Life history biology of early land plants: Deciphering the gametophyte phase

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The *ca.* 400-million-year-old Rhynie chert biota represents a benchmark for studies of early terrestrial ecosystems. The exquisite preservation of the organisms documents an ancient biodiversity that also includes various levels of biological interaction. Absent from the picture until recently has been detailed information about the development of the gametophyte phase and the alternation of generations of the macroplants in this ecosystem. Here, we trace the development of the gametophyte phase of *Aglaophyton*, an early land plant with an unusual complement of structural and morphological characters. Mature gametophytes consist of a fleshy protocorm attached to the substrate by basal rhizoids; arising from the upper surface are one to several upright gametangiophores bearing multiple gametangia. Stomata are present on the upper surface of the protocorm and gametangiophore, and endomycorrhizal fungi extend throughout the gametophyte. Gametophytes are unisexual, producing either antheridiophores or archegoniophores. There is no evidence that gametophytes later become hermaphroditic. The sexual dimorphism of the Rhynie chert gametophytes is inconsistent with theoretical ideas about the haploid phase of early land plants. The gametophyte phase of early land plants can now be considered within an ecological and evolutionary framework that, in turn, can be used to develop hypotheses about some aspects of the population dynamics and growth of these early land plants.

Early Devonian | fossil gametophytes | Rhynie chert | spore development | alteration of generations

The Early Devonian Rhynie chert Lagerstätte in Aberdeenshire, Scotland, has provided a wealth of information about organisms that inhabited early terrestrial ecosystems. Since the initial report of terrestrial land plants, microbes, and fungal spores in the Rhynie chert (1–5), numerous studies have described the biodiversity within the chert lenses, e.g., macroplants (1–3), fungi (6–8), animals (9, 10), algae (11), a lichen (12), and cyanobacteria (12, 13). Preservation is so exquisite that delicate structures such as sperm (14) and flagella (15) can be observed. In recent years, interest has expanded to studies documenting interactions between animals and plants (16), fungi and plants (17, 18), and fungi with other fungi (19), cyanobacteria (12), and algae (20). The Rhynie chert organisms include the oldest completely anatomically preserved terrestrial plants, which have been used as calibration points in the interpretation of both plant structure and function as well as in understanding the morphology and evolution of various tissue systems. One of the historically perplexing aspects of interpreting plant evolution has been the absence of fossils that represent the gamete-producing phase of the life cycle. Information presented here provides an up-to-date synthesis of what is known about early land-plant gametophytes from the Rhynie chert and places the life history of these plants within the context of the population dynamics in this early terrestrial ecosystem.

Materials and Methods

The Rhynie chert locality in Aberdeenshire, Scotland, consists of at least 10 plant-bearing beds that are represented as siliceous sinters (21, 22). The cherts are Lower Devonian (Pragian) in age and have been radiometrically dated at 396 ± 12 million years ago (23). The site is interpreted as a series of ephemeral freshwater pools within a hot springs ecosystem. The organisms were rapidly fossilized, perhaps as a result of a silica gel fixing. They are studied by petrographic thin sections in which a small piece of the chert containing plant material is cemented to a microscope slide, ground to a thickness of ≈50–150 μm, and then examined in transmitted light. Micrographs were prepared by using oil-immersion objectives directly on the polished rock surface without cover glasses.

Repository for Specimens

Preparations are deposited in the paleobotanical collections in the Forschungsstelle für Paläobotanik, Westfälische Wilhelms-Universität (Münster, Germany).

Results and Discussion

Development of the *Aglaophyton* Gametophyte. Information about the gametophytic phase in early land plants was speculative until the discovery of free-living gametophytes in the Rhynie chert (24). Since then, various aspects of the gamete-producing phase of several Rhynie chert macroplants have been described, and it is now possible to present a more complete picture of life histories in some early land plants (25–27). Here, we document the development of the gametophyte of *Aglaophyton major* from the germination of spores to the formation of mature gametangia on sexually dimorphic gametophytes.

Because fossil plants are typically disarticulated and, in the case of the gametophytic phase, not associated with the spore-producing phase, it is necessary to give different names to various parts until the entire plant or life history can be reconstructed. The sporophyte *A. major* is reconstructed as a system of upright, naked, dichotomizing axes <20 cm tall arising from a rhizome anchored by rhizoids (Fig. 1). Sporangia are terminal and produce trilete spores. Cells of the conducting strand lack wall thickenings like those of vascular plants and are more like those of certain bryophytes (28). Gametophytes of *A. major* were initially described as *Lyonophyton rhyniensis* (14). In the present study, gametophyte development for *Aglaophyton*/*Lyonophyton* is described in four stages: (*i*) globular, (*ii*) teardrop-shaped, (*iii*) protocorm, and (*iv*) gametangiophore (Fig. 1). Spores of *A. major* possess a conspicuous trilete mark on the proximal surface, and this mark may become widely separated as a result of gametophyte expansion (Figs. 1 and 2*A*). Many germinating spores in the Rhynie chert are associated with microbial mats that contain cyanobacteria and other microbes. In modern microbial mats extracellular polymeric substances are important components of the mat that are involved in attaching microbes to the substrate and forming a matrix around the organisms (29). Because the extracellular polymeric substances contain a large volume of water, we suggest that germination of the Rhynie chert

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Fig. 1. Life history of *A. major*/*L. rhyniensis* showing stages in the development of the dimorphic gametophytes. The mature sporophyte (lower left) bears sporangia with spores of two types. Blue spores develop into mature antheridiophores; orange spores develop into archegoniophores.

spores may have been facilitated by water trapped in these ancient microbial mats.

As spores germinate, the protoplast swells and becomes a globular mass that is initially irregular in outline and may extend well above the spore surface (Fig. 1). In some gametophytes at this stage of development, the spore wall is fragmented, suggesting rapid expansion of the spore protoplast before germination. The shape and general appearance are like that of the exosporic gametophytes of living nonseed vascular plants. The first cell division of the gametophyte is transverse and divides the globular mass into nearly equal segments (Fig. 2 *B* and *C*). The outer cell continues to divide to form a mass of cells that will ultimately give rise to the gametophyte proper (Fig. 2 *D* and *E*); the inner cell also divides a few times to form a small cell mass that remains within the confines of the spore wall. Oblique cell divisions of the outer cell give rise to an apical cell that subsequently divides to form cells of two types (Fig. 2*F*). On the outside is an outer ring of slightly flattened cells that will form the epidermis; on the inside is an inner core of cells that are more isodiametric in outline. During elongation, apical cells of the inner core continue to divide so that the gametophyte becomes teardrop-shaped and ≤10 cell layers thick.

The transition between the teardrop stage and the gametangiophore axis is slightly different in the development of the antheridiophore and archegoniophore (Fig. 1). The protocorm stage of the antheridiophore is first recognized as an expansion of the distal end of the teardrop-shaped gametophyte. As development continues, the protocorm becomes cup-shaped. Tissue differentiation is first recognizable in the transition zone between the protocorm and the teardrop stage. Epidermal cells differentiate to form rhizoids, which appear in tufts close to the protocorm base. Rhizoids rapidly elongate and can be traced out into the chert matrix. A distinct epidermis and delicate cuticle cover the protocorm. Stomata are rare in the teardrop stage but common in the protocorm close to the zone of rhizoids (Fig. 2*G*). They are simple and share common walls with the hypodermal cells beneath, which form a narrow channel that expands into the substomatal chambers. Arising from meristematic cells in the center or along the margin of the distal surface of the protocorm are two to several upright axes that subsequently differentiate into antheridiophores. A different pattern of development occurs for the archegoniophores. We are uncertain whether the teardrop stage simply elongates to become the archegoniophore

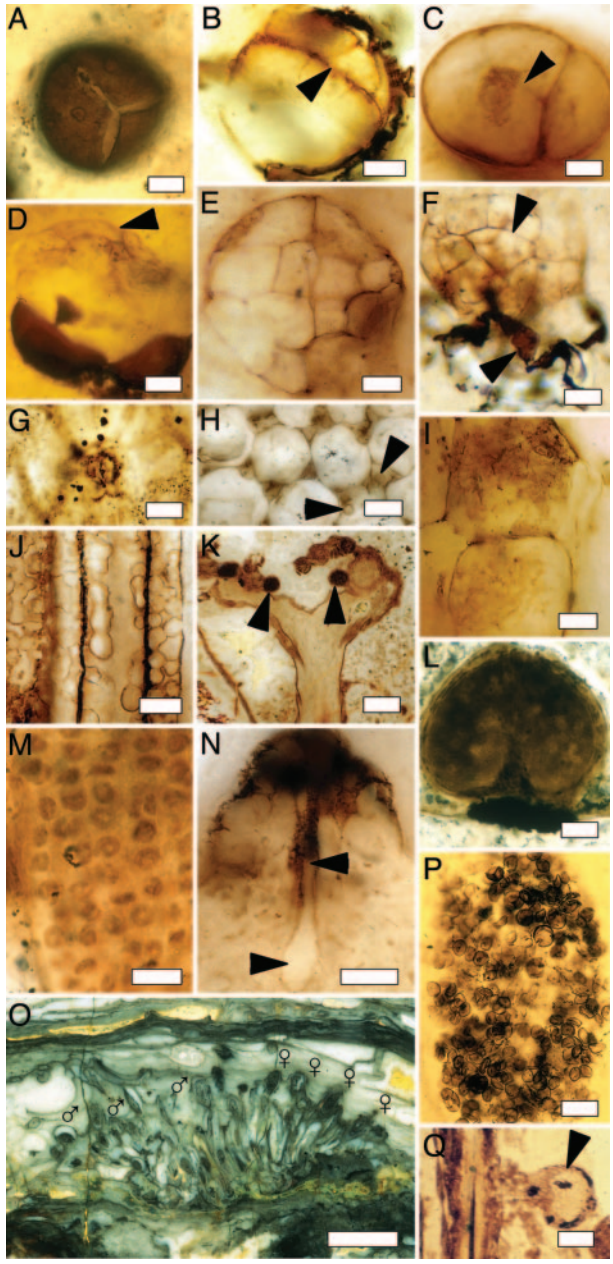

Fig. 2. Rhynie chert plants. (*A–L*) *Lyonophyton*. (*P*) *Horneophyton*. (*O*) *Remyophyton*. (*Q*) *Aglaophyton*. (*A*) Spore before germination with conspicuous trilete mark. (Scale bar, 15 μm.) (*B*) Early stage in gametophyte development showing initial transverse division (arrow). (Scale bar, 15 μm.) (*C*) Two-celled stage showing formation of a cross wall (arrow). (Scale bar, 13 μm.) (*D*) Multicellular gametophyte (arrow) protruding from broken spore coat. (Scale bar, 15 μm.) (*E*) Polar view of multicellular gametophyte showing arrangement of cells just beneath apical cell. (Scale bar, 15 μm.) (*F*) Gametophyte showing apical cell (upper arrow) and spore coat remnants (lower arrow). (Scale bar, 15 μm.) (*G*) Stoma on lower surface of gametangiophore. (Scale bar, 40 μm.) (*H*) Cortical cells with endomycorrhizal hyphae in intercellular spaces (arrows). (Scale bar, 50 μm.) (*I*) Cortical cells of gametangiophore containing arbuscules. (Scale bar, 12 μm.) (*J*) Longitudinal section of several conducting elements. (Scale bar, 7 μm.) (*K*) Longitudinal section of distal end of antheridiophore with two antheridia (arrows). (Scale bar, 2 mm.) (*L*) Longitudinal section of antheridium. (Scale bar, 100 μm.) (*M*) Section of antheridium showing sperm packets. (Scale bar, 5 μm.) (*N*) Longitudinal section of archegonium showing neck canal (upper arrow) and probable egg chamber (lower arrow). (Scale bar, 40 μm.) (*O*) Dense cluster of gametophytes showing size of antheridiophores and archegoniophores. (Scale bar, 6.5 mm.) (*P*) Spore aggregate. (Scale bar, 1.0 mm.) (*Q*) Bulbil (arrow) on vegetative axis. (Scale bar, 1.0 mm.)

and therefore lacks a clearly defined protocorm, or whether the protocorm is represented as the distal end of the teardrop stage. Archegoniophores branch dichotomously, whereas antheridiophores are unbranched (Fig. 1).

A cutinized epidermis covers both the antheridophores and archegoniophores; beneath this layer is a two-celled hypodermis. To the inside of this layer is a one- to two-cell-thick zone of slightly opaque isodiametric cells (Fig. 2*H*), some of which contain arbuscules (Fig. 2*I*) of the endomycorrhizal fungus *Glomites* (17). Hyphae enter the gametophyte at the base in the region of the rhizoids and extend through the intercellular spaces of the cortex (Fig. 2*H*).

Conducting elements are arranged in a terete strand that is first noticeable as the gametangiophore axes become conspicuous. The elements are slightly opaque, elongate cells characterized by wall thickenings that appear as a series of connected bubbles when viewed in transmitted light (Fig. 2*J*). Small lateral bulges of tissue that occasionally occur at the base of the immature gametangiophore axes may represent arrested apices, gametangiophore branches, or some type of haploid asexual propagule.

Gametophytes of *Lyonophyton* are unisexual and bear either antheridiophores or archegoniophores. None of the protocorms produce both types, nor is there any evidence that gametangia are mixed on a single gametangiophore. Gametangiophores are morphologically distinct depending on the type of gametangia produced. Mature antheridiophores are upright, unbranched, and ≈2.0 cm long. The distal ends are cup-shaped and lobed (Figs. 1 and 2*K*) and produce 10–40 antheridia, each spherical and ≤600 μm in diameter. Antheridia are stalked and columellate (Fig. 2*L*), with delicate parenchyma strands extending from the columella to the inner wall of the antheridium; on the distal surface, a small pore is formed in mature antheridia through which flagellated gametes (Fig. 2*M*) are released. The distal ends of the archegoniophores consist of several closely spaced dichotomies (Fig. 1), each possessing conducting strands. The tip is slightly expanded with a shallow central depression. Archegonia are slightly sunken on the inner surface of the depression or are subterminal along the axis just beneath the tip. Each archegonium is hemispherical and consists of ≤8–10 cell rows, with each row one to three cells thick and up to six cells high (Fig. 2*N*).

Postfertilization stages in Rhynie chert gametophytes are unknown. The only suggestion of developing sporophytes are small bulges of large-celled tissues that develop within archegoniophore tips of the Rhynie chert gametophyte *Remyophyton delicatum* (30), the presumed gametophyte of *Rhynia gwynne-vaughanii*. The difference in the general size of gametophyte cells and that of the adjoining bulge of tissue, together with the topographic position of the structure in the archegonia-bearing parts of the archegoniophores, suggest that these cells might represent a very early postfertilization stage in *Remyophyton*. The basal regions of *Horneophyton* axes have been proposed as the possible transition zone between gametophytic axes and a new sporophytic phase (31); however, the continuity of tissues, the presence of well developed rhizoids, and the total absence of gametangia in hundreds of specimens, as well as the description of the free-living gametophyte of *Horneophyton*, prove that this is the base of the sporophyte rhizome.

Comparison with Other Rhynie Chert Gametophytes. Free-living gametophytes are known for three of the Rhynie chert macroplants (*Aglaophyton*, *Rhynia*, and *Horneophyton*); to date, only antheridiophores are known for *Nothia*. All gametophytes consist of unisexual, axial, and upright structures (gametangiophores) that develop from the distal surface of a protocorm; on the base are tufts of rhizoids. Gametangiophores are small (<5 cm tall) and possess specialized conducting elements. The distal end of the gametangiophore is variously modified in different gametophyte taxa. Antheridia are large and stalked; archegonia are slightly sunken.

To date, the best-known mature population of gametophytes is represented by the fossil taxon *R. delicatum*, the gamete-producing phase of *Rhynia gwynne-vaughanii* (30). The biological relationship between gametophyte and sporophyte is based on the S-type secondary wall thickenings of the conducting elements. The tightly compacted cluster of *Remyophyton* gametophytes (Fig. 2*O*) consists of >200 globular to bowl-shaped protocorms, each bearing rhizoids on the basal surface and one to several upright unisexual gametangiophores on the distal surface. Of these, 27 are unbranched archegoniophores, and at least 69 additional ones are unbranched antheridiophores. Most gametangiophores grow orthotropically, however, some develop plagiotropically and display a more sinuous growth pattern. This pattern is common in many modern exosporic gametophytes, where crowding is the result of many spores germinating at approximately the same time and becoming intermingled during growth. *Remyophyton* antheridiophores are short (≤15 mm) with antheridia located on shield- to cup-like tips; archegonia-bearing axes are longer (≤2.0 cm) with slightly flared tips and archegonia either terminal or subterminal. The morphology and structure of antheridia and archegonia are similar to those of *Aglaophyton*/*Lyonophyton*.

A slightly different morphology is present in *Langiophyton mackiei*, the gametophyte of *Horneophyton ligneri*, and *Kidstonophyton discoides*, the antheridiophore-bearing gametophyte of *Nothia aphylla.* Both gametophytes are histologically and morphologically more complex, with the distal ends of the male and female gametangiophores modified into large cups that contain conducting elements. Evidence indicates that the elaboration of the distal ends of the gametangiophores has little if any phylogenetic significance but rather reflects the physiological complexity of the tips, because it appears that more gametangia are produced per gametangiophore in these gametophytes, compared with those of *Aglaophyton*/*Lyonophyton* or even *Rhynia*/*Remyophyton*. Very little is known about ontogenetic stages in the development of these two gametophyte types. Early stages in the germination of *Horneophyton* spores appear similar to those described in *Aglaophyton* (32).

There are other Devonian fossils interpreted as gametophytes based on the morphology of the cup-shaped distal ends of unbranched axes or lateral branches. However, in general, these fossils lack structural details; two of these are *Sciadophyton* (33) and *Calyculiphyton* (34). On the inner surface of most cups are stalked and/or sessile oval structures of two different sizes that are interpreted as gametangia. It is not known whether these structures represent different stages in gametangium development or are antheridia and archegonia that occur on the same gametangiophore cup, indicating that the gametophytes were bisexual. Another unresolved issue concerns the morphology of *Sciadophyton*. Although interpreted as rosette-like with axes arising from a central basal part, it is not known whether these axes arose from a single protocorm (thallus), or whether the rosette-like structure represents a stand of tightly packed independent plants in which the mode of attachment to individual protocorms is obscured by the tight packing. If the former interpretation is accurate, then these *Sciadophyton* gametophytes are different from other ones known in the Rhynie chert inasmuch as they may produce a much larger number of gametangiophores and gametangia per protocorm. However, *Sciadophyton* represents a form genus often with multiple growth forms intermingled on a single bedding plane. It is interesting to note that some of the gametangiophore axes contain conducting elements with thickenings of the S type.

Evolution of Alternation of Generation in Land Plants. Much has been written about the origin of land plants and the characteristics of

their charophycean ancestors (35). The ancestor of land plants is postulated as a haploid, flattened chlorophyllous gamete-producing thallus (36). Cladistic analyses have suggested that embryo-producing plants (bryophytes and vascular plants) are monophyletic, an assumption that is supported by molecular, structural, and morphological features in extant plants (37). Two principal theories have been proposed to explain the evolution of the dimorphic alternation of generations that characterizes vascular plants and bryophytes (38–40). The antithetic theory suggests that the sporophyte generation evolved from a haploid green algal thallus in which repeated mitotic cell divisions of a zygote resulting in an embryo retained on the thallus gave rise to the diploid phase (sporophyte). Evidence supporting this theory can be found in the life cycle of modern bryophytes in which the sporophyte is physiologically dependent on the gametophyte (39). Although generally not addressed, the antithetic theory necessitates that both types of gametangia were probably borne on the same thallus. The homologous theory assumes a green algal ancestor but with two nearly identical (isomorphic) phases, one producing gametes, the other producing spores. Various stages in the evolution of the sporophyte have been postulated, leading to the sporophyte becoming the dominant phase both morphologically and physiologically. The increasing size and complexity of the sporophyte phase results in more sporangia and spores being produced, and thus with potentially greater genetic variation represented among these spores, there is a corresponding increase in the probability of successful colonization of additional habitats (41). The gametophytic phase, although a less conspicuous part of the life cycle and not essential in local areas of clonal growth of the sporophyte, is equally essential for the long-term success of the species. Although hermaphroditic (bisexual) gametophytes may turn out ultimately to be the primitive type, all of the gametophytes thus far described from the Rhynie chert appear to be unisexual (Fig. 1).

Sexuality in Early Land-Plant Gametophytes. Perhaps the most interesting feature of the Rhynie chert gametophytes is the fact that they are unisexual with gametangia segregated on independent thalli. We find no evidence to suggest that sexual determination of the gametangia was related to the increased size or age of the gametophyte or, as in the case of many modern free-living gametophytes, a result of molecular and physiological cues that increase, delay, or otherwise alter the development of gametangia (42). Although we currently lack a large number of examples that span all stages of gametangiophore development, the presence of mature gametangia (defined on the basis of sperm release and lack of archegonial neck canal cells) suggests that sexual expression in these gametophytes was not labile like that in modern homosporous plants.

Aglaophyton, like all of the Rhynie chert sporophytes, is interpreted as homosporous, with spores ranging from 45 to 80 μm. In *Horneophyton*, another Rhynie chert sporophyte, spores are 42–74 μm (32). Although such variability is within the normal range for a population of spores, the unisexual gametophytes of the Rhynie chert plants suggest that sex determination may occur in the sporangium of the sporophyte. Intersporangial heterospory (anispory) is typically regarded as an early stage in the evolution of heterospory and is well documented in several groups of fossil plants (43). In most of these fossils, however, nothing is known about the gametophyte phase, and the assumption is made that the two sizes of spores each germinate to form a morphologically different type of gametophyte. We have examined populations of spores from a single sporangium of *Aglaophyton* and of *Rhynia* to determine whether there are any consistent differences in the size or ornamentation of the spores that might signal the sexuality of the mature gametophytes, but to date, we have found none. Because these spores produce unisexual gametophytes and, in the case of *Remyophyton*, both gametangia types occur at the same stage of development within a population, we are uncertain whether the sexually dimorphic gametophytes are the result of anispory or environmental control during development.

Three scenarios might be hypothesized to explain the occurrence of unisexual gametophytes in the life history of the Rhynie chert macroplants: (*i*) The sporophyte produces identical-appearing spores that are sex-determined (anispory), and there is some mechanism that ensures that gametophytes develop either antheridia or archegonia in close proximity within a dispersed population. (*ii*) Sex determination is sporangium-dependent, with a single sporophyte producing sporangia of both types (monoecy). (*iii*) Sex determination is dependent on sporophyte type, with different sporophytes producing either antheridia or archegonia (dioecy). The most compelling evidence to date supports the first hypothesis, but more than one system may have existed. It is difficult to envision a stand of $\approx$200 closely spaced gametophytes like that in *Remyophyton* as representing a random accumulation of spores from several sporangia or from the sporangia of several plants. We suggest that this population is natural and the result of spores being dispersed as an aggregate consistent with the discovery of isolated masses of spores within the chert matrix that represent the entire contents of a single sporangium. These spore aggregates (Fig. 2*P*) are known at least for the Rhynie chert sporophytes *Aglaophyton* and *Horneophyton*, and we suggest that they may represent a dispersal strategy that resulted in maintaining the close proximity of both types of unisexual gametophytes, thus ensuring fertilization. If *Aglaophyton* is functionally anisporous, then spore dispersal becomes an important factor in maintaining the next generation, because proximity of sperm and egg is critical.

Sexuality in Extant Spore-Producing Plants. Extant plants such as horsetails, club mosses, and most ferns are characterized by a haplodiplontic life history in which the sporophyte produces morphologically similar spores (homosporous) that germinate to form free-living, sometimes photosynthetic gametophytes. Sex expression in most is regarded as labile and determined by age and/or presence of an antheridiogen produced by an adjacent gametophyte (44). Slight differences in spore size in homosporous plants have been correlated with stored food, time to germination, and gametophyte development; large spores produce rapidly growing bisexual gametophytes that, in turn, influence smaller spores to produce slower-growing gametophytes with only antheridia. In *Equisetum*, patterns of sex determination are controlled by various environmental parameters (45). *In vitro* studies show that crowding, mineral deficiency, high temperatures, drought, and increased sucrose concentrations result in the production of more gametophytes with antheridia. In wild populations, environmental stress appears to have the same effect (46). In certain extant lycopods, the speed at which spores germinate results in gametophytes that occupy different microhabitats; rapidly germinating ones form surface-dwelling gametophytes with stomata, whereas some more slowly germinating spores produce subterranean, mycorrhizal gametophytes in which the fungus is restricted to a distinct tissue within the thallus.

Bryophytes also are homosporous but possess a life history in which the sporophyte is reduced and physiologically dependent on the gametophyte, which is the dominant part of the life cycle. Unisexual gametophytes occur in >50% of the taxa (47) and can be directly correlated with spores of two different sizes produced in the same sporangium (48). Some unisexual bryophytes have sex chromosomes, and for several, gametophytes that only produce archegonia are more common (49). Sex expression in bryophytes may be both labile and environmentally controlled (44), and all employ various modes of vegetative and asexual

reproduction (50). For example, in the modern liverwort *Marchantia inflexa*, antheridiophores and archegoniophores are produced on separate thalli, and individual thalli may grow continuously by various asexual methods. Field observations of *M. inflexa* (50) indicate that the appearance of antheridiophores, archegoniophores, spores, and gemma are seasonally cued. In modeling this system, it was noted that changes in sex ratios of antheridiophores and archegoniophores depended on levels of disturbance, and that the actual sex ratios within populations may be the result of metapopulation structure and dynamics.

Evolution of Bisexual Gametophytes. Most models that hypothesize the evolution of dioecy focus on flowering plants and thus may not be applicable to plants such as ferns and lycopods. Such studies generally conclude that dioecy evolved to reduce inbreeding and that arid or otherwise harsh conditions represent at least one environmental stimulus (51). The free-living, sometimes long-lived gametophytes of nonseed plants are, however, subjected to various ecological pressures that are not encountered in the highly reduced gametophyte generation of seed plants and thus represent an interesting model system in which to discuss the evolution of unisexual gametophytes.

In nonseed plants, hermaphroditic (bisexual) gametophytes would appear to represent an optimum breeding system in the sense that the close spatial relationship of the gametangia greatly increases the probability of successful fertilization. However, both theoretical and empirical data indicate that such a system results in strong inbreeding depression (52). This situation, together with the fact that the Rhynie chert plants can also reproduce by various asexual means, would result in a genetically homogeneous population with high levels of homozygosity in sporophytes in a given species. Such high levels of homozygosity could be offset by sexually dimorphic gametophytes, which may result in a slight shift toward outcrossing. As we note above, spores dispersed in mass and in areas of limited moisture, although closely related and thus increasing homozygosity in the sporophyte population, would ensure that gametophytes of both sexes would develop in close proximity, thus increasing the likelihood that fertilization would take place. On the other hand, if a few spores from an aggregation became mechanically disassociated and abiotically transported, the reproductive assurance in the breeding system of the Rhynie chert plants would shift in the direction of outbreeding. Because of gametophyte crowding and certain microenvironmental factors associated with the germination of spores, selection might have favored those spores able to germinate in less crowded conditions, thus further increasing chances of outbreeding. In what may have been a relatively patchy environment in the Rhynie chert ecosystem, sexually dimorphic gametophytes that produced either archegonia or antheridia would be under no selection pressure to allocate resources to one or the other, as is the case in hermaphroditic gametophytes in homosporous plants today. Perhaps this spatial segregation of gametangia served as a precursor to the evolution of the molecular and biochemical pathways in labile sex expression, which has become the norm in the majority of homosporous plants today.

Modern *Equisetum* may be viewed as an example of an analogous stage in the evolution of the breeding system that we hypothesize in the Rhynie chert plants. *Equisetum* spores contain a pair of hygroscopic elaters that function in dispersing spores from the sporangium. However, these appendages also become entangled with those of other spores so that clusters of spores are dispersed together. This event results in the development of multiple gametophytes in close juxtaposition and optimizes the opportunity for fertilization and the development of new sporophytes while reducing the chances for outbreeding. Gametophytes of *Equisetum* may be photosynthetic and long-lived and may produce either antheridia or archegonia initially; gametophytes with archegonia eventually develop antheridia, whereas those with antheridia rarely produce archegonia (53). Although there were certainly other dynamics taking place within a sexually dimorphic population of Rhynie chert gametophytes for which we currently have no information such as gametophyte longevity, the spatial distribution of gametangia may represent an early evolutionary adaptation that functioned to decrease inbreeding depression in early land plants.

Clonal Growth in Early Land Plants. All of the Rhynie chert plants can reproduce both sexually and by vegetative methods, and thus clonality may have served as the primary method of species invasion into new niches (54). Sections of rhizomes that supported a single sporophyte suggest that fragmentation and dieback may have been an important form of vegetative reproduction, as in some modern clonal plants (55). Other structures in the fossils that may have functioned in asexual reproduction include reduced branches as fragmentation propagules, bulbils (Fig. 2*Q*), and bulges containing poorly organized vascular tissue (56).

Much has been written about the evolution of a symbiotic relationship between fungi and early land plants during the transition to a terrestrial ecosystem. The association is often regarded as mutualistic, with the fungus gaining carbon and the plant gaining an increased ability to obtain water and nutrients. Recent studies, however, suggest that more complex interactions exist, and that these interactions are highly variable over time. For example, a well developed endomycorrhizal system in living plants can transfer carbon and phosphorus between plants (57), and recent studies indicate that certain symbiotic fungi increase growth in clonal plants (58). Fungal symbionts no doubt played a critical role in increasing water and nutrient uptake during the colonization of the land, but enhanced distribution of resources within a population of the Rhynie chert plants also may have served as an important survival strategy for the community, especially in one where clonality was high.

Conclusions

The Rhynie chert gametophytes represent an opportunity to examine the life history of early land plants within both evolutionary and ecological contexts. Many details remain unknown, but it is important to keep in mind that these uniquely preserved plants represent but a brief glimpse from a continuum of evolutionary innovations during the early colonization of the land. Several lines of evidence suggest that the earliest land plants were structurally and morphologically similar to extant liverworts and hornworts (59, 60) and moved into a terrestrial ecosystem as early as the mid-Ordovician (61). Both of these groups also contain arbuscular mycorrhizae assignable to the *Glomeromycota* (62), the same group, based on morphological and structural evidence, that is found in the Rhynie chert plants. Many molecular phylogenies also place the liverworts in a basal position relative to land plants.

By Lower Devonian time, both the gametophyte and sporophyte phases of at least some land plants were both autotrophic and mycorrhizal and thus had already evolved strategies to optimize available resources in the hot springs environment of the Rhynie chert. Both phases also were capable of several types of asexual reproduction, and thus clonality may have been the principal mechanism used in capturing new space. Although many specific details on the vegetative growth and reproductive potential of these plants remain to be quantified, including spacing between ramets, numbers of aerial axes, number of sporangia, spores per sporophyte, and sex ratios for gametophytes, we now possess more information than ever before about the life history of these plants.

Some of the ideas presented in this paper about early land-plant growth and life history have previously been beyond

the scope of paleobotany, to a large degree because complete life cycles were not known, or only a very limited number of specimens were available. Fossil ecosystems like the Rhynie chert continue to offer new insights into interactions among organisms and, because of the small size of the plants and manner in which preservation took place, also now make it possible to examine some aspects of population structure and dynamics. The unisexual gametophytes were part of a reproductive strategy that optimized fertilization likelihood vs. benefit of outbreeding in the context of what might have been clonal plants successfully adapted to highly specialized niches. These fossils further indicate that at least one taxon was constrained to keep different gametophytes in proximity through spore dispersal despite an inbreeding cost. Although postfertilization stages, including the developing sporophyte, are needed to complete our basic understanding of the life cycle of some of these unique plants, knowledge of the developmental stages leading to mature, sexually dimorphic gametophytes has opened up a new set of intriguing challenges in understanding aspects of the population biology within this early terrestrial ecosystem.

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