STUDIES OF DIOSPYROS KAKI. I

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 209

KONO YASUI

(WITH PLATES XII AND XIII AND ELEVEN FIGURES)

Although the Ebenaceae are recognized as a primitive family of the Sympetalae and possess several remarkable features, the only morphological investigation of them has been that of Miss HAGUE[1] on *Diospyros virginiana*. My purpose in undertaking the investigation of *D. Kaki* was to answer the following questions: (1) Are the Ebenaceae really primitive among the Sympetalae? (2) Are there cytological differences among the so-called garden varieties of *D. Kaki?* (3) How are the garden varieties related to one another? (4) How have they been obtained?

Material and method

D. Kaki is in very common cultivation in Japan and is represented by many so-called garden varieties. The fruits of these varieties differ in shape, size, and flavor, and also in their seeds. Each variety has also its characteristic flowers, leaves, etc., to which horticulturists paid little attention in classification, as compared with the differences in fruits and seeds. For example, the horticultural classification recognizes two principal divisions, dependent upon flavor of the fruit, as follows: (1) astringent kaki (shibu-kaki), and (2) sweet kaki (ama-gaki).

Each of these two divisions is divided into four or five groups, based upon the shape of the fruit, as follows. The astringent group contains (*a*) long, (*b*) round, (*c*) square, and (*d*) flat types; while the sweet group contains (*a*) gosho, (*b*) long, (*c*) round, (*d*) square, and (*e*) flat types.

In her report on *D. virginiana*, Miss HAGUE makes the following statement: "So far as the trees from which material was collected

[1] HAGUE, S. M., A morphological study of *Diospyros virginiana*. BOT. GAZ. **52**:34–44. *pls. 1–3.* 1911.

were observed, they were dioecious, and bore only perfect flowers. One possible exception has been found recently." In *D. Kaki*, on the other hand, there are many such exceptions, as follows.

1. In some varieties only the pistillate flowers occur in good condition, while all the staminate and some of the pistillate flowers are imperfectly developed.

2. In other varieties (gosho, egosho, yamagaki, jenjimaru) both kinds of flowers are always well developed.

3. A tree in Mr. YABE's garden in Tokyo (no. 1 of my material) has the same type of fruit as in some of the varieties in group 1, but has pistillate, staminate, and perfect flowers, and produces also two types of fruit from the two kinds of flowers.

4. No tree has been discovered by the writer on which only staminate flowers occur.

5. Some varieties have pistillate flowers only, when the trees are young, but as they become older or weaker (on account of poor nutrition or transplanting) they begin to produce staminate flowers also (hyakume-gaki).

6. Some trees show an alternation of fruiting and non-fruiting years, which means that during one year they produce many pistillate flowers and many fruits, while during another year they produce a few pistillate flowers and abundant staminate flowers. Under the latter conditions they bear very little fruit, or no fruit at all.

It may be added, in reference to the fourth condition referred to, that in Japan the staminate trees are being continually destroyed unconsciously, because the better varieties are produced always by grafting, and therefore many seedlings of staminate plants are destroyed, and pistillate plants take their place. The foregoing data gave me the suggestion that *D. Kaki* is naturally a monoecious plant (fig. 1), and that it is in process of losing this character, producing staminate flowers under cultivation.

I have selected five different varieties from one hundred or more for this study, as follows.

No. 1.—I am not certain of the name of this variety, but it resembles "yemon," which is an astringent, flat type. The difference is that yemon has no staminate flowers at the Okitsu station, but this form has three kinds of flowers upon the same individual.

No. 2.—Tenryubo, which is a sweet, round type. It has no staminate flowers at the Okitsu station. The fruit is rather small and has many seeds.

FIG. 1.—Branch of Tsuru-no-ko (one of the varieties of *D. Kaki*), showing the vigorous upper branchlets with pistillate flowers and the lower small branchlets with staminate flowers; $\times \frac{1}{2}$.

No. 3.—Jenjimaru is a sweet round type; but in this case there are always staminate flowers. The fruit is small and with many seeds, as in no. 2.

No. 4.—Tanenashi (meaning seedless) is an astringent square type. It has no staminate flowers and is generally seedless.

No. 5.—Fuyū is a sweet, gosho type. Usually it has no staminate flowers, but occasionally a very few may be produced. Occasionally the anthers of the perfect flowers contain a number of pollen grains, which germinate well on the stigma and in nutrient fluids.

I have also examined many other specimens, but the main results of the investigation were obtained from these five. The material of no. 1 was collected in Tokyo, but the others were obtained from the experiment station at Okitsu, in Central Japan. The material was fixed with Flemming's weaker solution, and chromo-acetic acid mixture was also used, giving better results than the former medium. The sections were stained with Flemming's triple stain and Haidenhain's iron-hematoxylin.

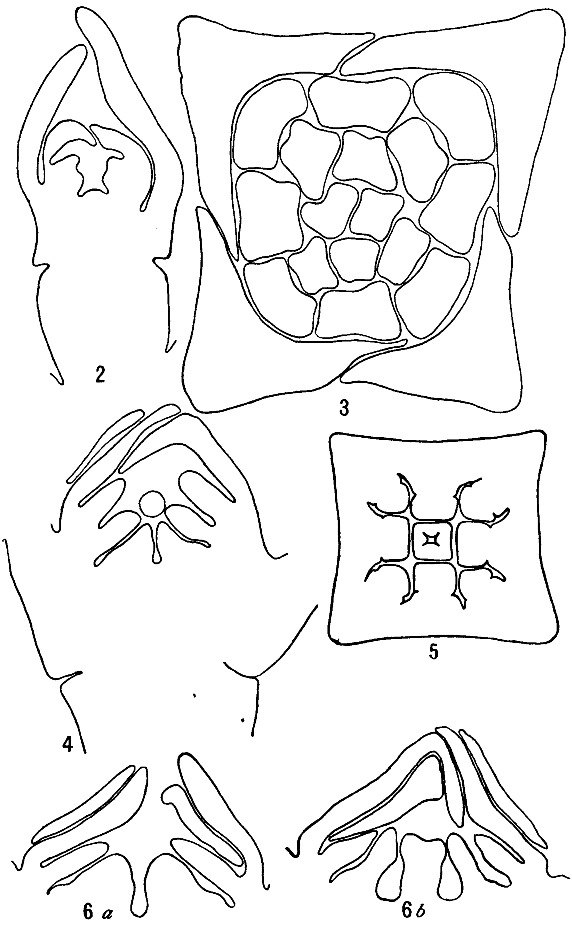
Development of staminate flower

The cluster of staminate flowers appears on young shoots in early spring. It consists usually of three flowers (fig. 1), but sometimes includes 7 or more. The very young shoot bends downward, and 2–4 of the lower leaves have nectaries on the under surface, near the base. The succession of members is strictly centripetal. The calyx is valvate, but the four petals are spirally arranged (fig. 3). The 16 stamens occur in four cycles (fig. 3), and appear in pairs from the earliest stage (fig. 2). The mature stamen has a very short filament, and the connective extends above the anther. The pistils complete their development very early. A few flowers were found which were 5-merous instead of 4-merous.

Development of perfect flower

The perfect flower always occurs at the top of the cluster. It has the same arrangement of members as the staminate flower when young (fig. 4), and the pistils of the perfect flower are smaller than those of the pistillate flowers. Occasionally they have less than 8 ovules, which is the ordinary number in the pistillate flowers, and sometimes they have no ovules. Abnormalities occur, not only in the number of ovules, but sometimes in the irregular forms of the pistils themselves. The fruit of the perfect flower is always smaller than that of the pistillate flower, and usually it has no seeds.

The foregoing facts suggested that the perfect flower is an abnormal one, produced by favorable conditions of nutrition; and that therefore it does not indicate a general tendency.



FIGS. 2–6.—*D. Kaki:* fig. 2, longitudinal section of a young staminate flower of no. 1; fig. 3, transverse section of a young staminate flower of no. 1, with calyx removed; fig. 4, longitudinal section of a perfect flower of no. 1, with calyx removed; fig. 5, transverse section of the lower part of a pistillate flower of no. 4, showing the 8 anthers and a pistil; fig. 6, flower of no. 4: *a*, radial section of a young pistillate flower through the two large opposite protrusions; *b*, longitudinal section through the two initial papillae on the large protrusion.

Microspore formation

There is no marked difference between the primary sporogenous cells and the primary wall cells before the latter begin to divide. Two periclinal divisions give rise to three outer wall layers and a tapetal layer. Two or three successive divisions separate the primary sporogenous cells from the mother cells. At the same time the uninucleate tapetal cells become very distinct between the sporogenous tissue and the wall layers, the latter of which become more and more flattened.

During the comparatively long resting period, the spore mother cell increases in size, its protoplasm becomes denser (but not so dense as that of the tapetal cells), and the nucleus becomes large (pl. fig. 1). The nucleus contains rather a small amount of chromatin granules associated with the fine linin network, and also a remarkably large nucleolus and several small ones (pl. figs. 2, 3).

In some cases I observed the larger chromatin granules appearing in pairs, but this did not seem to be the usual situation. The reticulum is denser at the outer part of the nuclear cavity than within. During presynapsis certain connections between the chromatin granules disappear, so that the reticulum gradually becomes simpler in structure. At the maximum of synapsis the chromatin substance appears like a mass of granules (pl. figs. 4 and 5).

There is no morphological connection between the chromatin reticulum and the nucleolus, although they seem to occur in very intimate relationship. A great many nucleolus-like bodies were observed close to the chromatin thread, near the large nucleus, but in other cases there was no such relationship (pl. fig. 4). In the presynapsis stage the double nature of the chromatin thread is not clear, but it becomes gradually evident with the loosening of the thread (pl. figs. 6, 7).

Before 1895, when MOORE called attention to the synapsis stage as an important period in the history of the nucleus, this stage was ignored. Since that time it has attracted chief attention, and is regarded in general as an important event in the history of the nucleus. Some investigators, however, do not regard it in this light. For example, LAWSON[2] makes the following statement.

> During their development, however, there is a great accumulation of sap within the nuclear cavity, which causes a great osmotic pressure. The pressure, acting from within, causes the nuclear cavity to expand. This expansion continues until the nuclear cavity grows to twice or even three times the original size. As the growth proceeds the membrane is gradually withdrawn from the chromatin mass within. The result of this withdrawal of the nuclear membrane is the formation of a large clear area of nuclear sap containing the mass of chromatin which has been left at one side. No evidence whatever was found to show that any contraction of the chromatin had taken place.

In my material of *D. Kaki* I could not recognize such a remarkable enlargement after the chromatin reticulum began to separate from the nuclear membrane. The great enlargement of the nucleus occurs in the resting stage, so that there is no conspicuous difference in the size of the nucleus at the synapsis stage and at the end of the resting stage, as LAWSON indicates.

The synapsis stage is a normal occurrence, and during it there is some rearrangement, but not fusion of chromatin bodies, which results in the spireme stage. At the beginning of the spireme stage the thread is distributed in the outer cavity of the nucleus, where thickening and shortening occur, resulting in the diakinesis stage (pl. figs. 6, 7, 8). There is no difference in the size of the pairing chromosomes, which are usually parallel, but sometimes X or V-shaped. At the same time there appear in the cytoplasm numerous fibers which penetrate the nuclear cavity and become attached to the chromosomes by one end, in connection with which the nuclear membrane begins to disappear. By the elongation of the fibers the chromosomes, which have been distributed on the surface of the nucleus, are drawn into the center of the nuclear cavity, and there are arranged in an equatorial plate by the gathering of the free ends of the fibers at the poles (pl. figs. 9, 10, 11). The nucleolus disappears entirely during metaphase.

The number of chromosomes is difficult to count by reason of their small size. Usually there are 28 in pairs, but sometimes

[2] LAWSON, A. A., The phase of the nucleus known as synapsis. Trans. Roy. Soc. Edinburgh **47**:591–604. *pls. 2.* 1911.

27 in no. 1 (pl. fig. 11). In *D. virginiana* Miss HAGUE counted 30 or more.

The longitudinal division of the chromosomes in preparation for the homotypic division occurs in metaphase, and at telophase the chromosomes become arranged in the spireme and spread over the outer cavity of the daughter nucleus. The number of chromatin granules is not the same as the x number of chromosomes; and there appear two or three nucleoli in the daughter nucleus (pl. fig. 12, *a*, *b*). The homotypic division of the microspore mother cell occurs in the usual way. The two axes of the spindle fibers of this division are not in any definite relation to one another (pl. figs. 13, 14). In wall formation the microspores are in tetrahedral arrangement (pl. figs. 15, 16), and the mother cell wall, which becomes mucilaginous and separates the four young microspores (pl. fig. 17), disappears gradually with the formation of the thick wall of the pollen grain.

At the same time the uninucleate tapetal cells enlarge and become multinucleate. These divisions of the tapetal nuclei are mitotic at first, but later they become amitotic, after which they begin to disorganize when the homotypic division of the mother cell has been completed (pl. figs. 20, 21, 22).

The mature pollen grain is spherical, with remarkable canals upon the surface of the wall (pl. fig. 18). The cytoplasm occurs as a thin layer surrounding a large central vacuole. In general only a single nucleus was observed in the pollen grain, pl. fig. 19 representing the only specimen in which two nuclei were observed.

The foregoing account of microspore formation was obtained from material of no. 1. I have investigated no. 3 also, but could not determine the number of chromosomes. The pollen grain is a little larger than in the case of no. 1. The anther of no. 3 contained many withered pollen grains.

Development of pistillate flower

The pistillate inflorescence usually appears upon an upper branch, consisting of one flower and two bracts that suggest the position of flowers that have disappeared. The bracts usually fall after blooming, but in some varieties they persist even after the ripening of the fruit.

The staminate flower begins to develop as early as the pistillate, but the pistils develop more rapidly than the stamens, resulting in the sterilization of stamens and in some cases in their disappearance.

The mature pistillate flower generally has two sets of stamens (nos. 1 and 2), but in some cases has more (nos. 1, 2, and some of 4) or less (no. 1 and some of no. 2).

I did not discover the following condition described by Miss HAGUE: "In the pistillate flower it is a common occurrence to find the number increased by the branching of one or more of the stamens."

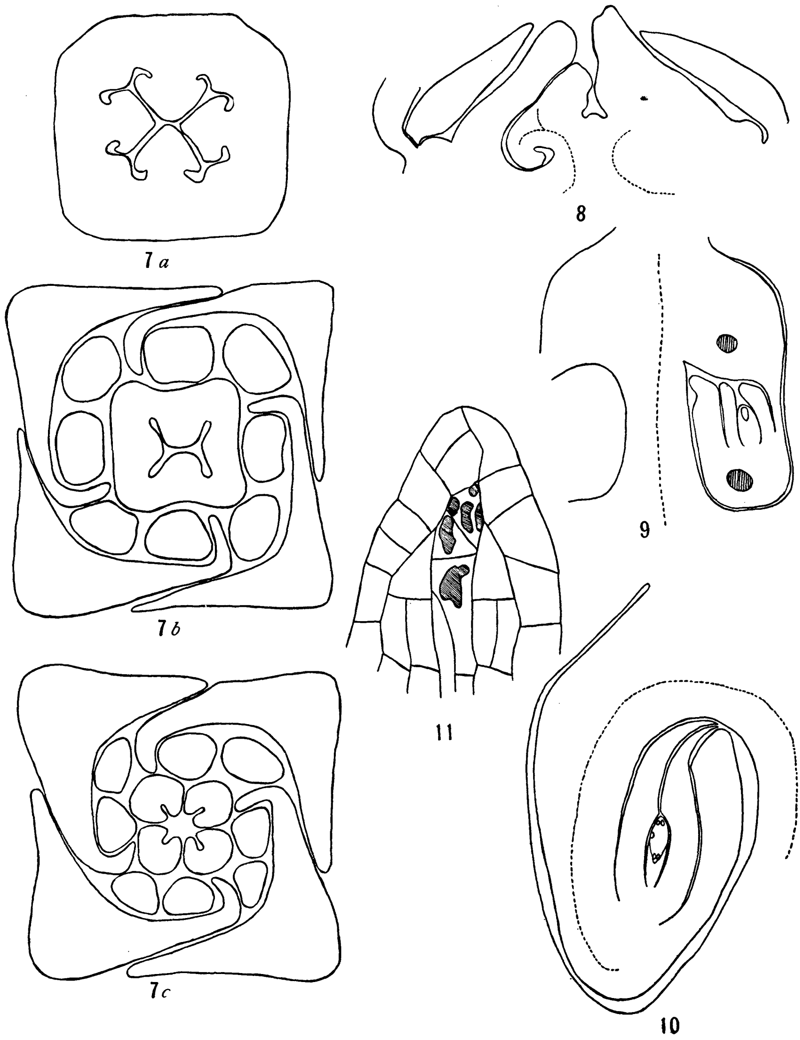
In the development of the pistil there appear first four large protrusions, and then four smaller ones between them (figs. 5 and 6, *a*, *b*). The larger ones give rise to two small papillae, which are the initials of ovules (figs. 6, *d*, and 7). The papillae grow at first toward the wall of the ovary, then curve downward toward the center, and finally upward (figs. 8 and 9). Simultaneously the small protrusions develop toward the center of the ovary, resulting, along with the development of larger protrusions, in 8 loculi for the 8 ovules.

The ovule has two integuments (fig. 9), which extend beyond the nucellus like a beak at the time the embryo sac is fully developed (fig. 10).

Megaspore formation

In all the varieties examined the archesporial cell is solitary and hypodermal. There is no division resulting in a parietal cell, so that the archesporial cell is the megaspore mother cell. The heterotypic division of this nucleus occurs in the same way as in the case of the nucleus of the microspore mother cell. After nuclear division, the cytoplasm organizes into two parts, the inner one being the larger. Sometimes a wall between these two parts does not appear before another division of nuclei occurs.

Wall formation in connection with the megaspores is variable in direction. Sometimes the two walls are perpendicular to one



FIGS. 7–11.—*D. Kaki:* fig. 7, cross-section of a pistillate flower of no. 4, at a little older stage than in figs. 5 and 6: *a*, lower part, showing 4 large protrusions which have papillae, and small protrusions among them; *b*, portion of the upper part, showing 4 petals, 8 anthers, and upper part of ovary; *c*, a still higher part, showing 4 petals, 8 anthers, and 4 young stigmas; fig. 8, longitudinal section of a young pistil of no. 4; fig. 9, longitudinal section of the central part of an ovary of no. 4, showing the longitudinal section of an ovule; fig. 10, longitudinal section of an ovule of no. 4; fig. 11, longitudinal section of the upper part of a nucellus of no. 4, showing the disorganizing embryo sac mother cell.

another, but at other times more or less parallel, resulting in different arrangements of megaspores (pl. figs. 23–26). The micropylar megaspores degenerate in connection with the maturation of the large innermost one in forming the embryo sac (pl. fig. 27). In my material I observed all of the megaspores disorganizing (fig. 11), and it is evident that this disorganization of the megaspore which ordinarily gives rise to the embryo sac is one reason for seedless fruit. In such a country as Japan, where many different kinds of persimmons are cultivated together, the difficulty of pollination is out of the question.

Summary

1. *Diospyros Kaki* is not a dioecious plant, but a monoecious one whose staminate flowers are disappearing under cultivation.

2. The monoecious habit might have been derived from a condition of perfect flowers; therefore this habit is not a primitive character in this species.

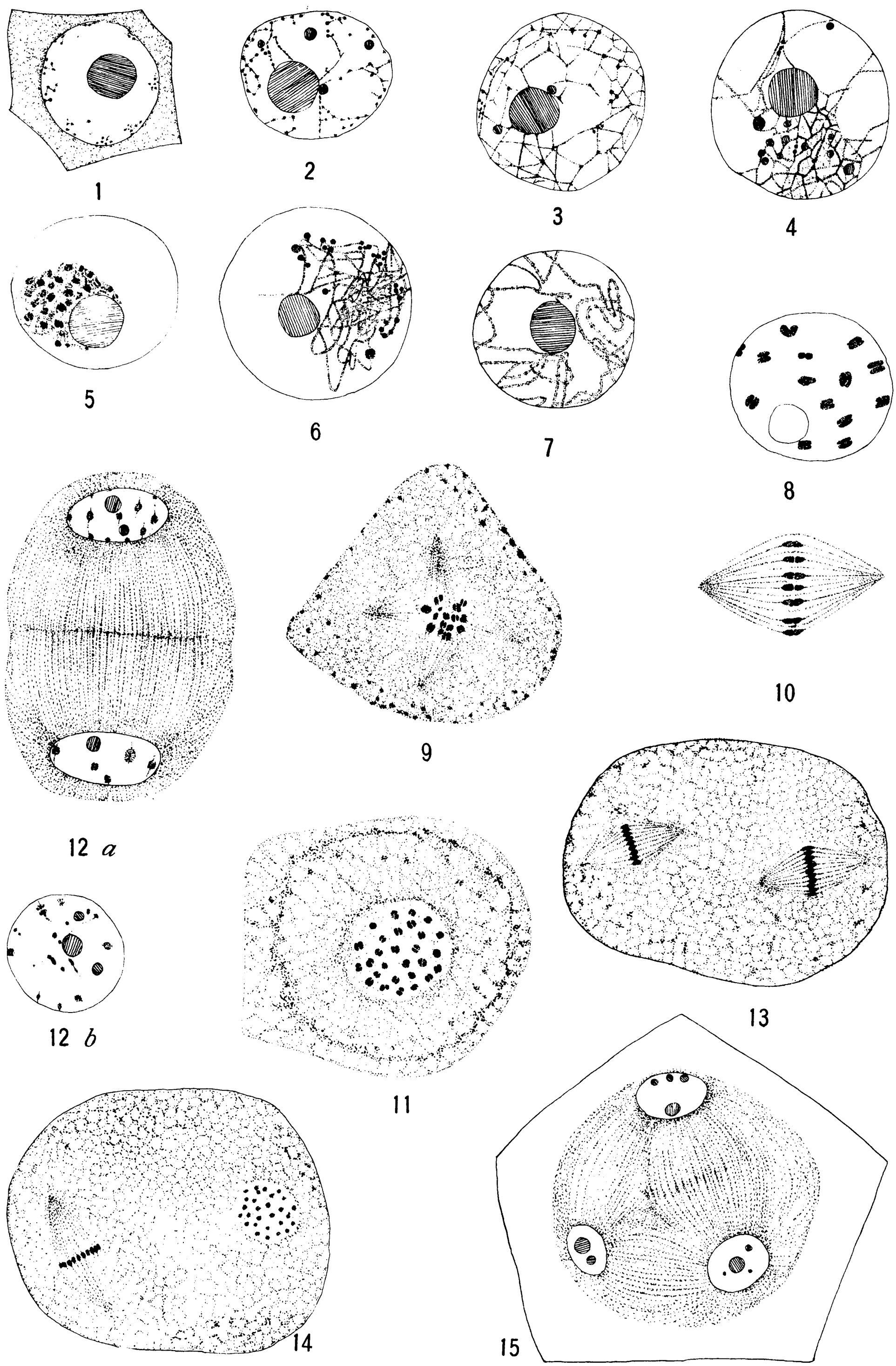
3. Perfect flowers do not indicate the primitive character of the variety in which they occur; they appear among other varieties only through restoration of lost parts.

4. The primitive character of Ebenaceae among Sympetalae is indicated by the spiral arrangement of petals; the stamen situation, although the number of stamens is not definite; and the two integuments.

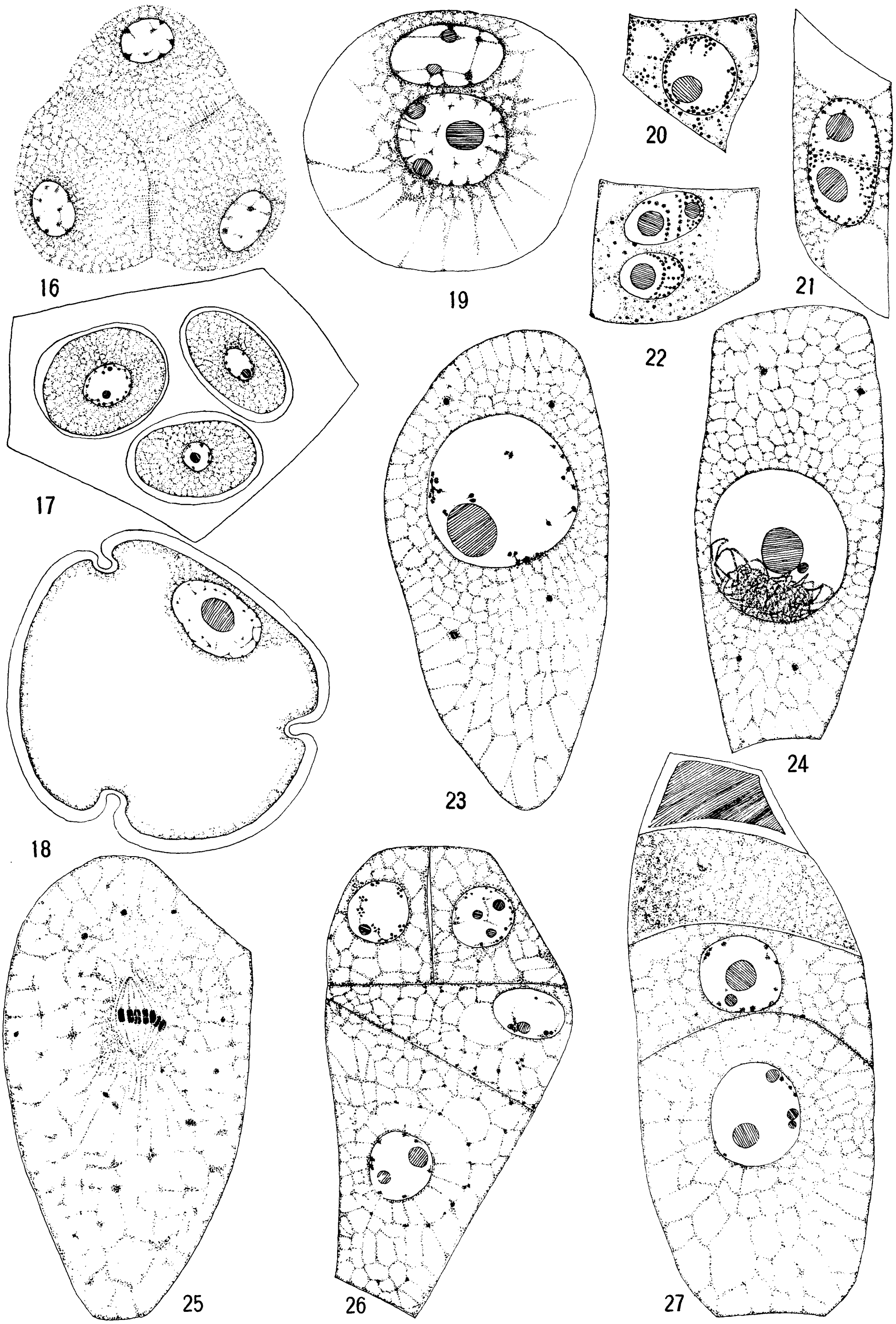
5. Megaspore formation is also of a primitive character, and suggests, along with other characters of the family, that it may have some relation to the Myrtiflorae.

6. There is no parietal tissue in the microsporangium, which indicates that Ebenaceae come from some higher family of Archichlamydeae, because in the lower families parietal tissue usually occurs in the megasporangium.

7. Embryo sac formation occurs in the usual way, and in general furnishes no evidence for the evolution of dicotyledons. It is true in general, of course, that the gametophytes of angiosperms are of less value for evidence concerning evolution than the sporophytes.



YASUI on DIOSPYROS



YASUI on DIOSPYROS

8. The $2x$ number of chromosomes is 56 or 54.

I desire to express my thanks to Professor JOHN M. COULTER, under whose direction the investigation was undertaken; to Professor C. J. CHAMBERLAIN, who gave much advice; and to Mr. Y. KUMAGAI and Professor Y. YABE, who assisted in securing material.

UNIVERSITY OF CHICAGO