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CONTRIBUTION TO THE MORPHOLOGY OF *ALBIZZIA LEBBEK*

BY

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Introduction.

Albizzia lebbek (Bentham and Hooker), a member of the family Mimosaceae, is a good sized deciduous tree planted extensively in the Indo-Gangetic Plains in gardens, roadsides and other places. The natural home of the tree is hard to determine, but it is believed to be wild in the sub-Himalayan tracts, Bengal, Chota Nagpur, the Indian Peninsula, Burma, and the Andaman and Cocos islands (TROUP, **30**). The white fragrant flowers appear in March and April on new shoots with the unfolding of the new leaves. The temperature in the Indo-Gangetic Plains during these months rises as high as 95° F. and the humidity falls to a minimum of about 30 per cent. Hot dry winds beginning at about 11 a.m. and continuing till about 4 or 5 o'clock in the evening accentuate the aridity of the hot season, and exercise a powerful dessicating influence on the vegetation.

The number of flowers in an inflorescence is variable but usually there are 20–30 flowers borne on a long peduncle in a globose head. Several peduncles bearing inflorescences of varying ages are produced in the axil of each leaf. In each head the terminal or central flower is distinct from the others and more prominent by being slightly larger and swollen at the base due to the presence of a nectary (fig. 1). Each flower has a five-toothed calyx, a tubular corolla with five lobes, a large number of protruding stamens with greenish filaments and relatively minute anthers, and a central ovary with a long thin style and a small stigma. In one flower two carpels were found joined together at the base by their flat sides. The fruits are quite large by the end of June, though they take months to reach full maturity. Fruits a year or more old are frequently found hanging on the tree.

A very large number of the inflorescences drop off entirely about 12 to 18 hours after the flowers have opened, and of those that remain only one flower from a head usually proceeds to fruit formation. It was suggested by DR. WINFIELD DUDGEON that an investigation of the morphology of the plant would be desirable as it was very likely to give a clue to the cause of the sterility of the flowers. The work was started with the idea that :

1. Probably the central flower alone is functionally bisporangiate and produces fruit.

2. The lateral flowers are functionally male or sterile and are hence abortive.

3. There are gradations from the central flower to the lowest lateral flowers, and it would thus be possible to trace and discover the method by which the bisporangiate flowers have become functionally diclinous in *Albizzia lebbek*.

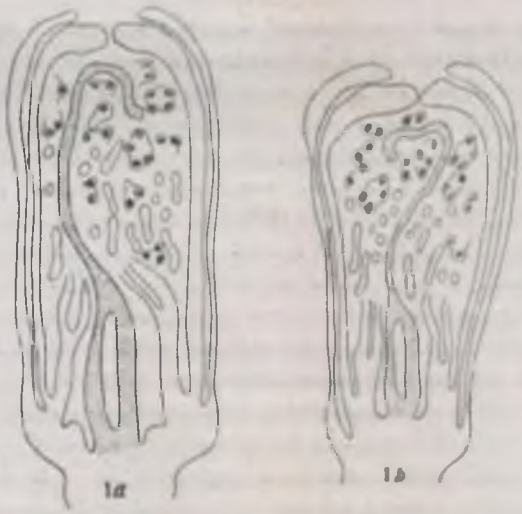

FIG. 1. a, b. Longitudinal sections of a terminal and a lateral flower. The terminal flower has a nectary at the base which is absent in the lateral. The ovary is stippled and the microsporogenous tissue cross-hatched for clearness. The filaments of the anthers have been cut in all directions. The stamens are monadelphous at the base. × 16.

This hope was however not fully realised. Indeed, it was subsequently found that it is not the terminal but one of the lateral flowers in an inflorescence that forms fruit. Widespread degenerations were, however, found in both the terminal and lateral flowers. If degenerations are the morphological cause of dichiny, then *Albizzia lebbek* has not proceeded far enough to give a very clear picture of the method.

According to the plan given here, the normal development is described first, then the degenerations, and finally a discussion of their importance and significance.

Previous Work.

A search through the available literature and reference works gives the impression that the Leguminosae have not received much morphological study in spite of their considerable economic importance. Among the earlier papers, the work of GUIGNARD (quoted in COULTER and CHAMBERLAIN, **6**) covering about 40 species of the Leguminosae is the most important. His account may be briefly summarised as follows. The archesporial cell divides to form the primary wall cell and the megaspore mother cell. The wall cell divides to form a wall tissue of varying thickness, usually large in the Mimosaceae and the Caesalpiniaceae. The megaspore mother cell produces an axial row of 2, 3, or 4 cells (megaspores ?) of which the innermost or the one next to it divides to form an eight-nucleate embryo sac. The antipodals persist till fertilisation in the Mimosaceae and the Caesalpiniaceae, but are usually shortlived in the Papilionaceae. The first division of the egg is transverse and further divisions may result in a massive proembryo. SAXTON (quoted in BROWN, **3**) described the embryo sac of *Cassia tomentosa* and found a deeply buried megaspore mother cell which forms the usual tetrad of megaspores. Of these the third gives rise to the embryo sac. He records an early fusion of the polars, and persistent antipodals. MARTIN in 1914 (**17**) published an account of the comparative morphology of *Medicago sativa*, *Vicia americana*, and 3 spp. of Trifolium. He noted a marked tendency toward sterility in the ovules of *Trifolium pratense* and found it to be related in some way to moisture conditions, since plants that were grown in the shade and were well-watered were completely sterile while those in dry places showed a varying percentage of fertile ovules. In a previous paper (**16**) he also called attention to the bursting of the pollen grains on the stigma of the same plant under moist conditions. Miss BROWN (**3**) in 1917 studied five varieties of *Phaseolus vulgaris*. She found that one of the daughter cells fails to divide and an axial row of only three megaspores is formed. The innermost megaspore functions and after three mitoses produces the usual eight-nucleate embryo sac. The antipodals disappear at the time of fertilisation and the synergids soon after. WEINSTEIN (**31**) in 1926 studied four varieties of *Phaseolus vulgaris* and found them to be closely similar to those studied by Miss BROWN. REED (**21**) in 1924 worked on *Arachis hypogea* and found the usual eight-nucleate embryo sac with ephemeral antipodals.

The embryo sac is filled with starch grains which are used afterward in the development of the endosperm. Sections of the anther showed that some of the pollen grains do not develop normally and are abortive. CASTETTER (4) in 1925 found vacuoles to play an important part in the cytokinesis in *Melilotus alba*. REEVES (23) studied *Medicago sativa* and found a many-celled archesporium and occasionally several linear tetrads in one ovule. In some embryo sacs, only one division occurs in the chalazal end so that the mature embryo sac is six-nucleate. The antipodals are ephemeral.

Material and Methods.

The first collections for this study were made in March and April, 1929. Various fixatives were used, but the best results were obtained with hot corrosive sublimate-formalin acetic acid-alcohol (CHAMBERLAIN, 5). The younger inflorescences were trimmed on both sides to secure easy penetration : in older inflorescences the flower buds were taken off singly and punched on one side; in flowers that were open or about to open the ovaries were dissected out and placed in the fixing fluid. The subsequent processes of dehydration and infiltration were as usual and the material was imbedded in paraffin of 56–58°C melting point. Considerable difficulty was experienced in sectioning the material, which was extremely refractory. Even after thorough infiltration and prolonged soaking of the imbedded material in water and all other precautions, the sections were invariably crumpled, badly scratched, and gave considerable trouble. Buds showing earlier stages were cut at 5–6 microns, and the older at 7 microns. Haidenhain's ironalum haematoxylin was used for staining. The short method (KAUFFMAN, 15) was found to give the best results.

As the results obtained from the first collections were not quite complete, another collection was made in the spring of 1930. The material was again fixed in corrosive sublimate-formalin-acetic acid-alcohol. For a greater part of the 1930 material, butyl alcohol was used in place of xylol, as it is said (ZIRKLE, 33) to have none of the hardening effects of the latter. The material was washed free from mercuric chloride in 50 per cent alcohol and then imbedded in paraffin after being taken through the butyl alcohol series. The results I obtained with this material were not appreciably superior to those with the usual xylol technique.

Normal Development.

MICROSPOROGENESIS.—The anther is first a mass of homogeneous cells which becomes four-lobed in cross section. The archesporial cells are rather late in differentiation and cannot be distinguished from the other cells till after a layer or two of wall cells has been formed.

Fig. 4 shows a part of a transverse section of a young anther with the archesporial cells at the lobes. There is a longitudinal row of two archesporial cells in each lobe and each of the cells divides once giving rise to four microspore mother cells. Fig. 5 shows a part of a transverse section of an anther with two microspore mother cells. The wall tissue beneath the epidermis is composed of the outermost layer or endothecium which develops the usual thickenings at maturity, usually two middle layers, and the tapetum. The tapetum attains its greatest conspicuousness at the beginning of the reduction division, but the cells always remain uninucleate (figs. 5, 6). This is in accordance with the observations of REEVES (**22**) who also found a uninucleate tapetum in the anthers of *Medicago sativa*. The filaments of the anthers enlarge considerably and as there is not enough space in the buds they are twisted in all directions. Thus in a single section of the flower one may get all views of the anther (figs. 1, a, b). The spore mother cells have a large nucleus with a prominent nucleolus. The cells are filled with dense cytoplasm which stains strongly. At synizesis the nucleolus lies in the middle of the chromatic knot and in favourable preparations the pairing of the chromosomes could be seen distinctly. The reduction divisions are passed through quickly and 16 microspores are produced. Fig. 6 shows the microspores in transverse section with the tapetum surrounding them. The middle wall layers have already disintegrated. The 16 microspores remain adhering together throughout the succeeding stages and form a single mass. Text-fig. 2a shows 6 of the 16 microspores; fig. 2b shows 12 of them in another view.

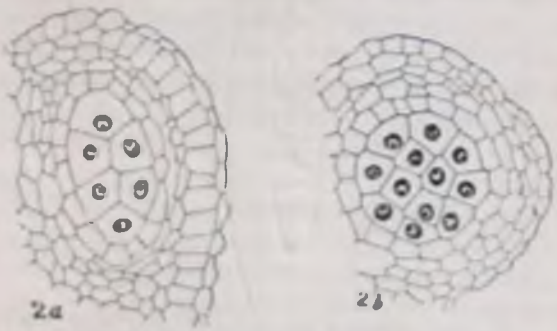

Fig. 2. a, b. Sections of one lobe of an anther showing two views of the microspores. × 320.

Because of their peculiar behaviour the microspores cannot completely round off as in most other plants. Only the outer wall is slightly rounded due to the release of pressure on this surface. The microspore nucleus divides (figs. 7, 8) and gives rise to the tube

and generative nuclei. A small generative cell is first cut off at the margin, but the wall soon dissolves and then the two nuclei lie free in the pollen grain (fig. 9). This is the shedding condition.

At the time of dehiscence the endothecium is the most prominent wall layer in the anther; the middle layers and the tapetum have already disintegrated and the epidermis is flattened out. The pollen grains escape in a mass of sixteen from each lobe of the anther.

MEGASPOROGENESIS.—The ovules are borne alternately in two rows on the two margins of the placenta. The number of ovules in an ovary varies considerably but usually it is 7-12. The young nucellus first arises erect and at right angles to the placenta, but when it approaches the dorsal wall of the ovary it begins to curve upward and all the ovules finally become half-campylotropous, curving toward the stigmatic surface, (fig. 3). At first they are

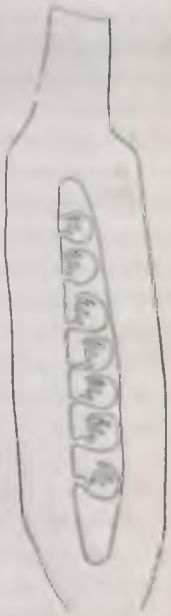

Fig. 3. Longitudinal section of an ovary, showing one of the two lines of ovules, × 20.

very much crowded but the ovary elongates rapidly and soon they become separated.

The young nucellus is a massive protuberance consisting of a group of homogeneous cells. A hypodermal cell enlarges as the archesporial cell (fig. 13). This divides into the primary wall cell and

the megaspore mother cell (fig. 14). The primary wall cell divide both anticlinally and periclinally to form a wall of about 6 or 7 layers in thickness (figs. 15, 16, 19, 20, 26). The nucelli in the Leguminosae have commonly been reported to be large, and according to several investigators a large number of wall cells are produced. Only two investigators, JOHNSON (1879) on *Lathyrus odoratus*, and GUIGNARD on *Orobus augustifolius* (quoted in DAHLGREN, 7) have reported absence of wall cells. Figs. 15, 16 show the young megaspore mother cells. Fig. 16 shows the appearance of the inner and outer integuments and the bending of the nucellus.

Usually there is only one archesporial cell as just described, but a number of young nucelli contained two and three, and in one case there were as many as five in a single nucellus. At first it was considered that these were merely ordinary cells of the nucellus which had attained greater conspicuousness for some reason, but further observations of more than one megaspore mother cell decided the point. Several archesporial cells have also been noted in the family by MARTIN in *Vicia* and *Trifolium*, BROWN in *Phaseolus*, and REEVES in *Medicago sativa*. This condition is quite common in the order Rosales.

The megaspore mother cell undergoes the usual synizesis and diakinesis stages and after the first reduction division a wall is laid down between the two nuclei. There is well defined interphase, and the daughter nuclei become well organised (fig. 17). Quite often the lower of the two daughter cells is slightly larger than the upper. The integuments are rather slow in their development and though the inner starts first, the outer grows faster and soon overtakes it. REEVES describes a similar situation in *Medicago sativa* where also the outer integument overtakes the inner. In both cases it is the outer integument and not the inner that covers up the nucellus and forms the micropyle.

The first reduction division is quickly followed by the second. Both cells usually divide at the same time (fig. 19), but sometimes the lower cell gets ahead of the upper. Fig. 18 shows the lower daughter nucleus in the metaphase of the second reduction division, while the upper is still undivided. Both the spindles usually lie parallel to the longitudinal axis of the cell, but the upper one may occasionally be slightly oblique (fig. 19) or even transverse to the first. In any case a tetrad of four megaspores is formed, all separated from one another by walls. Figs. 20 and 21 show a linear tetrad of megaspores. Fig. 32 shows a longitudinal section of a nucellus with a megaspore mother cell and a tetrad of megaspores in the same ovule. Both show signs of degeneration.

Out of the four megaspores produced the lowest functions and the upper three degenerate (fig. 22). Their degeneration seems to follow no fixed rule and any one of them may degenerate before the other two. Fig. 20 shows a tetrad in which the uppermost megaspore is already in an advanced stage of degeneration, while the other three are still sound. In fig. 32, the second megaspore from above has degenerated; in fig. 21, the two megaspores in the middle are degenerating; and in fig. 23, all but the lowest have degenerated. Such variations have been found to be quite common in this plant and are presumably due to the combined influence of the normal degeneration of the three distal megaspores, and the more serious degenerations of another type described later.

The proximal megaspore increases in size and the nucleus divides. The spindle has been observed to be slightly oblique to the longitudinal axis of the cell. Fig. 23 shows the daughter nuclei still lying close to each other. They soon move to the poles and the protoplasm becomes aggregated mostly in these regions leaving a big vacuole in the centre (fig. 24). In one case a feeble two-nucleate embryo sac was observed by the side of a megaspore mother cell (fig. 35). Apparently neither of these could get along well due to insufficiency of food and this may explain their degenerating appearance.

The two nuclei divide almost simultaneously to form four (fig. 25) which again divide to form the eight nuclei of the mature embryo sac, which is of the usual organisation (fig. 26). Due to the enlargement of the sac, the parietal and adjacent cells become flattened and crushed. The shape of the embryo sac varies, but usually the upper end is the broadest and the lower much narrower (figs. 26, 40). The egg apparatus is of the usual organisation. The synergids are hooked in some embryo sacs In embryo sacs that have waited long for fertilisation, the egg is sometimes unusually elongated and hangs down to a little above the middle of the embryo sac. The polar nuclei meet about the middle and remain together without fusing till fertilisation. The antipodals are quite conspicuous and begin to degenerate only after fertilisation.

Considerable amounts of starch were observed in a number of embryo sacs, but it was never found before the eight-nucleate stage. One slide was stained in iodine and it showed that starch grains are present in the adjoining cells of the nucellus also but they gradually decrease in number toward the outside. Even in fertilised embryo sacs with free endosperm nuclei but the egg still undivided, starch grains were present. REED also found an abundance of starch in the embryo sac of *Arachis hypogea* and his figures show that it is present as early as the 2-nucleate stage. REEVES also found an abundance of

starch in *Medicago sativa* from the beginning of the two-nucleate stage up to the development of the mature gametophyte. DAHLGREN (8) has recently published a summary of the literature on the occurrence of starch in the embryo sacs of angiosperms. He mentions that in general the content of starch in embryo sacs reaches its maximum shortly before fertilisation and from that time onward it is more or less rapidly consumed. JUEL (quoted in DAHLGREN'S paper) concluded from his studies on *Hippuris* that the occurrence of starch is due to delayed fertilisation, as the dissolved carbohydrates coming into the embryo sacs cannot be used and must be stored temporarily. SCHURHOFF (also quoted in DAHLGREN'S paper) also found large quantities of starch in old unfertilised embryo sacs of *Pelargonium zonale* and two spp. of *Geranium*, and gave a similar opinion. I cannot make any definite statement about *Albizzia lebbek* but it is probable that the same thing occurs here. I have never seen starch in embryo sacs of unopened flowers or even those that opened only an hour or two before the time of collection, while it was almost invariably present in varying amounts in flowers fixed the next morning. As I made no collections in the night, I am unable to say when the starch begins to appear.

POLLINATION AND FERTILISATION.—The flowers begin to open at about 4 P.M., are fully open by 5 or 6 P.M., and remain open all night. From about 7 A.M. the next morning, they begin to wither with the increasing heat and dessication, and large numbers fall off. The flowers emit a strong sweet smell during the night and early morning and bees have occasionally been found to visit the flowers for the nectar in the terminal flower. Bees may act as pollinators, but the adhering-together of the pollen grains in a mass makes pollen transfer rather difficult. Pollination by gravity seems to be equally likely. Out of a large number of inflorescences, some of the upper flowers are always erect, and the stigmas are slightly concave at the top so that a falling mass of pollen grains will fit in. That the pollination mechanism does not succeed very well is indicated by the fact that out of 2567 flowers examined on various days from 8–12 A.M., not more than 4 % of the stigmas had actually been pollinated.

In some plants it is known that failure of pollination is due to the unreceptivity of the stigmas. ROBINSOHN (quoted in SHOJI and NAKAMURA, **27**) used a mixture of sodium potassium tartarate 2.5 gms., silver nitrate 0.5 gms., and rain water 1400 gms., for comparing the receptivity of the stigmas. He treated the stigmas of the Compositae, Umbelliferae and other flowering plants and found that the non-functional stigmas were either not stained at all or at best took only a very light stain, while the functional stigmas were turned black. A

similar test was made on the stigmas of *Albizzia lebbek* using distilled water instead of rain water, to see if the stigmas were receptive and if so whether there was any difference between the receptivities of the terminal and lateral flowers. The test was made from the time of opening of the flowers to about 18 hours later. It was found that all the stigmas treated turned black and the degree of blackness was about the same. The test, if reliable, shows that in Albizzia the failure of pollination is not due to the unreceptivity of the stigmas. It would be interesting to try the same test on other plants of the family Mimosaceae, which have gone further toward the suppression of the female sexual organs in at least a part of their flowers.

Observation of stages in fertilisation is particularly difficult in a plant like this, because of the large number of degenerations to be described in the following pages and the uncertainty of pollination. It has not been found possible to tell from external observations whether a flower is perfect or in process of degeneration. In sections the small size of the mature embryo sac and the frequent presence of starch added further difficulties. Several collections were made at different times and a large number of ovaries were sectioned, but the required stages were not observed. However, from the presence of the pollen tubes in the micropyle and their remains in the upper end of the embryo sacs by the side of the young embryos, it may be inferred that fertilisation occurs.

ENDOSPERM AND EMBRYO.—Only a few of the fertilised ovaries were cut The fertilised egg divides late. In several preparations the primary endosperm nucleus had divided to form 6–8 free endosperm nuclei, but the egg was still undivided.

Though the plant blooms from about the middle of March to the middle of April or slightly later depending on individual trees, fruits are formed only toward the close of the flowering season. No fruits were observed on the trees kept under observation till the 7th of April in the year 1930. The reason why this should be so is not clear. It is possible that increased dryness favours the dehiscence of anthers and consequently increases the chances of pollination, STOUT (28) found an intermittent production of fruit in *Cleome spinosa*. He believes this to be due to the repeated morphological changes occurring in the flowers, *i.e.*, the plants pass through alternating periods when the flowers are bisexual or female to periods when they are only male. In the former case they produce fruit, in the latter they are sterile. No such phenomena have been observed in *Albizzia lebbek*. The earlier flowers and inflorescences are not morphologically different from the later ones.

Out of an inflorescence consisting of about 20–30 flowers usually only one or at most two or three flowers produce fruit. Only one case was found in which there were as many as five young fruits on a single peduncle. In most cases it is not the terminal flowers that produce fruit, but the lateral.

The actual number of seeds is often much less than the maximum that is possible. This is either because of the degenerations which may have been very serious in some of the embryo sacs, or of lack of fertilisation, or of both. Even the seeds already formed are badly attacked by insects and on a rough estimate only about 50 per cent of the seeds formed are viable.

Degenerations.

Degenerations in the anthers were observed only after the formation of the microspores. The earliest degenerations seen in the ovary were during the prophase of the megaspore mother cell. From this stage onward serious degenerations may be found at any stage, but are most frequent only after the formation of the tetrads. That these are real cases of degeneration and not artifacts due to poor fixation, is clear from the fact that other nuclei in the tissues beside these were perfectly normal and offered a strong contrast in appearance to the degenerating structures.

That degenerations are an important and significant feature of the life-history of this plant began to be clear very soon after the study was undertaken, but the expectation that they were to be found only in the lateral flowers, was not borne out. As in *Citrus* (OSAWA, **19**), *Daphne* (OSAWA, **20**), *Rumex crispus* (DUDGEON, **10**), *Prunus* (BRADBURY, **1**), *Rumex flexuosus* (JARETZKY, **14**), and *Asparagus officinalis* (SHOJI and NAKAMURA, **27**), they are characterised in their earlier stages by a decrease in the density and amount of the cytoplasm, and by an irregular outline of the nuclear membrane. Finally the cytoplasm and the nuclei both take a dense black stain and sink into an irregular structureless mass.

DEGENERATIONS IN THE ANTHERS.—As mentioned before, degenerations in the anthers have not been observed before the formation of the microspores. They are occasionally found after this stage. In earlier stages they are marked by an increasing vacuolation of the cytoplasm till it forms only a thin peripheral layer. In other cases the generative nucleus becomes uniformly granular and the nuclear membrane of the tube nucleus becomes obscure (fig. 10). In some cases it was observed that only the tube and generative nuclei took a homogeneous dense black stain while the cytoplasm still stained lightly. Frequently there are only a few dark

staining bits in the cytoplasm and the nuclei become entirely obscure (fig. 11). Finally in the most advanced cases the small amount of cytoplasm present is shrunken from the cell-wall towards the centre of the cell and stained so densely that the nuclei could not at all be distinguished (fig. 12).

DEGENERATIONS IN THE EMBRYO SACS.—Fig. 27 shows a megaspore mother cell in synizesis in the beginning of degeneration, as evidenced by the shrunken cytoplasm and the irregular outline of the nucleus. Figs. 28 and 29 show more advanced degenerations at still earlier stages, in which the cytoplasm has taken an intense almost uniform black stain with slightly lighter-stained strands attached to the cell wall. In fig. 28 the position of the nucleus can still be made out, while in fig. 29 the degeneration has advanced so far that a nucleolus is all that can be distinguished. Such cases of advanced degeneration in relatively young megaspore mother cells are indirect indications of the possibility of a beginning of degenerations at still earlier stages.

In most cases, however, the megaspore mother cell passes through the reduction divisions, and further degenerations are seen only after the tetrads have been formed. Fig. 30 shows an early stage of degeneration in a tetrad. Fig. 32 shows a linear tetrad and a megaspore mother cell in the same ovule; both are degenerating. One of the megaspores in the tetrad has already degenerated; degeneration in another is indicated by an irregular outline of the nuclear membrane and the aggregation of the chromatin in granular masses along the periphery of the nucleus. Fig. 31 shows a complete degeneration of all four megaspores with no trace of either the nucleus or the nucleolus. Fig. 33 shows a functioning megaspore in an early stage of degeneration; fig. 34 shows another at a more advanced stage.

At the two-nucleate stage, degenerations are rather frequent. Cases were observed in which either of the two nuclei showed signs of degeneration while the other was still normal. Fig 35 shows a feeble two-nucleate embryo sac by the side of a megaspore in the same ovule Here again the degeneration is evident by the aggregation of the chromatin granules along the side of the nucleus, and the small size of the sac. Figs. 36 and 37 show similar stages in degeneration An advanced case of degeneration is shown in fig. 38 where the two nuclei are almost indistinguishable and the cytoplasm has sunk into a structureless mass staining most deeply at the poles where the nuclei were situated.

At the four-nucleate stage degenerations are rather common. It seems that structures during the earlier stages of degeneration can continue development for some time. The degeneration, however,

slows down the development and finally entirely stops it. Fig. 93 shows an advanced case of degeneration at the four-nucleate stage in which all the four nuclei are merely densely staining masses with no trace of the original structure.

The most interesting cases of degeneration are met at the eight-nucleate stage. About 1,000 ovaries were cut and approximately 8–10,000 ovules were brought into review, but very few embryo sacs were found that may be called absolutely normal. There can be various combinations of degenerations. A number of cases were observed in which the two polar nuclei were lying in contact with each other but each showed signs of degeneration by its poor contents and the faint and irregular nuclear membrane. In some embryo sacs one of the polar nuclei was found to be at an advanced stage of degeneration, while the other was still much healthier (fig. 40). The antipodals usually hold out for a long time, though in some cases they are also involved early in the degenerations. It is very difficult to say which of the nuclei of the embryo sac is or are most likely to survive to the last. The egg is probably the last to be involved in a majority of cases, though fig. 41 shows an embryo sac in which the egg apparatus has undergone a complete break-down, while the polar nuclei are still in tact. Fig. 42 shows an embryo sac in which all the nuclei except the egg and the two polars have degenerated. Degenerations do not stop here, but may attack all the eight nuclei of the embryo sac (fig. 43).

Cases have been reported in other plants of degenerations in embryo sacs due to delay in pollination and a long wait for fertilisation. But the degenerations in *Albizzia* are not primarily due to this, for even unopened flowers show advanced degenerations. Figs. 42 and 43 have been drawn from preparations made from buds that would have opened only after about 8 hours from the time they were fixed.

INSECT ATTACKS.—The buds are often attacked by a species of Idiocerus, which bores holes through the perianth and deposits its eggs inside the flowers. The buds thus attacked wither and fall off very early. Whole inflorescences sometimes fall off because of holes bored by this insect in the peduncle. These degenerations are, however, quite different from those that have just been described.

Effects of Changes in Nutrition.

LEWITSKY (quoted in JARETZKY, **14**) was able to change the sex relations in the flowers of *Veratrum album* by interfering with food transporation by means of a cut in the stem; the lower flowers which usually have abortive anthers became normal bisexual, while

the upper, ordinarily bisexual, became functionally staminate. By suitable experimental conditions, SCHAFFNER (25) has been able to change the carpellate plants of *Arisaema triphyllum* into staminate plants and *vice versa*. Other investigators have also made similar experiments with interesting results. It was considered that if the degenerations in *Albizzia* are due to deficient nutrition, such experiments might give interesting results; it was expected that decreased nutrition would intensify the degenerations in both number and extent, and increased nutrition might promote healthier conditions To obtain a close insight into the plant and facilitate frequent observations, a scaffolding was built beside a large tree so as to keep a large number of branches within easy reach.

DECREASED NUTRITION.—An attempt to bring about a decreased food supply was made in the following ways:

1. Ringing of branches at different stages of development.
2. Removal of leaves while they were still very young and just preparing to unfold.
3. A combination of 1 and 2.

It has not been thought advisable here to discuss even briefly the theoretical possibilities of such experiments, and it does not seem necessary to do so since the results have not been very striking. There are some investigators who believe that the phloem cells are essential for food transportation, while others strongly affirm that upward translocation of food can take place with the water current. Still others believe that the cortex is the effective channel for pumping the water in an upward direction and hence according to them the ringing would disturb the ascent of water. It is enough to say here that in a general way the ringing of branches would tend toward decreasing the food supply going upward but only slightly disturb the water current: the removal of the leaves would deprive the plant of the newly manufactured food and limit its resources to the stored food that is being translocated upward. Both ringing and leaf removal should considerably intensify the effect as the resources of the plant would then be limited almost entirely to the food stored above the portion where the branch is ringed

It was found that in those branches that were ringed, the development of the buds was almost entirely arrested and the inflorescences dropped off without developing further. In only one case did the buds open, but the flowers produced were very much stunted in development and only about one-third of the usual size. The internal structure showed that in all cases the cells were very much smaller in size and had only small amounts of cytoplasm: the nuclei were much poorer in chromatin and smaller in size. This is plainly a

starvation effect and quite different in nature from the degenerations that have been described. Similar results seem to have been observed by other investigators. Thus SCHWARTZ (quoted in JARETZKY) found a considerable decrease in chromatin when his plants were placed in unfavourable external conditions, specially when they grew slowly, while in strong rapid growth the chromatin content was greater LEWITSKY (also quoted in JARETZKY'S paper) observed a total disappearance of chromatin in the root-tips of *Vicia faba* cultivated in poor soil and at a lower temperature.

In those branches from which the leaves were removed, it was found that the buds opened normally and showed no appreciable difference from the other flowers on the tree. Some of the flowers from these inflorescences produced fruit like the normal ones. This shows that a greater part of the food manufactured by the leaves is not used the same year, but is stored for next year's growth.

In those branches in which a combination of leaf removal and ringing was tried, the effects were the same as those produced by mere ringing, except that they were slightly more pronounced in some branches. The development of the buds was almost suddenly and completely arrested and none of the inflorescences opened.

INCREASED NUTRITION.—This was attempted in two ways:

1. A wooden box was taken and the lid and bottom removed. A big pan was filled with water and the box placed in it. A branch was then passed through a cut in the side of the box, which was finally covered with a glass plate to admit the light. The passage in the side was big enough to allow a free exchange of gases. Another box was fitted similarly and a branch inserted in it. This did not increase the food supply, but placed the branches in a more humid atmosphere and thus decreased the water loss to a considerable extent. More water was added in the pans every day to make up for the loss by evaporation. Collections were made from time to time. Unfortunately the branches were injured during a dust-storm, and broke off so that the results could not be followed to the end. The younger buds that could be collected and sectioned showed no appreciable differences from the normal buds.

2. In a number of cases the food supply available per flower was indirectly increased by decreasing the competition. From some branches a large number of inflorescences were carefully snipped off leaving a few, in others only one inflorescence was left on a branch, and in some cases all the flowers were removed from the inflorescences except one in each. The internal structure of these remaining flowers was found not to be appreciably different from the normal flowers, and degenerations were of about the same frequency.

These experiments indicate to a reasonable degree of certainty that neither excessive transpiration nor food deficiency is the primary cause of the degenerations. Indeed, in 1930, as already stated fruits were formed only toward the close of the flowering season, in April, when the temperature was *much higher* and the humidity *much lower* than in March. The decrease in the amount of chromatin when the branches were ringed is a natural enough response to an abnormal experimental condition, and seems to have little bearing on normal degeneration, specially as the type of degeneration in this case is different from that occurring under normal conditions.

Causes of Failure to Produce Fruits.

The following are the possible causes of the failure of the flowers to set seed in *Albizzia lebbek*:

1. Progressive degenerations in either stamens or carpels or both
2. Failure of pollination, due either to
   a. Failure of release of pollen mass, or
   b. Lack of pollinating agent and difficulty in the transportation of pollen, or
   c. Unreceptivity of the stigmas.
3. Failure of pollen tube growth and consequent failure of fertilisation.
4. Degenerations in the embryo.

From what has already been said, the degenerations are so widespread as to render most of the flowers functionless. Perfectly normal embryo sacs are rare, but it is possible that some of those in which degenerations have only begun might still get fertilised. It would be interesting to know what would be the result of fertilisation in an embryo sac otherwise normal but with only one healthy polar nucleus and the other degenerating.

Another important factor leading to sterility is difficulty in pollination. The sticking together of the pollen grains in a mass makes their transportation rather difficult. Out of the hundreds of flowers examined only about 4 per cent were actually pollinated. This combined with the degenerations in both the pollen grains and the embryo sacs makes the chances of normal development very rare.

If once the flowers are pollinated and the pollen grains and ovules are normal, there seems to be no hinderance in the growth of the pollen tube and fertilisation. It is possible that there might be degenerations in the young embryos also, but I have been able to cut very few of these and am not able to make any statement in this respect.

Discussion.

There are a number of plants in which many or even all of the flowers are known to be sterile. The causes of this may not be purely morphological or cytological, but may be due to or influenced by physiological or ecological conditions. They are usually discussed under four heads: environment, hybridisation, mutation, or orthogenesis.

ENVIRONMENT.—There is a number of plants in which sterility has been shown to be due to the influence of external factors. MUCKE (18) in 1908 found that *Acorus calamus* is always sterile in parts of Europe, while it freely produces fruit in the hotter parts of East Asia. He observed degenerations in the earlier stages of development in both the pollen grains and embryo sacs and concluded that they were due to unfavourable climate. MARTIN found a strong tendency towards sterility in the ovules of *Trifolium pratense* and found it to be related to moisture conditions. SCHURHOFF (26) found degenerations in the pollen grains of *Eichhornia speciosa* and thinks it to be a pathological condition due to unfavourable climate. STOW (29) working on several varieties of potato concludes that sterility is due to abnormalities in the reduction divisions resulting in abortive pollen grains. He finds that this is mainly caused by higher temperatures, for at lower temperatures (between 15 and 20 degrees C.) the reduction divisions are passed through normally and fertile pollen grains are produced. BORGENSTAM on *Syringa* and MICHAELIS on *Epilobium* (quoted in JARETZKY, 14) found that in the reduction divisions in the microspore mother cells there is a formation of many small nuclei. This they attribute to greater cold. While examples of this kind can be multiplied it seems pretty clear that environment plays an important part, and that at least quite a number of cases of sterility in plants is due to ecological influences. This does not, however, apply to *Albizzia* for reasons already given.

HYBRIDISATION.—Sterility in hybrids is quite common and well known. This is regarded as the result of an inharmonious blending of the characters of dissimilar parents which leads to a weakness of the association of the synaptic mates, and results in irregularities in gamete formation. The fact that most of the cases of abortive pollen grains have been reported in hybrids lends considerable support to this view. No indications of abnormalities in reduction divisions have, however, been noted in *Albizzia lebbek*.

MUTATION.—Transitions from the hermaphrodite to the monoecious condition in several families of the flowering plants have been attributed by some authors to mutation. It is possible that this

transition may always be bound up with degenerations. JARETZKY (14) believes this to be the case in *Rumex flexuosus*, *R. crispus*, and *R. maritimus*.

ORTHOGENESIS.—DUDGEON (10) from his studies on degenerations in *Rumex crispus* concludes that these are not necessarily related to deficient nutrition, or hybridisation, or mutation, but may be a hereditary tendency leading toward dicliny.

That degenerations may not at all be due to environment or hybridisation is shown by many facts, though it is probable that they may be influenced in their frequency, intensity, and course by environmental factors.

In the Tubiflorales there is a distinct tendency towards the suppression of at least one stamen, and it is well exemplified in the family Bignoniaceae, where there is almost constantly one staminode in addition to four perfect stamens.

In the Compositae the ray florets are quite often functionally carpellate and may not have even the rudiments of the stamens. In some species even the carpellate function is lost so that the ray florets serve merely for show.

A large number of plants of the family Anacardiaceae show degenerations in either stamens or carpels or both. In *Mangifera indica* (DUDGEON, 11) only one stamen develops normally, and the remaining four usually fail to differentiate the sporogenous tissue. Degenerations in the carpels may begin as early as the megaspore mother cell stage. As a result of these degenerations only a few flowers are physiologically bisporangiate, and these are towards the apex of the inflorescences. The lateral flowers are physiologically staminate. Toward the close of the flowering season, the flowers are often completely sterile and fall off in large numbers. In *Buchanania* and *Spondias*, out of the five carpels present only one is fertile and the remaining four sterile, presumably due to degenerations. Several species of the genera *Rhus*, *Pistachia*, and *Odina* have attained almost perfect dicliny, the male flowers have either a mere rudiment of an ovary or none, and the female flowers have only staminodes or even these might be absent.

In the family Mimosaceae itself there are several species of the genera *Parkia*, *Dichrostachys*, *Calliandra*, *Pithecolobium*, *Mimosa*, *Acacia*, *Neptunia*, and others in which the flowers are polygamous. *Dichrostachys cinerea* has long axillary spikes in which the upper flowers are fertile and yellow, while the lower are sterile with purple or rose-coloured staminodes (BRANDIS, 2). In some species of *Parkia* only the upper flowers in a head are bisexual, while the lower are male or sterile. In *Neptunia oleracea*, described by GEITLER (12),

the upper flowers are bisexual with the usual mimosacean characters, while the lower are morphologically sterile. The ovary in the latter is extremely reduced, and the 10 stamens are transformed into yellow petaloid staminodes. Between the two are transitional types with various intergradations. To the best of my knowledge, there has, however, been no morphological study with special reference to degenerations on any of these forms.

The tendency towards suppression of some of the sex organs occurs in places throughout the angiosperms, and YAMPOLSKY (39) has brought together a long list of such plants.

The bisporangiate flower has been regarded as characteristic of primitive angiosperms by several botanists. HUTCHINSON (13) has recently put forward a strong plea for the monophyletic origin of angiosperms and regards the Ranales and Magnoliales as the basal types from which the unisexual types have been derived by reduction. SCHAFFNER (24) says that there are three progressive steps of advancement of the angiosperm series in the line of sex determination: bisporangiate flowers, various degrees of monoeciousness, and finally dioeciousness. On the other hand, there are others who think that bisporangiate flowers are not necessarily primitive, but may be evolved from the unisexual condition. It is probably unnecessary to discuss here the relative merits of these views. There is a growing tendency among botanists today to regard the angiosperms as polyphyletic in origin, and it then becomes possible to recognise several distinct lines of evolution. While it is not safe to make any general statement, it seems clear that at least some flowers have become diclinous by a degeneration of one or the other set of sexual organs, and finally their entire elimination.

As mentioned before, it was expected at the beginning of this investigation that the terminal flower alone in *Albizzia lebbek* would be found to be functionally bisporangiate, and that the lateral flowers would be functionally staminate or sterile. It soon became clear that this expectation was wrong, for the degenerations are about equally common in both the terminal and lateral flowers. The reason why it is usually one of the lateral flowers in a head that forms fruit, apparently is because of their large number and consequently greater chances of pollination.

It is as yet difficult to say what is the significance of the degenerations in *Albizzia*. Probably they bear no casual relation to nutritional disturbances or hybridisation, but represent an inherent tendency towards an elimination of one or both of the sex organs in some flowers. The instability of the degenerations merely indicates that this tendency has not yet progressed very far in this plant.

A comparative investigation of other plants of the family probably would serve to clear the point, and I hope to continue this work.

In conclusion, I wish to express my gratitude to Dr. WINFIELD DUDGEON, under whose directions this work was carried out, for help and encouragement in various ways.

Summary.

1. *Albizzia lebbek*, a member of the family Mimosaceae, is a commonly planted tree in many parts of India.

2. The flowers are grouped in heads of about 20–30, which are borne on long peduncles in the axils of new shoots. The central flower in each head is distinct from the others by the presence of a nectary.

3. Most of the inflorescences fall off entirely, only about 12–18 hours after blooming, and of those that remain only one or two flowers in an inflorescence produce fruit.

4. The differentiation of the sporogenous tissue in the anthers is rather late. Sixteen microspores are produced in each lobe and these stick together in one mass. The tapetum is always uninucleate.

5. The microspore nucleus divides to form the tube and generative nuclei. This is the shedding condition.

6. There is usually a single archesporial cell in the ovule, but occasionally two or three and rarely more may be found. The primary wall cell divides to produce a six to seven-layered wall.

7. The megaspore mother cell undergoes the usual reduction divisions to form a linear tetrad of megaspores. The lowest megaspore functions.

8. The embryo sac is eight-nucleate with the usual organisation. The antipodals are conspicuous and the polars fuse only at the time of fertilisation.

9. Degenerations are met with in both the terminal and lateral flowers and are characterised in their early stages by a vacuolation of the protoplasm, indistinctness of the nuclear membrane, and a collection of the chromatin at the periphery of the nucleus. In the most advanced cases the nucleus and the cytoplasm take an intense black stain and sink into a structureless homegenous mass.

10. Degenerations in the anthers are met with only after the formation of the microspores. In the ovules they may occur at any stage beginning with the megaspore mother cell, but are commonest at the four and eight-nucleate stages. Any or all of the nuclei of the embryo sac may be involved.

11. The failure of such a large number of flowers to set seed is due partly to the widespread degenerations and partly to a failure of the pollination mechanism.

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Explanation of Plates.

All figures have been drawn with the aid of a camera lucida. Figs. 4 to 43 were drawn at an initial magnification of × 1600.

Figs. 1–3 are in the text.

Fig. 4. Transverse section of part of an anther showing two archesporial cells.

Fig. 5. Transverse section of part of an anther showing two microspore mother cells in one lobe.

Fig. 6. Same, showing the microspores surrounded by the uninucleate tapetum.

Fig. 7. Microspore nucleus in metaphase.

Fig. 8. Same; late anaphase.

Fig. 9. Mature pollen grain with the tube and generative nuclei.

Fig. 10. Pollen grain in degeneration; the generative nucleus is uniformly granular and the nuclear membrane of the tube nucleus is very indistinct.

Fig. 11. Pollen grain in degeneration.

Fig. 12. Same, more advanced stage; the nuclei and the cytoplasm have taken an intense black stain.

Fig. 13. Longitudinal section of the nucellus showing the archesporial cell.

Fig. 14. Archesporial cell divided into primary wall cell and megaspore mother cell.

Fig. 15. Young megaspore mother cell.

Fig. 16. Megaspore mother cell with nucleus in early prophase; the integuments have begun to develop.

Fig. 17. First reduction division; megaspore mother cell divided into two daughter cells.

Fig. 18. Second reduction division; lower cell in metaphase, upper still undivided.

Fig. 19. Second reduction division; both nuclei in telophase.

Fig. 20. Tetrad of megaspores; the uppermost has already degenerated.

Fig. 21. Another tetrad; the two megaspores in the middle are degenerating.

Fig. 22. Same, with the functioning megaspore enlarging.

Fig. 23. Young two-nucleate embryo sac.

Fig. 24. Older two-nucleate embryo sac.

Fig. 25. Four-nucleate embryo sac.

Fig. 26. Mature embryo sac; two of the antipodals have already degenerated, and the polars also show signs of degeneration.

Fig. 27. Megaspore mother cell in synizesis, beginning to degenerate.

Fig. 28. Megaspore mother cell ; advanced degeneration.

Fig. 29. Same ; in a still more advanced stage of degeneration.

Fig. 30. Tetrad of megaspores in an early stage of degeneration.

Fig. 31. Tetrad of megaspores in advanced stage of degeneration.

Fig. 32. Nucellus showing megaspore mother cell and a tetrad lying side by side ; both are degenerating.

Fig. 33. Functioning megaspore in an early stage of degeneration.

Fig. 34. Same ; more advanced stage.

Fig. 35. Megaspore mother cell and two-nucleate embryo sac in same nucellus ; both are degenerating.

Fig. 36. Two-nucleate embryo sac, degenerating.

Fig. 37. Same.

Fig. 38. Two-nucleate in an advanced stage of degeneration.

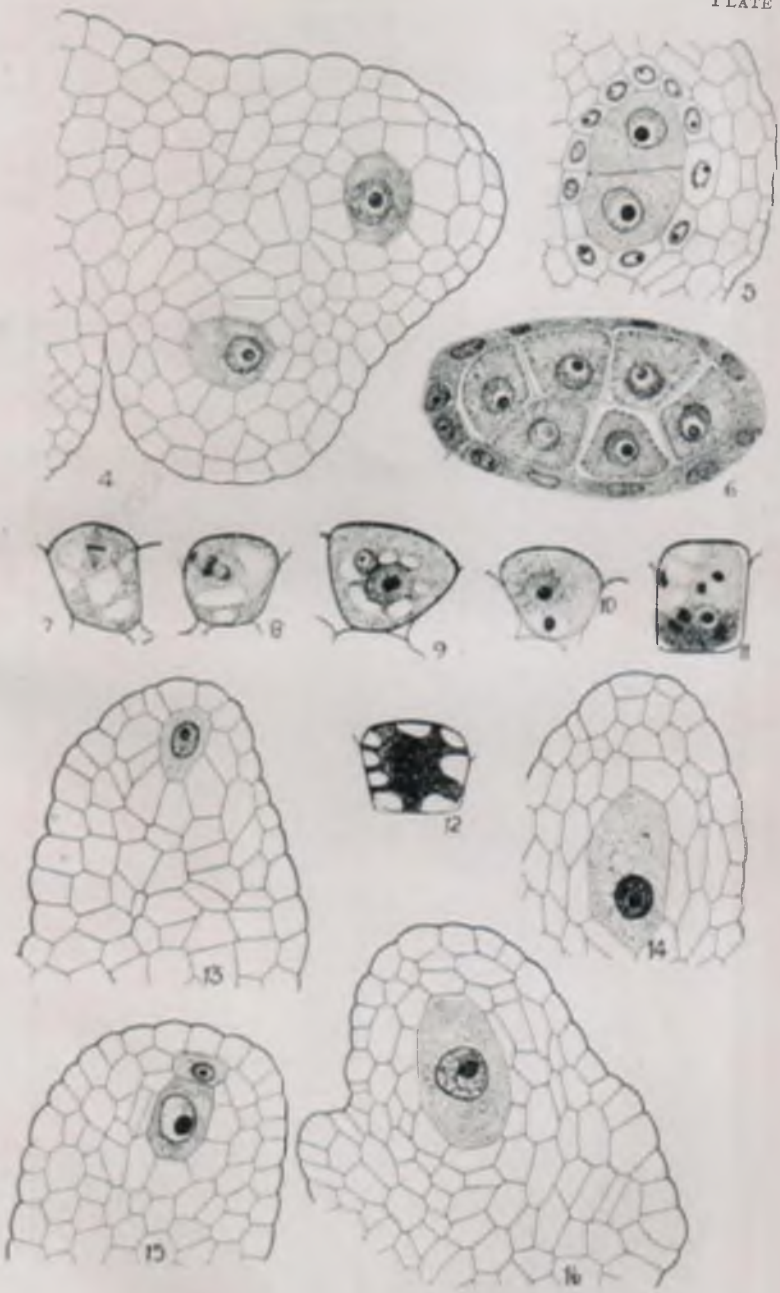
Fig. 39. Four-nucleate embryo sac in an advanced stage of degeneration.

Fig. 40. Mature embryo sac ; one of the polar nuclei is stained densely and is degenerating.

Fig. 41. Upper part of a mature embryo sac showing complete break down of the egg apparatus.

Fig 42. Embryo sac, showing degeneration of the synergids and the antipodals.

Fig. 43. Embryo sac, showing degeneration of all the nuclei.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