CLEISTOGAMY AND THE DEVELOPMENT OF THE EMBRYO SAC IN LESPEDEZA STIPULACEA

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INTRODUCTION

Lespedeza stipulacea Maxim., commonly known as Korean lespedeza, has become important as a hay, pasture, and soil-conserving crop (4). Although numerous investigations have been made on the culture and utilization of this annual, no detailed studies concerning the morphology and development of the flower have been reported. Cleistogamous flowers were first noted in Lespedeza in 1840 (9) and invariably have been designated as apetalous in contrast to the petaliferous flowers of this genus. McKee and Hyland (6) have observed that the occurrence of petaliferous and apetalous flowers is common to most if not all species of the Eulespedeza and Microlespedeza sections. However, little has been known concerning the conditions that are associated with the development of each flower type. Successful cross-pollinations, varietal or specific, have not been reported in either the annual or the perennial species. More complete knowledge of floral structure and development is desirable before proceeding with a breeding program. The studies on L. stipulacea herein reported have been made to determine (1) the morphology of the two types of flowers, (2) the development of the cells of the embryo sac, and (3) the effects of some environmental factors on flower formation.

METHODS

HISTOLOGICAL TECHNIQUE

Some of the material for the histological study was collected from plants growing in the field during 1941, the rest from plants grown in the greenhouse during the fall and winter of 1941-42. The best fixation was obtained by dipping the buds momentarily in Carnoy's fluid and then transferring them to a modification of Bouin's killing fluid, designated as Allen-Bouin II. Good fixation was obtained when the buds were left in the latter fluid 3 weeks. The material was dehydrated in butyl alcohol and infiltrated and embedded in paraffin according to the butyl alcohol method described by Johansen (5). Longitudinal sections were cut 8μ to 10μ thick and mounted serially. A combination of safranine and fast green gave the best staining results. Heidenhain's iron-alum haematoxylin stain, counterstained with orange G, was also used with some success.

1 Received for publication November 13, 1942. Cooperative investigation of the Division of Forage Crops and Diseases, Bureau of Plant Industry, and the Missouri Agricultural Experiment Station. Submitted to the Department of Botany, University of Missouri, in partial fulfillment of the requirements for the degree of master of arts. Missouri Agricultural Experiment Station Journal Series No. 861.

2 The writer is grateful to Roland McKee and Dr. E. Marion Brown, of the Division of Forage Crops and Diseases, to Dr. E. E. Naylor, of the University of Missouri, and to Dr. E. R. Sears, of the Missouri Agricultural Experiment Station, for advice and assistance in this investigation.

3 Italic numbers in parentheses refer to Literature Cited, p. 272.
PROCEDURE FOR DETERMINING THE EFFECT OF TEMPERATURE ON FLOWERING

Plants of three strains of *Lespedeza stipulacea* were grown to maturity in the thermo-regulated growth chambers described by Brown (2). Observations were made on the kinds of flowers that were formed at different temperatures during the summer under natural conditions of light and during the winter when supplementary lights were used. During the latter period supplementary illumination was supplied, at first by 250-watt Mazda lamps and later by double-unit 40-watt daylight fluorescent lamps, to extend the natural photoperiod to 15 hours. When the plants were 10 inches in height, the photoperiod was reduced to 12 hours to induce floral initiation.

OBSERVATIONS

FLORAL MORPHOLOGY

**GROSS MORPHOLOGY**

The flowers of *Lespedeza stipulacea* are axillary and are formed on compound racemes. In any one axil, apetalous or petaliferous flowers or a combination of both may be found. The older buds are found near the base of the raceme, but as flowering progresses new buds are formed in the old axils; young buds and mature seeds are commonly found in the same axil.

The petaliferous flowers are typical of those of the Leguminosae; their general morphological features are represented in figure 1, A to E. Subtending the flower and adhering closely to the calyx are 3 bracteoles (A). The calyx consists of 5 undiverged sepals, terminated by 5 lobes, into which the corolla is inserted. All members of the irregular corolla are free except the 2 keel petals (C), which coalesce slightly along their anterior margins and surround the pistil and the stamens. The diadelphous stamens (B) are 10 in number; the undiverged filaments of 9 of them form a tube, while the tenth is separate.

The apetalous flowers are also complete but differ from the petaliferous flowers in external morphological features by a marked reduction of the corolla, stamens, and pistil (fig. 1, G-K). These parts are confined within the tightly closed calyx, which does not open until the developing pod forces it apart. The ovary appears normal, but the style is much reduced and bends downward during development (fig. 2, A) so that at maturity it assumes a hooked position with the stigmatic surface pressed directly against the anthers (fig. 2, B). Young seed pods from the two types of flowers can usually be identified by the old petal and stamen tissue, which is more persistent though less prominent, on the pods from apetalous flowers (fig. 1, L). After this tissue has been discarded the tips of the pods from apetalous flowers usually differ from those of petaliferous flowers by being more hooked or curved. Since the apex of the pod represents merely the base of the style, this point of difference is probably significant only as a means of identification. Pods from both types of flowers are single-seeded, flat, roundish, and reticulate. The average length of a mature apetalous flower is approximately 1.5 mm., that of a petaliferous flower 7.5 mm.

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4 F. C. 19604, F. C. 19601, and Del. 2591.
FIGURE 1.—A-E, Parts of a typical petaliferous flower, X 5: A, Nature flower; B, androecium and gynoecium; C, keel; D, wings; E, standard; F, immature fruit from petaliferous flower, X 5. G-K, Parts of typical apetalous flower, X 5 except H, which is X 7½: G, Nature flower; H, androecium and gynoecium; I, keel petals; J, wings; K, standard. L, Immature fruit from apetalous flower, showing remains of corolla, X 5. M, Section of anther of apetalous flower, showing penetration of pollen tubes through anther wall, X 325. N-T, Megagametogenesis: N, Young pistil with ovule, showing archesporial cell, X 325; O, older ovule, showing primary parietal and megaspore mother cell, X 325; P, midanaphase of heterotypic division; Q, megaspores; R, surviving megaspore; S, four-nucleate embryo sac; T, mature megagametophyte of apetalous flower (a, antipodal cells; en, primary endosperm nucleus; e, egg cell; s, synergid). P-T, X 670. U, Formation of campylotropous ovule, X 40.
FIGURE 2.—A, Photomicrograph of longitudinal section of immature apetalous flower, showing a portion of the hooked style and the shriveled condition of the pollen grains.  × 325.  B, Photomicrograph of later stage, showing contact of stigma and anthers, and germinating pollen grains.  × 375.
An abscission layer is found at the distal end of the pedicel; a slight manipulation of the flower usually results in the severance of the flower at this point.

**Pollination**

The anthers of the petaliferous flowers open during anthesis, shedding the pollen grains against the stigma, which extends slightly beyond the anthers. Generally the stigma is confined within the keel, reducing the possibilities of wind pollination. The pollen grains are well filled and uniform in appearance.

The anthers of the apetalous flowers are indehiscent, the pollen grains germinating within the closed anther sacs (figs. 1, M, and 2, B). Some of the pollen tubes so formed enter the stigma after penetrating the anther wall. Pollen tubes were observed in anthers touching the stigma as well as in those not in direct contact with this organ. The pollen grains of the apetalous flowers have both a generative and a vegetative nucleus. They are more or less shriveled (fig. 2, A), only the better ones germinating. Indications of collapse of the pollen grain wall were observed as early as the time when the megaspore mother cell was being differentiated.

Under normal field conditions approximately 76 percent of the petaliferous flowers formed seeds during the 1941 season. When potted plants of *Lespedeza stipulacea* were grown during the winter months, however, the fertility of the petaliferous flowers was much less than that of the same strains grown during the summer under field conditions. The apetalous flowers are highly fertile during both the summer and winter.

**Development of the Ovule**

The single ovule develops as a mound of meristematic nucellar tissue arising from the inner surface of the ovary wall near its base. The young ovule is orthotropous (fig. 1, N), but as it develops it curves toward the base so that at maturity it is campylotropous (fig. 1, U). Shortly after the differentiation of the megaspore mother cell the inner and outer integuments appear as two rounded outgrowths of the epidermis on one side of the ovule (fig. 1, O). Shortly after the integuments on the other side develop. When the megagametophyte is mature, both integuments enclose the nucleus except at the micropyle; the inner integuments are two cells thick throughout their entire extent, the outer more than two.

**Development of the Megagametophyte**

Soon after the appearance of the ovule an archesporial cell is differentiated in the second layer of cells from the apex (fig. 1, N). This slightly enlarged cell has somewhat denser cytoplasm than the surrounding cells and divides to form a parietal and a primary sporogenous cell. The former divides at least once, as is indicated by the fact that the older embryo sacs are usually embedded 2 or more layers within the nucellus. The primary sporogenous cell enlarges, differentiating into a megaspore mother cell (fig. 1, O). Figure 1, P, represents the migration of 10 chromosomes to each pole of the spindle during the anaphase of the heterotypic division of the megaspore mother cell. The nuclei resulting from this division are increased by the second
meiotic division to 4 megaspores (fig. 1, Q). The observed haploid chromosome number of 10 is consistent with the somatic number reported by Cooper (3). The chalazal megaspore is functional, whereas the other 3 megaspores rapidly degenerate (fig. 1, R). The newly formed nuclei of the first division of the surviving megaspore migrate to opposite ends of the embryo sac, where, by 2 more divisions, the usual 8-nucleate condition is formed. Three of the nuclei at the chalazal end develop cell walls, becoming the antipodal cells, which soon degenerate. The fourth nucleus migrates toward the center of the sac to a position not far from the egg apparatus, where it is joined by a nucleus from the micropylar end. They fuse to form the primary endosperm nucleus before fertilization occurs.

The three remaining nuclei at the micropylar end develop cell walls and differentiate into the synergids and an egg cell. Evidence of degeneration in the synergids occurs soon after the fusion of the polar nuclei. The mature megagametophyte of an apetalous flower consists of three degenerate antipodal cells embedded in the nucellus; two synergids, which have begun to degenerate; an enlarged pear-shaped egg; and a large primary endosperm nucleus (fig. 1, T).

The general appearance of the mature ovules of petaliferous flowers indicates that fertilization occurs at a later stage than in the apetalous flowers. The nuclei of the synergids are no longer visible and the antipodal cells have so degenerated that they are visible only as darkly stainable material. The entire ovule is larger and the embryo sac has farther invaded the nucellar tissue.

FACTORS AFFECTING FLOWER FORMATION

Observations made on three strains of Lespedeza stipulacea grown in thermo-regulated growth chambers showed that both the ratio of petaliferous to apetalous flowers and the total number of flowers formed are conditioned by temperature (table 1). Petaliferous flowers predominated at 80° F. in the summer experiments and at 82° and 95° in the winter experiments; apetalous flowers predominated at 70° in both the summer and winter experiments. At 60° flowering was limited to a few apetalous flowers. At the more favorable temperatures the total number of flowers per plant was too large to count; furthermore, the relative numbers of the two types varied so greatly on the different branches of the same plant that fractional sampling would not have been more accurate than general observations.

<table>
<thead>
<tr>
<th>Period</th>
<th>Temperature °F.</th>
<th>Petaliferous flowers</th>
<th>Apetalous flowers</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 15—Sept. 15, 1941</td>
<td>80</td>
<td>Abundant</td>
<td>Few</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>None to few</td>
<td>Abundant</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>None</td>
<td>Very few</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>Abundant</td>
<td>Few to many</td>
</tr>
<tr>
<td>Oct. 29, 1941—Feb. 7, 1942</td>
<td>82</td>
<td>Abundant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>Very few</td>
<td>Abundant</td>
</tr>
</tbody>
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It has been observed throughout these experiments that temperature is not the only factor involved in the determination of flower type. The amount of development that the plant has undergone when the reproductive processes are initiated affects the proportion of each flower type. For instance, plants grown without the aid of supplementary light during the period of the winter when the days were shortest became reproductive when they were only about 2 inches in height and produced few if any petaliferous flowers at temperatures of about 80° F. When plants grown during the same period were first exposed to a 15-hour photoperiod until they had grown to a height of 10 inches and to a 12-hour photoperiod thereafter, numerous petaliferous flowers were formed.

Light intensity and length of day probably are factors also affecting the relative numbers of petaliferous and apetalous flowers. Cloudy weather seems to retard the formation of petaliferous flowers. It is commonly known that apetalous flowers are more numerous during the winter months, even though artificial lights are used.

Finally, individual potted plants frequently differ from one another in respect to flower formation when grown under controlled temperatures and identical light conditions. It is believed that the nutrition of the plant may be so altered by conditions in the soil as to have a marked effect on the ratio of the two flower types formed.

DISCUSSION

An unusual method of pollination has been described for the apetalous flowers of Lespedeza stipulacea. Madge (7) and others have reported similar mechanisms of pollination in the cleistogamous flowers of Viola. Parks (8) found that the anthers of the closed flowers of Commelinantia pringlei (S. Wats.) Tharp dehisce but that most of the pollen remains in the anther cavity.

One of the significant results of this investigation was the demonstration that the proportion of petaliferous and apetalous flowers is conditioned largely if not entirely by environmental conditions. When one or more of the factors become unfavorable for the formation of petaliferous flowers there is a reduction in the number of flowers of this type. Response to such a change is not always definite; gradations exist from plants producing only apetalous flowers to those that are highly petaliferous. McKee and Hyland (6) found that Lespedeza cuneata (Dum. de Cours.) G. Don formed 75 percent of its seed from petaliferous flowers in 1939 and only 31 percent in 1940. Differences in light intensity and day length during the flowering seasons were considered possible factors in this variation. Some of the species investigated by these workers produced more seeds from petaliferous flowers; others formed the greater proportion of their seeds from apetalous flowers.

Some of the factors that condition flowering in Lespedeza stipulacea have been found to affect flowering in other groups of plants where cleistogamy occurs. Bergdolt (1) found a decrease of cleistogamous flowers with increased soil fertility in Viola. Vöchting (10) reported that with some plants, such as Stellaria media (L.) Cyrill. and Lamium purpureum L., chasmogamous flowers were formed under ordinary
light conditions, whereas under feeble light the flowers remained closed with the conspicuous flower parts imperfectly developed. Parks (8) reported that cleistogamous flowers of *Commelinantia pringlei* would open if exposed to light. The association of reduced light with the appearance of cleistogamous flowers is consistent with the writer’s observations in *Lespedeza*.

**SUMMARY**

*Lespedeza stipulacea* Maxim. bears two kinds of flowers, the apetalous and the petaliferous.

The apetalous flowers are highly fertile; the fertility of the petaliferous flowers is variable, depending on the conditions under which they were formed.

The pollen of the apetalous flowers germinates in the unopened anther sacs, and owing to the proximity of the stigma to the anthers, some of the pollen tubes enter the stigma after penetrating the anther wall.

By two meiotic divisions the megaspore mother cell forms a row of four megaspores. The chalazal megaspore gives rise to an eight-nucleate, seven-celled embryo sac. The two polar nuclei fuse before fertilization takes place.

The proportion of apetalous and petaliferous flowers is determined largely, if not entirely, by environmental factors. Certainly temperature is a factor, since flowering is predominantly petaliferous at 80° F. and apetalous at 70°. Other factors, such as the initial development of the plant at the time of floral initiation, light intensity, day length, and soil conditions, are probably involved also.

**LITERATURE CITED**


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