and low temperature treatments in

early zygotic divisions following crossing, and later Raw (1939), Peto and Boyes (1940) and Peto and Young (1942) used seed and plumule immersion in colchicine solutions. The amphiploids produced in this way contained the entire haploid complement of each parent species in duplicate. A. intermedium hybrid material was used exclusively in these treatments, since doubling in the A. elongatum group had been successful only in crosses with monococcum wheats. Armstrong and McLennan (1944) listed thirteen strains produced between 1937 and 1944 from the crossing of different varieties of Triticum vulgare, T. dicoccum, T. turgidum, T. durum, T. pyramidale, and T. persicum with A. intermedium.

These authors found a considerable amount of sterility among these amphiploids. Among a group of four strains from crosses with T. vulgare var. "No. 49", which had been allowed to multiply for four or five generations without selection, the number of seeds per spikelet varied from about 1.0 to 2.7. These differences may have been influenced in some instances by extensive morphological disparity between strains, inherited from the wheat parents. The extreme divergence of fertility between strains, however, can only partly be explained on a morphological basis.

Besides this inter-strain variation, they found very great differences in fertility between different lines and between individual plants of a strain. The coefficient of variation for fertility per 10 spikelets in the "Vernal"

strain, for example, was 19.44 per cent (average fertility 26.8 ± 1.16), indicating a large variation in fertility.

This was true also of the "No. 49" (S 107) strain. Average spikelet fertilities in F_3's of a number of F_2 lines ranged from 0.45 to 1.29, and spikelet fertilities of individual F_3 plants of the strain ranged from 0.0 to 2.0 seeds per spikelet. The complete range (0.0 - 2.0) was found in one F_2 line, and other lines showed extensive variation.

Cytological investigations on F_2 plants of the "Vernal" strain reported by Peto and Boyes (1940), and on F_2 and F_3 plants of the "No. 49" strain reported by Armstrong and McLennan (1944), indicated that a close relation existed between chromosome numbers in these plants and the degree of sterility they showed. In both investigations, the chromosome number in all but a few plants was below 70, the expected 2n number for perfect bivalent pairing in the ancestry, and aneuploid numbers as low as 58 were found. Meiotic stages displayed varying numbers of univalents, and occasional multivalents. Armstrong and McLennan (1944) found a significant correlation between the number of chromosomes and fertility in the F_3, those plants having chromosome numbers closest to 70 being the most fertile.

A similar situation in crosses between T. timopheevi and T. durum varieties and A. tricophorum, a species closely related to A. intermedium, was reported by Pope (1950) who found that pairing was only occasionally completely bivalent in the F_1 amphiploids, and that their progenies showed a decrease in the number of synapsed chromosomes and a variable chromosome number.

Fertility was positively correlated in some cases with the number of bivalents and total synapsed chromosomes, but showed no correlation with the chromosome numbers. A closely analogous case has been reported for the cross T. turgidum x Aegilops speltoides by Thompson, Britten and Harding (1943). About 30 per cent of a random group of F_2, F_3 and F_4 amphiploids differed from the expected chromosome number of 42 by one or two chromosomes, and univalents as well as multivalent associations were almost universally present in meiotic stages. Plants with divergent chromosome numbers were less fertile than those with the normal number.

It is probable that the aneuploids found in Triticum - Agropyron progenies arose through unequal segregation of chromosomes to the sporocytes because of pairing irregularities at meiosis. Such irregularities might be caused by competitional pairing due to homoeology between Triticum and Agropyron genomes. Genome homoeologies varying from two genomes to one or less were found between A. intermedium and the hexaploid wheats by various workers (see Sears, 1948, for review). Peto (1936) found a much lower chiasma frequency associated with bivalents in a T. dicoccum var. Vernal x A. intermedium hybrid than in A. intermedium, indicating that the pairing affinity, and hence the homoeology, was probably weak.

It is not surprising, therefore, that such affinities did not appear to effect any appreciable competitional pairing in the amphiploids. Peto and Boyes (1940) found an average frequency of only 0.14 total tri- and quadrivalents in F_2 amphiploids of

the above cross, which was less than the number of quadrivalents found in the undoubled hybrids (0.2). On the other hand, they observed a relatively large number of univalents (7.7), and fewer bivalents (30) than expected (35). They attributed this large number of bivalents to a hindrance of pairing by genetically-controlled physiological characters or by increases in the spatial distribution of homologues at zygotene caused by chromosome doubling. This view was shared by Pope (1950) concerning the univalent formation found in the A. trichophorum group of amphiploids. However, Armstrong and McLennan (1944) suggested that the univalents might also be the result of a competitive effect as a result of chromosome homoeology. Homoeologous pairing might prevent homologous pairing, but yet be so weak that it did not persist until metaphase, thus leaving univalents at this stage.

The univalents in undoubled F_1 and doubled F_2 hybrids of the "Vernal" cross behaved similarly at meiosis: they lagged at M.I, divided equationally, and the daughter halves wandered at random during M.II. As a result, a consistently large number of micronuclei (averaging 4.05 per tetrad for the Vernal emmers x A. intermedium cross) were found in early stages of pollen development in the amphiploids. This indicated a considerable loss of chromosomes in the nuclei of the gametophytes. The viability of the pollen, nevertheless, did not appear to be much reduced, for the plants in which a high frequency of micronuclei were seen produced an abundance of morphologically normal pollen.

Armstrong and McLennan's (1944) study of F_2 and F_3 progenies of the "No. 49" strain revealed no strong tendency to revert to a

lower chromosome number (F_2's averaged 65.2 and F_3's 65.8). It is probable, therefore, that low chromosome number segregates following meiosis were discriminated against, either because of viability or reduced functionability of the gametophyte or by post-fertilization failures. Such a discrimination was found to occur in pentaploid wheat hybrids of T. vulgare with T. durum and T. dicoccum by Thompson and Cameron (1928), Thompson (1930) and Thompson and Arnason (1932). These hybrids produced apparently normal pollen which showed a complete range of chromosome numbers from 14 to 21. However, pollen with intermediate numbers was less frequent than expected from random distribution of the seven univalents found at meiosis, and showed a tendency to delayed development. In addition, backcross progenies with the parental species revealed a pronounced lack of plants produced from pollen or eggs with intermediate numbers, connotating a preferential fertilization by gametes with chromosome numbers close to 14 or 21.

Gametic elimination in these hybrids would be expected to be associated with a certain degree of female sterility, although elimination of eggs with intermediate numbers was reportedly not as extensive as that of the pollen. On the other hand, when pollen is abundant, microgametophyte elimination and preferential fertilization might be expected to increase rather than decrease the fertility in these plants.

Thompson (1930) was able to demonstrate that imbalance of chromosome sets in backcrosses produced irregularities tending to bring about an abortion of the kernel. The presence or

absence in the endosperm of complete sets of extra vulgare chromosomes (the univalents) from hybrid gametophytes caused at most a wrinkling, or a reduction in size of the seed. The presence of incomplete sets only, especially in increased dosages, produced extreme irregularities in the development of viable and nonviable kernels. In this respect certain chromosomes, when present alone, especially in high dosages, appeared to be more effective than others. The proportion of abortion of endosperms and embryos in the hybrids themselves was considered by Thompson to be the result of chromosomal imbalance, produced by a compounding of gametes of intermediate numbers, in the hybrid endosperm.

Thompson (1940) has pointed out, however, that "Hybrid sterility in plants is usually attributed to chromosomal causes because it is nearly always associated with chromosomal irregularities at meiosis. But since related species commonly differ not only in the structure or number of their chromosomes, but also in the numerous individual genes, it is to be expected that genic and chromosomal causes will operate together in many cases, and that it will be very difficult to disentangle their effects, or to be sure which is at work."

According to Stebbins (1950) "Genic sterility includes all types which are produced by failure of the sex organs to develop up to the point where meiosis can take place, or by genically controlled abnormalities of the meiotic process itself, such as spindle formation and genically controlled synapsis or desynapsis." In the wider sense, however, it should by definition include any other gene-induced abnormalities in a plant which render it sterile.

Such further abnormalities would include post-meiotic defects in gametophyte development and maternal influence on the whole process of seed formation from pollen-tube germination on the stigma onwards.

A number of instances of hybrid sterility in which genic control has been definitely established have been reported (see Stebbins, 1945, for review). However, the first clear evidence that hybrid sterility included both chromosomal and genic elements was provided by a comparison of sterile hybrids and their amphiploid derivatives. Greenleaf (1942) found that in amphiploids of Nicotiana sylvestris ♀ x N. tomentosiformis, N. sylvestris ♀ x N. tomentosa, and N. tomentosiformis ♀ x N. sylvestris the embryo sacs degenerated at or before the 4-nucleate stage in all ovules, although the meiotic pairing was normally bivalent and the pollen highly functional. In the undoubled hybrids of these crosses, little or no meiotic pairing was observed, and the resulting chromosomal sterility effectively masked the genic sterility, which had no opportunity of being expressed.

Stebbins, Valencia and Valencia (1946) found genic sterility superimposed on chromosomal sterility in A. trachycaulum x Hordeum nodosum and Stebbins (1950) quoted Walters (unpub.) as having found the same in A. parishii x Sitanion jubatum. Stebbins considered, moreover, that the partial asynapsis reported by Love and Suneson (1945) and Pope (1950) in Triticum-Agropyron amphiploids was caused by asynaptic genes, and presumably

amphiploids studied by Peto and Boyes (1940) and Armstrong and McLennan (1944) might, with equal reason, be included in this group. It should be pointed out, however, that even when the control of asynapsis is genic in nature, the effects subsequent to meiosis are identical with those caused by pairing failure because of non-homology.

Several types of sterility mechanisms, under genic control or under chromosomal influence in the form of imbalance, have been demonstrated as operating during the period lasting from deposition of functional pollen on the stigma to late stages in seed development.

Allelic series of incompatibility alleles affecting pollen-tube growth have been shown to cause self-sterility and limited cross-sterility in a number of cross-pollinated plants (see Lewis, 1949, for review). In selfs and incompatible crosses, downward growth of the pollen tube is slowed down or stopped in styles containing a certain allele identical with an allele in the pollen. Although the wheats are all highly self-fertile, Smith (1944) considered that there was a large amount of cross-pollination in Agropyron, and Armstrong (1936) found it to be cross-pollinated but not necessarily so. If such incompatibilities were inherited by the Triticum-Agropyron hybrids from the Agropyron parents, however, the amphiploids would be homozygous for the incompatibility alleles and therefore completely self-sterile, unless the chromosomes carrying the alleles were eliminated by homoeologous pairing, or conditions of doubling rendered the alleles inoperative. Maheshwari (1950) described

various abnormalities in fertilization processes in a number of plants. None of these was established as genically or chromosomally determined.

The influence of chromosome balance in determining normal and abnormal development of the seed following fertilization, established by Thompson and co-workers in wheat hybrids, has already been mentioned. In view of the specific effects of certain chromosomes, it may be inferred that certain genic combinations play an important part in kernel development. Definite chromosomal influence by imbalance of complete sets, without specific genic influence, has, however, been established in Zea Mays by Boyes (1950) and Cooper (1951). The material studied by Boyes was from crosses between inbred tetraploid lines and maternal diploids originating within them. Cooper's studies were on crosses between different diploid and tetraploid plants of a strain. Although some specific genic differences may have been involved in Cooper's material, there is little likelihood that such was the case in Boyes' material. Both workers found that extensive disturbances leading to collapse in early seed development occurred when the normal 2n (maternal tissue) : 3n (endosperm) : 2n (embryo) ratio was changed. Moreover, disturbances were less extensive and occurred later in development when the tetraploid was used as female parent.

Instances in which a genic influence on seed development has been shown have been reported by Lindstrom (1923) and by Mangelsdorf (1926) in Zea Mays, and by Cooper and Brink (1940) in Medicago sativa. After enforced selfing of these normally cross-

fertilized species, Lindstrom and Mangelsdorf found single recessive genes and Cooper and Brink a number of recessive genes for arrested seed development. Pearson (1935) studied a seedless strain of Vitis vinifera which formed functional embryo sacs that underwent normal fertilization but ceased development at an early stage. He concluded that this abortion of the seed was a function of the maternal genotype.

The mechanics of post-fertilization abortion have been described following intergeneric and interspecific hybridization in various cereals by Thompson and Johnson (1936), Brink and Cooper (1940), Boyes and Thompson (1937), Kihara and Nishiyama (1932) and Wakakuwa (1927). Similar conditions have been found following (4x - 2x) crosses in corn by Boyes (1950) and Cooper (1951), and following enforced selfing of rye by Landes (1939). In all cases studied, the primary factor in seed collapse was weak growth of the endosperm. Brink and Cooper considered that "the immediate effect of this endosperm hypofunction was .... maldistribution of nutrients moving into the seed, with the result that the endosperm received less, and the integument more than a normal share". Resulting overgrowth of the integument in most angiosperms tended to further depress endosperm growth. Studies in the Gramineae revealed that the integuments did not play a role in the abortion process. Brink and Cooper (1944), on the basis of an embryological study of the cross Hordeum jubatum x Secale cereale and Hordeum jubatum selfed, concluded that the antipodal group, long considered to have a nutritive function, played a role similar to that of the integuments in

the sterility mechanism. They found great differences in enlargement of the antipodals when rye sperms were substituted for barley sperms. Thompson and Johnson (1945) however, were able to find only slight differences in similar material and considered these to have been an effect rather than a cause of endosperm breakdown.

The post-fertilization phenomena in the partially sterile Triticum-Agropyron amphiploids have not been studied previously, and there has been considerable speculation concerning whether or not, and to what extent, post-fertilization abortion occurs in this group. This investigation was therefore planned and begun in 1947 by Dr. J.W. Boyes in an effort to obtain critical data concerning this question and to determine the nature of the abortion mechanism, if present.

MATERIALS AND METHODS

Plants of the following lines of Triticum-Agropyron intermedium amphiploids and their immediate progenies were used in this investigation:

1. Strain S 91 from the cross T. dicoccum var. Vernal emmers x A. intermedium; produced by Peto and Boyes at Ottawa in 1938 - 1939. F_5 generation.

2. Strain S 107 from the cross T. turgidum var. No. 49 x A. intermedium; produced by Armstrong and McLennan at Ottawa in 1939 - 1940. F_5 generation.

3. Strain S 147 from the cross T. vulgare var. Kharkov x A. intermedium produced by Peto at Ottawa in 1937. F_5 generation.

Representative samples of each of the strains were sown together in the field in 1948, and eight plants were chosen from the group as a whole to cover as great a range of sterility as possible on the basis of general observations and spikelet counts.

The chosen plants were selfed in 1949 within a period of 5 days; the period between emasculation and pollination ranged from 5 to 9 days. Emasculation was performed immediately after the head emerged from the sheath. Only the central spikelets were emasculated; any spikelets at the base or top of the head which appeared smaller than the central ones were removed. The central florets of each spikelet were removed, and anthers of the remaining two (oldest) side florets were removed from the side with a pair of fine-pointed forceps inserted between the palea and the lemma. Heads at the same stage of maturity as the emasculated heads were used for pollen. Individual ripe anthers were placed in the emasculated flowers as soon as they had begun to dehisce. Immediately after emasculation the heads were enclosed in cellophane bags, and these were replaced after pollination.

At periods of 1, 2, 3, 5, 7, 9, 14 and 21 days after pollination, portions of heads were removed, and the ovaries were dissected out by removing the lemma and palea and severing the ovary stalk with a razor blade. The ovaries were fixed in Allen's or Craf's fixative for 24 hours, and carried through the standard N-butyl alcohol series to paraffin after approximately one month's storage in 70 per cent alcohol. This material has

been designated "Group 1". Material from six plants of this group was chosen for the study of post-fertilization development. These plants are listed below in order of increasing fertility.

Plant No. 1: progeny of strain S 91.

Plant No. 2: progeny of strain S 91.

Plant No. 5: progeny of strain S 107.

Plant No. 6: progeny of strain S 107.

Plant No. 9: progeny of strain S 147.

Plant No. 10: progeny of strain S 147.

In 1950 a number of plants, grown from normal and abnormal seeds of these six plants, were placed in the field and allowed to overwinter. A group of four plants, belonging to progenies of plants 5 and 6 (strain S 107), were, selfed and polycrossed. These fulfilled the following requirements:

(a) chromosome numbers of each plant must be known;

(b) each plant must produce an abundance of good pollen;

(c) all plants must mature simultaneously;

(d) four plants were needed for polycrossing.

They were selfed and crossed according to the following schedule:

	Designation		Designation
S 107-6-8 selfed	A x	S 107-6-8 x S 107-6-11	A x B
S 107-6-11 "	B x	S 107-6-11 x S 107-6-8	B x A
S 107-5-3 "	C x	S 107-6-8 x S 107-5-3	A x C
S 107-5-4 "	D x	S 107-5-3 x S 107-6-8	C x A
		S 107-5-3 x S 107-5-4	C x D
		S 107-5-4 x S 107-5-3	D x C

Emasculation and pollination procedures were essentially the same as with the parental group, except that all emasculations were done the same day. The sampling periods chosen were 2, 5, and 9 days after pollination. Whole heads were removed for each sample. Samples were fixed in Craf's, and taken through an ethyl alcohol series to 70 per cent for storage. Ovaries were dissected out in 70 per cent alcohol, using the technique previously described, and carried through the N-butyl series to paraffin. This material has been designated "Group 2".

Ovaries in both groups were imbedded, sectioned and stained in the same manner. They were carefully aligned in series, end to end, for sectioning, with the abgerminal surfaces facing downward. It was thus possible to cut sagittal sections which paralleled closely the central axes of the ovaries. Sections were cut at 12 microns and mounted on 2" x 3" slides with Haupt's fixative. Heidenhain's iron-alum-hematoxylin schedule was followed for staining and a mixture of Canada balsam and xylol was used for mounting.

Nuclear counts on endosperms of Group 1 were made using an arbitrary standard of counting. It was found that counts on 3-day material made, (a) by tracing individual nuclei or cells which might appear in two or more sections, thus obtaining an accurate count, and (b) by counting all visible nuclei and cells appearing in each section and dividing the total nuclei by 2 and the total cells by 3, gave closely comparable results, for example, 208 and 464 for method (a) and 186 and 386 for

method (b). Counts were therefore made using method (a) for 1- and 2-day material, and method (b) for 3-day material, in which the large number of nuclei made method (a) infeasible. In advanced 5-day material it was possible only to estimate the numbers of nuclei and cells.

Measurements of antipodal volume were made in 1-to 3-day material of Group 1 using an ocular micrometer for plane dimensions (the long and short axes of the antipodal mass) and counts of the number of sections in which the antipodals appeared, for the vertical axes. Plane dimensions of the antipodal nuclei were determined with an ocular micrometer; where large nuclei appeared in two or more sections, only that section with the greatest dimensions was measured, and where nuclei were oval in cross-section long and short axes were measured.

In both groups, as an additional criterion of fertilization, measurements were made of the length of the ovule cavity in all ovaries analyzed.

Pollen counts to determine male fertility were made on anther smears fixed and stained in aceto-carmine and sealed with a beeswax-gum mastic mixture. Pollen fertility was determined as the percentage of viable pollen (that showing normal cytoplasm and normal dimensions and containing sperm nuclei).

Spikelet fertilities were determined for all plants represented in Groups 1 and 2. In Group 1, spikelet fertilities per 10 spikelets were found, (a) for total florets and (b) for 1st and 2nd florets only; in Group 2 only the latter method was used.

Chromosome counts were made on root-tips and pollen-mother-cells of Group 2 plants. Root-tips were fixed in Crafs' solution, dehydrated in butyl alcohol, imbedded in paraffin, sectioned at 15 microns, and stained with the crystal violet staining technique. Pollen-mother-cell material was fixed in absolute alcohol:acetic acid : 3 : 1, and mounted and stained with either Belling's iron-aceto carmine, or Zirkle's Certo, corn-syrup and aceto-carmine staining and mounting medium.

R E S U L T S

1. Male and Female Fertility, Spikelet Fertility and Percent Fertilization

A summary of pollen counts made on Group 1 and Group 2 plants is presented in Table 1.

Table 1

Percentage of Normal Pollen in Plants of Group 1 and 2

Group 1				Group 2			
Plant	Year	No. grains	% Normal	Plant	Year	No. grains	% Normal
1	1950	77	46.7	A	1951	173	83.2
2	"	112	72.3	B	"	150	70.8
5	"	152	94.7	C	"	150	60.8
6	"	111 250	81.1 86.4	D	"	150	64.6
9	1949	no material					
10	"	95 246	84.2 95.5				

All plants in both groups, with the exception of Plant 1, Group 1, showed a high degree of pollen fertility, and even in this plant it was high enough to assure effective pollination of all ovaries. Of course, for each group, plants which displayed an abundant amount of good pollen were chosen, since it was desired to eliminate partial male sterility as much as possible.

It was necessary to rely on semi-permanent slides for counts of some Group 1 plants. Slides made of plant 9 pollen were too deeply stained to identify sperm nuclei, but from morphological

appearances a high proportion of good pollen was produced.

In other plants (not cited) a considerable amount of male sterility was encountered. No. 8 plant of Group 1 was completely male-sterile, and three other male-sterile plants were found among sister progeny of strain 107. Besides Group 2, other plants in the progenies of plants 5 and 6 (not listed in Table 1) were above 60 per cent in pollen fertility.

A random group of stigmas, removed from ovaries of Group 2 while in 70 per cent alcohol, was stained with acid fuchsin and aceto-carmine (Chandler, 1931) and mounted in glycerine. Of the 54 styles examined, 52 had attached pollen grains with tubes growing down the stylar hairs.

Female sterility was estimated from a study of sectioned ovaries of the two groups (Table 2).

Table 2

Female Fertility in Plants of Groups 1 and 2 Expressed as Percentage of Normal/Total Embryo Sacs Analysed.

Plant	No. of ovaries analysed	No. of collapsed embryo sacs	No. of abnormal embryo sacs	Total normal embryo sacs	% Female fertility
Group 1: 1-, 2-, 3- and 5- day ovaries					
1 1	40	11	4	25	62.5
2	36	1	2	33	91.6
5	56	3	3	50	89.3
6	47	4	2	41	87.2
9	61	2	0	59	96.7
10	43	0	1	42	97.7
Group 2: 2- and 5- day ovaries					
A	155	3	8	144	92.9
B	102	11	3	88	86.3
C	102	4	2	96	94.1
D	84	0	3	81	96.4

Collapsed embryo sacs, resulting from failure in the development of the female gametophyte, were clearly recognizable, but

Table 3

Fertilization in Normal Ovaries of Plants of Groups 1 and 2.

Plant or cross	No. of normal ovaries examined	No. of tubes present without other signs of fertilization	No. of fertilized ovaries by other criteria	Total no. of fertilized ovaries	Percentage fertilization of normal ovaries *
Group 1: 1-, 2-, 3-, and 5- day ovaries					
1	20	3	13	16	80.0
2	32	4	26	30	93.8
5	54	4	35	39	72.2
6	45	6	24	30	66.7
9	56	5	45	50	89.3
10	43	1	35	36	83.9
Average fertility					
Group 2: 2- day ovaries					
A	18	0	13	13	72.2
B	22	0	18	18	81.8
C	12	2	10	12	100.0
D	20	1	13	14	70.0
Ao x B	20	0	14	14	70.0
Bo x A	17	0	11	11	64.7
Ao x C	15	0	5	5	33.3
Co x A	15	0	12	12	80.0
Co x D	7	0	6	6	85.7
Do x C	20	3	18	8	90.0

* see Table 12 for results of refined method of determining percentage fertilization in Group 2.

other abnormalities of the sac contents were sometimes difficult to distinguish from possible post-fertilization defects (see section 3). Such abnormalities included anomalies in the form of the egg-apparatus, position of polar nuclei, and degeneration of these elements, as indicated by dense staining.

In Group 1, Plants 1, 5 and 6 displayed a considerable amount of failure in embryo sac development. This is considered to play a significant part in the sterility of these plants, especially of plant 1. In the remaining plants of the group and in all plants of Group 2, however, the amount of female sterility is sufficient to account for only a small part of the total sterility. This is in fair agreement with the findings of W.P. Thompson (1940), who claims that about 2 per cent female sterility is found among Triticum-Agropyron amphiploids.

Data on the extent of fertilization in pollinated ovaries are given in Table 3. Fertilization was regarded as having been initiated, a) if a pollen tube was seen at the micropyle, b) if the triple-fusion nuclei were identified, either by tracing nuclear outlines or finding three or more nucleoli in the fusion group, c) if egg fusion was identified by nuclear outlines or the presence of two or more nucleoli, d) if division stages were seen in the egg or the polar group, and e) if embryos and/or endosperm nuclei were seen.

Results from both groups indicated that fertilization was initiated in a fairly high proportion of ovaries both in selfed and in crossed material. It is probable that an even higher fertilization frequency, as defined above, occurs, since even in

Table 4

Spikelet Fertility in Plants of Group 1 and Open-Pollinated Plants of Group 2 (First and Second Spikelets)

Plant	No. of spikes	Average no. of spikelets per spike	Average spikelet fertility		Average per cent fertility	
			Method a	Method b	Method a	Method b
Group 1.						
1	7	9.1 ± 0.7	0.44	0.43	22.0	21.5
2	5	10.0 ± 0.0	1.20	1.20	60.0	60.0
5	5	5.2 ± 1.2	0.85	0.81	42.5	40.5
6	6	5.0 ± 0.0	1.24	1.24	62.0	62.0
9	5	10.0 ± 0.0	0.84	0.84	42.0	42.0
10	5	10.0 ± 0.0	1.48	1.48	74.0	74.0
Group 2.						
A	4	6.7 ± 1.5	1.43	1.42	71.5	71.0
B	4	7.5 ± 1.7	1.14	1.16	57.0	58.0
C	2	8.0 ± 2.8	.95	.88	47.5	44.0
D	3	7.7 ± 0.6	1.35	1.08	67.5	54.0

early material the pollen tubes were frequently not distinguishable in fertilized sacs. It should also be noted that post-fertilization degeneration of ovules occurred as early as 5 days, preventing a diagnosis of whether fertilization had occurred (a more refined method for determing fertilization percentages is described for Group 2 material in section 6b).

Nevertheless, the results from certain plants indicate that the percentage of fertilization adequately accounts for their actual sterility after open-pollination in the field. Information concerning this sterility, obtained from spikelet counts, is summarized in Table 4. Average spikelet fertilities given were determined in two ways:

a) Average spikelet fertility = sum of spikelet fertilities for each of the heads divided by the no. of heads (the spikelet fertility of a head = total no. of kernels divided by the no. of spikelets).

b) Average spikelet fertility = total no. of kernels from all heads divided by total no. of spikelets of all heads.

The average percentage fertility (both methods) was calculated thus:

$$\text{Average percentage fertility} = \frac{\text{Average spikelet fertility} \times 100}{2 \text{ (i.e., florets per spikelet)}}$$

From a comparison of spikelet fertility with the results obtained from compounding female fertility and percentage fertilization (Table 5), it is seen that the yields obtained in the field are usually lower than those expected if these two mechanisms alone were the cause of sterility. However, in plant 6 of Group 1, and

Table 5

Comparison of Female Fertility and Percentage Fertilization with Spikelet Fertility in Groups 1 and 2.

Plant	Female fertility (S♀)	Percentage fertilization* (Sf)	S♀ x Sf	Spikelet fertility (Method a)
Group 1.				
1	62.5	80.0	50.0	22.0
2	91.6	93.8	85.9	60.0
5	89.3	72.2	64.5	42.5
6	87.2	66.7	58.1	62.0
9	96.7	89.3	86.4	42.0
10	97.7	83.9	82.0	74.0
Group 2.				
A	92.9	72.2	67.1	71.5
B	86.3	81.8	70.6	58.0
C	94.1	100.0	94.1	47.5
D	96.4	70.0	67.5	67.5

* data from Table 3.

in plants A and D of Group 2, there are strong indications that, as suggested above, female sterility and a reduced fertilization adequately account for their partial sterility.

The sections which follow present the results of embryological examination of selfed and crossed material in Groups 1 and 2 with the purpose of determining further mechanisms tending to reduce fertility.

2. Descriptions of Caryopsis Development in Group 1 Plants

The following descriptions are based on a study of the ovaries of Group 1 plants following self-pollination. A summary of the sectioned material and that examined but not sectioned is presented in Table 6. In the unsectioned material, development was diagnosed on the elongation of the ovaries alone, those ovaries which showed an appreciable elongation when compared with the smallest ovaries for a given period being classed as developed. Sectioned ovaries were classed as developing only if endosperm was present. After examining slides from 1- to 5-day material, it was considered by the writer that almost all major manifestations of post-fertilization abortion were to be found in developing seeds prior to 9 days after pollination. Material collected after 9 days following pollination was not examined. All collections but those of plant 10 contained appreciable proportions of ovaries which appeared to have failed to elongate or to have elongated only slightly. An attempt was made to incorporate a reasonably large sample of ovaries of this type, as well as of elongated ovaries, in the material examined from each collection.

With the exception of a brief assessment of female sterility,

Table 6

Summary of Superficial and Embryological Examination of Ovaries of Group 1 Plants.

	Period collected after self-pollination											
	1 day		2 days		3 days		5 days		7 days		9 days	
Sectioned Material:												
	Dev-eloped	Not dev-eloped	Dev-eloped	Not dev-eloped	Dev-eloped	Not dev-eloped	Dev-eloped	Not dev-eloped	Dev-eloped	Not dev-eloped	Dev-eloped	Not dev-eloped
Plant 1	0	9	2	10	6	4	0	9	2	10	2	6
Plant 2	0	0	2	12	4	4	6	9	5	6	0	0
Plant 5	5	7	3	14	7	8	8	8	4	5	3	5
Plant 6	2	15	5	5	3	4	9	12	12	3	0	0
Plant 9	9	5	5	9	9	8	11	10	9	4	0	0
Plant 10	11	3	6	5	13	1	5	0	5	2	0	0
Unsectioned Material:												
	Dev-eloped*	Not dev-eloped	Dev-eloped*	Not dev-eloped	Dev-eloped*	Not dev-eloped	Dev-eloped*	Not dev-eloped	Dev-eloped*	Not dev-eloped	Dev-eloped*	Not dev-eloped
Plant 1	0	0	0	5	0	8	0	2	0	1	0	4
Plant 2	0	0	0	0	0	2	0	0	7	0	6	8
Plant 5	0	0	1	2	0	0	0	0	4	0	6	3
Plant 6	0	0	0	0	0	0	0	1	3	0	5	2
Plant 9	0	4	0	1	0	1	1	1	0	0	4	2
Plant 10	0	4	0	0	0	0	11	0	16	1	14	1

* as determined by elongation of the kernel.

the following descriptions of caryopsis development include only references to ovaries in which either embryo or endosperm division was initiated. Sectioned ovaries in which endosperm or embryo development had not begun will be treated in section 3.

a. Normal Development: Plant 10.

Since this plant showed high fertility in the controlled selfs examined, and since almost without exception, it produced large, plump seeds, it was selected as most closely representing a fully-fertile plant. In subsequent descriptions of other plants, normal development is the characteristic development found in plant 10 for the given period. The most notable feature of caryopsis development in this plant was the very great uniformity shown among ovaries collected at each sampling period.

1 Day. - Almost all developing ovaries had 8 endosperm nuclei, and all zygotes were in late fusion or early division (Plate 1, Fig. 1). The antipodals were highly vacuolate and had expanded to about twice their original volume. The antipodal nuclei showed no significant increase in size and were very uniform in size range. Ovules had increased in length by approximately one-quarter to one-third. One ovary showed delayed endosperm development, the primary endosperm nucleus being still in late prophase.

2 Days. - Close to 120 endosperm nuclei and 4 embryo nuclei were found in almost all ovaries. One ovary showed 212 endosperm nuclei and an 8-nucleate embryo (Plate 1, Fig. 2). The antipodals remained highly vacuolate, and irregularity in size range accompanied a general further increase in their size. The antipodal nuclei at this stage showed a significant increase in size.

Ovules had extended to twice their original length. Endosperm consisted of free nuclei only. In one ovary, which in all other respects followed the general trend of development, endosperm development lagged considerably (22 nuclei).

3 Days. - Numbers of endosperm nuclei in most ovaries were distributed within the range, 330-550, and embryo nuclei within the range, 8-20. The antipodals had become flattened by the encroaching endosperm, and nuclei which could be measured did not appear to have enlarged further. The ovules had extended to three times their original length. Cell-wall formation in the endosperm had begun in the micropylar pocket.

5 Days. - Numbers of endosperm nuclei fell within the range 3000-4500, and embryo nuclei numbered from 33 to 57 (Plate 1, Fig. 5). Only remnants of the antipodals remained. Ovules had extended further, and were approximately 5 times the original length. Cellular development of the endosperm was almost complete. One ovary at this stage contained a degenerate endosperm, showing deeply-staining deposits in the micropylar pocket, together with a 3- to 4- nucleate embryo.

7 Days. - The embryo contained many nuclei, and had become pendulous. Cellular development was complete in the endosperm which formed an elongate mass from ten to fifteen times greater in length than width, and occupying the whole of the intra-integumental cavity. The ovule had extended to about 7 times its original length. Various irregularities appeared in ovaries at this stage, including an imbedding of the embryo by the endosperm and the presence, in the latter tissue, of groups of degenerate

nuclei. In two ovules the embryo and adjacent endosperm tissue was badly distorted as a result of a break in the ovary wall at the micropylar end. It is not known whether this is a result of mechanical injury or of the early deposition of an abscission layer.

b. Development in Plant 1.

This plant displayed extensive irregularities in embryosac formation, and so few regularly developing caryopses were encountered that the nature of development is not clearly definable. At one day no endosperm or embryo division was discerned, although in two ovaries sperm nuclei were found associated with egg and fusion nuclei. In one of these ovaries antipodals were absent and in the other they were extremely reduced (about 1/125 normal volume). Only slight vacuolization of the antipodals was evidenced in other, unfertilized sacs, and was associated with a considerable increase in volume. Maximum antipodal volumes were less than one-quarter normal.

2 Days. - Two developing ovaries which differed widely in development were found. In one, development lagged only slightly behind normal (80 endosperm nuclei, 4 nuclei in the embryo; antipodal vacuolization normal, volume 2/3 normal, and nuclear size normal). In the other, development of the embryo was normal, but the endosperm lagged (16 nuclei), and antipodal vacuolization, volume and nuclear size were more comparable with normal development at 1 day than at 2 days. Ovule elongation in these ovaries was comparable with the normal at 2 days and 1 day respectively. It appears that with the exception of the embryo, general development

lagged behind the normal by about 1 day in the second ovary.

3 Days. - The range of numbers of endosperm nuclei was 120-160; nuclei in the embryo varied from 8 to 14. This again indicates that while embryo development was comparable with the normal, endosperm development lagged by almost one day. Antipodal nuclei were slightly larger than normal for this stage. Elongation of the ovules was intermediate between that of 2- and 3- day normal ovaries.

7 Days. - As in 2- day material, two ovaries were again found to differ extensively in development. In both, embryo development was comparable with normal development at 5 days but showed signs of degeneration (vacuolization and loose organization of cells; Plate 4, Fig. 25). However, the most noteworthy feature in these ovaries was the extreme difference between the two endosperms. In one, development was quite regular in every respect; in the other, extreme abnormalities were seen. These abnormalities consisted of highly polyploid nuclei, and extensive deposits of nuclear debris throughout the cytoplasm. In addition, throughout a large part of the central region of the endosperm there was no wall-formation (Plate 5, Fig. 33). Elongation in the normal and abnormal sacs was equivalent to that of normal ovules at 7 and 5 days respectively, suggesting that, in the latter, division difficulties occurred at approximately 5 days.

c. Development in Plant 2.

This plant was completely regular in embryo sac production. Material of the 1- day collection has been lost.

2 Days. - Two ovaries in which development had taken place

yielded endosperm numbers of 34 and 16, and embryo numbers of 4 and 2. This represents delays in development of portions of a day. The one ovary lagged only in the extent of antipodal vacuolization, but the other also showed delays in antipodal volume and ovule length. Antipodal nuclei in these ovaries underwent a normal increase in size.

3 Days. - Extensive differences in development were found at this period among a group of four ovaries, all but one of which showed a distinct lag in endosperm development (from 22, 56, 106, to 229 nuclei). In the first of these the embryo was aborted, and approximately half of the endosperm nuclei were highly polyploid (Plate 2, Fig. 16). In the second, polyploidy was found in some of the endosperm nuclei, but to a less extent. Of the remaining embryos, the first two were quite normal, whereas the last was aborted but showed signs of having had more than the normal number of nuclei. From this it was inferred that abortion had occurred just previous to collection. An incomplete correlation between endosperm numbers and nuclear diameters in the antipodals (34, 39, 46, 40) was found. No correlation can be seen with ovule elongation although this character showed a range of development intermediate between normal material at 2 and 3 days.

5 Days. - Endosperm development was delayed somewhat more than the normal (317 to 1411 nuclei). On the other hand the embryos were at least as well advanced as, and in three ovaries were further advanced than in normal ovaries at this period. No correlation was found between variation in embryo and endosperm numbers. Embryo sac lengths were uniform, but were intermediate between 3- and 5- day lengths of normal sacs. In embryo sacs

showing an extreme reduction in number of nuclei, giant nuclei similar to those in the 3- day material were found.

7 Days. - The endosperms in these ovaries showed a wide variation in the degree of abnormality in development. Some of the endosperms were normally developed, while others showed extensive disturbances associated with interference of nuclear division. Knots of degenerate tissue, containing many irregular patches of deeply staining nuclear debris and strings of nuclear material, were concentrated in the central region of the endosperm, which exhibited a pronounced lack of wall-formation. Within these endosperms, polyploid nuclei were found. In one ovary, these nuclei were excessively large and cytoplasmic organization of the endosperm was almost completely lacking, portions of the sac with no nuclei being completely collapsed. Another ovary contained several highly polyploid nuclei together with a number of normal nuclei. Embryo development was variable (39 to 76 nuclei) but showed the same size range as did normal embryos at this period.

d. Development in Plant 5.

Only one ovary without an embryo sac was found. Two ovaries showed collapse of what appeared to have been mature sacs, and two displayed a wide separation of egg and synergids in the micropylar region.

1 Day. - Three ovaries had 4 endosperm nuclei and two others had 6 and 9 respectively. The zygotes in all ovaries were at varying stages from fusion to metaphase of the first division. There was a general lack of vacuolization in the antipodals but the antipodal volumes and the size of antipodal nuclei were normal.

In one ovary a single polyploid endosperm nucleus was found together with four smaller nuclei, indicating either an unequal post-fertilization division, or endopolyploidy. In a second ovary, a 4-nucleate endosperm was associated with an extremely small antipodal group (about 1/50 normal volume) with no vacuolization.

2 Days. - Endosperm and embryo development lagged considerably behind the normal, corresponding very closely to normal 1-day development, there being 8 endosperm nuclei and prophase zygotes in all developing ovaries. The endosperm nuclei were larger than normal, and antipodal nuclei had increased in size to the same extent as the normal. Antipodal vacuolization lagged behind normal.

3 Days. - Numbers of endosperm nuclei were as uniform as the normal; and occupied approximately the same range (350-600). Embryo nuclei numbers displayed a slightly higher range than normal (10-28). The size of the antipodal nuclei had increased to the same extent as in normal ovaries, and the elongation of the ovules was normal.

5 Days. - All developed endosperms were more extensive (5900 - 6300 nuclei) than the normal. This was also true of embryos, which contained approximately twice the normal number of nuclei (120-175). Ovules showed about the same increase in length as the normal, however. The endosperm showed fewer signs of abnormality resulting from endosperm failures than did the normal material; the single irregularity found was multipolar spindle formation in one ovary accompanied by an increase in size of the endosperm nuclei.

7 Days. - Endosperm size and embryo size in most ovaries appeared to be normal, and ovule length lagged only slightly behind normal. One out of five ovaries examined displayed aborted remains of the endosperm, which appeared to have stopped development at about 2 days, and the ovule length corresponded with that in normal ovaries at the 2- day period. In one of the "normal" ovaries there was pronounced degeneration within the endosperm similar to that described for both plant 1 and plant 2 at this stage.

e. Development in Plant 6.

In plant 6 there were three ovaries without embryo sacs, and three in which there appeared to be abnormality in the sac contents. No such abnormalities were found in any but the 1- day collection, although all material to 5 days was examined. It is probable that the abnormalities at one day are therefore not typical of this plant.

1 Day. - Two normally developing ovaries had 4 endosperm nuclei and zygotes in early fusion. All other features of these ovaries were normal, except for a reduction in antipodal volume and ovule length in one.

2 Days. - Most ovaries showed a regular development of endosperm and embryo, but both endosperm and embryo lagged slightly behind the normal (endosperm 56-96 nuclei; embryo 3-4 nuclei). In half the fertilized ovaries antipodal vacuolization was extensive, in the others it was slight; in all but one ovary the volume was comparable to that of normal ovaries, while the nuclei were some-

what larger. Ovules were only slightly shorter than those of normal ovaries.

3 Days. - In the two developing ovaries seen, endosperm and embryo development closely approached the normal (386-486 endosperm nuclei and 20 embryo nuclei). In one ovary, single fertilization was indicated by the presence of an 8-nucleate embryo with no associated endosperm (Plate 2, Fig. 10). That the elongation of the ovule may be influenced by endosperm growth is suggested by the fact that in this ovary the length had not increased past the one-day length of normal ovaries, whereas the lengths of the other two ovules were within the normal range for the 3- day period.

5 Days. - Developing endosperms and embryos in this plant were the most advanced of any member of the group at this stage. The number of nuclei in the endosperm averaged 7500 in three of the four ovaries counted, and 4000 in the fourth. In embryos, numbers averaged 100, with one at 30. In one ovary, division difficulties in the endosperm were indicated by the presence of strings of nuclear material and by a large variation in nuclear size, some nuclei being distinctly polyploid. The ovules were only slightly shorter than those of normal ovaries.

In the abnormal endosperm mentioned above, an important observation concerning the origin of polyploid nuclei and other forms of endosperm abnormality was made. One dividing nucleus contained many more chromosomes than normal. The metaphase plate was multiple, consisting of portions oriented in several directions. The division figure extended through 3 sections (36 microns),

one of which is shown in Plate 5, Fig. 34.

7 Days. - Three well-developed kernels of this group were found. These kernels were not sectioned, but compared very closely in appearance with normal ones at this period.

f. Development in Plant 9.

Plant 9 displayed only slightly greater female fertility than plant 10, only 2 collapsed ovaries being found in material to 5 days.

1 Day. - Endosperm development in ovaries was somewhat behind that in normal ovaries and varied from a 2-nucleate to a 5-nucleate condition, although in one ovary the normal 8 nuclei were found. Embryo development, on the other hand, appeared more advanced than normal, most zygotes being in early division stages. Antipodal vacuolization and volume were only slightly less than normal, and antipodal nuclei showed the same size range as in normal ovaries. Elongation of the ovule was only slightly less that normal.

2 Days. - Delay in endosperm development was further evidenced. The range of development in this tissue was fairly extensive (24 to 72 nuclei). Embryo development lagged slightly behind the normal at this stage, all embryos having two or three nuclei. Although the antipodal volume was somewhat less than normal, exhibiting a size range closer to that at 1 day, the vacuolization was quite normal. Both in size of the antipodal nuclei and in length of the ovules, these ovaries appeared to lag behind normal ovaries by about 1 day. In one ovary, a 2-nucleate embryo was found associated with an unfertilized polar-fusion

nucleus (Plate 2, Fig. 9).

3 Days. - A general delay in both endosperm and embryo development was evident. The endosperm yielded numbers of nuclei from 44 to 312, with most numbers ranging between 100 and 200; the embryo nuclei varied in number from 3 to 10. Two ovaries showed extensive embryo abortion associated with slight degeneration of the endosperm in the micropylar pocket (Plate 1, Fig. 5). The degree of elongation of ovules corresponded more closely with normal material at 2 days than at 3 days. A significant phenomenon occurred in two ovaries in which embryos were found. In one ovary, the primary endosperm nucleus, instead of dividing, had undergone a number of endomitotic divisions and was as a result highly polyploid; 140 nucleoli were counted in it (Plate 2, Fig. 15). In the other ovary the triple-fusion nuclei contained two large nucleoli and numerous other small ones, suggesting endomitotic divisions had occurred in one of the fusion nuclei. In both ovaries, the antipodals were significantly reduced in volume, and were only slightly vacuolate even when compared to normal ovaries at 1 day. These antipodals had not undergone the secondary collapse found in normally developing ovaries at this period, which is considered as caused by encroachment of the endosperm. It is notable that in the latter ovary only a very slight amount of elongation had occurred, less than that of normal ovules at 1 day; whereas in the former, elongation was within the normal range at 2 days.

5 Days. - Normally developing ovaries displayed numbers of nuclei ranging from 650 to 2500; these numbers indicated a delay

in development extending to 2 days behind normal. In these ovaries, embryos appeared slightly more advanced than normal, and ovules were only slightly shorter in length than normal. Another class of ovaries, larger in frequency of occurrence than the first, contained endosperms smaller than those of normal ovaries at 3 days (30 to 94 nuclei). In this group, the embryos were either normal but reduced in size (Plate 1, Fig. 8), or aborted (Plate 1, Fig. 7). The latter condition is much the same as that found in the same plant at 3 days. In ovule length, ovaries with reduced endosperms showed a considerable reduction in ovule length, and corresponded closely in this respect with normal ovaries at 2 days. In a third group of two ovaries, embryos but no endosperms were found; and in two others, endosperms were found associated with an undivided egg or zygote (Plate 1, Fig. 6). Ovule lengths of the latter ovaries fell within the range of normal ovaries at 1 day.

7 Days. - Developing ovaries compared fairly closely with the normal in size of the embryos and endosperms and in elongation of the ovules. A further group of embryos, displaying a reduced elongation of the ovule equivalent to normal elongation at 1 to 2 days, showed distinct endosperm abortion. This tissue had degenerated into a disorganized mass of cytoplasm or, along with the other components of the embryo sac except the embryo, had lost stainability. Embryos in this group were either absent or much retarded in development. One with only 11 nuclei was found.

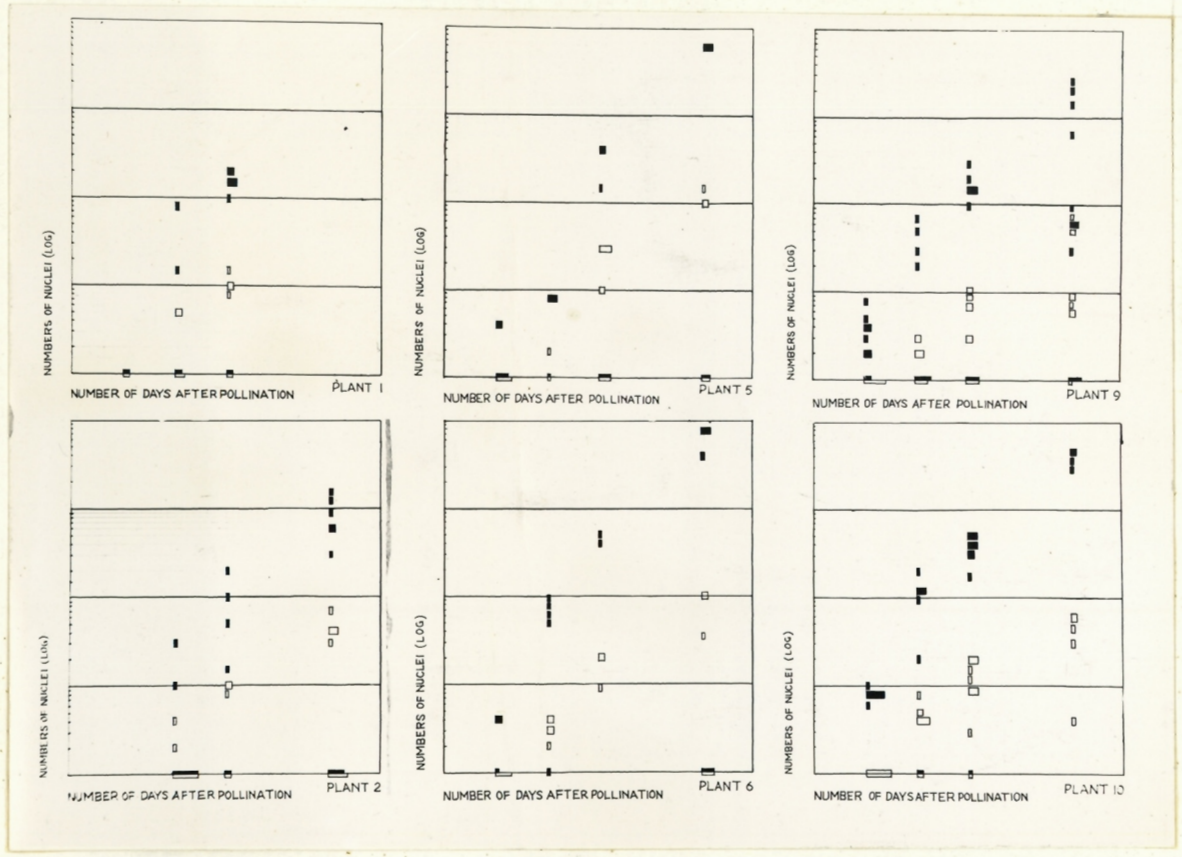
g. Summary of Descriptions.

In terms of endosperm and embryo division, the rates of development of caryopses in Group 1 plants are presented graph-

ically (Text Fig. 1). It can be seen from this that a considerable range in the rate of development is displayed by some plants, while in others the rate is more uniform. Those plants showing the greatest degree of irregularity in endosperm development were plants 2 and 9, plant 2 being more irregular in early stages and plant 9 at later stages of development. Most of the plants, with the exception of 9 and 10, show a fairly uniform development of the embryo. In plant 9, the extensive differences in embryo development at 5 days are correlated with differences in endosperm development, but in plant 10 this does not appear. Endosperm development in plants 5, 6 and 10,with the exception of a uniform delay shown in plant 5 at 2 days, was quite regular.

The development of the caryopsis in almost all ovaries of plant 10 closely follows in all details the descriptions given by Boyes and Thompson (1937) for the hexaploid wheats. Adequate descriptions of the development in Agropyron for comparison have not been found.

In Table 7 the foregoing descriptions have been summarized in terms of delay or advancement of different features of development, compared with normal as represented by plant 10. The sampling periods of normal development which most closely corresponded with maximum and minimum development of ovaries of each of plants 1, 2, 5, 6 and 9 are given. In some instances certain features were intermediate in development between those of normal ovaries at 2 sampling periods. In such instances a period intermediate between these 2 sampling periods is given, and the figure 1/2 is used to signify delayed development at 1 day.



Text Figure 1.

Rates of Growth of Endosperm and Embryos of Group 1 Plants, Expressed as Increase in Numbers of Nuclei. Major divisions of vertical axes: base line 1, other lines 10, 100, 1000, and 10,000. Collection periods 1, 2, 3 and 5 (days after pollination). Narrow rectangles are endosperms (solid) and embryos (blank); histograms are erected to right of ordinates at each collection period.

Table 7

Summary of Descriptive Comparisons of Development in Group 1 Ovaries with Normal Development (Plant 10).

Features of caryopsis development	Sampling periods for plants 1, 2, 5, 6, & 9	Sampling period (days after pollination) of plant 10 ovaries at equivalent stages of development.				
		Plant 1	Plant 2	Plant 5	Plant 6	Plant 9
Endosperm development	1 day	1	1	1	1	½
	2 days	1 to 1½	1½	1	1½ to 2	1½
	3 days	2	1½ to 2½	3	3	2 to 2½
	5 days		3 to 4	5 to 6	5 to 6	3 to 5
	7 days	5	2 to 7	7+	7	3 to 7
Embryo development	1 day	½	½	½ to 1	1	1½
	2 days	2	1½ to 2	1 to 1½	1½	1½
	3 days	3	3	3 to 4	3	1 to 2
	5 days		5 to 5+	5+	5	4½ to 5
	7 days	7	7	7		
Ovule lengths	1 day	½	1	1	1	½ to 1
	2 days	1 to 2	1 to 1½		2	1
	3 days	1 to 2	2 to 3	3	1 to 3	1 to 2
	5 days	4½	4 to 4½	5	4½	2 to 4½
	7 days	5		7		2 to 7
Antipodals;						
1. Volume	1 day	½		1 to ½	1	½
	2 days	1 to 1½	1½ to 2	1	1 to 2	1 to 2
2. Vacuolization	1 day	½		½	1	½ to 1
	2 days	1 to 2	1½	½ to 2	1½ to 2	2
3. Nuclear size	1 day	½ to 1		1	1	½ to 1
	2 days	2	2	2	2	1
	3 days	3	2 to 3	3		

An examination of this table reveals a correlation between lack of uniformity in endosperm development and a tendency to delay in the rate of increase in size of antipodal nuclei. Plants 5 and 6, which possess a high degree of uniformity in endosperm development also show a rate of increase in the antipodal nuclei comparable with,or greater than, the normal, whereas plants 3 and 9, which are irregular in endosperm formation, show considerable delays in size increase in these nuclei.

The delay in endosperm development is closely correlated with the amount of elongation of the ovules. The greatest delay in both endosperm development and ovule elongation occurred in plant 9, and it is notable that in these delayed ovaries, development appears to have ceased at the same period in all ovules. In plant 2, delays in endosperm development were also associated with delays in ovule elongation. In this plant there appeared to have been only a slowing down of development without any cessation. In both plants 2 and 9 the delays were associated with the formation of highly polyploid endosperm nuclei and with a higher incidence of other defects associated with failure in endosperm divisions.

Plants 5 and 6 showed an almost consistent uniformity in endosperm development, which was as fast as or more rapid than normal. Developmental failures were as rare as in plant 10. In these features there was also a high correlation with ovule elongation and with nuclear increases in the antipodal.

Plant 1 displayed a tendency in development similar to that of plant 2; however, female sterility in this plant prevented an

adequate sampling of developing kernels, so that a general characterization of development was possible only at 2 and 3 days. At these stages endosperm delays were associated with delays in ovule elongation.

In plants 6 and 9, single fertilization or single development following fertilization of the egg or polar nuclei had occurred, and this was associated also with delayed elongation of the embryo sac. These phenomena were rare (two examples of single fertilization of the egg were found in each of plants 6 and 9 (Plate 2, Fig . 9, 10, 11), and one of single fertilization of the endosperm in plant 9 (Plate 1, Fig. 6).

3. Appearance and Germinability of Kernels of Group 1 Plants.

Mature F_1 grains of Group 1 plants, harvested for spikelet fertility counts were classified in arbitrary grouplings according to the degree of abnormality. The results are presented in Table 8.

Table 8

Classification of Harvested Kernels From Group 1 Plants

Plant number:	1	2	5	6	9	10
Total no. of kernels classified:	40	186	70	120	44	90
Kernel classification						
% Plump, large	50.0	62.4	70.0	87.5	75.0	80.0
% Plump, small	30.0	22.6	21.4	8.3	13.6	11.1
% Large, shrunken	10.0	1.07	1.4	3.3	0.0	5.6
% Small, shrunken	10.0	10.2	5.7	0.8	11.4	3.3
% Aborted	0.0	3.8	1.4	0.0	0.0	0.0

A comparison of results in Table 8 with Table 7 reveals that those plants which displayed a greater tendency to lag behind normal in endosperm development also yielded more small, shrunken kernels. This correlation is shown in plants 1, 2 and 9. Plants 5 and 6, which showed a high degree of uniformity in endosperm development, also produced a high percentage of large plump kernels.

A comparison of aborted kernels in plants 2 and 5 with the descriptions of caryopsis development in these plants revealed a probable mechanism of abortion. Although the outer kernel

coat was hardened to some extent, aborted kernels among those harvested were completely collapsed and were about one-third to one-half the length of normal kernels. One ovary of plant 5 at 5 days showed endosperm and embryo abortion at this period, and compared very closely in length with the aborted kernels. One ovary of plant 2 at 7 days showed a very reduced number of endosperm nuclei. In the chalazal end of the embryo sac the nuclei were quite uniformly endopolyploid, and from the size of the nucleoli were judged to have remained for a considerable time without dividing. Large accumulations of enucleate cytoplasm appeared in this region. This ovary compared very closely in length with the aborted kernels from this plant. It is therefore considered highly probable that early failure of normal endosperm divisions, resulting in the production of a few highly polyploid nuclei, is a causal factor in the production of a certain proportion of aborted kernels in plants 2 and 5.

To test whether or not abnormalities in mature kernels of the type listed in Table 8 affected germinability of the kernels, a number of them harvested from open-pollinated heads of Group 1 plants were sown. A fairly equitable representation of normal and defective kernels was included in the test. A record of the appearance and germination of these kernels is presented in Plate 6. Out of 34 large plump kernels only one failed to germinate; out of 51 small, shrunken, or small and unshrunken kernels, 15 failed to germinate. It appears probable, therefore, that endosperm failure, indicated by wrinkling or reduction of the

kernel, is responsible for an inpaired ability to germinate.

It is improbable, however, that such irregularities account for decrease in spikelet fertility in Group 1 plants. In the first place, aborted, completely collapsed kernels with hardened outer coats were included in the spikelet counts. In the second place, with the possible exception of conditions in some plant 9 ovaries at 5 days, there were no clear indications that abortion occurred in developing endosperms at earlier stages than those seen in plants 2 and 5. On the contrary, ovaries in most aborted florets appeared to have regressed without having elongated extensively, and ovaries in later collections could be clearly separated into two distinct groups; those in which elongation was extensive and those in which only very slight or no elongation had occurred.

4. Failure During Fertilization

Group 1 ovaries which showed little or no elongation were subjected to a close examination to determine whether or not fertilization had occurred. The criteria used were those described in section 1. Without exception, in material collected later than 1 day after pollination, ovaries with no endosperm development could be distinguished from those with endosperm development by ovule lengths alone. Those ovaries in which no endosperm was produced are considered to correspond to the ovaries of "aborted" florets found in harvested material.

A few unfertilized ovaries were found in Group 1 (Table 3); however, many more were found to have been fertilized. The finding of such ovaries at late collection periods, supported by

the observation of instances of partial success of the fertilization process (section 4a), provides indisputable evidence that blocks in the fertilization process occur in these plants.

In Table 9 the results of a quantitative analysis of the frequency of this fertilization failure in Group 1 plants and of the effects expected upon total fertility are given.

In all plants excepting plant 10, the occurrence of fertilization failure is sufficiently frequent to provide a major sterility mechanism. In the latter plant, it has already been shown that the fertility is unusually high. A compounding of the percentage of sterility resulting from fertilization blockage with female fertility and percentage fertilization yields results considerably lower than those obtained from spikelet fertility counts. This is considered a result of artificial emasculation and pollination procedures.

a. Description of Anomalies of Fertilization and Their Relation to Kernel Abortion.

The anomalies observed in Group 1 material indicate stasis in the fertilization process at all stages from entry of the pollen tube into the embryo sac to division in the primary endosperm nucleus.

Sperms were seen within the pollen tubes in plant 9 at 1, 2, 3 and 5 days, and in plant 1 at 3 and 5 days (Plate 3, Fig. 20, 22). They were seen within the embryo sacs of plants 6, 9 and 10 at 2 days. They were seen apposed to the egg or polar nuclei in plants 1 and 5 at 1 and 2 days, in plant 6 at 1 and 5 days, and

Table 9

Percentage of Fertilized Group 1 Ovaries Showing

No Developmental Divisions

1- to 5-Day material

Plant	No. of fertilized ovaries*	No. ovaries with no endosperm or embryo divisions	% Success	% Success x $S_{♀}.Sf$**
1	16	8	50.0	25.0
2	30	18	40.0	34.4
5	39	16	58.9	38.0
6	30	11	63.3	36.8
9	50	16	68.0	58.7
10	36	1	97.2	79.7

* data from Table 3.
**data from Table 5.

in plant 9 at 2 days. A sperm was seen within the egg cytoplasm but separated from the egg nucleus in one ovary of plant 1 at 5 days (see also Plate 4, Fig. 29, for this phenomenon in a Group 2 plant at 2 days).

Zygotes and triple-fusion nuclei that had failed to enter division phases were identified by double and triple nucleoli respectively in ovaries from 2 days on. Both phenomena were seen in single ovaries of plants 2 and 9 at 2 days, plant 5 at 3 days (Plate 3, Fig. 24), and in one ovary each of plants 2 and 10 at 5 days. Triple-fusion nuclei were found associated with eggs in prophase in plants 2 and 9 at 3 days and in plant 2 at 5 days. They were also present with apparently unfertilized eggs in plant 2 at 2 days and plant 5 at 3 and 5 days. This apparent lack of correlation between the conditions found in the eggs and the polar groups of these ovaries suggests that there is an independent variability in the ability of each of the two sperm nuclei to impart the stimulus necessary for the initiation of division.

This suggestion is further supported by the observation of instances of single fertilization already mentioned in the descriptions of development in these plants. Ovaries in which the eggs had divided to form embryos in various stages of development but in which the polar fusion nuclei remained unfertilized were found in plant 6 at 3 days and in plant 9 at 2 days (Plate 2, Fig. 9, 10). Ovaries in which undivided eggs were associated with fairly well-developed endosperms were found in plant 5 at 3 and 5 days and in plant 9 at 5 days (Plate 1, Fig. 6).

To test the effect of this stasis in the fertilization process upon the condition of ovaries at late stages following pollination, undeveloped ovaries were checked in late material for normality of the embryo sac contents. At 5 days, no evidence of abortion appeared in either the eggs or the polar nuclei. At 7 days, however, in many ovaries which were judged by their elongation to have been fertilized, definite signs of abortion appeared. In plants 1 and 2 some embryo sacs were destained and shrunken away from the ovule walls; zygotes were difficult to identify and the polar nuclei were not seen, or appeared to have become completely disorganized.

A further group of ovaries in which no endosperm division occurred following fertilization has been mentioned previously in the descriptions of caryopsis development, but is included here because it represents partial failure of the fertilization process. Either a single highly-polyploid nucleus is produced because of spindle failure, or the three polar nuclei separate and develop into globular masses of chromatin each containing a single greatly-enlarged nucleolus. The former abnormality was observed in plant 9, and the latter in plant 5 at 3 days (Plate 2, Fig. 14, 15). The fact that similar clearly recognizable anomalies were not seen after 3 days suggests that degenerative processes probably occur early in abnormally developing embryo sacs of this type.

This is supported by the presence, in partly collapsed 7-day ovaries of plant 9, of abnormal masses of cytoplasm containing many small nucleoli and vacuoles. From their staining be-

haviour these masses were considered to contain dispersed nuclear material. They corresponded in size with the cytoplasmic sheath surrounding the polyploid nucleus shown in Plate 2, Fig. 15. This strongly suggests that the contained nucleoli, vacuoles and chromatin represent the dispersed remains of polyploid nuclei after breakdown of the nuclear wall.

These semi-collapsed ovaries also corresponded closely in ovule length with that illustrated in Plate 2, Fig. 15. All corresponded with normally developing ovaries of plant 10 at a period intermediate between one and two days after pollination. Moreover, ovaries with comparable lengths which showed no signs of endosperm or polar nuclei were found. Six ovaries of this type were found in plant 5, six in plant 6, five in plant 9 and one in plant 10 material collected from 3 to 5 days after pollination. This suggests that the phenomenon indicated in Plate 2, Fig. 15 may cause an appreciable amount of kernel abortion. On the other hand, the ovule illustrated in Plate 2, Fig. 14 was shorter than normal ovules at 1 day, and therefore appears to represent stasis at an earlier stage of the fertilization process. Ovaries of comparable lengths at periods from 3 to 7 days (with single exceptions in plants 1, 5 and 6, and two in plant 9) contained intact triple-fusion groups.

b. Relations Between Antipodal Development and Fertilization Failure.

A comparison of antipodal nuclear sizes between 1-, 2-, and 3-day fertilized ovaries with and without endosperm development

is made in Table 10. This comparison was made in order to determine whether any consistent relationship existed between antipodal development and success or failure in the initiation of endosperm divisions. The measurement used as a basis of comparison was the length (or diameter, if spherical) of the largest nucleus of the antipodal group. It was found that the size of this measurement was completely correlated with the size of the nucleus as indicated by the cross-sectional area.

It is seen that in the antipodal nuclei of ovaries wherein no division in the polar group occurred there was no significant increase in size, whereas in those ovaries in which endosperm was found, size-increases were extensive. Such increases in diameter would indicate an approximate doubling of the nuclear volume. The whole antipodal group was from two to three cell-layers in thickness, and it was found that the increases were confined largely to the layer of cells with surfaces contacting the cavity of the embryo sac.

In volume and in extent of vacuolization the antipodals were very variable in embryo sacs with and without endosperms. There were no indications that enlargement in these features was more extensive in fertilized than in unfertilized ovaries at 1 day. An arbitrary method of scoring vacuolization was used. Slight, intermediate, and maximum vacuolization were scored as +, ++, and +++ (see Plate 4, Fig. 32, Fig. 30, and Plate 2, Fig. 9, for the several examples). In averaging the results the average number of +'s was expressed numerically. The figures for antipodal volume are expressed as comparative values only,

Table 10

Average Maximum Linear Dimensions of Antipodal Nuclei in Fertilized Ovaries of Group 1 Plants.

Plant no.	Number of days after pollination					
	1 day		2 days		3 days	
	Undivided polars present	Endosperm present	Undivided polars present	Endosperm present	Undivided polars present	Endosperm present
1	20.0 (2)*	—	37.5 (3)	47.5 (2)	30.0 (3)	47.8 (6)
2	—	—	30.2 (8)	44.5 (2)	29.0 (3)	37.2 (4)
5	32.2 (4)	27.0 (2)	—	43.5 (2)	38.7 (4)	38.0 (1)
6	33.0 (3)	38.5 (2)	32.5 (2)	45.0 (4)	—	43.4 (3)
9	32.5 (2)	31.0 (7)	32.4 (5)	42.0 (3)	34.6 (3)	43.0 (4)
10	28.0 (1)	30.3 (6)	30.7 (4)	39.3 (6)	26.0 (1)	38.2 (6)
Average	29.14	29.13	32.66	43.63	31.65	41.27

* Figures in brackets are the numbers of ovaries in which measurements were recorded.

and not as actual dimensions. The average parameters for vacuolization and volume, respectively, of the antipodals for the entire group of 6 plants were:

at 1 day, in ovaries with endosperm, 2.6 and 1.27, and
in ovaries without endosperm, 2.3 and 1.36;
at 2 days, in ovaries with endosperm, 2.6 and 1.22, and
in ovaries without endosperm, 1.5 and 1.07.

At 1 day there appear to be no significant differences between ovaries with and without endosperm in either vacuolization or volume of the antipodals. However, at 2 days, antipodals in ovaries containing endosperm appear more vacuolate and larger than in those without. In neither group at 2 days was there indicated any increase in vacuolization or volume over that of 1 day. Moreover, at 2 days vacuolization and volume of antipodals in ovaries without endosperm appear to have undergone regression.

These findings suggest that vacuolization reaches a maximum at approximately one day following pollination. In view of the large portion of the antipodal cell volume occupied by the vacuole, the volume of the antipodals is considered to be dependent on the degree of vacuolization. That no significant differences occur between the two categories of 1-day ovaries suggests that failures in fertilization of the polar nuclei are not brought about as a result of antipodal malfunction. This is further supported by the observation of endosperm development in an ovary of plant 5 at 1 day, wherein the antipodals were non-vacuolate and only one-fortieth of the average volume of antipodals at this period.

The observations on some ovaries without endosperm at 3 days, however, suggest that antipodal vacuolization and volume, although reduced in ovaries which showed little elongation, had actually increased in some ovaries with an extensive elongation. In such ovaries, fertilization had occurred but had failed to proceed to endosperm formation. For example, in one ovary of plant 5 at 3 days, showing elongation comparable with the normal at 1 to 2 days, the comparative antipodal volume was 3.1, and in one ovary of plant 9 with elongation equivalent to normal at 2 days, it was 3.5. This represents almost a 3-fold increase over the average at 2 days (1.22). Both the aforementioned ovaries showed extensive vacuolization. In other ovaries, however, (for example, in plant 9 at 3 days, Plate 2, Fig. 15) the antipodals were much reduced in volume, and only slightly vacuolate. The explanation of these apparent contradictions has not been determined.

5. Comparative Development of the Endosperm and Embryo in Group 2 Plants.

Observations of caryopsis development in Group 2 plants were made principally upon collections at 2 and 5 days. The four plants involved in selfing and reciprocal crossing were:

Plants A and B -- F_1 progeny of Plant 6, Group 1;

Plants C and D -- F_1 progeny of Plant 5, Group 1.

An adequate sample of developing ovaries in each collection enabled a reasonably reliable characterization of embryo and endosperm development to be made in all selfs and crosses except one, C x D, in which no endosperms or embryos were found.

A summary of the sectioned material and that examined but not sectioned is presented in Table 11. Sectioned ovaries were classed as developed if they contained endosperm, and unsectioned ovaries were classed as developed on the basis of elongation of the kernel.

Endosperm development at 2 days proved to be variable within each class in which a sufficient number of ovaries were studied. Moreover, within some classes, the ovaries could be arranged in discrete groups according to the contained number of endosperm nuclei, indicating that some endosperms of these classes had undergone more divisions of some of their nuclei than had others. Ovaries were therefore classed according to the number of divisions which had occurred. Numbers of nuclei were grouped around 4, 11, 18, 24, and 35, and these were considered to signify the occurrence of a 2nd, 3rd, 4th and partial 5th or 5th division, respectively. Numbers of nuclei from 50 to 65, and from 75 to 133 were considered as representing partial or complete 6th and 7th divisions.

According to this classification, most ovaries of plants in the A-C group of selfs and crosses i.e., A selfed, A x C, C x A and C selfed , had undergone 4 or 5 divisions. Ovaries of the A-B group (A selfed, A x B, B x A, and B selfed), with the exception of A selfed, had undergone from 5 to 7 divisions. Of this group, plant B selfed ovaries had undergone from 5 to 6 divisions and those of B x A and A x B had undergone a partial or complete 7th division. Ovaries of plant C selfed and D x C of the C-D group (C selfed, C x D, D x C, and D selfed) had

Table 11

Summary of Embryological and Superficial Examinations of Self- and Cross-Pollinated Ovaries From Group 2 Plants.

a. Sectioned Material:

	Period collected after pollination					
	2 days		5 days		9 days	
	Developed *	Not developed	Developed *	Not developed	Developed *	Not developed
A selfed	7	18	10	8	4	9
B "	7	22	2	23	0	23
C "	6	13	4	11	0	14
D "	2	18	2	17	3	20
A x B	5	17	5	21	2	16
B x A	1	21	4	16	4	12
A x C	3	17	6	13	1	13
C x A	4	16	4	11	0	17
C x D	0	8	0	10	0	21
D x C	7	14	4	20	3	20

b. Unsectioned Material:

	Developed **	Not developed	Developed **	Not developed	Developed **	Not developed
A selfed	0	0	0	0	4	0
B "	0	0	1	0	0	0
C "	0	2	2	4	4	0
D "	0	0	0	0	0	0
A x B	3	0	2	1	1	8
B x A	0	0	0	6	4	2
A x C	0	4	7	0	9	0
C x A	0	0	0	1	6	1
C x D	0	0	0	0	0	0
D x C	4	0	0	0	0	0

* as indicated by presence of endosperm
** as indicated by elongation

undergone from 4 to 5 divisions, but those of plant D selfed had undergone only 3, and in the cross C x D no endosperm whatsoever was seen either 2-, 5-, or 9-day ovaries, although the degree of fertilization was high.

The development of endosperms at 5 days closely reflected the conditions found in 2-day ovaries. As was found in the 2-day material, a significant reduction in size of endosperms in several of the selfs and crosses compared with plant 10 endosperms. This was shown by measurements of length and width of the endosperms in median longitudinal sections. The reduction in size took the form of a marked decrease in numbers of nuclei in the central region of the kernel, so that only a single layer of nuclei appeared.

Reduction was found in all selfs and crosses which had shown not more than 5 divisions at 2 days (the A-C and C-D groups, with the exception of one normally developing ovary of the cross D x C). In plant D selfed material, which showed extreme delay in development at 2 days, this single-layering was accompanied by a considerable reduction in length. On the other hand, endosperms of the A-B group with the exception of A selfed again were comparable to normal in development. Those of plant B selfed and A x B were considerably thicker than the rest in the central region, while those of B x A were perfectly normal in thickness.

In plants A and C selfed and C x A of the C-A group, and in D x C, single ovaries were found at 2 days which had undergone only 2 divisions. These selfs and crosses, along with plant D selfed, appear to constitute a group which regularly produces

deficient endosperms, since all of them with the exception of D x C showed some endosperms with considerable distortion. This distortion did not appear in the other selfs and crosses. It consisted of strings of chromatin and deeply-staining irregular masses in the cytoplasm, considered to represent the remains of degenerate nuclei. These abnormalities occupied extensive areas of the endosperm cytoplasm where they were accompanied by a complete lack of wall-formation. In the A-C group they were restricted to the mid-region of the kernel, but in D selfed they extended into the micropylar pocket.

Embryos were regularly normal in development, the ratio between average maximum and minimum lengths being 1.0 to 1.4. Embryos of plant A, both in selfs and in crosses, were reduced in length, while ovaries in plant B selfed and crossed were longest. Although embryos of C selfed were next in length to those of B selfed, no differences between those of A x C and C x A were observed. The data from plants A and B suggest that these plants exert a maternal influence over kernel development. Differences in this maternal influence affect the development of the endosperm, and through this, the embryo. The data from the reciprocal crosses between plants A and C, however, suggest the possibility that pollen factors may have the effect of nullifying this maternal influence.

6. Fertilization Failure in Group 2 Plants.

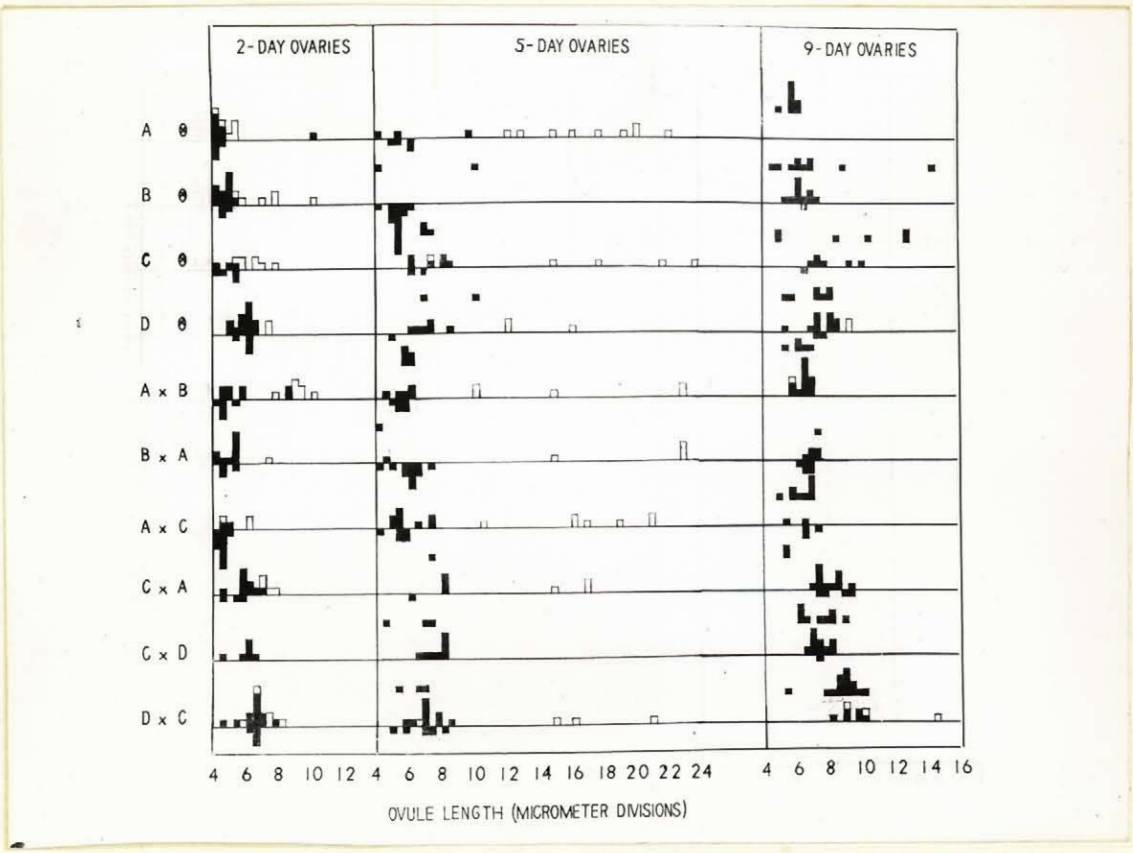
In considerable numbers of Group 2 material, fertilization had been initiated but had failed to bring about a division of the egg or the triple-fusion nuclei. In an attempt to determine

the relative importance of this occurrence as a cause of sterility, a closer analysis of the fertilization process was undertaken.

a. Relations between Fertilization and Ovule Elongation.

In order to determine the nature of the relationships between fertilization and ovule elongation, ovule lengths of four categories of Group 2 ovaries were compared. These categories were: (a) unfertilized ovaries; (b) fertilized ovaries without endosperm; (c) fertilized ovaries with endosperm; and (d) ovaries in which embryo-sac collapse or degeneration of elements of the embryo sac had occurred. The criteria for determining whether or not fertilization had occurred were the same as those used for Group 1 ovaries (section 1), except that pollen tubes were not used as evidence since they were difficult to detect and rarely seen.

The results of this comparison are presented graphically in Text Fig. 2. Micrometer-scale measurements of ovule lengths were plotted horizontally. Since the shortest normal, mature ovules were 4.0 divisions in length, this was used as the vertical axis for all graphs. Mature ovaries without endosperm are represented by the small solid squares, and those with endosperm by the blank squares. Fertilized ovaries were plotted above the abscissa for each of the selfs and crosses, and unfertilized ovaries were plotted below. Ovaries in which a diagnosis of fertilization could not be made from a study of embryo sac nuclei alone are represented by solid squares raised above the abscissa. Since only a few pollen tubes were seen, this was not used as a criterion of fertilization. No maturing kernels were found at 9 days in



Text Figure 2.

Ovule Elongation of Group 2 ovaries. Blank squares are single ovaries with endosperm; solid squares are ovaries without endosperm. Horizontal baselines opposite each self or cross (i.e. A⊕) separate three categories of the latter, (a) on baselines, fertilized ovaries, (b) underneath baselines, unfertilized ovaries, (c) raised above baselines, unclassified ovaries.

plant B selfed, in D x C or in C x D, and it was therefore decided to concentrate on a study of developmental anomalies occuring at early stages following fertilization. Hence, with the exception of a few embryo sacs showing distinctly retarded development, lengths of normally-maturing ovules were not included.

Histograms of 2-day and 5-day material showed that, throughout the whole group, unfertilized ovules did not appear significantly shorter than fertilized ovules. Moreover, some ovules containing endosperm nuclei were no longer than those designated as having been fertilized without having entered division stages.

A further group of ovaries is illustrated. In these the embryo sacs had become completely collapsed and non-stainable or the sac elements had degenerated. These conditions made it impossible, from a study of the embryo-sac nuclei alone, to decide whether or not fertilization had occurred. Such ovaries were absent at 2-days, and rare at 5-days. At both days a characteristic abortion of immature sacs accounted for most of the embryo-sac collapse.

In 9-day material this undefined group contains a considerable proportion of the ovaries examined, and from the extreme range of ovule lengths exhibited, it is judged to be a composite group representing fertilized and unfertilized mature and immature sacs. The observation that even the shortest ovules, with the possible exception of those of plants A and B selfed, showed considerable elongation suggests that a certain amount of elongation occurs after a time even in prematurely aborted ovaries. On the other hand, the rarity of these aborted ovaries,

as shown in 2- and 5-day material (Table 2) might easily account for their absence from samples of the size used in this study.

There appears to have been a marked variation between the individual groups in the length of mature ovules at the time of pollination. The mean ovule lengths of unfertilized ovaries of the A-B group at 2 days appear to be less than those of the C-D group, and in the A x C cross they appear less than in the C x A cross. This suggests that the ovules of plants A and B (progeny of plant 6) are uniformly shorter than those of plants C and D (progeny of plant 5). The same trend is also shown in 5- and 9-day ovaries, and in the mean lengths of fertilized ovaries without endosperm. The fact that both plants with the shorter ovaries are descended from plant 6 suggests that differences in ovule lengths of mature ovaries are inherited from the Group 1 plants.

b. Percentage Fertilization.

Results of an analysis of fertilized ovules is given in Table 12. This analysis was undertaken to determine the proportion of fertilized ovules in which the fertilization process progresses to the production of endosperm. Corrected values for fertilization percentages were obtained by combining the results of the first four columns of the table with the data from unsectioned material in Table 11, part (b). In this material, one or more unelongated ovaries remained unsectioned in A x B, B x A and C x A at 5 and 9 days, in C selfed at 2 and 5 days and A x C at 2 days. All of these ovaries were characterized as being unfertilized, but it is probable that some of them had undergone failure during fertilization instead. In some of these collections, therefore, the

Table 12

Summary of Analysis of Percentage Fertilization in Group 2 Plants.

Material		No. of mature ovaries	No. of evidently-fertilized ovaries	No. of collapsed elongated ovaries	Total no. of fertilized ovaries	% fertilized ovaries	% fertilized ovaries (corrected values)*
A x	2 dys.	19	12	0	12	63.7	63.7
	5 "	18	12	0	12	66.7	66.7
	9 "	12	0	8	8	66.7	75.0
B x	2 "	22	17	0	17	77.3	77.3
	5 "	20	3	1	4	20.0	23.8
	9 "	21	10	6	16	76.2	76.2
C x	2 "	15	10	0	10	66.7	58.8
	5 "	15	11	3	14	93.3	76.2
	9 "	14	6	4	10	71.4	77.7
D x	2 "	20	15	0	15	75.0	75.0
	5 "	18	9	2	11	61.1	61.1
	9 "	23	12	5	17	73.9	73.9
A x B	2 "	21	15	0	15	65.2	75.0
	5 "	23	10	5	15	71.4	61.5
	9 "	18	3	4	17	94.4	66.7
B x A	2 "	18	10	0	10	55.5	55.5
	5 "	17	7	0	7	41.2	30.4
	9 "	15	5	1	6	40.0	47.6
A x C	2 "	16	5	0	5	31.2	25.0
	5 "	17	15	0	15	88.2	91.7
	9 "	14	2	8	10	71.4	82.6
C x A	2 "	16	12	0	12	75.0	75.0
	5 "	14	6	1	7	50.0	46.7
	9 "	17	11	0	11	64.7	70.8
C x D	2 "	7	6	0	6	85.7	85.7
	5 "	10	8	2	10	100.0	100.0
	9 "	20	10	8	18	90.0	90.0
D x C	2 "	21	15	0	15	81.0	84.0
	5 "	24	13	4	17	70.8	70.8
	9 "	23	10	13	23	100.0	100.0

* Corrected percentages from combined data of this Table with those of Table 11, part (b).

corrected values for fertilization percentage may be somewhat low.

A definite relationship appears to exist between the numbers of ovaries at 2 days showing fertilization failure and the numbers of collapsed ovaries at 5 and 9 days. A striking illustration of this relationship is shown in 9-day ovaries of plant A selfed, in which the entire group of evidently fertilized but undeveloped ovaries, shown to be considerable at 2 and 5 days,is lacking, and appears to be represented by an equally large group of collapsed, destained and partially elongated ovaries in which the embryo sac details were so obscure that a direct diagnosis of fertilization could not be made. To a less extent, the same situation is shown in plant B selfed at 9 days, plant D selfed and B x A at 5 and 9 days, C x D at 9 days, and D x C at 5 days. In all 9-day classes but that of A x C, the addition of this collapsed, elongated group to the evidently fertilized group brought about a closer approximation to the percentage fertilization found for 2-day material (see corrected value for per cent fertilized ovaries). In the A x C material, the 5-day collection (which had no collapsed ovaries) is considered more reliable than the 2-day material and agrees fairly closely with the revised 9-day material.

Major inconsistencies in the results of the analysis (see Table 12) were found only for the above mentioned A x C material at 2 days and for plant B selfed material at 5 days. It is possible that, despite precautions taken to avoid inadequate pollination or pollination of immature ovaries, either may have

occurred.

Minor inconsistencies in some results were associated with development in some ovaries which, from a study of embryo sac nuclei, were diagnosed as unfertilized. In D x C material at 5 days, three such ovaries, included in Table 12 as unfertilized, showed extensive elongation corresponding to 7 and 8 divisions (Text Fig. 2). Classed as fertilized, these would bring the per cent fertility to 83.3, which closely approximates the 2-day value. This is also true of C x A material at 5 days; the inclusion of the extra five ovaries would bring the values to 85.7 per cent, somewhat higher than that recorded for 2-day material.

From an examination of the results from selfed material, fertilization appears to be uniformly close to 70 per cent. It is approximately the same in A x B and in C x A material. In B x A material it is close to 50 per cent, and in A x C, C x D, and D x C material it appears to be high, probably over 85 per cent.

A comparison of selfs and crosses reveals that in three crosses, A x C, C x D, and D x C, percentage fertilization was higher than in either parent selfed, and in one cross, B x A, it was lower. Reciprocal crosses between A and B and between A and C indicate that fertilization is higher when A is the female parent, whereas in the C x D cross no reciprocal differences appear.

c. Percentage Success in Endosperm Formation.

Following the determination of the numbers of fertilized ovaries in sectioned material of Group 2, percentages of fer-

tilized ovaries which contained endosperms were calculated. The results are presented in Table 13. Data from unsectioned material were included in the calculations, except for those collection groups which contained more than two undeveloped ovaries. As stated previously, such ovaries may or may not have been fertilized, and their inclusion would affect the validity of the results. In those groups of selfs and crosses containing one or two of them, they have been classed as unfertilized (Col. 3), and thus are not included in the calculations.

Complete sterility in ovaries, a very large proportion of which were definitely established as having undergone gametic fusion, was found in the cross C x D. On the other hand, in material of plant C selfed and of the cross C x A, appreciable numbers of ovaries developed endosperm. It is highly probable therefore, that reaction between plant D sperms and plant C gametophytes is responsible for the failure during fertilization.

The average level of success during the fertilization process is low. A comparison of these levels in selfed material of plants A, B, C and D with the spikelet fertilities (see Table 4) for the same plants after open-pollination shows that in plants A and C fertilization failure almost completely accounts for their partial sterility alone. On the other hand, in plants B and D it is much higher than would be expected from the harvesting results, suggesting that cross-pollination may take place more frequently in the field.

Table 13

Proportions of Fertilized Ovaries Containing Endosperms in Group 2 Plants.

Material	Sectioned Ovaries (a)		Unsectioned Ovaries		Totals		% Ovaries with endosperm
	(b) Fertilized	(c) Containing endosperm	Undeveloped, presumed unfertilized	Developed, presumed to have endosperm	Fertilized	Containing endosperm	
A selfed, 2-, 5-, 9 day	32	21	0	4	36	25	69.4
B " , 2-, 9-day	33	7	0	0	33	7	21.2
C " , 2-, 9 day	20	6	2	4	24	10	41.7
D " , 2-, 5-, 9 day	43	7	0	0	43	7	16.3
A x B, 2-, 5-day	30	10	1	5	35	15	42.9
B x A, 2-, 9-day	16	5	2	4	20	9	42.5
A x C, 5-, 9-day	25	7	0	16	41	23	56.1
C x A, 2-, 5-, 9-day	30	8	2	6	36	14	38.9
C x D, 2-, 5-, 9-day	34	0	0	0	34	0	0.0
D x C, 2-, 5-, 9-day	55	14	0	4	59	18	30.5

(a) Data from Table 11, part (b)
(b) Data from Table 12.
(c) Data from Table 11, part (a)

7. Chromosome Numbers of Group 1 and Group 2 Plants.

The results of a study of pollen mother-cells and root-tips of Group 2 plants are given below:

Plant A: 2n = 66 in two cells at anaphase of M1.

Plant B: 2n = 66 and 67 in two root-tip cells.

Plant C: 2n = 66 in two cells at metaphase of M1. Chromosome associations consisted of 2-3 univalents and 31-32 bivalents.

Plant D: 2n = 70 in two cells at metaphase of M1. Associations consisted of 3-4 univalents, 30-31 bivalents, 0-1 trivalents and 1 quadrivalent.

Other counts, made on plants 9 and 10 of Group 1 plants gave the following results:

Plant 9: 2n = 63 and 65 in two cells x at metaphase of M1. Associations consisted of 9-15 univalents, 23-27 bivalents, and 0-1 quadrivalents.

Plant 10: 2n = 55 to 58 in two cells at metaphase of M1. Associations consisted of 1-2 univalents, 26-27 bivalents, and 0-1 trivalents.

D I S C U S S I O N

The results obtained from an analysis of the causes of sterility in the two groups of Triticum-Agropyron amphiploids studied indicate that a number of mechanisms, rather than any one mechanism, are responsible for the failure to set viable seed. Moreover, the relative importance of these mechanisms appears to vary between strains and between different plants of a strain. The compounding of these various partial-sterility mechanisms makes it necessary to adopt a statistical approach to the problem of determining their relative importance.

1. Female Sterility

Female sterility in the form of the production of abnormal or collapsed embryo sacs was found to occur with varying frequencies throughout all the plants studied. However, in only one plant was its occurrence found to be high enough to materially influence the fertility. In this plant, (Plant 1, Group 1), some sacs appeared to have collapsed at or before the 8-nucleate stage. This was supported by the finding of one aborted ovary, among immature ovaries collected from this plant. In another ovary from pollinated material, a single polyploid nucleus was found in place of an embryo sac.

A similar type of abortion has been reported by Greenleaf (1942) in amphiploids of Nicotiana sylvestris crossed with N.tomentosiformis and N.tomentosa. This was not due to chromosome imbalance since meiosis in anthers of these amphiploids was

regular and normal pollen was produced. Amphiploids produced from hybrids of highly inbred strains produced completely female-sterile plants, whereas some wild strains as parents produced plants showing varying degrees of partial sterility. Greenleaf (1942) has advanced an hypothesis to explain his results. Within each species, gene complexes are built up which subtend, by an integrated series of reactions and reaction rates, a specific mode of embryo-sac development. Since these complexes are built up independently in different species, competition between the sets of genes results in developmental failure in the embryo sacs of the amphiploids.

The frequency of occurrence of embryo-sac abortion in plant 1 (11 out of 40 ovaries examined) suggests the possibility that it may be determined by a single recessive gene. However, it should be remembered that meiosis in these amphiploids is not regular; moreover, the highest degree of pollen abortion observed in these plants was found in plant 1. The association of both male and female sterility occurring after meiosis in this plant suggests that meiotic irregularity, rather than a sterility gene or genes, is responsible for both.

2. Miscellaneous Abnormalities of the Embryo Sac

Abnormalities of mature embryo sacs ranged from displacement and abnormal morphology of sac elements to multiple embryo sacs. All but one of the former defects were comparatively rare and no regularity of their occurrence in any plant could be detected. In two instances the egg and synergids were widely separated; polar fusion nuclei were occasionally found closely associated

with the antipodals rather than between this element and the egg apparatus; and in one instance a syncytium appeared in place of the separate egg and polar nuclei (Plate 3, Fig. 24).

Another morphological anomaly encountered was the presence of four nuclei in the polar group (one containing an extra sperm nucleolus - Plate 4, Fig. 29). More frequent was the occurrence in plants 1 and 6 of antipodals having a definitely embryo-like organization (Plate 4, Fig. 32). These were encountered only in the earlier collections however, it is unlikely that they represent elements of immature sacs. The fact that in the latter sac illustrated there was possibly triple-fusion with slight vacualization of antipodals suggests either that the antipodals were abnormal or matured more slowly than the remaining elements of the sac.

Multiple embryo sacs occurred with a high frequency in Group 2 plants. An analysis of ovaries (not mentioned above) revealed that they occurred with a greater regularity in certain plants. Out of 560 ovaries examined in Group 2, 18 (3.2%) showed duplication of at least one element. Of these, 9 were from Plant C, 6 from Plant D and 3 from Plant B. This represents 6.2%, 4.7% and 2.2% of the ovaries in Plants C, D and B. respectively. The multiple sacs regularly contained duplicate sets of eggs, polar nuclei and antipodals, but in a few sacs one or more of the pairs of elements was absent, suggesting differential development of twin embryo sacs. One egg and one polar group only was regularly fertilized, but in one instance two triple-fusion nuclei were found, with no visible fusion of the eggs. Nielsen (1946) found multiple embryo sacs in Poa pratensis and Maheshwari (1950)

stated "These may arise (1) either from the derivatives of the same megaspore mother cell, or (2) from two or more megaspore mother cells, or (3) from nucellar cells (apospory)."

It is doubtful that the multiple-sac condition increases sterility of amphiploids since there appears to be no consistent lowering of percentage fertilization in plants producing multiple sacs. It may be significant that plant A, in which no such multiple embryo sacs were found, displayed a much higher percentage of developing caryopses than did the other plants, in both self and crosses. The percentage (16.7%) of these sacs which contain endosperms is somewhat lower than that for normal and multiple sacs together, however. If, as appears to be the case in some ovaries, duplication of eggs or polar groups occurs at the expense of the antipodals, it is to be expected that endosperm development would be hindered by the absence of the nutritive group.

3. Sterility Caused by Failure during Fertilization

A second mechanism, heretofore unreported, plays an important role in the events leading to partial sterility in both groups. This takes the form of a complete cessation of the fertilization process at any of a number of stages before completion. In the ovaries of Group 1 plants it is clear that some sperms fail to reach the egg and the polar nuclei, and that others fail to penetrate to the egg nucleus. Other sperms either fail to impart the necessary stimulus for the initiation of mitotic division in the zygote or the primary endosperm nucleus or encounter eggs or polar nuclei which fail to respond. Finally, in some endosperms

chromosome division is initiated, but is endomitotic in nature.

Inability to function normally in fertilization appears to be independent in the two sperms entering an embryo sac. This is shown most clearly in the instances reported of single fertilization (i.e. Plate 2, Fig. 9, 10 and 11), and single failure during fertilization (Plate 2, Fig. 15). Several reported instances of this phenomenon in wheat are cited by Boyes (1936). Such independent failure in sperms from the same pollen grain suggests that different functions are involved in the two fertilization processes or that there was an instability in the division of the generative cell. Maheshwari (1950), although considering the question whether differences in sperm morphology are normally found an open one, cites several reported instances in which the two sperm nuclei were unequal in size and shape.

The validity of the method used to determine whether fertilization in undivided eggs and polars occurred is important. Although the writer has found no descriptions of the actual course of gametic fusion and triple-fusion in the Gramineae, it was found that the stages of fusion agree quite closely with those described and illustrated by Gerassimova (1933). According to her descriptions, soon after nuclear fusion in both egg and polars, the sperm nucleus loses its identity. However, it develops a nucleolus which after a time increases in size and finally fuses with the egg or the polar nucleoli. In the egg, therefore, a second nucleolus, and in the polars a third is considered to provide clear evidence that fusion has taken place.

Earlier stages of gametic fusion (i.e. coiled sperms in the egg cytoplasm and on the surface of the egg and polar nuclei) were only rarely seen (Group 1 material) and, even then, frequently

difficult to identify positively. In later material, it is not likely that they would be seen, since general stainability of sac elements decreases.

Fertilization failures, as indicated by the presence of sperm nucleoli, at two, three, and five days were found in all plants studied. Their frequency of occurrence varied considerably between plants. It was notably low in plant 10, and this fact together with the high spikelet fertility suggests that the major cause of steritity among other plants lies in fertilization failure. Moreover, in the cross C x D (Group 2) fertilization failure was found to cause complete sterility. However, appreciable fertility was found in Plants C and D when selfed. This indicates that functional eggs and polar nuclei were present in Plant C, and that functional sperms were produced by Plant D. It is therefore suggested that a reaction system similar to that proposed by East (1921) and Goodspeed and Clausen (1916), between gametic nuclei, causes this cessation of development in the cross C x D. Mutually incompatible complexes may be present in the two plants of this group, and their union in the cross produces profound disturbances in the functionability of the zygote and the triple-fusion or primary endosperm nuclei. Such differences would probably have their origin in the Agropyron parent rather than the highly homozygous wheat parent of the hybrid.

In certain instances degeneration of the egg (Plate 3, Figs. 17, 18 and 19) suggests that such disturbances in zygote functionability probably occur. In one instance (Plate 5, Fig. 36) a very marked erosion of the egg and synergids indicative of enzyme action, was seen at 5 days. Such action may be the re-

sult of a delayed immunological reaction, which might be accounted for by single or few gene differences. Kostoff (1929) has mentioned that evidences are found in Nicotiana of immunological reactions between male gametes and maternal tissue. East (1921) and Goodspeed and Clausen (1916), after detailed study of progenies of interspecific hybrids in this genus, concluded that a reaction system best explained partial sterility in some hybrid progenies. According to Goodspeed and Clausen, "if the N.tabacum and N.sylvestris systems display a high degree of mutual incompatibility, any gamete containing elements derived from both systems would give a reaction system subject to profound disturbances incident upon the inharmonious relations set up by the N.tabacum and N.sylvestris elements." A relatively slight admixture of one of the inharmonious elements does not affect the production of normal offspring, which are therefore like one or the other parents and fertile. "A slightly greater proportion of inharmonious elements in the reaction system would result in such profound disturbances as to produce the abnormal individuals of varying kinds which make up so large a proportion of the progeny from such parentage. When the proportions of inharmonious elements in the gametes becomes still greater, they fail to function at all. It is upon the formation of such non-functional gametes or the attempt to produce them that the partial sterility of the hybrid depends. . . ."

Such a system would apparently explain the abnormalities and failure in the production of embryo sacs and in fertilization in Triticum Agropyron amphiploids, although the proportions of such

failures apparently are much lower in these plants than in their material. Moreover, of the hybrids between wheat and couch grass, Verushkine and Schechurdine (1933), stated that "In all cases the F 1 plants showed the clear dominance of characters of the couch grass and only a few plants occupied an intermediate position with reference to the character of their ear structure." This suggests that the Triticum-Agropyron hybrids fulfil Goodspeed and Clausen's requirements for the workings of a reaction system. On the other hand, these abnormalities in both embryo sac production and fertilization might with equal probability be explained by a chromosome imbalance in the gametes and in the fusion nuclei.

4. Sterility Caused by Failure during Development of the Endosperm and Embryo

Following success in fertilization, further abnormalities in caryopsis development occur in some ovaries. Both the extent of the abnormalities and their frequency of occurrence varied considerably between individual amphiploids of the three crosses studied. In Group 1 plants, both representatives of the S 91 strain, (Plants 1 and 2) and Plant 9 of the S 147 strain showed extensive delay in endosperm formation in a large proportion of the maturing kernels, whereas those of the S 107 strain (Plants 5 and 6) and plant 10 of the S 147 strain produced a large proportion of well-formed endosperms of uniform size. The extent of delays in endosperm development was also variable. The evidence from a summary of the descriptions of Group 1 plants (Table 7) suggests that mitotic divisions cease at varying periods after pollination. This delay appears to be as extensive in the few retarded kernels observed in the S 107 strain

as in the many retarded kernels of the S 91 strain and plant 9 of the S 147 strain. This observation suggests that these phenomena are either governed by a very few genes as an all-or-none character, or is a chance feature determined by chromosomal imbalance. Mangelsdorf (1926) has described a number of gene-induced abnormalities in Zea Mays. However, the abnormalities found in defective endosperms of these amphiploids correspond closely with those described and illustrated by Kihara and Nishiyama (1932) for the cross Avena fatua X A.strigosa, by Boyes and Thompson (1937) for various interspecific crosses in the wheats, and by Landes (1939) for selfed rye. The explanations given by the former two groups of authors for the abnormalities in the developing endosperm is that of imbalance of chrososomes in the hybrid tissue because of chromosome-number differences in the parental species. Presumably, also, in the inbred rye chromosome irregularities occur at meiosis, according to Muntzing (1948). In the Triticum-Agropyron amphiploids varying numbers of univalents occur, and gametes with considerable differences in chromosome number must be produced. From a study of the chromosome numbers of progenies of the S 107 group, Armstrong and McLennan (1944) came to the conclusion that there was a tendency to stabilization at a number close below 70, the expected 2n number for perfect.

The chromosome number of plant D was found to be somewhat higher than those of the other plants. Plant D, moreover, was from a very shrunken kernel, whereas Plants A and B were from large, plump kernels, and Plant C was from a relatively normal, though slightly flattened seed. This suggests that the higher

chromosome number in Plant D may be correlated with some form of chromosome imbalance in the endosperm of the kernel from which it grew. Moreover, the A-B group of selfs and crosses, with the exception of Plant B selfed, produced a relatively high proportion of ovaries with endosperms, and, with the exception of Plant A selfed, relatively advanced endosperms, whereas Plant D selfed produced a very low proportion of ovaries with endosperm, and in these, development of the endosperm was very considerably delayed. It therefore appears possible that chromosome imbalance in a plant may be reflected in the endosperms of its offspring.

5. Relation of Antipodal Development and Ovule Elongation to Failure during Fertilization

The relation of the antipodal group to endosperm development is apparently a complex one. The regression shown in volume and vacuolization of the antipodals between 1 and 2 days in embryo sacs without endosperm suggests that a sustained stimulus is required from the developing endosperm, or possibly the embryo, for their continuing activity. Activity is initiated with great rapidity following fertilization. This is in agreement with similar findings by Brink and Cooper (1944) and by Thompson and Johnson (1945) in the cross Hordeum jubatum x Secale cereale.

In a one-day ovary of Plant 5, the antipodals were greatly reduced (about 1/40th normal volume) and almost non-vacuolate. It was therefore concluded that little or no antipodal activity was initiated. However, a normal 4-nucleate endosperm was present. This is evidence that the initiation of the earliest

mitotic divisions in the endosperm is independent of antipodal activity. A significant feature of antipodal development in Group 1 is the greater average maximum sizes of antipodal nuclei at 2 and 3 days in all other plants when compared with Plant 10. This suggests an over-stimulation of the antipodals, and recalls the somatoplastic hypothesis advanced by Brink and Cooper (1947) to explain endosperm failure. These averages appear to be increased by the presence of a few abnormally large antipodal nuclei in some ovaries of the former group.

Moreover, the extreme regularity in the size of antipodal nuclei at 1 day in plant 10 is contrasted with a high degree of variation in other plants. The following figures (not previously recorded) are for the average maximum diameters of antipodal nuclei in 2-day, fertilized, ovaries: Plant 5, 34.3 ± 5.5; Plant 6, 29.1 ± 6.7; Plant 9, 31.3 ± 6.0; and Plant 10, 30.0 ± 1.2. It is therefore suggested that these plants differed in the degree of variation among ovaries in the extent either of the stimulus provided the antipodals by the developing endosperm or of their capacity to react to a constant stimulus. This further suggests that this difference is due to chromosome - number differences following meiosis.

In examining ovaries of Group 2 material, many spheres of cytoplasm were noticed in some embryo sacs resembling those reported by Beaudry (1951) in embryo sacs following the cross Elymus virginicus x A.repens. In the aforementioned ovaries, however, these spheres appeared to originate in both endosperm and antipodals, whereas Beaudry considers those in his material to have arisen from the endosperm alone. Besides being grouped

around endosperm nuclei, they were also noted on the surfaces of antipodal cells (Plate 4, Fig. 31), usually near areas of vacuolization, and in one instance they were clearly seen in the vacuoles of antipodal cells. Moreover, there were indications that in these ovaries the antipodal vacuoles may burst and eject their contents into the embryo sac (Plate 1, Fig. 1). Therefore it is suggested that the antipodals eject vacuolar fluid, and probably spheres of cytoplasm, into the embryo sac. They may thus provide at least a portion of the cytoplasm surrounding the endosperm nuclei, thus tending to compensate for the rapid changes in nuclear-cytoplasmic ratio occurring during the period of intense nuclear multiplication in the endosperm up to about 3 days.

In all ovaries with endosperm seen in material past 2 days, the ovules had lengthened considerably. However, in some ovaries which showed failure during fertilization, considerable increases had occurred as well. Some of these embryo sacs observed in 3 and 5-day ovaries contained embryos (Plate 2, Fig. 9,10,11,12,13, 15), others had none and were generally as elongated as those with embryos. It is considered by the writer, therefore, that a stimulus to ovule-elongation is imparted to the surrounding maternal tissue by the fertilization process itself. No ovules were found which were more elongate than normal ovaries at 2 days after fertilization, and it is therefore assumed that the duration of effectiveness of the above-mentioned stimulus is approximately two days. Elongation past this stage is more dependent upon endosperm and/or embryo development. Several workers have found that parthenocarpy, in the form of elongation of the ovary, may

occur following pollination without endosperm or embryo development, and have been able to show that it is caused in some cases by pollination alone, and in other cases by fertilization without subsequent development of the embryo sac (see Gustafson, 1942, for review).

S U M M A R Y

Cytological studies were made of caryopsis development in a group of six Triticum-Agropyron amphidiploids, two from each of the following strains: F 5 of strain S 91, T.dicoccum var Vernal emmers x A.intermedium; F5 and F6 of strain S 107, T.turgidum var No. 49 x A.intermedium; and F5 of strain S 147, T.vulgare var Kharkov x A.intermedium. Quantitative data regarding the effects of various types of abnormality during and after fertilization were obtained from a group of four F6 plants, progeny of plants of the second strain. Fertility in these plants varied from 21.5 to 70.0 percent. Data obtained from a study of pollen and embryo-sac abortion, indicated that these forms of sterility caused only a small portion of the kernel failure. Percentage fertilization was generally high, and appeared to account adequately for partial sterility of only one plant of the first group.

Abnormalities in endosperm and embryo development were frequent and extensive in some plants, and infrequent and slight in others. These abnormalities appeared to be the cause of shrunken kernels, but only infrequently of aborted ovaries. Abnormalities of the fertilization process were also considerable. These resulted in complete failure to form endosperm and/or embryos, and the consequent collapse of ovaries. This type of abnormality was the major cause of reduced fertility in most of the plants studied.

Data concerning the relation of the antipodal group to kernel development indicated that they do not exert an influence on the fertilization but are stimulated to activity by it. They appear to require the presence of endosperm or embryos for

sustained activity. The fertilization process itself also appears to provide a stimulus to ovule elongation.

Data from chromosome numbers and the appearance of kernels giving rise to the second group suggested that imbalance of chromosomes in the gametes and endosperms may be responsible for abnormalities during and after fertilization.

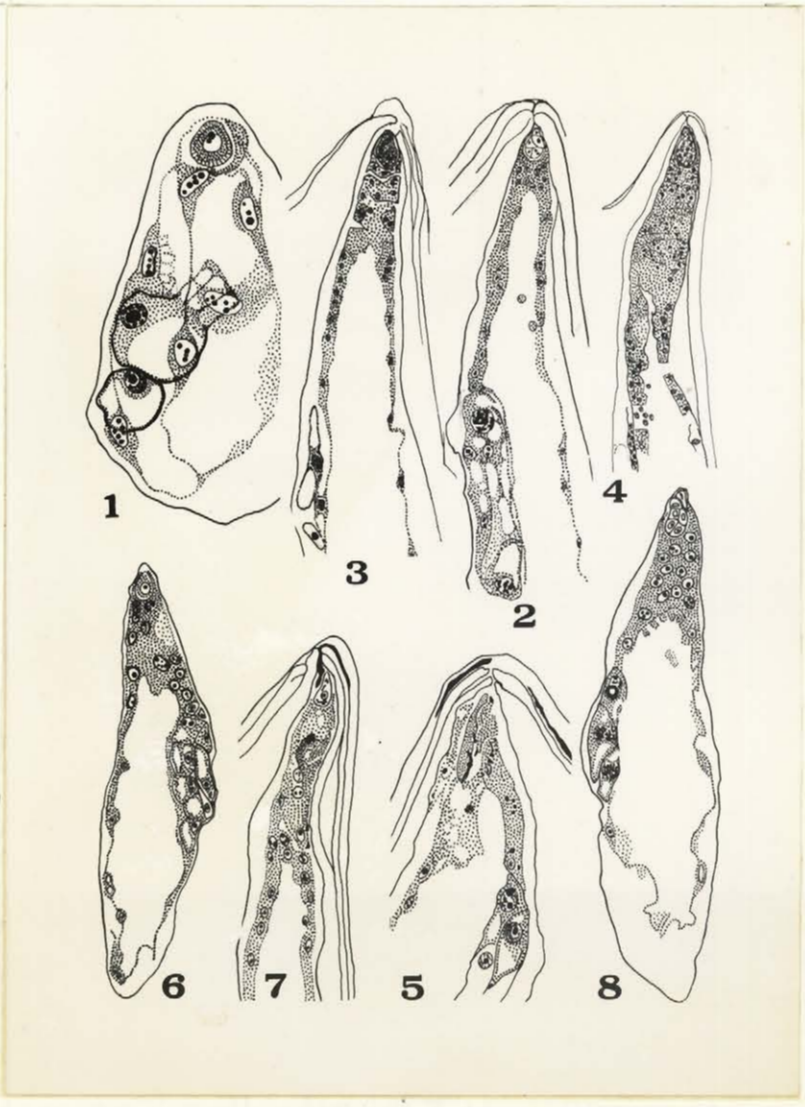
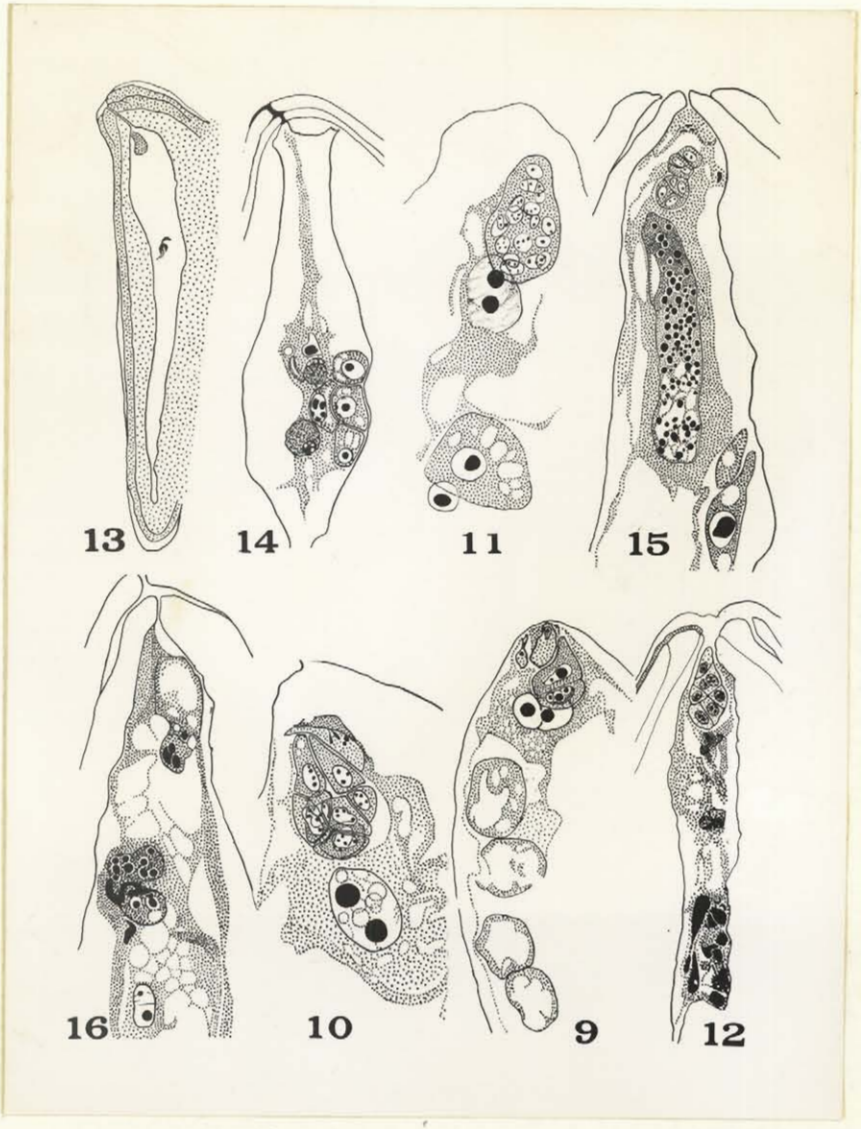


Plate 1



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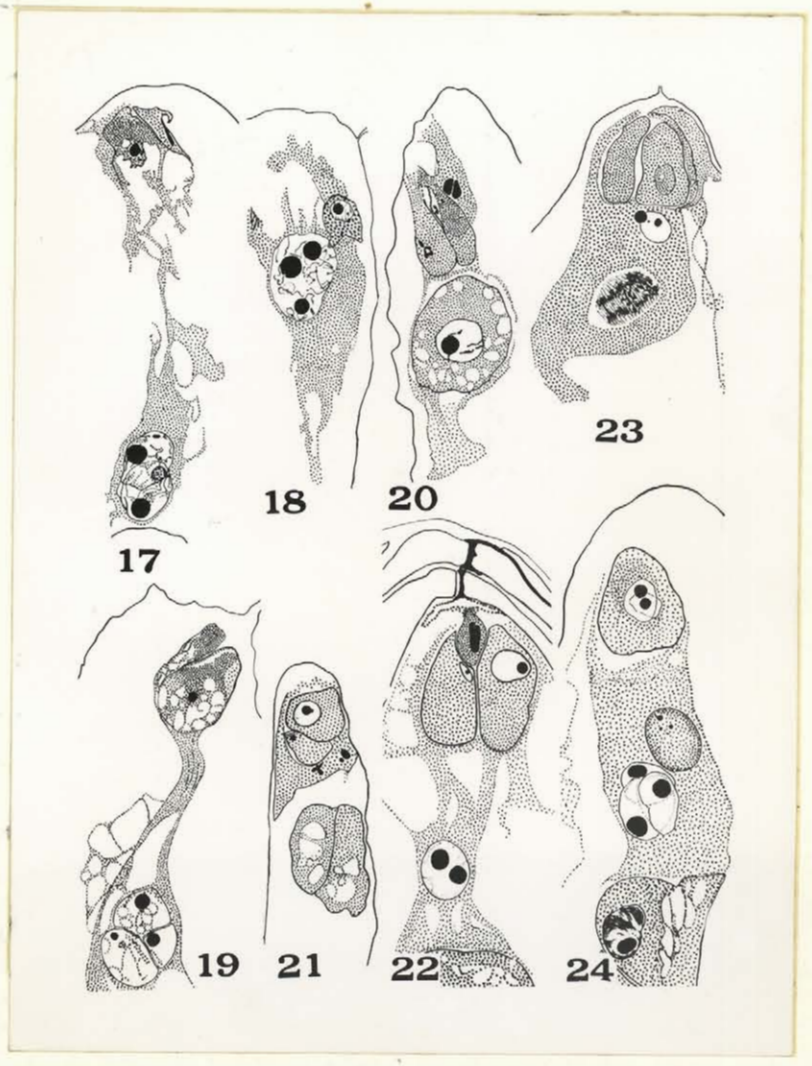

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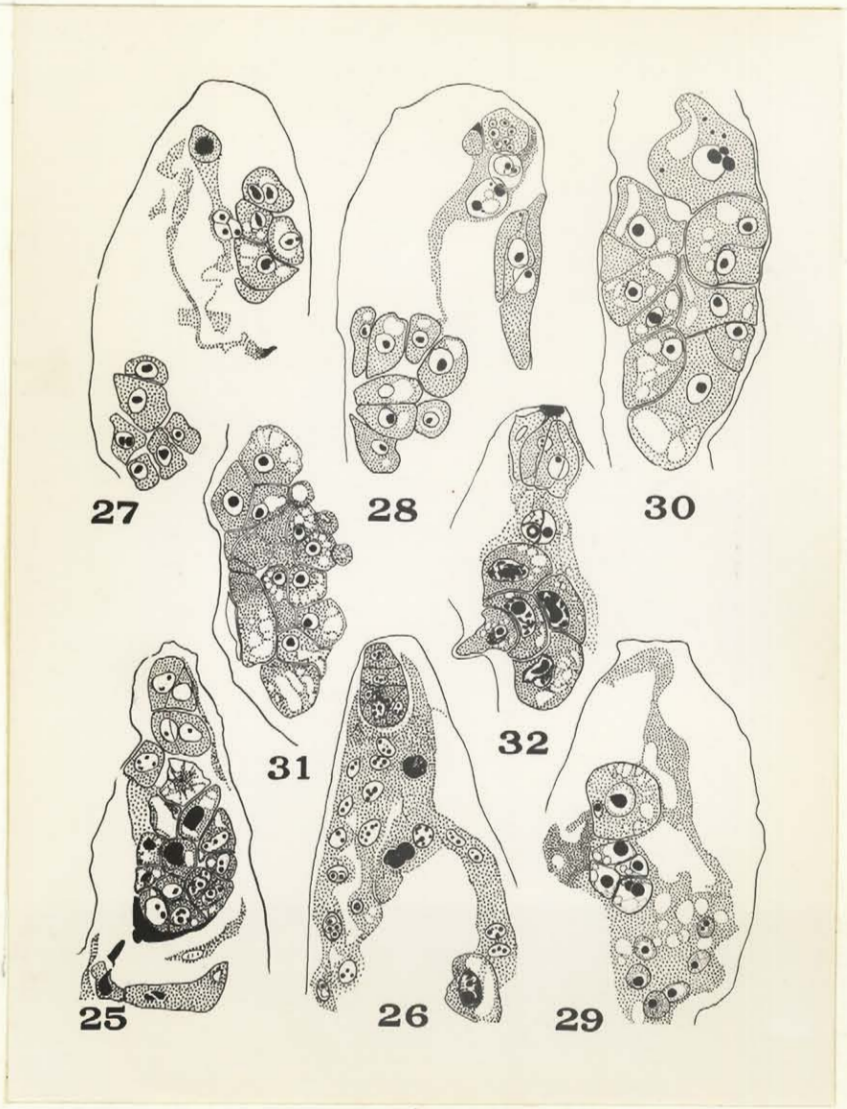

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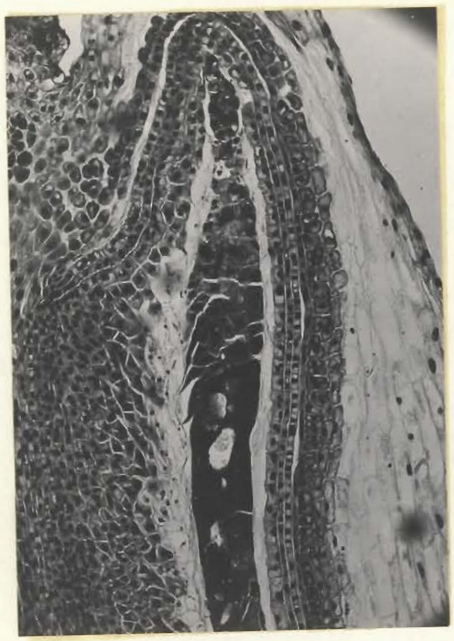

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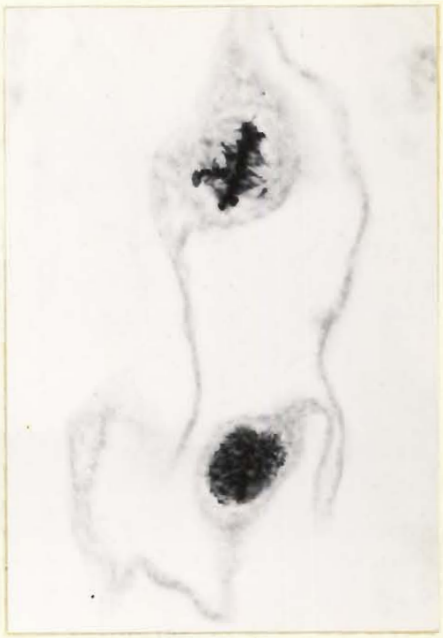

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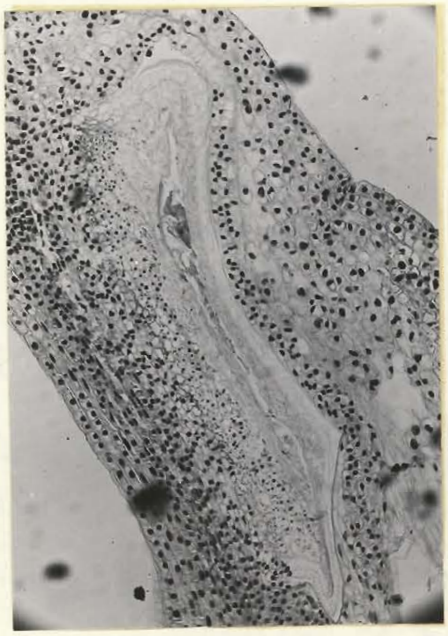

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Plate 5.

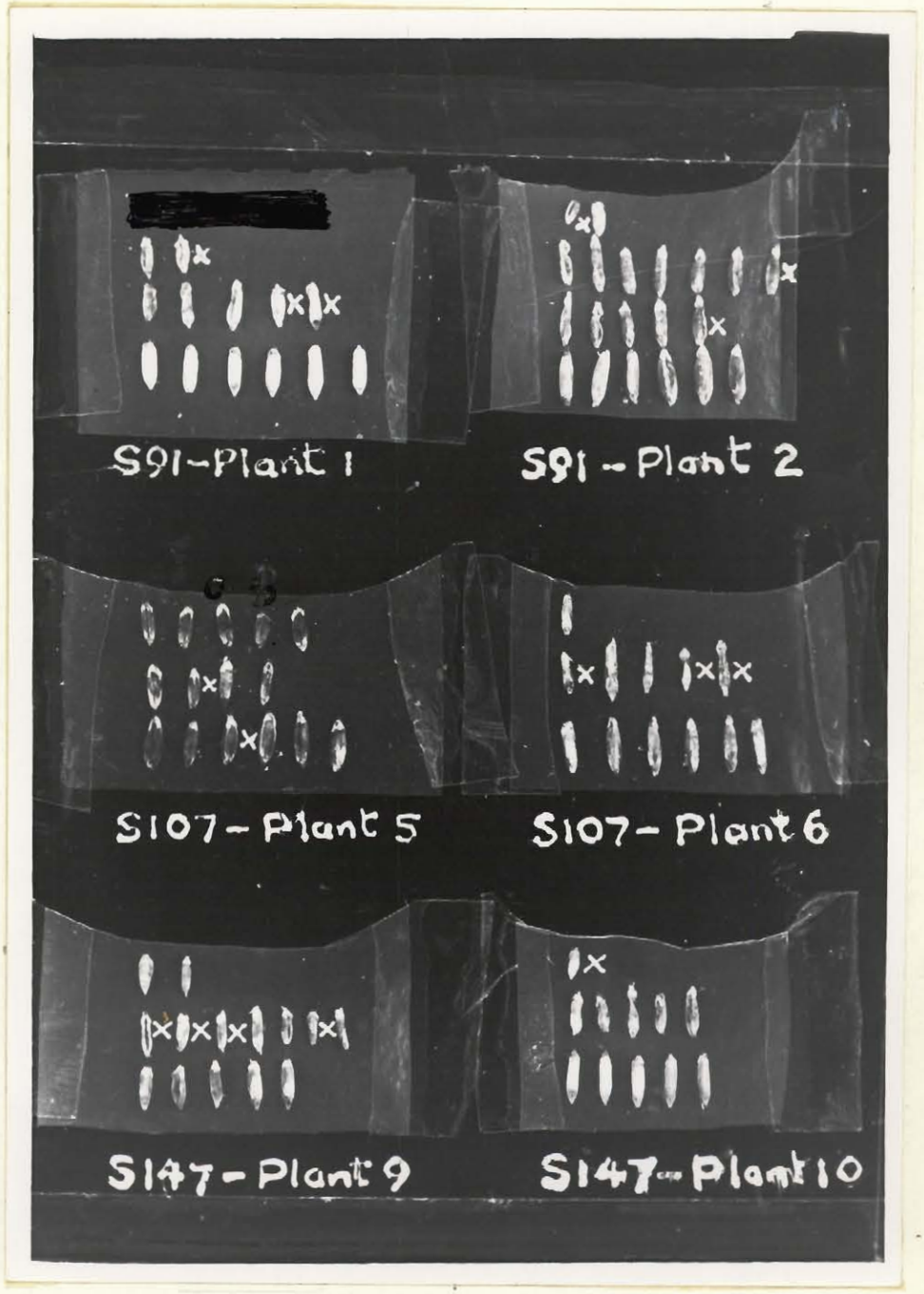
S91-Plant 1
S91 - Plant 2
S107 - Plant 5
S107 - Plant 6
S147 - Plant 9
S147 - Plant 10

Plate 6.

INDEX TO PLATES

Plate 1.

Fig. 1. Embryo sac of Plant 10 at 1 day; zygote nucleus with 2 nucleoli; endosperm 8-nucleate (2 nuclei not shown); maximum vacuolization in antipodals; X 120.

Fig. 2. Embryo sac of Plant 10 at 2 days, micropylar end; endosperm approximately 212-nucleate; embryo 8-celled; enlarged antipodal nuclei and spherical inclusions; X 60.

Fig. 3. Embryo sac of Plant 10 at 3 days, micropylar end; endosperm approximately 377-nucleate, embryo 18-celled; wall-formation in micropylar pocket; X 60.

Fig. 4. Embryo sac of Plant 10 at 5 days, micropylar end; endosperm approximately 4,600-nucleate; embryo 57-celled; wall-formation in micropylar pocket and near flattened remains of antipodals; X 45.

Fig. 5. Embryo sac of Plant 9 at 3 days; degeneration of endosperm (172 nuclei counted) in micropylar pocket; aborted embryo; remains of pollen tube; X 60.

Fig. 6. Embryo sac of Plant 9 at 5 days; endosperm 94-nucleate, zygote undivided; X 45.

Fig. 7. Embryo sac of Plant 9 at 5 days; endosperm approximately 645-nucleate, embryo aborted; pollen tube remains; X 60.

Fig. 8. Embryo sac of Plant 9 at 5 days; endosperm 72-nucleate; embryo 9-celled; X 60.

Plate 2.

Fig. 9. Embryo sac of Plant 9 at 2 days; embryo 2-celled unfertilized polar nuclei; antipodals highly vacuolate, (nuclei in other sections); end of pollen tube beside synergid; X120.

Fig. 10. Embryo sac of Plant 6 at 3 days; embryo 8-celled; unfertilized polar nuclei; end of pollen tube containing remains of nuclei (possibly sperms and X-bodies); X 180.

Fig. 11. Embryo sac of cross B x A at 5 days; embryo approximately 16-nucleate, with lack of wall-formation; unfertilized polar nuclei; slight vacuolization in antipodals; X 180.

Fig. 12. Embryo sac of Plant 9 at 5 days, micropylar end; embryo 9-celled; remains of triple-fusion nuclei near embryo; degener-

ated, heavily-stained antipodals in mid-region; remainder of sac collapsed; X 90.

Fig. 13. Ovary of Plant A selfed at 5 days; well-developed embryo; remains of triple-fusion or unfertilized polar nuclei; elongation of ovule equivalent to that of normal 2-day ovaries; X 30.

Fig. 14. Embryo sac of Plant 5 at 3 days; embryo not seen; separate remains of triple-fusion nuclei plus possible egg nucleus near antipodals; ovule elongation slightly less than normal at 1 day; X 90.

Fig. 15. Embryo sac of Plant 9 at 3 days, micropylar end; 10-celled embryo; primary endosperm nucleus highly endopolyploid, containing approximately 140 nucleoli; elongation slightly less than in normal ovaries at 2 days; X 90.

Fig. 16. Embryo sac of Plant 2 at 3 days, micropylar end; embryo aborted at approximately 4-cell stage; both normal and endopolyploid endosperm nuclei present; elongation equivalent to normal at 2 days; X 120.

Plate 3.

Fig. 17. Embryo sac of Plant D selfed at 2 days, micropylar end; vacuolate, degenerate zygote; triple-fusion nuclei; possible sperm on synergid; X 180.

Fig. 18. Embryo sac of Plant C selfed at 5 days, micropylar end; non-vacuolate, degenerate egg or zygote; triple-fusion nuclei; X 180.

Fig. 19. Embryo sac of Plant D selfed at 2 days, micropylar end; degenerate zygote; triple-fusion nuclei; X 180.

Fig. 20. Embryo sac of cross C x A at 5 days, micropylar end; x-bodies and sperms in apparently ramified pollen tube; unfertilized egg; polar nuclei not seen; X 240.

Fig. 21. Embryo sac of cross B x A at 5 days, micropylar end; fusion of sperm nucleolus with egg nucleolus; sperms in a supernumerary pollen tube; antipodals with medium vacuolization (nuclei in other sections); polar nuclei not seen; X 60.

Fig. 22. Embryo sac of Plant 1 at 3 days, micropylar end; sperms in pollen tube; unfertilized egg and polar nuclei; X 240.

Fig. 23. Embryo sac of Plant 9 at 1 day, micropylar end; division of highly polyploid primary endosperm nucleus; the zygote nucleus appears to be free in the cytoplasm of the sac; synergid nucleus degenerate; the end of the pollen tube appears next to synergid; X 180.

Fig. 24. Embryo sac of Plant 5 at 3 days, micropylar end; zygote nucleus in fusion stage; triple-fusion nuclei; a third body similar to those shown in Fig. 14, appears in the cytoplasm of the sac; X 120.

Plate 4.

Fig. 25. Section through aborted embryo of Plant 1 at 7 days; X 240.

Fig. 26. Embryo sac of Plant 9 at 5 days, micropylar end; degenerated endosperm nuclei associated with normal nuclei; endosperm 30-nucleate, embryo 15-celled, ovule-elongation compares with normal at 2 days; X 120.

Fig. 27. Abnormal embryo sac of cross C x D at 2 days; zygote degenerate; triple-fusion nuclei; 2 separate antipodal groups; X 90.

Fig. 28. Abnormal embryo sac of cross C x A at 5 days; zygote in fusion stage; "pseudo-embryo", probably of sperm or synergid origin; 2 separate antipodal groups; X 90.

Fig. 29. Abnormal embryo sac of Plant D selfed at 2 days; sperm nucleus in egg cytoplasm; polar group with 5 nucleoli; antipodals immature and lacking wall-formation; X 180.

Fig. 30. Antipodal group in ovary of Plant B crossed with Plant A; vacuolization intermediate; "micronuclei" in cytoplasm of one cell; X 120.

Fig. 31. Antipodal group of Plant B selfed at 5 days; intermediate vacuolization; spherical inclusions on the surface of two cells; X 120.

Fig. 32. Embryo sac of Plant 2 at 2 days; egg appears unfertilized; triple-fusion nuclei; the antipodals present an "embryo-like" appearance, and show minimum vacuolization; X 120.

Plate 5.

Fig. 33. Microphotograph of ovary of Plant 1, 7 days; endosperm abnormalities in the mid-region of the kernel, extending into the micropylar pocket; aborted ambryo.

Fig. 34. Microphotograph of ovary of Plant 9, 5 days; abnormal metaphase in endosperm nucleus; metaphase at top with plate oriented in 2 directions.

Fig. 35. Collapsed elongated ovary of Plant 9 at 7 days; elongation to normal ovaries from 1-day to 2-days.

Fig. 36. Microphotograph of Plant 2 at 5 days; erosion of cells (synergids) in micropylar pocket (light area). Maximum vacuolization of antipodals.

Plate 6. Kernels of Group 1 plants

S 91 - Plant 1
Row 1: small, shrunken kernels.
Row 2: shrunken kernels.
Row 3: large, plump kernels.

S 91 - Plant 2
Row 1: small, shrunken kernels.
Row 2 and 3: shrunken kernels.
Row 4: large, plump kernels.

S 107 - Plant 5
Row 1: flattened kernels.
Row 2: shrunken kernels.
Row 3: large, plump kernels.

S 107 - Plant 6
Row 1: small, plump kernels.
Row 2: shrunken kernels.
Row 3: large, plump kernels.

S 147 - Plant 9
Row 1: small, plump kernels.
Row 2: shrunken kernels.
Row 3: large, plump kernels.

S 147 - Plant 10
Row 1: small, shrunken kernels.
Row 2: shrunken kernels.
Row 3: large, plump kernels.

"x" to the right of a seed indicates failure to germinate. Seeds marked with letters are those from which Plants A, B, C and D originated.

A C K N O W L E D G M E N T S

The writer wishes to express his sincere thanks to Dr. J.W. Boyes for the planning and preliminary work of this study, and to Dr. J.M. Armstrong for helpful criticism; and to Mr. A. Kellett for the photographic work.

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