Permanent spore dyads are not ‘a thing of the past’: on their occurrence in the liverwort *Haplomitrium* (Haplomitiopsida)

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The liverwort *Haplomitrium gibbsiae* is shown to regularly produce spores released in the form of permanent dyad pairs. Developmental studies indicate that the dyads are produced via a unique half-lobed configuration of the developing sporocyte. Many fossil cryptophytes of Siluro-Devonian age, which are clearly embryophytes based on their morphology, contain permanent spore dyads in their sporangia, but this is the first demonstration of their occurrence in a living plant, a species belonging to Haplomitiopsida, which resolves in a clade that is considered to be sister to all remaining liverworts. Dispersed spore-like dyads are found in the rock record as far back as the mid-Cambrian, but most researchers still regard the first occurrence of isomorphic, tetrahedral tetrads in the mid-Ordovician as the benchmark age for the origin of land plants. Regardless of the geological antiquity of the embryophytes, it appears that *H. gibbsiae* has retained a non-simultaneous form of sporogenesis that may ultimately be traced to a charophytic origin. © 2015 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2015, 179, 658–669.


INTRODUCTION

The earliest evidence of land plants in the fossil record comes from Ordovician (Darriwilian) rocks and consists of isomorphic, tetrahedral spore tetrads, the topological arrangement of which represents an embryophyte synapomorphy (Edwards et al., 2014; Strother, Traverse & Vecoli, 2015). These tetrads always occur in association with permanent cryptospore dyads and monads, the systematic provenance of which is less certain. The occurrence of diverse assemblages of tetrad, dyad and monad cryptospores serves as a proxy for the existence of a land flora for nearly 40 million years prior to the appearance of plant megafossils in the mid-Silurian (Edwards, Feehan & Smith, 1983), supporting the interpretation that the evolution of resistant, thick-walled meiospores preceded the origin of the sporophytic plant body (Bower, 1908, 1935; Strother, 2010; Brown & Lemmon, 2011).

Although the vast majority of extant embryophytes produce spores that dissociate from their tetrad partners, occasional remnant species produce persistent and even permanent tetrads (Brown et al., 2015; Renzaglia, Lopez & Johnson, 2015). In contrast, there are no published accounts of permanent dyads in
extant plants. Spores in persistent tetrads found in extant plants typically adhere because of delayed degradation of the spore mother cell (SMC) wall. In such instances, the veil of the SMC wall in various stages of dissolution is evident across the mature sporoderm (Brown et al., 2015). This condition is reminiscent of the envelope-enclosed tetrads (e.g. Velatitetras N.D. Burgess) found in Silurian palynofloras (Strother & Traverse, 1979; Johnson, 1985; Burgess, 1991; Wellman, Edwards & Axe, 1998a, b). On the other hand, the rarer permanent free tetrads remain connected independent of the SMC wall (Renzaglia et al., 2015); these tetrads are dispersed and germinate as intact units. Thus, they are not enclosed in an envelope and may provide counterparts to even earlier fossil assemblages (Ordovician) that include abundant naked tetrads (Strother et al., 2015).

In comparison with tetrads, cryptospore dyads in the fossil record are perplexing in terms of both an understanding of the mechanics of sporogenesis and of the establishment of their affinity with extant land plants, none of which, up to now, have been known to produce permanent dyads. During a comprehensive study of sporogenesis and spore wall development in bryophytes, we discovered a species of the early divergent liverwort genus Haplomitrium Nees which routinely produces spores that remain united in dyads, even through germination. These are distinct from the persistent dyads described in the hornwort Leioclasporoceros Hässel, as the latter are the result of the peculiar isobilateral arrangement of spores (Brown et al., 2015). The permanent dyads in Haplomitrium are not covered by an envelope and thus bring to mind the naked dyads of the earliest plant fossil record. The first report of permanent dyads in an extant land plant and the nature of the interconnection between dyad spores are the subjects of this study. For comparison, new images of cryptospore dyads from the Cambrian, Ordovician and Silurian are also included. The occurrence of truly permanent dyads in the early divergent liverwort Haplomitrium provides a new avenue through which to explore the evolutionary implications of dyads as a primitive character in early embryophytes.

**MATERIAL AND METHODS**

Spores from disjunct populations of *H. gibbsiae* (Steph.) R.M. Schust s.l. were examined, including two from New Zealand, the type locality for the species, and one from Tierra del Fuego, Chile. Although Chilean plants have sometimes been referred to the species *H. chilensis* R.M. Schust. (Schuster, 1971), we follow Bartholomew-Began (1991) and treat populations from both localities as *H. gibbsiae*. Female plants of *H. gibbsiae* bearing sporophytes were collected from several sites on the South Island of New Zealand (Carafa, Duckett & Ligrone, 2003). The plants were maintained fresh in a plastic container in a growth cabinet under continuous illumination of 100 W m−2/s at 8 °C. For transmission electron microscopy (TEM), sporophytes were fixed in 3% glutaraldehyde, 1% fresh formaldehyde and 0.75% tannic acid in 0.05 M sodium cacodylate buffer, pH 7, for 3 h at room temperature. After several rinses in 0.1 M buffer, the samples were post-fixed in buffered (0.1 M, pH 6.8) 1% osmium tetroxide (OsO4) overnight at 4 °C, dehydrated in an ethanol series and embedded in TAAB low-viscosity resin via ethanol. Ultrathin sections were cut with a diamond knife, stained with methanolic uranyl acetate for 15 min and in Reynolds’ lead citrate for 10 min, and observed with a Hitachi H-7100 transmission electron microscope at 100 kV.

Collection details for plants from Chile are as follows: Isla Grande de Tierra del Fuego, Comuna Timaukel, Parque Nacional Alberto de Agostini, 54.5045S, 70.3472W, 5 m elevation, B. Shaw 13395 [F]. Samples were shipped live to Southern Illinois University, Carbondale, IL, USA (SIUC), where elongating and unopened capsules were processed for TEM according to the protocol in Renzaglia et al. (2015). Capsules were excised from elongated setae and fixed in 2% (v/v) glutaraldehyde in 0.05 M phosphate buffer, pH 7.2, for 1 h at room temperature, followed by three rinses in buffer. The specimens were post-fixed for 10 min in 1% (w/v) OsO4, rinsed three times in distilled water and then serially dehydrated in ethanol at room temperature. Ultrathin sections were collected on grids and post-stained with ethanolic uranyl acetate and Reynolds’ lead citrate. Observations were made and digital images were captured on a Hitachi H7650 transmission electron microscope at 60 kV.

Capsules from Chilean (Shaw 13395) and New Zealand (South Island: Hislop Creek, August 2011, Renzaglia 3439 [F]) samples were fixed for scanning electron microscopy (SEM) in buffered 2% glutaraldehyde, followed by post-fixation in 2% OsO4 or formaldehyde–acetic acid–ethanol (FAA). Specimens were transferred in a graded ethanol series to 100% ethanol, critical point dried using CO2 as the transitional fluid, mounted on stubs and sputter coated for 230 s (76.7 nm) with palladium–gold, and viewed using an FEI Quanta 450 scanning electron microscope.

Capsules from Chilean and New Zealand populations were surface sterilized using standard procedures, and the dispersed spore dyads were axenically placed on solidified culture medium. Images of germinating dyads were captured using compound microscopes (either a Zeiss Axioskop or a Leica CTR 5000) equipped with digital imaging cameras. Digital images of fossil dyads were taken with a Nikon D1x camera back attached to a Zeiss Universal

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RESULTS

Our studies confirm that, in New Zealand and Chilean populations, spores are typically released and germinate as dyads, although a few monads may also be seen. There are differences in dyad dimensions between the two localities, with those from Chilean samples measuring 56–61 μm across and 31–34 μm high, and those from New Zealand samples measuring 40–49 μm across and 23–29 μm high. Apart from these size and slight ornamentation differences, dyads from the two localities are identical.

The youngest tetrads of H. gibbsiae were observed in SEM and exhibit a peculiar half-lobed configuration (Fig. 1A). Irregular openings or pockets are visible in the SMC wall and these reveal the special spore wall that encircles the early expanding tetrads. Because the special wall begins to develop during meiosis in most hepatics, it is possible that these tetrads are late sporocytes in the process of dividing. The vast majority of capsules available for study contained mature tetrads that consisted of pairs of spores (dyads) in a staggered arrangement (Fig. 1B). The dyad arrangement corresponds with the half-lobing of the immature tetrads (cf. Fig. 1A, B).

The distal and proximal surfaces of the dyads are well defined (Fig. 2). Distal wall ornamentation comprises low tubercles with tiny perforations scattered throughout the surface (Figs 1B, 2A–C). In the Chilean material, tubercles terminate in rounded caps (Fig. 2A), whereas those in New Zealand dyads are apically flattened (Fig. 2C). The proximal walls consist of a minute reticulum in both populations, with tiny irregular bacculae prominent throughout (Fig. 2A, D). Spores do not exhibit a trilete mark and do not have apertures, although the proximal reticulum is denser in the centre of the wall (Fig. 2D).

Spores contain abundant peripheral oil droplets and a central nucleus surrounded by mitochondria and plastids (Fig. 3A, B). Occasionally, a dyad may display incomplete cytokinesis with clear cytoplasmic domains of two nuclei, each with tightly associated plastids and mitochondria (Fig. 3A).

The simple spore wall is three layered (Fig. 3). An intine, averaging 430 nm in thickness (N = 9), lies adjacent to the plasmalemma. The exine is two layered: the outer exine forms the sculptoderm and the inner exine consists of 1–15 layers of concentric tripartite lamellae (TPL). Sporopollenin impregnates the TPL that comprise the ‘stalk’ region of each tetrade (Fig. 3C, D). The densely sporopollenin-covered TPL are widely separated, longitudinally oriented in each tetrade and enclose a space that is similar in electron opacity to that surrounding the spores (Fig. 3B, D). Tubercle caps consist of short slips of TPL that are visible in a matrix of less dense sporopollenin (Fig. 3C, D). These caps are fragile and may break from the spore surface during specimen preparation (Figs 2A, B, 3A, C). The proximal wall is devoid of tubercles and contains only sporopollenin-covered

Figure 1. Scanning electron micrographs of tetrads of Haplomitrium gibbsiae. A, Young expanding two-lobed tetrad surrounded by spore mother cell (SMC) wall and spore special wall (arrow). B, Larger, mature tetrad showing dyad arrangement that corresponds with the two lobes in (A). Bars, 5.0 μm.

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Figure 2. Scanning electron micrographs of dyads and a tetrad of the two populations of *Haplotrichum gibbsiae*. A, Chilean dyad in side view showing flattened proximal and rounded distal surfaces. Tubercles with caps decorate the distal surface with small holes on the wall between. Arrow indicates cap that detached during specimen preparation. B, Chilean tetrad showing dyad arrangement. Arrow indicates entire tubercle that detached during specimen preparation. C, Distal surface of New Zealand dyad showing tubercles that are continuous across spore boundaries. D, Flattened proximal surface of New Zealand dyad with reticulate sculptoderm and small irregular upright rods. Arrow indicates exine elements connecting the two spores and arrowhead points to dense central reticulum of exine elements. Bars, 10 μm.
Figure 3. Transmission electron micrographs showing spore wall features. A, New Zealand tetrad with an incomplete cytokinesis of one dyad pair that contains cytoplasmic domains of two nuclei (n) and associated plastids (p) and mitochondria. Vacuoles and lipid droplets occupy the periphery of the cells. Bar, 5.0 μm. B, The spore of a Chilean dyad also contains abundant plastids (p) and mitochondria (m) around the nucleus (n), and peripheral lipid droplets (o). The wall has a prominent intine (i) and overlying thin exine (e) that comprises the ornamentation. Bar, 5.0 μm. C, In New Zealand plants, the exine consists of an inner continuous multilamellate layer (ie) and sporopollenin-impregnated stalks (s) that terminate in aggregates of slips of tripartite lamellae (TPL) embedded in sporopollenin. i, intine. Bar, 0.20 μm. D, In Chilean dyads, the exine overlies the intine (i), and consists of an inner single, rarely double (arrow), layer of TPL and sporopollenin-impregnated stalks (s) that terminate in aggregates of slips of TPL embedded in sporopollenin. Bar, 0.10 μm. E, F, G, Development of the inner exine of New Zealand dyads. E, TPL migrating through the intine (i) to be added to the multilamellate layer that comprises the inner exine. Bar, 0.20 μm. F, TPL emanate from the plasmalemma (short arrow) and migrate through the intine (i), whereas sporopollenin is added and contributes to the multilamellate layer of the inner exine (long arrow). Bar, 0.10 μm. G, Higher magnification of the fully developed multilamellate layer that is the inner exine (ie) overlying the intine (i). Sporopollenin in the outer exine (oe) is visible. Bar, 0.05 μm.
TPL that are densely packed and project outwardly from the intine (Figs 3A, 4). The inner exine contains 1–15 layers of TPL, depending on the stage of spore development. This layer completes development after the capsule elongates. In Chilean specimens, the inner exine contains one or two TPL layers (Fig. 3D), whereas those from New Zealand typically have well-developed multilamellate layers (MLLs) (Fig. 3C, G). These differences probably reflect slightly earlier stages of development in the Chilean capsules. During development, slips of TPL or slips of MLLs emanate from the plasmalemma, migrate through the intine and are added to the inner exine, progressively increasing the number of TPL in the MLLs (Fig. 3E, F). All of these stages are visible in a single capsule.

There is no intersporal septum between any two spores, regardless of whether or not they separate (Fig. 4). The intersporal zone in all instances consists of a network of exine elements similar to those in the tubercle stalks (Fig. 4B–E). Intersporal exine elements meet directly and fuse between spores that remain united in dyads (Fig. 4A–C). In contrast, the exine network across dyads in a tetrad, i.e. those that do not stay connected, is loose with only limited, easily broken, connection of elements across spores (Fig. 3D, E). A spotty, irregular zone of sporopollenin often remains after dyads separate (Fig. 4A, E). As seen in surface section, the outer exine on the proximal walls after dyad separation consists of a tight reticulum (Fig. 4F).

Following imbibition, the spore wall stretches, but the spores remain united in dyads (Fig. 5A). The first few divisions of germinating spores are endosporic (Fig. 5B, C). Following one or two cell divisions, the thin area of the dyad proximal wall adjacent to the shared dyad wall ruptures and the young sporeling emerges (Fig. 5C). Spores tethered in dyads are usually staggered in germination (Fig. 5C, D) or one of the pair may abort (not shown). During early sporeling development, the interconnection between spores in dyads remains until the globose sporelings are freed from their spore cases just after the stage shown in Figure 5D.

Fossil spore dyads are presented in Figure 6. Because descriptions of these dyads for the most part have been published elsewhere, the images in Figure 6 are integrated into the discussion below.

DISCUSSION

The unequivocal existence and simple manner by which dyad spores are connected in *H. gibbsiae* are of significance in interpreting the evolution of embryophytes and their spores. *Haplotomtrium* is one of three genera in Haplotomtripsida, the sister taxon to the remaining liverworts, and *H. gibbsiae* is resolved as sister to other species of the genus (Stech & Frey, 2004). The position of *H. gibbsiae* in the first divergent clade in phylogenetic trees of liverworts (Forrest et al., 2006; Cooper, Henwood & Brown, 2012) suggests that the developmental pathway leading to dyad production is ancient and may well represent a remnant condition from the earliest embryophytes. With liverworts often resolved as the first lineage of embryophytes in key molecular phylogenetic trees (Qi et al., 1998, 2006; Finet et al., 2010; Gao, Su & Wang, 2010; Karol et al., 2010; Chang & Graham, 2011), this finding is particularly significant. Even as recent studies (Cox et al., 2014; Wickett et al., 2014) have challenged both the paraphyly of the three bryophyte clades and the sister group relationship between liverworts and other embryophytes, the occurrence of permanent dyads in the clade that is sister to all other liverworts implies antiquity. Liverworts are now known to contain extant species that produce permanent tetrads and dyads, matching those seen in the earliest fossil record (Wellman, Osterloff & Mohiuddin, 2003; Stroter et al., 2015), prior to the first occurrence of upright axial plants (Kenrick et al., 2012). Edwards et al. (2014) pointed out that the dyad condition must now stand alongside tetrahedral tetrads (and their derivatives, trilete spores) as a plesiomorphic marker of embryophyte affinity in the fossil record, an assertion that was previously based on fossil data and is now grounded in fact with this first report of permanent dyads in a living taxon.

The structural simplicity of the connection between spores of the dyad pair in *H. gibbsiae* is reminiscent of the exine connections that unite spores in tetrads of *Sphaerocarpus* Boehm. (Renzaglia et al., 2015). In both plants, the spore surface ornamentation extends across spore boundaries, although, because of the regularity of the pattern, it is more prominent in *Sphaerocarpus* than in *H. gibbsiae*. Intersporal septa are lacking in both, and exine elements in adjacent spores connect directly to each other. In a phylogenetic context, tetrad-producing *Sphaerocarpus* represents one of the earliest diverging lineages of complex thalloid liverworts (Forrest et al., 2006; Crandall-Stotler, Stotler & Long, 2009), and these permanent tetrads may also be viewed as a relict of some of the earliest spore wall developmental processes that arose in land plants (Renzaglia et al., 2015).

The ultrastructure of spores and spore walls in *H. gibbsiae* is among the least complicated of those in liverworts, even compared with other species in the Haplotomtripsida. Both *Haplotomtrium hookeri* (Sm.) Nees and *Apotreubia nana* (S. Hatt. & Inoue) S. Hatt. & Mizut. have a well-developed intine and a two-layered exine, the inner of which is a continuous MLL (Brown et al., 2015). Contrary to expectations, spore
Figure 4. Transmission electron micrographs (TEMs) showing the absence of an intersporal wall and well-developed exine that connects the two dyad spores, but not dyad pairs. A, New Zealand tetrad with direct connection between the two exines of spores in a dyad, but separation between the exines between dyads. Well-developed plastids (p), numerous vacuoles (v) and lipid droplets (o) are visible in spore cytoplasm. Bar, 2.0 μm. B, The connection between Chilean spores in dyads consists of a network of fused outer exine tripartite lamellae (TPL) impregnated with sporopollenin that directly connects the intine (i) of the two spores. Bar, 1.0 μm. C, Lower magnification of connection between dyad spores in Chilean plants showing extensive connection between exines across spore boundaries. o, lipid droplets. Bar, 1.0 μm. D, Exines between the two dyads of Chilean plants do not fuse, but are progressively separated, leaving sporopollenin droplets between. o, lipid droplets. Bar, 1.0 μm. E, A spotty irregular zone of sporopollenin remains after dyad pairs separate, as seen in New Zealand plants. Bar, 1.0 μm. F, Surface section showing a tight reticulum of outer exine on the proximal wall after dyad separation in New Zealand plants. Bar, 0.5 μm.
walls of *H. gibbsiae* and other relictual bryophytes are thinner and provide less protection from desiccation than those of more derived taxa, such as *Fossombronia* Raddi (Brown & Lemmon, 1993). With abundant oil reserves and well-developed plastids, the spores of *Haplomitrium* germinate within a month of release and cannot survive drying for even a few days (Bartholomew-Began, 1991). In bryophytes, such as *Haplomitrium*, the resistant exine layer is so fragile and thin that the utility of spores in this species as perennating structures or in long-distance dispersal is unlikely. These features of the spore wall may be derived, despite the apparent retention of a primitive developmental pathway that results in united spores. Given that fossilization of spores favours those with robust, highly resistant walls that are capable of surviving fossilization, there is a strong bias in interpreting fossil spores as direct evidence of their natural selection as perennating structures important in dispersal and survival. With no fossil record of the more fragile spores, such as those produced by *H. gibbsiae*, the generalization of such a supposition across early land plants remains untestable.

In any case, the morphology and wall ultrastructure of extant permanent tetrads and dyads provides our clearest morphological link to the fossil record concerning embryophyte origins. The earliest fossil dyads from the mid-Cambrian (Fig. 6A) occur in mixed populations of spores that are bound together in packets with varying numbers of enclosed spores (Strother & Beck, 2000; Strother et al., 2004; Taylor & Strother, 2008). Individual spore walls are multilaminate and these laminae are typically extensively folded over each other creating an uneven ‘wrinkled’ appearance to the wall itself (arrow, Fig. 6A). Enclosing envelopes form a synoecosporal wall comprising up to four laminae c. 250 nm thick (Taylor & Strother, 2008). Ordovician dyads tend to be laevigate (smooth) and largely free of an envelope. They first occur in the Darriwilian (mid-Ordovician), as illustrated here in Figure 6B by *Didymospora luna* P.K. Strother et al. (Strother et al., 2015), and continue through the remainder of the Ordovician (Wellman, 1996).

Dispersed dyads from the lower Silurian (Fig. 6C) may exhibit spore surface textures similar to that of...
H. gibbsiae (Fig. 5A). Abbitusdyadus histosus C.H. Wellman & J.B. Richardson (Fig. 6D) from the lower Silurian is characterized by a reticulate wall that continues over the spore sutures in the dyad pair, as in the exine of H. gibbsiae. A parallel condition occurs with the Silurian enclosed cryptospore tetrad Velatitetas and permanent tetrads of extant Sphaerocarpos (Renzaglia et al., 2015). The MLL wall ultrastructure (reviewed in Taylor, 2009) of Silurian cryptospore dyads further draws comparisons with sphaerocarpalean and complex thalloid liverworts (Strother, 2010), suggesting that, by the Silurian, the massive layered exine of more derived taxa had evolved. Based on these comparisons, it is reasonable to suggest that naked, thinly ornamented dyads, similar to those of H. gibbsiae, may have originated extremely early in land colonization and at least by the Silurian, when ornamented dyads first became common.

Figure 6. Fossil cryptospore dyads from Cambrian, Ordovician and Silurian rocks. A, Closely associated dyads from a palynological assemblage recovered at 26.2 m from the base of the Bright Angle Shale at Sumner Butte in the Grand Canyon, Arizona (PKS sample GC97-23, mid-Cambrian, Glossopleura biozone age). These undescribed dyads are characterized by highly folded multilaminate walls (arrows), in both individual spore walls and synoecosomal walls (envelopes). B, A smooth-walled, free dyad, Didymospora luna Strother et al., 2015, from the Hanadir Shale Member of the Qasim Formation (mid-Ordovician, Darriwilian age) in Saudi Arabia. C, Unnamed cryptospore dyad from the lower Silurian (Llandovery, Aeronian) Tuscarora Formation, Millerstown, Pennsylvania. Although this specimen has a degraded wall, note the similarity to that of Haplomitrium gibbsiae in Figure 4A. D, Abbitusdyadus histosus Wellman & Richardson, 1996, recovered from the lower Silurian (Llandovery, Aeronian) Tuscarora Formation, Millerstown, Pennsylvania. In this species, a reticulate sculpture forms an envelope that encloses both spores of the dyad pair. Bars, 10 μm.

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There are no other known extant plants with permanent dyads. *Leiosporoceros* spp. may produce persistent, but not permanent, dyads, which are the result of the anomalous isobilateral tetrad characteristic of this hornwort. In this configuration, pairs of spores touch at their ends and remain connected at their intersporal walls (Brown et al., 2015). Spores adhere as pairs as a result of close contact and drying down of extraneous materials during capsule dehiscence. Thus, the dyad condition in *Leiosporoceros* is a result of a more or less mechanical condition that is not related to development during sporogenesis (Brown et al., 2015). Moreover, spore germination occurs in monads in this hornwort and not dyads as in *H. gibbsiae*.

The connection of pairs of spores, not tetrads, is perplexing in *H. gibbsiae*, given the predominance of simultaneous cytokinesis, which is characteristic of sporogenesis in most extant land plants. The only well-established example of successive cytokinesis in bryophytes is from the moss *Amblystegium* Schimp. (Brown & Lemmon, 1982) which, unlike other true mosses, has rounded sporocytes. Because of the lack of developmental stages, we were unable to determine whether cytokinesis is simultaneous in *H. gibbsiae*, but, based on the peculiar half-lobing of the young tetrad, we postulate that it is successive. The lack of separation of spores in each dyad pair supports the timing differences in the initiation of cytokinesis following meiosis I and II. At a minimum, there are mechanically distinct meiosis II events, each of which results in a dyad pair. In most bryophytes, cytokinesis is initiated through quadrilobing of the sporocyte and the establishment of spore domains in advance of meiosis (Brown & Lemmon, 2011, 2013; Brown et al., 2015). This process is controlled by unusual microtubule arrays that originate in the diploid sporocyte. Similarly, the spore wall ornamentation is predetermined in the sporocyte, further regulating and expediting spore development. The evolution of simultaneous cytokinesis was integrally involved in this process.

Given that *H. gibbsiae* is dioicous and dyads represent the end products of meiosis II divisions, it can be assumed that the cells of each dyad pair are either both male or both female. This is consistent with the tight association we have seen between plants of the same sex in wild populations. This was probably the case with early fossil dyads, even if dyads represent mitotic, not meiotic, products. However, the widespread occurrence of cryptospore monads, dyads and tetrads in the fossil record, as noted above, points towards their meiotic origin.

Previous authors have described the spores of *H. gibbsiae* commonly occurring as dyads or tetrads in seemingly mature capsules (e.g. Campbell, 1959; Schuster, 1967; Bartholomew-Began, 1991), but it was assumed that this was a transient phase and that the dyads would ultimately separate into monads prior to release or germination. In fact, Campbell (1959) illustrated germination in *H. gibbsiae* occurring from monads. This is puzzling because we observed germination occurring only from dyads in all Chilean and New Zealand populations studied, as illustrated in Figure 5. In naturally opened capsules, some monads were present, but the majority of the spores were dispersed as dyads. In addition to the dyad sporelings that developed in axenic culture, we also found a few germinated dyads of similar morphology on the leaves of a few plants in the Chilean population.

The documentation of developmental diversity in extant bryophyte sporogenesis (Brown & Lemmon, 2011; Brown et al., 2015; Renzaglia et al., 2015), in combination with the well-characterized spore topology and wall ultrastructure in Cambrian cryptospores (Strother et al., 2004; Taylor & Strother, 2008, 2009; Taylor, 2009), suggests that endoduplication prior to meiosis, much like that which occurs in *Coleochaete* Bréb. today (Hopkins & McBride, 1976), preceded the subsequent evolution of embryophytic sporogenesis, in which only four meiospores develop simultaneously. A similar explanation of endoduplication before meiosis was evoked by Taylor & Strother (2009) for the late Cambrian cryptospore *Agamachates* W.A. Taylor & P.K. Strother, which can produce three or more dyad sets within a common ‘envelope.’ In *Agamachates*, it is interpreted that varying numbers of genomic duplications in the 2n zygote, prior to meiosis, result in aggregations (packets) of differing numbers of spore dyads after successive cytokinesis and spore wall formation. Thus, both the fossil record and developmental studies support the occurrence of spore dyads as a characteristic element of the early phases of embryophyte evolution.

Presumably, during the adaptive radiation in subaerial environments, concomitant with the evolution of the plant embryo/sporophyte, the production of one tetrad per sporocyte was favoured over multiple spores per sporocyte. The physical conditions associated with subaerial life may well be correlated with timing shifts that initiated precocious cytokinesis and spore wall patterning in the sporocyte (Brown & Lemmon, 2011). This first unambiguous example of dyad formation in an embryophyte provides evidence that the unusual half-lobing of the young tetrad established domains only for the first meiotic division. Therefore, it is possible that sporogenesis in *H. gibbsiae* displays a developmental pattern that is a living remnant of an intermediary stage in the evolution of land plant meiosis, prior to the complete canalization of simultaneous meiosis in embryophytes.

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