A STUDY OF THE REPRODUCTIVE MECHANISMS IN CERTAIN SPECIES OF THE BOTHRIOCHLOA AND DICHANTHIUM COMPLEXES

By

MARGARET HOOVER BROOKS

Bachelor of Arts

Smith College

Northampton, Massachusetts

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Thesis Approved:

Jack R. Harlan
Thesis Adviser

James S Brooks

H. J. Featherly

Irvy V. Holt

Robert J. Siemy

Dean of Graduate School

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PREFACE

Because of some confusion in the literature as to the terminology of apomixis, it seems advisable to present the currently accepted scheme of classification used by Stebbins (63).

Chart showing the interrelationships of the processes of apomixis found in the higher plants -- modified from Gustafsson, 1946.

<table>
<tr><th colspan="3">Apomixis</th></tr>
<tr><th colspan="2">Agamospermy
(Reproduction by means of seeds)</th><th>Vegetative Reproduction
(No seeds produced when apomixis occurs)</th></tr>
<tr><td>Gametophytic apomixis

Sporophyte

archesporial cell — somatic cell

diplospory (somatic or partly meiotic divisions) — apospory (somatic divisions)

gametophyte (2n)

egg cell — cell other than egg

parthenogenesis* — apogamety*

sporophyte</td><td>Adventitious embryony

Sporophyte

Integument or nucellar cells

somatic divisions*

sporophyte</td><td>Reproduction by means of bulbs, bulbils, runners, layers, etc.</td></tr>
</table>

*The processes at this level can take place either autonomously or by pseudogamy, i.e., under the influence of pollen tubes or endosperm development.

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INTRODUCTION

Efficient use of the breeding potential of a plant species of agronomic importance can best be made when the reproductive mechanisms available to the species in question are well understood. Beyond being desirable to the breeder for obviously practical reasons, such fundamental information is also basic to any research which attempts to control the effect of the environment upon the expression of genetically controlled tendencies. These two reasons would by themselves provide sufficient stimuli for the study of the gametophytic generation of any angiosperm, but in addition the recognition of the phenomenon of apomixis as the apparently predominant means of reproduction in the complexes under consideration provides a third excellent reason for undertaking the present investigation. Apomixis, simply defined by Stebbins (63) as diverse means of asexual reproduction which "tend to replace or to act as substitutes for the sexual method" is widespread in the plant kingdom as a whole. Although part of the apomictic process, namely parthenogenesis, is to be found among representatives of the animal kingdom, it is far less prevalent here than in plants. Simpson (58) suggests that this may be correlated with the tendency shown by the former to "be less liable than plants to be hemmed in to evolutionary blind alleys", and Stebbins (63) points out that the structural and developmental growth patterns of plants have placed positive selection values upon certain modes of asexual reproduction therein. Moreover, whatever the association between apomixis and polyploidy may be, the

relative rarity of the latter in the animal kingdom may play a role in the relative absence of the former among the higher animals. Certain plant groups in which polyploidy, apomixis, and hybridization are closely interwoven form so-called agamic complexes (6) several of which have been extensively studied. In the Gramineae, in particular, apomixis has been found to be fairly widespread, and specifically within the tribe, Andropogoneae, Celarier and Harlan (20) have succeeded in demonstrating its presence in the section Amphilophiastrae of Stapf. Certain of the so-called complexes within this section lend themselves particularly well to studies of apomixis because of the existence of both diploid and polyploid chromosome numbers, the retention of wholly or partially sexual reproduction in some and the loss of such sexuality in other members of the complex, and the suspected relationships existing among these members. The researcher is definitely challenged to carry out some rather fundamental studies within a group of plants which based upon these considerations is probably still in an active stage of evolution. Certain hybrids obtained following hand emasculation and pollination within these groups (56) have suggested that facultative apomixis not only exists herein but exists to varying degrees from one accession to another. Simpson's reference above to the evolutionary blind alley of obligate apomixis is not applicable to such facultatives which, properly controlled by genetic, environmental, or even biochemical means, would seem to represent an ideal mechanism for combining the potentials of segregation with the conservatism of asexual reproduction.

It becomes, then, a matter of both theoretical and practical interest to attempt to determine exactly what constitutes this inherent facultativeness to begin with, what the internal situations and factors are

with which the breeder will have to deal, what potentials exist within the components of the reproductive system or systems which might offer possibilities for avoiding this evolutionary blind alley of obligate apomixis. The present avenue of approach is only one of many which suggest themselves, and the methods used in the present investigation are directed towards obtaining answers to only a few of the many questions which need to be asked before all the correct answers are obtained. A specific method not ordinarily employed in such studies has been utilized in this work under the assumption that the demonstration of the use and efficiency of such a method might be of value in itself, and secondly that the method might make feasible the obtaining of sufficient data upon which to more confidently base frequency determinations. Experience with such a method has been rewarding, and a discussion of the advantages and limitations of its use will be taken up later.

The principal purposes of this investigation have been threefold:

1. To obtain a better understanding of the mechanisms and developmental sequences of reproduction prevailing in selected species known to produce both sexual and apomictic progenies.
2. To make as many comparisons as possible as to the reproductive mechanisms operative in certain representatives of two very closely related genera at three ploidy levels.
3. To obtain estimates of sexual potential in these species with a view toward obtaining the background information requisite for selection of breeding materials and for any eventual attempt to control the alternation of sexual and apomictic cycles or to increase the frequency of sexual reproduction at will.

The accumulation of these data has been made without the use of controlled conditions, and if the assumption that such control might alter the frequencies is correct, these data obviously present the picture under only limited and non-induced environmental situations. Furthermore while a descriptive analysis, accurate to the best of our ability can be presented from such data, any interpretation of the significance is by necessity subjective and certainly plastic.

The investigations now to be discussed constitute, then, an attempt to shed more light upon the mechanisms of facultative apomixis not only because of the obvious need to better understand this method of reproduction from a practical standpoint, but also because the avenues of more theoretical study can only be opened up after the basic information is at hand.

REVIEW OF LITERATURE

Seed formation without fertilization was first observed, as far as is known, by J. Smith in 1841 (cited in 32) in the female plants of Alchornea ilicifolia, a dioecious Euphorbiaceae, the embryo originating from adventitious buds in nucellar tissue. The history of apomixis from this time up until the middle of the 20th century is comprehensively treated by Gustafsson (32)(33)(34) in his review of apomixis in higher plants published in 1946 and 1947. The scope and depth of this three volume monograph makes almost unnecessary any review of investigations previous to this time. In addition, several other exhaustive reviews of the general subject have been published including ones by G. L. Stebbins (62) and (63), dealing with apomixis in the angiosperms, and by Maheshwari (44) reviewing the embryology of apomixis. Publications of pertinent information in the Pteridophytes by Manton (45) and by Steil (64) are of interest also. In 1941, Nygren (50) published another review of apomixis in the angiosperms followed in recent years by a supplementary review article (53) of the same title. In spite of these excellent articles which are quite exhaustive in treatment, particular attention may be drawn to selected publications. Also certain pertinent data included in these reviews will be specifically mentioned, and finally brief summaries made of papers which have appeared since 1954. First of all, however, a brief historical account of research in apomixis as outlined by Gustafsson (32) may be of some interest.

The real beginning of the study of the problem of apomixis was embryological work on the cat's foot and dew cup genera by Juel in 1898 and 1900 and Murbeck in 1897 and 1901, these same authors extending their embryological investigations to Taraxacum and Hieracium in 1904-5. Actually, description of the oldest example of apospory in species with one archesporial cell is accredited to Rosenberg who in 1907 described an embryo sac originating from nucellar cells in Hieracium subg. Pilosella. A year later Winkler initiated terminology approximating that now used, substituting the collective term "apomixis" for the formerly misapplied term "apogamy". Ernst in 1918 was one of the first investigators to draw attention to the fact that in many ways apomicts behave like hybrids, but that this "hybrid nature" attends rather than causes apomixis. In 1920 Winkler again published on the nature and origin of apomictic phenomena, and in 1930, Rosenberg presented his general survey including most of the results which had been obtained to date in apomictic research, mainly embryological and cytological. Up to this time the principal plant researches in this area had been carried out in Rubus, Euhieracium, Taraxacum, Alchemilla, and Eupatorium; in the next fifteen years interest in apomixis increased considerably. New apomictic groups, so-called "polymorphic species-complexes" were studied in Potentilla, Poa, Sorbus, Crepis, Ranunculus, Calamagrostis, and Antennaria, the first two genera being worked on particularly by Müntzing. Embryological aspects of apomixis in Poa and Potentilla were studied simultaneously and independently in the early 1940s by Hakansson, Rutishauser, and Fagerlind, these same species serving for studies on the phenomena of pseudogamy. The first modern treatment of an agamic complex was presented by Babcock and Stebbins in 1938, a study of Crepis in particular and a discussion of species

formation and the polymorphism in apomicts in general.

Restricting attention at this point to apospory (terminology has been discussed in "Preface"), the original paper on this type of apomixis, as mentioned earlier, was Rosenberg's analysis in 1907 in Hieracium subg. Pilosella (cited in 32). Similar mechanisms in which the embryo sacs originated from a cell close to the macrospore mother cell, considerable growth of this somatic cell occurring previous to subsequent nuclear division, and characteristic vacuolization of the cytoplasm being noticeable were described in several species including Malus, Crepis, Hypericum, Poa, and Ranunculus (see 32). The number of divisional steps from the one-nucleate initial to the fully developed sac was three as a rule, but as Schnarf pointed out, agamospermous plants showed a conspicuous tendency to suppress the differences between the sac elements resulting in less differentiation between its component nuclei. Transitional forms between diplospory and apospory were reported by Gustafsson to have been observed in Alchemilla and Sorbus, but probably the best studied cases up to this time were in Poa (2)(3)(47), Potentilla by Muntzing, Christoff, Papasova, and Rutishauser, as cited by Gustafsson (33), and Parthenium (54). The only genera in Nygren's 1954 review (53) in addition to those already mentioned as exhibiting apospory followed by parthenogenesis are Atraphaxas, Elatostoma, and Paspalum, but several investigators contributed their work on new species, as this later review indicates. Within the Gramineae, approximately fifteen species (53) were known which exhibited diplospory, a few exhibited vivipary, and the only cases cited as exhibiting apospory plus parthenogenesis were: Paspalum dilatatum, Poa ampla, P. arida, P. arctica, P. compressa, P. herjedalica, and P. pratensis.

Because observations made in the course of the present work are in

essential agreement with numerous researches of long standing, it has seemed advisable in this review of literature to select certain references to which certain aspects of our work are pertinent. These will be enumerated at this time although in some cases a more detailed explanation of the terminology may be delayed until later.

1. The observation of multiple sacs within one ovule has been made by numerous investigators including Akerberg(2), Kiellander(40), and Nygren(52) on Poa, B. W. Smith (59) on Paspalum cilaratum, Gustafsson (32) in Hieracium, and Pratt and Einset (55) on American species of Rubus. Farquharson (26) discusses polyembryony in Tripsacum dactyloides where the multiple embryos may arise in separate sacs or from different nuclei within a single sac. He finds polyembryony in 43 out of 49 collections made, in one of which its frequency exceeds 50%. In his review of polyembryony Webber (73) distinguishes between false embryony, plural embryo sacs derived from several embryo sacs, and true polyembryony or plural embryos derived within or by projection into a single sac. The occurrence of twin seedlings from plural embryo sacs has been, according to Webber, attributed to the development of extra megasporocytes in Poa, to the development of sister megaspores in Poa, and to apospory in Malus, Poa, and other Gramineae. Webber states that although polyembryony is constitutional, environment determines the degree of its expression.

2. References to the crowding out of the sexual sac in apomicts is referred to in the early work of Rosenberg (cited in 32) on Hieracium subg. Pilosella in which he discusses the "conspicuous competition between aposporous and legitimate sacs" during which the sexual sacs are encroached upon. Akerberg (2) also comments that this competition has been observed

in Poa pratensis and in Crepis. In other cases, however, authors have reported that degeneration of the macrospore mother cell occurs independently of crowding out. Kiellander (40) working with Poa, states that this degeneration may occur at any stage, the disorganization of the macrospore mother cell not being caused by its being squeezed by aposporous cells but the role of the "legitimate embryo sac is taken over by one or more aposporous sacs". Emery (24) reports for Setaria macrostachya a similar degeneration of sporogenous gametophytic tissue and the subsequent formation of aposporous embryo sacs. In the common type of Bothriochloa ischaemum, Brown and Emery (14) report that two or three sac initials develop in the micropylar region of the nucellus either prior to degeneration of megaspore mother cells or shortly thereafter. In the oriental type of this species they state that more aposporous initials are produced and their initiation is spread over a longer period of time. Here, then, either a sterility factor or crushing from sac initials may cause degeneration of the megasporocyte. In Themeda triandra these same authors (14) suggest that the megasporocyte degenerates at an early stage and is followed by enlargement of one or more aposporous sac initials. In Paspalum, Synder (60) reports that the chalazal megaspore "often begins to degenerate before marked encroachment by the enlarging nucellar cells has occurred".

3. Numerous references to antipodals and their function are to be found in the literature. The bearing of these findings on the present observations of lack of antipodals in certain materials is of some concern. As early as 1934, Wakakuwa (68) comments on the degeneration of antipodals in wheat and their apparent function as nourishment for the endosperm. Beaudry (7) in discussing certain Elymus x Agropyron crosses

attributes the death of hybrid seeds to the disturbed physiology of the antipodals caused by stimulus from association of foreign sperm with the polars, the modified antipodals subsequently, in their secretory role, altering the nutrient supply to the endosperm and effecting its eventual breakdown. The high metabolic activity of the antipodals causes Maheshwari (44) to comment on their analogy with anther tapetum. Shadowsky (as cited in 12) records the antipodals in the Gramineae as ranging from three to forty three, the multiple numbers predominating, and states that they function in transmitting nutrients to other parts of the embryo sac, an idea propounded as early as 1890 by Westermaier (cited in 12). Brink and Cooper (12) report that the antipodals are normally stimulated to hypertrophy at fertilization, and that they function in secretion of substances needed for endosperm growth. Referring to Secale x Hordeum (13) crosses, the authors state that "the failure of the rye sperm to stimulate the antipodals to their normal secretory activity results in undernourishment of the endosperm mother cell and its immediate descendents". Hair (35) working with Agropyron also comments on the nutritive function of the antipodals.

4. References to numbers and behavior of polar nuclei are also frequent in the literature. Håkansson (36) records as many as 3 polars in Poa alpina, Farquharson (26) found 3-4 polars in some sacs of Tripsacum dactyloides, and Hair (35) observed multiple polars in Agropyron. As recorded by Brown and Emery (14) a single polar condition in aposporous sacs of Gramineae has been reported in Themeda triandra, Bothriochloa ischaemum, Cenchrus, Panicum, Paspalum, Pennisetum, and Setaria. A 2 polar condition in a 4-nucleate sac has been found by these authors to occur infrequently in Themeda, Bothriochloa, and in Setaria.

5. Attention should be drawn to the extensive literature on the fertilization of the unreduced egg nucleus in apomictic species. Gustafsson (32) affords references to this and selected additions may be mentioned here: Akerberg (2) in Poa and in Hypericum, especially by pollen of other species, Bergman (33) in Leontodon, Gentcheff and Gustafsson (33) in Potentilla, Burton (16) in Paspalum, Gerstel et al (28) in guayule, Löve (43), Müntzing (47), Tinney (67) and Nygren (52) in Poa, Powers (54) in guayule, Bremer, Naryanaswami, and Raghavan (cited in 53) in Saccharum, Grun and Triplett (cited in 53) in Poa. Nygren (53) commenting on this last work states that the morphology of the two types of hybrids, formed from reduced or unreduced egg cells, is quite different but that "transgressions may occur which is of particular interest in species with agamospermous reproduction". As Maheshwari (44) points out, timed pollinations may be the experimental approach towards production of higher ploidy embryos obtained from the fertilization of unreduced egg nuclei, bearing out Gustafsson's (32) statement that "the facility or difficulty with which the unreduced egg cells are fertilized is bound up with the time of the first egg cell division. The sooner this occurs or the slower the pollen tubes grow, the rarer will hybrid formation be".

6. The closely related subjects of developmental timing, synchronization of development in the male and female, and precocious embryo divisions are also extensively treated in the literature. Selected references would recall again Akerberg's (2) work on Poa where comparisons between sexual and apomictic synchronization of the two sexes indicated that maturity was reached later in the apomictic female. Bergman (according to Gustafsson, 32), however, records that in Hieracium the egg cell

of the apomictic type starts division ahead of the egg cell of sexual biotypes. Gustafsson (32) refers to the precocity of division of the apomictic egg nucleus as mentioned by Murbeck in Alchemilla and in Taraxacum and by numerous authors in Poa. Akerberg in particular feels that since the aposporous embryo has gone through several divisions before the pollen reaches it, parthenogenesis is maintained by this ability of the egg nucleus to begin to divide early. He agrees with Gentcheff and Gustafsson (cited in 2) from their work in Potentilla that the "egg nucleus in the aposporous embryo sac in Poa pratensis may be fertilized but from causes the genetic nature or origin of which cannot yet be explained an embryo is usually formed before fertilization can take place". Again Nygren (53) states that in Poa, 70% of the aposporous embryo sacs had egg cells which divided immediately after the sac was mature, and therefore they never had a chance to become fertilized. "Such a plant can be extensively used as a male parent and it might transfer the character of early division of the egg cell to its hybrids, a character which might in later generations be expressed in formation of a high percentage of apomictic maternal-type plants. These examples may suffice to show how an embryological study may save time for the breeder of apomictic strains and at the same time may enable him to predict the general magnitude of the variation to be expected in the offspring. The figures obtained in a given study may vary from one year to another and in different environments and even between different panicles on the same plant. The investigator needs to keep such variables in mind when he makes his predictions." The precocity of the endosperm as compared with the embryo is cited by Gustafsson (32) as occurring in Malus, Antennaria, and Eupatorium. In Parthenium (33), however, parthenogenetic

embryos are formed prior to fertilization. Brink and Cooper (13) indicate that in sexual species, the fertilization of the egg and central cell are parallel events, the endosperm nucleus dividing shortly thereafter; the zygote may divide concurrently but the endosperm normally divides before the embryo and its rate of mitosis exceeds that of the embryo. Considerable variation apparently exists but the authors suggest that the five known cases in sexual species recorded as having zygote division preceding endosperm division (Najas minor, Limnocharis emarginata, Elodea canadensis, Limnophyton obtusifolium, Vallisneria spiralis) should be reinvestigated since the last two named species are known to be erroneously analyzed. In Paspalum in which Synder (60) reports that "lack of correlation between development of the archesporial cell and comparable events in microsporogenesis is readily apparent", the embryo only starts division when the endosperm is sixteen to thirty two nucleate. Synder states, "Gustafsson has stressed the existence of a strong tendency to preclude fertilization through a forward shift in the time at which embryo development is initiated. Here and in other apomictic Paniceae (Warmke, Synder et al) initiation of embryo development usually does not occur until 15-30 hours after growth of the pollen tube into the sac. A more plausible explanation for the failure of fertilization in these species and probably in the initial establishment of apomixis in many other species would be a physiological condition of the egg cell and male nuclei acting to prevent the union of these gametes."

7. The importance to fertility of the ratio of maternal tissue: endosperm: embryo ploidy, normally 2:3:2 in sexual organisms, has been stressed by several authors. Müntzing (47) suggests that this may not be as critical in apomicts as in sexuals. However Brink and Cooper (13)

feel that the change in this 2:3:2 ratio may be an important cause of seed collapse and refer to Thompson's work in which crosses between species differing in chromosome number are more likely to yield germinable seed if the parent with the larger chromosome number is used as the female. Warmke (72) in his work on Panicum suggests also that a 2:3:2 ratio would be selectively favored over a 2:5:2 which would result from pseudogamous 2 polar unreduced sacs.

8. Finally there are several references in the literature to polyspermy. Artschwager (4) finds this phenomenon occurring in Sorghum vulgare and Warmke (71) in Taraxacum, and Webber (73) refers to work in Saccharum in which polyembryony results from fertilization by extra-generative nuclei.

While admittedly the above review of literature is a selected one, an attempt has been made to therein indicate, superficially at least, some of the parallel researches to that to be reported here.

At this time attention will be drawn to several specific papers which have appeared since 1954. One is an account by Fisher, Bashaw, and Holt (27) presenting evidence for apomixis in Pennisetum ciliare and Cenchrus setigerus, in which material the megaspore undergoes two divisions and is then crowded out by aposporic sacs produced from enlarged nucellar cells. Approximately 22% twin embryos are obtained, the proembryos developing both in the chalazal and micropylar ends of the ovule. The authors postulate that both genera probably belong to a single agamic complex.

An excellent paper appeared also in 1954 by Warmke (72) on apomixis in Panicum maximum, a species exhibiting facultative apospory combined with pseudogamy. Both 8-and 4-nucleate sacs are produced, the 4-nucleate type having 2 synergids, 1 egg, and 1 polar. Warmke mentions Narayan's (48)

finding of similar 4-nucleate embryo sacs in Pennisetum rueppellii and P. villosum. Enlargement of the nucellar cell is independent of sporogenous cell degeneration which is not, at an early stage, due to crowding. Later on, however, crowding of multiple sacs becomes a factor in the ability to reach maturity. Fertilization occurs 4.5 to 8 hours after anthesis; the first endosperm division occurs 9 hours after anthesis, the first division of the embryo, 19 hours after anthesis at which time the endosperm has 16-32 free nuclei, differing in this respect from the autonomous and precocious embryo development in Poa. Occasionally two 8-nucleate sacs were found in a single ovule indicating the possibility that 8-nuclei did not always represent a reduced condition. However, Warmke points out that an 8-nucleate unreduced sac, containing 2 polars would result after fertilization in a 2n embryo, 5n endosperm and 2n maternal tissue, apparently less balanced and less fertile (13) than a 2:3:2 condition resulting from fertilization in a reduced sac or fertilization of the single polar of an unreduced 4-nucleate sac. Fertility studies indicated that the lowest fertility was obtained from material having the largest number of sacs per ovule. A low fertility obtained from material with the largest number of 8-nucleate sacs is also probably significant, whereas the highest fertility was obtained in material with the lowest number of sacs and few if any 8-nucleate sacs.

In 1955 Synder, Hernandez, and Warmke (61) published on the mechanism of apomixis in Pennisetum ciliare. They found that the development of the embryo sac followed two different patterns, one which resulted in a 4-nucleate type with 2 synergids, 1 egg, and 1 polar; another resulting in an 8-nucleate type with 2 synergids, 1 egg, 2 polars and 3 plus antipodals. The number of sacs per ovule differed in different strains,

varying from 1-8 with means varying from 1.5 to 4.6. In strains with the highest number of 8-nucleate sacs, the enlarging nucellar cells were frequently not well defined until after meiosis was completed in the megasporocytes, whereas in strains showing a lower number of such sacs, enlarged aposporic initials were commonly found at a similar stage of development. The authors conclude that variations in the timing of the development of nucellar embryo sac initials and in the number of sacs per ovule indicate considerable differences in the factors responsible for aposporic phenomena. Here, too, embryo divisions are stimulated by endosperm development and usually occur after the endosperm has reached a 16-32 nucleate condition. Polyembryony, i.e. Webber's false polyembryony, occurs in about 20% of ovules of one strain, resulting in 17.7% twin seedlings.

Mention should be made of several points emphasized by J. B. Hair (35) in his paper dealing with subsexual reproduction in Agropyron. In this material at the time of anthesis, the unreduced egg had often developed precociously into a small proembryo and "put itself out of reach of fertilization", whereas in sexuals the free nucleate endosperm was initiated in advance of divisions in the young zygote. The author, agreeing with Esau's (25) data in Parthenium, states that in apomictic plants there is very little correlation between the rate of development of the embryo and that of the endosperm. An interesting observation of multiple polars was made in this material, the more apomictic material exhibiting a decrease in the number of synergids and an increase in the number of polars, eggs, or antipodal groups. Chiarugi and Francini, cited by Hair (35), had observed similar irregularities in apomictic Ochna serrulata, reporting 25% of sacs unanalyzable and recording difficulties with antipodals and questionable synergids.

Two papers appeared in 1957 on apomixis in Gramineae. Emery (24), studying Setaria, reported that 5 perennial species form polyploid members of an agamic complex. Two types of embryo sacs are formed, an 8-nucleate sexual type and a 4-nucleate apomictic type, the 4 nuclei being 1 synergid, 1 egg and 2 polars. He finds, in addition, that the sexual members of this agamic complex, as of the majority of such complexes, are usually self-incompatible and hence cross fertilized, but that this condition is not without exceptions.

Brown and Emery (14) reported on apomixis in the tribe Andropogoneae. In Themeda triandra, although reduced sexual sacs were occasionally possible, aposporous 4-nucleate sacs predominated, a 1-polar type being about five times as frequent as a 2-polar 4-nucleate type. Apparently these 2-polar aposporous sacs are not functional, as endosperm chromosome counts revealed only a 3n or 2n, unfertilized condition. The precocious development of either the embryo or endosperm was not constant. In Bothriochloa ischaemum, common type, 2n = 40,60, the ratio of 2-polar to 1-polar types, both apomictic, was 1:7; that in the oriental type, 2n = 50,60, was 1:8 with no sexual sacs observed. Here usually initial divisions of the egg are reported to follow the initial endosperm divisions although two accessions, King Ranch and Triangle City, are reported to show the reverse precocity. The authors suggest that Themeda represents a rather young and still evolving group, an agamic complex of sexual diploids and more or less aposporous polyploids. In Bothriochloa ischaemum, on the other hand, apomixis has probably existed for a longer time, and it may represent one obligate apomictic species belonging to a larger agamic complex.

MATERIALS AND METHODS

The plant materials for this investigation have been maintained in an experimental nursery at the Oklahoma Agricultural Experiment Station for the past several years. These and other related accessions are part of a collection of Old World Bluestems used in the forage crops breeding program. The six accessions studied belong to the tribe Andropogoneae, subtribe Bothriochloae (Keng.). In the following table, the species names, accession numbers used at this station, the place of origin of the collections, and their chromosome numbers are listed:

Species	Accession Number	Place of Origin	Chromosome Number
Dichanthium annulatum	3242	Calcutta, India	2n = 20 (21)
Dichanthium annulatum	3182	Israel	2n = 40 (21)
Dichanthium annulatum	4099	Punjab, India	2n = 40 (21)
Dichanthium annulatum	4083	South Africa	2n = 60 (21)
Bothriochloa intermedia gangetica type	2655	British Guiana[1/]	2n = 40 (20)
Bothriochloa ischaemum	2582	Formosa	2n = 60 (17)

In general, field material has been used whenever possible, collections being made from row plantings established at the Agronomy Farm Andropogon garden (19) west of Stillwater, Oklahoma. At times it has proven necessary to use greenhouse material, but no attempt has been made as yet to undertake a comparative study of the effect of the two

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[1/] Bothriochloa intermedia, gangetica type, is an introduction from British Guiana where it occurred as an escape from cultivation; it is probably an Indian type.

environments; however relatively few of the data reported here were obtained from greenhouse plants. Inflorescences were collected almost entirely between 8:30 and 11 A.M. during the flowering season and fixed immediately in the field. The fixative employed was that suggested by Bradley (11), and the method utilized exclusively for the study of the female gametophytes was the Bradley method, modified only slightly to the requirements of this material. Inflorescences were preserved in a fixative of 4 parts chloroform : 3 parts absolute alcohol : 1 part glacial acetic acid in which they keep satisfactorily for many months under slight refrigeration. At a magnification of 18x, the stamens and pistil of the sessile floret were dissected out intact and transferred to a small wire basket affixed to a wire loop (see diagram) to facilitate transfer from one beaker to the next.

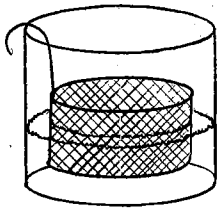


Figure 1. Basket used to transfer ovule through solutions.

The florets of a single raceme were usually worked with as a group, each group being assessed a separate series number for convenience of recording. The wire basket containing the material was transferred through the following solutions at the indicated intervals:

1. 4% aqueous iron alum at 75° C. for 3 minutes
2. Distilled water at 75° C. for 2 minutes
3. " " " " " " "
4. 50% HCl for 10 minutes
5. Distilled water for 10 minutes
6. " " " " "

Studies of the anthers of each floret were made from simple aceto carmine smears, and the stigmas, especially at post fertilization stages, were examined for germinating pollen. The ovaries, placed on a slide in a drop of water, were worked with under a 18x dissecting microscope; the ovule was removed by using fine sewing needles inserted in corks as dissecting tools. All debris and excess water were removed from the slide; a drop of acetocarmine placed on the material, and coverslip pressure applied. Careful manipulation with a needle on top of the coverslip while observing the preparation under 125x magnification and slight application of heat caused the embryo sac, or initial, to pop out intact in a high percentage of cases. During the later stages of this work it became increasingly easy to obtain the sac or sacs entirely free from any extraneous tissue before staining, thus greatly simplifying analysis.

The principal disadvantage of this method encountered in the present investigation is that in a smear preparation it is difficult to determine the orientations, positions, and/or places of origin of the sacs. Obviously, to effectively analyze the contents of a sac, the sac has to be pushed free of surrounding tissues, and its original position cannot be easily or accurately determined. Consequently, difficulties were met with particularly in attempts to analyze the origin of sac initials or megaspores; and it is suggested that while this technique is completely adaptable to study of these earlier stages, considerable perseverance is required to obtain results in which one has confidence. The more

commonly used sectioning techniques have disadvantages also, particularly that of interpretation through different levels. The beauty of the technique used in this investigation is not only its simplicity and speed but the possibility of more accurate interpretation, since the sacs in their entirety are present in a single microscopic field. In the hands of an expert technician, this method certainly offers considerable potential for studies of this kind.

A few observations on pollen grain germination and pollen tube growth have been made. While this is not a major feature of the research, mention should be made of the satisfactory technique employed in these preliminary studies. A method suggested by J. Wilson (personal communication to Dr. J. R. Harlan) is the so-called CL method, involving fixation in FAA, bleaching in dilute sodium hypochlorite from 3 to 24 hours depending on the pigmentation of the stigmas, and staining in aqueous lacmoid.

Detailed analyses were always made with an oil immersion lens at a magnification of 1125x. A Bausch and Lomb E research microscope was used. A Bausch and Lomb K camera was used for photography. The purchase of the photomicrographic equipment used and the installation in the laboratory of darkroom facilities for this work were of great assistance, and the author wishes particularly to thank the Agronomy department of Oklahoma State University for these research aids.

RESULTS

The data obtained for each accession in this investigation are presented in Tables I through VI.

Table I. Dichanthium annulatum A-3242, a diploid.
Table II. Dichanthium annulatum A-3182, a tetraploid.
Table III. Dichanthium annulatum A-4099, a tetraploid.
Table IV. Dichanthium annulatum A-4083, a hexaploid.
Table V. Bothriochloa intermedia, gangetica type, A-2655, a tetraploid.
Table VI. Bothriochloa ischaemum A-2582, a hexaploid. 2/

Data for Each Accession, Tables I through VI

Stage of microspore or pollen. Column I.

The lefthand column of each table is an arbitrarily chosen means of classification which helps to pocket the analyses into comparable age or time brackets, but it is not intended that undue emphasis be placed upon conditions existing at one stage as opposed to another. Although as will be seen by vertical scanning of Table VIII, developmental processes can be viewed under such a system, it has been utilized here primarily as a tool for better recording and analysis of the data. The approximate age of an embryo sac was classified into one of four groups according to the stage of development of the pollen in the anthers of the same floret. The four groups chosen were:

1. microspore or pollen with 1 nucleus or showing signs of division of the 1 nucleus. In the Tables, this

2/ In the presentation of results and discussion of them to follow, the accessions will be referred to by number only to avoid constant repetition of genus and species names. For example, Dichanthium annulatum A-3242 will be referred to simply as 3242.

TABLE I

Dichanthium annulatum 3242

1 Stage of Microspore or Pollen	2 Number of Ovules Analyzed	3 Number of Sacs	4 Average Number of Sacs Per Ovule	5 Sacs Not Analyzable	6 Immature or Undeveloped Sacs	7 4-Nucleate Sacs	8 5-Nucleate Sacs	9 Embryos in Apomitic-Type Sac	10 Embryos in Sexual-Type Sac	11 Embryos not Typed
1 nucleate and 1 - 2	8	8	1		4		4			
2 - 3 and 3 nucleate	29	29	1				29			
Starch Formed	9	9	1				9			
Pollen Shed	10	10	1				6		4	
Totals	56	56	1		4		48		4	

TABLE II

Dichanthium annulatum 3182

1 Stage of Microspore or Pollen	2 Number of ovules Analyzed	3 Number of Sacs	4 Average Number of Sacs Per Ovule	5 Sacs Not Analyzable	6 Immature or Undeveloped Sacs	7 4-Nucleate Sacs	8 5-Nucleate Sacs	9 Embryos in Apomictic-Type Sac	10 Embryos in Sexual-Type Sac	11 Embryos not Typed
1 nucleate and 1 - 2	23	27	1.1	4	9	12	2			
2 - 3 and 3 nucleate	40	52	1.3	8	1	35	8			
Starch Formed	36	63	1.8	13		46	3			1
Pollen Shed	28	45	1.6	10		20	6	3		6
Totals	127	187	1.5	35	10	113	19	3		7

TABLE III

Dichanthium annulatum 4099

1 Stage of Microspore or Pollen	2 Number of Ovules Analyzed	3 Number of Sacs	4 Average Number of Sacs Per Ovule	5 Sacs Not Analyzable	6 Immature or Undeveloped Sacs	7 4-Nucleate Sacs	8 5-Nucleate Sacs	9 Embryos in Apomictic-Type Sac	10 Embryos in Sexual-Type Sac	11 Embryos not Typed
1 nucleate and 1 - 2	24	27	1.1		4	18	5			
2 - 3 and 3 nucleate	20	34	1.7	4		17	13			
Starch Formed	51	112	2.2	16	1	74	20		1	
Pollen Shed	41	77*	1.9	4		6	19	20	12	7
Totals	136	250	1.8	24	5	115	57	20	13	7

* 9 cases of endosperm only

TABLE IV

Dichanthium annulatum 4083

1 Stage of Microspore or Pollen	2 Number of Ovules Analyzed	3 Number of Sacs	4 Average Number of Sacs Per Ovule	5 Sacs Not Analyzable	6 Immature or Undeveloped Sacs	7 4-Nucleate Sacs	8 5-Nucleate Sacs	9 Embryos in Apomictic-Type Sac	10 Embryos in Sexual-Type Sac	11 Embryos not Typed
1 nucleate and 1 - 2	44	48	1.1	1	7	33	7			
2 - 3 and 3 nucleate	39	51	1.3	2	3	37	8			1
Starch Formed	11	12	1.1			9	2			1
Pollen Shed	9	13	1.4	1		2	1	5	3	1
Totals	103	124	1.2	4	10	81	18	5	3	3

TABLE V

Bothriochloa intermedia 2655

1 Stage of Microspore or Pollen	2 Number of Ovules Analyzed	3 Number of Sacs	4 Average Number of Sacs Per Ovule	5 Sacs Not Analyzable	6 Immature or Undeveloped Sacs	7 4-Nucleate Sacs	8 5-Nucleate Sacs	9 Embryos in Apomictic-Type Sac	10 Embryos in Sexual-Type Sac	11 Embryos Not Typed
1 nucleate and 1 - 2	22	30	1.4	2	20	8				
2 - 3 and 3 nucleate	89	149	1.7	12	7	111	19			
Starch Formed	24	45	1.8	4	2	25	7	5		2
Pollen Shed	66	119*	1.8	13	6	22	7	18	6	45
Totals	201	343	1.7	31	35	166	33	23	6	47

* 2 cases of endosperm only

TABLE VI

Bothriochloa ischaemum 2582

1 Stage of Microspore or Pollen	2 Number of Ovules Analyzed	3 Number of Sacs	4 Average Number of Sacs Per Ovule	5 Sacs Not Analyzable	6 Immature or Undeveloped Sacs	7 4-Nucleate Sacs	8 5-Nucleate Sacs	9 Embryos in Apomictic-Type Sac	10 Embryos in Sexual-Type Sac	11 Embryos not Typed
1 nucleate and 1 - 2	22	50	2.3	22	11	16	1			
2 - 3 and 3 nucleate	22	35	1.6	8		24	2			1
Starch Formed	9	9	1.0	1		6	2			
Pollen Shed	55	112	2.0	29		32	12	8	3	28
Totals	108	206	1.9	60	11	78	17	8	3	29

stage is designated as 1-nucleate and 1-2 (1 vegetative and 1 generative nucleus).

2. pollen showing division stages of 2-3 or containing 3 nuclei (1 vegetative and 2 generative nuclei). This stage is designated as 2-3 and 3 nucleate.

3. pollen grains showing starch formation.

4. pollen shed stage.

Number of sacs studied. Column 3.

Every sac in Column 3 is accounted for in some column to its right, Column 5 through 11. If a preparation provided the investigator with no information at all, it was discarded, and no record appears on these Tables.

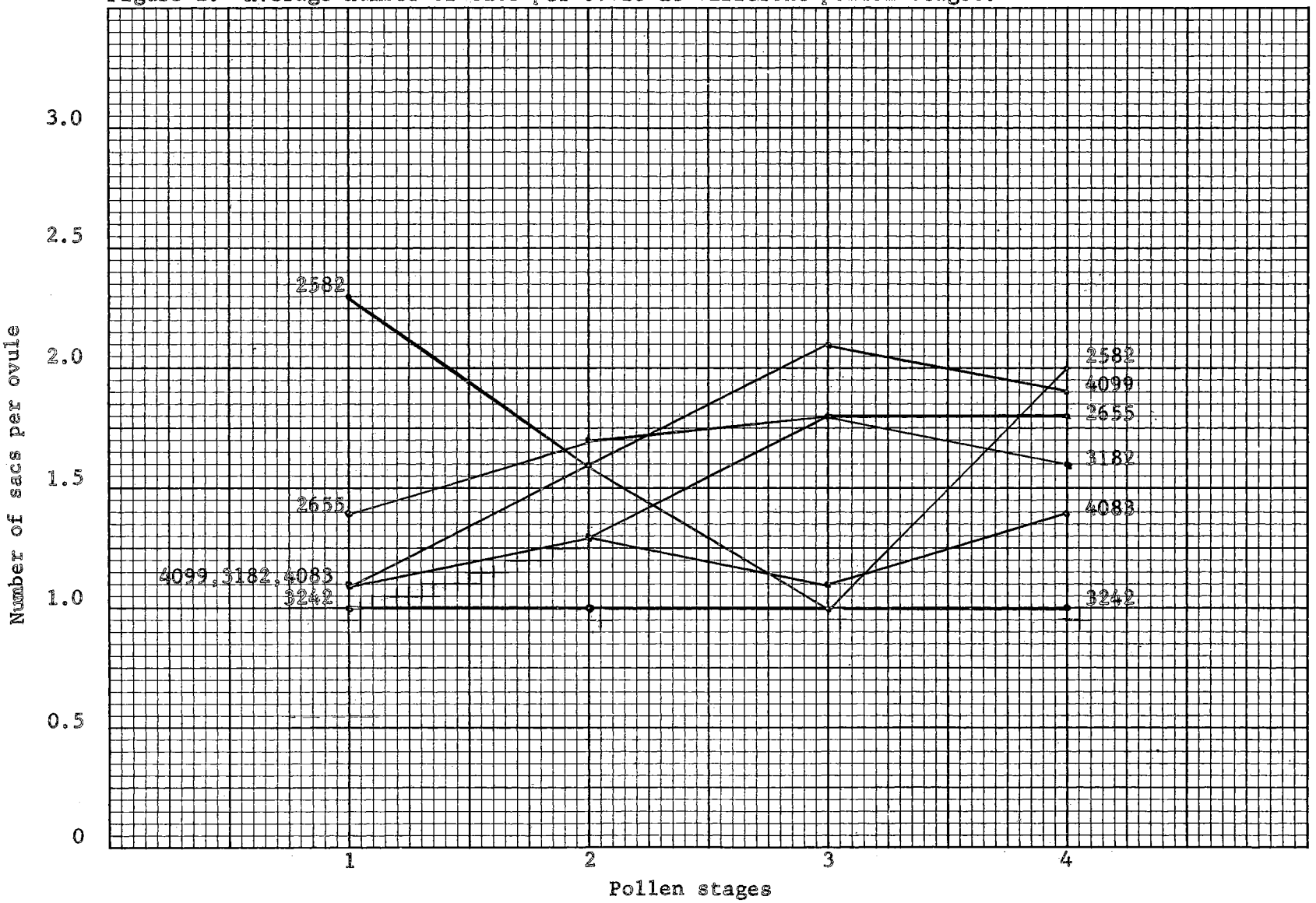
Average number of sacs per ovule. Column 4.

These averages represent the ratio:

$$\frac{\text{number of sacs studied (Column 3)}}{\text{number of ovules analyzed (Column 2)}}$$

3242 is the only accession consistently having 1 sac per ovule. The other accessions show from 1 to several sacs within a single ovule, as many as 7 having been observed in 4099 and 2582. Compared with the constancy in number of sacs per ovule evident in the diploid, the tetraploids all appear to show varying degrees of increase in sac number up to the starch formation stage of the pollen. The hexaploids both show a decrease between the 3-nucleate and starch formation stage of the pollen, 4083 showing the least overall variation but 2582 appearing very erratic. In the following graph, the abscissa represents the 4 pollen stages; the ordinate, the number of sacs per ovule. The decrease in number of sacs per ovule in both hexaploids at pollen stage 3 may be due to the relatively low numbers of sacs available for those particular analyses. In addition, variability is evidenced in 2582 in several ways to be indicated later. However, the necessity for discarding much material and retaining only the analyzable preparations,

Figure 2. Average number of sacs per ovule at different pollen stages.



which might possess the fewer number of sacs, probably accounts in great part for this record in 2582. A higher number of sacs per ovule actually exists at the two intermediate pollen stages in this accession.

Sacs not analyzable. Column 5.

If one or more sacs of an ovule could be analyzed, and it was observed that other sac material was present but partially obscured or torn, these later sacs were counted and tabulated as "unanalyzable". Since the number of sacs per ovule (Column 4) is based on the total sacs present, it was felt that some recognition of these should be made.

Immature or undeveloped sacs. Column 6.

This is a tabulation of sacs which were intact and entire but in which less than 4 nuclei were found. 2655 appears to be the only accession in which an appreciable number of such immature sacs persisted into and beyond the starch stage of the pollen, and this accession, and 4083, the only ones to show significant numbers of immature sacs beyond the earliest pollen stage studied.

Types of embryo sacs. Column 7 and 8.

Two types of sacs were found in all the accessions except 3242 and are tabulated in Columns 7 and 8 respectively. It has become convenient to refer to these as the 4-nucleate and 5-nucleate types, but certain explanations are in order. The 4-nucleate condition in these materials appears to be 2 synergids, 1 egg nucleus, and 1 polar nucleus. The 5-nucleate type differs by having 2 polar nuclei and a group of antipodals. The finding of 5 nuclei plus antipodals in all the embryo sacs of 3242, which reproduces by sexual means only, was taken as evidence that a 2 polar condition could be associated with sexuality. The finding also that the group of multi-antipodals, bunched and often extruding from

the chalazal end of the embryo sac, became easily detached, even to some extent in 3242 material in which only a single sac per ovule is formed and particularly in multi-sac ovules, led to the decision that a search for antipodals in every preparation was not necessary.[3/] It was thought that the presence of 2 polars, particularly in cases where in addition 2 synergids and 1 egg nucleus were clearly seen, was sufficient evidence for classifying such sacs as of a potentially sexual type. Since all preparations are not perfect, the finding of 2 polars was considered adequate by itself. In the light of Brown and Emery's (14) report of an occasional 2 polar condition in a 4-nucleate sac, it is possible that a small percentage of the sacs in this material has been misclassified. If 2 polar apomictic sacs do occur in this material, as will be pointed out later, more types of sacs occur here than have been reported in other apomictic genera. In the present investigation, sacs containing 2 polars are classified as of a potentially sexual type. The presence of 2 polars is quite evident when it occurs, although it is not always easy to distinguish between 2 nearly fused polars and one binucleolate polar, the nucleoli being extremely large and densely staining. Critical study usually reveals the true condition however, the few slides not lending themselves to accurate analyses being discarded.

Embryo types. Columns 9 through 11.

Certain decisions were necessary also in classifying embryos. The following system was used:

[3/] Although the antipodals are not always lost in the course of the technique employed, periodical checks were made for the presence of antipodals by using a simple aceto-carmine smear technique. This simpler technique is not adequate for careful analyses of sac nuclei, but the antipodals were less likely to be lost. It was quite obvious that sacs with antipodals occurred in all the accessions studied, a conclusion supported by the finding of antipodals in postfertilization sacs, a stage which can be studied without the use of the Bradley technique.

1. Apomictic type embryos. Column 9.
   a. embryos in sacs containing 1 polar.
   b. embryos in sacs in the same ovule as a sac containing a sexual-type embryo.

2. Sexual type embryos. Column 10.
   a. embryos in sacs containing 2 polars. The finding of embryos in 2 polar sacs will be discussed later.
   b. embryos in sacs containing antipodals. Again it is possible that sacs containing antipodals are not sexual (71). However, from material containing the two principal types of embryo sacs discussed here, maternal type and hybrid progeny have been recovered (20), and it is logical to assume that what is seen at an embryo sac stage is representative of the two types of reproduction.

3. Embryos not typed. Column 11.
   a. embryos in sacs not containing antipodals and in which the endosperm was developed so that the number of polars could not be determined. This represents an appreciable number of cases, particularly in the Bothriochloa spp. 2655 and 2582, and in the Dichanthium 4099. Where embryos can be typed, by polar number or antipodals, this stage of maturity, since it represents a more terminal stage in development, has some advantages as the preferable stage at which to determine frequencies of the sexual type sac. The high frequency of untypable embryos, however, might detract considerably from confidence in such determinations.

Summary of Data for All Accessions, Table VII

Table VII represents a summary in percentages of the data for all six accessions. Again Column 3 shows the average number of sacs per ovule, and, in addition the range of numbers encountered. The true picture is probably not represented in 2582, since as previously explained, preparations are not tabulated from which no analyses were obtained; whole series were discarded here because the numerous, large and twisted, vacuolated sacs could not even be accurately counted. The average number of sacs here is doubtless well over 2 per ovule. Adding Columns 4, 5, 6, and 7 horizontally for each accession should total 100% and show

TABLE VII

SUMMARY OF DATA FOR ALL ACCESSIONS STUDIED

1 Accession Number	2 Total Number of Sacs Studied	3 Average Number of Sacs Per Ovule and Range	4 4-Nucleate Sacs plus Apomictic-Type Embryos as % of 2	5 5-Nucleate Sacs plus Sexual-Type Embryos as % of 2	6 Immature Sacs	7 Unanalyzable Sacs or Untyped Embryos as % of 2
3242	56	1 (1)	0.0	92.9	7.1	0.0
3182	187	1.5 (1-4)	62.0	10.2	5.3	22.5
4099	250	1.8 (1-7)	54.0	28.0	2.0	16.0
4083	124	1.2 (1-3)	69.4	16.9	8.1	5.6
2655	343	1.7 (1-5)	55.1	11.4	10.2	23.3
2582	206	1.9 (1-7)	42.0	9.7	5.3	43.0

TABLE VII (Continued)

Accession Number	8 Total Number of Mature Analyzable Sacs or Embryos	9 4-Nucleate Sacs plus Apomictic-Type Embryos as % of 8	10 5-Nucleate Sacs plus Sexual-Type Embryos as % of 8	11 % of Sexuals Present As Single Sac	12 Multinucleolate Condition	13 Presence of Polyspermy
3242	52	0.0	100.0	100.0		
3182	135	85.9	14.1	33.0	≠	
4099	205	65.8	34.2	37.1	≠	
4083	107	80.4	19.6	85.0	≠ ≠	
2655	228	82.3	17.7	33.3	≠	≠
2582	106	81.1	18.9	40.0	≠ ≠ ≠	≠

the average composition of the total sacs studied. These percentages are based upon the totals in Column 2.

Column 4 and Column 9 are both summations of the sacs and embryos considered to be apomictic. However, the percentages in Column 4 are based on total number of sacs, Column 2; whereas the percentages in Column 9 are based on totals of mature analyzable material only, Column 8. Similarly Column 5 and Column 10 are summations of sacs and embryos considered to be sexual and are again based upon the totals of Columns 2 and 8 respectively.

The percentages of sexual type sacs which exist as single sacs in an ovule are indicated in Column 11. The very high percent in 4083 is doubtlessly related to the high number of single sacs of all types, and as already indicated, all the sacs in 3242 are both single and of a sexual type. The other accessions show similarity here, from 33 - 40% of the sexual type sacs being singles. Column 12 indicates the occurrence of multi-nucleoli. Both hexaploids show this in striking fashion. Binucleolate nuclei are the most frequent type, but 3 or 4 nucleoli per nucleus are not uncommon. All sac nuclei, synergids, egg nucleus and polars have been found at one time or another in this condition. In 4083 when multi-nucleoli exist, particularly in the polar nucleus, they are usually of quite different sizes (average measurements indicate .0198 mm. for the larger and .0079 mm. for the smaller); whereas in 2582 they are both more frequent and more equal in size. Polyspermy, or the presence of extra-generative nuclei has been observed in both Bothriochloa spp. Its possible significance will be discussed later on.

TABLE VIII

COMPARATIVE RATINGS OF ACCESSIONS AT VARIOUS POLLEN STAGES(VALUES EXPRESSED AS PERCENT)

1		2		3		4	
Unanalyzable Sacs and Untyped Embryos		Immature Sacs		4-Nucleate Sacs and Apomictic Type Embryos		5-Nucleate Sacs and Sexual Type Embryos	
1 Nucleate and 1 - 2							
2582	44.0	2655	66.6	4083	68.8	3242	50.0
3182	14.8	3242	50.0	4099	66.6	4099	18.5
2655	6.6	3182	33.0	3182	44.4	4083	14.6
4083	2.1	2582	22.0	2582	32.0	3182	7.4
4099	0.0	4099	14.8	2655	26.6	2582	2.0
3242	0.0	4083	14.6	3242	0.0	2655	0.0
2 - 3 and 3 Nucleate							
2582	22.2	4083	5.9	2655	74.5	3242	100.0
3182	15.4	2655	4.7	4083	72.5	4099	38.2
4099	11.8	3182	1.9	2582	68.6	4083	15.7
2655	8.1	4099	0.0	3182	67.3	3182	15.4
4083	3.9	2582	0.0	4099	50.0	2655	12.8
3242	0.0	3242	0.0	3242	0.0	2582	5.7
Starch Formation							
3182	22.2	2655	4.4	4083	75.0	3242	100.0
4099	14.3	4099	0.9	3182	73.0	2582	22.2
2655	13.2	2582	0.0	2655	66.6	4099	18.8
2582	11.1	4083	0.0	2582	66.6	4083	16.6
4083	8.3	3182	0.0	4099	66.0	2655	15.5
3242	0.0	3242	0.0	3242	0.0	3182	4.8
Pollen Shed							
2582	50.9	2655	5.0	4083	53.9	3242	100.0
2655	48.7	4099	0.0	3182	51.0	4099	40.3
3182	35.5	2582	0.0	2582	35.7	4083	30.8
4099	26.0	4083	0.0	4099	33.7	2582	13.4
4083	15.4	3182	0.0	2655	33.6	3182	13.3
3242	0.0	3242	0.0	3242	0.0	2655	10.9

Comparative Ratings of Accessions at Various Pollen Stages. Table VIII

In Table VIII these same data have been presented in slightly different form. Each heading of a column indicates a classification of embryo sacs, the four conditions accounting for all the sacs studied. The Table was set up in this fashion:

The percentages are all based on figures in Column 3 (Tables I - VI).
Percentages of Column 1 (Table VIII) are taken from figures of Columns 5 plus 11 (Tables I - VI).
Percentages of Column 2 (Table VIII) are taken from figures of Column 6 (Tables I - VI).
Percentages of Column 3 (Table VIII) are taken from figures of Columns 7 plus 9 (Tables I - VI).
Percentages of Column 4 (Table VIII) are taken from figures of Columns 8 plus 10 (Tables I - VI).

The percentages for any one accession read horizontally in any one section should total 100% and indicate the average composition of these sacs at any one stage. The four sections of Table VIII represent the four pollen stages used, so that comparisons of the figures of any one accession read vertically should indicate in general a developmental sequence. Each box is ranked from high percentage to low so that shifts in comparative standing can also be studied.

Estimates of Sexual Potential. Table IX

This Table has three parts to indicate a comparison of estimates of the sexual potential made (A) as percentage of total sacs, (B) as percentage of mature analyzable sacs, and (C) as percentage of ovules. These results will also be discussed further in a later section.

In the course of this investigation several observations have been made which do not lend themselves to presentation in tabular form.

Since, however, these observations may be of some interest, they

TABLE IX

ESTIMATES OF SEXUAL POTENTIAL

Stage of Pollen	ACCESSIONS					
	3242	3182	4099	4083	2655	2582
(A) % of Total Sacs Having 5-Nuclei or Sexual Type Embryos						
1 Nucleate and 1-2	50.0	7.4	18.5	14.6	0.0	2.0
2-3 and 3 Nucleate	100.0	15.4	38.2	15.7	12.8	5.7
Starch Formed	100.0	4.8	18.8	16.6	15.5	22.2
Pollen Shed	100.0	13.3	40.3	30.8	10.9	13.4
(B) % of Mature Analyzable Sacs Having 5-Nuclei or Sexual Type Embryos						
1 Nucleate and 1-2	100.0	14.3	21.7	17.5	0.0	5.9
2-3 and 3 Nucleate	100.0	18.6	43.3	17.7	14.6	7.7
Starch Formed	100.0	6.0	22.1	18.2	18.9	25.0
Pollen Shed	100.0	20.7	54.4	36.4	24.5	27.3
(C) % of Ovules Having 5-Nuclei or Sexual Type Embryos						
1 Nucleate and 1-2	50.0	8.7	20.8	15.9	0.0	4.5
2-3 and 3 Nucleate	100.0	20.0	65.0	20.5	21.3	9.1
Starch Formed	100.0	8.3	41.2	18.2	29.2	22.2
Pollen Shed	100.0	21.4	75.6	44.4	19.7	27.3

will be mentioned at this time.

1. Relative position of embryo sacs in an ovule.
2. Measurements of sac size.
3. Precocious initiation of embryo development.

As discussed under materials and methods, relative positions of the sacs are difficult to determine with this technique. In the polyploids, the sexual type sac with antipodals has often been observed fitted tightly into the micropyle in the same relative position and relationship as the single sexual sac of the diploid. The apomictic type sacs appear usually to be derived from initials close to the micropylar region, although occasionally either because of the technique used or as depicting the actual story, they appear more chalazal in position in relation to the position of the sexual type sac. The best identified 1-nucleate sac initials found in this study were apparently megaspores judging by position. It was admittedly much simpler to find a single enlarged cell and other disintegrating megaspores in the region of the micropyle than to observe after smearing a single enlarged somatic cell and to be sure it was of nucellar origin. Single celled sacs have been observed not only in 3242 where this determination is relatively simple but in 2655, 3182, and 4083, also. It is known from later stage analysis that in all these materials, sexual type sacs are produced; it might, however, be of theoretical interest to know if the process of meiosis always occurs to produce a megaspore which may or may not continue to function.

A few measurements of sac dimensions (length by width) were made on the diploid, on a tetraploid, and on both hexaploids. The following averages in mm. were obtained:

3242, diploid (sexual sacs)	.108 x .076
3182, tetraploid (apomictic-type sacs)	.149 x .098
4083, hexaploid (apomictic-type sacs)	.173 x .130
2582, hexaploid (apomictic-type sacs)	.245 x .157

With a few notable exceptions, the sexual type sacs, as would be expected if they are reduced in chromosome number, at both ploidy levels were somewhat smaller than the apomictic type sacs of the same material. Measurements were also made of nuclear and nucleolar diameters in 4083 and the following averages in mm. obtained:

synergid nucleus	.0154
synergid nucleolus	.0077
egg nucleus	.0286
egg nucleolus	.0011
polar nucleus	.0506
polar nucleolus	.0198

The size differences between the various nuclei has been helpful in their identification. A few instances have been noted where very little differentiation in size has occurred between the nuclei of a 4-nucleate sac, but in the vast majority of cases these differences are very easy to detect. It is somewhat questionable, however, how much measurements on fixed and smeared material may mean. The technique employed although "gentle" is still a squash type method. The existence of multiple sacs also makes the measurements less reliable particularly, for example, in 2582, where size determinations of the crowded, twisted, and vacuaolated sacs is nearly impossible.

Finally, the precocious initiation of embryo development should be considered. The finding in these materials of multi-celled embryos coexisting in sacs with 2 polars and antipodals forces the conclusion that here precocious development of the embryo is not restricted to an apomictic mode of reproduction. The data for the sexual diploid are meagre

at this stage, but both conditions have been found here, the cases in which the endosperm had undergone more divisions than the embryo certainly predominating, but instances observed of the embryo (up to 19-celled) present in a sac where the polars showed no signs of fertilization. In these cases either fertilization of egg cell and polars is not always completely synchronized or fertilization has not occurred at all, and embryo initiation is not dependent upon it. This type of behavior which can occasionally occur in the sexual diploid, occurs more frequently in 4099 and 3182, in both of which approximately 50% of sacs studied at this stage showed the embryo to be more advanced than the endosperm. In 4099, 70% of the sacs which showed a more advanced endosperm were sacs with antipodals. In 2655, 24 out of 32 sacs studied, or 75% showed an embryo further advanced in divisions than the polar(s) or endosperm; in 4083, this percentage was approximately 80% and in 2582 over 90% of the sacs with embryos showed embryo precocity.

DISCUSSION

When this investigation was initiated, apomixis had not been proven to exist in Dichanthium and Bothriochloa species. It was, however, strongly suspected principally because of the morphological uniformity of progeny obtained in the breeding work. Proof of apomixis in both complexes represented here was eventually obtained (20), and the present investigation not only provides supporting evidence to this finding but sheds some light on the mechanisms involved.

General Pattern of Development

In general, apospory (see Preface) has been reported to be the mode of gametophytic agamospermy followed in these genera (14). The existence in these materials of both sexual-type sacs of probable archesporial origin and apomictic-type sacs of presumably nucellar origin would make diplospory somewhat unlikely. In addition, the position of the sexual sacs directly above the micropyle in the ovule and the general position of the apomictic sacs either to one side of the micropyle or occasionally apparently quite far removed from it would point towards apospory as the most likely pathway of gametophyte formation. These materials are also considered to be pseudogamous. Not only are most aposporic genera also pseudogamous, but considerable direct and indirect evidence was at hand (Celarier, pers. comm.) to point to such an association existing here. Breeding experiments had indicated that some of the material reproduced exclusively by sexual means; some were facultative apomicts, and some

appeared to behave as obligate apomicts. The specific accessions chosen then represented these three modes of reproduction as well as happening to represent three ploidy levels and two complexes. 3242, the diploid, showed evidence of sexual reproduction only, the tetraploids, 3182, 4099, and 2655 were considered facultative; and the hexaploids, 4083 and 2582 had not given rise to any hybrid progeny.

Comparison of Sexual and Apomictic Methods of Reproduction

The characteristics of the female gametophyte in the sexually reproducing Dichanthium contrast beautifully with the situation in the apomicts, and in these materials, these general embryological conditions may be considered to constitute the principal basic differences between the two modes of reproduction.

1. Number of sacs per ovule

The most obvious comparison involves the production of a single embryo sac per ovule in the obligate sexual type versus multiple embryo sacs per ovule in the apomicts. At the time this research was initiated, D. annulatum 3242 was the only available sexual diploid in these two species. However, a D. sericeum diploid and a sexually reproducing tetraploid produced at this station were examined, and both were found to possess a single sac per ovule. In all the apomictic Dichanthium species studied an average of 1.1 sacs per ovule (see Figure 2 and Table X) was obtained at the earliest pollen stage studied. At this same stage both Bothriochloa species showed a tendency towards more sacs per ovule. 4099 "ends up" with more sacs than 2655, however, exhibiting as seen in the table below (Table X) the greatest degree of change of any of the accessions.

4083, with its low number of sacs per ovule (average of 1.2), retains throughout development a relative constancy which 3242 (average of 1.0) exhibits to an extreme. Of the apomicts studied, 4099 and 2582 have the highest average number of sacs per ovule. 4099 has the highest frequency of sexual type sacs and 2582, the lowest, if these frequencies are based upon total sacs. 4099 still retains its position, but the large number of unanalyzable sacs raises the rating of 2582 when the frequencies are based on analyzable sacs only. From these data, however, the frequency of sexual types in the apomicts does not seem to have much relation to the number of sacs per ovule, and one would question, therefore, that competitive crowding plays a significant part in the non-functioning of the sexual type sac. The rise in the frequency of sexual type sacs in 2582 at a later stage may indicate either that these sacs are initiated later in development than the apomictic type or that competition between the apomictic sacs themselves at an early stage increases the frequency of the sexual type later. That both factors probably play a role is suggested by the following table, since the net change in the number of sacs does not appear to be large in 2582. Viewed in this fashion the hexaploids appear to vary the least of the apomicts, with respect to number of sacs during development through these stages, one (4083) showing a slight increase in number of sacs per ovule concurrent with a decrease (68.8 - 53.9) in percentage of apomictic type sacs, and an increase (14.6 - 30.8) in percentage of sexual types, and the other (2582) showing a slight decrease in the number of sacs per ovule concurrent with an increase in frequency of both apomictic (32.0 - 35.7) and sexual (2.0 - 13.4) types.

TABLE X

CHANGE IN NUMBER OF SACS PER OVULE BETWEEN EARLIEST AND LATEST POLLEN STAGES STUDIED

Accession	No. sacs/ovule at earliest stage studied	No. sacs/ovule at latest stage studied	Net Change
3242	1.0	1.0	0
3182	1.1	1.6	+.5
4099	1.1	1.9	+.8
4083	1.1	1.4	+.3
2655	1.4	1.8	+.4
2582	2.3	2.0	-.3

The average number of sacs per ovule does not seem, however, to be correlated with this ploidy level. The one hexaploid, 2582, shows the highest average, the other, 4083, shows the lowest average of the apomicts. Except for 4099 which shows some other exceptional behavior patterns, the Dichanthium species, as a group, exhibit a tendency towards fewer sacs per ovule than the Bothriochloa species and one might suspect that there is some correlation here between genus and sac numbers. With the exception of 4099 again the Dichanthium species rank in order of sexual potential as they do in average number of sacs. Whether then 4099 is under control of genetic, physiological, or other type factors enabling the sexual sac to more often persist and function or whether sexuality is not correlated with number of sacs per ovule remains at this time unknown. Certainly when the obligate sexual is considered and the high frequency of sexual type sacs in 4083 is recognized, the potential for sexuality appears to be in part conditioned by low number of sacs per ovule; additional factors may be operative in 4099, and still other factors doubtless determine how much of the potential will be ultimately realized.

2. Embryo sac constituents

The principal difference between the obligate sexual accession and the apomicts, besides that of sac number, involves the sac constituents. The sexually reproducing plants show only sacs of the 5-nucleate type plus antipodals. One would expect to find this type to different degrees in facultative apomicts and this is true, the predominant type being, however, the 4-nucleate type. The finding, however of 5-nucleate sacs in what had been thought to be obligate apomicts was somewhat unexpected. As mentioned earlier 2-polar types with and without antipodals are classified as sexuals. There appears to be strong justification here for associating a 2-polar type with sexuality both because of such an association in 3242 and because the 2 polars are usually found in what we have called the 5-nucleate, not the 4-nucleate type. If 2 polar, 4-nucleate sacs occur in these materials, they are rare, and three types of sacs would have to be recognized, 2 types of 4-nucleates and one type of 5-nucleate.

3. Precocious embryo development and synchronization mechanisms

The finding of precocious embryos developing in 2 polar sacs is not evidence for apomixis since as already indicated, this condition occurs in both apomicts and obligate sexuals. Good association of antipodals with these embryo-2 polar sacs is also frequent. Without this evidence one might suspect precocious embryos in 2 polar sacs to indicate either 2 polar apomictic sacs or possibly the presence of multi-nucleolate nuclei; with such evidence, however, we do not feel at the present time justified to do other than regard such sacs as sexual.

It would appear more likely that there is some tendency in these

materials towards autonomous initiation of the embryo and that this tendency, which increases with ploidy level is genetic. It certainly appears to be negatively correlated with ability to produce hybrid progeny and may be a large factor in the failure of all sexual potential to be realized. It may be that the condition in these materials is that the female tends to develop slightly faster than the male. This would be supported by the fact that they are protogynous (Dewald, pers. comm.). There is also a tendency for autonomous initiation of development of the egg cell. For development to proceed, however, fertilization of the polar is required; when this happens, the apomictic embryo can proceed in its development, but if the sexual embryo is already "out of reach of fertilization" its development stops even when the endosperm is formed. These apomictic tendencies towards faster development in the female and initiation of embryo development prior to fertilization are probably enhanced at the higher ploidy levels so that even though a sexual potential is present, it cannot be realized. These timing and synchronization mechanisms are probably quite delicately balanced and may well be under both genetic and environmental influence. Such mechanisms may be a partial explanation of the association of apomixis and polyploidy, and in our materials it may be possible to visualize here a trend from sexuality through facultativeness to obligate apomixis on such a basis. In addition to observing this precocious embryo development, however, a reverse trend was observed in 2582 which could also conceivably play a part in its obligate apomictic behavior in spite of sexual potential. This is the fact that at the late pollen shed stage the majority of its sexual type sacs

were still present in the 5-nucleate stage. Since some evidence from the earlier stages could be interpreted as indicating later initiation of the sexual type sac, and since competition with the larger more developed apomictic sacs could conceivably occur to delay development, it might be that these sexual sacs are not ready for fertilization when it occurs. Since both events occur, precocious embryos and 5-nucleate sacs at pollen shed, it may be assumed that the failure of sexual potential to be realized in some of these species is due primarily to some upset in synchronization or timing as has been suggested by various investigators for other materials. Subject as these materials are to environmental influences, it would seem entirely possible then that very slight changes could tip these mechanisms one way or the other. The finding in some materials particularly 2655 of embryos and endosperm in the same ovules that contain 5-nucleate sacs is also suggestive that these sexual sacs, which presumably are in a better position for fertilization, may lag behind in development and not be ready to accept this early fertilization which in all of these cases had occurred before the shedding of the floret's own pollen. If fertilization of these sacs could possibly occur subsequently, the apomictic embryos would obviously better the sexual ones competitively. The failure to find sexual type sacs at the earliest pollen stage in 2655 is further support for this late initiation and lag of sexual sacs. It is interesting that both the Bothriochloa species show this feature. It is suggested here then that the data provide some evidence that in apomictic materials there is a genetic and/or physiological tendency for upsets in timing and synchronization of the two sexes,

that usually the apomictic egg nucleus can initiate development autonomously and precociously and that this tendency is occasionally exhibited also in the sexual type sacs. In addition in Bothriochloa there may be a tendency for the sexual type sacs to be initiated later than the apomictic sacs, and that this retardation in development may result in their failure to reach maturity in time for fertilization.

Additional Observations

1. Multi-nucleoli

One other possible factor contributing to confusion concerning the number of sac nuclei is the tremendous size and dark staining of nucleoli. A multinucleolate condition of one or more sac nuclei is very prevalent in some materials. This phenomenon has not been observed in 3242, and it is considered herein to be a function of ploidy level since its prevalence increases in the hexaploids, 2582 and 4083, where occasionally three or more nucleoli per nucleus are found. Any significance of this condition over and beyond correlation with ploidy level is not apparent at this time. The observation, already mentioned, of nucleoli of very different sizes in the polar nucleus of 4099 is, however, interesting. Until we know more about the basic physiology of the chromosomes and nuclei within the sac, this and the fact that not all the nuclei show this multi-condition at the same time remain unexplained.

2. Polyspermy and fertilization of unreduced gametes

The phenomenon of polyspermy has been observed in both Bothriochloa accessions studied. Instead of the usual 1 vegetative and 2

generative nuclei normally observed in functional pollen, multiple generative nuclei are occasionally present here. It is not known if or how these function, but 4 generative nuclei frequently occur previous to starch formation. It might be possible to explain in this way a hybrid obtained by Celarier (unpubl.) from crossing a 2655 female with a 4099 male in which the progeny plant was a hexaploid and strongly resembled the paternal parent. Multiple embryos observed within one sac in 2655 might be due to fertilization of a nucleus other than the egg nucleus by sperm nuclei from more than one pollen grain or by multiple generative nuclei such as these. The finding also of hexaploid hybrids (Celarier, pers. comm.) from crossing of two tetraploids, a phenomenon which apparently occurs frequently in some materials (50% of analyzed hybrids with 2655 as female) would seem to be too common to be accounted for by polyspermy, although this is conceivable. It is more probable in the opinion of these investigators (Celarier and Harlan, pers. comm.) that these hexaploid hybrids represent the product of fertilization of the unreduced apomictic sac. Two cases were found in 2655 of proembryos with 50 +, probably 60, chromosomes in open pollinated materials which, because of their field position, were not likely to have been fertilized by pollen other than from a 20 or 40 chromosome species; it is presumed that these cases represent instances of fertilization of the unreduced egg nucleus by a reduced sperm. This again would indicate that studies need to be undertaken to concentrate on the timing and synchronization mechanisms at work in facultative apomicts. If these mechanisms were thoroughly understood, it might be possible to more or less control the production of hybrid embryos

from unreduced apomictic sacs.

Estimation of Sexual Potential

The frequency of the sexual and apomictic potential has been estimated in these materials by use of the following scheme:

1. Apomixis (Column 4 or 9, Table VII): 4-nucleate embryo sacs (1 polar) plus apomictic embryos.

2. Sexuality (Column 5 or 10, Table VII): 5-nucleate sacs (2 polar) plus sexual embryos.

Which figures, those of Column 5 or those of Column 10, are better indications of potential sexuality is problematic. At present we have no way of knowing what the composition of the unanalyzable and immature sacs is. If we choose to consider the mature analyzable sacs as a representative random sample, the percentages in Column 10 are probably more usuable. The ranking in order of sexual potential remains the same in either case except for the position of 2582 which apparently suffers from the huge numbers of unanalyzable sacs it contains. It is hoped that these frequency determinations carry some significance even if errors of interpretation may have been made in some analyses. One might ask why these average frequencies have been emphasized instead of simply the situation existing at the final pollen stage. A comparison of Column 5 with the lower righthand box of Table VIII may be of some interest in this connection. The generally higher frequencies obtained at this final pollen stage could be due to:

A. Late initiation of sexual sacs, which is believed to occur in some materials, and decrease through competition of the frequency of apomictic sacs.

B. The untypable and unanalyzable sac group may contain a higher proportion of apomictic sacs than expected in a random sample.

This second possibility makes it inadvisable to use this last stage as the best picture of potential sexuality, although this stage is obviously closer to the ultimate realization of what type a seed will become. One is forced to fall back upon the averages. In the second place, the averages give a better overall picture of what an investigator would have to work with if he were to try controlled methods for increasing hybrid production.

Disintegration of Embryo Sacs

Although observations of more than one embryo in one endosperm is rare, finding several embryos in one ovule is common in all the apomicts except 4083 where the frequency of multiple sacs is low. Ultimately, however, one seed per ovule is ordinarily produced from these plants. From this viewpoint the estimates of sexual potential as recorded in Table IX-C may be of interest. Although no systematic frequency accounts of twinning have been made, such seedlings are apparently recovered in only a low percentage of cases (Celarier, pers. comm.), approximately 3% having been recovered in a related accession. Obviously when two or more sacs are produced per ovule, disintegration of all but one must be the rule. This disintegration is most easily observed at a post anthesis stage. Disintegrating sacs have been seen previous to this, and occasionally even sacs which have progressed as far as endosperm formation show obvious signs of going to pieces. In the vast majority of cases, however, the sac that is shrivelled and disintegrating accompanies a sac containing a developing embryo and endosperm. This was particularly easy to study in 4099 where small disintegrating sacs carrying antipodals were extremely common in association with large, well developed apomictic

sacs with endosperm and an embryo. At this station, Richardson (unpubl.) reports that crosses using 4099 as the female yield approximately 22.2% hybrids. Not only is this a lower figure than any of the estimates given in this investigation for the sexual potential of this accession, but it might be presumed also that cytological analyses will reveal that not all of the 22.2% are sexual type hybrids. Obviously the discrepancies between these two sets of data can be accounted for in part by these disintegrating sexual sacs, which presumably might be regarded as results of the upsets in timing and synchronization mechanisms and/or simply the products of competitive defeat.

Realization of Sexual Potential

There is considerable evidence from the breeding data that Bothriochloa as a complex tends towards a greater degree of obligate apomixis than Dichanthium. To show such a comparison we might have done better to select an accession other than 2655 which apparently in many respects admirably represents its name of intermedia. It has served (Richardson, unpubl.) as a crossing bridge between the two complexes used in this study, and although as mentioned above, not all the hybrids obtained using it as a female are from sexual type sacs, it still reproduces facultatively. B. ischaemum of which 2582 is a representative is generally regarded as an obligate apomict. It is hoped that various characteristics of 2582 have been sufficiently emphasized to provide partial explanations for a reproductive behavior quite different from that of an obligate sexual such as 3242. Although it would be difficult to deny that certain accessions behave as obligate apomicts, it certainly is of interest that even some of these show an inherent potential for sexuality. Two possible

factors to cause a higher percent of hybrid progeny from 2655 than from 2582, which from the present data do not differ greatly in sexual potential, would be the greater possibility of fertilization by a reduced sperm nucleus of an unreduced egg nucleus due in turn to less disturbed timing mechanisms (in 2655), and unknown physiological and/or genetic factors involved at a hexaploid level (in 2582). The potential for sexuality exists also, as we have seen, in 4083 which like 2582 produces primarily maternal type progeny. More factors to reduce the realization of sexuality are obvious in 2582 than in 4083 such as number of sacs per ovule, later initiation of sexual type sacs, possibly less basic potential to work with. The late flowering habit of 4083 has made it more difficult to obtain observations at the pollen shed stages, but one can postulate that genetic imbalance due to ploidy level may be in part responsible, and that timing mechanisms are again effective. In both, the high frequency of precocious embryos is evident. One can only speculate as to what factors play a part following the stages we have considered in this report, but whatever the cause or causes, one is certainly tempted to work out methods for developing the potentials. A comparison again of the potentials in the hexaploids just mentioned with those of 3182 and 2655 are interesting. As far as averages of sexual sacs are concerned, there is surprisingly little difference. One has to assume, since more hybrids have been recovered using the tetraploids as females, that differential disintegration of sexual sacs occurs, that a large proportion of the hybrids from the tetraploids are of the unreduced female x reduced male type, whereas the higher frequencies of precocious embryo initiation in the hexaploids may reduce this, and that we know hardly anything about what happens between pollen shed and seed germination.

Behavior of Bothriochloa ischaemum 2582

A few words should be said concerning 2582 which in many ways shows more erratic behavior than the other accessions. The following abnormalities have been observed here: polyspermy, adventitious embryony, two pistils per floret, two ovules per pistil, multiple synergids per sac, as well as the numerous contorted and distended sacs already mentioned. Presumably 2582 is very subject to environmental influence, and again various environmental controls should be tried to increase the realization of sexual potential.

General Remarks

1. Implications of this investigation

If any factors at all have been discovered or suggested in this investigation as playing a part in the complex and interrelated physiological system of an embryo sac, it is very likely that many more such intricate factors remain still unknown and misunderstood. If this work has done little else, it will perhaps have indicated that it is not always easy to know what questions to ask particularly if the answers be the fundamental ones concerning which guesswork is futile, and if they be asked of a cell or group of cells as complex as an embryo sac. This investigation has attempted to obtain basic information concerning the methods of facultative apomixis for three principal reasons:

A. To better visualize the actual processes going on at the reproductive level and to use this information in more intelligent breeding and selection experimentation.

B. To better visualize and plan experimentation at some control level, environmental, genetical, physiological, biochemical or all of these.

C. To provide the basic information for some theoretical thinking and eventual research into problems of an even more fundamental nature.

Obviously numerous unsolved problems have been left at loose ends, and this particular investigation may have opened up more questions than it has provided answers for. This is perhaps not regrettable. Certain information is at hand:

A. A method has been applied that could be used to screen breeding materials for sexual potential.

B. The need for and advantages of rather thorough and painstaking analyses indicated.

C. Certain comparative studies have been possible through the use of three ploidy levels, two complexes, and two principal modes of reproduction.

D. Certain patterns and behaviors have been observed and data accumulated to lend support to certain hypotheses.

2. Future work

But beyond this perhaps this investigation will help to indicate work that needs to be done. This present type of research, visual and descriptive to a large extent, can best be utilized as the foundation upon which to build two bridges, one to better practical agricultural efficiency, and one to deeper basic knowledge of life processes. The imagination of any scientist is more stimulated by an experimental approach and several roads would seem to open out from this point in this particular research.

A. Experimentation with controlled environments, both physical and biochemical.

B. Embryo culture work as a tool to better understanding of the processes outlined here and those that follow. As Wardlaw (70) states, "The activation of the ovum at fertilization, and the nutrition, growth and development of the embryo, undoubtedly afford a wide field for study."

C. Pollination studies involving chemical extracts and culture methods to increase our understanding of pseudogamy and fertilization.

D. Finally, in its very broad aspects, it seems to the author that in the embryo sac one has an ideal place in which to tackle in some way the immense problem of differentiation. The embryo sac meets many of the requirements for such complex studies since "in embryos we are dealing with cells and tissues, either in the primary embryonic condition, or in the process of becoming differentiated." (70)

These problems are complex but they constitute a meeting ground for the embryologist, the geneticist, the morphologist, the physiologist, and the biochemist. Perhaps by cooperative efforts of all of these, more answers to the questions of differentiation will be forthcoming.

SUMMARY AND CONCLUSIONS

In the course of this investigation, an attempt has been made to carry out a detailed analysis of the methods of reproduction followed by selected accessions of the Bothriochloa and Dichanthium complexes by studying conditions and processes within the female gametophyte. Bradley's (11) smear method was exclusively utilized in order to demonstrate the practicality of this procedure for studies in the female. Stages of pollen development were used as a means for determining comparable ages of the embryo sacs. Three ploidy levels were represented. The diploid was found to be an obligate sexual, containing only one embryo sac per ovule at all developmental stages studied after maturity. This sac was consistently of the 5-nucleate plus antipodals type. At a pollen shed stage, the endosperm generally divided more rapidly than the embryo, although the reverse situation was also occasionally observed.

The ovules of the polyploids all contained an average of more than one embryo sac per ovule, and the sacs were of two types, 5-nucleate (see Types of embryo sacs, RESULTS) and 4-nucleate. At a pollen shed stage, there was evidence of more precocious initiation of development of the embryo, and in some accessions, some evidence for lagging in initiation and rate of development of the sexual sacs. These data are interpreted as contributory to the theory that timing and synchronization mechanisms are somewhat upset in apomictic reproduction, so that the sexual potential inherent in a species fails to be realized. The following figure may help in summarizing some of the data:

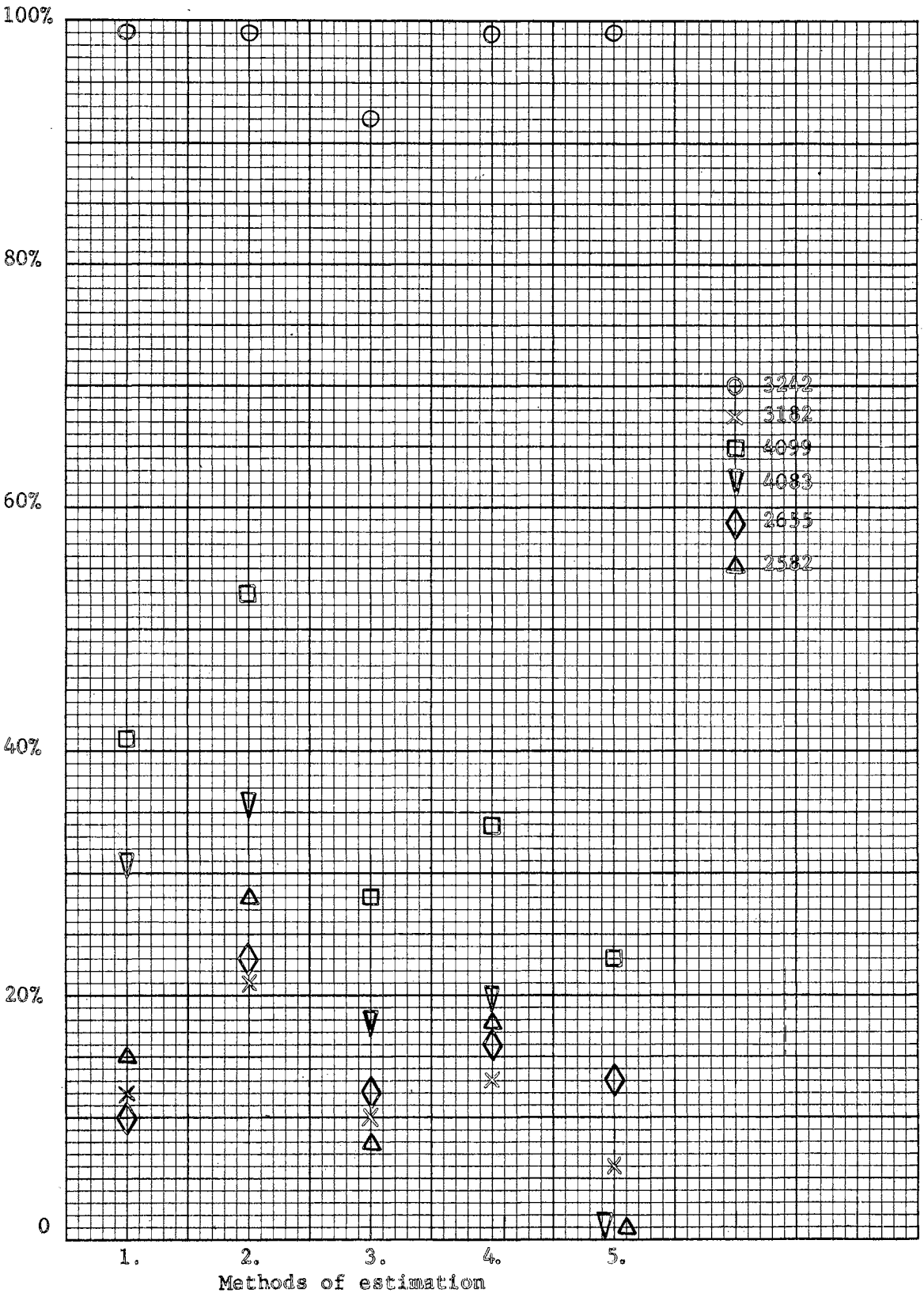


Figure 3. Estimation of sexual potential.

Legend for Figure 3.

The following data were used to plot points in successive columns:

Column 1. Frequency of sexuals at pollen shed stages as percentage of total sacs studied at this stage (bottom righthand box, Table VIII).

Column 2. Frequency of sexuals at pollen shed stages as percentage of analyzable sacs studied at this stage (computed from Table VIII).

Column 3. Frequency of sexuals computed as averages of all sacs (Column 5, Table VII).

Column 4. Frequency of sexuals computed as averages of analyzable sacs (Column 10, Table VII).

Column 5. Average percentage non-maternal progeny obtained in pollinations of emasculated florets using the accessions studied as the female (Richardson, 2 years data, unpublished).

As will be noticed, 3242, 4099, 4083, 2655, and 3182 are consistently ranked in this order in Columns 2, 3, and 4; 3242, 4099, and 4083 are consistently ranked in 1, 2, 3, and 4; 3182 and 2655 switch positions in 1 and 2; 2582 remains in fourth position in three columns and drops to sixth in one. It appears that the overall averages of all stages and those of pollen shed stages only render at least similar rankings. The difference in the sexual potential between the diploid and the polyploids is the most striking, but the high rating of 4099 is also conspicuous. More unexpected, however, was the finding of sexual potential in the hexaploids, particularly 4083. Since, as indicated in Column 5, both hexaploids breed as obligate apomicts, the search for the factors which inhibit the realization of potential presents exciting challenges to the

research worker.

Several observations made on these materials are mentioned in this report which we cannot at present judge as being or not being significant. The present data suggest that some of the factors conditioning sexual potential are number of sacs per ovule, type of embryo sac, frequency of the sexual-type sac, and additional factors such as may be operative in 4099.

Certain factors determining the ultimate expression of potential sexuality are indicated to include disintegration of sexual-type sacs due to the unbalance of ploidy and to upsets in timing mechanisms resulting in precocious embryo initiation and/or late initiation and lagging development of sexual sacs. One is led to speculate concerning the biochemical factors, both genetic and physiological, which form the basis for these mechanisms and their significance in evolution as expressed in sexual, facultative, or obligate apomixis. The possible experimental approaches to the further study of these processes are numerous and complex but worthwhile both from a practical and a fundamental or theoretical viewpoint.

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A P P E N D I X

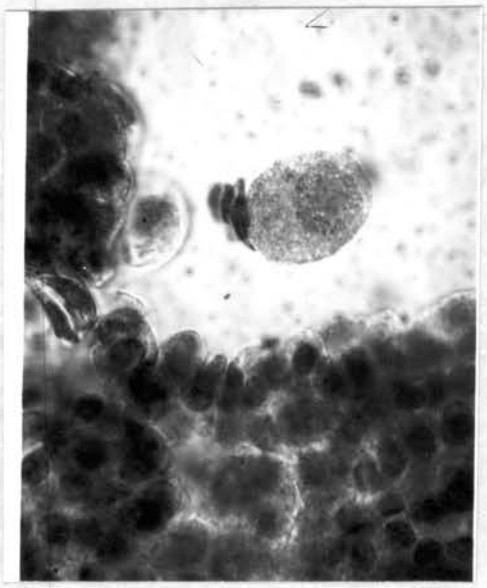
Plate I

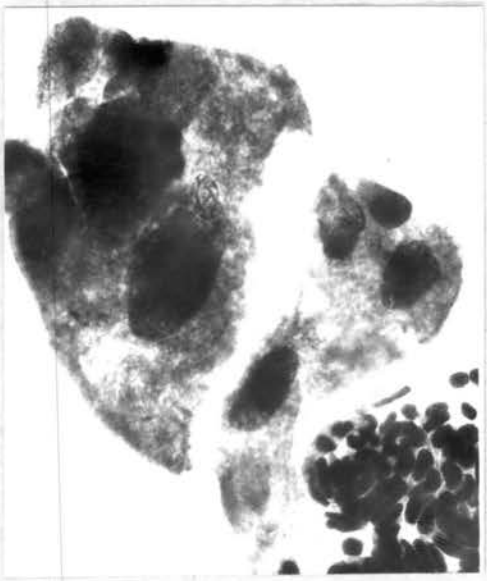
Upper left: Embryo sac initial. B. intermedia 2655. 650X

Upper right: Entire ovule showing one embryo sac near micropyle. B. intermedia 2655. 70X

Lower left: Two 4-nucleate sacs from one ovule. B. intermedia 2655. 650X

Lower right: Two sacs from one ovule. B. intermedia 2655. 360X





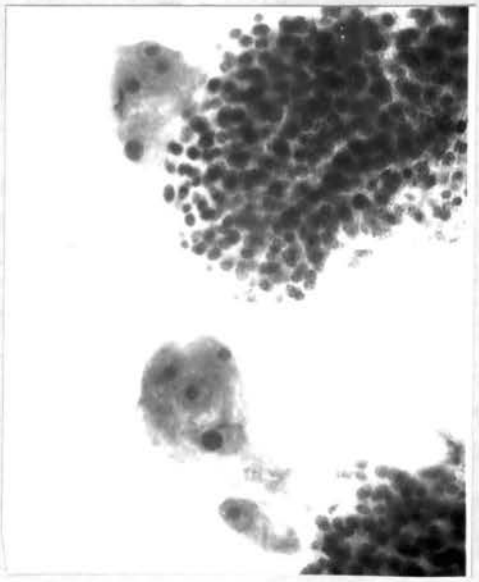


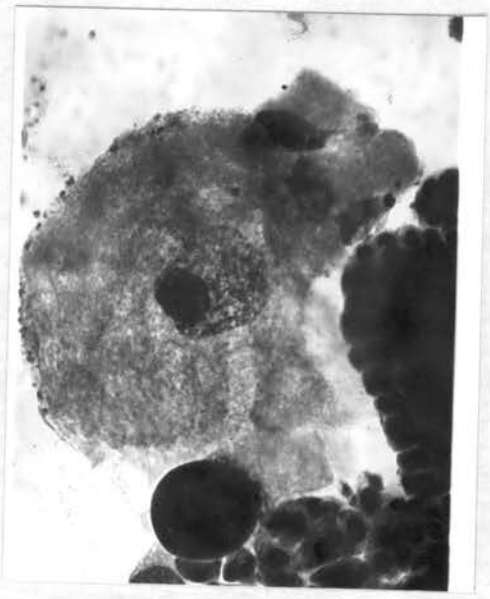
Plate II

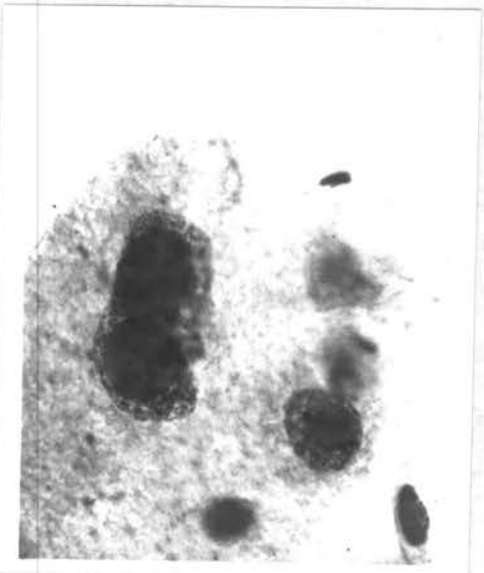
Upper left: 4-nucleate embryo sac. D. annulatum 4099. 650X

Upper right: 4-nucleate embryo sac. D. annulatum 4099. 650X

Lower left: 5-nucleate embryo sac. B. intermedia 2655. 650X

Lower right: 5-nucleate embryo sac. D. annulatum 4099. 650X





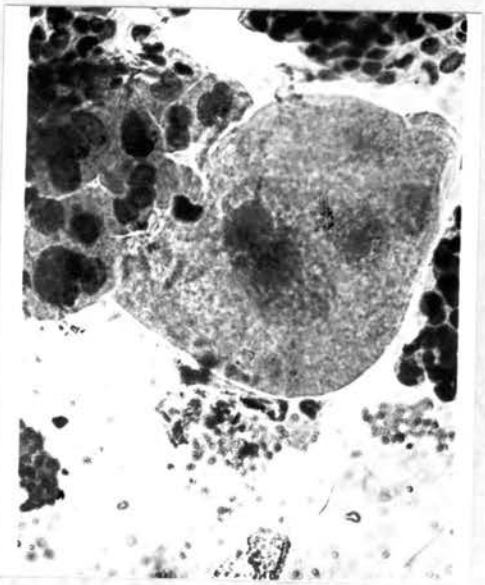


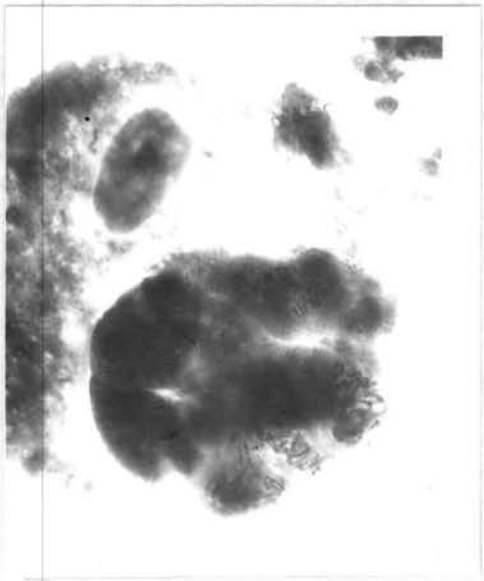
Plate III

Upper left: Embryo and 1 polar nucleus. B. intermedia 2655. 650X

Upper right: Endosperm of apomictic sac and disintegrating sexual sac. D. annulatum 4099. 360X

Lower left: Ovule with one apomictic-type embryo and endosperm (upper right) and one sexual-type sac (lower left). B. intermedia 2655. 70X

Lower right: Embryo and endosperm. D. annulatum 4099. 650X



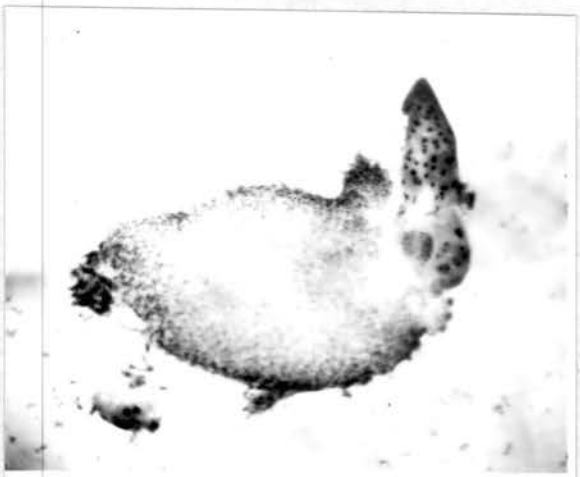


Plate IV

Upper left: 3-nucleate pollen grain. B. ischaemum 2582. 650X

Center left: Germinating pollen grain. B. intermedia 2655. 650X

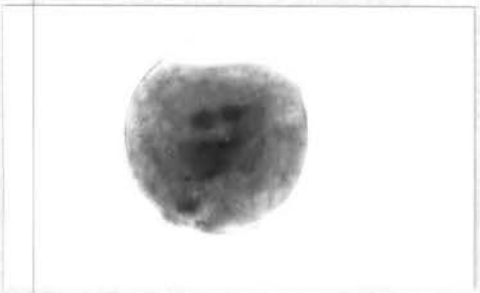
Lower left: Multi-generative nuclei in pollen grain. B. ischaemum 2582. 650X

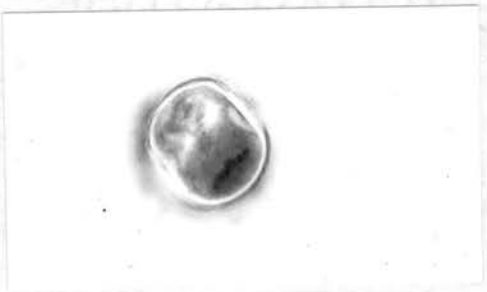
Upper right: 1 - 2 nucleate pollen grain. D. annulatum 4099. 650X

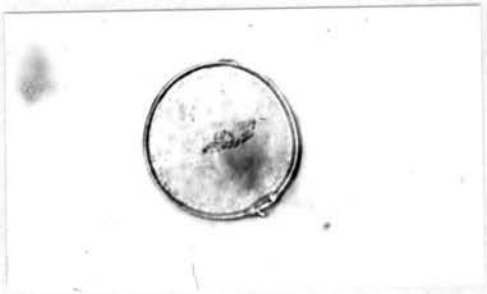
Center upper right: 2 - 3 nucleate pollen grain. B. intermedia 2655. 650X

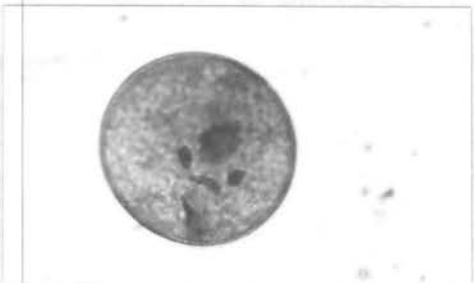
Center lower right: 2 - 3 nucleate pollen grain. D. annulatum 4099. 650X

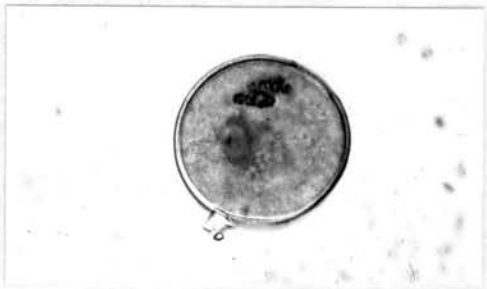
Lower right: 3-nucleate pollen grain. B. intermedia 2655. 650X











VITA

Margaret Hoover Brooks

Candidate for the Degree of

Doctor of Philosophy

Thesis: A STUDY OF THE REPRODUCTIVE MECHANISMS IN CERTAIN SPECIES OF THE BOTHRIOCHLOA AND DICHANTHIUM COMPLEXES

Major Field: Plant Breeding and Genetics

Biographical:

Personal data: Born in Talas, Turkey, February 6, 1913, the daughter of Alden Robbins and Esther Finger Hoover.

Education: Grade school education both abroad and in this country; graduated from Vail-Deane School, Elizabeth, New Jersey; received the Bachelor of Arts degree from Smith College, Northampton, Massachusetts, with a major in Zoology in June, 1933; attended graduate school at Brown University, Providence, Rhode Island, 1933-34, major in Zoology; attended graduate school at Johns Hopkins University, Baltimore, Maryland, 1936-37, major in Zoology; completed requirements for the Doctor of Philosophy degree in May, 1958.

Professional experience: Graduate assistantship in Zoology at Brown University, 1933-34; research assistant to Dr. C. W. Metz, Carnegie Institution of Washington, Baltimore, Maryland, 1936-37; research assistant to Dr. M. Demerec, Carnegie Institution of Washington, Cold Spring Harbor, Long Island, 1934-36, 1937-38; National Science Foundation predoctoral fellowship, 1955-56.

Honors: Graduated from Smith College, magna cum laude, 1933; Phi Sigma Graduate Award, Oklahoma State University, 1956.

Organizations: American Botanical Society; American Genetics Society.

Honorary organizations: Phi Beta Kappa, Smith College, 1933; Sigma Xi, Johns Hopkins University, 1937; Phi Kappa Phi, Oklahoma State University, 1958.