Proc. Natl. Sci. Counc. ROC(B)
Vol. 25, No. 2, 2001. pp. 97-118

Development of Ovulate Cones from Initiation of Reproductive Buds to Fertilization in *Cephalotaxus wilsoniana* Hay.

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(Received March 27, 2000; Accepted September 13, 2000)

ABSTRACT

Cephalotaxus wilsoniana Hay. is endemic to Taiwan. This study was performed morphologically and anatomically to investigate reproduction in this species for the purpose of conservation. The duration from reproductive bud formation to fertilization in *C. wilsoniana* lasts about one year and five months. Buds are initiated in late January and differentiate into one vegetative bud and 3 female cones in late February. A female cone is constructed with 4 pairs of decussate opposite bracts. A small ridge-like secondary axis sits on the axil of each bract. Two ovules are borne on both sides of each secondary axis. A lysogenous pollen chamber begins to be formed from the degenerative tissues on the top end of the nucellus in early March. In late March the megasporogenous tissue is differentiated in the core center of the nucellus, and the micropyle closes gradually after pollination. By late July, pollen tubes have developed in the pollen chamber, and the megaspore mother cell appears. Then the functional megaspore becomes active in mid-October. The 8 free nucleate macrogametophyte appears in late December. From January to late March of the following year, the elliptical cyst-like female gametophyte keeps growing through continuous divisions of its free nuclei. The cyst layer of protoplast thickens in early April. In mid-April, cell walls begin to form among free nuclei. The archegonia are initiated in late April. Pollen tubes extend their tips to the macrogametophyte in early May, and each tube with 2 spermatozoids reaches a mature archegonium with an egg needed to perform fertilization in late May. Generally, only 1–(3) ovules in each cone can become mature.

Key Words: *Cephalotaxus wilsoniana* Hay., ovulate cones, ovules, fertilization

I. Introduction

The genus *Cephalotaxus* earlier included six species (Pilger and Melchior, 1954) but now includes nine species (Fu, 1984), seven of which are distributed around Mainland China and one is found in Taiwan. *Cephalotaxus wilsoniana* Hay. is the only species found to be endemic to Taiwan (Li and Keng, 1954, 1994).

Very early, Sokolowa (1890) described the development of the female gametophyte in gymnosperms, including the *Cephalotaxus* species. Arnoldi (1900), Coker (1907) and Buchholz (1925) studied *C. fortunei* to investigate the development of the ovule with embryogeny. In the other species, *C. drupacea*, Lawson (1907) observed role played by the female gametophyte in fertilization. Sugihara (1947) briefly described 2 male gametes. Favre-Duchartre (1957) and Singh (1961) conducted comprehensive studies on the reproduction biology in the same species, but the materials they collected were separated by great distances. Kaur (1958) briefly reported the proliferation of the megaspore mother cell. Concerning the female cones, Worsdell (1901) and Hirmer (1936) considered a small ridge-like outgrowth of the cone axis to be a secondary axis. Florin (1938-1945) termed the 2 ovules and the outgrowth between them a "seed-scale complex".

In the genus *Cephalotaxus* as a whole, Johansen (1950), Sterling (1963), Maheshwari and Singh (1967) and Konar and Oberoi (1969) reviewed the development of the male and female gametophytes as well as fertilization.

The ovulate cone structure of *C. wilsoniana* was described by Li and Keng (1954, 1994), Liu (1960), Gong and Chiang (1971), Liu and Liao (1980) and Liu *et al*. (1988), but their observations were limited, and there were differences among them. It was the purpose of this study to inventory the development of ovulate cones up to fertilization.

II. Materials and Methods

Reproductive buds to seeds of *Cephalotaxus wilsoniana* were collected during various stages of development from Dec. 23, 1994 to May 29, 1996 in Nan-tou County, in the central part of Taiwan. There are monoecious trees producing ovulate cones in three sites for collection: in the Experimental Forest of National Taiwan University, Chi-tou; on the Montane Experimental Farm of National Taiwan University, Mei-fen; and in the Natural Protection Area of Ruei-yen Brooke, Tsuei-fen (as shown in Table 1). The latter two lo-

Table 1. Number of Ovulate Cones Collected on Dates (mm/dd/yy) in Different Development Stages (I-XIX) from the Localities "Chi-tou" (CT), "Tsuei-fen" (TF) or "Mei-fen" (MF), Followed by Sectioning

date	collection	section	stage
12/23/94 (CT)	6	2	
01/17/95 (CT)	6	2	
01/27/95 (CT)	9	2	
01/31/95 (CT)	10	2	(I)
02/08/95 (CT)	10	3	
02/18/95 (CT)	8	2	
02/28/95 (CT)	15	3	(II)
03/10/95 (CT)	10	2	(III)
03/16/95 (CT)	6	3	(IV)
03/24/95 (CT)	6	3	(V)
03/28/95 (CT)	9	2	
04/03/95 (CT)	5	2	
04/15/95 (CT)	8	2	(VI)
05/12/95 (CT)	10	2	
05/30/95 (TF)	13	4	(XIX)
07/30/95 (TF)	14	4	(VII)
10/11/95 (TF)	11	3	(VIII)
12/27/95 (MF)	11	4	(IX)
01/04/96 (MF)	6	2	
01/14/96 (MF)	6	2	
01/24/96 (MF)	5	2	(X)
02/07/96 (MF)	8	2	
02/14/96 (MF)	6	2	(XI)
02/29/96 (MF)	7	2	
03/06/96 (MF)	6	2	(XII)
03/13/96 (MF)	6	2	
03/20/96 (MF)	7	2	(XIII)
04/10/96 (MF)	4	1	(XIV)
04/17/96 (MF)	4	1	(XV)
04/24/96 (MF)	4	1	(XVI)
05/01/96 (MF)	4	1	(XVII)
05/08/96 (MF)	4	1	
05/15/96 (MF)	4	1	
05/22/96 (MF)	4	1	(XVIII)
05/29/96 (MF)	4	1	(XIX)

calities are close to each other. It was confirmed after checking by microscopy that ovulate cones collected in late spring, 1995 at Chi-tou did not develop well. Therefore, collections of healthy ovulate materials as well as young seed-like ovules were performed at Tsuei-fen and Mei-fen, but Mei-fen is more accessible than Tsuei-fen.

Microtechniques for sectioning materials followed Johansen (1940) and Chiang-Tsai (1975). Buds or cones with ovules and stalk were immediately fixed in FAA (formalin: acetic acid:50% ethanol = 1:1:18 in volume) after collection. Materials were then dehydrated in TBA (tertiary butyl alcohol)-series and embedded in paraffin for sectioning. Sections were obtained on a sliding microtome (Schlittenmikrotom Om E, C. Reichert, Wien, Austria) with a thickness of 12 – 15 μm. After double staining with safranin-O and mehtyl green or fast green, followed by dehydration, sections were mounted in Canada balsam for observation and photography under a light microscope (BH2, Olympus, Tokyo, Japan). Morphological photography was performed using a stereomicroscope (SZ40, Olympus).

III. Results and Discussion

1. Initiation of Reproductive Buds in Stage I

In general, reproductive buds of *Cephalotaxus wilsoniana* are slightly bigger than vegetative ones. A vegetative shoot always bears 3 reproductive buds at its end (Fig. 1(a)). Immediately beside the terminal bud, there are 2 lateral ones at axils of 2 opposite vegetative leaves.

Reproductive buds are globose and 2 mm in diameter, subsessil and covered with scale leaves, which are spirally arranged. In a longitudinal section, the bud body is composed of an apical meristem with many young scale leaves on an axis. Of the apical meristem (Fig. 1(b)), a few cells, which are located either immediately beneath the outmost layer or in the core, are divided periclinally or obliquely.

There are a few apical meristems that are axillary to scale leaves and are convex-shaped (Fig. 1(c)). The meristem is a few cells thick and consists of a outmost layer and a core. They possibly give rise to organs, such as ovulate cones. A few protodermal cells of scale leaves undergo anticlinal division. The female cones of *C. drupacea* are initiated in the middle of October (Singh, 1961). They arise in the axils of the lower 2 – 4 bud scales and are subtended with scale leaves.

2. Differentiation of Reproductive Buds and Development of Ovulate Cones in Stage II

Three reproductive buds are located at the end of a vegetative shoot (Fig. 2(a)), one terminal and the other two axillary. Reproductive buds are globose, clearly showing a short, thick stalk. The bud and the stalk are covered with spirally arranged scale leaves. Each of reproductive buds consists of a vegetative bud at the terminal end and up to 3 ovulate cones around it (Fig. 2(b)). Two of these cones are opposite each other, and the other one is between them.

The cones are ca. 2 mm thick. Few ovules are visible in the top view of each cone. Gong and Chiang (1971) described that 1 – 3 "pistillate strobili" and a vegetative bud on the shoot terminal end of *C. wilsoniana*. Singh (1961) observed that the winter buds in the female tree of *C. drupacea* produced either a leafy shoot, a leafy shoot and 2 – 4 female cones or only "a few" female cones. In the second type of bud, after producing female cones, the shoot apex gives rise to a foliage (vegetative) shoot.

The cones have a short stalk and are small in size. The stalk of a developing ovulate cone is elongated (Fig. 2(c)) and is constructed with vascular tissues with divergences toward the lateral organs. Each young secondary axis is present in the axil of a bract. The secondary axes are thick and conical in form. Their epidermal cells are small. Hypodermal cells at

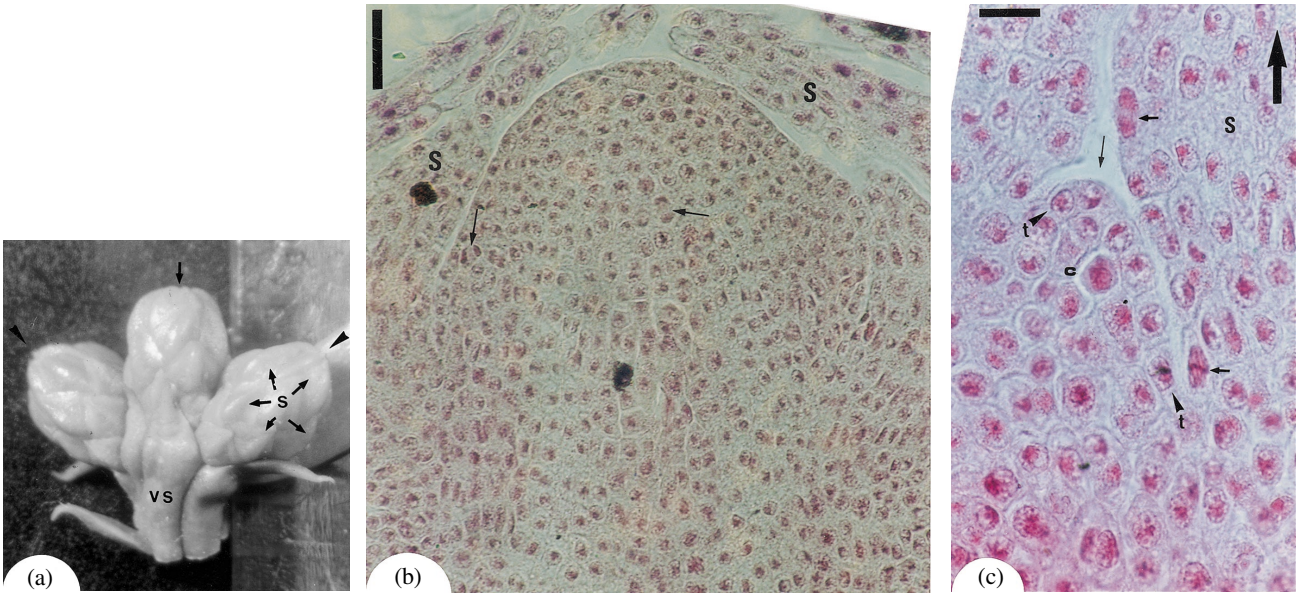


Fig. 1. Initiation of reproductive buds in stage I. (a) (SV) One terminal (arrow) and 2 lateral (arrowheads) reproductive buds are located at the end of a vegetative shoot (vs). Buds are subsessil and covered with scale leaves (s), which are spirally arranged. (b) (LS) (Bar = 50 μm) One apical meristem is surrounded by many scale leaves (S). A few cells (arrows), which are located in the core center or in the hypodermal layer, undergo division periclinally or obliquely. (c) (LS) (Bar = 22.5 μm) A meristem (longer, small arrow) axillary to a scale leaf (S) is convex-shaped and is a few cells in thickness, with an outmost layer (t) and a core (c). A few protodermal cells of the scale leaf undergo anticlinal division (shorter, small arrows). The axial direction of the bud is indicated by large arrow.

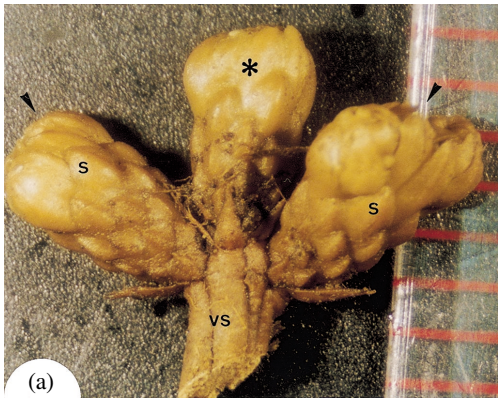
Notes: (1) Observation or orientation views of sections: (TV) = top view; (SV) = side view; (CS) = cross section; (LS) = longitudinal section; (OLS) = oblique longitudinal section. (2) Ratio of magnification in Figs.: scale unit of a linear = 1 mm under stereomicroscopy or bar in μm under light microscopy.

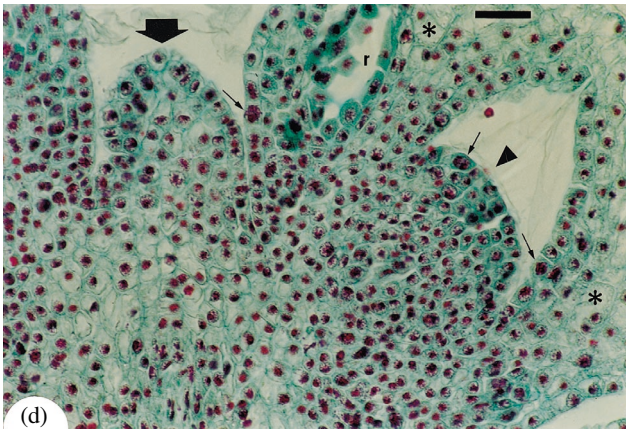
the axis apex have a big nucleus and a darkly stained cytoplasm. Singh (1961) noted that an outgrowth arises in the axil of every fertile bract and acquires the typical organization of a vegetative shoot apex, i.e., 4 cytohistological zones. The outgrowth represents a secondary axis, from which 2 ovules arise as lateral protuberances. In this study, two juvenile ovules were found to sit subopposite on both sides of a secondary axis.

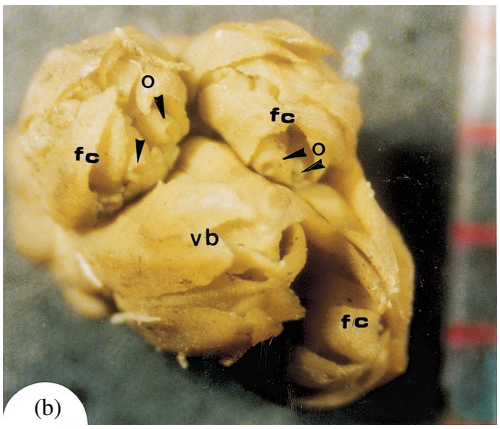
Each of the juvenile ovules is composed of a nucellus at the center and 2 integuments on both sides in the longitudinal section (Fig. 2(d)). Cell division occurs very frequently in the young ovule, *e.g.*, anticlinal divisions in the protodermal cells of the integuments and the nucellus. Tissues of the nucellus and the basal zone of the integuments have meristem characteristics while the cells in the upper part of the integuments are large and loosely arranged. The upper part of the nucellus is ca. 4 – 5-cells high. Resin ducts appeared for the first time in the integuments in this stage, but they were found for the first time up to the first of April in *C. oliveri* (Li *et al.*, 1986). Two ovules are found in the axil of each bract except for the lowest (basal sterile) pair. Gong and Chiang (1971) described two ovules at the axil of each "scale." Ovules were found to be elliptic, and the micropylar opening was circular. However, they failed to point out presence of secondary axes. The mass of nucellus arose mainly from the parietal layer through repeated, periclinal divisions that characterize a leaf primodium (Singh, 1961). However, Fig. 2(d) of this work shows that the cell divisions in the young nucellus occur in multiple orientations.

3. Initiation of the Pollen Chamber in Stage III

Ovulate cones are elliptic-ovate in form, ca. 4.0 mm long and 3.0 mm thick (Fig. 3(a)). Their stalks are glabrous, ca. 2.5 mm long. Each cone usually contains 8 opposite bracts. The bracts are slender at the base and obovate with a triangle apex in the upper part. They are ca. 2.5 mm long. In *C. drupacea* (Singh, 1961), each cone bears 5 – 7 pairs of opposite and decussate bracts. The cone axis is fleshy, so the arrangement of bracts is concealed. There are few reports on the structure of the so-called "pistillate flowers" in *C. wilsoniana* by authors in Taiwan. The strobilus is an elliptic short spike (Li and Keng, 1954; Liu, 1960; Gong and Chiang, 1971). A pistillate flower contains a few (Liu, 1960) or 5 – 6 (Liu and Liao, 1980) paired opposite or 5 – 7 spiral (Gong and Chiang, 1971) fleshy carpels (ovuliferous scales) and 2 ovules at the inner side of each fleshy scale (Li and Keng, 1954; Liu, 1960; Gong and Chiang, 1971; Liu and Liao, 1980). The term "carpels" or "ovuliferous scales" used in previous works should be correspondent to "fertile bracts" in this study. The spiral arrangement of the "scales" described by Gong and Chiang (1971) might be the scale leaves found here. The axillary







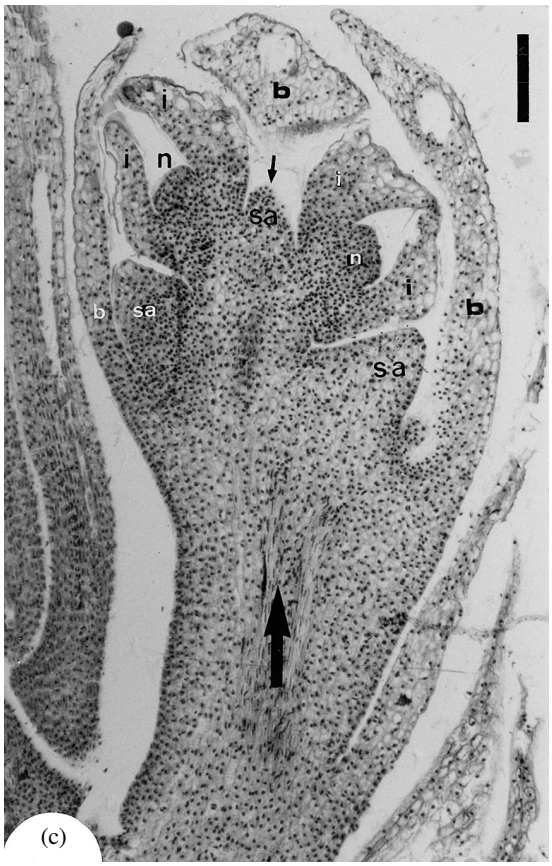
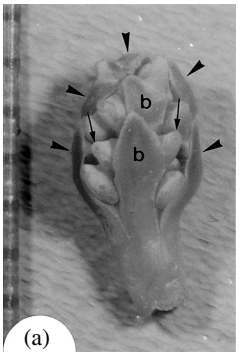


Fig. 2. Differentiation of reproductive buds and development of ovulate cones in stage II. (a) (SV) One terminal reproductive bud (asterisk) and 2 lateral ones (arrowheads) are located on a vegetative shoot (vs). The bud stalks become slightly elongated and are covered with spirally arranged scale leaves (s). (b) (TV) Each reproductive bud consists of a vegetative bud (vb) at the terminal end and 3 female (ovulate) cones (fc) around it, two of which are opposite each other while the other one is between them. A few ovules (o) are visible in the top view of the cones. (c) (OLS) (Bar = 225 μm) A developing ovulate cone consists of ovules, bracts (b) and secondary axes (sa). The stalk of one ovulate cone is long and vasculated (big arrow). A secondary axis is axillary to each bract. Two ovules are subopposite set at both sides of a secondary axis (small arrow). Each ovule is composed of integuments (i) and a nucellus (n). (d) (OLS) (Bar = 50 μm) A young ovule beside a secondary axis (big arrow) contains a nucellus (arrowhead) and integuments (asterisks) with a resin duct (r). The entire nucellus and the basal zone of the integuments have the appearance of a meristem; *e.g.*, a few cells (arrows) are undergoing division.



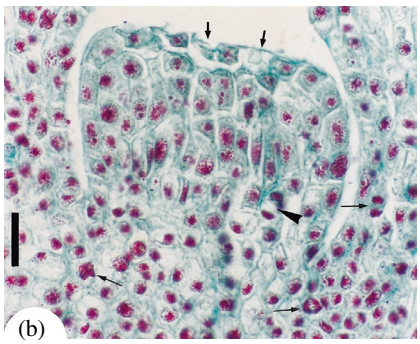


Fig. 3. Initiation of the pollen chamber in stage III. (a) (SV) An ovulate cone is elliptic-ovate shaped and has 8 opposite bracts (b and arrowheads) on a glabrous stalk. Two ovules (arrows) are axillary to each bract and are spread out sideways. (b) (LS) (Bar = 45 μm) The upper part of the nucellus has developed further and is about 6 – 8 cells in height. There are 5 – 6 cells (shorter arrows) in epidermal and hypodermal layers, which are located at the top of the nucellus, are collapsed and lack a visible nucleus. A pollen chamber begins to arise in this lysogenous space. Lower cells in the upper part of the nucellus are longitudinally elongated. One of them in the basal part is undergoing horizontal division (arrow head). A few cells (longer arrows), which are distributed in the base of integuments, are undergoing divisions with different orientations.

secondary axes with ovules extend and are visible in this stage.

Ovules are ca. 600 × 600 μm in size. The upper part of the nucellus is bigger, about 6 – 8 cells in height and 14 – 16 cells in width (Fig. 3(b)). Collapse occurs in about 5 – 6 cells of the epi- and hypodermal layers at its plain top end, beneath which cells are longer and longitudinally orientated. The lysogenous space becomes the pollen chamber. A few days before pollination, 2 – 3 layers of nucellar cells in the micropylar region start to degenerate and form a rudimentary pollen chamber in *C. drupacea* also (Singh, 1961). In *C. wilsoniana*, cells may undergo divisions with horizontal and other orientations in the basal zone of the nucellus and the integuments (Fig. 3(b)). This zone appears to be characteristic of intercalary meristem.

4. Development of Integuments and Secondary Axes in Stage IV

Ovulate cones (ca. 5.0 × 3.5 mm) and their stalks (ca. 3.5 mm long) grow further (Fig. 4(a)). Ovules and secondary axes are visible from the side after they extend from the axils of the bracts. Bracts are still ca. 2.5 mm long. Secondary axes are short and thick (Fig. 4(b)). Basically, it is a mass of ground tissue, in which a few cells have thick and lignified walls. Epidermal cells are larger in the upper part than those in the basal part.

Ovules are ca. 900 μm long and 700 μm thick. The entire integument is like a deep cup in appearance, inside which the nucellus sits at the bottom (Fig. 4(c)). The tissues in the basal zone of the ovule still appear to be characteristic of meristem. The edges of the integuments at the micropyle are thin with about 2 – 4 layers of cells. Its middle part is slightly thicker, containing 6 layers of cells. The base of the integuments is ca. 12 – 14-cells thick. The inner epidermis, which faces the micropylar canal, and parts of the hypodermis of the integuments are meristematic in morphology. These cells are small with a relatively large nucleus. The outer epidermal cells are much larger than those of the inner epidermis and might have a large vacuole. The ground tissue of the integument, which is between the inner hypodermis and the outer epidermis, is parenchyma; in it, cells are large, axially elongated, occasionally vacuolated, and contain starch grains. The nucellus is 18 – 20 cells wide and ca. 10 – 15 cells high. Cells in its upper half are larger than those in its lower half. There

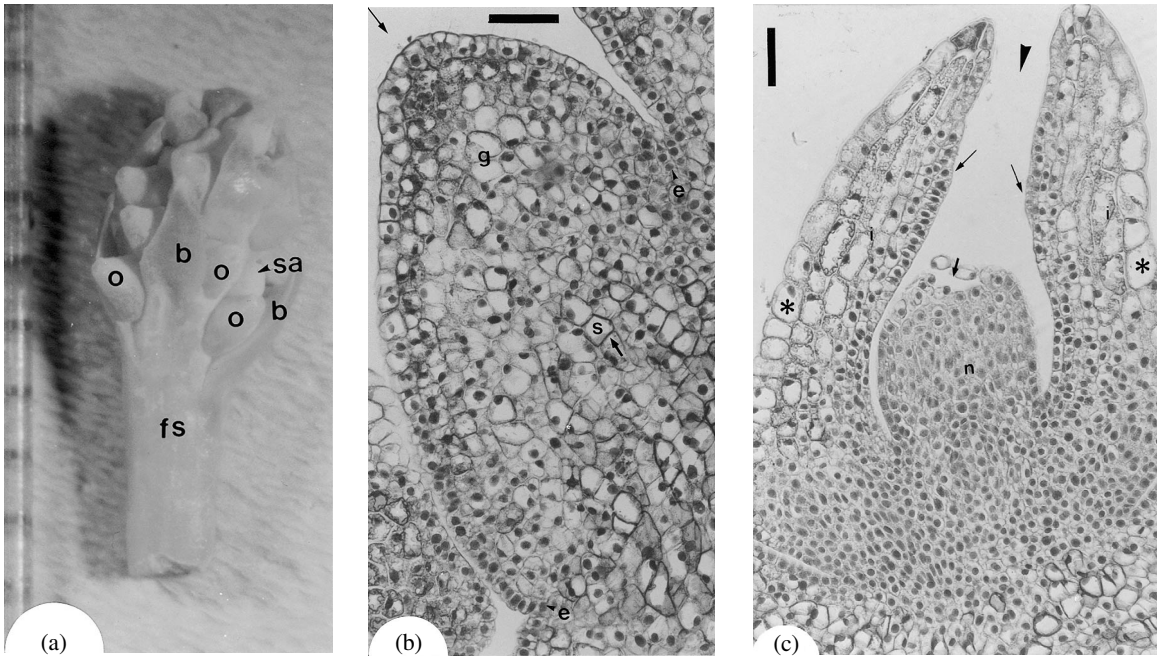


Fig. 4. Development of integuments and secondary axes in stage IV. (a) (SV) Ovules (o) and secondary axes (sa) of an ovulate cone emerge from the axils of the bracts (b). The female (ovulate) cone stalk (fs) has grown longer. (b) (LS) (Bar = 90 μm) A secondary axis is short and thick. The epidermal cells (e), which are located in the apical part (long arrow) of the secondary axis, are bigger than those in the basal part. The ground tissues (g), which are inside of the epidermis, are basically a mass of parenchyma tissue, a few cells (s) of which have thick, lignified walls. (c) (LS) (Bar = 90 μm) One entire integument (i) is like a deep cup in appearance, inside which the nucellus (n) sits at the bottom. Tissues in the basal zone of the ovule still appear to be characteristic of a meristem. The upper part of the nucellus is ca. 10 – 15 cells high. There is a small intercellular space (short arrow) between the cells at the top of the nucellus. The basal part of integuments is ca. 12 – 14 cells thick, the middle is ca. 6 cells thick and the edge at the micropyle (arrowhead) is acute. The inner epidermis (long arrows), with part of the hypodermis, of the integuments is meristematic in appearance. These cells are small with a relatively large nucleus. The outer epidermal cells (asterisks) are much larger than those of the inner epidermis and might have a large vacuole. The ground tissue of the integument, which is between the inner hypodermis and the outer epidermis, is parenchyma; cells are large, axially elongated, occasionally vacuolated, and contain starch grains.

is a small intercellular space in the loose tissue, which is 1 – 2 cells deep and located at the plain top end of the nucellus.

5. Closure of the Micropylar Canal and Differentiation of the Megasporogenous Tissue in Stage V

An ovulate cone is ca. 5.0 mm long and more than 4.0 mm thick. Its stalk becomes much longer than in the previous stage and up to ca. 7.0 mm long (Fig. 5(a)). The basal parts of each conical ovule and each bract expand, and the cone axis and secondary axes become thicker, so that the cone body appears to be bigger. Bracts are ca. 3.0 mm long.

Ovules are about 1200 μm long and 800 μm thick. The integuments become thicker, especially in the section around the micropylar canal, i.e., between the micropyle and the top end of the nucellus (Fig. 5(b)). In that segment of the integuments, four types of tissue are found. The outer epidermal cells are large, having a nucleus and a thick outer wall with a cuticular layer. Beneath the outer epidermis, there are about 3–(4) layers of cells, which are medium-large in size and longitudinally elongated. This is the ground tissue of the integuments. The inner epidermis, instead of a thin layer one cell thick, has become a thick layer, which is 2 – 4 small but elongated cells in depth. These long cells are joined in rows orientated against the micropylar canal. Compared to the previous stage, the inner epidermal cells have undergone division (hyperplasy) periclinally and elongation (hypertrophy) anticlinally to the micropylar canal, so that only a narrow space is left in the canal.

Similar to other *Cephalotaxus* species, a band of inner epidermal cells in the integument along the micropylar canal becomes active. After pollination, these cells grow inward, undergo a few transverse divisions and close the micropylar opening, thus effectively sealing off the freshly arrived pollen (Singh, 1961; Li *et al*., 1986). As illustrated in Fig. 5(b), however, the inner epidermal cells proliferate transversely through "periclinal divisions" and elongation against the micropylar canal so that the micropylar canal becomes almost closed. Between this inner thick layer of tissues and the ground tissue, there are 1–(2) longitudinal layers of small cells with walls that are thickened and lignified. These layers appear to originate from the inner hypodermis. Resin ducts are clearly

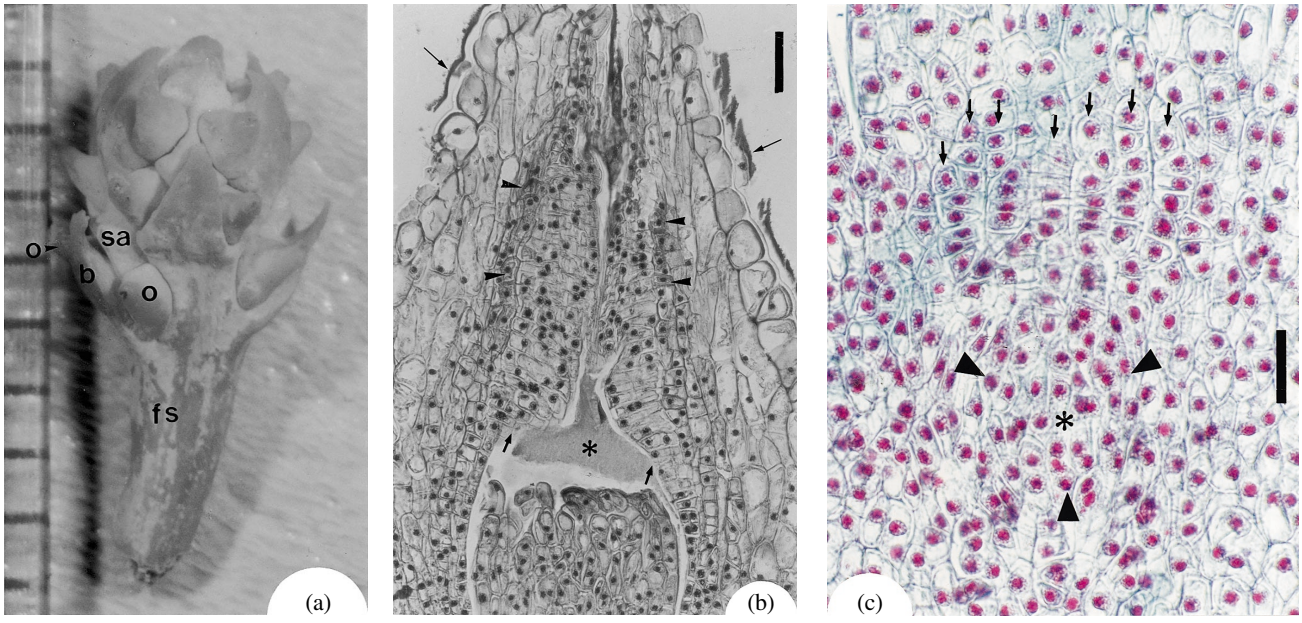


Fig. 5. Closure of the micropylar canal and differentiation of the megasporogenous tissue in stage V. (a) (SV) Each conical ovule (o) has broadened at the base, and the short secondary axes (sa) have thickened, so that the bracts (b) are now spread out. The female cone stalk is denoted by fs. (b) (LS) (Bar = 90 μm) Integuments have become thicker so that the micropylar canal, i.e., the region between the micropyle and the top of the nucellus, is now almost closed. There are four types of tissue, which are now differentiated in that segment of the integuments. The outer epidermal cells (long arrows) are large and have a nucleus and a thick outer wall with a cuticular layer. Beneath the outer epidermis, there are about 3–(4) layers of cells, which are medium large in size and longitudinally elongated. This is the ground tissue of the integuments. The inner epidermis can no longer be identified as a single layer of cells. There are tissues (short arrows) in a thick layer, which is 2 – 4 small but elongated cells in depth. These long cells are joined in rows, which are orientated against the direction of micropylar canal. Between this thick, inner layer of tissues and the ground tissue, there are 1–(2) longitudinal layers of cells (arrow heads) that are small with thickened and lignified walls. Amorphous substances (asterisk), which may be stained, have accumulated in the space over the top of the nucellus. The tissue in the top part of the nucellus is loose with few intercellular spaces. Cells in this part are larger or longer than those in the lower part. Some of them might be vacuolated and lack nuclei. (c) (LS) (Bar = 45 μm) There are 6 – 7 longitudinal, neighboring rows (arrows) of cells, which are flat-shaped. Along the direction of these cell rows downwards in the core center (asterisk), there is a group of cells (among 3 arrowheads) with a circular arrangement and a thickness of about 7 cells.

present in the lower part of the integuments.

Amorphous substances, which can be stained, accumulate in the space over the top of the nucellus. This substance might play a role as the pollination drop as Singh (1961) reported. Pollen grains were caught by the pollination drop, which was exuded from the micropyle and later "sucked into" the pollen chamber of the nucellus. Then the pollen grains germinate and form pollen tubes.

The upper part of the nucellus is 13 – 15 cells wide and 15 – 17 cells high, and the tissue is loose with few intercellular spaces. Some of the large cells might be vacuolated without a nucleus (Fig. 5(b)). Cells in this part are larger or longer than those in the lower part. In the lower part, there are 6 – 7 longitudinal, neighboring rows of cells, which are flat in shape (Fig. 5(c)). Along the orientation of these cell rows downwards in the nucellar core center, there is a group of cells, which have a dense, circular arrangement with a thickness of ca. 7 cells. This group of cells is the megasporogenous tissue.

The sporogenous tissues are quite distinct in the nucellus (Singh, 1961). These tissues are located near the chalaza and made up of cells in a concentric arrangement (Li *et al*., 1986). About 3 – 6 archesporial cells are hypodermal but not always in a single layer. The primary parietal layer and the sporogenous layer originate through periclinal division in the hypodermal layer. These tissues can be recognized in the ovule at an early stage, but Singh (1961) did not give detailed morphological descriptions of the primary parietal layer and sporogenous layer. As a whole, the basal zone of the integuments and of the nucellus is still characteristic of intercalary meristem.

6. Formation of Sclerenchyma in Integuments in Stage VI

An ovulate cone body is up to ca. 6.0 mm long and less than 4.5 mm thick. The elongated stalk extends to a length of ca. 7.5 mm (Fig. 6(a)). Bracts remain ca. 3.0 mm in length and are adaxially thickened at the base. Secondary axes appear to be adaxially very adnate to the cone axis.

Ovules are ca.1200×800 μm in size. Resin ducts extend into the lower part of the integuments and have a wider duct space than in the previous stage (Fig. 6(b)). Below that level, the ovule is abruptly constricted, and a short ovule stalk appears. Many sclereids with thick, red-stained cell walls appear in the inner, thick layer of the integuments, which is newly formed tissue along the micropylar canal (Fig. 6(c)). These cells are arranged in anticlinally orientated rows. Similar to other *Cephalotaxus* species, as the ovules mature, these elongated cells acquire a very thick wall (Singh, 1961; Li *et al*., 1986).

Not all nucelli develop further. The cells in the entire

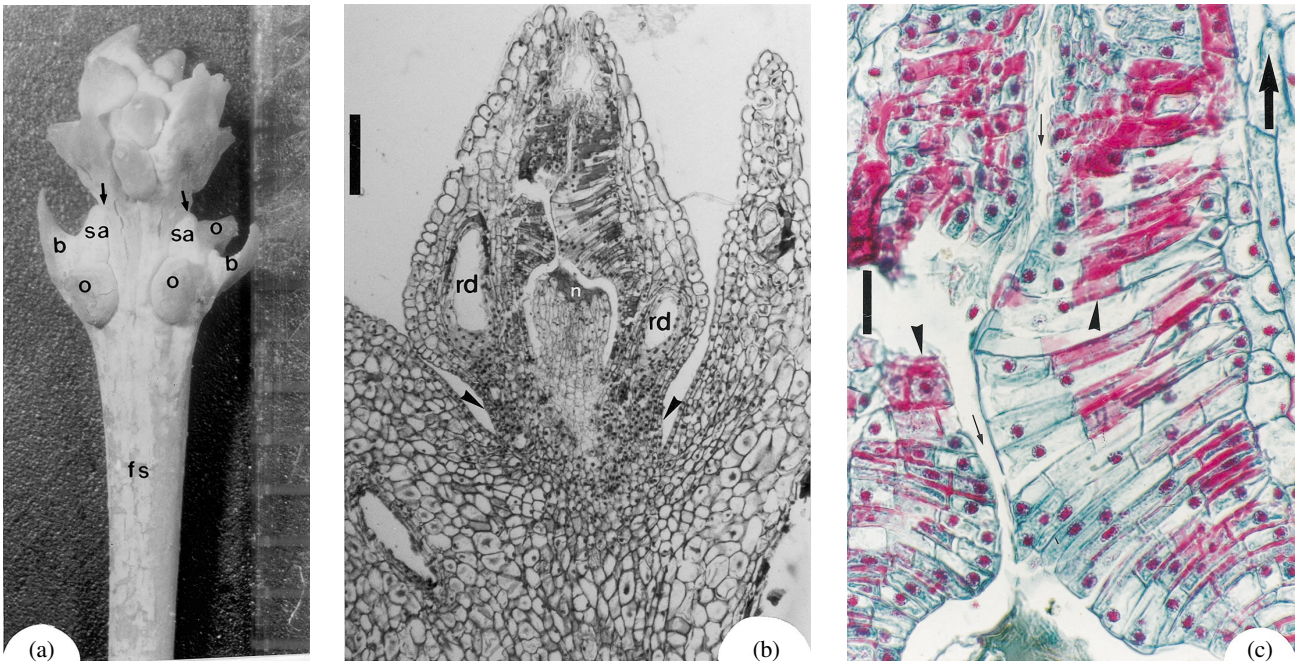


Fig. 6. Formation of sclerenchyma in integuments in stage VI. (a) (SV) Ovules (o) have thickened. Secondary axes (sa) are adaxially very adnate to the cone axis (arrows). Bracts (b) are spread out. The female cone stalk is denoted by fs. (b) (LS) (Bar = 225 μm) Wide resin ducts (rd) are found in the lower part of the integuments where the thickness of the ovule is greatest. Below that level, the ovule is abruptly constricted, and a short ovule stalk (arrowheads) appears. The nucellus (n), from the top to the core base consists of lightly stained, degenerative tissues. (c) (LS) (Bar = 45μm) Magnification of the integuments shown in Fig. 6(b). Many sclereids (arrowheads) with thick, red-stained walls are found in the thick, inner layer of the integuments along the micropylar canal (small arrows). The axial direction is indicated by the large arrow.

nucellus, from the top to the core base, are light-stained with almost no protoplast (Fig. 6(b)). This nucellus appears to be aborted in this stage, i.e., 2 weeks after pollen shedding (Lo and Wang, 1999). Also, some of the cells, which are arranged in 1 – 2 layers regularly around the core base of the nucellus, are full of dark-stained material in their lumens. This material appears to consist of tannins.

7. Development of Pollen Tubes in the Pollen Chamber and Initiation of the Megaspore Mother Cell in Stage VII

An ovulate cone reaches a length of ca. 6 mm and a thickness of 4.5 mm (Fig. 7(a)). The bracts are thick at the base. Ovules grow to different sizes within a cone. The bigger ones can be ca. 1300×1000 μm. A mass of sclerenchyma is found in the inner, thick layer of the integuments (Fig. 7 (b)). Each anticlinally orientated rows is composed of about 4 – 7 cells, the walls of which are 5 to 7 μm thick. There are a few free sclereids in the ground tissue of the integuments as well as in the cone axis below the ovule. The ovule stalk is longer. Resin ducts have expanded in the lower part of the integuments.

Two parts of pollen tube(s) are visible in the pollen chamber. One of them contains 2 free nuclei (the tube nucleus and the stalk nucleus) (12 μm in dia.), a cell (the body cell) with a nucleus (12 μm in dia.) and cytoplasm (Fig. 7(c)). The body cell and 2 nuclei are located in the leading end of pollen tube, which extends toward the chalaza. The 2 free nuclei are similar to each other in morphology. The nucleus of the body cell is just behind these 2 free nuclei.

It is commonly thought that there are no prothallial cells in *Cephalotaxus* species since their pollen grains contain a generative (antheridial) cell and a tube cell (Strasburger, 1872, 1879; Thibout, 1896; Coker, 1907; Lawson, 1907; Khoshoo, 1957a, 1957b; Favre-Duchartre, 1957; Kaur, 1958; Singh, 1961; Li *et al*., 1986; Chen *et al*., 1987; Lo and Wang, 1999). According to Singh (1961), the pollen settles on the degenerative top of the nucellus (a weakly developed pollen chamber). The intine enlarges at the tube cell end

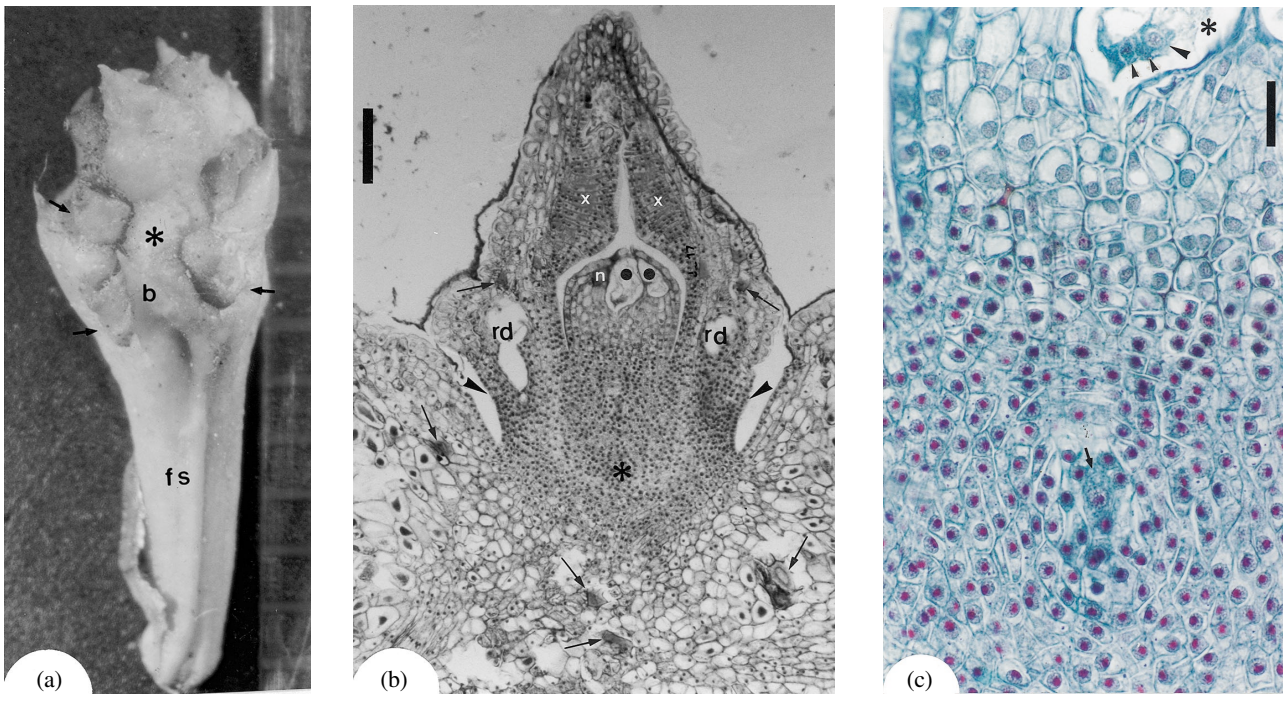


Fig. 7. Development of pollen tubes in the pollen chamber and initiation of the megaspore mother cell in stage VII. (a) (SV) Shown are the female cone stalk (fs), bracts (b), secondary axes (asterisk) and ovules (arrows) in a developing cone. (b) (LS) (Bar = 225 μm) Integuments with resin ducts (rd) are thick. The solid sclerenchyma (x) is found in the thick, inner layer of the integuments. There are a few free sclereids (arrows) in the ground tissue of the integuments as well as in the cone axis below the chalaza (asterisk). Two sections of pollen tubes (rounded dots) are visible in the pollen chamber at the top end of the nucellus (n). The ovule stalk (arrowheads) has become longer. Magnification of the nucellus is shown in Fig. 7(c). (c) (LS) (Bar = 45 μm) One section of a pollen tube in the pollen chamber (asterisk) contains 2 nuclei (small arrowheads), a body cell with a nucleus (big arrowhead) and limited cytoplasm. All 3 units of this pollen tube are located in the area extending from the leading end downwards to the core center of the nucellus. The nucleus of the body cell is just behind these 2 free nuclei. Cells beneath the pollen chamber are large with a large, lightly stained nucleus and are vacuolated. Under them, the tissue shows meristematic characteristics. The megaspore mother cell (arrow) in the nucellar core center is longitudinally elliptic, slightly larger than those of the neighboring cells and contains thick cytoplasm. A few layers of cells are arranged more or less in an elliptic circle around the megaspore mother cell. Under this circle and above the chalaza, a few cells are arranged more or less in 3–(4) longitudinal rows and are compacted closely together.

to form a short but broad pollen tube, which penetrates the nucellus. There never are more than 4 pollen tubes in the same nucellus (Singh, 1961) but as many as 12 have been seen by Favre-Duchartre (1957) and 2–5–(8) by Li *et al*. (1986).

The tube nucleus migrates into the pollen tube. During tube growth, the antheridial cell enlarges considerably and divides into a body (spermatogenous) cell and a stalk (sterile) cell (Singh, 1961). Thus, the pollen tube, i.e., the male gametophyte, contains a large body cell, a stalk nucleus and a tube nucleus (Arnoldi, 1900). The body cell is ca. 14 μm in diameter, and the sterile cell, which is equal to the tube cell in size, is ca. 7 μm in diameter in *C. oliveri* (Li *et al*., 1986). Both of the cells move down and lie free in the cytoplasm of the tube after the stalk cell set its nucleus free from cell wall. The stalk nucleus and the tube nucleus are similar to each other, and they usually precede the body cell, which remains close to them (Singh, 1961). However, Chen *et al*. (1987) observed that the spermatogenous cell extends ahead of these two nuclei in *C. fortunei* and *C. oliveri*. In *C. drupacea*, the contents of the pollen tube lie at its tip (Singh, 1961), but Favre-Duchartre (1957) observed some pollen tubes in which the contents lay far behind the tip.

Cells beneath the pollen chamber are slightly different in appearance from those in the nucellar core (Fig. 7(c)). The former are large and have a large, lightly stained nucleus and may be vacuolated. They appear to be degenerative in cytology. The latter have meristematic characteristics. In the core center of the nucellus, there is a longitudinally elliptic (ca. 30 $\times$ 20 μm) cell, which is slightly larger than the neighboring cells and contains a thick cytoplasm. A few layers of cells are arranged more or less in an elliptic circle around the cell. This cell is the megaspore mother cell.

However, in the earliest stage observed by Favre-Duchartre (1957) in *C. drupacea*, a megaspore mother cell was found to be situated several layers below the epidermis. Kaur (1958) also recognized a single megaspore mother cell organized in *C. drupacea var. pedunculata*. The ovule is homogenous until the megaspore mother cell differentiates (Singh, 1961). One of the sporogenous cells at the center enlarges considerably, especially along its longer axis, and acquires a large nucleus and frothy cytoplasm (Singh, 1961; Li *et al*., 1986). The cell is ca. 48 μm long and 17 μm wide, and the nucleoplasm of the nucleus is thin (Li *et al*., 1986).

A few cells under the megaspore mother cell and above the chalaza are arranged more or less in 3–(4) longitudinal rows and are compacted closely together (Fig. 7(c)). The nucellar cells at the chalazal end have undergone periclinal divisions and have formed such rows, designated by Singh (1961) as "the pavement tissue."

8. Activity of the Functional Megaspore in Stage VIII

An ovulate cone (6 mm in length and thickness) changes slightly in appearance beginning at the end of July (Fig. 8(a)). Bracts thicken and become fleshy at the base. Secondary axes and ovules become thicker. Ovules reach ca. 1400 $\times$ 1300 μm.

There is a large, rounded-elliptic cell in the core center of the nucellus (Fig. 8(b)). Its nucleus (ca. 14 $\times$ 8 μm) and cytoplasm are lightly stained, and the cell wall is thin, so that its outline can not be clearly identified. This is a functioning megaspore, which is morphologically very similar to that in *C. drupacea* (Singh, 1961). A few cells in its neighborhood each have a nucleus flat in shape and/or red in color after staining while the others are without any nucleus. These cells appear to be degenerative while the active megaspore develops.

Usually, the megaspore mother cell undergoes meiosis through horizontal division, forming dyads and then linear tetrads (Singh, 1961; Li *et al*., 1986). Linearly arranged tetrads form, but the upper dyads mostly remain undivided (Kaur, 1958) or only the chalazal megaspore of the tetrads functions (Singh, 1961). The meiosis and the development of the linear tetrads were not studied in this work.

9. The Eight Free Nucleate Macrogametophyte in Stage IX

An ovulate cone is 5 mm long and more than 5 mm thick (Fig. 9(a)). The cone axis has thickened locally where the developing ovules set. Ovules do not develop synchronically in size; a few are bigger (up to ca. 1400 $\times$ 1200 μm)

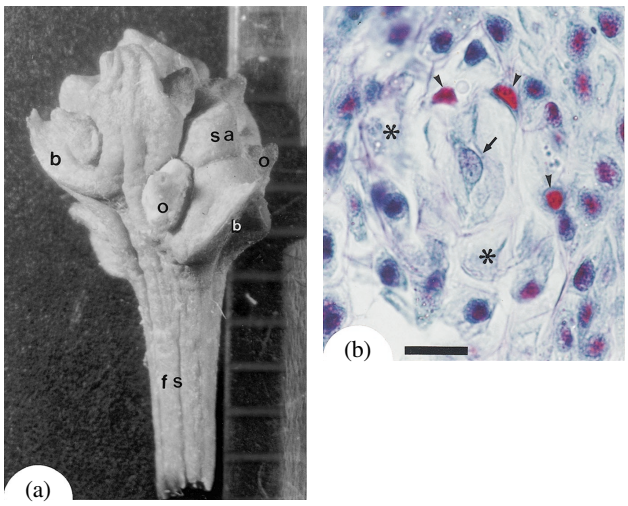


Fig. 8. Activity of the functional megaspore in stage VIII. (a) (SV) Bracts (b), secondary axes (sa) and ovules (o) of an ovulate cone have all grown further, and the cone body is, consequently, thicker than before. The female cone stalk is denoted by fs. (b) (OLS) (Bar = 22.5 μm) The megaspore, which is located in the core center of the nucellus, has a lightly stained nucleus (arrow) as well as cytoplasm and a thin cell wall so that its outline is not clearly identifiable. A few cells (arrowheads) in its neighborhood each contain a nucleus that is flat in shape and/or red in color after staining while a few others (asterisks) have no nucleus.

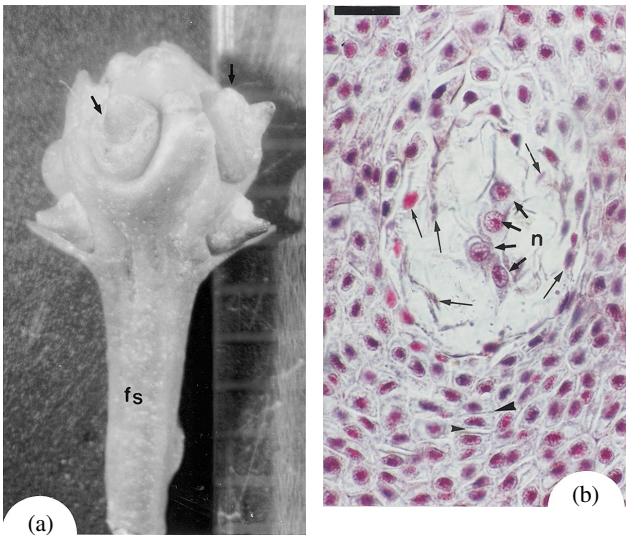


Fig. 9. The 8-nucleate macrogametophyte in stage IX. (a) (SV) The cone axis has thickened locally where the ovules are located. Ovules have not developed synchronically; i.e., a few (arrows) are larger than the others. The female cone stalk is denoted by fs. (b) (LS) (Bar = 45 μm) There is a longitudinally elliptical-rounded space in the core center of the nucellus, within which a macrogametophyte is visible. The macrogametophyte contains 4 large, free nuclei (n) in this section along with 4 other nuclei in 2 other serial sections. These nuclei are connected by few cytoplasm-strand. A few degenerative cells (longer arrows) around the space either contain a lightly stained nucleus or are collapsed with a flat shape and are arranged in circular layers. A few longitudinal rows of cells with horizontal walls (arrowheads), similar to a sidewalk (pavement) in construction, are found under the macrogametophyte.

while others are smaller. The small ones appear to have ceased development.

In the nucellar core center of an ovule that is developing well (Fig. 9(b)), there is a longitudinally elliptic-rounded space, ca. 160 μm long and 120 μm wide. It envelops 8 free, relatively large nuclei (ca. 14 μm in dia.), which are tightly connected by few cytoplasm strands (ca. 70 μm long). This is the female (macro-) gametophyte. Around this space, cells contain a lightly stained nucleus or have collapsed into a flat shape and are arranged in circular layers. The pavement tissue under the macrogametophyte becomes more conspicuous.

In *C. drupacea*, the functional megaspore becomes vacuolated, and its nucleus divides to form a 8- or 16-nucleate, somewhat elongated female gametophyte, which continues to enlarge by consuming the surrounding nucellar cells (Singh, 1961).

10. The Juvenile Macrogametophyte in Stage X

Ovulate cones look almost the same as in the previous stage (Fig. 10(a)). One cone body may be less than 6 mm long and ca. 5 mm thick. A few ovules are bigger (2000 μm long and thick) while others are smaller. Compared to the

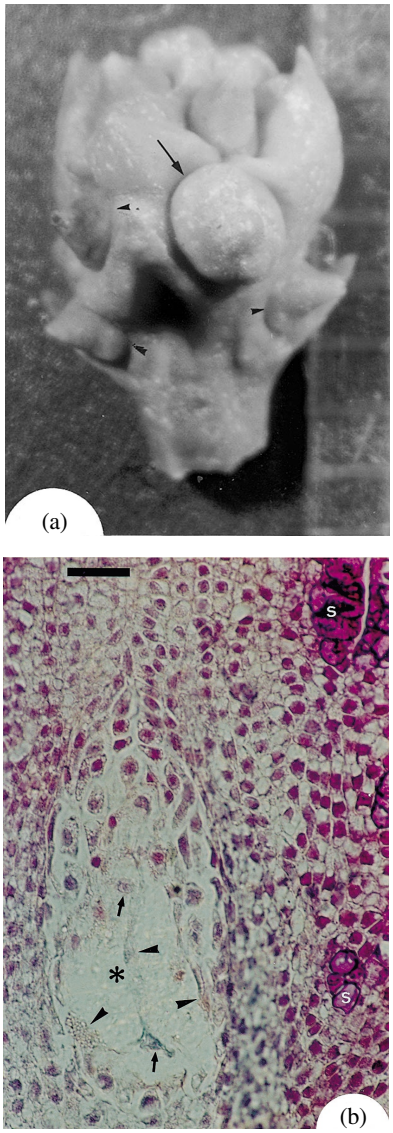


Fig. 10. The juvenile macrogametophyte in stage X. (a) (SV) One of the ovules (arrow) is large, while others are small (arrowheads). (b) (LS) (Bar = 50 μm) A few cytoplasm strands (arrowheads) with free nuclei (arrows) are distributed within a longitudinally elliptic space (asterisk). A few clusters of sclereids (s) with thick, lignified cell walls are found at the junction between the integuments and the nucellus as well as around the macrogametophyte in the ovule.

previous stage, integuments become longer and thicker while the upper part of nucellus remains almost the same size. The spaces in resin ducts in the integuments are longer and wider than before. The ovule stalk becomes more conspicuous.

Pollen tubes are present in the pollen chamber. A few clusters of sclereids with thick and lignified cell walls are found at the junction of the integuments and the nucellus as well as around the macrogametophyte in the ovule (Fig. 10(b)). However, in other *Cephalotaxus* species, some epidermal cells at the junction of the nucellus and the integument accumulate tannin (Singh, 1961; Li *et al.*, 1986).

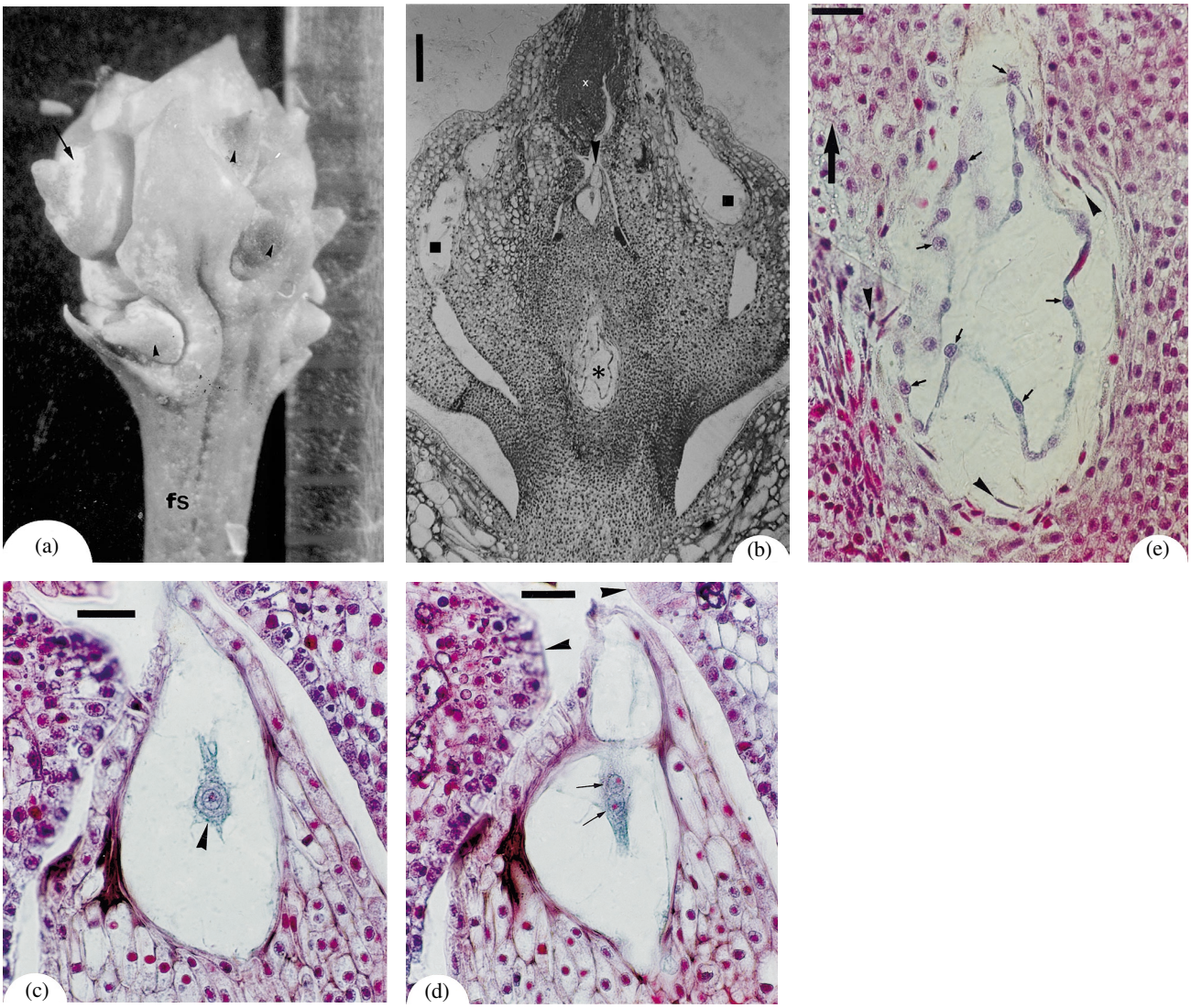


Fig. 11. The 150-nucleate macrogametophyte in stage XI. (a) (SV) One of the ovules (arrow) is large, while the others (arrowheads) appear to have ceased developing. The female cone stalk is denoted by fs. (b) (LS) (Bar = 250 μm) One ovule body has thickened, and the stalk has become more conspicuous. Wide resin ducts (square dots) are present in the thickened integuments and extend from the apex of the integuments to the base of the ovule. The solid sclerenchyma (x) has developed well in the inner parts of the integuments along the narrow micropylar canal. A pollen tube is present in the pollen chamber (arrowhead). A macrogametophyte (asterisk) is developing in the core base of the nucellus. The tissues around the macrogametophyte appear to be meristematic. Magnifications of the pollen tube and the macrogametophyte from 2 serial sections of the same ovule are shown in (c), (d) and (e) respectively. (c) (LS) (Bar = 45 μm) The pollen tube (microgametophyte) contains a cell (arrowhead), which has a nucleus with a conspicuous nucleolus and some surrounding cytoplasm. (d) (LS) (Bar = 45 μm) The pollen tube contains 2 nuclei (arrows), each with a conspicuous nucleolus, above the cell. These 2 nuclei are equal in size but smaller than that of the cell. The inner layer tissues (arrowheads) under the sclerenchyma of the integuments have extruded horizontally inwards towards the top part of the nucellus. Each cell in the tissues is small and has a relatively large nucleus surrounded by dark-stained cytoplasm. (e) (LS) (Bar = 50 μm) A macrogametophyte contains many free nuclei (arrows). These nuclei are distributed sparsely within a more or less elliptic space and are connected by cytoplasm in a long strand. The space has extended at the top into a small concave subunit. There are a few collapsed cells (arrowheads) at the periphery of the space, outside of which the tissues are composed of small cells densely compacted. The axial direction is indicated by the large arrow.

There is an elliptic space that is longitudinally orientated, ca. 180 μm high and 120 μm wide (Fig. 10(b)). A few free nuclei are distributed at the periphery of the space and are connected with cytoplasm strands. This macrogametophyte becomes slightly larger compared to the previous stage during the winter.

11. The 150 Free Nucleate Macrogametophyte in Stage XI

A developing ovulate cone is almost 7 mm long and ca. 5.5 mm thick (Fig. 11(a)). An ovule that develops well reaches a length of 2700 μm including the stalk and has a thickness of

2500 μm. The ovule stalk becomes more conspicuous (Fig. 11(b)). Wide resin ducts are present in the thickened integuments, extending from the apex of the integuments to the base of the ovule. The tissues in the inner part of the integuments along the micropylar canal are completely differentiated, solid sclerenchyma. Also, tissues under this sclerenchyma extrude horizontally inwards against the nucellar top part (Fig. 11(d)). Each cell in the tissues is small and has a relatively large nucleus with a dark-stained cytoplasm. These tissues appear arise from the inner epidermis after hyperplasy.

In the pollen chamber, a pollen tube (microgametophyte) contains a body cell (Fig. 11(c)), and there are 2 nuclei (the tube and the stalk nucleus) (ca. 16 μm in dia.) in the vicinity (Fig. 11(d)). The body cell has a nucleus (ca. 18 μm in dia.) with a conspicuous nucleolus and a few cytoplasms surrounding it. Those 2 nuclei are equal in size, and each has a conspicuous nucleolus.

A macrogametophyte is much larger (Fig. 11(e)) and contains ca. 150 free nuclei (ca. 14 μm in dia.). Nuclei are distributed sparsely within a more or less elliptic space (ca. 480 μm long and 240 μm wide) and are connected side by side with a single circular protoplast strand in the longitudinal section. The space extends at the top into a small concave subunit. Also, in other *Cephalotaxus* species, the free nuclei are arranged in a single layer along the periphery, leaving a central vacuole (Singh, 1961; Li *et al*., 1986). However, in the 32-nucleate stage, earlier than in *C. wilsoniana* studied here, the gametophyte has a 2-nucleate finger-like projection at the micropylar end in *C. drupacea* (Singh, 1961).

There are a few collapsed cells around the macrogametophyte, outside of which the tissues are composed of small, densely compacted cells (Fig. 11(e)). Proliferation seems to have happened to the tissues surrounding the developing macrogametophyte (Fig. 11(b)), and these cells are characteristic of meristem.

12. The 230 Free Nucleate Macrogametophyte in Stage XII

A developing ovulate cone is almost 7 mm in length and less than 5.5 mm in thickness (Fig. 12(a)). The ovule body is compressed globose and may reach ca. 2400 × 2400 μm on an elongated stalk (600 μm long) (Fig. 12(b)). The apical end of the integements has a short, conical shape, consisting of a thick layer of outer epidermis, a few layers of ground tissues in the middle and a solid mass of sclerenchyma inside. The conical apex of the integements remains the same size as in the previous stage.

A newly formed section of the micropylar canal is found between the basal border of the sclerenchyma and the top of the nucellus. The inner layer tissues of the integuments along this newly formed section are meristematic with periclinal

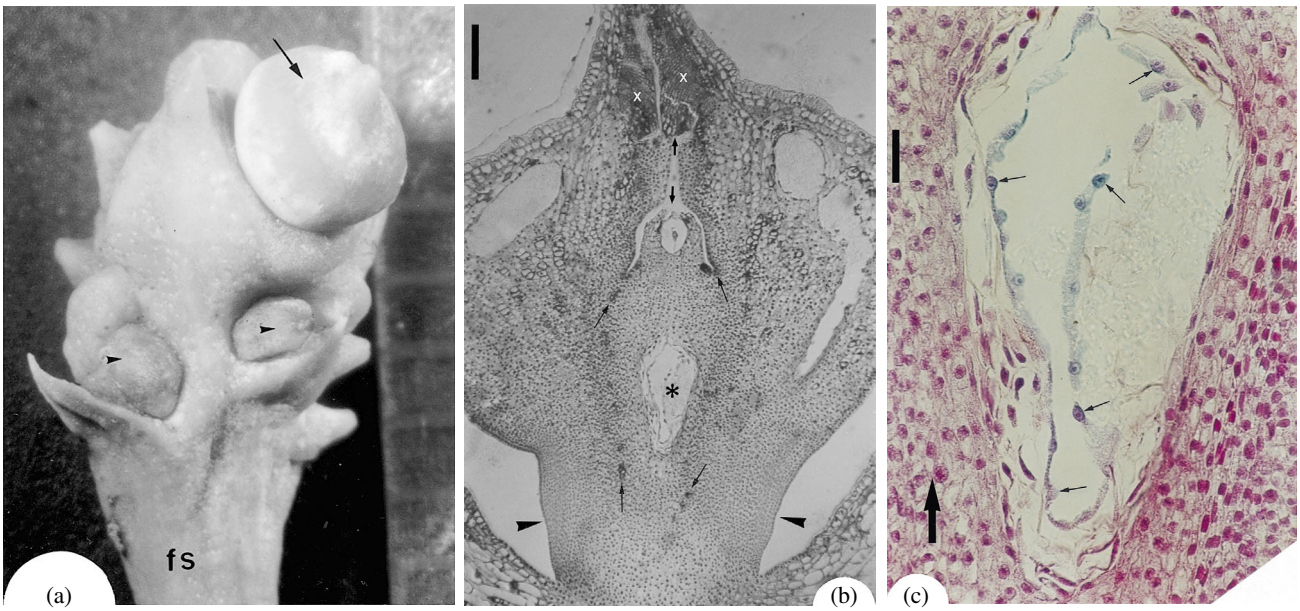


Fig. 12. The 230-nucleate macrogametophyte in stage XII. (a) (SV) One ovule (arrow) is larger than the others (arrowheads), which remain almost the same size as in the previous stage. The female cone stalk is denoted by fs. (b) (LS) (Bar = 250 μm) One ovule body is compressed globose on an elongated stalk (arrowheads). The apical ends of the integements are short and conical in shape, and are composed of a thick layer of outer epidermis, a few layers of ground tissues in the middle and a solid mass of sclerenchyma inside (x). A newly formed section of micropylar canal (the extension between the 2 short arrows) is found between the basal border of the sclerenchyma in the integuments and the top of the nucellus. A few small areas of tissue (long arrows) composed of dark-stained cells are scattered along the border between the integuments and the nucellus. The top of the macrogametophyte space (asterisk) is rounded. The macrogametophyte is magnified in the following figure. (c) (LS) (Bar = 50 μm) Many free nuclei (arrows) of the macrogametophyte are connected by a long cytoplasm strand and are distributed in a large space. The axial direction is indicated by the large arrow.

cell divisions. They are 6 – 8 cells deep and extend 12 – 14 cells along the canal. The extension of the micropylar canal appears to result from elongation of the thick integuments.

A pollen tube is present in the pollen chamber and contains 2 nuclei and a cell. A few small areas of tissue, which are composed of dark-stained cells, are scattered along the border between the integuments and the nucellus. These cells probably contain tannins.

The macrogametophyte is now larger in the core base of the nucellus. A single protoplast membrane containing ca. 230 free nuclei (ca. 14 μm in dia.) (Fig. 12(c)) is distributed at the periphery of a large space (ca. 520×260 μm). The top of the space is rounded, no longer concave (Fig. 12(b)) as in the previous stage. Similarly in *C. drupacea*, the finger-like projection of the macrogametophyte becomes level and is no longer conspicuous later after the stage of ca. 128 nuclei (Singh, 1961). During the development of the macrogametophyte with free nuclei, the nucellar cells contain many starch grains (Li *et al*., 1986), which were not examined in our study.

13. The Macrogametophyte of a Thin Even Membrane in Stage XIII

A still developing ovulate cone body is ca. 9.5 mm long. Ovules reach various sizes and have a prominent conical apex in the integuments (Fig. 13(a)). The biggest ovule body may reach 5000×3800 μm while others cease growing.

The newly formed section of the micropylar canal is further elongated. Along this new section of the micropylar canal, the inner layer tissues in the integuments are still meristematic in morphology and are 10 – 13 cells deep. A few segments of layered sclerenchyma, the cells of which have thick, dark-stained cell walls, are distributed in the area of the junction of the nucellus and the integuments basipetally and around the entire macrogametophyte (Fig. 13(b)).

The macrogametophyte consists of a smooth thin membrane of protoplast (numerous free nuclei and cytoplasm) which envelops a huge, elliptic space (ca. 1600×880 μm). Nuclei distributed evenly in the matrix of cytoplasm (Fig. 13 (c)) are slightly larger than those in the nucellar cells outside, around the macrogametophyte.

In a later stage, the macrogametophyte also becomes oblong and has a thin membrane in other *Cephalotaxus* species (Singh, 1961; Li *et al*., 1986). Sokolowa (1890) and Favre-Duchartre (1957) also observed such a membrane. Within this thin layer of cytoplasm, the free nuclei are usually distributed unevenly, but sometimes evenly, and are connected with spindle fibers (Li *et al*., 1986). Spindle fibers, however, occur later up to the stage of walls formation among nuclei in this work.

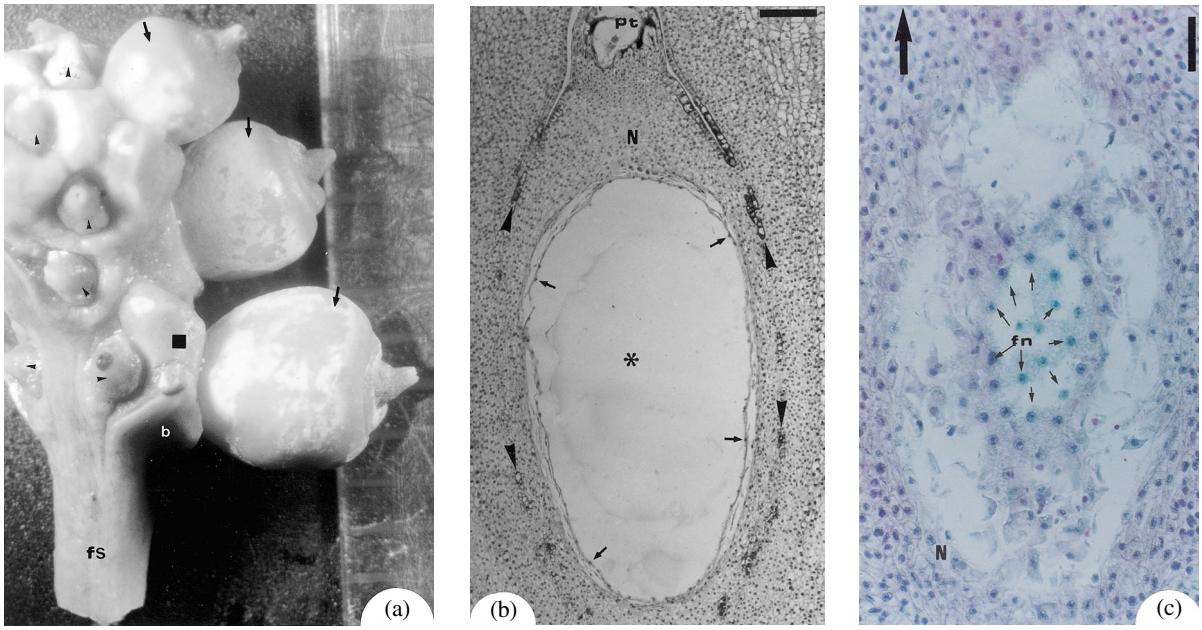


Fig. 13. The macrogametophyte of a thin even membrane in stage XIII. (a) (SV) Larger ovules (arrows) vary in size while others (arrowheads) have ceased growing. The secondary axis (square dot) beside the largest one is thick. A bract is denoted by b and the female cone stalk by fs. (b) (LS) (Bar = 225 μm) The picture is focused on the center of the entire macrogametophyte. The macrogametophyte consists of a thin, smooth membrane of protoplast, numerous free nuclei (arrows) and cytoplasm, which envelops a huge, elliptic space (asterisk). A few segments of layered sclerenchyma (arrowheads), cells in which have thick, darkly-stained cell walls, are distributed from the junction of the nucellus (N) and the integuments basipetally around the entire macrogametophyte. The pollen tube is denoted by pt. (c) (LS) (Bar = 90 μm) A feature of the macrogametophyte is magnified from another section of the same ovule shown in (b). The picture is focused on the side periphery of the macrogametophyte. Numerous free nuclei (fn) are distributed evenly in the matrix of the cytoplasm. These nuclei are slightly larger than those of the nucellar cells (N) located outside, around the macrogametophyte. The axial direction is indicated by the large arrow.

14. The Macrogametophyte of a Thickened Uneven Layer in Stage XIV

Ovules may reach 7.0 × 5.0 mm in size. The space occupied by the macrogametophyte has expanded to 4000 × 1600 μm. The macrogametophyte consists of a solid protoplast layer with an irregular thickness (20 – 40 μm) of 1 – 3 nuclei (Fig. 14(a)). Free nuclei in the macrogametophyte are distributed unevenly in the cytoplasm on the surface (Fig. 14 (b)). Singh (1961) reported that the distribution was uneven until after the last division. Theoretically, twelve times of successive mitosis in the functional megaspore results in 4096 free nuclei in the peripheral cytoplasm around the central vacuole. Actually, 4234 nuclei were counted by Li *et al*. (1986).

A pollen tube is found in the pollen chamber. Its rounded body cell (ca. 36 μm in dia.) with a nucleus (ca. 20 μm in dia.) is located in the tube behind while the tube and stalk nuclei (ca. 22 μm in dia.) are in front of the body cell.

15. Cell Walls Formation among Free Nuclei of the Macrogametophyte in Stage XV

The biggest ovule of a cone is ca. 7.0 × 5.5 mm. The space occupied by the macrogametophyte reaches 3700 × 1700 μm.

The entire macrogametophyte appears to be a collapsed balloon (Fig. 15(a)). The protoplast layer of the macrogametophyte is reversed from one pole to the other pole, therefore, two layers meet together. This may be artifact of dehydration of material during the microtechnical process.

The macrogametophyte protoplast becomes a layer with uninucleate even thickness. At the micropylar end of the macrogametophyte, anticlinal cell walls have separated all free nuclei from one another completely to form 5 – 6-gonal cells on the surface (Fig. 15(b)). At the basal end of the macrogametophyte, the protoplast begins to form anticlinal cell walls with spindle fibers among a few free nuclei, which are distributed evenly (Fig. 15(c)). Singh (1961) noted that the spindle fibers also involved in wall formation. Wall formation takes place by means of centripetally advancing proliferated cells (Singh, 1961; Li *et al*., 1986).

There are two pollen tubes in the pollen chamber (Fig. 15(a)). The composition of the pollen tubes is similar to that in the previous stage. (The body cell is 34 – 40 μm with a nucleus of ca. 20 μm; the tube and stalk nuclei are ca. 20 × 18 μm.)

16. Initiation of Archegonia in Stage XVI

Ovules develop further (up to 10.0 × 6.0 mm) and leave a space, ca. 5000 × 2100 μm, for the macrogametophyte. The border of the macrogametophyte is clear and is composed of a thick wall (Fig. 16). The matrix of the solid macrogametophyte is composed of parenchymatous tissue with no intercellular space. Cells are of various forms in the longitudinal section, from square to long rectangular, and are arranged more or less in centripetal-orientated rows. In the last stage, the macrogametophytes of other *Cephalotaxus* species are also filled with cells that are arranged in rows converging toward the center (Fujita, 1961; Singh, 1961; Li *et al*., 1986). These cells contain scanty amounts of cytoplasm and have thin walls in *C. drupacea* (Singh, 1961) and in *C. wilsoniana* as found here.

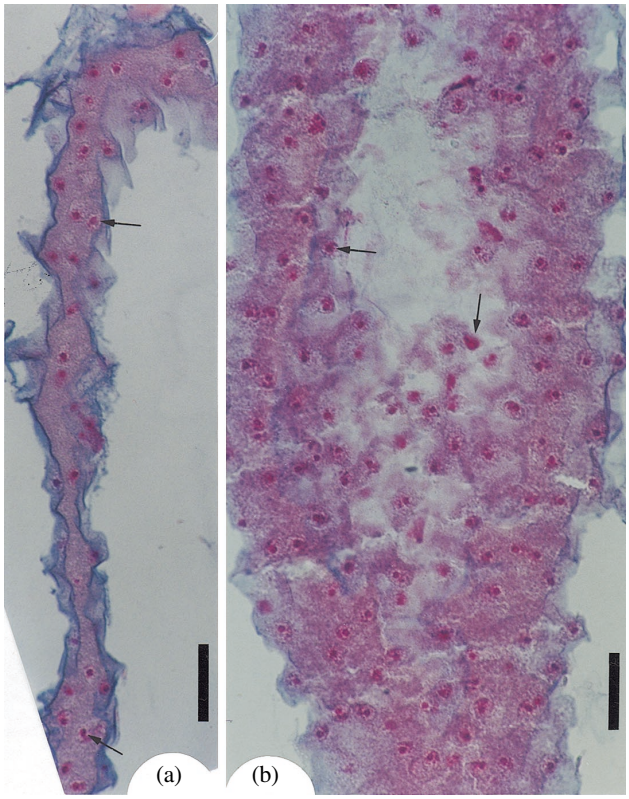


Fig. 14. The macrogametophyte of a thickened uneven layer in stage XIV. (a) (LS) (Bar = 40 μm) A macrogametophyte is composed of a solid protoplast layer of 1 – 3-nucleate irregular thickness in the cross view. Nuclei are indicated by arrows. (b) (LS) (Bar = 40 μm) Free multinuclei (arrows) of the female gametophyte are distributed unevenly in the cytoplasm in the surface view.

A rare larger cell (ca. 130 × 70 μm) is found at the micropylar end of the macrogametophyte right below the pollen chamber (Fig. 16). It has a relatively large nucleus (ca. 20 μm in dia.) attached to the macrogametophyte wall; from the nucleus, a cluster of cytoplasm strands radiates outwards. Its neighboring cells each contain a smaller nucleus (ca. 16 μm in dia.). There are totally 3 such cells each with a large nucleus in the same macrogametophyte. They are the archegonial initials.

The macrogametophyte gives rise to the archegonium at the micropylar end (Coker, 1907). The archegonial initials consist of a few (Li *et al*., 1986) or 2 – 5 (Singh, 1961) surface cells at the micropylar end of the macrogametophyte. The

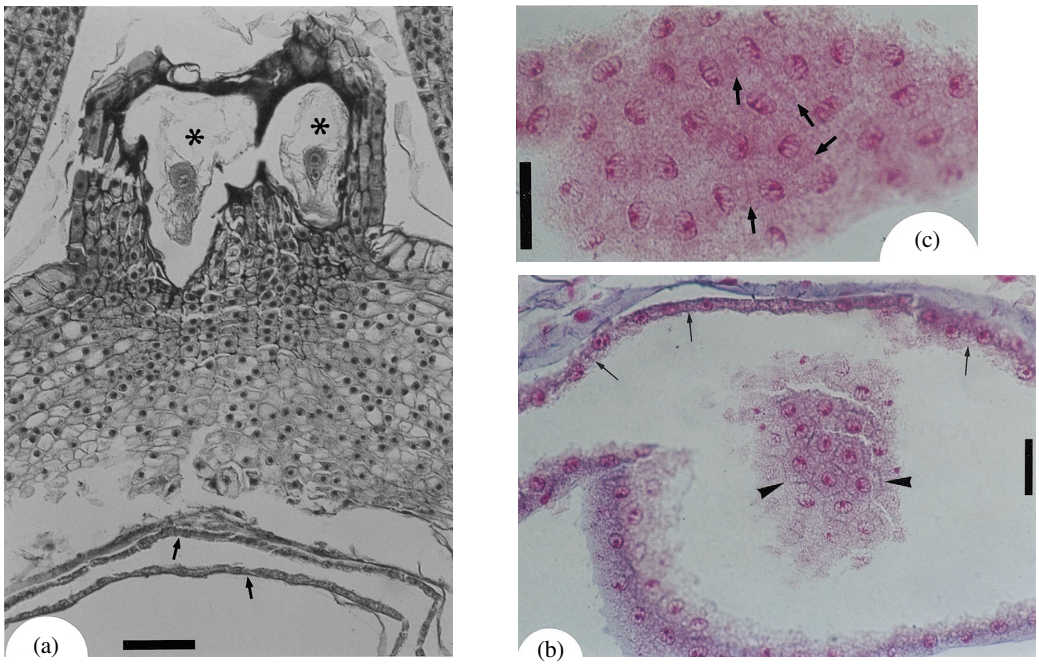


Fig. 15. Cell wall formation among free nuclei of the macrogametophyte in stage XV. (a) (LS) (Bar = 80 μm) One entire macrogametophyte appears to be a collapsed balloon. The protoplast layer of the macrogametophyte is reversed from one pole to the other pole, therefore, two layers (arrows) meet together. There are two pollen tubes (asterisks) in the pollen chamber. (b) (LS) (Bar = 40 μm) A macrogametophyte protoplast has become a layer with uninucleate even thickness. At the micropylar end of the gametophyte, anticlinal cell walls (arrows) have separated all free nuclei completely to form 5 – 6-gonal cells (arrowheads) in the surface view of the protoplast. (c) (LS) (Bar = 40 μm) At the basal end of the female gametophyte, the protoplast is beginning to form anticlinal cell walls (arrows) with spindle fibers among a few free nuclei, which are distributed evenly.

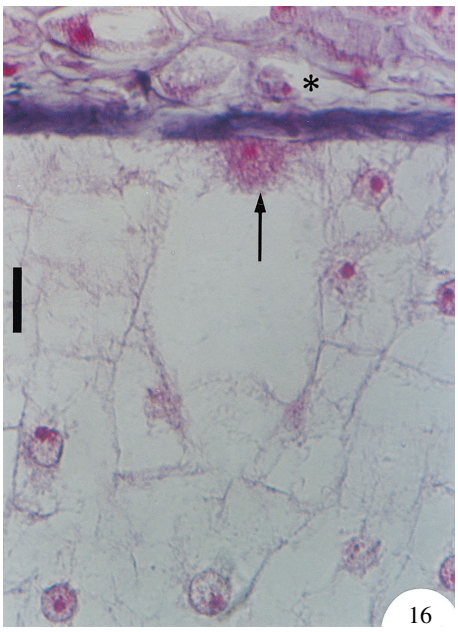


Fig. 16. Initiation of archegonia in stage XVI. (LS) (Bar = 20 μm) A rare, larger cell is found in the micropylar end of the solid parenchymatous macrogametophyte right below the pollen chamber. It has a relatively large nucleus (arrow) attached to the gametophyte wall; from the nucleus, a cluster of cytoplasm strands radiates outwards, while the other neighboring cells each contain a smaller nucleus. Nucellar tissues are indicated by the asterisk.

initials are ca. 70 μm long (Chen *et al*., 1987). In the immediate vicinity of the archegonial initials, a few cells undergo anticlinal division and differentiate into the jacket layer (Singh, 1961; Li *et al*., 1986). Such cell divisions were not observed in this study, but several cells neighboring the archegonial initial appeared to be ready to initiate the jacket (Fig. 16).

The pollen tubes remain in the pollen chamber. The body cell (ca. 50 μm in dia.) and its nucleus (ca. 28×16 μm) may become larger than in the previous stage while the tube and stalk nuclei (ca. 22×16 μm) remain the same size.

17. Development of the Archegonium and Pollen Tubes Reaching the Macrogametophyte in Stage XVII

Ovules expand in size up to 12.0×7.0 mm. Cells of the macrogametophyte around the archegonia undergo division frequently and contain only a nucleus each as usual (Fig. 17(a)).

There are (2)–4–(7) archegonia per macrogametophyte. Usually, there are 2 – 5 archegonia in *C. drupacea*, situated singly at the micropylar end of the macrogametophyte (Singh, 1961). The archegonia are constructed in the form of a cyst, which is embedded in the matrix of the macrogametophyte (Fig. 17(a)). The cyst has a wall layer, which envelops a large cell within a cavity. The wall consists of a smooth, closed

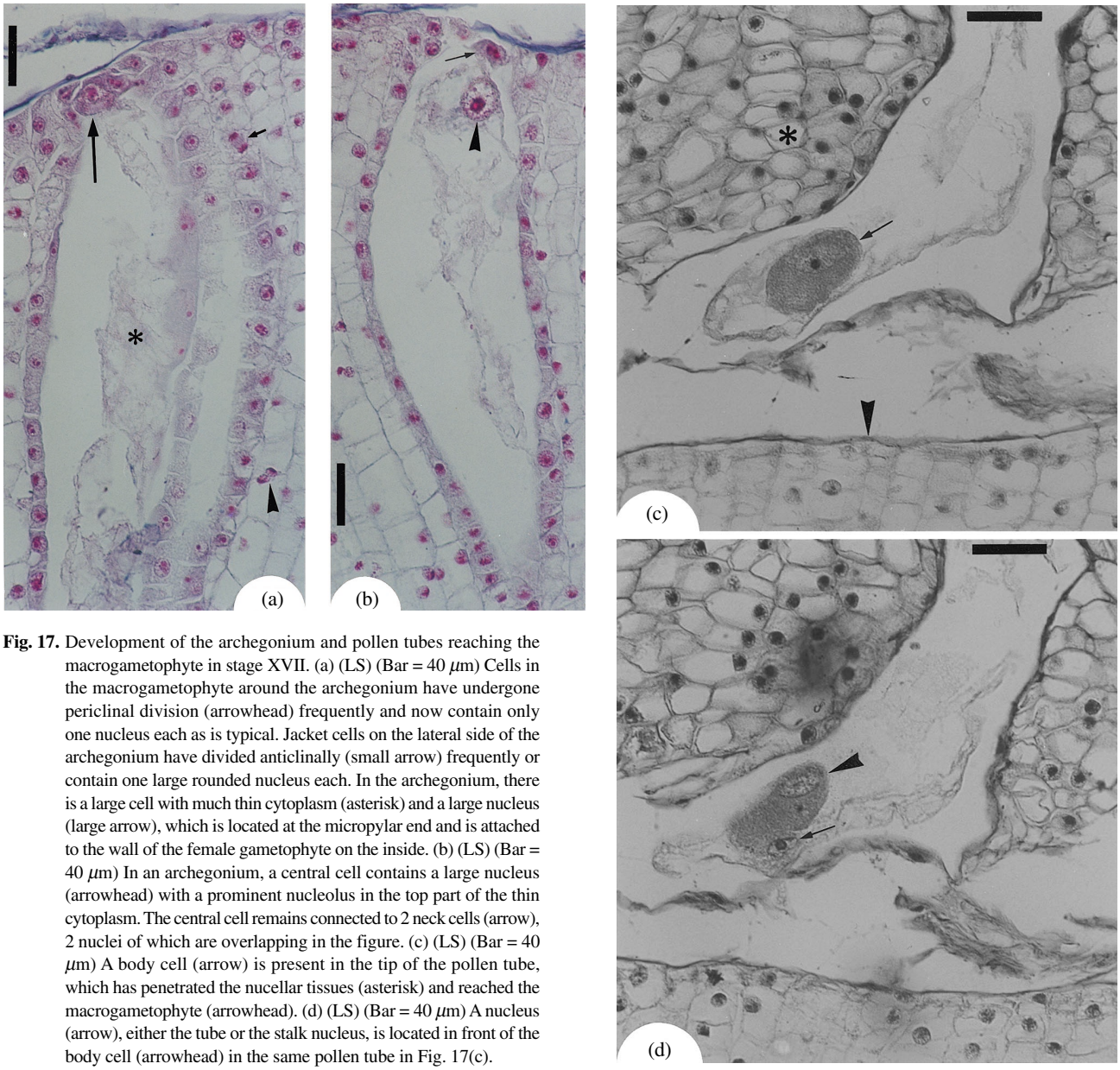


Fig. 17. Development of the archegonium and pollen tubes reaching the macrogametophyte in stage XVII. (a) (LS) (Bar = 40 μm) Cells in the macrogametophyte around the archegonium have undergone periclinal division (arrowhead) frequently and now contain only one nucleus each as is typical. Jacket cells on the lateral side of the archegonium have divided anticlinally (small arrow) frequently or contain one large rounded nucleus each. In the archegonium, there is a large cell with much thin cytoplasm (asterisk) and a large nucleus (large arrow), which is located at the micropylar end and is attached to the wall of the female gametophyte on the inside. (b) (LS) (Bar = 40 μm) In an archegonium, a central cell contains a large nucleus (arrowhead) with a prominent nucleolus in the top part of the thin cytoplasm. The central cell remains connected to 2 neck cells (arrow), 2 nuclei of which are overlapping in the figure. (c) (LS) (Bar = 40 μm) A body cell (arrow) is present in the tip of the pollen tube, which has penetrated the nucellar tissues (asterisk) and reached the macrogametophyte (arrowhead). (d) (LS) (Bar = 40 μm) A nucleus (arrow), either the tube or the stalk nucleus, is located in front of the body cell (arrowhead) in the same pollen tube in Fig. 17(c).

layer of cells, i.e., the jacket. The jacket cells are small and contain a relatively large, rounded nucleus each with thick cytoplasm. The jacket consists of a single layer of inconspicuous cells (Singh, 1961; Li *et al*., 1986). These cells are isodiametric to begin with but later become elongated and uninucleate. Favre-Duchartre (1957) noted, however, that the jacket cells frequently contain 2 nuclei. Jacket cells frequently divide anticlinally to the archegonium in this stage (Fig. 17 (a)), which means that the archegonium is growing.

The archegonia, including the jacket, may grow up in size to 720 × 200 μm. A week later after initiation, on 10th May, an archegonium 700 μm long was found in *C. fortunei* (Chen *et al*., 1987). In this study, most of the archegonia were independently arranged within the top part of the macrogametophyte, but two neighboring archegonia occasionally shared a common lateral layer of the jacket. Rarely, 2 or 3 archegonia are adjacent to one another and sometimes have a single common jacket (Li *et al*., 1986) or have a common archegonial cavity (Singh, 1961).

In the archegonium, there is a large cell having very thin cytoplasm and a big nucleus, which is located at the micropylar end, attached to the wall of the macrogametophyte on the inside (Fig. 17(a)). This large cell hangs from the "ceiling" in the archegonial cavity and appears to become the female reproductive cell. In another archegonium, the large cell contains a large nucleus (ca. 36 × 30 μm) with a prominent nucleolus (ca. 13 × 10 μm) in the top part of the thin cytoplasm (Fig. 17(b)). The large cell is connected to neck

cells at the top. The large cell is the central cell.

In general, the archegonial initials divide periclinally to form a large central cell and a small neck initial in the *Cephalotaxus* species (Singh, 1961; Li *et al*., 1986). The nucleus of the central cell has a prominent nucleolus and lies just below the neck cells. The neck initial divides anticlinally to form a 2 – 5-celled neck composed of one tier (Singh, 1961). The outer tangential walls of the neck cells are always thick. Since the neck cells are located at the top of the macrogametophyte on the inside, their outer walls are actually the wall of the macrogametophyte, as Fig. 17(b) shows. Occasionally, however, no neck cells are found in the other archegonium. There are degenerative nucleolus-like bodies or remnants of cell walls at the location where the neck is usually found in an archegonium. In this case, the central cell has no large nucleus inside.

A body cell is present inside the tip of the pollen tube, which has penetrated the nucellar tissues and reaches the macrogametophyte (Fig. 17(c)). The body cell (ca. 70×40 μm) with thick cytoplasm and its nucleus (ca. 36×20 μm) are larger than in the previous stage while the tube and the stalk nucleus remains the same size (22×16 μm) (Fig. 17(d)). The latter are located in front of the former in the same pollen tube.

Singh (1961) noted that in the spring of the second year, the tubes grew quickly and reached the archegonia, which were almost mature. The body cell enlarges and reaches ca. 58 μm along the long axis in *C. oliveri* (Li *et al*., 1986). Its cytoplasm is thick and is arranged radially at the center as a blepharoplast. The body cell nucleus is ca. 22 μm in dia. and has a nucleolus 3 μm thick. The nucleus is lightly stained (Chen *et al*., 1987).

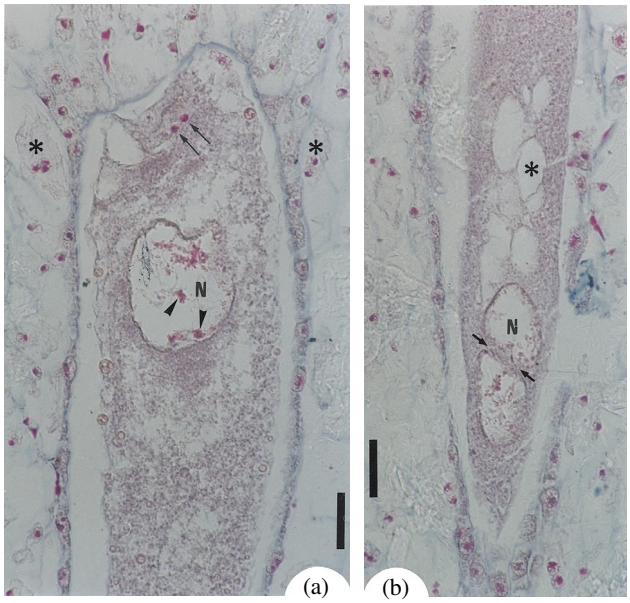


Fig. 18. Differentiation of the egg cell in stage XVIII. (a) (LS) (Bar = 40 μm) One archegonium is embedded deep inside the female gametophyte, and its top is open to the archegonium chamber. 2–(3)-nucleate parenchyma cells (asterisk) of the macrogametophyte are commonly found around the archegonia. The egg cell has more and thicker cytoplasm, almost filling the entire archegonium, than before. Two red-stained nucleolus-like bodies (arrows) are scattered in the top part of the cytoplasm. A large, nearly rounded nucleus (N) is present in the upper part of the egg cell. The nucleus has a thick membrane and contains a few nucleolus-like bodies (arrowheads) or amorphous substances. (b) (LS) (Bar = 40 μm) In another archegonium, the egg cell contains a nucleus (N) in the lower part and several elliptical vacuoles (asterisk) in the middle part. The large nucleus is elongated longitudinally with a constriction (arrows) in the middle, which separates the nucleus into 2 parts at the focus point of this figure.

18. Differentiation of the Egg Cell in Stage XVIII

The fecund ovules enlarge gradually (14.5×9.5 mm). There frequently are 2–(3)-, but not more than 3-, nucleate parenchyma cells around the archegonia in the macrogametophyte (Fig. 18(a)). Fujita (1961) defined the macrogametophyte as the endosperm in *C. drupacea*. Its cells each contains (1)–4–8–(15) nuclei but only one nucleus during the early stage.

An archegonium may be embedded deeper in the macrogametophyte, thus, it may be open at the top in the archegonial chamber (Fig. 18(a)). Singh (1961) noted that the cells of the macrogametophyte at the micropylar end lengthen upwards and divide periclinally during the formation stage of the archegonium. The neck cells appear sunken so that each archegonium now has its own archegonial chamber. However, no periclinal divisions in those cells of the macrogametophyte were noted by Favre-Duchartre (1957) in the same species, *C. drupacea*, and studied here.

The archegonia, including the jacket, may reach ca. 1120×200 μm, the maximum size ever observed, in this stage. Chen *et al*. (1987) described the mature egg as being shaped like a carrot; i.e., the upper end is obtuse and the lower end is pointed. It is 1000 – 1100 μm long and 100 – 108 μm wide in *C. fortunei*; ca. 910 μm long and 85 μm wide in *C. oliveri*. The latter size data, however, were obtainted for a mature archegonium by the same team (Li *et al*., 1986). It seems that Chen *et al*. (1987) considered the size of an egg to be equal to that of an archegonium, excluding the jacket.

The egg cell has more and thicker cytoplasm, almost filling the entire archegonium, than the central cell did before (Fig. 18(a)). In the top part of the cytoplasm, there are two red-stained nucleolus-like bodies, which might arise from the degenerative ventral canal nucleus. The central cell divides to form the egg nucleus and the ventral canal nucleus (Coker, 1907). Both nuclei are equal in size and are not separated by a wall (Singh, 1961). However, the ventral canal nucleus is ephemeral and is soon reduced in size (Singh, 1961; Chen *et al*., 1987). The ventral canal nucleus was not observed in this study. In the middle part of the cytoplasm of another archego-

nium, several elliptical vacuoles were found (Fig. 18(b)). At this stage in *C. fortunei*, large vacuoles in the cytoplasm of the egg become gradually smaller, become scattered at the periphery of the cytoplasm and finally disappear (Chen *et al*., 1987).

An egg nucleus is present in the upper part (Fig. 18(a)) or the lower part (Fig. 18(b)) of the cell and is nearly round in shape (Fig. 18(a)) (ca. 110 × 90 μm) or is elongated longitudinally with a constriction (Fig. 18(b)). It has a thick membrane and contains only a few red-stained nucleolus-like bodies or amorphous substances, instead of the nucleolus and chromatin. While the ventral canal nucleus is reduced, the egg nucleus is considerably enlarged and has migrated downward (Singh, 1961) and stayed in the upper to middle part of the egg (Li *et al*., 1986). Meanwhile, minute darkly staining bodies appear along the inner surface of the nuclear membrane, the nucleolus disappears and the chromatin becomes indistinct. This was also observed here in *C. wilsoniana*.

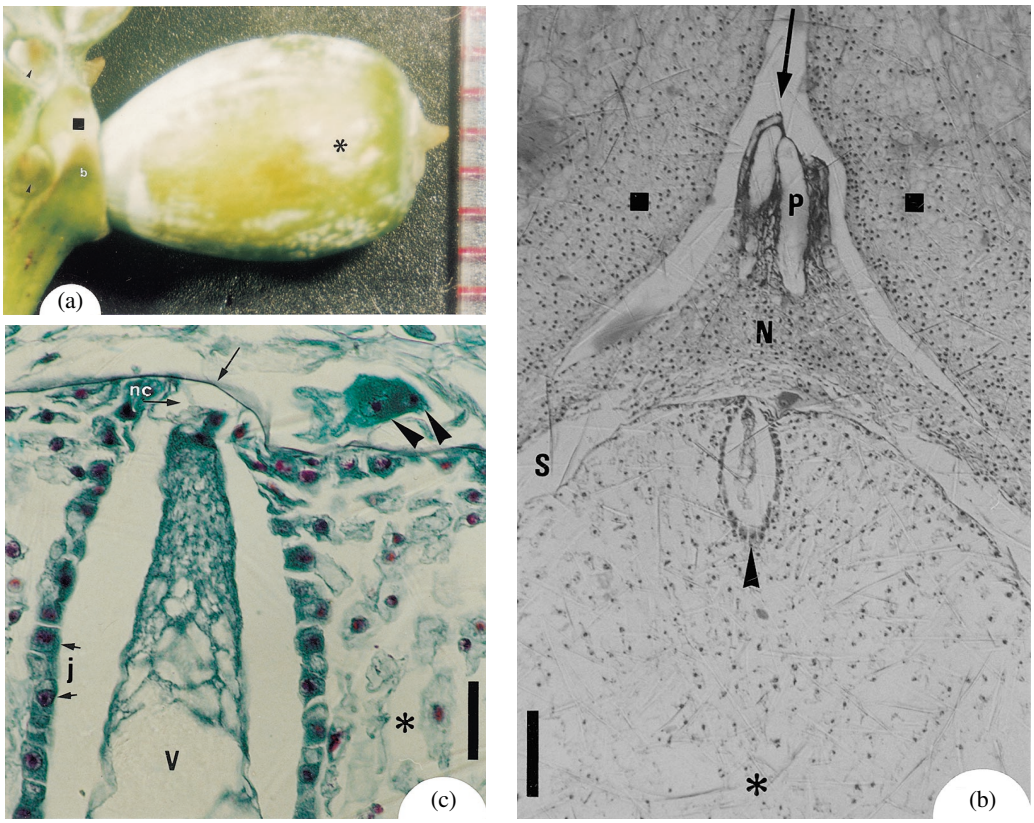
19. Male Gametes Reaching the Mature Archegonium and Fertilization in Stage XIX

Usually, only one ovule, occasionally up to three, in a cone grow further and reach 17.0 × 10.0 mm in size (Fig. 19 (a)). Similarly, only one or two ovules mature per cone were found in other studies (Liu and Liao, 1980; Liu *et al*. 1988) or in *C. drupacea* (Singh, 1961).

Nucellar tissues around the pollen chamber, which may enclose pollen tubes, appear to become degenerative (Fig. 19(b)). The other tissues beneath the pollen chamber and the inner layers of the integuments are composed of small cells densely compacted. A large elliptic, solid macrogametophyte (ca. 7.0 × 3.0 mm) lies below. It has contact with the nucellus only at its micropylar end while its other part has a smooth surface and is completely separate from the nucellus with a vast space in between. The nucellar tissues around the macrogametophyte have become relatively thin. They are pressed into a thin layer after the macrogametophyte expands (Li *et al*., 1986).

As archegonia mature, they become long, narrow, and pointed at the lower end (Singh, 1961; Li *et al*., 1986) as is also the case in *C. wilsoniana* (up to ca. 960 × 175 μm.). A large, long egg cell (440 – 520 × 110 – 120 μm) contains less cytoplasm than in the previous stage and has a net-like construction with many rounded to elliptic vacuoles of various sizes (Fig. 19(c) and (h)).

The egg nucleus is located at the top of the cell and beneath the neck cell (Fig. 19(d)). The nucleus (ca. 40 × 30 μm) with a large nucleolus (ca. 14 μm in dia.) is almost equal in size and similar in morphology to the central cell, but quite different from that in the previous stage a week before. Favre-Duchartre (1957) found that the egg nucleus had a prominent nucleolus as the central cell did. A mature egg nucleus may reach a size of ca. 85 × 68 μm in *C. oliveri* (Li *et al*., 1986). It



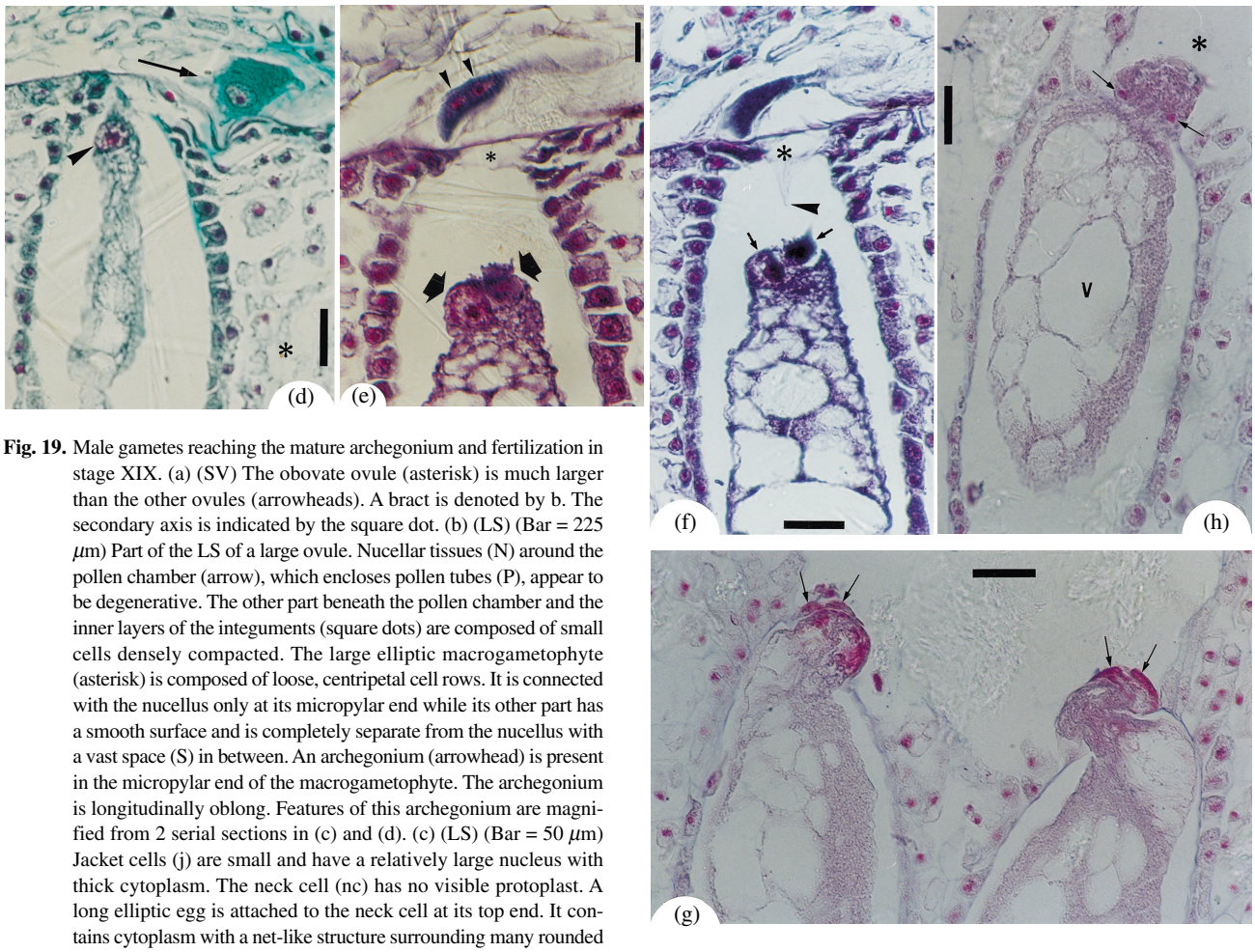


Fig. 19. Male gametes reaching the mature archegonium and fertilization in stage XIX. (a) (SV) The obovate ovule (asterisk) is much larger than the other ovules (arrowheads). A bract is denoted by b. The secondary axis is indicated by the square dot. (b) (LS) (Bar = 225 μm) Part of the LS of a large ovule. Nucellar tissues (N) around the pollen chamber (arrow), which encloses pollen tubes (P), appear to be degenerative. The other part beneath the pollen chamber and the inner layers of the integuments (square dots) are composed of small cells densely compacted. The large elliptic macrogametophyte (asterisk) is composed of loose, centripetal cell rows. It is connected with the nucellus only at its micropylar end while its other part has a smooth surface and is completely separate from the nucellus with a vast space (S) in between. An archegonium (arrowhead) is present in the micropylar end of the macrogametophyte. The archegonium is longitudinally oblong. Features of this archegonium are magnified from 2 serial sections in (c) and (d). (c) (LS) (Bar = 50 μm) Jacket cells (j) are small and have a relatively large nucleus with thick cytoplasm. The neck cell (nc) has no visible protoplast. A long elliptic egg is attached to the neck cell at its top end. It contains cytoplasm with a net-like structure surrounding many rounded vacuoles (v). There are 2 small nuclei (arrowheads) in a pollen tube immediately outside the thick wall (arrow) of the macrogametophyte and in the vicinity of the archegonium. Each of these 2 nuclei has a prominent nucleolus, and both are surrounded by very dark-stained cytoplasm. (d) (LS) (Bar = 50 μm) The long egg cell contains a large nucleus (arrowhead) with a nucleolus and cytoplasm. The nucleus is located at the top end of the egg cell and beneath the neck cell. There is a spermatozoid (arrow) in the same pollen tube, which is slightly closer to the neck cell than these 2 nuclei shown in (c). The spermatozoid is a medium large nucleus with a conspicuous nucleolus and is surrounded by dark-stained cytoplasm. (e) (LS) (Bar = 25 μm) and (f) (LS) (Bar = 45 μm) Magnified features of another archegonium from 2 serial sections. (e) Two large nuclei (broad arrows) are joined closely together at the top end of an egg cell. Two smaller nuclei (arrowheads), which are located immediately outside the neck cell (asterisk), are surrounded by the dense cytoplasm of a pollen tube. Each of these 2 nuclei has a conspicuous nucleolus. (f) A conical wall structure is found to be extruding from the neck cells (asterisk) into the archegonial cavity. Its apex (arrowhead) almost reaches one of those 2 large nuclei (arrows) at the top of the egg. This structure appears to be a part of the pollen tube. (g) (LS) (Bar = 40 μm) There are 2 neighboring archegonia; at the top of each one, a cluster of cyto- and/or nucleo-plasm is merging into the archegonial chamber through the area where the neck is usually located. Each plasm-cluster contains a few relatively large, red-stained lens-shaped nucleolus-like bodies (arrows) at the top in the periphery. (h) (LS) (Bar = 40 μm) Many vacuoles (V) of various sizes are densely distributed in the cytoplasm. The cluster of thick plasm is also merging into the archegonial chamber (asterisk) and includes 2 smaller red-stained nucleolus-like bodies (arrows) in its basal part.

is about 1.5 times the size of the nucleus of central cell.

There is a pollen tube located immediately outside the border of the macrogametophyte and in the vicinity of the neck cell of the archegonium (Fig. 19(c)). The neck cell appears to lack a protoplast. This pollen tube contains a cluster of very dark-stained cytoplasm (ca. 55×18 μm in area), which surrounds 2 small nuclei (ca. 14 μm in dia.), the tube and the stalk nuclei, with a conspicuous nucleolus (ca. 6 μm). Also, in the same cluster of dark-stained cytoplasm (more than $70 \times$ 50 μm in area), there are two male gamete nuclei (spermatozoids), also with one conspicuous nucleolus (ca. 8 μm) each. They are not exactly equal in size; one of them (Fig. 19(d)) is smaller (ca. 28×22 μm) and closer to the neck cell while the other nucleus is slightly larger (ca. 30×24 μm) and slightly lagged behind the smaller one. Both the male gametes were followed by the tube and stalk nuclei, and are larger than these 2 naked nuclei but smaller than the nucleus of egg cell. They were mature and ready for fertilization.

In other *Cephalotaxus* species, there are many reports about the morphology of microgametophytes. Rarely, the pollen tube is found near the macrogametophyte when the archegonia are not yet mature but are still in the foam stage (Singh, 1961). The nucleus of the body cell undergoes division to form 2 male gametes (Arnoldi, 1900). Accounts differ as to the structure of the male gametes: they were described uninucleate cells by Coker (1907) and Favre-Duchartre (1957) but naked nuclei by Arnoldi (1900), Lawson (1907), Sugihara (1947), Kaur (1958) and Singh (1961). In order to answer whether male gametes are either cells or naked nuclei, Gianordoli (1974) examined them with electro-microscopy; inside the pollen tube, both spermatozoids depend on each other because there is no actual wall between them. Two gametes in a tube were equal in size as reported by Lawson (1907), Favre-Duchartre (1957), Singh (1961) and Chen *et al*. (1987) while Coker (1907), Sugihara (1947), Kaur (1958), Gianordoli (1974) and Li *et al*. (1986) described them being unequal. The gametes are 60 – 65 μm in dia. with a nucleus ca. 40 μm thick in *C. fortunei* (Chen *et al*., 1987) while they are 43 or 48 μm in dia. in *C. oliveri* (Li *et al*., 1986).

Fertilization in *C. wilsoniana* occurs in another archegonium in this stage. Two nuclei are joined together at the top of an egg cell (Fig. 19(e)). The upper one is smaller (over 20 μm in dia.) and close to nuclei of mature spermatozoids in size while the lower one is larger (ca. 32 μm in dia.) with a prominent nucleolus (ca. 14 μm dia.) and close to nuclei of mature eggs in size. Chen *et al*. (1987) noted that egg cells had a large nucleolus during fertilization. Coker (1907) found the male nuclei being about one-third the size of the archegonial nucleus. Sometimes, 2 large nuclei are seen below the neck, having arisen through division of the egg nucleus (Favre-Duchartre, 1957; Li *et al*., 1986). Both nuclei are similar in morphology, but the lower one is slightly larger (Li *et al*., 1986). It is pity that the sizes of these nuclei have not been recorded.

Two smaller nuclei in a pollen tube are also present immediately outside the neck cell, which has no protoplast (Fig. 19(e)). These 2 nuclei each have a conspicuous nucleolus and are surrounded by very dense cytoplasm. Except for these 2 nuclei, there are no other cells or nuclei in this tube. A conical wall structure is found to extend from the neck cell inwards into the archegonial cavity (Fig. 19(f)). This structure appears to be a part of the same pollen tube, the apex of which projects downwards and hardly reaches the upper, smaller one of these 2 neighboring nuclei. This smaller nucleus seems to have been released from the pollen tube apex through the neck cell onto the egg nucleus where it performs fertilization.

Archegonia open due to degeneration of the neck cells (Singh, 1961). The latter probably abort due to the incoming pollen tube, and a passage is formed. The neck cells begin to degenerate as the pollen tube comes into contact with them (Li *et al*., 1986). The tube tip enters the archegonium and bursts (Singh, 1961). One or both of the male nuclei together with some cytoplasm of the body cell are released inside the archegonium. Lawson (1907), Favre-Duchartre (1957), Gianordoli (1974) and Li *et al*. (1986) observed that both male gametes enter the archegonium while Kaur (1958) reported the entrance of only one male nucleus.

In addition, there are 2 other neighboring archegonia, which show variation in fertilization (Fig. 19(g)). Egg cytoplasm with many vacuoles is present within the archegonium as usual. On the top of each archegonium, a cluster of cyto- and/or nucleoplasm extends into the archegonial chamber through the area where the neck is usually located. Each plasm-cluster contains a few relatively large, red-stained lens-shaped nucleolus-like bodies, instead of a nucleus, at the top on the periphery. It seems that, these bodies have been added to each egg at the top from each male gamete outside. This is similar to *C. drupacea*, where the male and female gamete nuclei come into contact with each other, and the male one assumes a lenticular shape (Lawson, 1907; Singh, 1961).

A feature of another archegonium is slightly different from that described above in the same ovule (Fig. 19(h)). The cluster of thick plasm includes 2 smaller, red-stained nucleolus-like bodies (ca. 8 μm in dia.) in its basal part, which are equal in size to the nucleolus of the male gametes (spermatozoid). The plasm-cluster with the bodies might be mixtures of cyto- and/or nucleoplasm composed of the egg and spermatozoid since Singh (1961) noted that the zygote nucleus becomes enclosed by the cytoplasm of the body cell. The fertilization process may happen within only one week, i.e., from the previous stage to this stage, on the top of the egg cell.

Fusion of the male and the female gametes occurs in the center of the archegonium in other *Cephalotaxus* species (Coker, 1907; Lawson, 1907; Buchholz, 1925). After fertilization, the tube and stalk nuclei usually stay in the pollen tube behind and are never seen in the cytoplasm of the egg (Singh, 1961) or have degenerated (Li *et al*., 1986). A pollen tube can fertilize only a single archegonium (Singh, 1961).

Based on the continuous observations (Dec. 1994 – May 1996) described above, the entire process, including different stages from initiation of reproductive buds to fertilization, is given in Table 1. A phenological comparison of the development of ovules with macro- and microgametophytes in *C. wilsoniana* with that in other *Cephalotaxus* species is summarized in Table 2.

As a whole, the developmental processes of ovules with macro- and microgametophytes in 3 species are similar in phenology except that initiation of megaspore mother cells happens in late July in *C. wilsoniana*, about 3 months later than in the 2 other species (Table 2).

Pollen tubes stay in a resting phase for about one year (Singh, 1961) or over a winter (Li *et al*., 1986). According to our findings, the pollen tubes developed in the nucellus for at least 10 months, from their first appearance on 30th July of

Table 2. Phenological Comparison of the Development of Ovules with Macro- and Microgametophytes in *C. wilsoniana* in the Present Study with that in *C. drupacea* (Singh, 1961) and in *C. oliveri* (Li *et al*., 1986)

	Taxon		
Development stage	*C. drupacea*	*C. oliveri*	*C. wilsoniana*
1. Initiation of reproductive bud			late Jan.[a] (1st winter)
2. Initiation of ovulate cone	mid. – late Oct.		
3. Initiation of ovule	mid. Nov. (1st winter)	(1st winter)	late Feb.
4. Pollen chamber initiation	early March		early March
5. Differentiation of megasporogenous tissue	late March	late March	late March
6. Pollination	mid. – late March	late March – early April	late March – early April
7. Closure of micropyle	early April	early – mid. April	late March
8. Initiation of mega-spore mother cell	mid. – late April	early – mid. April	late July
9. Pollen grain germination	mid. – late April	early April	
10. Antheridial cell division	late April – early May	early – mid. April	before late July
11. Megaspore mother cell meiosis	mid. Nov.	(2nd winter)	after mid. Oct.
12. 8 free nucleate macrogametophyte	late Nov. (2nd winter)	mid. March	late Dec. (2nd winter)
13. 150 free nucleate macrogametophyte			mid. Feb.
14. 230 free nucleate macrogametophyte			early March
15. Cell wall formation in macrogametophyte	late March	mid. May	mid. April
16. Initiation of archegonium	early April	mid. – late May	late April
17. Development of archegonium and pollen tube reaching gametophyte	early April	late May	early May
18. Differentiation of egg cell	early April	late May	late May
19. Maturation of gametes anb fertilization	mid. April	late May – early June	late May

[a]Dating in three sections per month: early, mid. and late.

the previous year until they reach mature archegonia in the last stage (Table 2).

According to most authors, the development of ovules from initiation of reproductive buds to pollination passes through just one winter. Gong and Chiang (1971) noted that the pistillate flower buds of *C. wilsoniana* became mature for pollination during the period from January to March, just as we observed.

Arnoldi (1900) reported that the megaspore mother cell in an ovule differentiates one year after pollination, and that the macrogametophyte starts to develop about 2 months later in *C. fortunei*. Coker (1907) differed with Arnoldi (1900) with regard to the same species and stated that in the second winter, the ovules contain one megaspore each; i.e., the mother cell undergoes meiosis before the resting period begins.

Lawson (1907) stated that the ovules pass the second winter in the mother cell stage, and that meiosis takes place one year after pollination in *C. drupacea*. Favre-Duchartre (1957) agreed with Lawson with regard to the same species; according to him, the mother cell differentiates six months after pollination, and during the second winter, the ovules stay in this stage. The mother cell undergoes meiosis only at the beginning of spring (the middle of February). Also, for the same species, Singh (1961) stated that the mother cell differentiates just one month after pollination; it undergoes meiosis, and a free nuclear macrogametophyte with 8 – 16 nuclei is formed before the second winter begins. Initials of archegonium appear in the first week of April. Fertilization takes place in the middle of April.

Acknowledgment

This research was supported under a grant (NSC 86-2313-B-002-120) provided by the National Science Council of the Republic of China. The authors are grateful to Mr. M.C. Tsai, senior specialist, Montane Experimental Farm, and to Dr. N.C. Chung, associate researcher, Experimental Forest, National Taiwan University, for collecting ovulate cones at Mei-Fen and Chi-Tou, respectively.

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