

REVIEW

A NEW CLASSIFICATION OF THE GAMETOPHYTE DEVELOPMENT OF HOMOSPOROUS FERNS, FOCUSING ON MERISTEM BEHAVIOUR

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ABSTRACT

Different shapes of fern gametophytes may be the result of adaptive evolution to different habitats where the gametophytes grow. In homosporous ferns, gametophytes are cordate-thalloid (terrestrial) in many taxa but can also be strap-like, ribbon-like, and filamentous (epiphytic) or tuberous (subterranean) in other taxa. Recently developed long-term observational techniques of the same individual gametophyte during growth have led to a new classification of development types for planar gametophytes. We recognise five basic types (*Lygodium*, *Elaphoglossum*, *Anemia*, *Colysis*, and *Vittaria*). The five types reflect different combinations of the three meristems: apical cell-based, marginal and multicellular meristems. In the *Lygodium*-type, the apical cell-based meristem is immediately followed by the multicellular meristem phase, resulting in typical cordate gametophytes. In the *Elaphoglossum*- and *Anemia*-types (i.e. subtypes of the *Lygodium*-type) strap-shaped and asymmetric cordate gametophytes, respectively, are formed. In the *Colysis*-type, the marginal meristem phase occurs between the apical cell-based meristem and the multicellular meristem phases, and in the *Vittaria*-type, the multicellular meristem phase is absent. In both of these latter types, irregularly branched ribbon-like gametophytes form.

INTRODUCTION

Gametophytes of homosporous ferns are generally cordate-thalloid in shape, but in some taxa they are strap-like (in e.g. Grammitidaceae, Polypodiaceae), ribbon-like (*Vittariaceae*, some of the *Hymenophyllaceae*), filamentous (some of the *Hymenophyllaceae*, *Schizaea*) or tuberous (*Ophioglossaceae*, *Psilotaceae*; Orth, 1936; Nayar and Kaur, 1971; Raghavan, 1989). The strap-like gametophyte is several times longer than broad with a cordate apex, whereas the ribbon-like gametophyte is narrow and markedly elongate, with nearly parallel sides and a round apex. The filamentous gametophyte consists of branched uniseriate filaments, and the tuberous are usually subterranean, nearly cylindrical or irregular in shape (Nayar and Kaur, 1971). Fern gametophytes are much smaller in size and have less distinguishing morphological characters than sporophytes. According to Bower (1923), the vegetative characters of fern gametophyte are deficient in stability and in variety of detail, and are consequently of minor importance in comparative studies. This general belief was unfortunate but

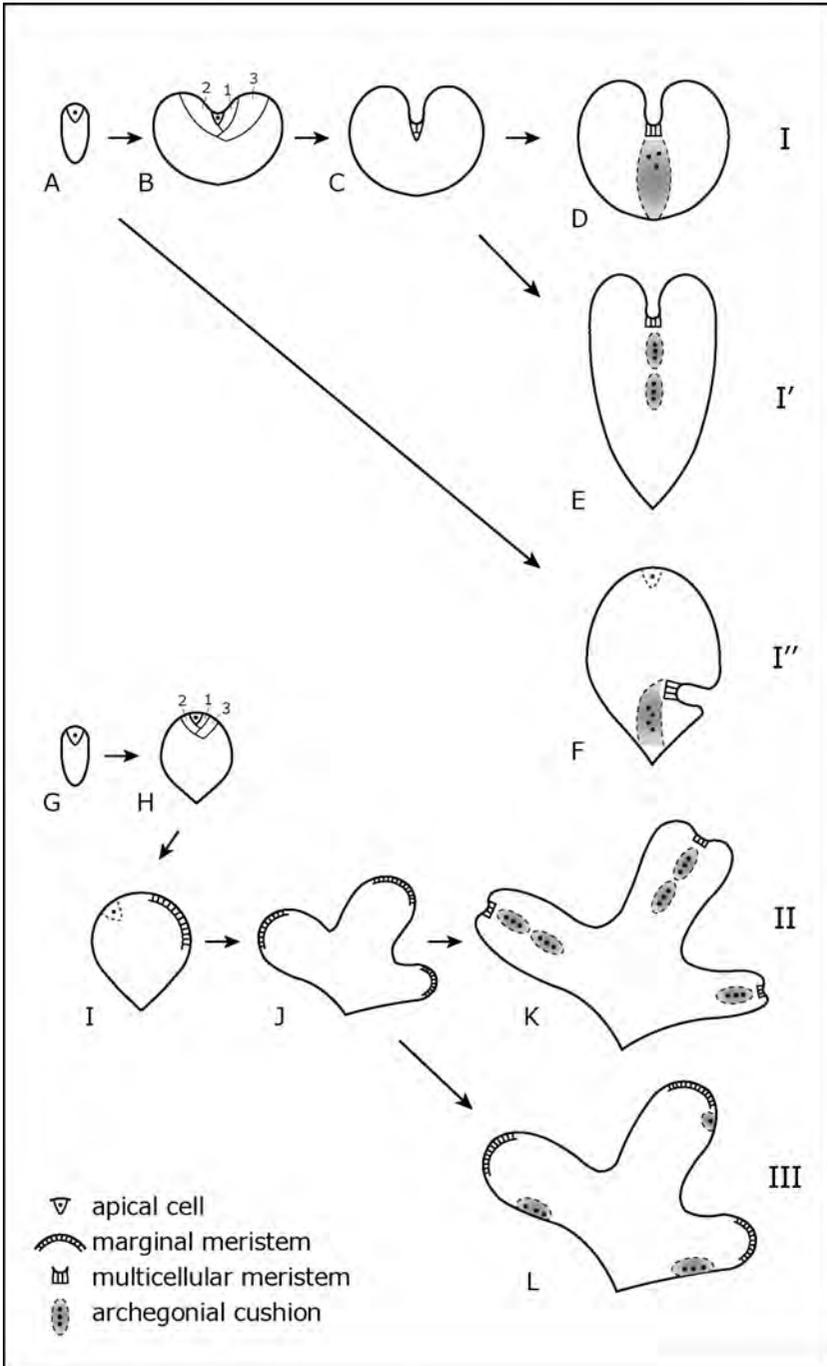


Figure 1. Diagrammatic illustrations of five developmental types of gametophytes of homosporous ferns. I, *Lygodium*-type; I', *Elaphoglossum*-type; I'', *Anemia*-type; II, *Colysis*-type; III, *Vittaria*-type. A-L indicate various stages of development. Some of the merophytes that constitute the gametophyte are shown in B and H. 1-3, the youngest to third youngest derivatives, respectively.

understandable at the time, prior to sufficient knowledge of the systematics and ecological patterns, which has now been accumulated. Since the 1950s, however, gametophyte development was targeted for research by fern taxonomists for use in classification efforts (Atkinson and Stokey, 1964), and the number of relevant publications increased (see references in Nayar and Kaur, 1971).

Based on data prior to the 1970s, Nayar and Kaur (1971) published an extensive review on the comparative development of the gametophytes of homosporous ferns, addressing the evolution of gametophyte shapes in relation to phylogeny. Various shaped gametophytes are attributable to differences in the meristem. Tuberos gametophytes have an apical meristem with either a single apical cell or a group of apical cells (Whittier, 1983; Whittier and Thomas, 1993; Takahashi and Imaichi, 2007). Filamentous gametophytes also bear an apical cell (the terminal cell) that acts as an initial cell (Nayar and Kaur, 1971). Planar gametophytes (cordate, strap- or ribbon-shaped) basically have both the apical cell and the multicellular meristem in most cases, with distinct developmental types: *Adiantum*, *Aspidium*, *Ceratopteris*, *Drynaria*, *Kaulinia*, *Marattia* and *Osmunda* (see Figure 2 in Nayar and Kaur, 1971). Since the review by Nayar and Kaur (1971), research in this field has drastically declined. Of note, however, is continued research into the remaining taxa, which is thought to be necessary for comparative study (e.g. Mendozoa-Ruiz and Pérez-García, 2005; Chou *et al.*, 2007; Gabriel y Galán and Migliaro, 2011; Testo and Watkins, 2011; references in Pérez-García and Riba, 1998).

In ferns and lycophytes, sporophytes and gametophytes are equally subjected to environmental conditions and then adapt to the habitats where they grow. To better understand the life history of ferns, recent botanists have widely accepted that information concerning gametophytes is essential (Farrar *et al.*, 2008). Field research of fern gametophytes has indicated that the life span of the gametophyte itself (annual or perennial) and its ability to reproduce vegetatively are directly related to gametophyte shape (e.g. Dassler and Farrar, 1997, 2001; Farrar, 1998; Watkins *et al.*, 2007).

As mentioned above, gametophyte shape is closely related to its life span, and the shape is a result of meristem activity. Therefore, accurate analyses of meristem behaviour are crucial for ecological as well as evolutionary studies of gametophytes. To examine meristem behaviour, Takahashi *et al.* (2009) recently conducted long-term observations of the same individual gametophyte, termed the sequential observation method. These authors captured light microscope images at set intervals (e.g. every 24 h) of the same gametophyte individual during growth, enabling an unprecedentedly easy and accurate analysis of cell lineages (cf. Figures 3 and 4 herein). Details of the methods are outlined in Takahashi *et al.* (2009). Based on meristem behaviour, five basic gametophyte types have thus far been recognised: *Lygodium*, *Elaphoglossum*, *Anemia*, *Colysis* and *Vittaria*. This classification is quite different from that proposed by Nayar and Kaur (1971). In this article, I describe these five types and discuss their developmental relationships as well as adaptation to the habitat.

Five development types

1. *Lygodium*-type (Figure 1, type I)

Previous studies have documented that the developmental process of the cordate gametophyte is quite similar among various fern taxa (references in Nayar and Kaur, 1971). At its early stage, the young narrow spatulate gametophyte forms a triangular ('obconical' or 'wedge-shaped') apical cell (Figure 1A). The apical cell produces a

limited number of derivative cells from two lateral division faces (Figure 1B) and is then replaced by the multicellular meristem (the ‘pluricellular meristem’ by many authors, or the ‘marginal meristem’ by authors before Stokey and Atkinson, 1956; Figure 1C herein). A multilayered midrib, on which archegonia arise, then develops behind the multicellular meristem (Figure 1D). The archegonial midrib is generally called the ‘cushion’ (Raghavan, 1989). At this stage, the gametophyte attains sexual maturity.

The commonly accepted idea that the apical cell and the multicellular meristem act as the initial cell or cells is an assumption formed by comparisons of merophyte (a clonally related cell packet) arrangements in different individuals at different developmental stages or by computer analysis (Döpp, 1927; Orth, 1936; Korn, 1974; von Aderkas and Cutter, 1983). To provide evidence for this assumption, we conducted sequential observations of the gametophyte of *Lygodium japonicum* (Thunb.) Sw. (Lygodiaceae) (Takahashi and Imaichi, in prep.), which is known to be strikingly cordate, with a deep notch overlapped by equally sized anterior wings (Momose, 1967). The apical cell of *L. japonicum* actually cuts off 6-8 derivatives in two lateral facets with a

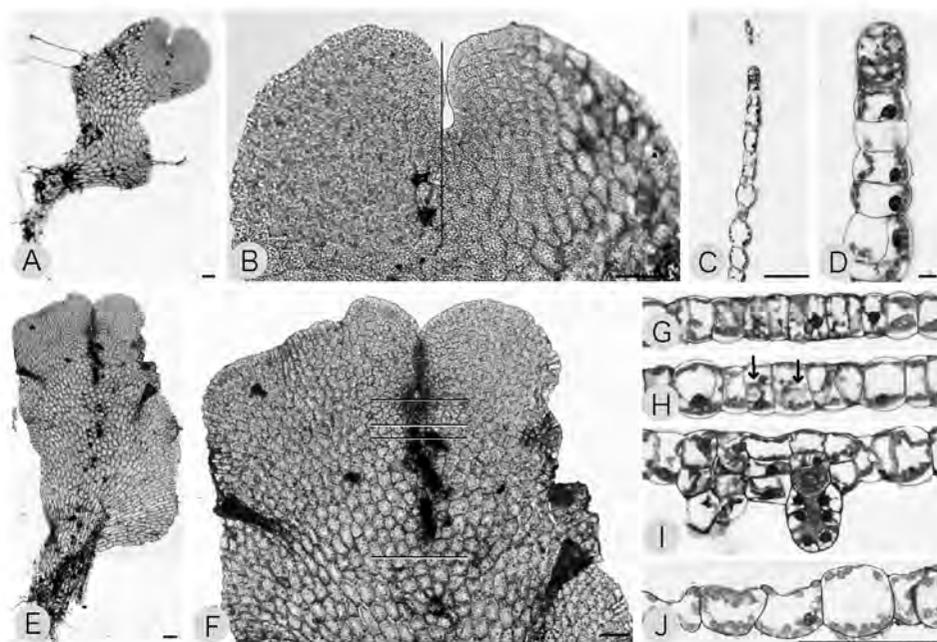


Figure 2. Gametophyte of *Selligiea hastata* (Thunb.) Fraser-Jenk. **A.** Relatively young gametophyte that has not yet developed the archegonial cushion. **B.** Enlarged figure of the apical portion of A. Vertical line indicates the site of the longitudinal section. **C, D.** Longitudinal section shown by the vertical line in B. Enlarged figure of the apical portion is shown in D. Terminal two or three cells are meristematic. **E.** Mature gametophyte with archegonial cushion. **F.** Enlarged figure of E. Four horizontal lines indicate sites of sections shown in G-J, respectively. **G.** Multicellular meristem with central narrow cells. **H.** The midrib portion that is two-cells thick. Arrows indicate cell walls formed in an orientation parallel to the substratum. **I.** Cushion with two young archegonia. **J.** One-cell layer portion below archegonial cushion. Scale bars: (A-C, E,F,J) = 100 μ m; G-J all at same magnification; (D) = 10 μ m.

left-right alternation of cell plate orientation. These derivative cells undergo repeated periclinal and anticlinal divisions to form large rectangular or fan-shaped merophytes, which are regularly arranged in a zig-zag manner and occupy most of the young gametophyte (Figure 1C). Here, the apical cell and its immediate derivatives constitute an apical meristem, called the apical cell-based meristem. After the apical cell-based meristem phase is completed, the apical cell of *L. japonicum* undergoes a transverse cell division to give rise to an anterior rectangular cell and an inner triangular cell, the former of which further undergoes anticlinal and periclinal divisions (Figure 1D), and the latter of which also divides in various orientation. In *L. japonicum*, the multicellular meristem composed of a row of 2-4 narrow cells is established at the site of the original apical cell. In this sense, the apical cell is replaced by the multicellular meristem.

The Lygodium-type of development is commonly found in cordate gametophytes of most terrestrial fern families, including Osmundaceous ferns, Gleichenioid ferns, Tree ferns, and Polypod ferns (sensu Pryer, 2004). In addition, cordate gametophytes of some epiphytic taxa, e.g. Drynarioid ferns (Polypodiaceae), also show the Lygodium-type development (Nayar, 1965; Chandra, 1979). It needs to be clarified whether cordate gametophytes of other epiphytic taxa, e.g. *Davallia* (Davalliaceae, Nayar and Kaur 1971), *Pyrrosia* (Polypodiaceae, Nayar 1961), show the Lygodium-type of development.

2. *Elaphoglossum*-type (Figure 1, Type I')

Gametophytes of epiphytic Polypodiaceous taxa often exhibit a strongly elongated, strap shape [e.g. *Elaphoglossum* (Lomariopsidaceae), and *Lepisorus*, *Christiopteris*, and *Crypsinus* (Polypodiaceae); Nayar and Kaur, 1971; Figure 1E herein]. According to the literature, the strap-shaped gametophyte also forms the apical cell and subsequently the multicellular meristem, as does the typical cordate gametophyte. However, the longer duration of the activity of the multicellular meristem compared with the typical cordate gametophyte could result in an elongated shape and a longer life span (Stokey and Atkinson, 1957 for *Elaphoglossum*; Nayar and Raza, 1970 for *Lepisorus*; Nayar, 1967b; Chiou *et al.*, 1997 for *Christiopteris*). Another characteristic to note for the strap-shaped gametophyte is that the cushion is relatively thin (2-6 cells thick for *Elaphoglossum*; Stokey and Atkinson, 1957) and is often discontinuous and intermittent (Figure 1E).

Figure 2 shows wild gametophytes of *Selliguea hastata* (Thunb.) Fraser-Jenk. (Polypodiaceae), which was growing on rocks in Kiyotaki, Kyoto, Japan. The ferns were identified using a DNA barcoding technique (Ebihara *et al.*, 2013; Ogura-Tsujita *et al.*, 2013) at the species rank. Similar to other epiphytic strap-shaped gametophytes, the *S. hastata* gametophytes are strongly elongated with a cordate apex and have marginal hairs that are reddish brown in colour (Figure 2A, B, E, F). The multicellular meristem consists of a row of narrow cells (Figure 2G), and the archegonial cushion behind the meristem is only two cells thick (Figure 2H, I). Notably, relatively young gametophytes that have already developed the multicellular meristem often do not yet have a multilayered archegonial cushion behind the meristem (Figure 2B-D). Such young gametophytes are entirely one-cell thick with no midrib. Even in mature gametophytes (Figure 2E, F), the portion below the proximal end of the central archegonial cushion is also one-cell thick (Figure 2J), perhaps indicating that the gametophyte did not produce an archegonial cushion until long after the multicellular meristem was formed. This pattern contrasts with that of the typical cordate gametophyte, in which the archegonial cushion begins to form just after the multicellular meristem is established. Such a delay in the initiation of the multilayered cushion could cause the multicellular meristem phase to be longer.

Based on the comparative development mentioned above, strap-shaped gametophytes can be regarded as a sub-type of the cordate type, i.e. highly elongated cordate gametophytes. Some intermediate shapes can be assumed to exist between the elongate-cordate and the strap-shaped gametophyte. Therefore, future sequential observations

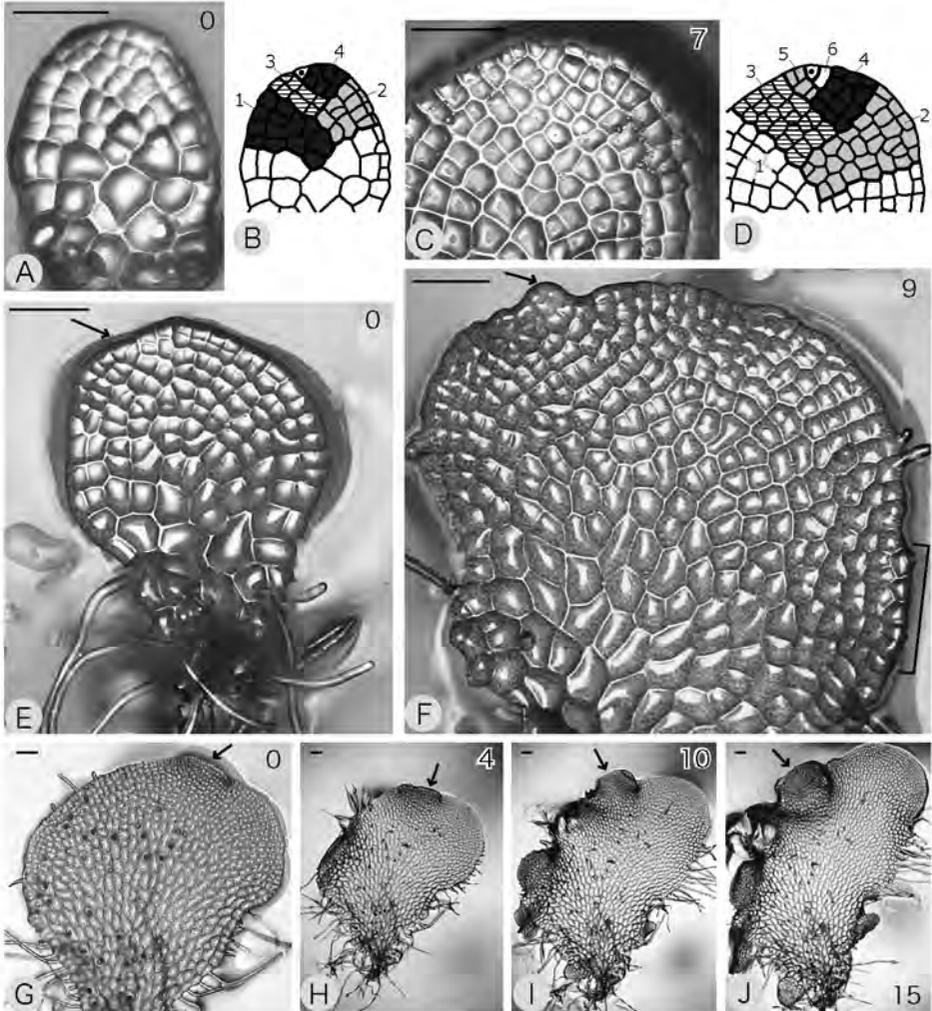


Figure 3. Gametophyte development of *Loxogramme salicifolia* (Makino) Makino. A, C, E-J. Epi-illuminated micrographs. B and D. Line drawings of A and C, respectively. Numbers at the upper (A-I) and lower (J) right corners indicate the number of days passed after observations began. **A-D.** Two early stages of the same gametophyte. Triangular cell marked by a dot regularly produces derivatives (1-6) as an apical cell. **E and F.** Two young stages of the same gametophyte. The apical cell (arrow) has stopped cell division in F. The gametophyte has been expanded in the region away from the original apical cell. The region indicated by the bracket gives rise to the marginal meristem. **G-J.** Four images of the same growing gametophyte individual at the marginal meristem stage. Arrows show the same site on the gametophyte. Due to marginal meristem activity, several lobes are growing. Scale bars = 100 μ m.

should focus on the growing elongate-cordate or strap-shaped gametophytes of several taxa, including *Elaphoglossum* and *Crypsinus*.

The Elaphoglossum-type of development is found in epiphytic taxa of Polypodiaceae and Lomariopsidaceae.

3. *Anemia*-type (Figure 1, type I’)

In cordate gametophytes, both semicircular wings are of nearly equal size in typical cases, but they can be strongly asymmetrical with one wing larger than the other, especially when young, in some taxa such as *Anemia* and *Mohria* (Anemiaceae), *Ceratopteris* (Ceratopteridaceae), *Acrostichum* (Pteridaceae) and *Onychium* (Adiantaceae; Nayar and Kaur, 1971). In the literature, the apical cell was once reported as present (Banks *et al.*, 1993 for *Ceratopteris*), but it is typically considered to be absent (Nayar and Kaur, 1971 for the *Ceratopteris*-type; Momose, 1949; Atkinson, 1960, 1962; Pray, 1971 for *Anemia* and *Mohria*; Pal and Pal, 1963; Nayar and Kaur, 1969; Nester and Schedlbaur, 1981 for *Ceratopteris*; Nayar and Kazmi, 1964 for *Acrostichum*; Momose, 1967; Nayar and Kaur, 1971 for *Onychium*). In sequential observations of *Anemia phyllitidis* (L.) Sw., Takahashi *et al.* (2012) found that a triangular apical cell forms, as in other symmetric cordate gametophytes but, in some instances, it stops dividing just after the formation of one or, in some instances, two derivatives. Without the functional apical cell, the young gametophyte expands by intercalary growth, and a meristem with a row of narrow rectangular cells is later formed at the lateral site adjacent to the multilayered base (Figure 1F). The meristem contributes to expansion of both wings and the archegonial cushion. This meristem was once called the lateral meristem, but Takahashi *et al.* (2012) indicated that the meristem is comparable with the multicellular meristem of typical cordate gametophytes in its cell division pattern. We conclude that due to the deficiency of a functional apical cell, the initiation site of the multicellular meristem is shifted to the lateral side in the *Anemia*-type. *Ceratopteris* and *Acrostichum* also exhibit the same developmental process as the *Anemia*-type (R. Imaichi, unpublished data). In many cases, the deeply asymmetric cordate shape becomes symmetrically cordate through gametophyte growth.

The *Anemia*-type of development is commonly found in Ceratopteridaceae and Anemiaceae, and some genera of Pteridaceae and Adiantaceae.

4. *Colysis*-type (Figure 1, type II)

Some epiphytic Polypodiaceous taxa have irregularly branched gametophytes, which have been reported to be either strap-shaped (Nayar, 1962 for *Colysis* (Polypodiaceae); Takahashi *et al.*, 2009 for *Colysis*) or ribbon-shaped (Stone, 1960 for *Grammitis* and *Ctenopteris* (Grammitidaceae); Nayar, 1963 for *Leptochilus* and *Paraleptochilus* (Polypodiaceae); Nayar, 1967a for *Loxogramme* (Polypodiaceae); Wagner and Farrar, 1976 for *Hyalotricha* (Polypodiaceae) and *Grammitis* (Grammitidaceae)). The development of *Colysis decurrens* (Wall. ex Hook & Grev.) Nakaike was documented by Takahashi *et al.* (2009). Contrary to previous reports suggesting that *Colysis* gametophytes do not have single apical cells or any organised meristems (Nayar, 1962; Nayar and Kaur, 1969; the Kaulinia type of Nayar and Kaur, 1971), the gametophyte possesses an apical cell (Figure 1G, H) and a multicellular meristem (Figure 1K). However, the development of *Colysis* gametophytes differs greatly from that of cordate gametophytes; after the apical cell ceases to function, a new meristem (i.e. a marginal meristem; Figure 1I) arises before the multicellular meristem phase. The marginal

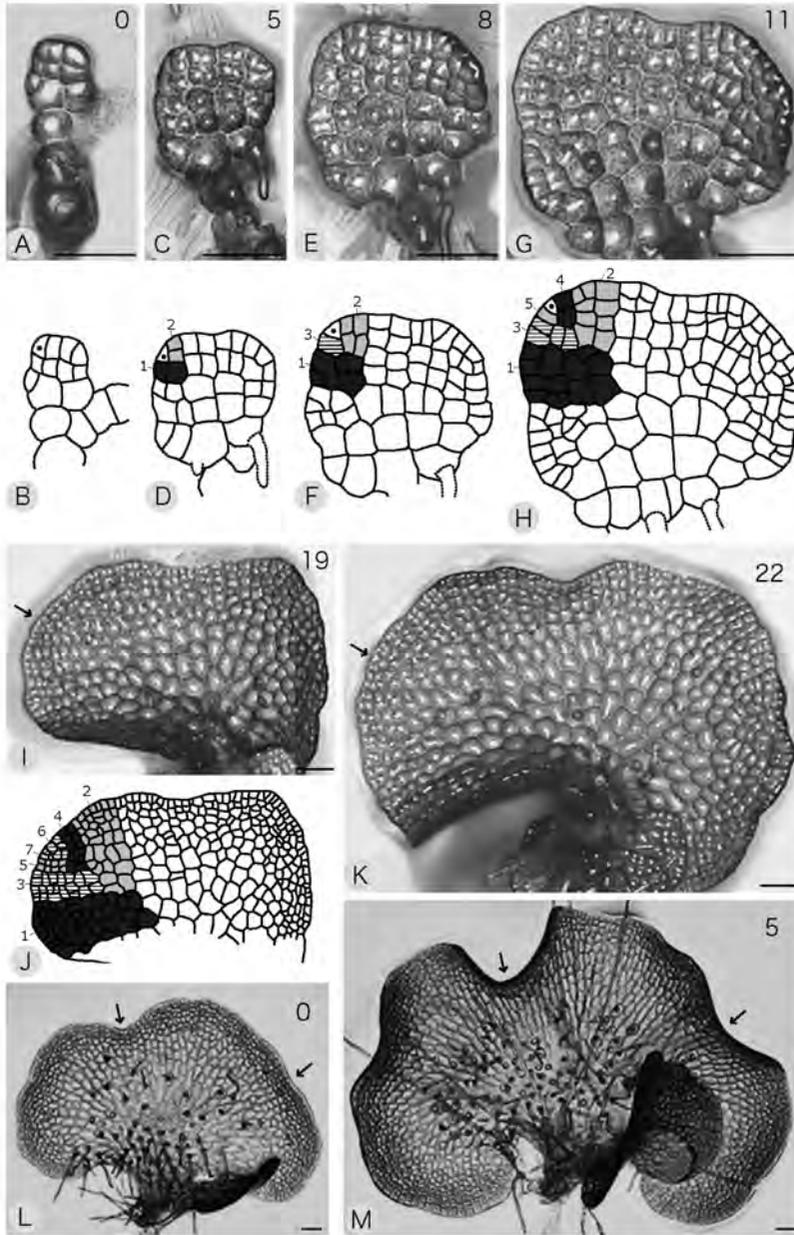


Figure 4. Development of *Vittaria* sp. A, C, E, G, I, K-M. Epi-illuminated micrographs. B, D, F, H, J. Line drawings of A, C, E, G, and I, respectively. Numbers at the upper right corners indicate the number of days passed after the observations began. **A-K.** Images at several developmental stages of the same growing gametophyte individual. Triangular cell marked by a dot indicates an apical cell. Numbers 1-9 indicate merophytes derived from the derivatives of the apical cell. Arrows in I and K indicate the site of the original apical cell. **L and M.** Two images of the same growing gametophyte at the marginal meristem stage. Left and right arrows indicate the same sites in both images where growth has been stopped. Scale bars = 100 μ m.

meristem consists of small rectangular cells with no growth centre, and it covers the anterior margin of the gametophyte (Figure 1I). The marginal meristem often divides by cessation of activity in the middle, resulting in gametophyte branching (Figure 1J). During the marginal meristem phase only antheridia form, and archegonia do not arise. As a result, some strap-shaped lobes are elongated. Ultimately, a multicellular meristem is established on the anterior end of the lobe, and thin superficial cushions with archegonia are later formed (Figure 1K).

Colysis-type development also occurs in the irregularly branched gametophytes of *Loxogramme salicifolia* (Makino) Makino (Polypodiaceae). The triangular apical cell forms at the anterior end of young spatulate gametophytes. This apical cell cuts off several derivative cells and then ceases to function (Figure 3A-E). Concomitantly, cells distinct from the original apical cell become active and contribute to gametophyte growth (Figure 3E, F), and the marginal meristem occurs sporadically. In both *Colysis* and *Loxogramme*, the marginal meristem similarly develops with no relation to the original apical cell. During the marginal meristem phase, the *Loxogramme* gametophyte branches frequently to form many lobes (Figure 3G-J). No rules appear to exist in terms of branching site or frequency. During our culture period, the archegonial cushion had not yet formed in *Loxogramme* gametophytes. However, several studies have reported that the archegonia are formed on thin patchy cushions (2-4 cells thick) behind the multicellular meristem or at the anterior end in *Loxogramme* (Momose, 1967; Nayar, 1967a).

The Colysis-type of development is found in some epiphytic taxa of Polypodiaceae, and commonly found in Grammitidaceae.

5. *Vittaria*-type (Figure 1, Type III)

Another developmental type of irregularly branched gametophytes occurs in the Vittariaceae and some of the Hymenophyllaceae. Previous reports have claimed the absence of the apical cell in Vittariaceae (Nayar and Kaur, 1971), whereas the sequential observation method has documented the occurrence of an apical cell that acts as the initial cell for *Vittaria* sp. (see Figure 4). Two triangular cells are formed at the anterior end of spatulate young plates, and one of these cells is selected to be the functional apical cell (Figure 4A-D). The apical cell cuts off several (seven, in the individual observed) derivatives and ceases to divide (Figure 4D-J). After this stage, cells in the peripheral region located away from the site of the original apical cell become meristematic and gives rise to the marginal meristem (Figure 4I-K). Notably the apical cell-derived merophytes were observed to occupy only less than half of a growing gametophyte (Figure 4J). The remainder of the gametophyte grows mainly through intercalary growth. The marginal meristem then covers nearly the entire margin of the growing gametophyte (Figure 4K). The marginal meristem is involved in gametophyte branching, resulting in the production of several lobes (Figure 4L, M). After this stage, several lobes grow each to form a ribbon-like lobe, maintaining the marginal meristem at the anterior end of each lobe. In the irregularly branched gametophytes of Hymenophyllaceae, the occurrence of both a wedge-shaped apical cell and the marginal meristem has also been reported (Stokey 1940; Stone 1965).

The developmental process of Vittariaceae and Hymenophyllaceae gametophytes is similar to that of the Colysis-type up until the marginal meristem phase, as mentioned above (Figure 1 G-J). However, the location of the archegonial cushion differs greatly between the Colysis- and *Vittaria*-types (Figure 1 K,L). The archegonial cushion is

superficial and central in the Colysis-type, whereas it is marginal or lateral in the Vittaria-type (Farrar, 1974, 1978; Farrar *et al.*, 2008). In some taxa of Hymenophyllaceae, archegonia also arise on the two-cell-thick cushion along the margin of the thallus (Stokey, 1948 for *Hymenophyllum*; Dassler and Farrar, 1997 for *Callistopteris*), whereas in other taxa, they arise on specialised archegoniophores, which develop as lateral, or rarely terminal, outgrowths from the filaments (Stokey, 1948 for *Trichomanes*).

Figure 5 shows the irregularly branched ribbon-like gametophytes of *Hymenophyllum*

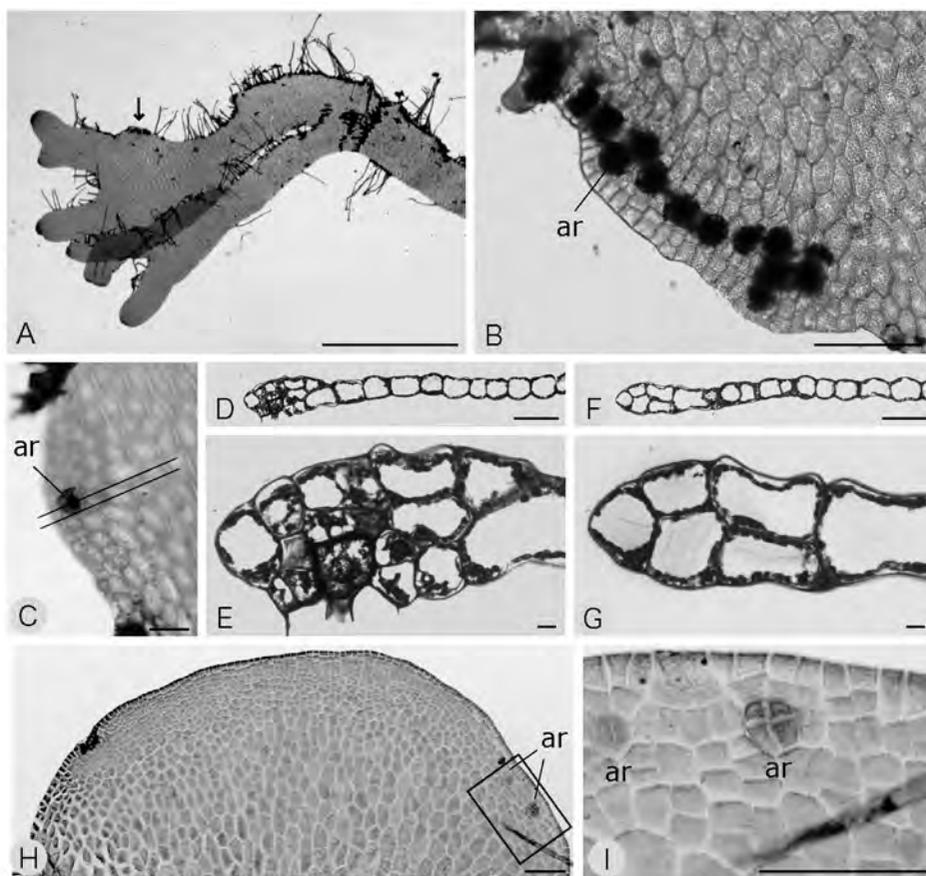


Figure 5. Wild gametophytes of *Hymenophyllum barbatum* (Bosch) Baker. A-C are light micrographs, and D-G are sectional views. H and I are light micrographs of the anterior end of a lobe, stained with Trypan blue. **A.** Irregularly branched gametophyte. Arrow indicates an archegonial cushion lobe. **B.** Enlarged figure of a marginal cushion lobe with many archegonia. **C-G.** Young lobe with an initiating archegonium. The two lines indicate sites of longitudinal sections. **D, E.** Sectional views cut at the site indicated by the upper line in C. E is an enlarged figure of archegonium-bearing region of D. **F, G.** Sectional views cut at the site indicated by the lower line in C. G is an enlarged figure of the peripheral region. **H, I.** Anterior region of a lobe covered by marginal meristem. Area demarcated by a rectangle is magnified in I. Note that newly initiating young archegonia are found in the flank of marginal meristem. ar, archegonium. Scale bars: (A-D, F, H, I) = 100 μm ; (E, G) = 10 μm .

barbatum (Bosc) Baker (collected in Okutama, Tokyo, and identified using the DNA barcoding technique). A series of archegonia appears on a marginal lobe along the basal portion of the gametophyte lobe (Figure 5A, B). The surface view of the archegonial lobe shows that it is composed of many small rectangular cells, where, unlike the multicellular meristem, a row of narrow elongate cells expected to act as initial cells cannot be distinguished (Figure 5B). Transverse sections of a young archegonial lobe show that a two-cell-thick cushion is located just behind one or two outermost cell layers that are one-cell thick (Figure 5C-G). This structure suggests that the cells just below the outermost cell layer of the marginal meristem change their division orientation from vertical to parallel to the substrate, resulting in the two- or three-cell-thick cushion. Similar changes in the orientation of cell division also occur when the archegonial cushion is formed behind the multicellular meristem in the Colysis-type.

In the surface view of the growing lobe, newly initiating archegonia are observed on the flank of the terminal marginal meristem, with the younger archegonia in the region closer to the marginal meristem (Figure 5H, I). This pattern is consistent with Stone's (1965) description: "Young archegonia were also observed on recently formed small cushion areas very close behind the apical initial cells of narrow ribbons of *Mecodium australe*. . . ." Thus, in the Vittaria-type, the archegonial lobe probably begins to develop due to the activity of the marginal meristem. In the Colysis-type, the multicellular meristem appears to be originated from the marginal meristem, and hence the multicellular meristem is regarded as a modified marginal meristem (Takahashi *et al.*, 2009). In comparing the multicellular meristem of the Colysis-type and the marginal meristem of the Vittaria-type, the main difference distinguishing the latter from the former type lies in the site where the archegonial cushion forms (Figure 1 K,L). Notably, the archegonial cushion never forms on the marginal lobe in Colysis-type, and it never forms on central midrib in Vittaria-type, as far as we know. Future studies should more precisely compare the developmental processes of these two types.

The Vittaria-type of development is commonly found in Vittariaceae and Hymenophyllaceae.

CONCLUSION AND PERSPECTIVE

Significance of understanding development of gametophyte forms

Gametophyte biology is necessary to understand adaptation and evolution of ferns, because the gametophyte plant body (that is independent of the sporophyte plant body) is inevitably subjected to environmental conditions and then adapted to the habitats where they grow. Different habitats may select for different growth forms and different growth rates. For example, growth on a vegetation-free but temporary soil habitat is quite different from that on a long-lived tree limb in competition with bryophytes and other epiphytes. In the former habitat cordate gametophytes are generally found, while in the latter habitat slow-growing, ribbon-like gametophytes are predominant. The present review shows that such different growth forms are actually controlled by meristems. To understand gametophyte ecology and reproduction, we have to understand the gametophyte form and its basis in development.

Recent development of flora-wide barcode identification of wild gametophytes (Ebihara *et al.*, 2013; Tsujita *et al.*, 2013) helps us understand gametophyte/habitat relationships more clearly. Now gametophyte research has entered a new era involving ecological and/or evolutionary aspects. To understand the whole story of the gametophyte evolution not only the gametophyte ecology, focusing on the gametophyte-habitat

relationships, but also the comparative development, focusing on the meristem behaviour, should be investigated.

Roles of the three different meristems

Planar fern gametophytes show diversified shapes, depending on the presence or absence of the three following meristem types and how and to what extent they contribute to gametophyte development: apical cell-based meristem, marginal meristem, and multicellular meristem. Regardless of the gametophyte shape, the apical cell-based meristem is always formed as the first step of development. The apical cell-based meristem contributes to the early expansion of the gametophyte. The number of derivatives cut off from the apical cell is nearly the same among differently shaped gametophytes, i.e. 6-8 for *Lygodium* (cordate), 5-6 for *Colysis* (ribbon) and 7 for *Vittaria* (ribbon). The reason that the presence of the apical cell was previously missed in ribbon-like gametophytes (cf. Nayar and Kaur's *Kaulinia* type, 1971) is likely to be related to the shape of the anterior end of the gametophyte: it is round for the *Colysis*- and *Vittaria*-types but notched for cordate gametophytes. In the round apex of the *Colysis*- and *Vittaria*-types, each merophyte that constitutes a gametophyte is elongate-rectangular in shape (Figure 1H), whereas in the notched apex of the *Lygodium*-type, each merophyte is fan-shaped, with the longest outer margin and the shorter inner margin (Figure 1B).

The multicellular meristem has two main functions. It acts as initial cells to contribute to the expansion of both wings and formation of a multilayered archegonial cushion. In addition, the multicellular meristem is also involved in apical branching of the gametophyte. The multicellular meristem is divided into two independent meristems via the cessation of activity in the middle portion of the once extended meristem (*Lygodium japonicum*, Takahashi *et al.*, in prep.). Note that the apical cell-based meristem never divides in two.

The main role of the marginal meristem is to extend the entire periphery of the gametophyte and to contribute to the formation of many lobes through division of the marginal meristem. Owing to the marginal meristem, an irregularly branched shape is established. The marginal meristem does not contribute to the archegonial cushion in the *Colysis*-type but probably does in the *Vittaria*-type.

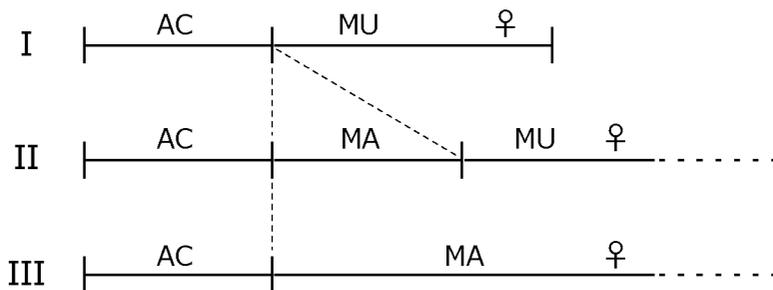


Figure 6. Diagrammatic illustrations of developmental phases that form three types. I. *Lygodium*- and *Elaphoglossum*-type for cordate gametophytes. II. *Colysis*-type. III. *Vittaria*-type. Dotted lines continuous to solid lines in II and III indicate that the multicellular and the marginal meristem phases continue indefinitely, respectively. AC, apical-cell based meristem phase; MA, marginal meristem phase; MU, multicellular meristem phase. ♀, archegonium.

Three meristem behaviours resulting in diversified gametophyte shapes

If the apical-cell based meristem phase is immediately followed by the multicellular meristem phase (Lygodium-type), the typical cordate gametophyte results (Figure 6I). In this case, the multicellular meristem begins to produce an archegonial cushion soon after the meristem is established. If the multicellular meristem does not produce the archegonial cushion for a while (i.e. the formation of the archegonial cushion is delayed; Elaphoglossum-type), a strongly elongate-cordate, strap-shaped gametophyte is formed. If the apical cell does not function and the multicellular meristem is formed on the lateral side and not on the apical end (Anemia-type), an asymmetrically cordate gametophyte results. Therefore, the Elaphoglossum-type, which forms strap-shaped gametophytes, and the Anemia-type, which forms asymmetric cordate gametophytes, are both considered to be subtypes of the Lygodium-type, which forms the typical cordate gametophyte.

If the marginal meristem phase intervenes between the apical cell-based meristem phase and the multicellular meristem phase, the Colysis-type is established (Figure 6II). During the marginal meristem phase, the gametophyte branches repeatedly, forming many lobes. This process may lead to a much longer life span of the irregularly branched gametophyte compared to the cordate gametophyte. If the multicellular meristem phase is absent, the Vittaria-type results (Figure 6III). In both the Colysis- and Vittaria-type, the gametophyte becomes irregularly branched during the marginal meristem phase. Thus, so-called ribbon-like gametophytes involve two different development types, Colysis and Vittaria. The difference between these two types involves the site of the archegonial cushion: in the former type, it is superficial and lies at the centre of the lobe, whereas in the latter type, it is located on the lateral margin of the lobe (cf. Figure 1K, L). In the Colysis-type, the archegonial cushion is formed by the activity of the multicellular meristem, which is considered modified marginal meristem (Takahashi *et al.*, 2009). In the Vittaria-type, however, the initiating archegonial lobe is found in the flank of the marginal meristem, suggesting that the marginal meristem of the Vittaria-type could have gained the ability to form a multilayered archegonial cushion near the margin because the multicellular meristem phase was lost. The developmental process of the archegonial cushion in the Colysis- and Vittaria-types should be compared further.

Farrar *et al.* (2007) classified planar fern gametophytes into three ecologically functional groups based on form, type of meristem, type of proliferation and longevity: annual cordate gametophytes with the meristem in the notch, perennial strap-shaped with the meristem in the notch and perennial ribbon-shaped that have the meristem in the margin. Missing from this classification is the Colysis-type, which has meristem in both the margin (marginal meristem) and the notch (multicellular meristem). Still other types of development may also occur, especially for irregularly branched, ribbon-like gametophytes. For example, the very narrow gametophytes of *Pleurosoriopsis* (Polypodiaceae) appear always to retain the triangular apical cell and have superficial archegonial cushions (Masuyama, 1975; R. Imaichi, personal observation). Gametophytes of *Phanerosorus* (Matoniaceae) are also ribbon-shaped with an apical cell and a superficial archegonial cushion (Yoroi and Kato, 1987). Whether these gametophytes possess the multicellular meristem when the archegonial cushion forms remains uncertain. In some *Trichomanes* (Hymenophyllaceae) species, the gametophyte uniquely shows two sharply defined growth forms: a filamentous, branching phase that gives rise to an upright, blade-like phase (Farrar and Wagner, 1968 for *Trichomanes*).

The examination of gametophyte development should be especially extended to non-cordate gametophytes that mainly occur in epiphytic taxa.

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REFERENCES

- ATKINSON, L.R. 1960. The Schizaeaceae: the gametophyte of *Mohria*. *Phytomorphology* 10: 351-367.
- ATKINSON, L.R. 1962. The Schizaeaceae: the gametophyte of *Anemia*. *Phytomorphology* 12: 264-288.
- ATKINSON, L.R. & STOKEY, A.G. 1964. Comparative morphology of the gametophyte of homosporous ferns. *Phytomorphology* 14: 51-70.
- BANKS, J.A., HICKOK, L. & WEBB, M.A. 1993. The programming of sexual phenotype in the homosporous fern, *Ceratopteris richardii*. *Int. J. Plant Sci.* 154: 522-534.
- BOWER, F.O. 1923. The ferns (Filicales). Vol. 1. Analytical examination of the criteria of comparison. Cambridge Univ. Press, London, UK.
- CHANDRA, S. 1979. Gametophyte morphology of the fern genus *Drynariopsis* (Polypodiaceae). *Amer. Fern J.* 69: 111-118.
- CHIOU, W.-L. & FARRAR, D.R. 1997. Comparative gametophyte morphology of selected species of the family Polypodiaceae. *Am. Fern J.* 87: 77-86.
- CHOU, H.M., HUANG, Y.M., WONG, S.L., ISIEH, T.H., HSU, S.Y. & CHIOU, W.L. 2007. Observations on gametophytes and juvenile sporophytes of *Archangiopteris somai* Hayata (Marattiaceae), an endangered fern in Taiwan. *Bot. Stud.* 48: 205-213.
- DASSLER, C.L. & FARRAR, D.R. 1997. Significance of form in fern gametophytes: clonal, gemmiferous gametophytes of *Callistopteris baueriana* (Hymenophyllaceae). *Int. J. Plant Sci.* 158: 622-639.
- DASSLER, C.L. & FARRAR, D.R. 2001. Significance of gametophyte form in long-distance colonization by tropical, epiphytic ferns. *Brittonia* 53: 352-369.
- DÖPP, W. 1927. Untersuchungen über die Entwicklung von Prothallien einheimischer Polypodiaceen. *Pflanzenforschung* 8: 1-58.
- EBIHARA, A., YAMAOKA, A., MIZUKAMI, N., SAKODA, A., NITTA, J.H. & IMAICHI, R. 2013. A survey of the fern gametophyte flora of Japan: frequent independent occurrences of noncordiform gametophytes. *Am. J. Bot.* 100: 735-743.
- FARRAR, D.R. 1974. Gemmiferous fern gametophytes—Vittariaceae. *Am. J. Bot.* 61: 146-155.
- FARRAR, D.R. 1978. Problems in the identity and origin of the Appalachian *Vittaria* gametophyte, a sporophyteless fern of the eastern United States. *Am. J. Bot.* 65: 1-12.
- FARRAR, D.R. 1998. The tropical flora of rockhouse cliff formations in the eastern United States. *J. Torrey Bot. Soc.* 125: 91-108.
- FARRAR, D.R. & WAGNER, W.H., Jr. 1968. The gametophyte of *Trichomanes holopterum* Kunze. *Bot. Gaz.* 129: 210-219.

- FARRAR, D.R., DASLLER, C., WATKINS, J.E. & SKELTON, C. 2008. Gametophyte ecology. In: RANKER, T.A. & HAUFLER, C.H. (Eds) Biology and evolution of ferns and lycophytes. Cambridge Univ. Press, Cambridge, UK. pp. 222-256.
- GABRIEL y GALÁN, J.M. & MIGLIARO, G. 2011. Comparative study on the gametophyte morphology and development of three paramo species of *Jamesonia* (Pteridaceae, Polypodiopsida). Nord. J. Bot. 29: 249-256.
- KORN, R.W. 1974. Computer simulation of the early development of the gametophyte of *Dryopteris thelypteris* (L.) Gray. Bot. J. Linn. Soc. 68: 163-171.
- MASUYAMA, S. 1975. The gametophyte of *Pleurosoriopsis makinoi* (Maxim.) Fomin. J. Jpn. Bot. 50: 105-114.
- MENDOZA-RUIZ, A. & PÉREZ-GARCÍA, B. 2005. Análisis comparativo de la fase sexual de dos especies de *Microgramma* (Polypodiaceae, Pleopteltoideae). Acta Botanica Mexicana 71: 1-10.
- MOMOSE, S. 1949. On the prothallium of *Lygodium* and *Anemia*. J. Jpn. Bot. 24: 128-132 (in Japanese).
- MOMOSE, S. 1967. Prothallia of the Japanese ferns (Filicales). Univ. of Tokyo Press, Tokyo, Japan (in Japanese).
- NAYAR, B.K. 1961. Studies in Polypodiaceae. VII. *Pyrrosia*. J. Indian Bot. Soc. 40: 164-186.
- NAYAR, B.K. 1962. Morphology of spores and prothalli of some species of Polypodiaceae. Bot. Gaz. 123: 223-232.
- NAYAR, B.K. 1963. Contributions to the morphology of *Leptochilus* and *Paraleptochilus*. Am. J. Bot. 50: 301-308.
- NAYAR, B.K. 1965. Gametophytes and juvenile leaves of Drynarioid ferns. Bot. Gaz. 126: 46-52.
- NAYAR, B.K. 1967a. The gametophyte and juvenile leaves of *Loxogramme*. Am. Fern J. 58: 19-29.
- NAYAR, B.K. 1967b. Morphology of the spores and prothallus of *Christiopteris tricuspis*. Am. Fern J. 57: 15-27.
- NAYAR, B.K. & KAUR, S. 1969. A reinvestigation of the morphology of the gametophyte and juvenile sporophyte of *Ceratopteris thalictroides*. Can. J. Bot. 47: 395-404.
- NAYAR, B.K. & KAUR, S. 1971. Gametophytes of homosporous ferns. Bot. Rev. 37: 295-396.
- NAYAR, B.K. & KAZMI, F. 1964. The gametophyte of *Acrostichum aureum* L. Proc. Indian Acad. Sci. 59: 185-194.
- NAYAR, B.K. & RAZA, F. 1970. The prothalli of some Polypodiaceae—II *Lepisorus loriformis*, *L. thunbergianus*, *Polypodium vulgare* and *Weatherbya accedens*. J. Indian Bot. Soc. 49: 81-86.
- NESTER, J.E. & SCHEDLBAUER, M.D. 1981. Gametophyte development in *Anemia mexicana* Klotzch. Bot. Gaz. 142: 242-250.
- OGURA-TSUJITA, Y., SAKODA, A., EBIHARA, A., YUKAWA, T. & IMAICHI, R. 2013. Arbuscular mycorrhiza formation in cordate gametophytes of two ferns, *Angiopteris lygodiifolia* and *Osmunda japonica*. J. Plant Res. 126: 41-50.
- ORTH, R. 1936. Morphologische und physiologische Untersuchungen an Farnprothallien. Planta 25: 104-150.
- PAL, N. & PAL, S. 1963. Studies on morphology and affinity of the Parkeriaceae. II. Sporogenesis, development of the gametophyte, and cytology of *Ceratopteris*

- thalictroides*. Bot. Gaz. 124: 405-412.
- PÉREZ-GARCÍA, B. & RIBA, R. 1998. Bibliografía sobre gametofitos de helechos y plantas afines. 1699-1996. Monographs in systematic botany from the Missouri Botanical Garden. 70. Missouri Botanical Garden Press, St. Louis, MO, USA.
- PRAY, T.R. 1971. The gametophyte of *Anemia colimensis*. Am. J. Bot. 58: 323-328.
- PRYER, K.M. SCHUETTPELZ, E. WOLF, P.G. SCHNEIDER, H. SMITH, A.R. & CRANFILL, R. 2004. Phylogeny and evolution of ferns (Monilophytes) with a focus on the early leptosporangiate divergences. Amer. J. Bot. 91: 1582-1598.
- RAGHAVAN, V. 1989. Developmental biology of fern gametophytes. Cambridge Univ. Press, Cambridge, UK.
- STOKEY, A.G. 1940. Spore germination and vegetative stages of the gametophytes of *Hymenophyllum* and *Trichomanes*. Bot. Gaz. 101: 759-790.
- STOKEY, A.G. 1948. Reproductive structures of the gametophytes of *Hymenophyllum* and *Trichomanes*. Bot. Gaz. 109: 363-380.
- STOKEY, A.G. & ATKINSON, L.R. 1956. The gametophyte of the Osmundaceae. Phytomorphology 6: 19-40
- STOKEY, A.G. & ATKINSON, L.R. 1957. The gametophyte of some American species of *Elaphoglossum* and *Rhipidopteris*. Phytomorphology 7: 275-292.
- STONE, I.G. 1960. Observations on the gametophytes of *Grammitis billardieri* Willd. and *Ctenopteris heterophylla* (Labill.) Tindale (Grammitidaceae). Aust. J. Bot. 8: 11-37.
- STONE, I.G. 1965. The gametophytes of the Victorian Hymenophyllaceae. Aust. J. Bot. 13: 195-224.
- TAKAHASHI, N. & IMAICHI, R. 2007. Developmental morphology of young gametophytes of *Botrychium microphyllum* in axenic culture. J. Jpn. Women's Univ. Fac. Sci. 15: 45-49.
- TAKAHASHI, N., HASHINO, M., KAMI, C. & IMAICHI, R. 2009. Developmental morphology of strap-shaped gametophytes of *Colysis decurrens*: a new look at meristem development and function in fern gametophytes. Ann. Bot. 104: 1353-1361.
- TAKAHASHI, N., KAMI, C., MORITA, N. & IMAICHI, R. 2012. Comparative development of heavily asymmetric-cordate gametophytes of *Anemia phyllitidis* (Anemiaceae) focusing on meristem behavior. J. Plant Res. 125: 371-380.
- TESTO, W.L. & WATKINS, J.E. Jr. 2011. Comparative development and gametophyte morphology of the hart's-tongue fern, *Asplenium scolopendrium* L. J. Torrey Bot. Soc. 138: 400-408.
- VON ADERKAS, P. & CUTTER, E.G. 1983. The role of the meristem in gametophyte development of the osmundaceous fern *Todea barbara* (L.) Moore. Bot. Gaz. 144: 519-524.
- WAGNER, W.H. & FARRAR, D.R. 1976. The Central American fern genus *Hyalotricha* and its family relationships. Syst. Bot. 1: 348-362.
- WATKINS, J.E. Jr., MACK, M.K. & MULKEY, S.S. 2007. Gametophyte ecology and demography of epiphytic and terrestrial tropical ferns. Am. J. Bot. 94: 701-708.
- WHITTIER, D.P. 1983. Gametophytes of *Ophioglossum engelmannii*. Can. J. Bot. 61: 2369-2373.
- WHITTIER, D.P. & THOMAS, R.D. 1993. Gametophytes and young sporophytes of *Botrychium jenmanii* in axenic culture. Int. J. Plant Sci. 154: 68-74.
- YOROI, R. & KATO, M. 1987. Wild gametophytes of *Phaneroglossum major* (Matoniaceae). Am. J. Bot. 74: 354-359.