CYTOLOGY OF PARTHENOGENESIS IN POA PRATENSIS[1]

By FRED W. TINNEY[2]

Formerly *assistant agronomist, Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Since Müntzing (*8*)[3] announced in 1932 that seed is set without gametic union (apomixis) in certain Swedish biotypes of *Poa pratensis* L. and *P. alpina* L., and with gametic union (amphimixis) in certain Swiss biotypes of *P. alpina,* several other investigators have reported evidence that apomixis occurs in *P. pratensis.* The cytological studies heretofore published, however, do not adequately explain apomixis in these species. The observations herein described pertain to the cytology of apomictic seed development in *P. pratensis.*

REVIEW OF LITERATURE

The studies of Müntzing (*8*) on Swedish biotypes of *Poa pratensis* showed that plants of different biotypes (or progeny from different plants) might have different somatic chromosome numbers, whereas plants of the same biotype (or the progeny from one plant, in one known instance) possess the same maternal chromosome number. He found also that plants with a common maternal origin were extremely uniform in morphological type and that the chromosome behavior in the meiotic divisions of the microsporocytes was frequently irregular although this irregularity had no apparent effect on fertility. Müntzing (*9*), in a study of twin seedlings, found that the two plants obtained from one seed usually had approximately the same number of chromosomes but that it was not uncommon for one member of such a pair to be approximately triploid in respect to the diploid chromosome number of its twin.

Nilsson (*10, 11, 12*) found that self-fertility might vary from 0 to 78.9 percent in different plants of *Poa pratensis* and that the fertility of open-pollinated plants might vary from 2.6 to 78.1 percent. He suggested that fertility in individual plants is affected by the environment and that in some plants sterility is due to defective sex organs. He concluded that the seeds of *P. pratensis* are set apomictically in nearly all cases. This species, however, presents a case of pseudogamy since pollination must be effected to induce seed development. Occasional intermediate forms appear, which Nilsson suggested result from gametic union.

[1] Received for publication July 1, 1939. The investigation herein described is a part of a project on the improvement of pasture grasses that is being conducted cooperatively between the Department of Agronomy, Wisconsin Agricultural Experiment Station, and the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] The author lost his life in the sinking of the S. S. *Athenia* on September 3 or 4, 1939, while on his return from the Seventh International Congress of Genetics, which met at Edinburgh, Scotland, August 23-29, 1939. In submitting the manuscript of the present paper the author asked that acknowledgment be made of the assistance of Arnold Lueck and Paul Ozanne, who aided in making the cytological preparations; of Prof. O. S. Aamodt, who contributed in many ways toward the completion of the work; and of Prof. C. E. Allen, who aided in the final preparation of the manuscript.

[3] Italic numbers in parentheses refer to Literature Cited, p. 360.

Åkerberg (*3*) also concluded that apomixis commonly occurs in *Poa pratensis*, although he has found four biotypes that regularly reproduce sexually. He found pollination necessary to initiate apomictic seed development; the pollen of *P. alpina* was nearly as effective in providing the stimulus to the development of the ovule of *P. pratensis* as was its own pollen. Fertilization is thought to occur occasionally in apomictic plants, since intermediate types appear. It is suggested from the chromosome number of one hybrid, that it arose from a fusion of an unreduced egg and a reduced male gamete (*1*). Another hybrid that Åkerberg obtained from pollinating unemasculated florets of *P. pratensis* with pollen from *P. alpina* suggested by its chromosome number that it arose from fertilization of a reduced egg by a reduced male gamete (*2*).

Cytological studies on the embryo-sac development have been made by Andersen (*4*) and Armstrong (*5*). Since Andersen's study was made prior to the suggestion that apomixis occurs in *Poa*, she naturally did not consider seed development from this point of view. She stated, however, that "fertilization was not observed by the author, although the slides were carefully examined for this detail."

Armstrong (*5*) found that plants from different strains of Canadian and European origin might have different chromosome numbers. All the strains that he studied gave evidence of the occurrence of meiosis in macrospore mother cells. He concluded, from the presence of paired chromosomes at diakinesis in macrospore mother cells and from the good germination of pollen, that fertilization occurs in two strains, Mammoth from Ontario and No. 994 from Aberystwyth. It is interesting to note that he found the plants of the Mammoth strain morphologically uniform.

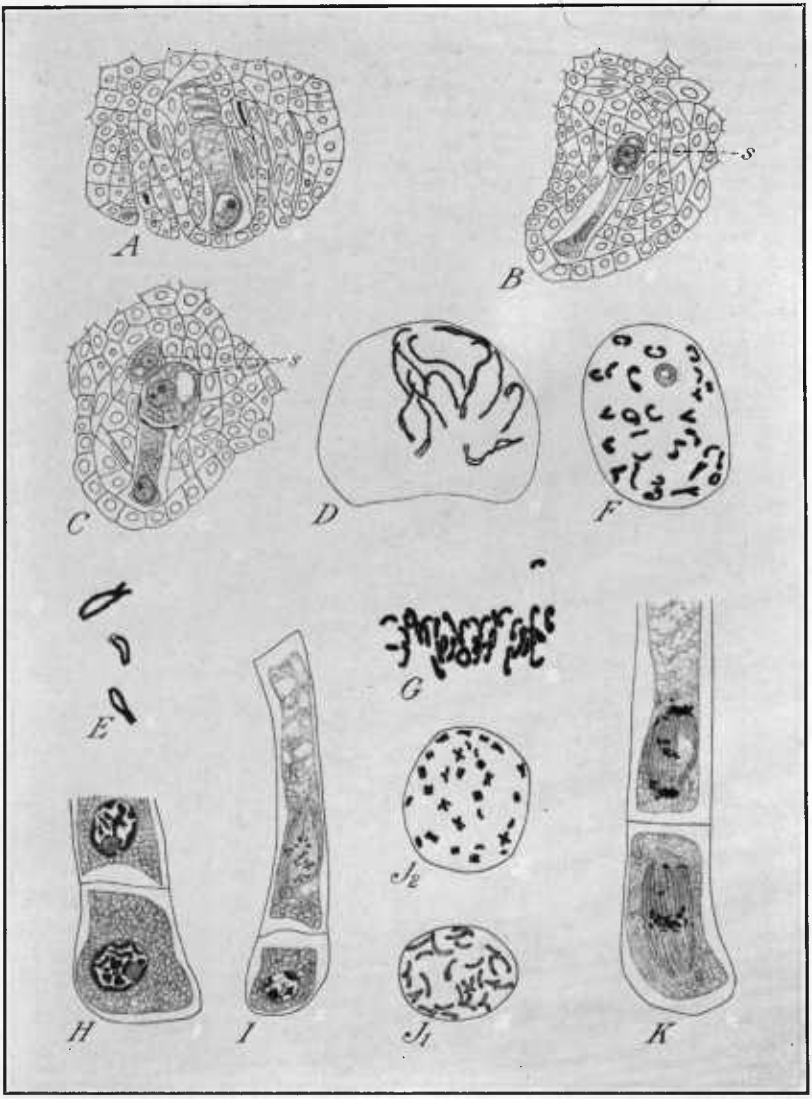
Other reported apomictic species in the Gramineae are: *Poa palustris* L. (*P. serotina* Ehrh.) (*7*); *P. alpigena* (E. Fries) Lindm., *P. glauca* Vahl., *P. arctica* R. Br. (*6*); *Calamagrostis obtusata* Trin. (*15*); and *Nardus stricta* L. (*14*). The complete cytological facts are not known for any of these species.

MATERIALS AND METHODS

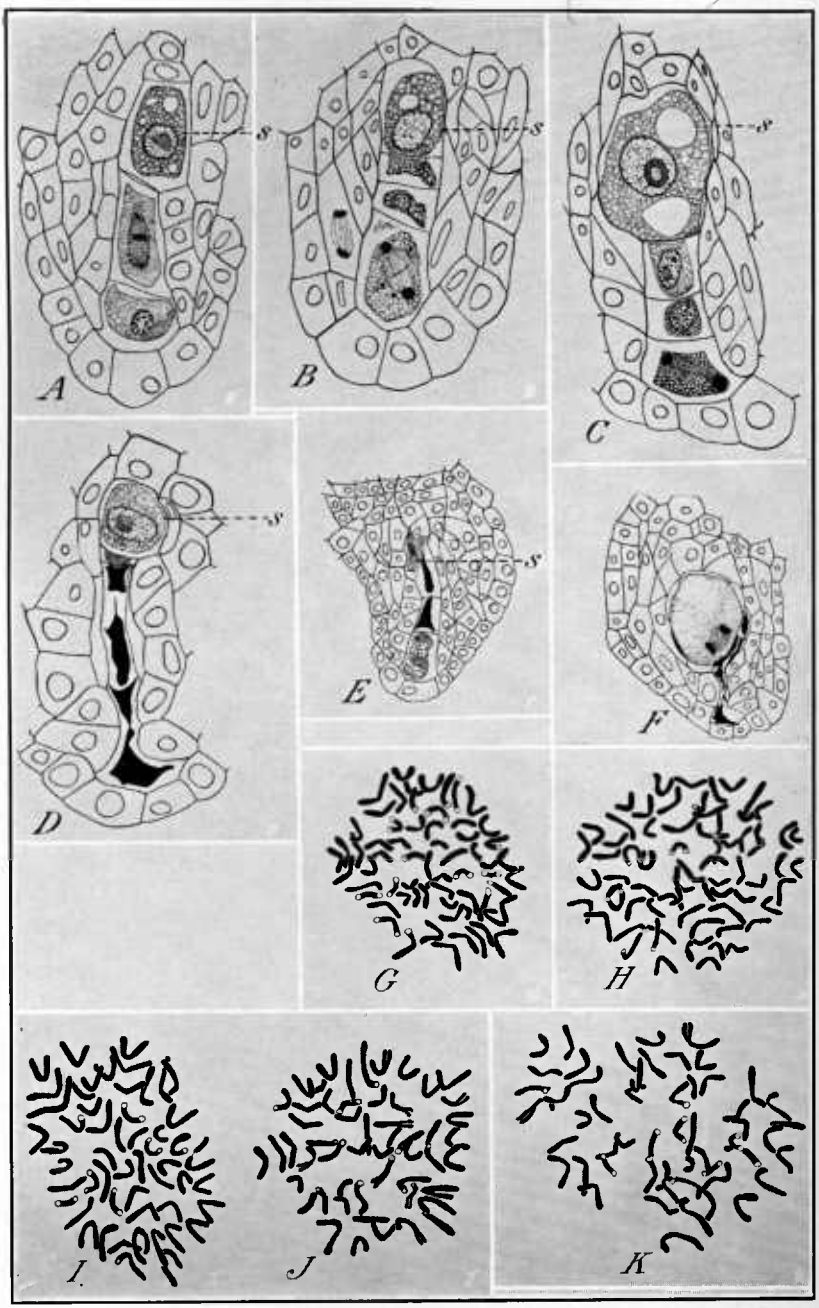
Seed of *Poa pratensis* collected from some of the oldest permanent pastures in Wisconsin was grown as single clones in the agronomy greenhouses and on the university farm at Madison, Wis. Most of the material for cytological study was collected from plants growing in the greenhouses during the past three winters, although some material was studied from collections made during summers in the field nursery.

In order to avoid confusion due to collecting material from plants differing among themselves in their method of reproduction, as many stages as possible were studied in material collected from the same clone. Altogether 5 clones of different biotypes have been studied in detail. In addition, material from 1 clone from each of 12 different biotypes was collected in the field nursery for a study of embryo development in the embryo-sac just before pollination.

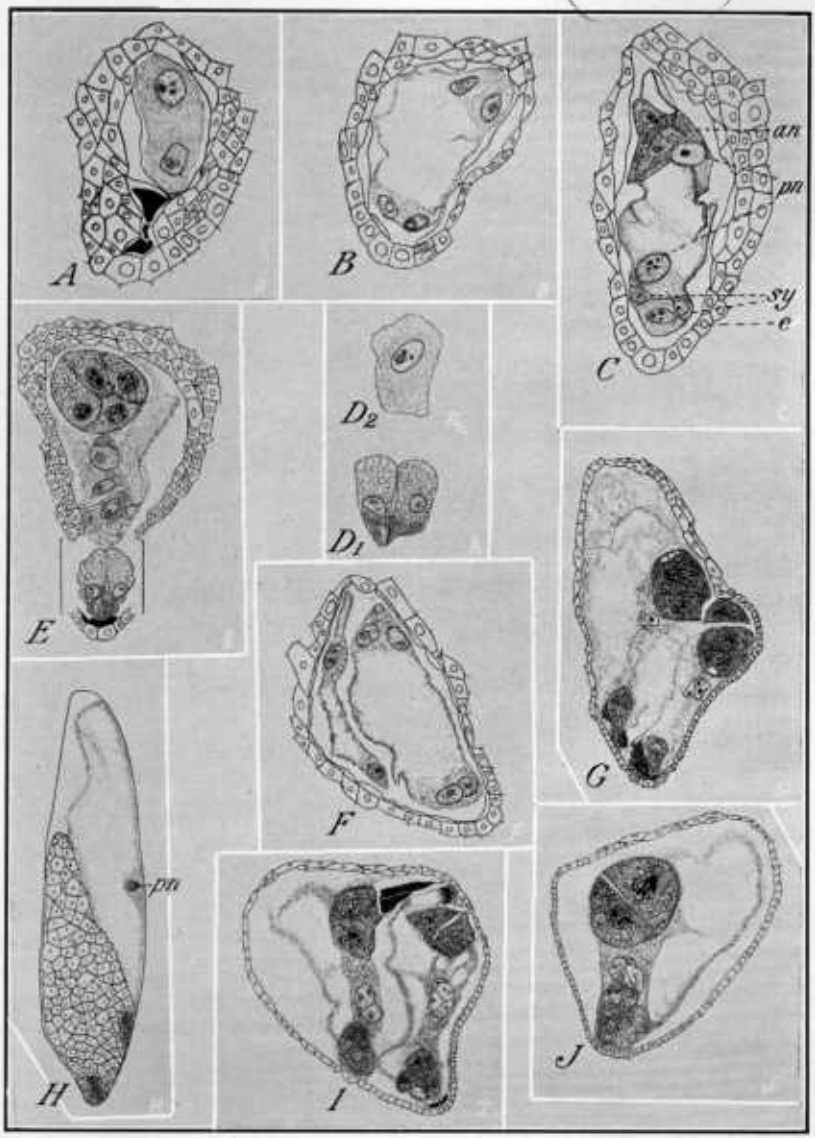
Individual florets were separated from the spikelets with the aid of a dissecting microscope, submerged for not more than 1 second in a solution of Carnoy's solution (6 parts absolute alcohol : 1 part glacial acetic acid : 3 parts chloroform), and then placed directly into Münt-



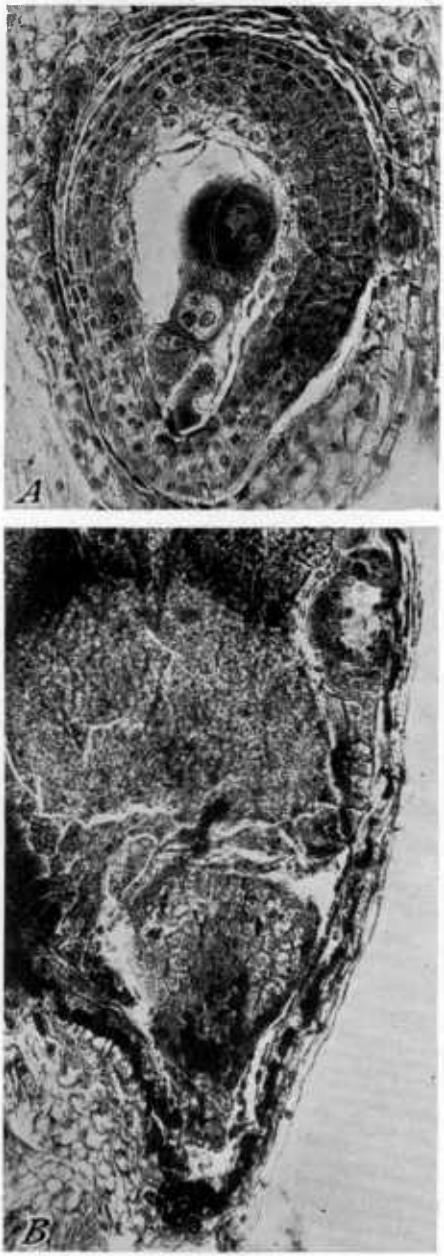
A, Young ovule with developing integuments and macrospore mother cell. × 218 *B*, Portion of nucellus, showing macrospore mother cell and differentiated somatic cell (*s*). × 218. *C*, Same as *B*, but showing macrospore mother cell and two differentiated somatic cells (*s*). × 218. *D*, Section through nucleus of macrospore mother cell illustrated in *A*, showing paired chromosome at diplotene. × 1,640 *E*, Portion of nucleus of a macrospore mother cell in early diakinesis. × 1,640. *F*, Nucleus of macrospore mother cell in late diakinesis. × 1,640. *G*, Metaphase of first meiotic divisions in macrospore mother cell (taken from plant shown in plate 2, *I* and *J*). × 1,640. *H*, Portion of daughter cells formed by first meiotic division; nuclei in interkinesis. × 629. *I*, Daughter cells; micropylar nucleus in interkinesis; chalazal nucleus in anaphase. × 462. J_1 and J_2, Second meiotic division; micropylar nucleus in early prophase, showing approximately 25 chromosomes (J_1); chalazal nucleus in late prophase, showing approximately 29 chromosomes (J_2). × 1,640. *K*, Portion of daughter cells, second meiotic division; micropylar nucleus in anaphase, chalazal nucleus in telophase. × 629. Drawings made with camera lucida at table level. All figures arranged with micropylar end of embryo sac toward bottom of page.



A, Portion of nucellus, showing two cells formed as result of first meiotic division; nucleus of micropylar cell in interkinesis and other in metaphase of second division; differentiated somatic cell (*s*). × 342. *B*, Same as *A*, but showing three cells formed as result of meiotic divisions (micropylar cell failed to complete second division); differentiated somatic cell (*s*). × 342. *C*, Same as *B*, but showing further enlargement of somatic cell (*s*). × 342. *D*, Same as *B*, but showing four disintegrated macrospores and differentiated somatic cell (*s*) separated from macrospores by vegetative cell. × 342. *E*, Same as *B*, but showing three cells in process of disintegration (micropylar cell failed to complete second division); early differentiation of somatic cell (*s*). × 180. *F*, Same as *B*; first division of nucleus in embryo sac and disintegrated macrospores. × 180. *G*, Metaphase; first division of nucleus in embryo sac, showing approximately 63 chromosomes. × 1,112. *H*, Metaphase; root tip of same plant as *G*, showing approximately 70 chromosomes. × 1,112. *I*, Same as *G* from another biotype, showing approximately 53 chromosomes. × 1,112. *J*, Metaphase; root tip from same plant as *I*, showing approximately 53 chromosomes. × 1,112. *K*, Metaphase; root tip from same plant as in plate 1, J_1 and J_2, showing approximately 54 chromosomes. × 1,112.



A, Portion of nucellus, showing two-nucleate embryo sac and three disintegrated macrospores. × 199. *B*, Same as *A*, showing four-nucleate embryo sac. × 199. *C*, Same as *A*, showing complete embryo sac, antipodals (*an*), polar nuclei (*pn*), synergids (*sy*), and egg (*e*). × 199. D_1, Synergids, and D_2, egg, from same embryo sac. × 199. *E*, Portion of nucellus, showing embryo sac with two-cell proembryo; two synergids, displaced by amount indicated to permit of their being drawn. × 141. *F*, Portion of nucellus with two developing embryo sacs: one binucleate, the other four-nucleate. × 199. *G*, Composite drawing from two sections, showing two complete embryo sacs each containing a proembryo. × 97. *H*, Composite drawing of later stage in seed development, showing two proembryos surrounded by endosperm of one embryo sac; two polar nuclei (*pn*) of second sac are unfused. × 26½. *I*, Composite drawing showing portion of nucellus and two embryo sacs, one of which possesses two polar nuclei; the other, five. × 97. *J*, Portion of nucellus with single embryo sac, showing four polar nuclei. × 97.



A, Photomicrograph of section of ovule collected just before pollination, showing proembryo with several cells. × 231. *B*, Photomicrograph of section of ovule collected after anthesis, showing two embryos. × 150.

zing's (*8*) modification of Navashin's solution or another modification composed of equal parts of two solutions, one of which consisted of 90 cc. of water, 10 cc. of glacial acetic acid, and 1½ gm. of chromic acid, and the other, of 40 cc. of formalin and 60 cc. of water. The air was removed with an aspirator. Fixation may be further enhanced by completely removing all floral parts and quickly submerging the pistils in the Carnoy fluid before placing them in the final fixing solution. This latter procedure was found especially useful when it was necessary to cut the ovules at particular angles in order to obtain polar views of the reduction divisions or of the first nuclear division in the embryo-sac mother cell. Root tips were fixed in Müntzing's solution. All material was left in the fixing fluid for from 18 to 24 hours and then washed in three or four changes of 70-percent alcohol (*13*). It was dehydrated in the usual manner, cleared in cedarwood oil, and embedded in paraffin. Root tips were cut at 10μ and stained in crystal violet iodine. Florets and pistils were cut from 10μ to 20μ, stained in Heidenhain's iron-alum haematoxylin, and destained in a saturated aqueous solution of picric acid.

OBSERVATIONS

The ovule in the basal florets of the spikelets, at the time the panicle begins to emerge from the sheath, consists of an outer and inner integument, each composed of two layers of cells, and a nucellus with a well-differentiated macrospore mother cell (pl. 1, *A*). A median section of an ovule at this stage invariably shows a single elongated, very conspicuous macrospore mother cell with the nucleus located usually near the micropylar end, or in some instances near the chalazal end. The cytoplasm is usually denser at the micropylar end than at the chalazal end of the cell.

In some instances at this early stage, one cell (pl. 1, *B*), or less frequently two cells (*C*), of the nucellus, near the chalazal end of the macrospore mother cell, differ conspicuously from the surrounding cells of the nucellus. The differentiated nucellar cell (or cells) is spherical, stains more darkly and, except for one or two characteristic vacuoles, contains denser cytoplasm than the surrounding cells of the nucellus. The significance of this cell will be discussed later.

The nucleus of the macrospore mother cell undergoes, so far as can be determined, a typical first meiotic division. Some tangential sections (pl. 1, *D*) clearly show paired chromosomes at diplotene. At later stages (*E*) the paired chromosomes appear to be held together by chiasmata. Stages in diakinesis (*F*) show that the chromosomes in most instances are associated in pairs, although univalents are frequently present. In one metaphase figure of the first reduction division (*G*) most of the chromosomes are associated in pairs, but there are three trivalent chains so arranged on the spindle that the two end chromosomes of the chain in each case will pass to the same pole; one chain of either three or four chromosomes is so arranged that the end chromosomes will pass to opposite poles; and three or four univalents are present. The first meiotic division forms two cells of which the one nearer the micropyle is usually the smaller (*I*). The nucleus of each daughter cell passes into interkinesis (*H*).

During the second meiotic division the chalazal cell precedes the micropylar cell in division. In one instance (pl. 1, *I*) the nucleus in the micropylar cell is in interkinesis when the one in the chalazal cell

is in anaphase; in another (J_1), the micropylar nucleus is in an early prophase, and the chalazal nucleus (J_2) is in a late prophase. J_1 and J_2 show that the number of chromosomes in each daughter nucleus is approximately one-half the somatic number of approximately 54 in the root tip of the same plant (pl. 2, *K*). In another instance (pl. 1, *K*), the nucleus of the micropylar cell is in anaphase and the nucleus of the chalazal cell in telophase. Univalents are frequently observed either passing precociously to the poles or remaining at the equator. In either case they frequently fail to be included in a daughter nucleus. Either daughter nucleus, then, may not receive a complete chromosome complement.

The second meiotic division in the chalazal cell regularly forms two haploid macrospores, both of which soon distintegrate. In most of the observed cases this second division is not completed in the micropylar cell because of the precocious disintegration of the cell (pl. 2, *B*, *C*, *E*). Consequently, only three cells are formed from the macrospore mother cell, although in one instance (*D*) four macrospores were observed. In all observed instances all the macrospores thus formed from the macrospore mother cell disintegrate and do not function in the development of the embryo sac.

A cell of the nucellus near the chalazal end of the macrospore mother cell has been described (p. 353) as being conspicuously different from the surrounding cells. In some instances (pl. 1, *B*, *C*) one cell or occasionally two cells are differentiated as early as the beginning of meiosis in the macrospore mother cell. Not infrequently, differentiation is not conspicuous until the meiotic divisions are completed (pl. 2, *E*). Plate 2, *A*, shows that of the two cells toward the micropyle, the nucleus of one is in interkinesis and the nucleus of the other is in metaphase, while the third cell, largest and inward, has been separately differentiated. In plate 2, *B* and *C*, the three micropylar cells that were derived from the macrospore mother cell are beginning to disintegrate. In the one nearest the micropyle, nuclear division has just been completed. The fourth and innermost cell has been differentiated separately. In another instance (pl. 2, *D*), four macrospores had been formed by two completed divisions and had completely collapsed. At the chalazal end of the macrospore row is a separately differentiated cell, separated from the row of disintegrated macrospores by a vegetative nucellar cell.

The differentiated nucellar cell just described is destined to function as the initial cell of the embryo sac (pl. 2, *F*). This conclusion is confirmed by the chromosome counts during the division of the primary nucleus of the embryo sac. In one instance (*G*) approximately 63 chromosomes were observed in this division. The chromosome number in cells of a root tip (*H*) of the same plant is approximately 70. In consequence of the high number of chromosomes and the resultant difficulty in distinguishing them, it is probable that the remainder of the diploid complement in the nucleus of the embryo sac was hidden from view. In another case, approximately 53 chromosomes were observed in nuclear division both in the initial cell of the embryo sac (*I*) and in a cell of a root tip (*J*) of the same plant. In several other cases a sufficiently accurate count of the chromosomes in the initial cell of the sac could be made to demonstrate the presence of the diploid number.

A two-nucleate embryo sac and three disintegrated macrospores are shown in plate 3, *A*, a four-nucleate sac in plate 3, *B*, and a completed eight-nucleate seven-cell sac in plate 3, *C*.

The mature embryo sac consists of three large, darkly staining antipodal cells at the chalazal end, a single central cell containing two polar nuclei, and the egg apparatus (egg and two synergids) at the micropylar end. The nuclei of the antipodal cells may divide and if cell division does not occur the cell in question remains binucleate. In some cases cell division follows to form ultimately five or six antipodal cells.

The synergids (pl. 3, D_1 and *E*) enlarge and become pear-shaped, with the apex of each projecting toward the micropyle. They lie side by side directly beside the egg. The cytoplasm in the basal portion of each synergid becomes very fibrous in appearance, constituting the filiform apparatus often described for synergids. A nucleus lies near the center of each synergid, and as the two cells disintegrate the nuclei maintain their structure longer than does the cytoplasm. In some instances it appears that the two synergids in the process of disintegration fuse into one darkly staining body.

The cytoplasm of the egg (pl. 3, D_2) becomes coarsely vacuolated; the nucleus lies near the center of the cell surrounded by denser cytoplasm. At the apical end the cytoplasm assumes regularly a more coarsely alveolar appearance, which seems to disappear when the cell divides.

The egg divides to form a proembryo in many of the first, second, or third florets of the spikelet before anthesis begins (pl. 3, *E*). The two synergids shown in *E* have been displaced by the amount indicated to permit of their being drawn. The proembryo shown in the photomicrograph (pl. 4, *A*) is larger than the one in the drawing (pl. 3, *E*), but the florets from which these figures were obtained were at approximately the same stage of flowering when fixed. In this instance the synergids have begun to disintegrate and have lost their identity. These observations have been made repeatedly in material fixed in the greenhouse under conditions that excluded the possibility of the presence of pollen in the air, and in material fixed in the field 1 or 2 days before the floral parts had opened to permit the exposure of the stigma. There seems no doubt that pollen is unnecessary to initiate development of the egg into an embryo. Since it has been shown that the nuclei of the embryo sac, including the egg nucleus, are all diploid, it is apparent that the functioning of the egg is not dependent on gametic union. The external stimulus to the egg, if one is necessary, is apparently exerted from within the embryo sac.

The two polar nuclei (pls. 3, *H*; 4, *A*) lie together in the dense cytoplasm of the embryo sac midway between the antipodals and the proembryo. Fusion of the polar nuclei is not completed until the proembryo consists of several cells, and occurs invariably after anthesis. Fusion is accomplished by dissolution of the adjacent membranes to form the primary endosperm nucleus, which later divides to form the endosperm nuclei. The cells of the endosperm, thus formed, would possess a $4n$ number of chromosomes in contrast to the $3n$ number usually found in sexually reproducing species.

It is not certain whether pollination is associated with endosperm development. Pistils examined a few hours after pollination show pollen grains germinating freely on the stigma and pollen tubes

growing down the style. In no case, however, have pollen tubes been observed in tissue of the ovary, or in the embryo sac. Florets that were emasculated and not polinated have in a few instances developed seeds, but it is possible that in these instances the stigma was accidentally pollinated during the act of emasculation. Florets that were open-pollinated in the greenhouse occasionally contained aborted ovules, and examination has shown proembryos but no endosperm. The two unfused polar endosperm nuclei were still frequently visible in such cases. The failure of the polar nuclei to fuse and to bring about the development of an endosperm may have been due to a failure of pollen tubes to stimulate endosperm development.

POLYEMBRYONY AND ATYPICAL EMBRYO SACS

Sections of young ovules not infrequently show two embryo sacs in course of development (pl. 3, *F*). The initial cells from which such sacs develop have not been observed in division, but it seems very probable from observations of ovules containing single embryo sacs that two sacs may arise independently from two differentiated nucellar cells, such as are sometimes present (pl. 1, *C*). Consequently, the nuclei in both embryo sacs would be expected to possess identical diploid chromosome complements. Andersen (*4*) concluded that polyembryony results from the functioning of two embryo sacs and that each embryo sac develops either from two macrospores in the same row or from two macrospores derived from separate macrospore mother cells.

One embryo sac, presumably that favored by position, may grow faster than its twin; the larger one develops in the usual manner, while the smaller develops irregularly and consequently may not produce a proembryo. If the development is not too irregular, each will produce a proembryo (pl. 3, *G*). In all observed instances in which two embryos were present there was evidence of the previous presence of two embryo sacs; for example, two sets of antipodals, or two pairs of polar nuclei, or both. The ovule illustrated in plate 3, *H*, contains two proembryos and a mass of endosperm tissue formed by division of the primary endosperm cell of one embryo sac. The previous presence of a second sac is evidenced by the two polar nuclei incompletely fused.

The embryo nearer the micropyle may be large and typical in shape; its twin embryo smaller, atypical, and located in an unusual position (pl. 4, *B*). Such instances, however, do not necessarily imply a difference in origin of the smaller embryo. These instances may be explained by the more rapid growth of one embryo and of its endosperm, in consequence of which the smaller embryo, in a less favorable position for growth, is crowded against the wall of the nucellar cavity.

There remains the possibility that one of the twins may have developed parthenogenetically from a haploid egg, in which case it would be expected to be weaker than the diploid twin. Müntzing (*9*) found one haploid plant which he suggested arose as a twin from the parthenogenetic development of a haploid cell. He found also in some instances that the two members of a pair, in the seedling stage, differ in size and vigor, perhaps in consequence of the more favorable position of the larger. He found the chromosome number of twin seedlings to be usually the same, but occasionally one was approximately triploid

with respect to its diploid twin; and if there was a difference in size, the triploid seedling, more frequently than the diploid, was the weaker of the two. Müntzing suggested that the triploid arose from the fertilization of a diploid egg.

The egg in each embryo sac (in case two sacs are present) frequently develops into a proembryo before pollination, and under such circumstances fertilization of the egg in either embryo sac obviously could not occur. The chromosome number would be the same in the two embryos (omitting the possibility of the development of a haploid embryo sac from a macrospore). If one or both eggs did not begin to develop until sufficient time had elapsed for the pollen tubes to reach the embryo sacs, one or both eggs might be fertilized. Müntzing (*9*) found both members of one set of twin seedlings to be approximately triploid. It seems reasonable to assume that the eggs in the weaker embryo sac might delay parthenogenetic development longer than those in the more vigorous one, and consequently provide opportunity for gametic union. Under such circumstances triploidy will be more frequently associated with the smaller of twin seedlings than with the larger.

There is no evidence that embryos originate by sporophytic budding from the nucellus.

Embryo sacs, when occurring either singly or doubly, occasionally contain more than the eight nuclei. In some instances an apparently functional embryo sac, in addition to the antipodals, two-nucleate primary endosperm cell, and egg apparatus, consists of single cells or nuclei lying in the cytoplasm of the nuclear cavity. It is probable that the condition might become too atypical for an embryo sac to function.

More than two polar nuclei are occasionally seen in process of fusion. In one instance (pl. 3, *J*) there were four polar nuclei; and in another (pl. 3, *I*), there were five polar nuclei in one embryo sac and two in a companion sac. The origin of these additional nuclei or cells is not known, but it seems probable that they result from nuclear divisions during embryo-sac development. It seems improbable that any of the nuclei shown fusing with the two polar nuclei came from a pollen tube.

DISCUSSION

The evidence presented by Müntzing (*8*), Nilsson (*12*), and Åkerberg (*3*) indicates that gametic union does not regularly occur in Swedish biotypes of *Poa pratensis*. According to Åkerberg and Nilsson, however, pollination is necessary to initiate seed development, and in a few cases they found evidence that pollination was followed by a union of male and female gametes. The chromosome number of one hybrid plant suggests that it arose from the union of an unreduced egg and a reduced male gamete (*1*). Müntzing (*9*) found that occasionally one member of a twin pair was approximately triploid and suggested that in such a case a diploid egg was fertilized by a haploid male gamete. In two instances the egg was thought to have been haploid. In one of these instances the egg was presumably fertilized by a haploid male gamete of *P. alpina* (*2*), while in the other the haploid egg presumably developed parthenogenetically (*9*). Åkerberg (*3*) found four biotypes that appeared to reproduce regularly by gametic union.

The cytological observations on *Poa pratensis* herein described show that in the biotypes studied the single macrospore mother cell underwent meiosis in the usual manner and formed haploid macrospores (usually three, since the second meiotic division was often incomplete). In all the observed instances the three or four macrospores so formed disintegrated. Andersen (*4*) reported, without presenting evidence, that the macrospore mother cell formed a row of four macrospores and that usually the chalazal macrospore formed the embryo sac although in several observed ovules the micropylar macrospore functioned. Armstrong (*5*) observed meiosis and concluded that one of the macrospores thus formed develops a haploid embryo sac and that meiosis ipso facto implies sexual reproduction.

The initial cell of the embryo sac is differentiated from among the vegetative cells of the nucellus while the meiotic divisions are under way, and develops into a typical eight-nucleate, seven-cell embryo sac. Consequently the nuclei of the embryo sac, including the egg nucleus, possess the same diploid chromosome complement as do the nuclei of the maternal parent. The egg is capable, consequently, of developing into an embryo without restoration of the diploid chromosome number by fertilization. Under these circumstances all the plants grown from seeds collected from a single plant possess the same diploid complement of chromosomes, and obviously are, therefore, similar in morphological type. Müntzing (*8*) has found that 10 plants examined in a progeny from a single plant all had the same maternal chromosome number. Observations made by the author indicate that all the plants (with few possible exceptions) grown from seeds collected from a single plant are alike in growth form and habit. Since the embryo sac develops from a vegetative cell of the nucellus without meiosis, its origin may be considered an instance of apospory. The embryo develops from the egg by parthenogenesis.

Cytological observations have not confirmed the conclusions of Åkerberg (*1*) and Nilsson (*12*) that pollination is necessary to induce seed development. These investigators based their conclusions on the lack of seed setting in emasculated florets, and obviously were not able to separate the two phases of the development of a seed, i. e., embryo development and endosperm development. The embryo of *Poa pratensis* develops in many instances before pollination, but since endosperm development does not begin until after pollination the effect of pollen or pollen tubes on endosperm development is not easy to determine. A comparatively few florets have been emasculated by the author, and some of these have produced seeds. In the emasculation the floret may have been injured or the pistil unintentionally pollinated. According to Åkerberg (*1*) and Nilsson (*12*), the environment also may affect seed development. Consequently, negative results might be due to any of several causes. There is some evidence to indicate that seed abortion is due to the lack of endosperm development, and it may be that in such instances pollen failed to germinate on the stigma.

The egg frequently begins development before pollination is effected, but occasionally development does not begin until after pollination. Since pollen tubes have been observed in the style, although not in the embryo sac, it is possible that occasionally one reached the embryo

sac before parthenogenetic development began and that a diploid egg was then fertilized. Circumstances such as this may lead to the production of an occasional hybrid as reported by Åkerberg (*1*), and of triploid twin plants such as were found by Müntzing (*9*). Chromosome disjunction during meiosis in the microsporocytes is extremely irregular, and in many instances chromosomes are lost from the daughter nuclei. It is not known whether chromosome-deficient pollen grains germinate on the stigma, although a deficiency appears to have no effect upon the development of pollen grains. If a gamete possessing a deviating chromosome number effects fertilization, the resulting zygote will be only approximately triploid. Müntzing (*9*) is not certain of the exact chromosome number in twin plants of *Poa pratensis*, but he suggests that the number is only approximately triploid. Once the hybrid is produced, the newly combined chromosome complements, either euploid or aneuploid, may be transmitted to future generations by parthenogenesis. Diploid parthenogenesis and occasional fertilization of the egg by a sperm possessing a varied chromosome number can produce the singular characteristics of *P. pratensis:* (1) Extreme polymorphism and varying euploid and aneuploid chromosome numbers of different biotypes and (2) constant morphological type and chromosome number in plants of the same biotype.

SUMMARY

Median sections through the micropylar region of young ovules of *Poa pratensis* invariably show a single, elongated, very conspicuous macrospore mother cell. The nucleus of this cell undergoes meiosis, and haploid macrospores are formed—usually three, since frequently the micropylar cell does not complete the second meiotic division. In observed instances, all the macrospores subsequently disintegrate.

The embryo sac develops, without meiosis, from a cell of the nucellus, which is located near the chalazal end of the macrospore mother cell. The typical mature embryo sac consists of three antipodal cells, a primary endosperm cell containing two nucelli, and the egg apparatus (egg and two synergids).

The diploid egg develops into a proembryo by parthenogenesis; the development begins frequently before pollination. (Possible exceptions are noted in the text.) Since endosperm development was not observed to begin until after pollination, it may be that pollination or the growth of pollen tubes in stylar tissue is necessary for endosperm development and consequently for seed development. Pollen tubes have not been observed in the embryo sac.

Diploid parthenogenesis and occasional fertilization of the egg by a sperm possessing a varied chromosome number can produce the singular characteristics of *Poa pratensis:* (1) Extreme polymorphism and varying euploid and aneuploid chromosome numbers between different biotypes and (2) constant morphological type and chromosome number in plants of the same biotype.

Two embryos are occasionally produced by the functioning of two embryo sacs. Each embryo sac appears to develop independently from separate somatic cells of the nucellus. There is no evidence that embryos arise by sporophytic budding from the nucellus.

LITERATURE CITED

(1) ÅKERBERG, ERIK.
1936. STUDIEN ÜBER DIE SAMENBILDUNG BEI POA PRATENSIS L. Bot. Notiser 1936: [213]–280. [English summary, pp. 268–269.]
(2) ———
1936. BASTARD MELLAN POA PRATENSIS L. × P. ALPINA L. ARTIFICIELLT FRAMSTÄLLD. Bot. Notiser 1936: 563–566.
(3) ———
1938. SEED PRODUCTION OF THE POA SPECIES. Svensk Frötidning 7: 79–83, illus. [Translation by R. Peter Jones in Imp. Bur. Pastures and Forage Crops, Herbage Rev. 6: 228–233, illus.]
(4) ANDERSEN, ALICE M.
1927. DEVELOPMENT OF THE FEMALE GAMETOPHYTE AND CARYOPSIS OF POA PRATENSIS AND POA COMPRESSA. Jour. Agr. Res. 34: 1001–1018, illus.
(5) ARMSTRONG, J. M.
1937. A CYTOLOGICAL STUDY OF THE GENUS POA L. Canad. Jour. Res. (C) 15: 281–297, illus.
(6) FLOVIK, KARL.
1938. CYTOLOGICAL STUDIES OF ARCTIC GRASSES. Hereditas 24: [265]–376, illus.
(7) KIELLANDER, C. L.
1935. APOMIXIS BEI POA SEROTINA. (VORLÄUFIGE MITTEILUNG). Bot. Notiser 1935: 87–95.
(8) MÜNTZING, ARNE.
1932. APOMICTIC AND SEXUAL SEED FORMATION IN POA. Hereditas 17: 131–154, illus.
(9) ———
1937. POLYPLOIDY FROM TWIN SEEDLINGS. Cytologia Fujii Jubilaei Volumen, June 1937: 211–227, illus.
(10) NILSSON, FREDRIK.
1933. SJÄLV- OCH KORSBEFRUKTNING I RÖDSVINGEL (FESTUCA RUBRA L.), ÄNGSGRÖE (POA PRATENSIS L.) OCH ÄNGSKAVLE (ALOPECURUS PRATENSIS L.). (With an English summary.) Bot. Notiser 1933: 206–230. [In Swedish. English summary, pp. 221–223.]
(11) ———
1934. STUDIES IN FERTILITY AND INBREEDING IN SOME HERBAGE GRASSES. Hereditas 19: 1–162.
(12) ———
1937. FRÖSÄTTNINGEN HOS ÄNGSGRÖE, POA PRATENSIS. Bot. Notiser 1937: 85–109. [In Swedish. English summary, pp. 106–108.]
(13) RANDOLPH, L. F.
1935. A NEW FIXING FLUID AND A REVISED SCHEDULE FOR THE PARAFFIN METHOD IN PLANT CYTOLOGY. Stain Technol. 10: 95–96.
(14) SCHNARF, KARL.
1929. EMBRYOLOGIE DER ANGIOSPERMEN. *In* Linsbauer, K., Handbuch der Pflanzenanatomie . . . , Abt. 2, t. 2, 689 pp., illus. Berlin.
(15) STENAR, HELGE.
1933. PARTHENOGENESIS IN DER GATTUNG CALAMAGROSTIS EMBRYOBILDUNG BEI CALAMAGROSTIS OBTUSATA TRIN. UND CALAMAGROSTIS PURPUREA TRIN. Arkiv. för Bot. 25A, No. 6, 8 pp., illus.

U. S. GOVERNMENT PRINTING OFFICE: 1940