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Multicellular spores and false anisospory in Bryowijkia ambigua (Musci: Trachypodaceae)

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All observed capsules of *Bryowijkia ambigua* (Hook.) Noguchi, contain small unicellular spores (38–58 μm) that are aborted, and large multicellular spores (145–180 μm) that are presumably viable. Regardless of maturation stage of a capsule or geographical origin, spore size variation within a capsule follows a consistent bimodal pattern. The two types of spores are in a constant 1:1 ratio, with about 230–250 spores of each type per capsule. This ratio is established early in spore development, after a phase of granule deposition. In each tetrad, two spores abort while still unicellular and the other two spores remain viable and become multicellular. These observations suggest that the spore mass in *Bryowijkia ambigua* represents a very unusual instance of false anisospory, since it involves unicellular and multicellular spores. As a phylogenetic trait, these multicellular spores are interpreted as a derived feature, as compared with the unicellular spores in *Trachypus*, other genera in the Trachypodaceae, and the majority of species in mosses.

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Все наблюдаемые урны Bryowijkia ambiqua (Hook.) Noguchi содержат маленькие одноклеточные споры (38 58 мм), которые являются бесплодными, и большие многоклеточные споры (145 180 мм), которые, вероятно, являются жизнеспособными. Невзирая на стадию созревания урны или географическое происхождение, изменчивость величины спор внутри урны следует последоваыельному бимодальиому паттерну. Оба типа спор находятся в постоянном отношении один к одному, примерно с 230 - 250 спорами каждого типа на урну. Данное отношение устанавливается рано в развитии спор, после фазы гранулярного осаждения. В каждой тетраде две споры являются бесплодными и одноклеточными, а остальные две споры остаются жизнеспособными и становятся многоклеточными. Данные наблюдения предполагают, что масса спор в Bryowijkia ambiqua представляет собой весьма необычный пример ложной анизоспории, так как она охватывает одноклеточные и многоклеточные споры. Как филогенитический признак, данные многоклеточные споры интерпретируют в качестве производной особенности в сравнении с одноклеточными спорами в Trachypus, другими родами в Trachypodaceae, и с большинством видов во мхах.

Bryowijkia is a monotypic genus endemic to continental southeast Asia (Gangulee 1976). In an evaluation of its systematic position, Vitt and Buck (1984) concluded that *Bryowijkia ambigua* (Hook.) Noguchi should be classified in the Trachypodaceae, contrary to Brotherus (1925) who placed the genus as *Cleistostoma* in the Hedwigiaceae. Recent systematic studies also indicate that *Bryowijkia* does not belong to the Hedwigiaceae, because it lacks all the characters that circumscribe that family: a globular protonema, short leaf cells with papillae, and rugulate spores (De Luna, unpubl.).

The morphology of *Bryowijkia* is unusual in several ways. Portier de la Varde (1924) described in detail the differentiated marginal cells in the leaves, the very short seta, and the ornamentation of the reduced peristome. Recently Vitt and Buck (1984) pointed out that the

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elongate leaf cells with longitudinally seriate papillae in *Bryowijkia* are known elsewhere only in a small number of species in the genera *Trachypus* (Trachypodaceae) and *Papillaria* (Meteoriaceae). *Bryowijkia* was classified in the Trachypodaceae because it shares additional features with *Trachypus*, e.g., quadrate alar cells and plicate leaves with a single costa (Vitt and Buck 1984).

The spores of *Bryowijkia ambigua* also exhibit characteristics not commonly found in mosses. The spores in *B. ambigua* are dimorphous, i.e., each capsule contains small unicellular and large multicellular spores (Brotherus 1925, Vitt and Buck 1984). Only two additional species among all bryophytes are known to have small unicellular and large multicellular spores within the same capsule, *Leucodon atrovirens* (Muraoka 1968, Akiyama 1988) and *Mesotus celatus* (Allen 1987a). A review of the pertinent literature indicates that such spore dimorphism has not been studied in detail in *Bryowijkia* and *Mesotus*. During the course of revisionary studies of the Hedwigiaceae, many specimens of *Bryowijkia* have been examined. The purpose of this paper is to report data on spore size frequency, spore viability, and spore output per capsule, in order to characterize adequately the peculiar spore mass of *B. ambigua*.

Multicellular spores and false anisospory in mosses

In most bryophytes, the spores are unicellular, more or less spherical, and 10 μm – 50 μm in diameter (McClymont 1955, Miyoshi 1962, Boros and Jarai-Komlodi 1975, Clarke 1979). However, multicellular spores have been reported in a few mosses and in several liverworts and hornworts (Herzog 1917, Nehira 1983, 1987, Schofield 1981, 1985). In mosses, the best known examples of multicellular spores are found in the Dicnemonaceae (Allen 1987b), *Muelleriella* Dusén (Vitt 1976), and *Drummondia* (Vitt 1972, Allen 1987c).

Other mosses that have multicellular spores are: *Leucodon atrovirens* Noguchi (Akiyama 1988), *Mesotus celatus* Mitt. (Allen 1987a), *Sphaerotheciella* Fleisch. (Manuel 1977), and *Ephemeropsis trentepohlioides* (Renn.) Sainsb. (Sainsbury 1955). Schofield (1981) reported that "*Ephemeropsis tjibodensis* Goeb. also possesses multicellular spores". However, according to Brotherus (1925), Tixier (1974), and my observations from herbarium specimens (Touw 20903, 21058, DUKE), the spores in *E. tjibodensis* are unicellular. Multicellular spores were also observed in *Symphysodon vitianus* (Sull.) Broth. (Smith 7357, DUKE), a case that apparently has not previously been reported in the literature.

Multicellular spores are different from spores that undergo endosporic germination after dispersal, e.g., those of *Andreaea, Glyphomitrium*, and various liverworts. In these instances, although initial mitosis results in a "massive" endosporic protonema, the spores are unicellular when shed from the capsule (Nehira 1983). In contrast, multicellular spores develop while still in the capsule, i.e., mitosis is endosporic and endothecial (cf. Niedhart 1979). These multicellular spores do not continue protonemal development within the capsule. Rather, they are shed and would require rehydration to reassume protonemal and shoot development (Allen 1987c, Nehira 1983).

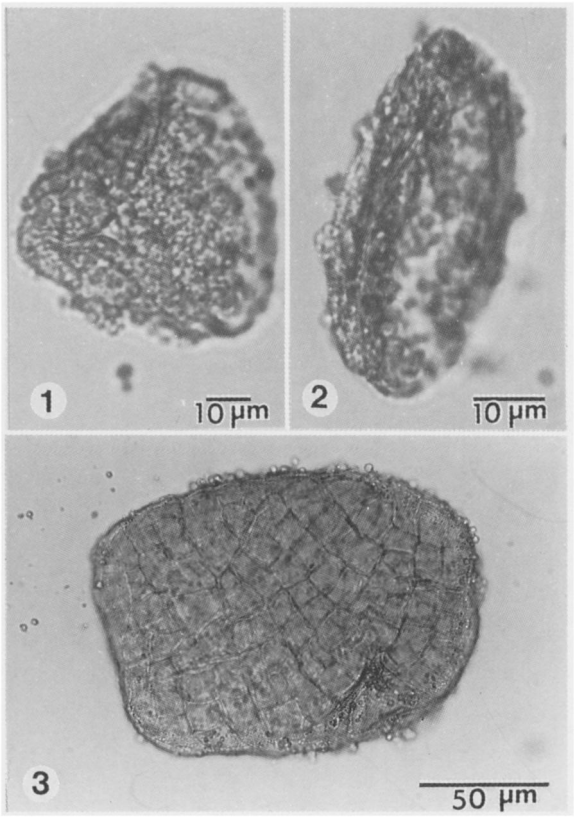
Spore size variation within a species is usually unimodal (isospory, cf. Mogensen 1981). Spore size dimorphism apparently is not known in liverworts (Schuster 1984). However, it has been reported in a few moss species, for example in *Cinclidium* spp., *Fissidens cristatus* (dubious), *Leucodon* spp., *Macromitrium* spp., *Orthotrichum lyellii, Rhizomnium magnifolium*, and in *Schlotheimia* spp. (Ernst-Schwarzenbach 1938, Vitt 1968, Mogensen 1978a, b, Ramsay 1979, Akiyama 1988).

In most instances of spore dimorphism, unicellular spores of two different sizes are produced in the same capsule, usually in a 1:1 ratio. According to Vitt (1968), Mogensen (1978a, 1983), and Ramsay (1979), if the two size fractions consist of living spores, the dimorphism is defined as anisospory. Additionally, Mogensen (1978a) proposed that when the small size fraction, composed of 50% of total spores, includes only aborted spores and the large spore fraction consists only of living spores, the dimorphism should be defined as false anisospory or pseudoanisospory. Mogensen (1981, 1983) also suggested that other types of variation in spore size frequency exist when there are more than two mean spore sizes, and when the ratio of aborted/living spores is variable. Most previous reports of intraspecific spore size variations are instances of anisospory or pseudoanisospory, always involving unicellular spores (Mogensen 1983). This report documents an unusual example of pseudoanisospory that involves unicellular and multicellular spores.

Materials and methods

A total of 65 herbarium specimens of *Bryowijkia ambigua* were studied to collect data on spore dimorphism, spore development, sexuality, and geographical distribution. Capsules from seven herbarium specimens (populations 1–7) were examined to estimate variation in spore size and spore output. Additionally, capsules from one specimen (pop. 8) were dissected and the spores were cultured to estimate germination percentages. The eight primary specimens are listed below with standard reference numbers:

1. – Thailand, Chieng Dao. 1960. Ps. Sukhaul (MO, US). 2. – Thailand, Payap, Doi (Mt) Suthep, granitic massive W of Chiengmai. 28 Nov., 1965. A. Touw 8502



Figs 1–3. Unicellular and multicellular spores of *Bryowijkia ambigua*. 1. – Polar view of an unicellular spore, showing scars in spore wall. 2. – Lateral view of a collapsed unicellular spore; note the granulae deposited on the spore surface. 3. – A multicellular spore also with granulae on the spore surface.

(MO). 3. – Thailand, Payap, limestone massive Doi (Mt) Chiengdao. Dec 6, 1965. A. Touw 9124 (MO, NY). 4. – Thailand, same locality as above. 7 Dec., 1965. A. Touw 9299 (MO). 5. – Thailand, Doi chong, northern part. 17 Feb., 1968. B. Hansen & T. Smitinand 13019 a (MO). 6. – Burma. Dec. 1906. D. O. Mills et al. (NY). 7. – Siam, W Chieng Mai. 1922. E. Smith (US). 8. – Nepal, in a forest near Chitre. 7 Jul., 1972. Z. Iwatsuki 2404 (MO).

A mature, but still operculate, capsule was selected from each of specimens 1–7. The capsule was placed in a drop of water on a microscope slide, and its walls were punctured to allow water to enter and rehydrate the spores. Then the capsule was dissected and all spores were kept on the slide and mounted in Hoyer's medium. The size of every spore was recorded measuring the longest diameter under a compound microscope, using a 10 × objective. At the same time all the spores were classified as unicellular or multicellular, and living or dead.

Spore viability was estimated with acetocarmine staining. According to Mogensen (1978a, b), living spores are stained, while dead spores do not stain at all. Although it is not necessarily true that all stained spores are viable, it is safe to assume that non-stained spores are dead. Other criteria for estimating spore viability, such as germination percentages, were also attempted.

A modified Hoagland's culture medium (cf. Mishler 1985) was used to germinate spores from two capsules from one herbarium specimen (pop. 8). Nine Petri dishes were prepared with culture medium at three different pH values (6.4, 7.0, 7.6) and were placed in three different growth conditions. Two sets of dishes were placed in growth rooms, one at 17°C and the other at 20°C. The third set was placed on a window sill of a room at about 25°C.

Preliminary data on spore development and germination pattern was recorded from herbarium specimens. Few capsules in different maturation stages were dissected to examine tetrads and the development of unicellular and multicellular spores. Also, germinated spores with young developing gametophytic shoots were found established on the perichaetial leaves in several specimens of *Bryowijkia ambigua*. Some of these germinated spores were mounted on a slide with Hoyer's medium.

Results and discussion

The unicellular spores of *Bryowijkia ambigua* are more or less isodiametric, or trilete. Frequently, however, the spores are collapsed (Figs 1, 2). In contrast, the multicellular spores are subspheric to irregularly obloid (Fig.

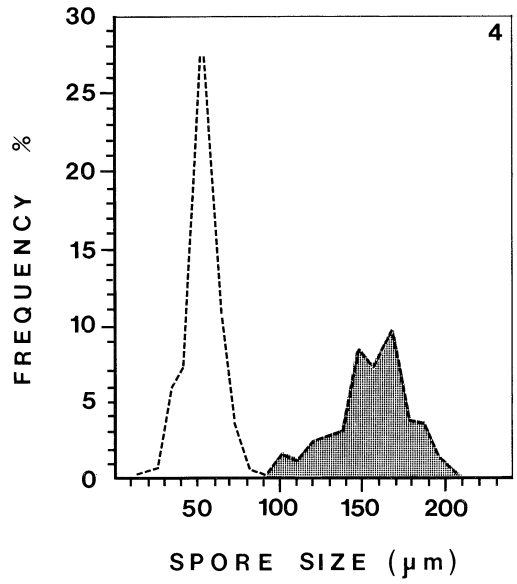


Fig. 4. Size variation in the spore mass of *Bryowijkia ambigua*. The graph shows spore size frequency in one mature capsule taken from population 3. Shaded area indicates size variation of multicellular spores.

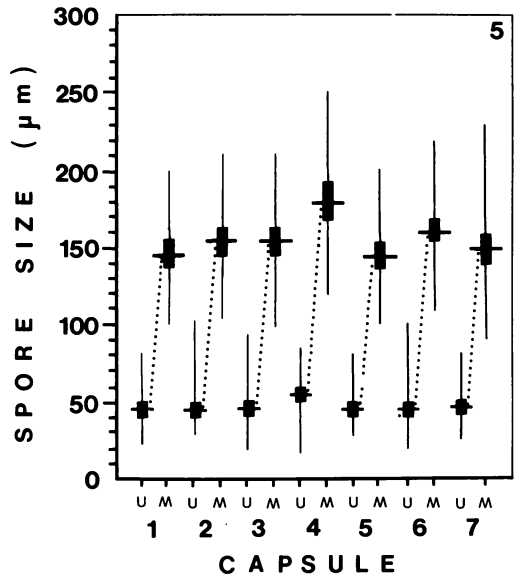


Fig. 5. Spore size variation in *Bryowijkia ambigua*. The graph shows spore size in seven mature capsules taken from populations 1–7. The mean sizes (horizontal line), confidence intervals (verical bars), and ranges (vertical lines) are indicated for unicellular spores (U), and for multicellular spores (M). The dotted line unites observations from the same capsule.

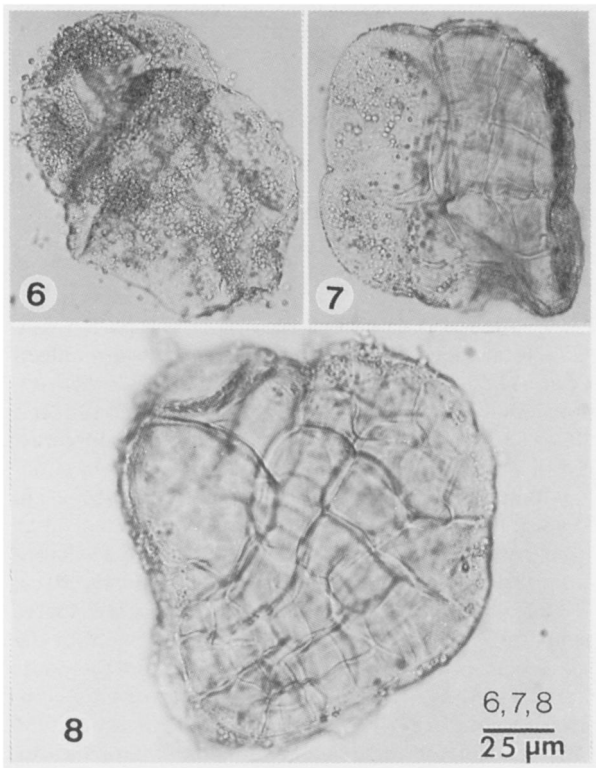
3), are about 6–8 cells wide, 12–15 cells long, and 5–7 cells in depth. The surface of both types of spores is covered by granules (Figs 1–3).

The multicellular spores in *B. ambigua* are relatively large and well-developed compared with most multicellular spores known in other mosses. For example, spores in *Leucodon atrovirens* consist of about 8 to 16 cells in one or two layers (Muraoka 1968, Akiyama 1988). The multicellular spores of *Ephemeropsis tentrepohlioides* (Sainsbury 1955), *Muelleriella* (Vitt 1976), *Sphaerotheciella* (Manuel 1977) and *Symphysodon vitianus* also consist of few cells. Only spores of the Dicnemonaceae (Allen 1987a), *Mesotus* (Allen 1987c), and *Drummondia* (Vitt 1972), consist of many cells and are tridimensional or "massive", like the multicellular spores in *Bryowijkia*.

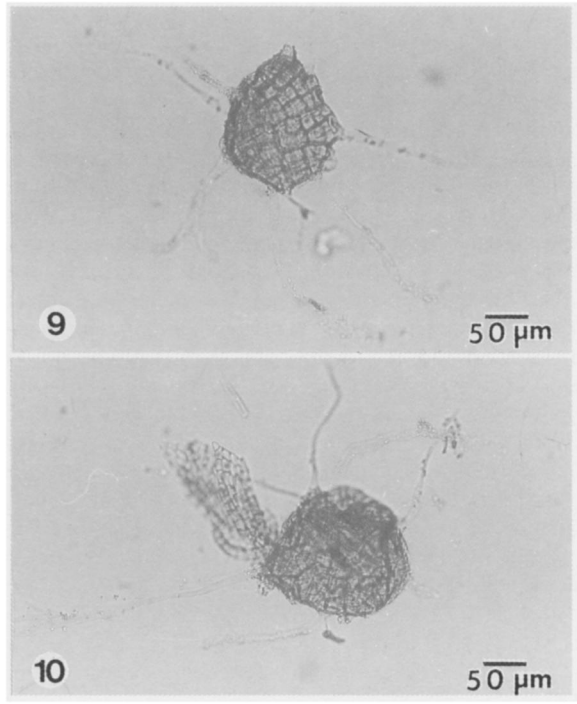
A graph of spore size frequency showing two approximately bell-shaped curves indicates that two statistical populations of spores are represented in a capsule of *Bryowijkia ambigua* (Fig. 4). Bimodality of spore size was present in all capsules studied (Fig. 5). Without exception, the fraction of small spores included only unicellular spores, whereas the fraction of large spores consisted only of multicellular spores. An analysis of variance followed by a multiple range test (Scheffe method, evaluated at a significant level of 1%) indicated that there are no significant differences either among the seven mean sizes (µm) of unicellular spores ($x_1 = 49.0$, $x_2 = 49.1$, $x_3 = 46.8$, $x_4 = 51.6$, $x_5 = 49.8$, $x_6 = 49.7$, $x_7 = 43.3$), or among the seven means of multicellular spores ($x_8 = 145.4$, $x_9 = 154.2$, $x_{10} = 154.7$, $x_{11} = 181.5$, $x_{12} = 148.4$, $x_{13} = 161.8$, $x_{14} = 149.2$). The only significant difference is between size means of unicellular and multicellular spores.

The spore sizes reported here are similar to the dimensions given by Vitt and Buck (1984). They stated a range from 45 to 55 µm for unicellular spores, and 150–210 µm for multicellular spores. In all capsules examined in the present study, unicellular spores ranged from 20 to 100 µm in diameter, but they most frequently measured from 38 to 58 µm (Fig. 5). The fraction of multicellular spores exhibited a wider size range than did the unicellular spores. Multicellular spores measured between 100 and 250 µm, but most spores fell in the range from 145 to 180 µm.

The wide size range of multicellular spores might conceivably be correlated with different maturation stages of the spores within a mature capsule. However, the same degree of developmental variation in spore size seems to be present in all capsules examined. Measurements from seven populations also suggest that the same size range of each spore type is present in each capsule (Fig. 5). Since the specimens are from different localities, the same analysis also suggests that spore



Figs 6–8. Morphology during late sporogenesis in *Bryowijkia ambigua*. 6. – A young tetrad with all spores still unicellular. Spore walls are covered by fine granulae. 7. – A tetrad in a later stage shows two spores already multicellular while the other two remain unicellular. 8. – Still in a tetrad, multicellular spores continue developing while the two unicellular spores abort and collapse.



Figs 9–10. Protonemal development in *Bryowijkia ambigua*. 9. – Massive group of cells with several rhizoidal filaments. 10. – In a later stage, buds are differentiated directly from the massive group of cells. These sporelings were found attached to perichaetial leaves in herbarium specimens.

dimorphism in *B. ambigua* is not sporadic within certain populations, but is indeed characteristic of the species.

Regardless of developmental variation, or geographical origin, unicellular and multicellular spores are consistently present in a 1:1 ratio, within each capsule. There are about 230–250 spores of each type per capsule. Total counts of the spores in seven capsules ranged from 439 to 509 spores per capsule. However, for several reasons, in some slides it was not possible to count all spores. Therefore, it is probable that the spore output in *B. ambigua* is about 500–520 spores per capsule.

Although there are few data on spore output in mosses, in most species spore output per capsule varies from one thousand to several hundred thousand (Kreulen 1972, During 1979, Longton and Schuster 1983). Only a small number of species produce as few spores per capsule as does *Bryowijkia*. For example, species of *Archidium* are noted for their capsules containing less than 20 spores (Snider 1975). Also, capsules in the Dicnemonaceae (Allen 1987b) and *Drummondia* (Vitt 1972) produce few spores. In *Mesotus* no counts were available, but two capsules that I examined contained about 1000 spores (Vitt 8238, DUKE) each. The same counts suggested that a 1:1 ratio of unicellular and multicellular spores occurs in *Mesotus* as well as in *Bryowjikia ambigua*.

Observations of gross morphological changes during spore development and maturation in *Bryowijkia ambigua* are presented in Figs 6–8. The earliest developmental stage observed consisted of unicellular spores still united in tetrads (Fig. 6). At this stage, all spores in a tetrad have the same nearly spherical shape, are about the same size, and have smooth surfaces. In a later stage, these unicellular spores become densely covered by granules (Fig. 6). Presumably, these granules constitute the perine (Mogensen, pers. comm.).

After the phase of granule deposition, two spores of each tetrad remain unicellular, while the other pair of spores becomes multicellular (Fig. 7). The shape of the unicellular spores changes from nearly spherical to trilete or completely flattened (Figs 7, 8; cf. Figs 1, 2). This suggests that abortion of 50% of the spores within a capsule probably happens during this developmental phase. The observation that no unicellular spores in a mature capsule stained when treated with acetocarmine is congruent with the assumption that these spores are aborted.

The shape of the multicellular spores also changes as endosporic mitosis continues. Multicellular spores first become ovoid, oblong, and finally reach their mature irregular obloid shape (Figs 3, 7, 8). These spores stained with acetocarmine suggesting that they are viable. Viability could not be directly demonstrated, since neither the unicellular nor the multicellular spores germinated under the growth conditions attempted here. This is in spite of the fact that such conditions have been adequate for spore germination in numerous other moss species. The age of the spores (cf. pop. 8), and possible fumigation treatments might account for these negative results. Nevertheless, sporelings were found established on perichetial leaves in several herbarium specimens. All sporelings consisted of a massive endosporic protonema, with rhizoids and buds developing directly from cells of the massive protonema (Figs 9–10). According to these observations, protonemal development in *B. ambigua* is of the *Drummondia*-type (Nehira 1983).

In summary, the features that define false anisospory (sensu Mogensen 1983) have been documented in *Bryowijkia ambigua*. First, it has been shown that spore size frequencies are consistently bimodal. Second, total spore counts indicate that unicellular and multicellular spores are in a 1:1 ratio. Third, this ratio is apparently established early during spore development, when only two spores in a tetrad become multicellular. Also, acetocarmine staining suggests that the unicellular spores are aborted, and that only multicellular spores remain viable. Based on these observations, the spore mass in *B. ambigua* is interpreted as an instance of false anisospory, and so far it is the only documented example involving unicellular and multicellular spores. The spore mass in *Mesotus* is apparently an additional example, which still needs to be confirmed with detailed spore counts and observations of spore development and viability.

At present it is difficult to adequately interpret the

biological significance of spore dimorphism in relation to the immersed capsules, reduced peristomes, a possibly dioecious sexual system, and epiphytic habitat of *B. ambigua*. Such an extraordinary combination of specialized features merits further developmental, ecological, and evolutionary study.

The actual causes of spore dimorphism (genetical, developmental, physiological, etc.) in *Bryowijkia ambigua* are impossible to determine at present. A genetic cause of pseudoanisospory has been proposed based on examples which only involve unicellular spores (Mogensen 1978a, 1981, 1983). The complementary action of dominant and recessive homallelic pairs (A + B, a + b) of a system of two genes in different chromosomes has been proposed to cause the 50% of spore mortality per capsule in *Cinclidium*. In each tetrad, only the heteroallelic combinations (A + b, a + B) would produce viable spores, and eventually gametophores. Dioecy and the location of one gene in sex chromosomes are necessary conditions to restore heterozygous (Aa + Bb) sporophytes (Mogensen 1978a). Although a genetic cause may also explain pseudoanisospory in *Bryowijkia*, the extrapolation of the same genetic system, still untested, or the proposition of alternative explanations seem unwarranted at present.

Longton and Schuster (1983) and Schuster (1984) speculated that, in the evolution of adaptations to hard, dry surfaces, pluricellular spores are "a secondary development", at least in hepatics and anthocerotes, where "generalized" groups have unicellular spores. In mosses, since the species with multicellular spores are either epiphytic or epilithic, Buck (1980) commented that "it appears that multicellular spores are an adaptation to xeric conditions and are not an indication of relationships".

Based on the criteria summarized by Patterson (1982) to evaluate character homology, similar multicellular spores occurring in genera unrelated to *Bryowijkia* are homologs as protonema, but cannot be interpreted as taxic homologs, because of differences in development, and because their phylogenetic incongruence with other characters. Thus, multicellular spores are assumed to have originated independently in several lineages of mosses: in the Dicranales (Dicnemonaceae and *Mesotus* in the Dicranaceae), Eubryales (*Muelleriella* and *Drummondia* in the Orthotrichaceae), Leucodontales (*Bryowijkia ambigua* in the Trachypodaceae, *Leucodon atrovirens* in the Leucodontaceae, *Sphaerotheciella* in the Cryphaeaceae, and *Symphysodon vitianus* in the Pterobryaceae), and in the Hookeriales (*Ephemeropsis trentepohlioides* in the Ephemeropsidaceae). Whether these multicellular spores are apomorphic or plesiomorphic at a particular phylogenetic level is a polarity decision that needs a case by case evaluation. For example, under any out-group comparison within the Leucodontales, it is feasible to interpret multicellular spores in *Bryowijkia ambigua* as a derived feature within the Trachypodaceae.

Several authors have speculated on the adaptive significance of spore features in relation to reproductive ecology in bryophytes. For example, During (1979), Longton and Schuster (1983), and Mogensen (1983) considered spore size in relation to spore number and dispersal distance. According to them, small spores are correlated with large spore numbers and long-range dispersal. Also, Nehira (1983, 1987), Schofield (1981), and Schuster (1984) have suggested that endosporic protonema and multicellular spores, in general, are ecological adaptations to xeric habitats. The multicellular spores and presumably short protonemal development directly producing gametophytic shoots are viewed as an "advantage to a moss that colonizes such well-drained sites as tree bark or rock faces" (Schofield 1981). However, these adaptive hypotheses have remained untested, and *B. ambigua* provides a potential model for experimental studies, within a phylogenetic framework. For example, putative ecological advantages of multicellular spores can be tested in comparison to unicellular spores of the other species in the Trachypodaceae.

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