Sigeru DAIGOBO*: Morphological variation in the gametophytes of *Lycopodium clavatum* in Japan**

大悟法 滋*: ヒカゲノカズラ配偶体の変化性

The morphology of the gametophyte of *Lycopodium clavatum* from Europe was studied in considerable detail by Bruchmann (1898, 1910) and Lang (1899). Later workers, Spessard (1917), Degener (1924), Stokey & Starr (1924), Barrows (1935), Gauthier & Dumais (1938), Eames (1942) and Bruce & Beitel (1979), also found the gametophytes in North America. In this paper, the morphology of gametophytes of *L. clavatum* L. var. *nipponicum* Nakai was described in order to confirm earlier reports and to add details of the anatomical features special to this variety collected in Japan.

Materials and methods The gametophytes of *L. clavatum* var. *nipponicum* were collected in Sugadaira, Nagano Prefecture, Japan, during 1977–1981. Gametophytes supporting sporophytes were found in a grove of *Quercus mongolica* on the mid-slope about 10 m below the site where adult sporophytes were growing. Young gametophytes without sporophytes were located 1–3 cm below the surface of leaf mold adjacent to the region where the young sporophytes were found. Gametophytes were fixed in CRAF I solution, and embedded in paraffin for serial sectioning. The most satisfactory stain was a combination of Heidenhain's hematoxylin and fast green.

Observations About 70 gametophytes of *L. clavatum* var. *nipponicum*, including about 50 gametophytes with young sporophytes, were observed. They varied in shape and size depending on their degree of maturity.

In the early 1–2 mm diameter stages the gametophyte was obconical in shape and had a rim-like margin on the top (Fig. 1). A meristematic region was located inside the upcurved rim (Figs. 22–24, m). In mature stages, the gametophytes usually became flattened plates of tissue 1–3 mm thick (Figs. 3, 5–7, 9 and 10). The primary conical tubercle pointed downward in the middle of the ventral side (Fig. 10, arrow). Mature gametophytes were often irregularly

* Institute of Biological Sciences, University of Tsukuba, Sakura-mura, Ibaraki-ken, 305. 筑波大学生物科学系.

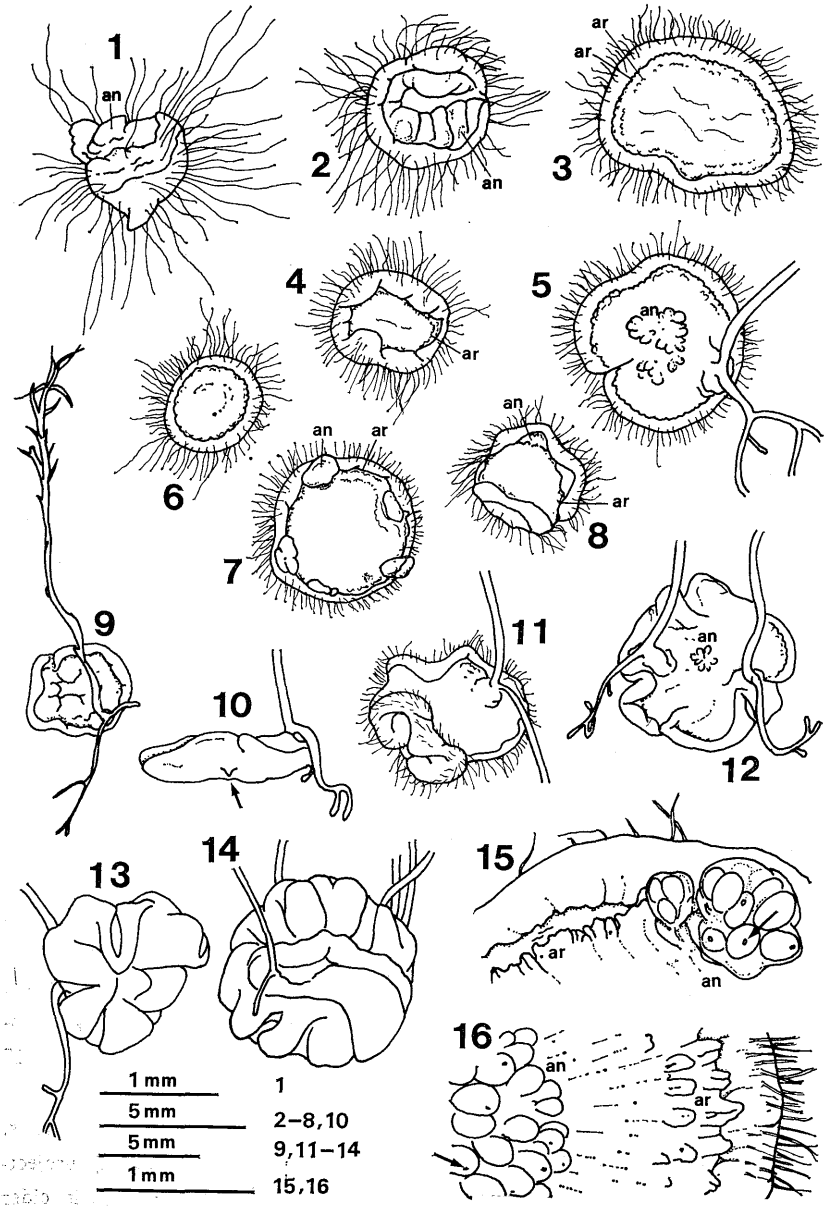
** Contributions from the Sugadaira Montane Research Center, No. 70.

lobed, and became convoluted in the older gametophyte which supported sporophytes (Figs. 11–14). Many old gametophytes had multiple sporophytes. Five were found on a single large gametophyte (Fig. 14). The gametophytes were generally brownish in appearance, although one of the mature gametophyte which was found on the soil surface was green in part of the dorsal side. Numerous rhizoids 1–3 mm long projected from the ventral surface, especially along the margin. They were much shorter in older gametophytes.

In longitudinal sections, several layers of tissue were distinguished by particular cell types and the localized distribution of an endophytic fungus (Figs. 17, 18 and 21–26). The outermost layer of the ventral side was composed of 2–3 layers of slightly flattened cell without any associated fungus (Figs. 21 and 26, a). Three to five, rarely seven, layers parallel to the outermost layer were heavily infected with intracellular fungi (Figs. 21 and 26, b). The next single or rarely two layers filled with mycelia were composed of elongate pallisade cells aligned with their long axes at right angles to the surface (Figs. 21 and 26, c). The layer of pallisade cells was immediately followed by fugus-free, 8-10 layers consisting of relatively thick-walled cells (Figs. 21, and 26, d). The uppermost layer of the dorsal side of the gametophyte usually made up more than half of the thickness and consisted of parenchyma cells (Fig. 21, e). These cells were relatively large, but those surrounding the sexual organs and near the meristematic regions were much smaller. The cells which formed the boundary line between the uppermost (d) and the pallisade (e) layers were often shrunken (Fig. 21, arrow).

Four types of the gametophytes were recognizable according to the distribution of their sexual organs: male, female, protandrous hermaphrodite and protogynous hermaphrodite. Male gametophytes were relatively small and 1–3 mm in diameter. They were obconical in shape and disc-like on the top with well-developed rims. The young gametophyte showed an antheridial ridge on the upper surface (Fig. 1), and the larger one was surrounded with a thick rim-like margin (Fig. 2). Median longitudinal sections of the male gametophytes showed a large number of antheridia on well-developed ridges (Figs. 17 and 22).

Female gametophytes were usually disc-shaped with slightly up-curved rims. Archegonia were scattered in 2 or 3 rows along the edge of a slightly projecting cushion (Fig. 23). The archegonia were distributed in double rings in older


1
an
2
an
ar
ar
3
4
ar
5
an
6
an
ar
7
an
ar
8
9
10
11
an
12
13
14
15
ar
an
16
an
ar
1 mm
5 mm
5 mm
1 mm
1
2–8,10
9,11–14
15,16

gametophytes, because new archegonia developed on the outside (Fig. 3). The old female gametophytes, 7-12 mm in diameter, sometimes bore sporophytes (Fig. 11). The margin of rim of the gametophytes was often relatively slightly curved (Figs. 3 and 6) but sometimes strongly curved (Figs. 4 and 18).

Protandrous hermaphroditic gametophytes probably developed from male gametophytes by the horizontal growth of the margin and relatively slow-forming archegonia. They were disc-shaped, more than 5 mm in diameter, and had many functional archegonia on the edge of flattened surface with old antheridia located near the center (Figs. 5, 12, 16 and 24). The antheridial ridges were not well-developed. These gametophytes usually supported sporophytes (Figs. 5, 12 and 24).

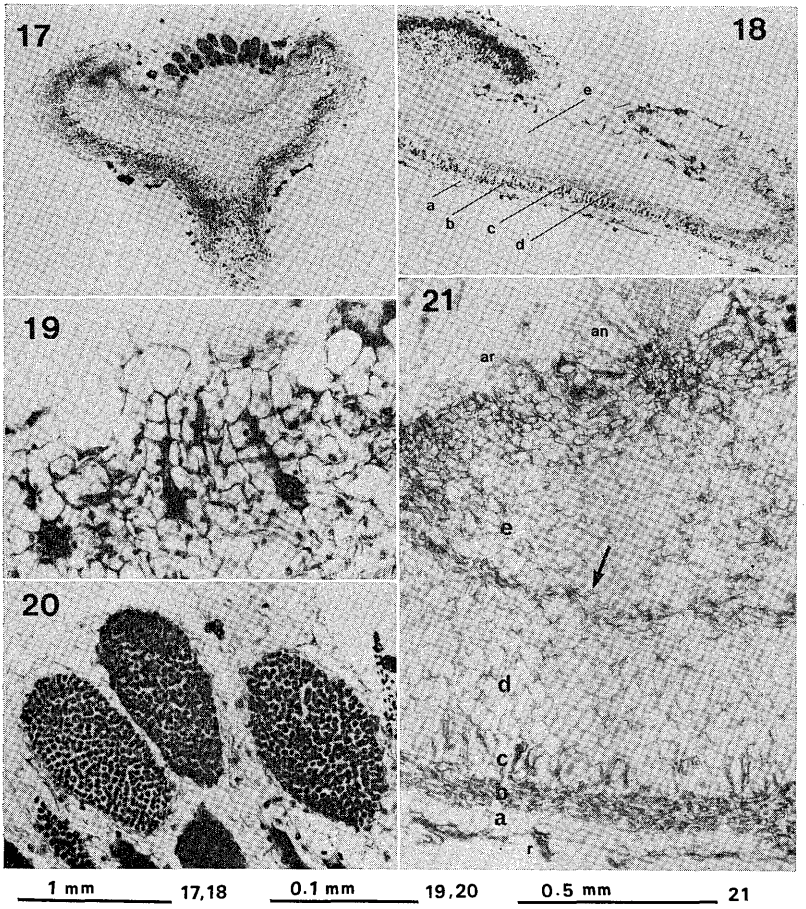
Protogynous hermaphroditic gametophytes (Figs. 7, 8) obviously developed from female gametophytes by the development of antheridial ridges in the lateral margin. Old archegonia were on the lateral ridge and developing antheridia were near the margin (Figs. 15 and 25). They were disc-shaped and more than 4 mm in diameter. In many cases, they supported sporophytes, and often became convoluted with maturity (Figs. 13 and 14).

Antheridia were produced in several rows on ridges near the margin. In protandrous hermaphroditic gametophytes old antheridia were observed in the central part of the surface. A large ellipsoidal mass of spermatocytes were completely or sometimes partially embedded in gametophyte tissues (Figs. 20 and 27). Free swimming spermatozoids were not observed. The jacket of the mature antheridium consisted of 2 or 3 cell layers (Fig. 27). Frequently empty antheridia with degenerated opercular cells were observed (Figs. 15 and 16, arrow). In cross section, the operculum consisted of one cell (Fig. 28, arrow). Various stages of the development of the antheridia (Figs. 30-32) as well as the first vertical division of a inner cell divided from the initial (Fig. 29) were observed.

Archegonia were usually produced on the edge of the flattened or hollow upper surface. In rare cases of the large protogynous hermaphrodite, many

Figs. 1-16. Gametophytes of *L. clavatum* var. *nipponicum* from the upper view, except 10 from the side view, showing antheridia (an) and archegonia (ar). 1 and 2. Male gametophytes. 3, 4, 6 and 11. Female gametophytes. 5 and 12. Protandrous hermaphroditic gametophytes. 7-10, 13 and 14. Protogynous hermaphroditic gametophytes, showing the primary conical tubercle in 10 (arrow). 15 and 16. Distribution of sexual organs of protandrous hermaphrodite (15) and protogynous hermaphrodite (16), showing opercular cells (arrow).

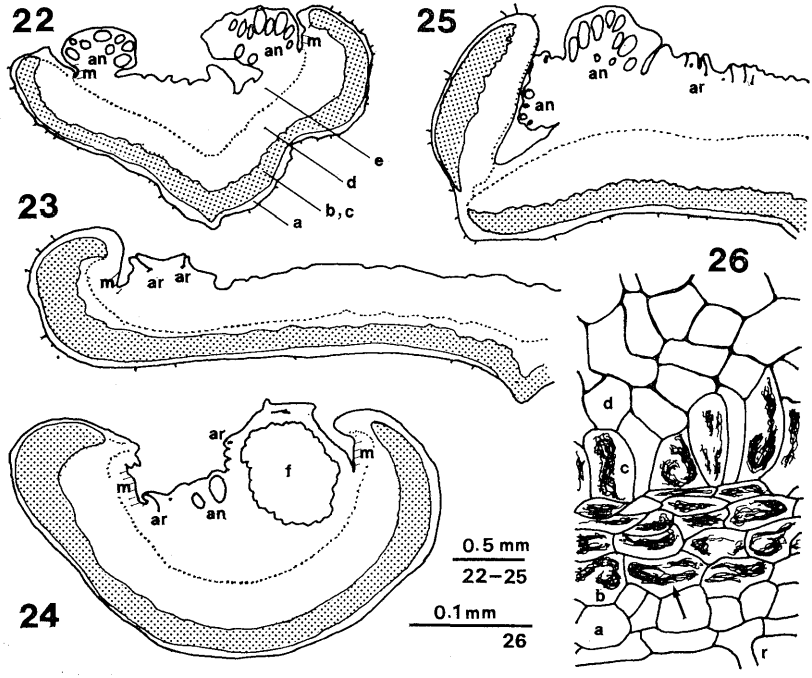
old archegonia were located in the central part of the gametophytes. Mature archegonia projected necks from the surface of gametophyte tissue. Neck cells were usually composed of a single layer in the upper portions. The upper neck cells were in 3-6 tiers as seen from the surface view (Figs. 33 and 34), and 4 or 5 rows as seen in cross section (Figs. 35 and 36). They were fragile, and



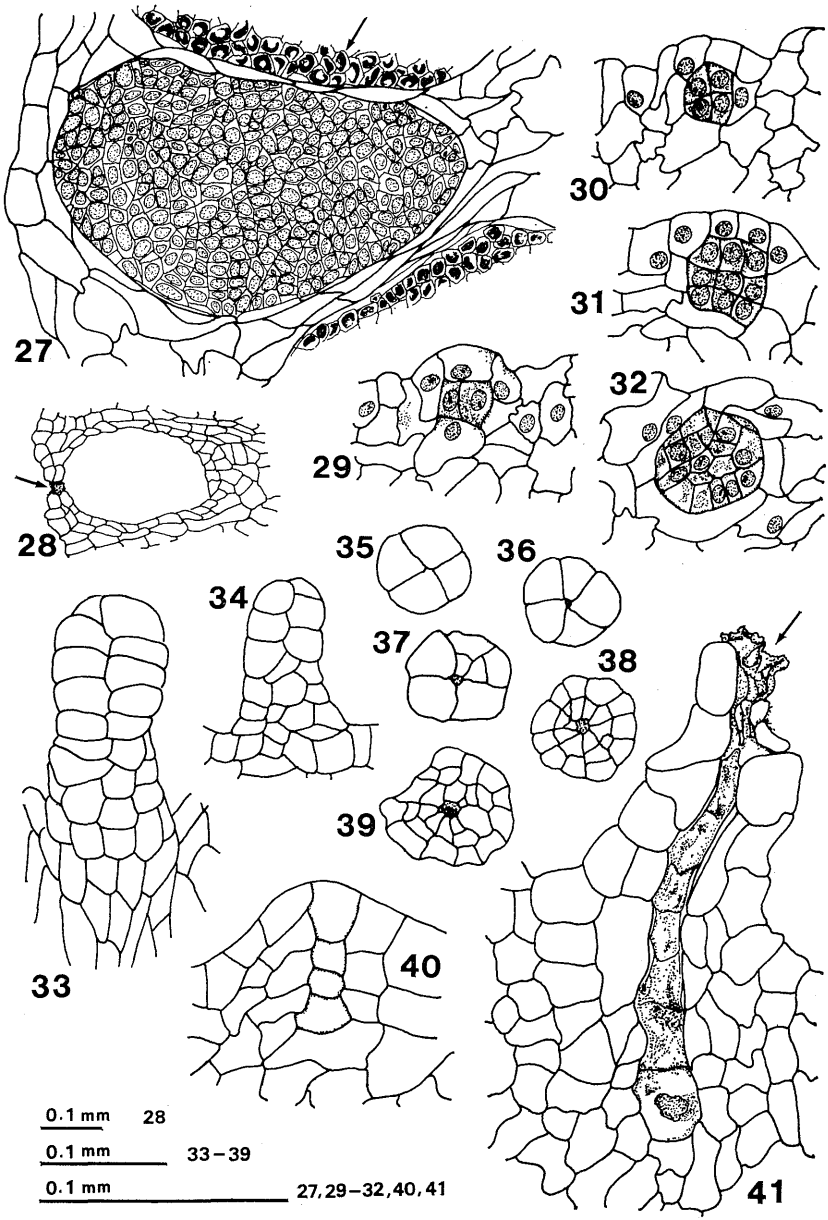
Figs. 17-21. Longitudinal sections of the gametophytes of *L. clavatum* var. *nipponicum*. 17. A male gametophyte. 18. A female gametophyte. 19. Archegonia. 20. Antheridia. 21. A protogynous hermaphroditic gametophyte, showing layers of tissue (a-e), antheridium (an) and archegonium (ar).

usually incomplete in older archegonia (Figs. 19 and 41). The lower portion was composed of more than one cell layer (Figs. 37-39) of 3-5 tiers of cells projecting from the surface (Figs. 33 and 34). The venter of the archegonia and the lower portion of the canal were surrounded by a layer of small cells cut off from gametophyte tissue (Fig. 41). The canal was occupied by 8-10 cells, although it was difficult to be certain because of the fragility of the upper portion of neck cells. The young stages of the archegonium (Fig. 40) were not followed in detail.

Discussion The gametophytes of *L. clavatum* var. *nipponicum* were obconical in early stages and disc-shaped in mature stages. As the disc diameter



Figs. 22-26. Drawings of the longitudinal sections of *L. clavatum* var. *nipponicum*, showing antheridia (an), archegonia (ar), meristematic regions (m) and fungus-infected rayers (dotted). 22. Median section of a male gametophyte. 23. A half of median section of a female gametophyte. 24. Median section of a protandrous hermaphroditic gametophyte, showing the foot of the sporophyte (f). 25. A part of convoluted edge of protogynous hermaphroditic gametophyte. 26. Under portion of gametophyte, showing the layers of tissue (a-d), fungal hyphae (arrow) and rhizoid (r).


27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
0.1 mm 28
0.1 mm 33–39
0.1 mm 27, 29–32, 40, 41

increased, the edges curled upward or downward and the plants became furrowed and saddle-shaped. With continued growth the curling caused convolution. These features are much the same as those illustrated for *L. clavatum* by Bruchmann (1898) and Lang (1899).

Two markedly different types of gametophyte have been known in *Lycopodium*. The first type lives on the surface of the substrata, is green, develops rapidly, and is short-lived and minute such as *L. cernuum* (Treub 1884) and *L. inundatum* (Goebel 1887). The second is subterranean, nongreen, slow developing, long-lived and much larger such as *L. annotinum* (Bruchmann 1899) and *L. obscurum* (Spessard 1922). The gametophyte of *L. clavatum* var. *nipponicum* obviously belongs to the second type in habitat. However, one gametophyte having a green colour in part of the dorsal side was found from the surface of soil such as in the case of *L. selago* (Bruchmann 1910). The form and structure of the green gametophyte were similar in detail to the other ones.

In the internal structures with several cell layers, this variety resembles the European species described by Bruchmann (1898) and Lang (1899). Slight differences were observed in the cell numbers of the outermost ventral layer and the lower fungal layer.

Bruchmann (1889) reported that *L. clavatum* gametophytes were monoecious, but Lang (1899) reported they were dioecious. Later Bruchmann (1910) mentioned that young gametophytes were mostly male, rarely female, and that old gametophytes were monoecious. In this study, four types of gametophytes were distinguishable: male, female, protandrous hermaphrodite and protogynous hermaphrodite. The distribution of the sexual organs on the gametophyte seems to be caused by irregularities following the marginal growth and development of the antheridial ridge. Sexual organs were similar to the structures described by Bruchmann (1898) and Lang (1899), except that the upper parts of neck cells were often composed five cells rows, and the neck cells and neck canal cells were constructed of many more tiers of cells.

I would like to express my sincere thanks to Dr. Seiji Tokumasu for his

Figs. 27–41. Antheridia and archegonia of *L. clavatum* var. *nipponicum*. 27. An antheridium having a large number of spermatocytes, and in the adjoining antheridia nearly mature spermatozoids (arrow). 28. An old empty antheridium, showing an opercular cell (arrow). 29–32. Several young stages of antheridia. 33 and 34. Surface views of archegonia. 35–39. Cross sections of archegonia necks. 40. A young stage of archegonium. 41. A mature archegonium, the upper portion is missing (arrow).

help in collecting the material used in this study. Grateful thanks are also due to Professor Larry B. Liddle who kindly corrected English of this paper.

References

Barrows, F. L. (1935) Propagation of *Lycopodium* I. Contrib. Boyce Thompson Inst. **7**: 267-294. Bruce, J. G. & Beitel, J. M. (1979) A community of *Lycopodium* gametophytes in Michigan. Amer. Fern Journ. **69**: 33-41. Bruchmann, H. (1898) Über die Prothallien und die Keimpflanzen mehrerer europäischer Lycopodien. Friedrich Andreas Perthus, Gotha. 1-119. —— (1910) Die Keimung der Sporen und die Entwicklung der Prothallien von *Lycopodium clavatum* L., *L. annotinum* L., und *L. selago* L. Flora **101**: 220-267. Degener, O. (1924) Four new stations of *Lycopodium* prothallia. Bot. Gaz. **77**: 89-95. Eames, A. J. (1942) Illustrations of some *Lycopodium* gametophytes. Amer. Fern Journ. **32**: 1-12. Gautier, R. & Dumais, R. (1938) Les prothalles de Lycopodes dans le Québec. Nat. Canadian **65**: 280-284. Goebel, K. (1887) Ueber Prothallien und Keimpflanzen von *Lycopodium inundatum*. Bot. Zeit. **45**: 161-168, 177-190. Lang, W. H. (1899) The prothallus of *Lycopodium clavatum* L. Ann. Bot. **13**: 279-318. Spessard, E. A. (1917) Prothallia of *Lycopodium* in America. Bot. Gaz. **63**: 66-76. —— (1922) Prothallia of *Lycopodium* in America II. Bot. Gaz. **74**: 392-412. Stokey, A. G. & Starr, A. M. (1924) *Lycopodium* prothallia in western Massachusetts. Bot. Gaz. **77**: 80-88. Treub, M. (1884) Études sur les Lycopodiacées I. Ann. Jard. Bot. Buitenz. **4**: 107-138.

* * * *

長野県菅平で採集された約70個体のヒカゲノカズラの配偶体について，その外部形態と解剖学的な観察を行った。この地中生，塊状の配偶体は，発達初期には逆円錐形で，成熟すると円板状になる。古い配偶体ではしばしばその周辺部がわん曲し，とくに大型のものでは複雑に折りたたまれた形になる。また，内部組織は，内在する菌糸と関連して数層に分化している。このような形態は，組織や生殖器官を構成する細胞数などに多少の違いは認められるが，Bruchmann (1898) と Lang (1899) によるヨーロッパ産のヒカゲノカズラについての報告とよく一致する。配偶体の形態は配偶体の大きさ，古さによって異なっているが，生殖器官の形成，とくに造精器を形成する組織の盛り上りによっても影響を受ける。この生殖器官形成の状態は個々の配偶体によって異なっており，その形成様式から雄性配偶体，雌性配偶体，雄性先熟型両性配偶体，雌性先熟型両性配偶体の4型が認められた。