A CYTOLOGICAL STUDY OF CLEISTOGAMOUS STIPA LEUCOTRICHA

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Introduction

The genus *Stipa* is of some importance as a range grass in most of the temperate-zone grasslands. One species, *S. leucotricha,* is of considerable economic importance in Texas. This species is common throughout Texas east of the Pecos River and south of the Red River. It occurs in the very southern part of Oklahoma but is absent from the extreme eastern part of Texas and most of the coastal plain. In the Austin region of central Texas it is very abundant and can be described as a weed-type of grass which rapidly occupies disturbed soil and overgrazed pastures. It often continues in the succession from beginning to the end for it is frequently a minor constituent of the dominant grassland climax. However, since little of the central Texas range is occupied by the dominant association because of nearly continuous overgrazing, it usually results that *Stipa leucotricha* is one of the common good native forage grasses in this region. It is eaten by cattle and is highly regarded by many ranchers because of its abundance, long growing season and capacity to cure well on the ground. *Stipa leucotricha* is essentially a winter-growing perennial. Because it grows vigorously from September to June and after each rain during the summer, it may offer forage throughout the year.

Hitchcock (1935) reports that, "Cleistogamous spikelets with glumes obsolete and lemma nearly awnless are borne in basal sheaths just after maturity of the panicle." Dyksterhuis (1945) made a study at Fort Worth, Texas, of these axillary, often subterranean, cleistogenes and of their rôle in nature. He found that these cleistogenes were formed whether or not the normal panicle was produced and also that they were produced by plants kept clipped to one and one-half inches. He states that the species may behave under heavy grazing as a winter annual without producing flowering culms. This is accomplished by fall seedlings of cleistogamous origin producing new cleistogenes in the spring and then succumbing to summer drought. These cleistogenes, then, give this species a great advantage over other grasses, especially in heavily grazed areas, for a crop of seed is assured every year.

The present study began with an attempt to determine the chromosome number of this species from root tip mitosis but for technical reasons the exact number was not determined. While searching for anthers with pollen mother cells in the proper stage of meiosis it became apparent that the florets of the panicle are unusual and so a detailed study was made of these florets.

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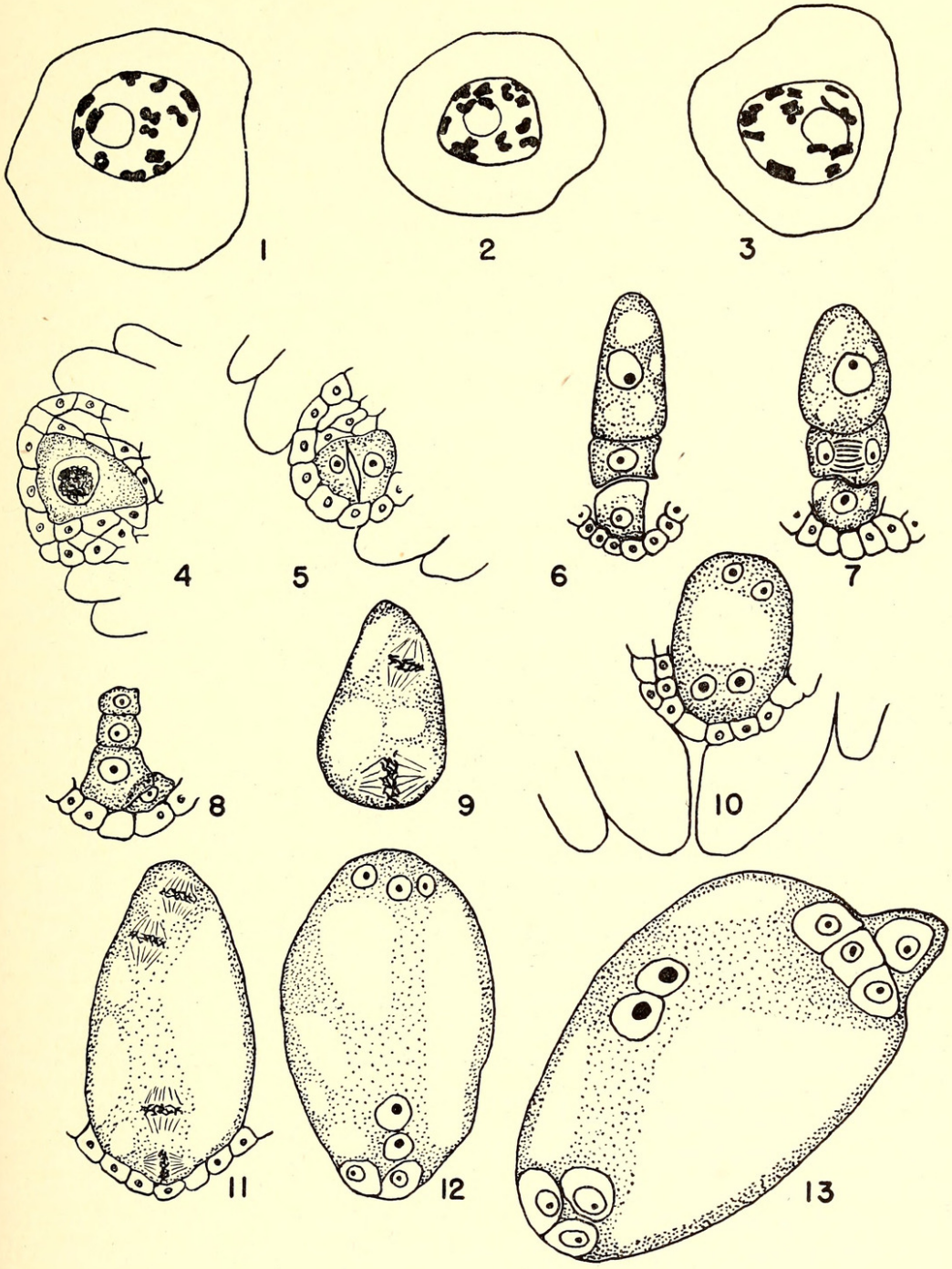
Material and Methods

In this work, panicle florets only were studied. Inflorescences were collected at various stages of development and florets were fixed in absolute-acetic fixative. Young florets were clipped across to permit the penetration of the fixative inside the tube formed by the lemma and palea. Older florets were dissected, and the lemma and palea were removed. Material was embedded in paraffin and sectioned longitudinally at 15 microns. Two methods of staining were employed, Heidenhain's hematoxylin, and a combination of Feulgen and fast green. The hematoxylin stain was more satisfactory.

Chromosome Number and Meiosis

The chromosome number could not be determined from root tip mitoses by the usual method employing Craf Navashin fixative and Heidenhain's hematoxylin stain. A search for meiosis in pollen mother cells yielded no results by the smear method. Fortunately one flower was sectioned in which the pollen mother cells of one anther sac were in the diakinesis stage. There were only eight pollen mother cells in this sac and one was distorted by sectioning. The remaining seven were used in this study. Love, in Myers' text (1947) reports his material, growing under cultivation in California, to have a count of 2n = 28. In the seven pollen mother cells studied the following results were obtained. In each of four cells 13 diakinesis bivalents were found (fig. 1), in two cells there were 11 bivalents and one quadrivalent (fig. 2) and in one cell there were 12 bivalents and two univalents (fig. 3). It is probable that this one plant had a 2n chromosome number of 26 which is different from Love's material. It is evident, however, that meiosis in this particular plant is quite irregular and it may be that different plants of this species have different chromosome numbers. Further study is necessary before a definite basic chromosome number can be assigned to this species.

The presence of occasional quadrivalents and univalents at meiosis in diploid species is not uncommon in the grasses. Myers (1947) lists five species that have been reported to show associations of four or more chromosomes at diakinesis. These conditions have been attributed by the authors to structural hybridity. In the present study it was not possible to determine whether the cause was structural hybridity or the presence of two pairs of homologous chromosomes. No explanation is offered for the occurrence of a pair of univalents in one pollen mother cell. Myers lists 10 species of grasses that have shown occasional univalents at first meiotic division. This list includes both diploid and polyploid species. *Stipa leucotricha* is probably diploid, for other basic numbers that have been reported in the genus are 10, 11, and 12 (Myers 1947).



FIGS. 1–13. Embryo sac development of *Stipa leucotricha*. FIGS. 1–3, Diakinesis in pollen mother cells: 1, 13 bivalent chromosomes; 2, 11 bivalents and one quadrivalent; 3, 12 bivalents and two univalents. FIG. 4, Meiotic prophase in archespore. FIG. 5, Secondary megasporocytes. FIG. 6, Three "spore" stage. FIG. 7, Transverse division of middle "spore". FIG. 8, Enlargement of a micropylar megaspore. FIG. 9, Second division in the embryo sac. FIG. 10, Four-nucleate embryo sac. FIG. 11, Third division in the embryo sac. FIG. 12, Eight-nucleate embryo sac, the synergids with cell walls. FIG. 13, Mature embryo sac with four antipodal cells.

Development of the Embryo Sac

The archesporial cell (fig. 4) lies immediately under the epidermal layer of the nucellus. At this stage the two integuments do not cover the terminal portion of the nucellus. The archesporial cell acts as the megaspore mother cell and meiosis takes place in it. Figure 4 shows a prophase stage of meiosis. The two cells produced are oriented so that the smaller is against the epidermis and the larger is embedded more deeply in the nucellus (fig. 5). Figure 6 shows a peculiar three-celled stage which was seen in a number of ovules. The origin of the middle cell was not determined but it arises from division of either the larger or the smaller cell of figure 5. Figure 7 shows another common occurrence, the lateral division of this middle cell. This lateral division was observed repeatedly and may well be typical of this species. Usually the spore toward the chalaza develops into the embryo sac as in figure 7 but in two cases the micropylar cell was enlarged (fig. 8). At the second division of the developing embryo sac (fig. 9) it is in contact with the epidermis and the nonfunctional spores are undetectable. In the four-nucleate embryo sac stage (fig. 10) the inner integument has grown around the nucellus except for the micropyle. The last embryo sac division results in eight nuclei, four at each end of the embryo sac. Figure 11 shows an orientation of the spindles which would suggest that the two synergids would be sister cells as would be also the egg and micropylar polar nucleus. This situation appears to be the rule in angiosperms for Brink and Cooper (1947) state that " . . . wherever definite observations have been made, the two synergids, on the one hand, and the egg cell and upper polar nucleus, on the other hand, represent sister nuclei, and no reliable observations are at hand to substantiate any other origin." Maheshwari (1948) makes a similar statement.

The mature embryo sac (fig. 12) is of the monosporic 8-nucleate, or normal type, which according to Maheshwari, "occurs in at least 70 percent of the angiosperms so far studied." All Gramineae have this normal type of embryo sac development (Schnarf, 1929). In the Gramineae, however, there is typically a further development of the antipodal cells to form a large tissue within the embryo sac. Shadowsky (1926) studied this tissue in 16 species of grasses in 7 tribes and found considerable antipodal development in all species studied. He classified them as to

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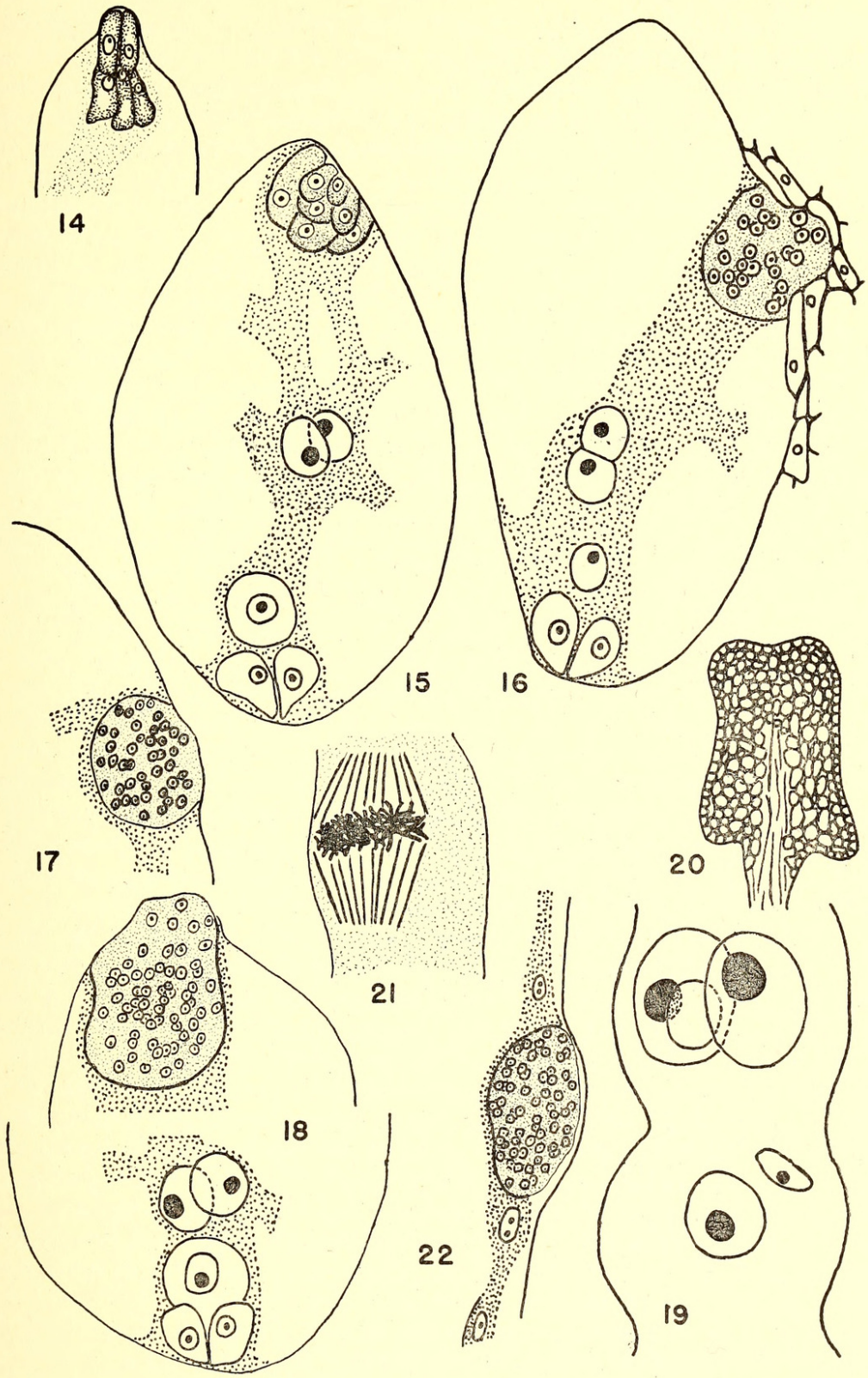
Explanation of Figures 14–22.

Figs. 14–22. Embryo sac development of *Stipa leucotricha*. Fig. 14, Antipodal tissue of five cells. Fig. 15, Embryo sac with antipodal tissue of seven cells. Fig. 16, Embryo sac with antipodal tissue of 24 nuclei. Fig. 17, Antipodal tissue of 42 nuclei. Fig. 18, Embryo sac at time of fertilization with antipodal tissue at end of sac with 56 nuclei. Fig. 19, Double fertilization. Fig. 20, Section of a sterile anther. Fig. 21, Division of the primary endosperm nucleus. Fig. 22, Antipodal tissue and free nuclear endosperm.

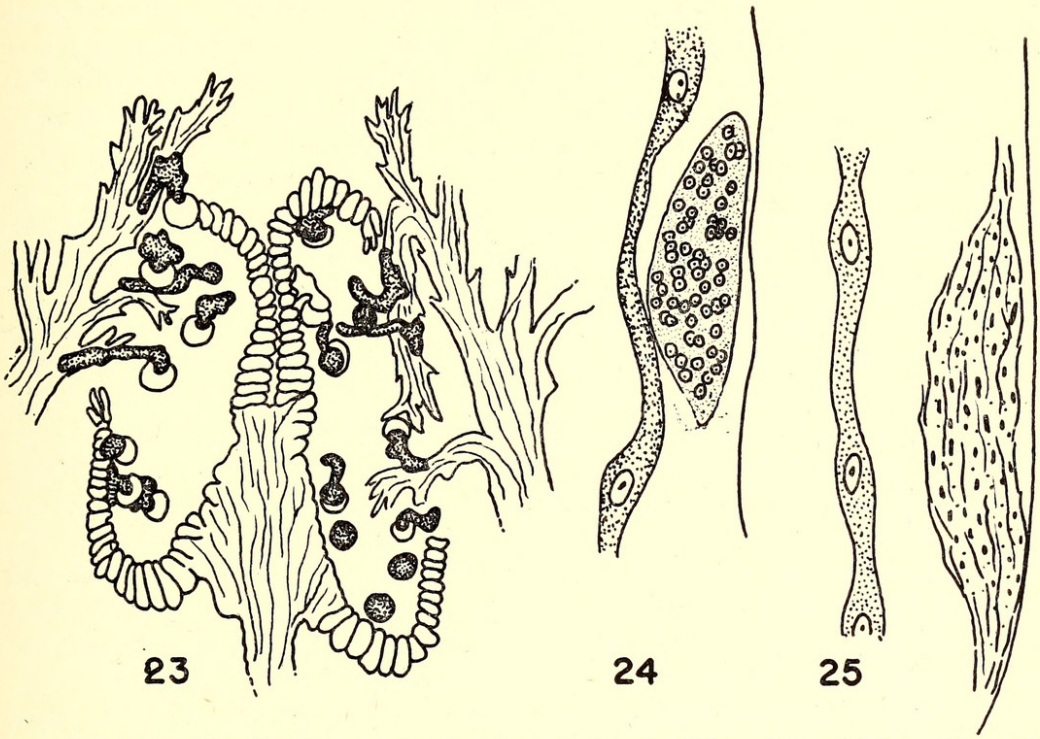


FIGS. 14–22. Embryo sac development of *Stipa leucotricha*.

whether this tissue was at the end or at the side of the embryo sac. In *Stipa leucotricha* growth of this tissue begins immediately after the 8-nucleate stage has been reached, usually by the formation first of one additional cell (fig. 13) which penetrates the chalazal tissue. It is assumed that this fourth cell is derived from one of the antipodal cells although the division was never seen nor could the chromosome number of the antipodal tissue be determined. At the same time the two polar nuclei have made contact with each other to one side of the middle of the embryo sac. These two nuclei remain in close contact in this position until just before fertilization. Figure 14 shows the 5-celled stage of the antipodal tissue, the tissue being still at the chalazal end. Figure 15 is of an embryo sac showing the 7-celled condition. The embryo sac has lengthened at the chalazal end so that the antipodal tissue is becoming lateral in position. Figure 16 shows the antipodal tissue on the lateral wall and penetrating the nucellus. There are 24 nuclei in this mass but cell walls, if they exist, could not be distinguished. The tissue is still covered by the protoplasm of the embryo sac on the inner surface. Figure 17 shows an antipodal mass with 42 nuclei. The embryo sac has increased greatly in size so that this mass is on the wall at about the middle of the sac. Rarely the antipodal mass remains terminal and moves along with the enlarging embryo sac as seen in figure 18. This mass contains 62 nuclei. The maximum number in this species seems to be about 60 nuclei since counts of 62, about 60, and 56 nuclei have been made. This compares with 30 or more in *Zea* and *Euchlaena* (Cooper, 1937), 300 in *Sasa paniculata* (Yamaura, 1933), and the various numbers reported in 16 species by Shadowsky (1926). At the time of fertilization the two unfused polar nuclei move to a position close to the egg cell (fig. 18).

Before fertilization of the egg can be effected, cleistogamous pollination (autogamy) takes place. Figure 23 represents a section of an anther and a few branches of the stigma during this process. Part of the outer wall of each anther sac disintegrates [as Uphof (1938) reports for *Cardamine chenopodifolia*] and a few stigma branches enter. The movement of these stigma branches is not caused by growth. Before the walls of the anther disintegrate these branches are confined between the palea and the anther. The disintegration of the anther walls allows the stigma branches to expand or straighten out and by so doing to enter the pollen sacs. The pollen grains germinate in place and the pollen tubes grow in various directions, some making contact with the stigma but many others never making contact. Pollen tube growth was not studied but was assumed to be normal since double fertilization took place. Figure 19 shows an egg with a male nucleus within it and shows the other male gamete in contact with the two still unfused polar nuclei. The endosperm is

triploid as a result of this triple fusion. Figure 21 represents the first division of the primary endosperm nucleus. The exact chromosome number could not be determined. Thirty-four chromosomes were counted and there were a few chromosomes remaining but uncountable. This is a few less than the expected triploid number, 39.



FIGS. 23–25. Embryo sac development of *Stipa leucotricha*. FIG. 23, Germination of pollen within anther sacs. FIG. 24, Antipodal tissue and free nuclear endosperm. FIG. 25, Disintegrated antipodal tissue and free nuclear endosperm.

The growth of the endosperm is much more rapid than that of the embryo. When the proembryo consists of four cells the endosperm consists of 28 free nuclei scattered in a thin layer of cytoplasm. Figure 22 shows the appearance of the antipodal mass at this stage. There are about 60 nuclei in this mass which is located on the lateral wall about midway of the sac. The cytoplasm of the embryo sac, endosperm at this stage, covers the inner surfaces only of the antipodal mass. At a later stage, when the proembryo consists of 8 cells and the endosperm of 73 free nuclei, the antipodal mass has assumed a lens shape and is definitely outside of the endosperm cytoplasm as shown in figure 24. The antipodal mass and the number of nuclei (56 in fig. 24) do not increase during or after fertilization. At a still later stage, when the embryo consists of 22 cells and the endosperm of 288 free nuclei, the antipodal mass is considerably flattened, the cytoplasm has largely disappeared and small scattered masses of chromatin are

all that remain of the nuclei. Thin cell walls are visible at this time so it is probable that cell walls existed in the antipodal mass at previous stages. This remnant of the antipodal tissue is definitely outside of the endosperm layer (fig. 25).

Discussion

Cleistogamy. The phenomenon of cleistogamy is common in the Gramineae (Chase, 1918). Uphof (1938) in reviewing the subject, devoted two pages to the grasses. In some species, as *Leersia oryzoides,* cleistogamous spikelets are normal in structure, and are produced in normal inflorescences. Other species produce normal chasmogamous spikelets in terminal inflorescences but cleistogamous spikelets in the axils of lower leaves (Chase 1918). Usually these axillary cleistogamous spikelets (cleistogenes of Chase and cleistogames of Uphof) are much reduced spikelets, so greatly modified that they would not be classified in the same genus or tribe if their origin were not known. It is in this group that *Stipa leucotricha* has been included by Hitchcock (1935) and Dyksterhuis (1945). Observations on plants studied in the region of Austin, Texas, in the spring of 1948 show that all stages of development up to milky endosperm take place while the spikelet is included in the upper leaf sheath. Normal anthesis in grasses takes place some time after the inflorescence has emerged from the sheath and so it would be reasonable to assume that an emerging inflorescence of *Stipa leucotricha* would consist of florets in preanthesis. It is here shown that the panicle spikelets were cleistogamous in the plants studied cytologically and those observed in the field in 1948. The presence of axillary cleistogenes in many of the plants studied was confirmed. It is significant that the two years 1947 and 1948 were severe drought years in central Texas whereas the spring of 1949 had more than average rainfall.

Uphof cites many cases in which environmental factors have been shown to produce cleistogamy in plants although no case is cited in the Gramineae. It is evident that some such factors, especially available soil water, are responsible for cleistogamy in *Stipa leucotricha,* since plants of this species that produced chasmogamous florets with open pollination in the spring of 1949, had been in 1948, completely chasmogamous. Nevertheless, even in 1949, there were cleistogamous florets scattered among the chasmogamous. The grass *Bromus catharticus* behaved much the same way and in both cases inflorescences produced late in the spring under dryer, hotter conditions contained a larger proportion of cleistogamous florets or were completely cleistogamous.

Reduction of flower parts, often correlated with cleistogamy, occurs in the florets of this grass. The awn of the axillary cleistogenes is greatly reduced but the condition of other structures has

not been studied. In the panicled florets the awn is normal in development but the three anthers are small, less than 1 mm. in length. Usually two of these anthers are smaller than the third and are sterile, producing no pollen (fig. 20). In a few florets one or both of these small anthers produced a few pollen grains in one or both of the anther sacs but this was unusual. The fertile anther itself may produce as few as eight pollen grains though usually more. Such small anthers are found in cleistogamous florets of other grasses; *Bromus catharticus, Triodia pilosa, Chloris andropogonoides,* etc., whereas chasmogamous florets of the same species or related species are much larger.

Embryo sac development. The development of the embryo sac of *Stipa leucotricha* is normal and typical of grasses. Reduction division takes place in the archesporial cell. Although the full course of meiosis was not seen, one early prophase stage has been drawn in figure 4. That reduction does take place in megasporogenesis is established almost conclusively. Figure 9 shows the metaphases of the second division in the embryo sac. In one of these the chromosomes are so spaced that nearly all can be counted. Eleven chromosomes plus a few (2 to 4) were found. In figure 11 the chromosome number could be estimated as between 10 and 15. This was true also in another embryo sac observed in nearly the same stage. Finally, the first division of the primary endosperm nucleus showed 34 plus a few chromosomes (fig. 21).

Double fertilization takes place normally. Brink and Cooper find that in *Hordeum jubatum* the antipodal mass at the time of fertilization occupies about one-quarter of the space in the embryo sac and that during the course of gametic union (two hours or less) this mass increases to over six times its former volume. This is not the case in *Stipa leucotricha.* At fertilization the antipodal mass never reaches a volume one-quarter of that of the embryo sac. Furthermore the antipodal mass of *Stipa* does not increase in volume at all during or after fertilization as Brink and Cooper report in *Hordeum.* In *Stipa* this mass reaches its full development shortly before fertilization and remains of the same volume until its protoplasm disappears. Shadowsky, Brink and Cooper, Schnarf and others have postulated a glandular or food transport function for the antipodals. In *Stipa* it appears more likely that protoplasm is formed and food materials are stored in this tissue during a period when there is no growth of any other structure within the embryo sac except perhaps some cytoplasm and a great deal of vacuole. Following fertilization, however, there is a slow growth of the embryo and a rapid growth of the endosperm. Much of the food for the rapidly growing endosperm is probably derived from the embryo sac cytoplasm, from the contents of the large vacuole and, in the case of grasses, from the antipodal tissue.

It is difficult to assign to the antipodal tissue of *Stipa leucotricha* the rôle of the conducting tissue between the ovule and the embryo sac, as Schnarf has done for angiosperms in general and Shadowski and Brink and Cooper have done for a number of grasses. The antipodal tissue is located on the side of the embryo sac adjacent to the funiculus but removed from it by many parenchyma cells, as Brink and Cooper also show in their figures. However, these authors state that Schnarf attributes a significant nutritive role to the antipodals. They quote three statements to this effect, among them, "the position of the antipodals at the base of the embryo sac, where in general the conducting tissue of the ovule terminates, points to the assumption that the incoming material must pass through the antipodal region." This hardly applies since in many grasses, *Hordeum jubatum* and *Stipa leucotricha* included, the antipodal mass is about midway of the lateral wall of the embryo sac and not at the base of the sac. This antipodal tissue may be glandular as the latter authors indicate but it is not proved. Certainly many plants form endosperm without this tissue, in fact, with no antipodals at all. It is true, however, that in *Stipa* there is not the rapid growth of the antipodals following fertilization. This may be a basic difference between this species and *Hordeum jubatum* for it is at that time, when the antipodals increase to a volume six times their former volume, that Brink and Cooper speak of these "activated antipodals" and "aroused antipodals" with secretory activity. The antipodals of *Stipa leucotricha,* then, are not glandular, as are those of the intergeneric cross of Brink and Cooper. Another difference between *Stipa* and *Hordeum* is that in *Hordeum,* as Brink and Cooper point out, "The antipodals are a prominent and presumably active tissue in normal *H. jubatum* throughout the period when the endosperm is free nucleate. They quickly decline when the rapidly develop- young endosperm becomes cellular." In *Stipa leucotricha,* on the other hand, there is nothing left but cell walls while the endosperm is still free nucleate. From the present study it seems most reasonable to conclude that the antipodal tissue is storage tissue, built up when there is little growth of other tissue within the embryo sac and then used as food by the rapidly growing endosperm.

Summary

The panicled spikelets in all plants of *Stipa leucotricha* that were observed at Austin, Texas, in the spring of 1948, were cleistogamous. These same plants produce axillary cleistogenes also. Of the three anthers produced in each floret, two are generally reduced and sterile while the third is reduced but produces good pollen.

Embryo sac development is of the normal, monosporic 8-nucleate type. The chalazal spore normally functions as the

embryo sac mother cell although the micropylar spore may do so occasionally. Commonly three "spores" are produced, the middle one then dividing transversely.

As is characteristic of the Gramineae, a large antipodal tissue is produced in the embryo sac. In this species the maximum number of nuclei is approximately 60. Cell walls could not be observed. The antipodal tissue reaches its maximum size before fertilization and does not increase in size thereafter. By growth of the embryo sac this tissue eventually comes to lie on the lateral wall next to the funiculus. During early stages of endosperm development this tissue lies outside of the endosperm cytoplasm. When the embryo consists of 22 cells and the endosperm of 288 free nuclei the antipodal mass has lost its protoplasm and only thin cell walls (not visible in earlier stages) remain. It appears that the antipodal tissue grows after maturity of the embryo sac when no development, other than the enlargement of the embryo sac, is taking place. After fertilization the antipodal tissue functions as stored food for the rapid growth of the endosperm.

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Literature Cited

Brink, R. A. and D. C. Cooper, 1944. The antipodals in relation to abnormal endosperm behavior in Hordeum jubatum × Secale cereale hybrid seeds. Genetics 29: 391–406.

———. 1947. The endosperm in seed development. Bot. Rev. 13: 423–541.

Chase, Agnes, 1918. Axillary cleistogenes in some American grasses. Am. Jour. Bot. 5: 254–258.

Cooper, D. C. 1937. Macrosporogenesis and embryo sac development in Euchlaena mexicana and Zea mays. Jour. Agric. Res. 55: 539–551.

Dyksterhuis, E. J. 1945. Axillary cleistogenes in Stipa leucotricha and their rôle in nature. Ecology 26: 195–199.

Hitchcock, A. S. 1935. Manual of the grasses of the United States. U. S. Dept. of Agric. Misc. Publ. No. 200. Washington, D. C.

Maheshwari, P. 1948. The angiosperm embryo sac. Bot. Rev. 14: 1–56.

Myers, W. M. 1947. Cytology and genetics of forage grasses. Bot. Rev. 13: 319–421.

Schnarf, K. 1929. Embryologie der Angiospermen. Berlin. Borntraeger.

Shadowsky, A. E. 1926. Der antipodiale Apparat bei Gramineen. Flora 120: 344–370.

Stebbins, G. L. and R. M. Love. 1941. A cytological study of California forage grasses. Am. Jour. Bot. 28: 371–382.

Uphof, J. C. Th. 1938. Cleistogamic flowers. Bot. Rev. 4: 21–49.

Yamaura, A. 1933. Karyologische und embryologische Studien uber einige Bambusa-Arten (vorläufige Mitteilung). Bot. Mag. Tokyo 47: 551–555.