Takasi YAMAZAKI*: **Embryological studies in Ebenales (4)**

山崎 敬*: カキノキ目の胚発生 (4)

Ebenaceae

The embryological studies in the Ebenaceae are very poor and imperfect. Only the formation of the embryo sac of *Diospyros virginiana* (Hague 1911) and *Diospyros kaki* (Yasui 1915) are reported. In this paper, I report the embryology of *Diospyros kaki* and the relationship of the Ebenales. The materials were collected from the cultivated plant in Tokyo.

Ovule and embryo sac. The superior ovary has eight locules, with an anatropous pendulous ovule in each locule. The ovule is bitegmic and tenuinucellate. The inner integument being consisted of 10 cell layers forms the micropyle. The innermost layer of the inner integument represents the endothelial layer with the quadrilateral cells containing prominent nucleus and dense cytoplasm. The outer integument is massive, with the cell walls of the outermost few layers thickly lignified and tinged with brown (Fig. 35).

A single hypodermal archesporial cell differentiates into young nucellus (Fig. 1). The nucellus represented by a single layer of cells degenerates early. The archesporial cell functions directly as the megaspore mother cell and gives rise after divisions to a linear tetrad of four megaspores (Fig. 2). Only the chalazal one is functional and gives rise to the embryo sac. The functional megaspore undergoes three nuclear divisions leading to the formation of two, four, and eight-nucleate gametophyte stages (Figs. 3 and 4). The mature embryo sac is linear-oblong in shape, and of nomal organization (Fig. 7). Three antipodal cells penetrating into the chalazal region lie in a linear row and degenerate before the final formation of the embryo sac (Fig. 5).

Formation of the endosperm. The endosperm is the cellular type. The first divison of the primary endosperm nucleus takes place with a transverse

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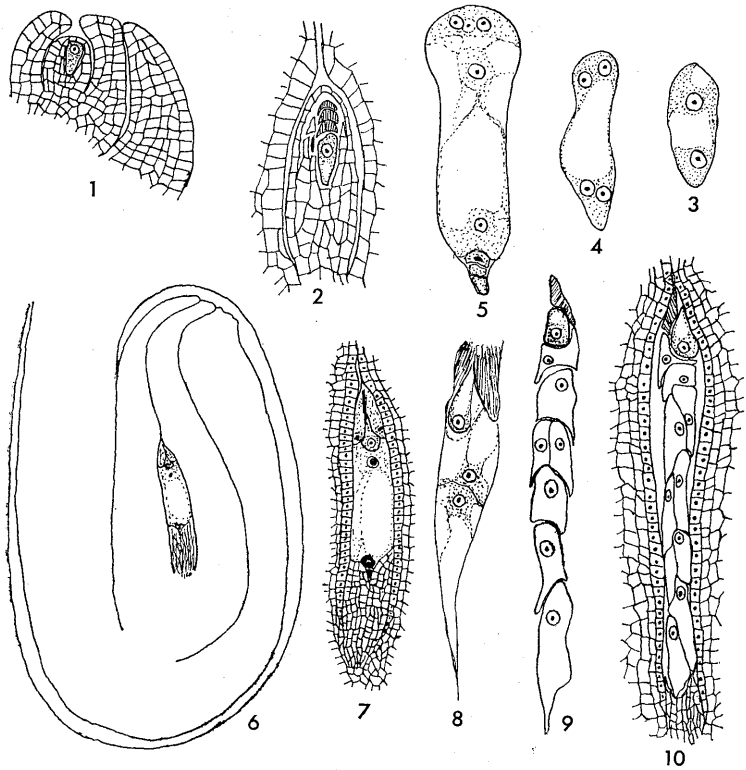
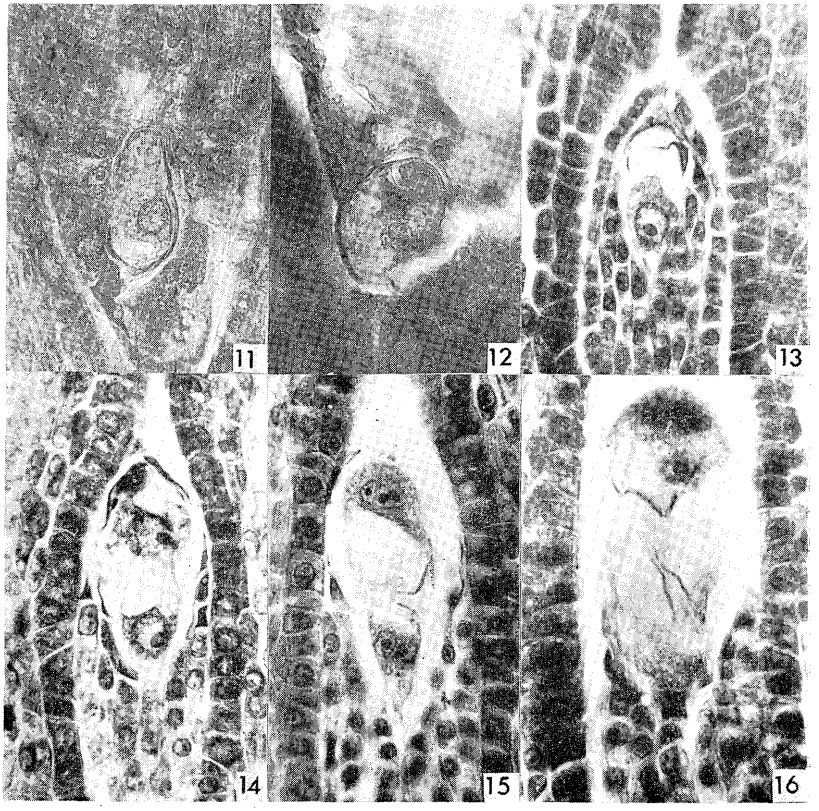


Figs. 1-10. *Diospyros kaki.* Stages in development of the embryo sac and endosperm. 1-5: ×100. 6: ×50. 7-10: ×100.

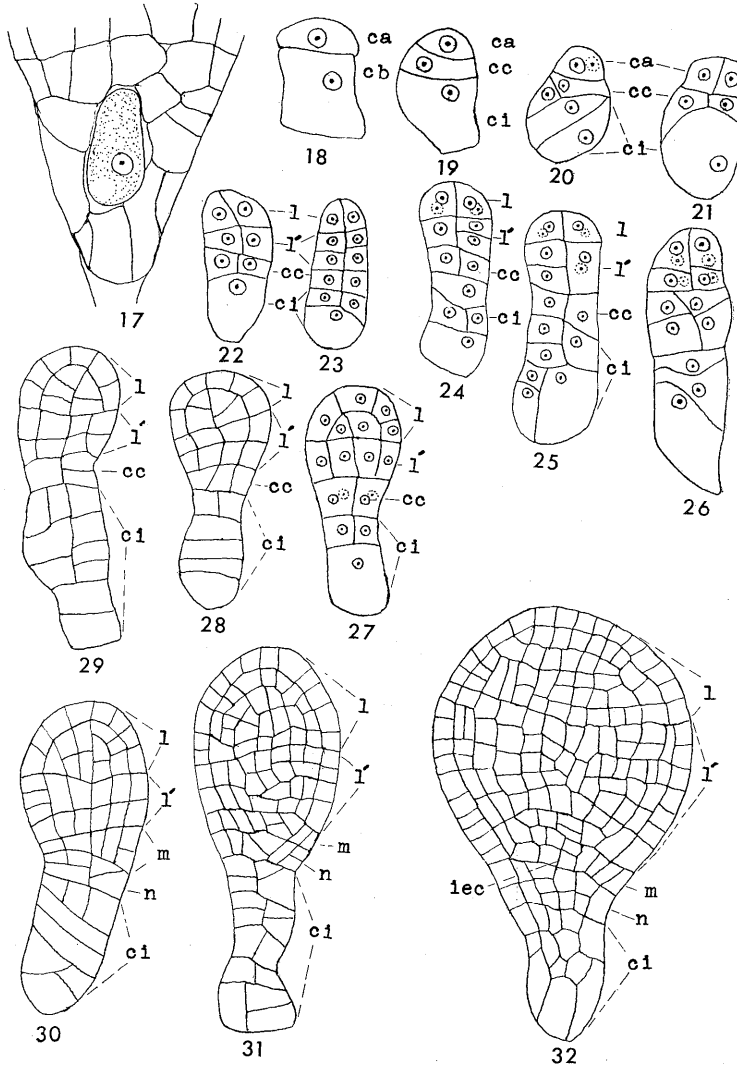
thin wall to form a micropylar and a chalazal chambers (Fig. 8). The second divisions in these two chambers are mostly transversal and the third are mostly longitudinal respectively. Thus the endosperm cells have a tendency to be arranged in a linear row of six tiers (Figs. 9 and 10). Subsequent divisions are irregular. By further divisons, all the cells contribute to the endosperm formation. The endosperm develops irregularly to form a ruminate shape in young stage (Fig. 34). The mature endosperm is an elliptical body with cells containing much oil but no starch. The endosperm haustorium is not formed at all.

Embryogeny. After the fertilization, the zygote swells and forms an

elliptical body containing a large vacuole (Figs. 11 and 17). The first division is transverse resulting in an apical cell *ca* and a basal one *cb* (Fig. 18). The upper cell *ca* divides longitudinally followed by transverse division to from the tiers *l* and *l'* (Figs. 20-23). The tier *l* divides by vertical walls oriented at right angles to the first longitudinal walls to give rise to four cells (Fig. 24). Four cells of the upper tier *l* are divided by tangential walls to form outer and inner daughter cells (Fig. 27). In rare cases they are divided once more transversely to form two tiers of four cells each (Fig. 26). The outer daughter cells are divided only by anticlnial walls and



Figs. 11-16. *Diospyros kaki*. 11. Zygote, one celled stage. 12. Zygote, three celled stage. 13. One nucleate megaspore. 14. Two nucleate megaspore. 15. Four nucleate megaspore. 16. Eight nucleate megaspore. The antipodals are degenerating. All ×200.

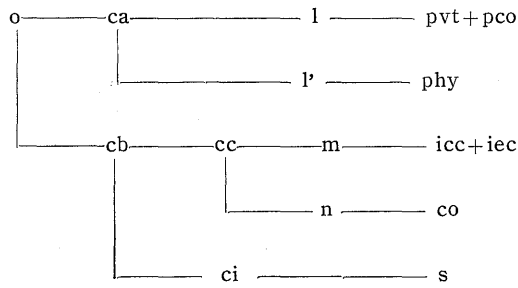


Figs. 17–32. *Diospyros kaki*. Stages in embryo formation. All ×200.

give rise to the epidermal initiais. The inner daughter cells of the tier *l* differentiate both into the mother cells of the cotyledonary initials in the outer side and into the elements which constitute the stem apex in the inner side (Fig. 29–32). The lower tier *l'* is divided by longitudinal walls differentiating into the outer and inner cell layers (Fig. 27). The resultant outer daughter cells of the tier *l'* repeatedly divide anticlinally and give rise to the epidermal initials, and the inner cells by continuous periclinal and anticlinal divisions form the plerome and periblem initials (Figs. 28–32).

The cell *cb* divides transversely to form two tiers *cc* and *ci* (Figs. 12 and 19). The tier *cc* divides longitudinally to form two cells (Figs. 20–22), and again divides rather slowly to form two tiers *m* and *n* (Fig. 30). The upper tier *m* differentiates to form the initials of the central cylinder of the hypocotyl *icc* and the initials of the root cortex *iec* (Figs. 30–32). The lower tier *n* differentiates to form the initials of the central part of the root cap *co*. The lower cell *ci* undergoes a few longitudinal and transverse divisions, and contributes to the formation of the suspensor *s*. A mature embryo comprises two large cotyledons and a large thickened hypocotyl.

The process of the embryo formation of *Diospyros kaki* is as follows:

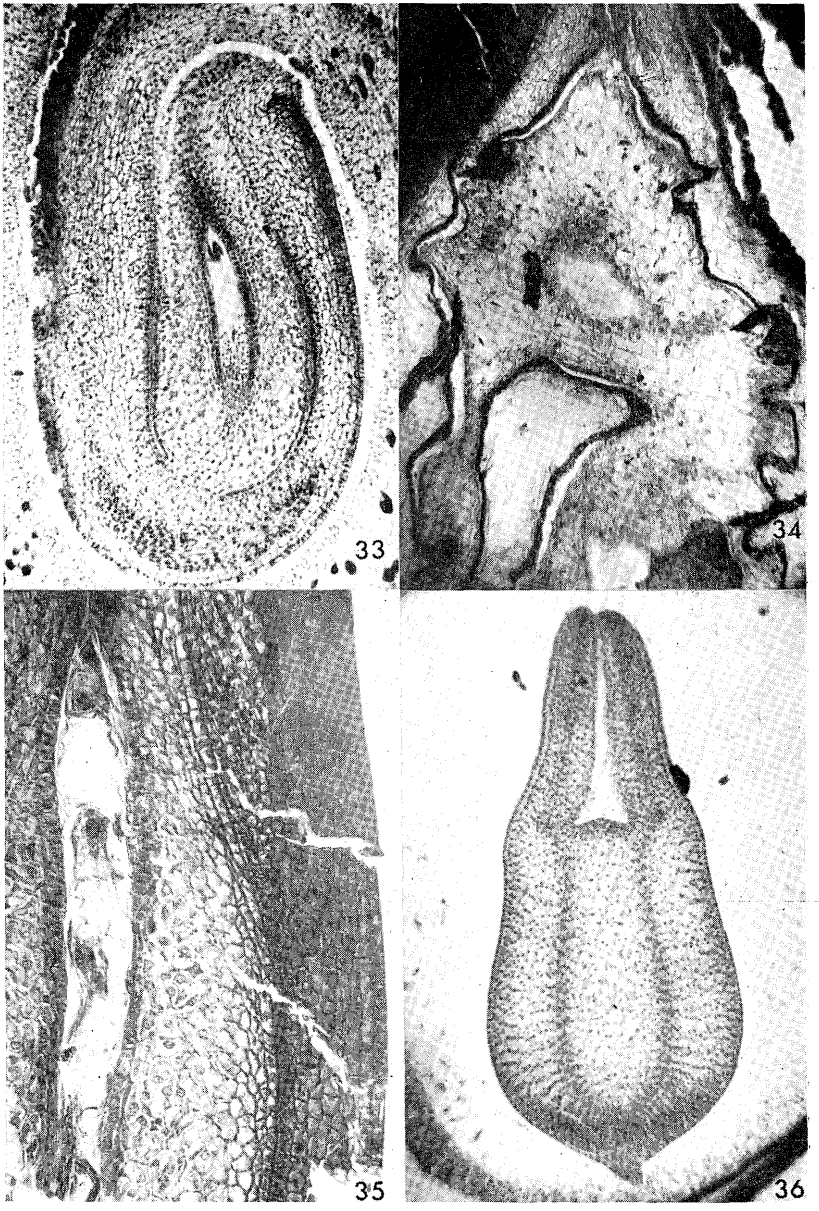


Seed coat. In the early stage of the endosperm formation, the cells of the outer integument are lignified and tinged with brown. At this stage, the inner integument is organized with strongly vacuolate thin-walled cells (Fig. 35). In mature seed, the cells of the inner integument are completely disintegrated and remain as a thin membrane. Thus the seed coat is derived mainly from the outer integument of about 10 layers consisting of elongated lignified cells rich in tanin.

Table 1. Features of the ovule and seed in families of Ebenales.

	Sapotaceae	Ebenaceae	Stylacaceae	Symplocaceae
Ovule	Anatropous	〃	〃	〃
	Tenui-nucellate	〃	〃	〃
Integument	Unitegmic	Bitegmic	Bitegmic	Unitegmic
Endothelial layer	Absent	Present	Absent	Absent
Embryo sac	Normal type	〃	〃	〃
Endosperm	Nuclear type accompanied by a large vacuole	Cellular type no large vacuole	〃	〃
	No starch	〃	〃	〃
Embryogeny	Chenopodiad type	〃	〃	〃
	Slow differentiation	〃	Simplified differentiation	〃
Mature embryo	Two large cotyledons and a short thick hypocotyl	Two large cotyledons and a massive straight hypocotyl	〃	Two small cotyledons and a curved elongated hypocotyl
Seed	Endosperm absent, large embryo	Massive ruminate emdosperm, embryo of moderate size	Less massive endosperm, large embryo	〃
Seed coat	Massive layers differentiated into three zones	Lignified cells derived from the outer integument	Massive layers of stone cells derived from the outer integument	Thin membrane

Discussion The seed fromation of the Ebenales is variable as shown in the comparative table 1. But they are fundamentally similar in several characters as described below. a) The ovule is tenuinucellate. b) The embryo sac fromation is Polygonum type. c) The cell division of the early endosperm formation is irregular. The endosperm has no haustorium and contains no starch but much oil. d) The embryogeny is Chenopodiad type; namelly, the initials of the root cortex (*iec*) and the initials of the central cylinder of the hypocotyl (*icc*) differentiate from the upper tier of the basal



Figs. 33-36. *Diospyros kaki*. 33. Ovule. ×55. 34. Ruminate endosperm. ×110. 35. Young seed showing the lignified outer integument and the thin walled inner integument. ×110. 36. Young embryo ×45.

cell (*cb*) of the two celled stage of the embryo formation.[1)]

The embryogeny of the Ebenales is similar to that of the Ericales. In both orders, the cells *icc* and *iec* are differentiated from the same tier, that is to say, from the tier *m* itself or from the derivatives of *m*. These features in the formation of the embryo have important significance in the phylogenetic standpoint. Thus, Ebenales shows the closest resemblance to Ericales in embryogeny and not to Guttiferales. But in the former the endosperm is formed by the irregular cell divisions in early stage and has no haustorium. The differentiation of the initials of the root cortex is slow. In the Ericales the endosperm is formed by four celled layers in early stage and normally has the endosperm haustorium (except Diapensiaceae). The differentiation of the initials of the root cortex is comparatively early. These embryological characters of Ebenales are similar to those of Diapensiaceae of Ericales. These facts seem to indicate that the Ebenales is more primitive than Ericales.

Literature

Hague, S.M., A morphological study of *Diospyros virginiana*. Bot. Gaz. **52**: 34-44 (1911). Yasui, K., Studies of *Diospyros kaki* 1. Bot. Gaz. **60**: 362-373 (1915). Yamazaki, T., Embryological studies in Ebenales. 1. Styracaceae. Journ. Jap. Bot. **45**: 267-273 (1970). 2. Symplocaceae. Ibid. 353-358 (1970). 3. Sapotaceae. Ibid. **46**: 161-166 (1971).

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カキノキの子房は上位で，4枚の心皮からなり，各心皮は2室を作り，各室1個の下垂する胚珠をもつ。したがって8個の胚珠があるが，普通はこのうち数個のみが種子にまで発達する。胚珠は2枚の珠皮をもつ。内珠皮は胚乳形成の初期に退化し，外珠皮が木質化して厚い種皮を作る。胚嚢形成は普通形であるが，反足細胞は胚嚢形成直

1) The embryogeny of the Symplocaceae was reported in the previous paper (Journ. Jap. Bot. **45**: 353-358). The author described that the cells *iec* and *icc* are differentiated from the derivatives of *ca*. It was, however, not correct. These cells are in fact derived from the uppermost tier of the derivatives of the cell *cb*. It is quite the same case as seen in the other families of the order. Thus, the abbreviation of Fig. 3 of *Symplocos chinensis* should be corrected as follows: *cc*→*l*, *cd*→*l'*, *l*→*l'*, *l'*→*m*, *m*→*n*, *n*→*ci* (The letters of the left hand of the arrow should be replaced with the right hand ones).

前に退化して消失する。胚乳形成は，細胞膜がごく薄く，nuclear type とまちがわれやすいが，明らかに cellular type である。初期には横分裂の傾向が強く，1～2細胞からなる6層の細長い組織ができ，その後不規則に分裂して錯道質の胚乳が作られる。錯道は胚乳が成熟するにつれて消失するが，錯道が現われることはカキノキ目の他の科にはみられない特徴である。胚形成は chenopodiad type で他の科と同じであるが，アカテツ科同様，原根層の分化は不明瞭である。

カキノキ目で報告したエゴノキ科，ハイノキ科，アカテツ科，カキノキ科の4科の，種子形成のさいにみられる差異はかなり大きく，その詳細は表1に示してある。全体に共通してみられる特徴として，胚形成がすべて chenopodiad type であること，胚乳には吸収器官が分化しないことがあげられる。カキノキ目は胚形成の点からいうとツツジ目に近く，しばしば近縁とされるツバキ目とは全く異る。しかしツツジ目の胚乳形成は初期に4細胞からなる細長い組織ができ，それから多方向に分裂して胚乳を作り，上下の一部の細胞は吸収器官に分化する。また胚形成も，比較的早期に原根層が分化してくるなど，分化の規則性が強まっている点が，ツツジ目はカキノキ目より進んだ形をとっているといえる。

イワウメ科はツツジ目に入れられたり，独立の目とされたりしているが，胚形成，胚乳形成はツツジ目と異り，カキノキ目のものと一致する。イワウメ科の子房はツツジ目の他のものと同様に多数の胚珠をもち，カキノキ目のものが子房の各室に1～2個の胚珠をもつのと異るが，ツツジ目よりもカキノキ目により近い群といえよう。

カキノキ目の4科の相互関係は，胚形成の点からいうと，早い時期に組織の分化が明らかになるエゴノキ科とハイノキ科とは，比較的あとまで分化がはっきりしないアカテツ科とカキノキ科よりも進んだ群といえる。ハイノキ科は完全な子房下位であり，珠皮が1枚であるなど，エゴノキ科より進んだ群といえよう。アカテツ科とカキノキ科とでは，アカテツ科は胚形成の初期に前胚形成に似た行動をとる点で，カキノキ科より原始的なものとみられるが，珠皮は1枚であり，胚乳形成は nuclear type であり，種子が成熟すると殆んど胚乳がなくなり，胚のみで種子の内容が占められるなど，かならずしも原始的とのみはいえない特殊化した特徴をもっている。これらの特徴が科全体のものなのか，あるいは属や種の特徴なのかはより広い調査を必要とする。

○植物採集覚書（其三十一）（奥山春季）　Shunki OKUYAMA: Tentative list of plants for collectors (31).

北海道地方（其一）

○函館　原標本植物　*Viola laciniosa* A. Gray〔エゾノタチツボスミレ〕Perry, Jap. Exped. **2**: 308 (1856).　*Lonicera Morrowi* A. Gray〔キンギンボク〕l. c. 313