

THE SEPARATION OF GENERATIONS: BIOLOGY AND BIOGEOGRAPHY OF LONG-LIVED SPOROPHYTELESS FERN GAMETOPHYTES

Jerald B. Pinson,^{1,*} Sally M. Chambers,* Joel H. Nitta,† Li-Yaung Kuo,‡ and Emily B. Sessa*

*Department of Biology, University of Florida, Box 118525, Gainesville, Florida 32611, USA; †Department of Organismic and Evolutionary Biology, Harvard University, 22 Divinity Avenue, Cambridge, Massachusetts 02138, USA; and ‡Institute of Ecology and Evolutionary Biology, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 10617, Taiwan

Editor: Erika Edwards

Premise of research. Ferns (monilophytes) and lycophytes are unique among land plants in having two independent life stages: the gametophyte generation, which is generally small, cordiform, and short-lived, senescing after fertilization, and the sporophyte generation, which is considered the dominant, long-lived portion of the life cycle produced following fertilization. In many species of epiphytic ferns, however, the gametophyte generation is capable of sustained vegetative growth, and some are able to reproduce asexually via gemmae. These two characteristics have increased the independence of these gametophytes, so much so that some species never produce sporophytes at all, while other species produce sporophytes only in parts of their geographic range, a trend we term here the “separation of generations.”

Pivotal results. Long-lived fern gametophytes have evolved independently in several families and can be found around the world. We present a comprehensive review of the long-lived fern gametophytes that are able to forgo the production of a sporophyte, including accounts of their discovery, taxonomy, biology, ecology, and biogeography. We also present several hypotheses concerning why these species do not produce sporophytes, identify gaps in our knowledge about these organisms, and suggest areas of future study.

Conclusions. While several populations of independent gametophytes have been identified and characterized in temperate regions, it is likely that the bulk of species with spatially separated generations occur in the tropics, where little work has been done. Additionally, virtually no studies have been undertaken that attempt to determine the underlying factors inhibiting sporophyte production in ferns. As 2017 marks the fiftieth anniversary of the first comprehensive study published on independent fern gametophytes, we can think of no better time for a review on their biology and an assessment of the work that still needs to be done.

Keywords: alternation of generations, life cycle, gametophyte, sporophyte, fern, gemmae.

We are accustomed to see and to marvel at the great varied form and adaptation of the sporophytes, which are the ferns as we know them, but indeed there must be nearly as much variety of adaptation among the gametophytes. (Holtum 1938, pp. 421–422)

Introduction

By definition, all land plants (embryophytes) cycle between diploid sporophyte and haploid gametophyte stages, known as the “alternation of generations.” In the two largest groups of land plants, bryophytes and spermatophytes (seed plants), one stage is nutritionally dependent on the other; however, in ferns (monilophytes) and lycophytes, the two life stages are in-

dependent and can live freely from one another. In ferns and lycophytes, as in seed plants, the diploid sporophyte is traditionally defined as the “dominant” generation. The fern sporophyte produces haploid spores via meiosis that are dispersed into the surrounding environment once they mature. If these spores land in a suitable environment, they will germinate into haploid gametophytes. The gametophyte is the sexual stage of the life cycle, producing antheridia and archegonia via mitosis, which produce sperm and eggs, respectively. Once successful fertilization has occurred, a diploid sporophyte grows directly from the fertilized egg cell within the archegonium on the haploid gametophyte, and eventually the gametophyte tissue senesces after the new sporophyte is established.

Although most ferns follow the standard alternation of generations cycle closely, the independence of these two life stages allows for both the gametophyte and the sporophyte to explore novel ecological niches (Sato and Sakai 1981) and life-history strategies (Dassler and Farrar 2001). In some species, the gametophyte can be long-lived, a pattern that can be seen multiple times among epiphytic ferns, which have stretched the limits of physiological tolerance by taking to the canopy, a

¹ Author for correspondence; e-mail: jbp4166@ufl.edu.

Manuscript received April 2016; revised manuscript received July 2016; electronically published November 23, 2016.

stressful environment that imposes various constraints on plant growth and establishment (Watkins and Cardelús 2012). At the extreme, some ferns have done away with the sporophyte generation altogether, surviving exclusively in the gametophyte stage. Globally, three known species of ferns fall into this category, and 24 others have gametophytic ranges that extend the species' overall geographic ranges into regions where sporophytes are produced infrequently or not at all (table 1). We term this spatial disjunction between gametophytic and sporophytic ranges the "separation of generations."

Most epiphytic ferns occur in tropical habitats (Gentry and Dodson 1987; Dubuisson et al. 2009), where competition can be high. In response to the challenges of these environments, epiphytic fern gametophytes have evolved a three-dimensional morphology that is much more complex than the typical two-dimensional cordiform (heart-shaped) shape seen in terrestrial fern gametophytes (Dassler and Farrar 2001; Pitterman et al. 2013). Epiphytic gametophytes are often branching and dissected and are capable of sustained vegetative or clonal growth, allowing them to prolong their life spans (fig. 1). This branching morphology is thought to increase the likelihood of the thalli of two individuals coming into close enough proximity for outcrossing to occur (Farrar and Dassler 2001), and the longevity of these gametophytes also means that one individual can persist for an extended period of time before another spore lands nearby and produces another gametophyte (Watkins and Cardelús 2012), thus increasing the chances for out-

crossing. Additionally, this morphology may promote desiccation tolerance, the crevices created by the three-dimensional morphology helping to retain water for a longer period of time, thus slowing the drying rate and allowing for tolerance of the stressful canopy conditions. A study conducted to examine the degree to which gametophytes of tropical ferns are desiccation tolerant indicated that gametophytes with this morphology were capable of withstanding greater levels of desiccation than their terrestrial, cordate counterparts (Watkins et al. 2007b).

This complex morphology has evolved independently in members of at least six separate fern families (Hymenophyllaceae, Polypodiaceae, Pteridaceae, Lomariopsidaceae, Dryopteridaceae, and Schizaeaceae; fig. 2). With a few exceptions, the majority of species in the first five families that have this elongated and branched morphology are epiphytic, indicating the importance of this morphology in tropical canopies. The genera *Schizaea* and *Bolbitis* are notable outliers, which are, for the most part, terrestrial ferns. In the first three families, several species have further evolved the ability to reproduce asexually via small vegetative propagules called gemmae that are produced mitotically from gametophyte thallus tissue (figs. 1H, 1J, 1M, 3). Gemmae are thought to enhance the likelihood of establishment of fern populations in the canopy (Ebihara et al. 2008), as only one gemma needs to be dispersed to give rise to an entirely new, albeit clonal, population. These gemmae also have the ability to produce antheridia, thus promoting outcrossing (Emigh and Farrar 1977).

Table 1

Species Discussed in This Review

No.	Family	Species	Supporting publication(s)
1	Hymenophyllaceae	<i>Crepidomanes intricatum</i>	Ebihara et al. 2008
2	Hymenophyllaceae	<i>Didymoglossom petersii</i>	Farrar 1990
3	Hymenophyllaceae	<i>Hymenophyllum tayloriae</i>	Raine et al. 1991
4	Lomariopsidaceae	<i>Lomariopsis kunzeana</i>	Possley et al. 2013
5	Polypodiaceae	<i>Moranopteris nimbata</i>	Farrar 1967
6	Pteridaceae	<i>Vittaria graminifolia</i>	Farrar and Landry 1987
7	Pteridaceae	<i>Vittaria appalachiana</i>	Farrar and Mickel 1991
8	Hymenophyllaceae	<i>Hymenophyllum wrightii</i>	Duffy et al. 2015
9	Hymenophyllaceae	<i>Callistopteris baldwinii</i>	Dassler and Farrar 1997
10	Hymenophyllaceae	<i>Callistopteris apiifolia</i>	Ebihara et al. 2013; Nitta et al., forthcoming
11	Hymenophyllaceae	<i>Hymenophyllum recurvum</i>	NA
12	Hymenophyllaceae	<i>Vandenboschia cyrtotheca</i>	NA
13	Pteridaceae	<i>Vaginularia paradoxa</i>	Nitta et al., forthcoming
14	Hymenophyllaceae	<i>Hymenophyllum badium</i>	Ebihara et al. 2013
15	Hymenophyllaceae	Unknown	Ebihara et al. 2013
16	Hymenophyllaceae	Unknown	Ebihara et al. 2013
17	Hymenophyllaceae	<i>Vandenboschia kalamocarpa</i>	Ebihara et al. 2009
18	Pteridaceae	<i>Antrophyum henryi</i>	Chen et al. 2013b
19	Pteridaceae	<i>Antrophyum parvulum</i>	Chen et al. 2013b
20	Pteridaceae	<i>Haplopteris heterophylla</i>	Chen et al. 2013a, 2013b
21	Pteridaceae	<i>Haplopteris</i> sp.	Kuo et al., forthcoming
22	Pteridaceae	<i>Haplopteris</i> sp.	Kuo et al., forthcoming
23	Lomariopsidaceae	<i>Lomariopsis lineata</i>	Li et al. 2009
24	Lomariopsidaceae	<i>Lomariopsis</i> sp.	Ebihara et al. 2013
25	Hymenophyllaceae	<i>Vandenboschia speciosa</i>	Rumsey et al. 1990

Note. A complete list of the 25 species discussed in this review, along with the publication in which a given species was first mentioned (or named) in a scientific study. For those taxa reported here through personal communication, the publication cell is listed as nonapplicable (NA).

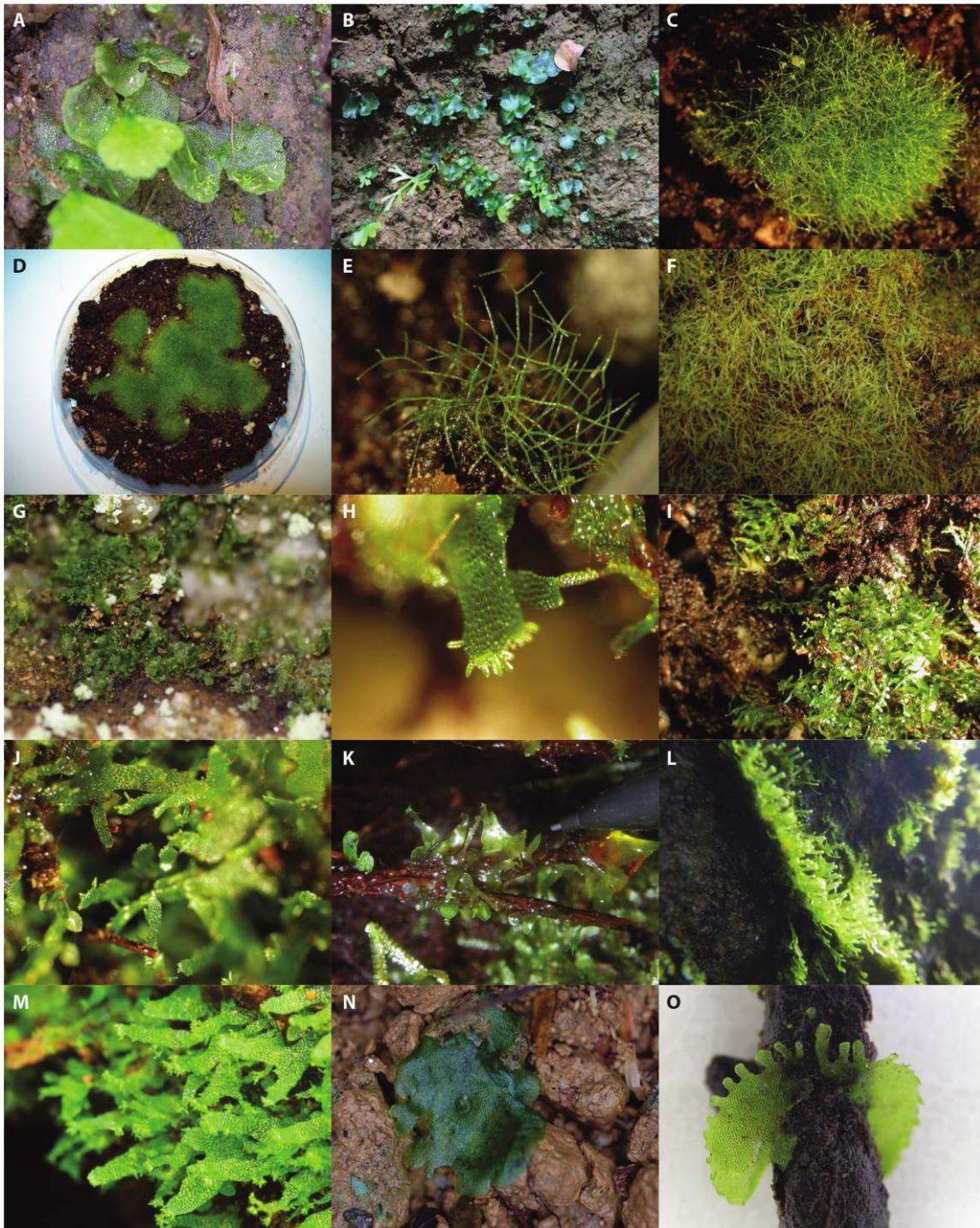


Fig. 1 Photographs illustrating the morphological diversity of fern gametophytes. A, B, Typical cordiform gametophytes (species unknown). The left-hand gametophyte has an emergent young sporophyte slightly out of focus in the foreground. C–E, *Crepidomanes intricatum* (Hymenophyllaceae). F, *Hymenophyllum tayloriae* (Hymenophyllaceae). G, *Vittaria appalachiana* (Pteridaceae). H–J, *Hymenophyllum wrightii* (Hymenophyllaceae). K, *Callistopteris apiifolia* (Hymenophyllaceae). L, *Vaginularia paradoxa* (Pteridaceae). M, *Haplopteris heterophylla* (Pteridaceae). N, *Danaea nodosa* (Marattiaceae). O, *Anetium citrifolium* (Pteridaceae). A, N, and O by J. E. Watkins Jr. B and F by E. Sessa. C–E and H–J by A. Duffy. G by S. Chambers. K and L by J. Nitta. M by C.-W. Chen.

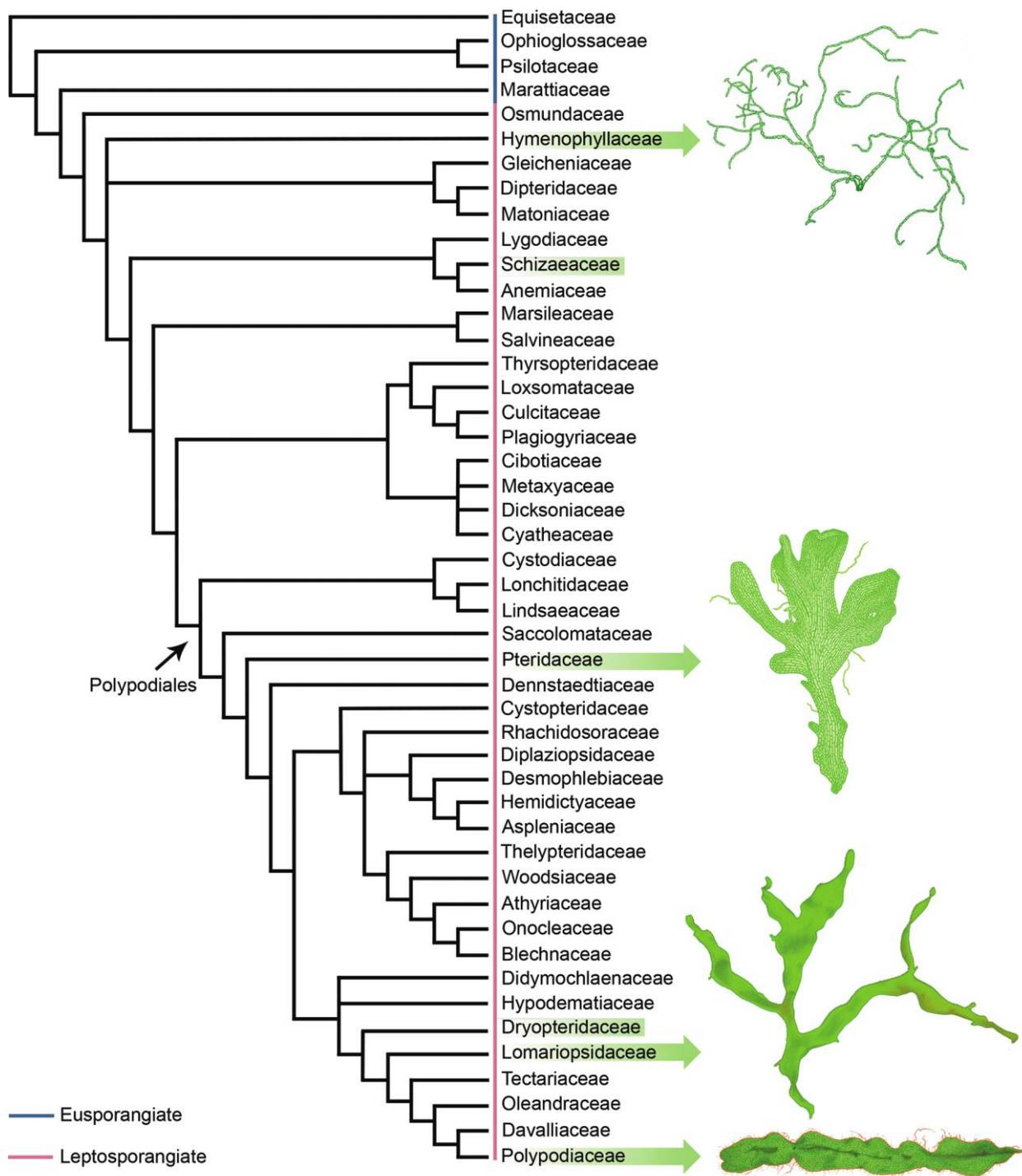


Fig. 2 Phylogeny of extant ferns with representative drawings of gametophyte morphology in four families that include members displaying the separation of generations pattern. Note that there is no illustration for Schizaeaceae or Dryopteridaceae. The topology is based primarily on Smith et al. (2006) but is also an amalgamation of several other studies. The position of Equisetaceae follows that of Knie et al. (2015) and Rothfels et al. (2015). Families within Eupolypods II are based on Rothfels et al. (2012). The recognition of Cystodiaceae and Lonchitidaceae as separate from Lindsaeaceae is based on Christenhusz et al. (2011). The families Didymochlaenaceae and Desmophlebiaceae were established by Zhang and Zhang (2015) and Mynssen et al. (2016), respectively. The systematic position of Dennstaedtiaceae was resolved by Lu et al. (2015) and Rothfels et al. (2015), and the position of Lomariopsidaceae was established by Kuo et al. (2011).

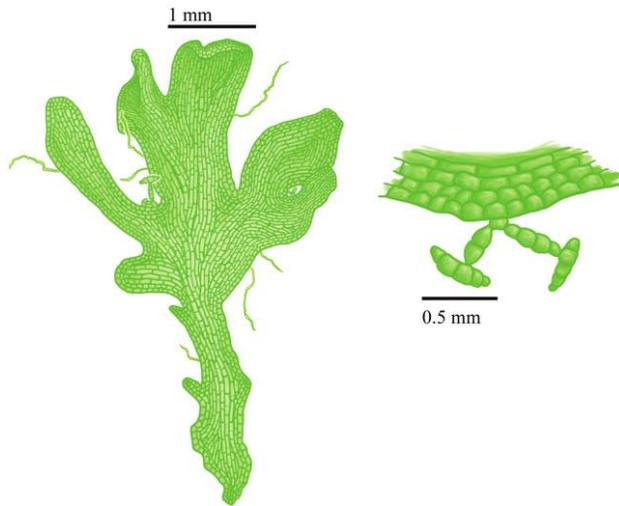


Fig. 3 *Vittaria graminifolia* (Pteridaceae) with a magnified illustration of gemmae. Illustrated by Simon Parsons.

The evolution of independent, morphologically complex, perennial gametophytes capable of asexual reproduction has several corollaries. Nayar and Kaur (1971) noted that fern gametophytes and sporophytes share nearly the same niche space requirements, because a sporophyte will form only if a gametophyte is able to persist long enough for fertilization to occur. However, it has been shown since that gametophytes of some species are able to withstand a wider range of environmental conditions than the sporophytes of their own species, and there are several well-documented cases where gametophytes extend the species' geographic range beyond that of their respective sporophytes (Nauman 1986; Farrar and Landry 1987; Farrar 1990; Rumsey et al. 1998a; Dassler and Farrar 1997; Ebihara et al. 2013; Duffy et al. 2015). In terrestrial ferns with cordiform gametophytes, any individuals growing beyond the range limits of conspecific sporophytes would likely not last more than a few growing seasons (Watkins et al. 2007a). Populations of perennial gametophytes capable of asexual reproduction, however, can persist for virtually indefinite amounts of time. In the three most extreme examples, *Crepidomanes intricatum* (Farrar) Ebihara and Weakley, *Hymenophyllum tayloriae* Farrar and Raine, and *Vittaria appalachiana* Farrar and Mickel, a viable sporophyte has never been observed (Farrar and Mickel 1991).

The goal of this article is to provide an overview of the biology and natural history of the various fern species exhibiting the separation of generations, which we define as species that have gametophyte populations spatially separated from conspecific sporophyte populations or that have no sporophytic counterpart. Although ferns with these unique life histories can be found around the world, they have been documented and studied most thoroughly in eastern North America and western Europe; thus, the information presented here focuses most heavily on species from these areas. We review the morphology, ecology, reproductive strategies, biogeographic patterns, and habitat specificity of these organisms, and we discuss future

directions for research along with probable causes for the spatial separation of the two generations.

Morphology

Fern gametophytes are often depicted in textbooks (e.g., Evert and Eichorn 2013; Reece et al. 2014) as the small, short-lived, cordiform portion of the life cycle. The cordiform thallus is common among terrestrial taxa of the Polypodiales, but a variety of gametophyte morphologies exist (fig. 1). The quote above serves to illustrate just how varied fern gametophyte morphology can be. If we consider only leptosporangiate ferns, which make up the majority of the approximately 9000 taxa (Smith et al. 2006), gametophyte morphology can be broadly categorized into four types sensu Farrar et al. (2008): cordiform, strap shaped (sometimes referred to as “elongate-cordate”; fig. 4), ribbon shaped (fig. 3), and, recognized here as a distinct morphology, filamentous (fig. 6).

Cordiform, or “heart-shaped,” gametophytes (fig. 1A, 1B) grow from a single apical meristem located in a notch at the apex of the thallus. These gametophytes grow quickly but remain small and are generally short lived, lasting a year or less (Farrar et al. 2008). It has been demonstrated that establishment of many terrestrial fern gametophytes requires disturbance, which creates an environment relatively free of competition and may expose spore banks in the soil (Watkins et al. 2007a). But this proclivity toward disturbed habitats means that terrestrial gametophytes must grow and produce sporophytes quickly (r selected) before another disturbance destroys their populations. Experimentally, however, it has been shown that, when gametophytes of this type are grown separately to prevent fertilization in a laboratory setting, some can live for indefinite periods of time. For example, gametophytes of *Osmundella claytonia* L. and *Pteris nodulosa* Nieuw. were kept alive for over 3 yr (Mottier 1927). Walp and Proctor (1946) grew cordate gametophytes of several species together as a demonstration for freshman biology students, but when they failed

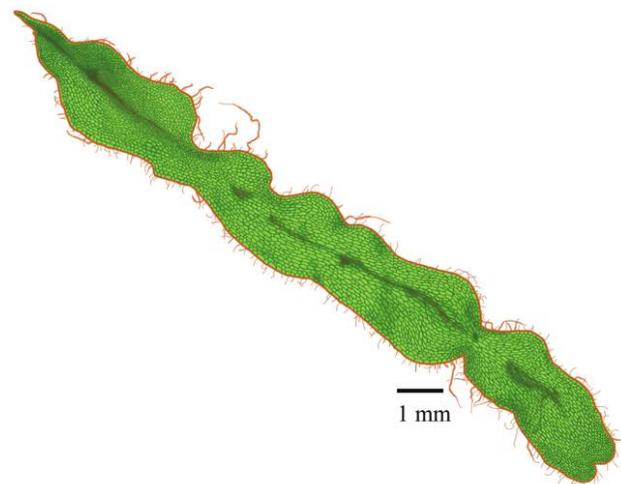


Fig. 4 *Prosaptia contigua* (G. Forst.) C. Presl (Polypodiaceae). Illustrated by Simon Parsons.

to produce sporophytes, possibly due to overcrowding or the addition of potassium manganite, they kept the culture growing for at least 8 yr.

Epiphytic gametophytes generally live in a more stable environment with high levels of competition. They invest more time and resources into growth (*K* selected), and sporophyte production can be delayed if the gametophyte is growing in less than optimal conditions. Strap-shaped gametophytes (fig. 4) retain a defined apical meristem and develop a notch at the tip of the growing region, similar to cordiform gametophytes (Farrar et al. 2008); however, this morphology differs in having multiple meristems and proliferation points that grow more in length than they do in width. These gametophytes grow slowly and are long-lived, capable of sustaining indefinite meristematic activity. As new thallus projections proliferate, each with the ability to produce gametangia and sporophytes, older portions of the thallus tend to die back. Thus, multiple sporophytes may be produced from a complex, clonal collection of thalli that were initially one gametophyte (Farrar et al. 2008). Strap-shaped gametophytes are characteristic of epiphytic taxa belonging to Polypodiaceae and Dryopteridaceae (e.g., *Elaphoglossum*; fig. 2), as well as less proliferate types in some eusporangiate ferns (e.g., Osmundaceae).

Ribbon-shaped gametophytes (figs. 3, 5) have disrupted marginal meristems along the length of the thallus rather than the typical apical meristem (Farrar et al. 2008). The meristem increases the length of the gametophyte, but at certain points growth ceases, leading to the formation of several distinct ribbon-like sections that continue to grow and branch on their own. Like the strap-shaped type, these gametophytes are also perennial and can grow indefinitely. The ribbon-shaped morphology commonly occurs in epiphytic or epipetric ferns belonging to the families Hymenophyllaceae, Polypodiaceae, Lomariopsidaceae, and Pteridaceae (fig. 2).

Filamentous gametophytes (fig. 6) occur in the families Hymenophyllaceae and Schizaeaceae. Gametophytes of this type are highly reduced, bearing a strong resemblance to the vege-

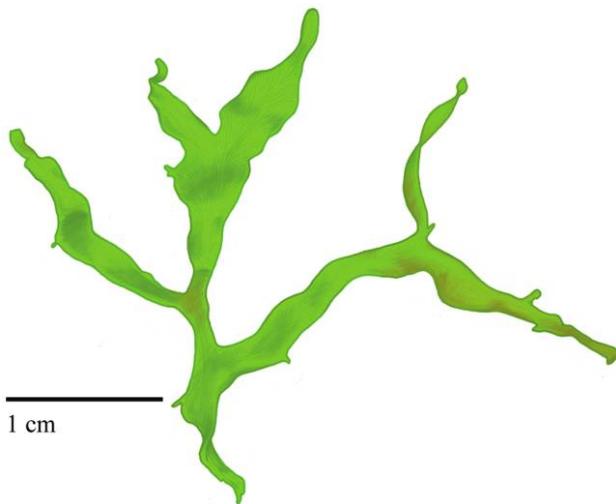


Fig. 5 *Lomariopsis lineata* (Lomariopsidaceae). Illustrated by Simon Parsons.

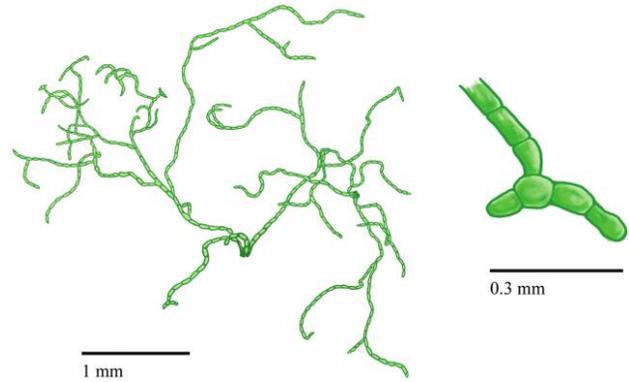


Fig. 6 *Didymoglossum petersii* (Hymenophyllaceae) with a magnified illustration of the uniseriate geometry of the cells. Illustrated by Simon Parsons.

tative cells of algae. These gametophytes produce uniseriate filaments that are capable of repeated branching and indefinite growth, and some are capable of producing gemmae (Farrar 1992).

Asexual Reproduction: Gemmae

Sporophytes in a number of fern species are capable of vegetative reproduction, usually via the process of budding (McVeigh 1937; Johns and Edwards 1991). Gametophytes, as well, have a number of mechanisms that allow them to reproduce asexually. Multiple species in several unrelated families produce proliferations that bud from the main portion of the thallus, which, either upon physical detachment in a laboratory setting or as older portions of the thallus begin to senesce in nature, can grow into clonal gametophyte individuals. This type of growth can be observed in species of the Polypodiaceae (Chiou and Farrar 1997), Dryopteridaceae (Chiou et al. 1998), Plagiogyriaceae, and Cyatheaceae (Atkinson and Stokey 1964). Another type of proliferative growth is the production of filamentous strands from otherwise cordate thalli that eventually broaden to form new adult gametophytes, which can be observed in the genus *Asplenium* (Testo and Watkins 2011) and the species *Stegnogramma burksiorum* (J.E. Watkins & Farrar) Weakley (Watkins and Farrar 2002). Distinct from both of these types, and perhaps the most well-studied form of asexual reproduction in fern gametophytes, are the vegetative propagules termed gemmae.

The production of gemmae by fern gametophytes is relatively rare and is predominantly restricted to gametophytes with strap- and ribbon-shaped morphologies. In groups with these morphologies, some species have the ability to produce gemmae along the margins of the thallus, which occurs most commonly in the grammitid (Polypodiaceae; Stokey and Atkinson 1958) and vittarioid (Pteridaceae; Goebel 1888) ferns, as well as in some filmy ferns (Hymenophyllaceae; Bower, 1888). Gemmae add a new dimension to the independence of long-lived fern gametophytes, allowing them to produce extensive colonies of clones and aiding in short-distance dispersal. Outside of these groups, the fern genera *Ophioglossum* and *Psilotum*, both eusporangiate ferns (fig. 2), have been found to

produce gemmae in subterranean portions of sporophytes, gametophytes, or both (Bierhorst 1971; Farrar and Johnson-Groh 1990). The in vitro production of gemmae has also been observed in cultured gametophytes of *Osmunda regalis* L. (Fernández 1997; Magrini and Scoppola 2012).

Developmentally, gemmae are often borne on short stalks called gemmifers (fig. 3), which grow directly from the margin and the surface of the gametophyte (Chen et al. 2013b). The size of the gemmifer varies among families and species, from as little as one cell in the vittarioids (Farrar 1974) to four to six cells in *Trichomanes alatum* Sw. (Bower 1888). In *Hymenophyllum eximium* Kunze, gemmifers do not appear to be present, and gemmae are instead produced from a protruding cell (Goebel 1888). Additionally, the number of gemmae produced per gametophyte varies highly among species, as does their arrangement and the number of cells per individual gemma. Gemmae grow linearly from the gemmifer and may occur in pairs (one attached to the other), as in *Vittaria graminifolia* Kaulf. (fig. 3) and *Vittaria lineata* (L.) Sm., or as solitary propagules, as in *Radiovittaria stipitata* (Kunze) E.H. Crane (Farrar 1974). Gemmifers can hold from one to multiple sets of gemmae or gemma pairs. The gemmae can be composed of anywhere from two to 16 cells in different species, and they generally have two smaller terminal cells that act as the rhizoidal primordia, which can contain chlorophyll or starch (Farrar 1974). Once the gemmae are mature, an abscission layer forms and the propagules naturally detach. From there, they can either develop into new genetically identical gametophytes, or, in the presence of a gibberellin-like hormone that promotes the development of antheridia, called antheridiogen, they can begin to directly grow antheridia, theoretically increasing the chances for outcrossing (Farrar 1974; Emigh and Farrar 1977).

Gemmae range in size from about 0.2 to 1.0 mm in length (Farrar 1990) and are therefore relatively large compared with spores, which in homosporous ferns range from 15 to 150 μm (Tryon and Lugardon 1991). This makes long-distance dispersal of gemmae unlikely. Field studies of the gametophyte-only fern *Vittaria appalachiana* have shown that, when transplanted beyond its northern range boundary, gametophytes of this species are capable of surviving, suggesting that dispersal ability defines the northern range limit of this species (Stevens and Emery 2015). *Vittaria appalachiana* is also absent from recently anthropogenically disturbed sites, such as rockfaces that have been cut for roads and tunnels, suggesting slow rates of dispersal and establishment (Farrar 1990).

Despite evidence suggesting that the long-distance dispersal capabilities of gemmae are limited, these propagules are quite effective dispersers over short distances. For example, *V. appalachiana* frequently occurs in dense, clonal populations within the rockhouses it inhabits. Abiotic factors, such as water, may act as dispersal agents to some extent, and Farrar (1985) postulated that animals might aid in dispersal as well. This has been demonstrated to be true for bryophytes, with slugs (Kimmerer and Young 1995) and potentially ants (Rudolphi 2009) dispersing various vegetative propagules, but these types of studies, even for bryophytes, are few and far between. To gain a better understanding of how gametophyte-only ferns obtained their current distributions, further investigations into the dispersal ability of fern gemmae will be needed. Currently, the assumption is that the distributions of widespread gametophyte-

only taxa are the result of ancient spore dispersal that occurred before these ferns lost their sporophyte counterparts (Farrar 2006; see “Drivers of Geographic Separation” below).

Biogeographic Patterns: The Separation of Generations

We are aware of 25 fern species in which gametophytes persist indefinitely in the absence of conspecific sporophytes (table 1), and this number is increasing rapidly. In some cases, gametophytes and conspecific sporophytes may cover the same geographic range, but independent gametophyte populations can be observed in microhabitats that appear to be unsuitable for sporophyte production, likely due to fine-scale environmental differences or the inability of a gametophyte to self fertilize. Alternatively, gametophyte populations may exist allopatrically, apart from conspecific sporophytes and as extensions of the species' geographic range. At the extreme, sporophytes may not exist at all.

In the sections below, the 25 species are organized broadly into areas of occurrence, beginning in eastern North America and proceeding westward to western North America, the Pacific Islands, Asia, and ending in Europe. The greater number of species in temperate zones likely reflects sampling bias—most fern species capable of producing long-lived gametophytes occur in families typically found in the tropics, and recent studies suggest that many additional independent gametophytes await discovery in tropical areas.

Eastern North America

Temperate regions have by far the most thoroughly studied populations of long-lived gametophytes, due largely to the efforts of Donald Farrar, Frederick Rumsey, and Elizabeth Sheffield. To date, we are aware of eight fern species, belonging to five families (table 1), showing the spatial separation of gametophyte and sporophyte generations in eastern North America: Hymenophyllaceae: *Crepidomanes intricatum* (Farrar) Ebihara and Weakley, *Didymoglossum petersii* (A. Gray) Copel., and *Hymenophyllum tayloriae* Farrar and Raine; Lomariopsidaceae: *Lomariopsis kunzeana* (Underw.) Holttum; Polypodiaceae: *Moranopteris nimbata* (Jenman) Proctor; and Pteridaceae: *Vittaria graminifolia* Kaulfuss and *Vittaria appalachiana* Farrar and Mickel.

Several of the species listed above have geographic ranges that extend, at least in part, into the Appalachian Mountains and Plateau, where they can often be found growing in scattered rock outcrops, which are the eroded remains of the Cretaceous uplift of the Plateau (Miller and Duddy 1989). Known colloquially as “rockhouses” or “rock shelters,” these outcrops generate environmental conditions characterized by extremely low light levels (0–5.99 $\mu\text{mol m}^{-2} \text{s}^{-1}$; S. Chambers, unpublished data) and high relative humidity (85%–95%; Chambers and Emery 2016). One unique characteristic of this habitat is its ability to buffer seasonal and daily temperature variation, creating warmer conditions in the winter, cooler conditions in the summer, and generally a more stable thermal environment relative to surrounding conditions (Farrar 1998; Chambers and Emery 2016).

Although the species in eastern North America occupy similar habitats, there is disparity in the size of their ranges. For example, gametophytes of *V. graminifolia* are currently known from two locations: St. Helena Parish in Louisiana (Farrar and Landry 1987) and Broxton Rocks Preserve in southern Georgia (J. B. Pinson and S. M. Chambers, unpublished data), while *Moranopteris nimbata* is found in only a single site in Macon County, North Carolina (Farrar 1990). In contrast, the two species with the most extensive ranges in North America, *C. intricatum* and *V. appalachiana*, both grow throughout the Appalachian Mountains, with similar habitat specificity and almost identical geographic ranges. Below, we summarize what is currently known about each of these species.

Hymenophyllaceae

1. *Crepidomanes intricatum* (fig. 1C–1E) completely lacks a sporophyte and is by far the most common of the Hymenophyllaceae in the continental United States. This species can be found throughout much of the Appalachian Mountains and Plateau, from Georgia and Alabama, through parts of Illinois and Indiana, and as far north as New England and Vermont. The gametophytes are filamentous, and given that their appearance is similar to that of algae, the first formal description of this species was actually included in an 1887 book on the freshwater algae of North America (Wolle 1887). The author correctly theorized, however, that it might be a fern on the basis of a few scant but defining features that separate it from filamentous algae (Farrar 1992). Originally described as *Trichomanes intricatum* Farrar (Farrar 1992), it was unknown whether sporophytes of this species existed at all. In 2008, however, a team of investigators discovered that *T. intricatum* had the exact same *rbcL* sequence as an accession of the Asian fern *Crepidomanes schmidtianum* and differed in only one base pair for a second accession, indicating a relatively recent formation of this species (Ebihara et al. 2008). Accordingly, the species has been transferred to *Crepidomanes* (Weakley et al. 2011).

There are a number of plausible hypotheses for how this species formed and came to occupy its current distribution. In one scenario, long-distance dispersal of *C. schmidtianum* from Asia to eastern North America initially gave rise to populations with the normal alternation of generations (Ebihara et al. 2008). Then, in response to glacial expansion, this species lost its sporophytic generation and retreated into rock shelters, resulting in the current distribution and allowing for reproductive isolation that promoted speciation. *Crepidomanes intricatum* occupies habitat that extends well north of the Pleistocene glaciation boundary, so if a fertile sporophyte was responsible for its current distribution, that sporophyte must only recently have gone extinct as well (<12,000 yr ago), assuming poor dispersal ability of gametophyte-only populations. It is also possible that *C. intricatum* and *C. schmidtianum* are the product of separate hybridization events involving the same maternal progenitor or that one gave rise to the other via hybridization, which would explain the matching *rbcL* sequences. Efforts to determine the population structure of *C. intricatum* are currently under way at Utah State University (A. Duffy, personal communication), which may help to determine its origins.

2. *Didymoglossum petersii* (fig. 6) grows mostly in the Neotropics, including Guatemala, Honduras, Costa Rica, and Nicaragua (Mickel and Smith 2004), but it has several populations in eastern North America as well in the southern Appalachians, with additional small populations in Florida and Louisiana (Farrar 1993a). Gametophytes are filamentous and can be found growing independently of sporophytes in certain parts of its range in North America, such as at Broxton Rocks Preserve in Georgia (J. B. Pinson and S. M. Chambers, unpublished data), as well as at several sites in Arkansas, where gametophytes were documented growing up to 50 km from the nearest sporophyte population (Farrar 1992; Bray 1996). This species is both epiphytic and epipetric; in Louisiana, gametophytes and sporophytes were observed growing on the trunks of beech (*Fagus grandifolia* Ehrh) and magnolia (*Magnolia grandiflora* L.) trees (Allen 1975; Farrar and Landry 1987). Despite its wide range, very little work has been done on this species.

3. *Hymenophyllum tayloriae* (fig. 1F) is endemic to the United States and is extremely rare, occurring in only a few counties in North Carolina and Alabama (Farrar 1998). This species was first reported in 1936, when Mary Taylor collected a small juvenile fern sporophyte in Pickens County, South Carolina. The identity of the collection remained somewhat intractable, as it was originally identified as *Hymenophyllum hirsutum* (L.) Sw., but on closer examination, it was determined to be a new species. It would be another 55 yr, however, until it was given the specific epithet of *tayloriae*, commemorating its initial collector (Raine et al. 1991). The gametophytes are ribbon shaped and produce copious amounts of gemmae, but juvenile sporophytes are rare, and, to date, a mature sporophyte for this species has never been observed. The single juvenile sporophyte collected by Taylor in 1936 was the only observed occurrence of a sporophyte until 1993, when several were discovered at a single site in Alabama by the bryologist Paul Davison. About 50 sporophytes were found at this location, all in microhabitats of small crevices in sandstone rock outcroppings (Farrar and Davison 1994). All of these sporophytes were juvenile, however, and it is unknown whether they ever matured or produced spores.

Lomariopsidaceae

4. *Lomariopsis kunzeana* grows on the tropical islands of Cuba and Hispaniola but is also present in small numbers in solution holes throughout southern Florida, where it is considered endangered (Gann et al. 2006). In some of these solution holes, *L. kunzeana* has been observed growing as both a sporophyte and ribbon-shaped gametophytes, but in others, it has been observed growing only as independent gametophytes (Possley et al. 2013). Solution holes are formed when water collects at the surface of carbonate rock and subsequently percolates down to subterranean aquifers, dissolving the rock as it goes (Ford and Williams 2007). These depressions can vary in size and depth, some spanning several meters in width and/or reaching below the water table (Kobza et al. 2004). The differences in depth among sinkholes and solution holes likely produce differing fine-scale environmental conditions, which may drive the lack of sporophyte production in certain depressions. More study of this phenomenon and of its effects on fern distributions in these microhabitats is needed.

Polypodiaceae

5. *Moranopteris nimbata* has strap-shaped gametophytes (Farrar 1967). The species is generally found in the tropics of the Caribbean, including the islands of Cuba, Hispaniola, and Jamaica (Smith 1993), but a single population at a single site in North Carolina was discovered in 1966 (Farrar 1967, 1971, 1985). This population was growing epipetrically and intermixed with bryophytes about 800 mi from the nearest documented occurrence of the species in the tropics. Unlike other long-lived gametophytes endemic to the Appalachians, *M. nimbata* was growing where it received continuous spray from a waterfall. Although sporophytes have been observed growing in this North Carolina population, the species is considered to be an independent gametophyte, because the dozen or so sporophytes observed at the site over the course of 4 yr were all juvenile and infertile, whereas the gametophytes were present in copious numbers. Long-distance dispersal, possibly of even just a single spore, is the likeliest explanation to account for the occurrence of this species in the United States (Farrar 1967). A recent attempt to observe the species at the same location was unsuccessful (F. W. Li, personal communication); thus, it is unclear whether the population still exists.

Pteridaceae

6. *Vittaria graminifolia* (fig. 3) is common in the Neotropics, growing in several Central and South American countries (Mickel and Smith 2004). In the United States, it can be found as both independent, ribbon-shaped gametophytes and sporophytes. Edgar Wherry (1964) first reported *V. graminifolia* as occurring in the United States, but the occurrence was based on a misidentified collection of sporophyte tissue (Gastony 1980; Farrar 1993b). Farrar and Landry (1987) would later find independent gametophytes of *V. graminifolia*, as determined by enzyme electrophoresis, growing in a single county in Louisiana on beech and magnolia trees. More recently, Frankie Snow and Carl Taylor found specimens of *V. graminifolia* in Broxton Rocks Preserve, Georgia (J. B. Pinson and S. M. Chambers, unpublished data). The specimens here were locally abundant but restricted to small, moist, shaded sandstone outcrops of the Altamaha Grit formation (Edwards et al. 2013). A few sporophytes were found at this site, but large gametophyte populations without sporophytes could also be seen. These are the only two confirmed recordings of *V. graminifolia* in the United States. It is unknown how long the population has existed at Broxton Rocks, but both instances in the United States are likely the result of long-distance dispersal. The species has reported chromosome counts of $2n = 120$ and $2n = 240$, and both appear to be fertile (Gastony 1977; Smith and Mickel 1977).

7. *Vittaria appalachiana* (fig. 1G) is the independent gametophyte with the second-largest distribution after *C. intricatum* in the eastern United States, occurring in 12 states, from Alabama to New York (Farrar 1993b). Gametophytes are ribbon shaped (Farrar and Mickel 1991), and mature sporophytes have never been observed for this species, although Farrar (1978) found three juvenile sporophytes (<1 cm tall) in Jackson County, Ohio. These seemed to have been produced

apogamously due to the presence of budding protrusions on other gametophytes at the same location that lacked gametangia. Similarly, Alma G. Stokey grew gametophytes in culture that produced apogamous growth, but most of these developing sporophytes died before even producing vascular tissue. The largest of these, according to her notes, was less than a centimeter tall (Farrar 1978). Because the sporophytes died, she never published this work. Caponetti et al. (1982) had similar results, and their cultures produced six sporophytes (all <1 cm) before an air conditioner failure killed all of their cultured specimens. Although gametangia and mobile sperm have been observed (Farrar 1978), it appears that the species has lost the ability to produce sporophytes via fertilization or apogamy.

Recent work has focused on elucidating the origin of this species. Farrar (1990) found fixed heterozygosity at several allozyme loci, which could be interpreted as evidence of hybridization in the ancestry of *V. appalachiana*. Pinson and Schuettpelz (2016) subsequently tested for hybridization using a suite of plastid markers and the nuclear marker *det1*. They found that, rather than grouping with two putative parental species, as would be expected in the case of hybridization, the alleles for *V. appalachiana* all nested within a clade containing two genetically distinct groups of *V. graminifolia*, making the later paraphyletic. These results suggest that either the two species are conspecific or *V. graminifolia* sensu lato is actually composed of two separate species. *Vittaria appalachiana* is a polyploid ($n = 120$; Gastony 1977), and the molecular results suggest that it is likely of autopolyploid origin.

Farrar (1990) also used allozymes to uncover population structure within *V. appalachiana*. For the gene PGI, six genotypes were recovered from a total of 92 populations located in seven states. Of those, only three states (Alabama, Ohio, and North Carolina) had multiple genotypes, and the remaining four states each contained only a single genotype among all sampled populations. When comparing multilocus genotypes in the same study, the same relative pattern of diversity was recovered in Ohio and Alabama, and homogeneity was found elsewhere. This suggests that Ohio and Alabama may have been the center of diversity from which gametophyte populations were established in outlying areas. Alternatively, *V. appalachiana* may have been equally diverse throughout the Appalachian Mountains, but certain genotypes and alleles became extirpated due to bottlenecking events (Farrar 1990). Since genetic drift and bottlenecking can have an inordinate effect on asexual populations, this seems to be a plausible hypothesis for many of the species with independent fern gametophytes.

The northern range limit of *V. appalachiana* is in the southwestern portion of New York (Farrar 1978). This range corresponds with the southern limit of the Illinoian glacial boundary, the last of the major glacial incursions of the Pleistocene. Stevens and Emery (2015) recently determined that transplanted individuals from populations throughout the geographic range of the species could successfully survive in latitudes to the north of contemporary range limits. This suggests that dispersal limitation drives the geographic range boundary of the species and that the contemporary boundary reflects historical limitations established during the Pleistocene glaciation (Stevens and Emery 2015). Additionally, the previously discussed *C. intricatum* has very similar habitat requirements to that of *V. appalachiana*,

and its distribution extends well beyond the glacial boundary, into Vermont (Farrar 1992). This indicates that neither temperature limitation nor lack of suitable available habitat is the cause of *V. appalachiana*'s truncated range; rather, the species is physically unable to migrate into more northern areas where suitable habitat exists. By proxy, this further suggests that *V. appalachiana* lost the ability to produce mature sporophytes before the last glaciation event, whereas *C. intricatum* lost this ability sometime after the glaciers receded and after it had already extended its range northward (Farrar 1978).

Western North America

8. *Hymenophyllum wrightii* Bosch (Hymenophyllaceae) has ribbon-shaped gametophytes and is the only known fern species in western North America that displays the pattern of spatially separated generations (fig. 1H–1J). First collected as a sporophyte by Herman Persson on the Queen Charlotte Islands (who published the record “almost as a footnote” in 1958), *H. wrightii* had previously been known only from Asia (Persson 1958; Taylor 1967). Although Persson had obtained a sporophyte specimen, it became apparent through later collections that sporophyte production was rare. Duffy et al. (2015) recently collected specimens from several populations and found that the gametophytes have a wide distribution, from Washington State up through parts of British Columbia and Alaska; however, documented occurrences of the sporophyte have only ever been reported from the Queen Charlotte Islands. Genetic analysis of the gametophyte samples collected by Duffy and colleagues showed no variation between populations at two plastid loci (*rbcl* and *rps4-trnS*) but showed two to three nucleotide differences from Asian *H. wrightii* accessions. Sporophytes from the Queen Charlotte Islands were not included in that analysis.

Besides growing much farther north than the independent gametophytes in eastern North America, *H. wrightii* also has slightly different habitat requirements. It can be found growing in shady areas of moist cliff faces, but gametophytes are also commonly found growing epiphytically on living conifers, around the tree base or on exposed roots. It can also be found growing on decaying plant matter, especially in the dark recesses of fallen trees. Given the exposed nature of these habitats and lack of consistent water availability, *H. wrightii* is found only within about a 1.5-km distance from the Pacific, where maritime weather keeps humidity high and temperatures moderated (Duffy et al. 2015). The gametophytes are ribbon shaped and produce copious gemmae. This, combined with the rare occurrence of sporophytes, indicates that individual populations of independent gametophytes are likely sustained by vegetative reproduction. Since they can be found growing on isolated logs and trees, this suggests that the gemmae may be relatively successful short-distance dispersers, and *H. wrightii* perhaps represents an ideal system for the study of gemma dispersal.

Pacific Islands

The Pacific Islands, along with the Paleo- and Neotropics, likely harbor large numbers of independent fern gametophytes,

but researchers are just now beginning to survey in these areas. In this section, we report on five species that have independent populations of gametophytes. The only currently available data come from the Hawaiian Islands and French Polynesia.

Hymenophyllaceae

9. *Callistopteris baldwinii* Copel. is a terrestrial filmy fern with gemmiferous, ribbon-shaped gametophytes that displays a trend of sporophyte reduction and loss along elevational gradients. On tropical oceanic islands, rainfall generally increases with increasing elevation, a trend caused by the orographic uplift of trade winds (Loope and Giambelluca 1998). As a result, cloud forest ecosystems are common at higher elevations. The sporophytes of this species are highly sensitive to desiccation and appear to be restricted to these cloud forests (Dassler and Farrar 1997). Sporophytes are rarely observed at lower elevations, and dwarfed sporophytes observed at mid-elevation in Hawai'i were not mature (Dassler and Farrar 1997). In contrast, gametophytes are present at all elevations. At high elevation, where fog cover and rainfall are consistently high, gametophytes were observed growing abundantly on a number of substrates, including rocks, fallen logs, living tree trunks, and soil banks (Dassler and Farrar 1997). Near sea level, the gametophytes appeared to be restricted to vertical rocks and soil banks, forming large, thick mats, presumably maintained primarily by dispersal of gemmae, as sporophytes were absent. Dassler and Farrar (1997) determined that gametangia were present in sufficient numbers to sustain sexual reproduction, and embryos were found at all elevations in Hawai'i. It would therefore seem that sporophyte production and development are limited by the death of either embryos or juvenile sporophytes in drier conditions.

10. *Callistopteris apiifolia* (C. Presl) Copel. (fig. 1K; see also “Asia”), a species currently considered to be closely related to *C. baldwinii*, has also been observed by Nitta and colleagues (Nitta et al., forthcoming) to show a pattern of reduced sporophyte production along an altitudinal gradient on the islands of Mo'orea and Tahiti, French Polynesia, with ribbon-shaped gametophytes distributed over a wide range of elevations but sporophytes confined to moist cloud forest habitats at high elevation. Furthermore, Nitta and colleagues (J. H. Nitta, J. E. Watkins, N. M. Holbrook, R. Taputuarai, T. Wang, C. C. Davis, unpublished data) tested the ability of *C. apiifolia* gametophytes to withstand desiccation and found that they were no more tolerant than sporophytes, suggesting that these gametophytes are exploiting protected microhabitats rather than relying on desiccation tolerance to survive beyond the range of sporophytes.

11. *Hymenophyllum recurvum* Gaudich. is a species endemic to the Hawaiian Islands that has ribbon-shaped gametophytes, which can be found growing independently in honeycomb-like indentations on the surfaces of boulders deposited by basaltic lava flows. Within these hexagonal pockets, populations are often found without any associated sporophytes (D. R. Farrar, personal communication). Yet both gametophytes and sporophytes of this species can be found growing sympatrically throughout the islands in epiphytic communities. Although

the two generations overall are geographically sympatric, the occurrence of independent gametophyte populations in a radically different habitat than that of sporophytes underscores the potential for ecological differences between the generations and the sometimes-narrow environmental conditions required for sporophyte production. It is also possible that the gametophytes growing in the lava flow crevices rarely receive the free water necessary for fertilization, which may explain the lack of sporophytes in that environment.

12. *Vandenboschia cyrtotheca* Copel. grows as both gametophytes and sporophytes at high elevations on the Ko'olau Mountains on the eastern side of the island of O'ahu. This species appears to exhibit a similar elevation-dependent distribution as *C. baldwinii*. Farrar conducted an isozyme analysis on gametophyte populations collected from the Waianae Mountains in eastern O'ahu and determined that independent gametophytes found at lower elevations also belonged to *V. cyrtotheca* (D. R. Farrar, personal communication). No further study of this species has been conducted.

Pteridaceae

13. *Vaginularia paradoxa* (Fée) Mett. (= *Monogramma paradoxa* (Fée.) Bedd.) is a small vittarioid fern distributed primarily in the South Pacific with ribbon-shaped gametophytes (fig. 1L). Nitta and colleagues (Nitta et al., forthcoming) used DNA bar codes (short diagnostic DNA sequences that can discriminate between species; Hebert et al. 2003) to compare ranges of sporophytes and gametophytes along an elevational gradient from 200 to 2000 m on the islands of Mo'orea and Tahiti, French Polynesia. They found over 20 populations of *V. paradoxa* gametophytes growing on trees or rocks from ca. 200 to 600 m but never observed any sporophytes of this species, despite intense effort. Sporophytes of *V. paradoxa* are apparently extremely rare in Tahiti and Mo'orea, having been collected only a handful of times (only three specimens from Mo'orea, all from the 1850s, and nine specimens from Tahiti, with the most recent collection in the 1980s at P). Additional gametophyte populations have also been discovered on other islands in the Society Islands from which sporophytes of *V. paradoxa* have never been recorded (J. H. Nitta, unpublished data). As in other independent vittarioid gametophytes, *V. paradoxa* produces copious gemmae and forms clonal mats up to several square centimeters. It is unknown how it maintains these large, frequent populations despite sporophytes being extremely rare or lacking sympatrically, although it is possible that long-distance dispersal from other islands plays a key role.

Asia

Asia has been the focus of recent efforts to find and identify populations of independent gametophytes, and several have been discovered in the last few years. Similar to the study on *V. paradoxa* conducted on the islands of Mo'orea and Tahiti described above, recent studies in Japan and Taiwan have also employed DNA bar coding to conduct surveys of local gametophyte populations and identify them to species without having to rely on morphological characters (Ebihara et al. 2010,

2013; Chen et al. 2013b; Kuo et al., forthcoming). There are currently 12 known instances of independent gametophyte populations that grow at least 20 km (and more often >100 km) away from any known populations of sporophytes. Unfortunately, some of these cannot be identified to species or even genus due to a lack of genetic studies. In addition, although many populations have been identified, little additional research on their ecology or natural history has been conducted; thus, we know relatively little about these ferns' habitat requirements and whether they are able to produce even juvenile sporophytes. Below, we describe what is known about these species' ranges and occurrence.

Hymenophyllaceae

14. *Hymenophyllum badium* (Hook. and Grev.) is an epiphytic fern with ribbon-shaped gametophytes distributed throughout southeast Asia, southern China, Vietnam, Taiwan, and Japan. In Japan, it occurs primarily in the southeastern half of the country. Ebihara et al. (2013) conducted a DNA bar code survey of gametophytes at eight sites throughout Japan and compared these with sporophyte records. Gametophytes of *H. badium* were found growing in Saitama Prefecture, Japan, 100 km from the closest-known sporophyte. These gametophytes were growing beyond the northern limit of the sporophyte in Japan, which is itself the northern edge of the geographic distribution of this species.

(10). *Callistopteris apiifolia* (fig. 1K) was observed as gametophyte populations without nearby sporophytes on the island of Iriomote by Ebihara et al. (2013). The sporophyte of this species is extremely rare in Japan and is considered endangered. The species is primarily distributed on islands in the tropical Pacific and in Southeast Asia (see Pacific Islands), and it is possible that subtropical Iriomote represents the northern limit of the sporophyte. That the gametophytes can still thrive there may be another example of fern gametophytes able to live at or beyond the northern range boundaries of their related sporophytes.

15, 16. In addition to *C. apiifolia*, gametophytes of two more filmy fern species were observed growing on Iriomote by Ebihara et al. (2013) that did not match any known fern species from Japan for the DNA bar code marker *rbcl*. Efforts are ongoing to locate sporophyte matches for these taxa, should they exist (A. Ebihara, personal communication).

17. *Vandenboschia kalamocarpa* (Hayata) Ebihara is a member of the *Vandenboschia radicans* species complex in Japan. Ebihara et al. (2009) investigated a single site in Shizuoka Prefecture where three hybrid sporophytes in this complex occurred: diploid *Vandenboschia* × *stenosiphon* (H. Christ) Copel. (genotype $\alpha\beta$), triploid *Vandenboschia* × *quelpaertensis* (Nakai) Ebihara (genotype $\alpha\alpha\gamma$ or $\alpha\gamma\gamma$), and tetraploid *Vandenboschia orientalis* (C. Chr.) Ching (genotype $\alpha\alpha\gamma\gamma$). Of these, only *V. orientalis* is fertile, so it was unclear how the hybrid taxa were being produced at the site. By carefully sampling and sequencing filamentous gametophytes found at the site, Ebihara et al. (2009) discovered haploid *V. kalamocarpa* (genotype α) gametophytes, which they inferred were contributing the α type genome to the hybrids but were themselves incapable of producing nonhybrid sporophytes. The closest

known location of *V. kalamocarpa* sporophytes is the Izu Islands, located 50 km off the coast of Japan. Ebihara and colleagues speculated that this species was able to contribute to hybrid formation but was unable to produce nonhybrid sporophytes due to differences in environmental conditions on the Izu Islands compared with Honshu. To our knowledge, this is the only documented example of an independent gametophyte contributing to the formation of hybrid species outside the range of conspecific, nonhybrid sporophytes.

Pteridaceae

18, 19, 20. *Antrophyum henryi* Hieron., *Antrophyum parvulum* Blume, and *Haplopteris heterophylla* C. W. Chen, Y. H. Chang and Y. C. Liu (fig. 1M). Chen et al. (2013a, 2013b) also used DNA bar coding to identify field-collected gametophytes to species, but they focused on vittarioid ferns in Taiwan. They found gametophytes of these three species growing at least 20 km away from the nearest known conspecific sporophytes and in different elevations or habitats than typically observed for the sporophytes.

21, 22. *Haplopteris* species. In another study in Taiwan using DNA bar codes, Kuo and colleagues (L.-Y. Kuo, C.-W. Chen, W. Shinohara, A. Ebihara, H. Kudoh, H. Sato, Y.-M. Huang, W.-L. Chiou, unpublished data) comprehensively sampled gametophytes every 2 mo for a year at a single site in the Fushan area (northern Taiwan). They found that species richness of gametophyte populations changed over the course of the year and exceeded that of sympatric mature sporophytes by a factor of 2–3 overall. Furthermore, they documented two species of *Haplopteris* with independent gametophyte populations at this site. The first lacked gametangia and is likely conspecific with *Monogramma (Haplopteris) capillaris* Copel., a species with a distribution in Southeast Asia, owing to their near-identical (>99%) chloroplast DNA (cpDNA) sequences (*chlL* + *matK* + *ndhF*). Two additional populations were discovered of the second *Haplopteris* species, one in northern Taiwan and one in Japan (Yakushima). At one of the sites in northern Taiwan, the authors observed both male and female gametangia along with juvenile sporophytes. In the other two populations, however, gametangia and juveniles were absent. Interestingly, both the gametophytes and juvenile sporophytes in the former population were epiphytic but were observed to be epipetric or terrestrial at the latter two populations, implying that microhabitat conditions may have an effect on the production of gametangia and/or sporophytes. It is currently unknown whether this second species has a mature sporophyte counterpart, although the occurrence of distant populations in Taiwan and Japan (and divergence times of less than 0.5 mya) suggests recent spore dispersal. Further population genetic studies are needed to shed light on their geographic origin and phylogenetic relationships.

Lomariopsidaceae

23. *Lomariopsis lineata* (C. Presl) Holttum (fig. 5) is a species that was originally thought to be a liverwort, and its identification as a fern gametophyte is atypical, since independent gametophyte populations of this species are known only from

the aquarium trade. In 2001, Christel Kasselmann, an avid aquarium designer, propagated the plants and shared the propagules with other aquarium enthusiasts, after which it became popular in the aquarium market under the name Süßwassertang, German for “freshwater seaweed” (Kasselmann 2010). Li et al. (2009) sequenced several plastid loci for the species, resolving it as being most closely related to *L. lineata* (Holttum), an epiphytic fern that grows in several countries in Asia (Barcelona et al. 2006; Ke et al. 2013). Little is known about this species, and to date, we are unaware of any record in which the gametophytes of *L. lineata* have been observed naturally growing in aquatic environments. Kasselmann (2010), however, reported that specimens have been found growing on rocks in seasonally dry riverbeds. During the time that it has been in the aquatic trade, there are no reports of sporophyte production, even though archegonia and antheridia have been observed. It is currently unknown whether the ornamental represents an independent occurrence of *L. lineata* gametophytes or whether it represents a unique species (Li et al. 2009). The gametophytes lack gemmae, which is likely why they have not been observed independently in nature. Because there was only one known introduction by Kasselmann into the aquatic trade, it can be reasonably assumed that all of the plants currently being sold are exact clones of each other and have reproduced entirely by continuous meristematic growth.

24. Gametophytes belonging to the genus *Lomariopsis* sp. were discovered by Ebihara et al. (2013) on Iriomote Island. Genetic analysis revealed that the species failed to match *rbcL* sequences from any *Lomariopsis* known from Japan, indicating its potential as an independent gametophyte. Current efforts are focusing on sampling a broader range of *Lomariopsis* from Asia to try to find a match for this species. Thus, a total of four apparently gametophyte-only species (*C. apiifolia*, the two unidentified filmy ferns, and *Lomariopsis* sp.) have been documented on Iriomote Island, again suggesting that its location as the northern limit for many tropical taxa and its isolated nature could promote occurrence of gametophyte-only populations.

Europe

Hymenophyllaceae

25. *Vandenboschia speciosa* (Willd.) Kunkel (Hymenophyllaceae), known colloquially as the Killarney fern, has filamentous gametophytes and is the only fern in Europe known to display the pattern of spatially separated generations. First collected in 1724, populations of *V. speciosa* may have suffered severe depletion as avid collectors scoured the countryside for ferns during the Victorian fern craze (~1850–1890; Allen 1969; Rumsey et al. 1998a, 1998b; Whittingham 2009). The last reported observation of sporophytes at the site of its initial collection was in 1785, the area having undergone extensive disturbance. Farrar, however, returned to this location in 1989 and found a gametophyte population still thriving. He had predicted that gametophytes of *V. speciosa* might persist in the absence of sporophytes and was the first to discover such independent colonies (Rumsey et al. 1998b). The species

is primarily located in eastern Europe (e.g., Poland) and several Mediterranean islands (Rumsey et al. 1998b), but populations also occur in central and northern Europe (Rasbach et al. 1993; Vogel et al. 1993; Krukowski and Swierkosz 2004). The sporophytes, however, appear to be mainly restricted to the wetter and warmer climates in the south and are sparse to effectively nonexistent in the drier portions of central Europe (Rumsey et al. 1998b; Krippel 2001). Additionally, many of the sporophytes that are produced in northern Europe, particularly in Great Britain and Ireland, never reach maturity or produce spores (Ratcliffe et al. 1993).

Two cpDNA (*trnL*) haplotypes can be found among *V. speciosa* populations, which correspond to different geographical regions, the first occurring in the Azores and the second prevalent in other Mediterranean islands, from Madeira to the Canaries (Rumsey et al. 1996). These islands are the only places where populations of *V. speciosa* are abundant and regularly complete their life cycle, and therefore, presumably, the populations on these islands serve as source populations for those found in Europe. The haplotype present in the Azores can be found in central Europe, while the haplotype from Madeira and the Canary Islands corresponds to populations in Spain and Italy. Both haplotypes, however, can be found in Great Britain and Ireland (Rumsey et al. 1996).

Further genetic analyses of allozymes conducted by Rumsey et al. (1999) found a total of seven multilocus polymorphisms (MLPs) among populations in southwestern Scotland, whereas complete homogeneity was uncovered in individual populations, thus suggesting little dispersal among populations. In Great Britain, where most populations are gametophyte only, the few observed sporophytes appear to be produced apogamously based on identical sporophyte MLPs with surrounding gametophytes (Rumsey et al. 1999). In Spain, there is evidence for some dispersal as MLPs are shared among populations. Additionally, intergametophytic reproduction may occur in this region as population-specific MLPs also exist (Rumsey et al. 2005). Conversely, in central Europe, the presence of multiple MLPs among distant populations suggests that sporophytes likely existed in the region at one time. Long-distance dispersal to various locations may have established populations after the Pleistocene glaciations, followed by secondary dispersal to nearby areas (Rumsey et al. 1998b). Finally, in Italy, a unique allele of the PGM enzyme locus has also been found for *V. speciosa*, which has yet to be detected in any other population, suggesting that these may be relict Pleistocene populations (Rumsey et al. 2005).

Drivers of Geographic Separation

Field observations and common-garden studies have shown that some gametophytes can tolerate a wider range of environmental conditions, particularly colder temperatures, compared with their respective sporophyte counterparts (Farrar 1978; Sato and Sakai 1981). Plant responses to desiccation and cold temperatures invoke a similar metabolic pathway; thus, plants that can physiologically tolerate cold temperatures are likely tolerant of desiccating environments as well (Knight and Knight 2001; Sinclair et al. 2013). Tolerance of these cold temperatures may be one reason that ferns with

temperate distributions can exist without a sporophyte. Specifically, reductions in temperature associated with Pleistocene glaciation expansions may have created cold, dry conditions that the sporophyte could not survive. Farrar (1978) hypothesized that warmer temperatures during the early Tertiary allowed some species of tropical epiphytic ferns to occupy habitats in North America and that subsequent climatic conditions associated with the Pleistocene glaciations contributed to the loss of sporophytes in these species. The remaining sporophyteless ferns would therefore be considered ancient relicts of once flourishing populations. This seems particularly plausible for *Vittaria appalachiana* and *Crepidomanes intricatum*, both of which have ranges throughout the Appalachian Mountains and Plateau.

Earth's climate over the past 50 Myr has, for the most part, been much warmer than that of the ice-capped planet we are familiar with today. Even parts of the Pliocene (~3 Myr ago) had average global temperatures between 2° and 3°C higher than preindustrial temperatures (Jansen et al. 2007), and while the generic vegetation schemes in eastern North America closely resembled those seen at present, temperatures were still slightly elevated compared with those seen today at midlatitudes (Cronin et al. 1994) and along the Atlantic Coastal Plain during parts of the Pliocene and into the Quaternary (Groot 1991).

With the onset of the glaciers in the Pleistocene, any species that could not adapt would have relocated to suitable climates or protected refugia or gone extinct (Davis and Shaw 2001; Beatty and Provan 2010). For many bryophyte and monilophyte species in the Appalachians, these colder temperatures may have led to aberrations in the alternation of generations, where sporophytes and specific sexes are limited to certain portions of the geographic range or are entirely nonexistent. In bryophytes, the Appalachians harbor several taxa that appear to lack one sex or the other; dioicous liverwort genus *Plagiochila* has ca. 22 species in the Appalachians, many of which exist only as a single sex (Longton and Schuster 1983). In the tropics, however, these species grow as both sexes and undergo the normal alternation of generations (Farrar 1978; Schuster 1983, 1992). Yet again, this pattern may be driven by stress tolerance, specifically in response to desiccating environments. In the dioicous desert moss, *Syntrichia caninervis*, studies have shown that sexes respond differently to stressful environmental conditions generated by low moisture availability and high light levels, with female plants being significantly more tolerant than their male counterparts (Stark and McLetchie 2006; Stark et al. 2005). This pattern is also apparent in dioicous bryophyte taxa in the Appalachians, because there is evidence to suggest that, in suboptimal conditions, functional archegonia are produced, but males fail to produce antheridia (Longton and Schuster 1983; Schuster 1992), a pattern the bryologist R. M. Schuster termed “sexual regression.” To date, no studies have been conducted to determine whether this pattern, termed “spatial segregation of sexes” (Bierzychudek and Eckhart 1988), occurs in fern gametophytes or whether this may be one reason for the lack of sporophyte production in disjunct populations.

Although rare in some gametophytes with filamentous morphology (i.e., *C. intricatum*), long-lived ribbon-shaped gametophytes in the Appalachians often retain the ability to produce gametangia (i.e., *V. appalachiana*), but sporophytes either are

found in small numbers or are not produced at all. Following the hypothesis that these ferns are relictual populations from the Pleistocene, their inability to produce sporophytes seems to indicate the presence of selective pressures on the sporophyte generation. For example, it is possible that individuals that did not allocate resources to the production of a sporophyte that was likely to die had higher survival rates. Thus, only those individuals with deleterious alleles associated with sporophyte production survived historical environmental pressures and subsequently generated large populations via asexual gemmae. To date, there have been no genetic studies attempting to test this hypothesis.

There are varying degrees of sporophyte loss in some species. As seen in the Pacific Island species *Callistopteris baldwinii*, it is likely that the first stage in sporophyte loss is a reduction in size. *Lomariopsis kunzeana* in southern Florida rarely produces mature sporophytes, but when it does, they are generally smaller than those produced in Cuba and Hispaniola. *Stegnogramma burksiorum*, endemic to Winston County in Alabama, produces dwarfed sporophytes compared with its sister species *Stegnogramma pilosa* (M. Martens & Galeotti) K. Iwats., which occurs throughout central Mexico (Watkins and Farrar 2005). In culture studies, *S. burksiorum* has been noted above for the ability of its cordate gametophytes to grow thin filaments that later develop into widened prothalli, a form of asexual reproduction. If the cold temperatures of the Pleistocene had continued, it is possible that this species may have lost the ability to produce sporophytes as well.

However, although the Pleistocene relict hypothesis offers a plausible explanation for the existence of gametophyte-only species in eastern North America and appears to work well in cases of dioicous bryophytes, it cannot suitably account for the majority of fern species with spatially separated generations. In Europe, for example, the distribution of populations of *Vandenboschia speciosa* with and without sporophytes does not appear to reflect glacial patterns. In the United Kingdom, there are gametophyte-only populations not far removed from populations with sporophytes, suggesting that there may be genetic restrictions underlying the inability of some populations to produce sporophytes or fine-scale microclimatic conditions that inhibit the production of sporophytes. In western North America, gametophytes of *Hymenophyllum wrightii* can be found in the United States, Canada, and Alaska, but sporophytes are produced only on the Queen Charlotte Islands, a distribution pattern that cannot be explained by range expansions/contractions concomitant with glacial movement. Additionally, many of the independent gametophytes discussed in this review were discovered in tropical regions that were devoid of glaciers in the Pleistocene.

It seems that environmental effects may thus be extremely important in explaining the separation of generations pattern. To test this hypothesis, transplant and common-garden studies, similar to those employed by Chambers and Emery (2016), may be utilized. Specifically, gametophyte explants may be transplanted into populations in which sporophyte production is known to occur or placed in simulated environmental conditions (i.e., growth chambers) that reflect natural conditions associated with sporophyte-bearing populations. Furthermore, specific environmental factors believed to be driving the lack of sporophyte production may be isolated in manipulative studies

conducted in growth chambers, thus allowing for the examination of environmental thresholds to sporophyte production.

It should be noted that there are limits to the establishment of sporophytes after the dispersal of a single spore to a previously uncolonized environment or the migration of clonal gametophyte propagules, whether by gemmae or portions of the thallus. Not all fern gametophytes are hermaphroditic, and despite recent evidence that the gametophytes of a large number of fern species are capable of selfing, many appear to have genetic barriers to the production of a completely homozygous sporophyte (Sessa et al. 2016). So while gametophytes with strap, ribbon, or filamentous morphologies are able to produce extensive vegetative and clonal growth from a single spore, that clonal gametophyte population may not have the capacity to produce sporophytes, due to an inability to self-fertilize. Currently, we are aware of a few instances in which gametophytes translocated from the tropics to greenhouse environments in North America have failed to produce sporophytes, despite being established for many years. J. B. Pinson and S. M. Chambers (unpublished data) determined that independent gametophytes that have thrived in the greenhouse of orchid biologist Mark Whitten (personal communication) for 20 yr without any known instances of sporophyte production are closely related to *Polytaenium lineatum* (Sw.) J. Sm. (96% GenBank match for *rbcL*). Additionally, Charles Alford, a fern horticulturalist in south Florida, has dense populations of gametophytes closely related to *Hecistopteris pumila* (Spreng.) J. Sm. (97% GenBank match for *rbcL*) growing in his greenhouse as mats of gametophyte thalli without sporophytes (C. Alford, personal communication). Both species grow as sporophytes in Central and South America. Although it has yet to be experimentally tested for these species (or any long-lived gametophyte), it seems likely that the gametophytes in the examples above are simply unable to produce sporophytes because they each comprise only a single genotype.

Conclusions and Future Directions

Until recently, fern gametophytes were often thought of as the Achilles' heel of the fern life cycle (e.g., Page 2002). Gametophytes lack stomata and roots, have no vascular tissue, are mostly one cell layer thick, and require free water for fertilization, making them seem ill-equipped to survive in fluctuating environments. But "natural selection does not tolerate mistakes" (Farrar, as quoted in Watkins and Cardelús 2012, p. 695), and researchers have recently begun to discover the unique innovations and evolutionary adaptations that fern gametophytes possess. Nowhere is this more evident than in the long-lived epiphytic gametophytes discussed here. With the capacity for increased longevity and asexual reproduction, some species have even jettisoned the sporophyte generation entirely and yet still manage to maintain large populations and distributions. Although these species have been relatively well studied and characterized in temperate regions, where they are the most conspicuous, we are only just now beginning to uncover patterns of spatially separated fern generations in the tropics. There are several obstacles that will need to be overcome to thoroughly document and understand this pattern in tropical regions, not the least of which is successful identification of gametophytes.

With technological advances in DNA sequencing, such as the DNA bar-coding approaches used in Japan, Taiwan, and Tahiti described above, we can now identify fern gametophytes to the species level without having to rely on morphology. These molecular tools may be especially useful in identifying novel species with independent gametophytes in the tropics. For example, in the tropical forests of Central and South America, one can easily find dense patches of fern gametophytes, but it is unclear how these relate to nearby species and populations that readily produce sporophytes. DNA bar coding can be successful, however, only with a well-sampled reference library, as highlighted by the inability to identify several of the putative independent gametophytes in Asia described above. Next-generation sequencing technologies, such as sequence capture technology that can recover genetic data from degraded DNA sources, including herbarium specimens (Straub et al. 2012), would greatly increase the coverage of the reference library and may enhance our ability to confidently identify gametophytes to species. Results from studies such as these will likely uncover novel species and identify even more disjunct populations, increasing the number of ferns that display the separation of generations. These genetic resources can also be used to examine phylogenetic relationships, biogeographic patterns, patterns of gene flow, and genetic structure among populations.

Beyond fundamental issues of identification, our understanding of the basic ecology and demography of many of these fern species is sorely lacking. Given the possibilities of microclimatic variation to restrict sporophyte production, further research should be conducted to determine what environmental factors may be driving this pattern and at what threshold sporophyte production is inhibited. Classic ecological approaches, including common-garden and manipulative experiments, can be used to directly test the role of microclimate. These are critical questions to address, because changes in the Earth's climate may make many areas unsuitable for these gametophyte-only

populations and may also limit sporophyte production, which would compromise species' ability to colonize new habitats if they have small populations and/or gametophytes that lack gemmae. For those fern species that appear to have lost the ability to produce sporophytes entirely, quantitative trait loci or association genetic studies may be of use in determining what genes code for the production of sporophytes and which genes have been lost in these species.

The first account in which independent fern gametophytes were formally recognized came with the description of *Vittaria appalachiana* by Wagner and Sharp (1963), which graced the cover of *Science*. This was followed soon after by Farrar's (1967) first publication of four independent gametophytes in eastern North America, making 2017 the fiftieth anniversary of this fundamental work. Since then, it is clear that, although we have made great strides in cataloging and understanding these species around the world, there is still much to learn. It is our hope that this review will serve as a primer for future studies to both identify new species with independent gametophytes and quantify the conditions that prohibit their sporophytes from forming.

Acknowledgments

We thank Simon Parsons for contributing the illustrations of fern gametophytes (figs. 3–6), as well as James Watkins Jr. and Aaron Duffy for contributing photographs in figure 1. We would also like to thank all of those who supplied information via personal communication, including Dr. Donald Farrar, who pointed us in the direction of unpublished occurrences and has contributed significantly to the study of independent fern gametophytes. We are also thankful for the helpful comments of the two anonymous reviewers, as well as Dr. Walter Judd, who supplied comments on the earliest version of the draft.

Literature Cited

- Allen CM 1975 *Trichomanes petersii* in Louisiana. *Am J Bot* 65:97–98.
- Allen DE 1969 The Victorian fern craze: a history of pteridomania. Huthinson, London.
- Atkinson LR, AG Stokey 1964 Comparative morphology of the gametophyte of homosporous ferns. *Phytomorphology* 14:51–70.
- Barcelona JF, NE Dolotina, GS Madroñero, WG Granert, DD Sopot 2006 The ferns and fern allies of the karst forests of Bohol Island, Philippines. *Am Fern J* 96:1–20.
- Beatty GE, J Provan 2010 Refugial persistence and postglacial recolonization of North America by the cold tolerant herbaceous plant *Orthilia secunda*. *Mol Ecol* 19:5009–5021.
- Bierhorst DW 1971 Morphology of vascular plants. Macmillan, New York.
- Bierzuchudek P, V Eckhart 1988 Spatial segregation of the sexes of dioecious plants. *Am Nat* 132:34–43.
- Bower FO 1888 On some normal and abnormal developments of the oophyte in *Trichomanes*. *Ann Bot* 1:269–305.
- Bray J 1996 Microhabitat diversity of *Trichomanes petersii* in Arkansas. *Am Fern J* 86:125–126.
- Caponetti J, M Whitten, M Beck 1982 Axenic culture and induction of callus and sporophytes of the Appalachian *Vittaria* gametophyte. *Am Fern J* 72:36–40.
- Chambers SM, NC Emery 2016 Population differentiation and countergradient variation throughout the geographic range in the fern gametophyte *Vittaria appalachiana*. *Am J Bot* 103:86–98.
- Chen CW, YM Huang, LY Kuo, YM Chang, YC Liu, WL Chiou 2013a A new vittarioid fern species, *Haplopteris heterophylla* (Pteridaceae). *Syst Bot* 38:901–909.
- Chen CW, YM Huang, LY Kuo, QD Nguyen, HT Luu, JR Callado, DR Farrar, WL Chiou 2013b *trnL-F* is a powerful marker for DNA identification of field vittarioid gametophytes (Pteridaceae). *Ann Bot* 111:663–673.
- Chiou WL, DR Farrar 1997 Comparative gametophyte morphology of selected species of the family Polypodiaceae. *Am Fern J* 87:77–86.
- Chiou WL, DR Farrar, TA Ranker 1998 Gametophyte morphology and reproductive biology in *Elaphoglossum*. *Can J Bot* 76:1967–1977.
- Christenhusz M, XC Zhang, H Schneider 2011 A linear sequence of extant families and genera of lycophytes and ferns. *Phytotaxa* 19:7–54.

- Cronin TM, DA Willard, HJ Dowsett, SE Ishman, TR Holtz Jr 1994 The Yorktown Formation of Virginia: implications for late Pliocene climate and sea level history. *Geol Soc Am Abstr Program* 25: A334.
- Dassler C, D Farrar 1997 Significance of form in fern gametophytes: clonal, gemmiferous gametophytes of *Callistopteris baueriana* (Hymenophyllaceae). *Int J Plant Sci* 158:622–639.
- 2001 Significance of gametophyte form in long-distance colonization by tropical, epiphytic ferns. *Brittonia* 53:352–369.
- Davis M, R Shaw 2001 Range shifts and adaptive responses to quaternary climate change. *Science* 292:673–679.
- Dubuisson JY, H Schneider, S Hennequin 2009 Epiphytism in ferns: diversity and history. *C R Biol* 332:120–128.
- Duffy A, M Stensvold, D Farrar 2015 Independent gametophytes of *Hymenophyllum wrightii* in North America: not as rare as we thought. *Am J Bot* 105:45–55.
- Ebihara A, D Farrar, M Ito 2008 The sporophyte-less filmy fern of eastern North America *Trichomanes intricatum* (Hymenophyllaceae) has the chloroplast genome of an Asian species. *Am J Bot* 95:1645–1651.
- Ebihara A, S Matsumoto, M Ito 2009 Hybridization involving independent gametophytes in the *Vandenboschia radicans* complex (Hymenophyllaceae): a new perspective on the distribution of fern hybrids. *Mol Ecol* 18:4904–4911.
- Ebihara A, J Nitta, M Ito 2010 Molecular species identification with rich floristic sampling: DNA barcoding the pteridophyte flora of Japan. *PLoS ONE* 5:e15136.
- Ebihara A, A Yamaoka, N Mizukami, A Sakoda, J Nitta, R Imaichi 2013 A survey of the fern gametophyte flora of Japan: frequent independent occurrences of noncordiform gametophytes. *Am J Bot* 100:735–743.
- Edwards L, J Ambrose, LK Kirkman, eds 2013 Coastal Plain ecoregion. Pages 347–510 in *The natural communities of Georgia*. University of Georgia Press, Athens.
- Emigh VD, DR Farrar 1977 Gemmae: a role in sexual reproduction in the fern genus *Vittaria*. *Science* 198:297–298.
- Evert RF, SE Eichorn 2013 *Raven biology of plants*. 8th ed. WH Freeman, New York.
- Farrar DR 1967 Gametophytes of four tropical fern genera reproducing independently of their sporophytes in the southern Appalachians. *Science* 155:1266–1267.
- 1971 The biology of ferns with asexually reproducing gametophytes in the eastern United States. PhD diss. University of Michigan, Ann Arbor.
- 1974 Gemmiferous fern gametophytes—Vittareaceae. *Am J Bot* 61:146–155.
- 1978 Problems in the identity and origin of the Appalachian *Vittaria* gametophyte, a sporophyteless fern of the eastern United States. *Am J Bot* 65:1–12.
- 1985 Independent fern gametophytes in the wild. *Proc R Soc Edinb B* 86:361–369.
- 1990 Species and evolution in asexually reproducing independent fern gametophytes. *Syst Bot* 15:98–111.
- 1992 *Trichomanes intricatum*: the independent *Trichomanes* gametophyte in the eastern United States. *Am Fern J* 82:68–74.
- 1993a Hymenophyllaceae. Pages 190–197 in *Flora of North American Editorial Committee*, eds. *Flora of North America: north of Mexico*. Oxford University Press, New York.
- 1993b Vittariaceae. Pages 187–189 in *Flora of North American Editorial Committee*, eds. *Flora of North America: north of Mexico*. Oxford University Press, New York.
- 1998 The tropical flora of rockhouse cliff formations in the eastern United States. *J Torrey Bot Soc* 125:91–108.
- 2006 Conservation assessment for Appalachian vittaria (*Vittaria appalachiana* Farrar & Mickel). US Department of Agriculture Forest Service, Eastern Region, Milwaukee, WI.
- Farrar DR, C Dassler, JE Watkins Jr, C Skelton 2008 Gametophyte ecology. Pages 222–256 in TA Ranker, CH Haufler, eds. *Biology and evolution of ferns and lycophytes*. Cambridge University Press, Cambridge.
- Farrar DR, PG Davison 1994 *Hymenophyllum tayloriae*: sporophyte and gametophyte together again after 58 years. *Am J Bot* 81(suppl.): 128 (abstract).
- Farrar DR, CL Johnson-Groh 1990 Subterranean sporophytic gemmae in moonwort ferns, *Botrychium* subgenus *Botrychium*. *Am J Bot* 77:1168–1175.
- Farrar DR, GP Landry 1987 *Vittaria graminifolia* in the United States, again. *Am J Bot* 74:709–710.
- Farrar DR, JT Mickel 1991 *Vittaria appalachiana*—a name for the Appalachian gametophyte. *Am Fern J* 81:69–75.
- Fernández H, AM Bertrand, R Sánchez-Tamés 1997 Gemmation in cultured gametophytes of *Osmunda regalis*. *Plant Cell Rep* 16:358–362.
- Ford D, PW Williams 2007 *Karst hydrogeology and geomorphology*. Wiley, Chichester.
- Gann GD, KN Hines, EV Grahl, SW Woodmansee, CS Smith, J Bell-Willcox 2006 Rare plant monitoring and restoration on Long Pine Key, Everglades National Park: year end report, year 3. Institute for Regional Conservation, Miami, FL.
- Gastony GJ 1977 Chromosomes of the independently reproducing Appalachian gametophyte: a new source of taxonomic evidence. *Syst Bot* 2:43–48.
- 1980 The deletion of *Vittaria graminifolia* from the flora of Florida. *Am Fern J* 70:12–14.
- Gentry AH, CH Dodson 1987 Diversity and biogeography of neotropical vascular epiphytes. *Ann Mo Bot Gard* 74:205–233.
- Goebel K 1888 Morphologische und biologische studien. II. Zur keimungsgeschichte einiger farne. *Ann Jard Bot Buitenzorg* 7:74–119.
- Groot JJ 1991 Palynological evidence for late Miocene, Pliocene and early Pleistocene climate changes in the middle U.S. Atlantic coastal plain. *Quat Sci Rev* 10:147–162.
- Hebert PDN, A Cywinska, SL Ball, JR DeWaard 2003 Biological identifications through DNA barcodes. *Proc R Soc B* 270:313–321.
- Holtum RE 1938 The ecology of tropical pteridophytes. Pages 421–422 in F Verdoorn, ed. *Manual of pteridology*. M Nijhoff, The Hague.
- Jansen E, J Overpeck, KR Briffa, J-C Duplessy, F Joos, V Masson-Delmotte, D Olago, et al 2007 Palaeoclimate. Pages 435–497 in S Solomon, D Qin, M Manning, Z Chen, M Marquis, KB Averyt, M Tignor, HL Miller, eds. *Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge.
- Johns RJ, PJ Edwards 1991 Vegetative reproduction in pteridophytes. *Curtis's Bot Mag* 8:109–112.
- Kasselmann C 2010 *Aquarienpflanzen: 450 Arten im Porträt*. Ulmer, Stuttgart.
- Ke TJ, X Fuwu, W Faguo, Z Xianchung, M Kato, D Barrington 2013 Lomariopsidaceae. Pages 725–726 in ZY Wu, PH Raven, DY Hong, eds. *Flora of China*. Science, Beijing.
- Kimmerer RW, CC Young 1995 The role of slugs in dispersal of the asexual propagules of *Dicranum flagellare*. *Bryologist* 98:149–153.
- Knier N, S Fischer, F Grewe, M Polsakiewicz, V Knoop 2015 Horsetails are the sister group to all other monilophytes and Marattiales are sister to leptosporangiate ferns. *Mol Phylogenet Evol* 90:140–149.
- Knight H, MR Knight 2001 Abiotic stress signalling pathways: specificity and cross-talk. *Trends Plant Sci* 6:262–267.
- Kobza RM, JC Trexler, WF Loftus, SA Perry 2004 Community structure of fishes inhabiting aquatic refuges in a threatened Karst wetland and its implications for ecosystem management. *Biol Conserv* 116:153–165.

- Krippel Y 2001 Aire de répartition et statut de *Trichomanes speciosum* Willd. (Hymenophyllaceae) au Luxembourg. *Bull Soc Nat Luxem* 102:3–13.
- Krukowski M, K Swierkosz 2004 Discovery of the gametophytes of *Trichomanes speciosum* (Hymenophyllaceae: Pteridophyta) in Poland and its biogeographical importance. *Fern Gaz* 17:79–84.
- Kuo LY, CW Chen, W Shinohara, A Ebihara, H Kudoh, H Sato, YM Huang, WL Chiou Forthcoming Not only in the temperate zone: independent gametophytes of two vittarioid ferns (Pteridaceae, Polypodiales) in East Asian subtropics. *J Plant Res*.
- Kuo LY, FW Li, WL Chiou, CN Wang 2011 First insights into fern matK phylogeny. *Mol Phylogenet Evol* 59:556–566.
- Li FW, BC Tan, V Buchbender, RC Moran, G Rouhan, CN Wang, D Quandt 2009 Identifying a mysterious aquatic fern gametophyte. *Plant Syst Evol* 281:77–86.
- Longton R, RM Schuster 1983 Reproductive biology. Pages 386–462 in RM Schuster, ed. *New manual of bryology*. Hattori Botanical Laboratory, Nichinan, Japan.
- Loope LL, TW Giambelluca 1998 Vulnerability of island tropical montane cloud forests to climate change, with special reference to East Maui, Hawaii. *Clim Change* 39:503–517.
- Lu JM, N Zhang, XY Du, J Wen, DZ Li 2015 Chloroplast phylogenomics resolves key relationships in ferns. *J Syst Evol* 53:448–457.
- Magrini S, A Scoppola 2012 First results from conservation studies of chlorophyllous spores of the Royal fern (*Osmunda regalis*, Osmundaceae). *Cryobiology* 64:65–69.
- McVeigh I 1937 Vegetative reproduction of the fern sporophyte. *Bot Rev* 3:457–497.
- Mickel J, AR Smith 2004 The pteridophytes of Mexico. New York Botanical Garden, Bronx.
- Miller DS, IR Duddy 1989 Early cretaceous uplift and erosion of the northern Appalachian basin, New York, based on apatite fission track analysis. *Earth Planet Sci Lett* 93:35–49.
- Mottier DM 1927 Behavior of certain fern prothallia under prolonged cultivation. *Bot Gaz* 83:244–266.
- Mynssen CM, A Vasco, RC Moran, LS Sylvestre, G Rouhan 2016 Desmophlebiaceae and *Desmophlebium*: a new family and genus of Eupolypod II ferns. *Taxon* 65:19–34.
- Nauman CE 1986 *Trichomanes* in Florida. *Am Fern J* 76:179–183.
- Nayar BK, S Kaur 1971 Gametophytes of homosporous ferns. *Bot Rev* 37:295–396.
- Nitta JH, J-Y Meyer, R Taputuarai, CC David Forthcoming Life cycle matters: DNA barcoding reveals contrasting community structure between fern sporophytes and gametophytes. *Ecol Monographs*.
- Page CN 2002 Ecological strategies in fern evolution: a neopteridological overview. *Rev Palaeobot Palynol* 19:1–33.
- Persson H 1958 The genus *Takakia* found in North America. *Bryologist* 61:359–361.
- Pinson JB, E Schuettpelz 2016 Unraveling the origin of the Appalachian gametophyte, *Vittaria appalachiana*. *Am J Bot* 103:668–676.
- Pittermann J, CB Brodersen, J Watkins Jr 2013 The physiological resilience of fern sporophytes and gametophytes: advances in water relations offer new insights into an old lineage. *Front Plant Sci* 4:285.
- Possley J, J Maschinski, S Hodges, E Magnaghi, D Powell, K Weclawska, S Wright, V Pence 2013 Year 11 report: biological monitoring for plant conservation in Miami-Dade County natural areas. Miami-Dade County Resolution R-808-07. Fairchild Tropical Botanic Garden, Miami, FL.
- Raine CA, DR Farrar, E Sheffield 1991 A new *Hymenophyllum* species in the Appalachians represented by independent gametophyte colonies. *Am Fern J* 81:109–118.
- Rasbach H, K Rasbach, C Jérôme 1993 Über das Vorkommen des Hautfarns *Trichomanes speciosum* (Hymenophyllaceae) in den Vogesen (Frankreich) und dem benachbarten Deutschland. *Carolinaea* 51:51–52.
- Ratcliffe DA, HJB Birks, HH Birks 1993 The ecology and conservation of the Killarney fern *Trichomanes speciosum* Willd. in Britain and Ireland. *Biol Conserv* 66:231–347.
- Reece JB, LA Urry, MI Cain, SA Wasserman, PV Minorsky, RB Jackson 2014 *Campbell biology*. 10th ed. Benjamin Cummings, Menlo Park, CA.
- Rothfels CJ, FW Li, EM Sigel, L Huiet, A Larsson, DO Burge, M Ruhsam, et al 2015 The evolutionary history of ferns inferred from 25 low-copy nuclear genes. *Am J Bot* 102:1089–1107.
- Rothfels CJ, MA Sundue, LY Kuo, A Larsson, M Kato, E Schuettpelz, KM Pryer 2012 A revised family-level classification for eupolypod II ferns (Polypodiidae: Polypodiales). *Taxon* 61:515–533.
- Rudolphi J 2009 Ant-mediated dispersal of asexual moss propagules. *Bryologist* 112:73–79.
- Rumsey FJ, JA Barrett, M Gibby, SJ Russell, JC Vogel 2005 Reproductive strategies and population structure in the endangered pteridophyte *Trichomanes speciosum* (Hymenophyllaceae: Pteridophyta). *Fern Gazette* 17:205–215.
- Rumsey FJ, AC Jermy, E Sheffield 1998a The independent gametophytic stage of *Trichomanes speciosum* Willd. (Hymenophyllaceae), the Killarney fern and its distribution in the British Isles. *Watsonia* 22:1–19.
- Rumsey FJ, SJ Russell, J Ji, JA Barrett, M Gibby 1996 Genetic variation in the endangered filmy fern *Trichomanes speciosum* Willd. Pages 161–165 in JM Camus, M Gibby, RJ Johns, eds. *Pteridology in perspective*. Royal Botanic Gardens, Kew.
- Rumsey FJ, E Sheffield, DR Farrar 1990 British filmy-fern gametophytes. *Pteridologist* 2:40–42.
- Rumsey FJ, JC Vogel, SJ Russell, JA Barrett, M Gibby 1998b Climate, colonization and celibacy: population structure in central European *Trichomanes speciosum* (Pteridophyta). *Plant Biol* 111:481–489.
- 1999 Population structure and conservation biology of the endangered fern *Trichomanes speciosum* Willd. (Hymenophyllaceae) at its northern distributional limit. *Biol J Linn Soc* 66:333–344.
- Sato T, A Sakai 1981 Cold tolerance of gametophytes and sporophytes of some cool temperate ferns native to Hokkaido. *Can J Bot* 59:604–608.
- Schuster RM 1983 Phytogeography of the Bryophyta. Pages 463–626 in RM Schuster, ed. *New manual of bryology*. Hattori Botanical Laboratory, Nichinan.
- 1992 On *Megaceros aenigmaticus* Schust. *Bryologist* 95:305–315.
- Sessa EB, WL Testo, JE Watkins 2016 On the widespread capacity for, and functional significance of, extreme inbreeding in ferns. *New Phytol* 211:1108–1119.
- Sinclair BJ, LV Ferguson, G Salehipour-Shirazi, HA MacMillan 2013 Cross-tolerance and cross-talk in the cold: relating low temperatures to desiccation and immune stress in insects. *Integr Comp Biol* 53:545–556.
- Smith AR 1993 Grammitidaceae. Pages 309–311 in *Flora of North America* Editorial Committee, eds. *Flora of North America: north of Mexico*. Oxford University Press, New York.
- Smith AR, JT Mickel 1977 Chromosome counts for Mexican ferns. *Brittonia* 29:391–398.
- Smith AR, KM Pryer, E Schuettpelz, P Korall, H Schneider, PG Wolf 2006 A classification of extant ferns. *Taxon* 55:705–301.
- Stark LR, DN McLetchie 2006 Gender-specific heat-shock tolerance of hydrated leaves in the desert moss *Syntrichia caninervis*. *Physiol Plant* 126:187–195.
- Stark LR, DN McLetchie, BD Mishler 2005 Sex expression, plant size, and spatial segregation of the sexes across a stress gradient in the desert moss *Syntrichia caninervis*. *Bryologist* 108:183–193.
- Stevens SM, NC Emery 2015 Dispersal limitation and population differentiation in performance beyond a northern range limit in an asexually reproducing fern. *Divers Distrib* 21:1242–1253.
- Stokey AG, LR Atkinson 1958 The gametophyte of the Grammitidaceae. *Phytomorphology* 8:391–403.

- Straub SC, M Parks, K Weitemier, M Fishbein, RC Cronn, A Liston 2012 Navigating the tip of the genomic iceberg: next-generation sequencing for plant systematics. *Am J Bot* 99:349–364.
- Taylor TM 1967 *Mecodium wrightii* in British Columbia and Alaska. *Am Fern J* 57:1–6.
- Testo WL, JE Watkins Jr 2011 Comparative development and gametophyte morphology of the hart's-tongue fern, *Asplenium scolopendrium* L. *J Torrey Bot Soc* 138:400–408.
- Tryon AF, B Lugardon 1991 Spores of the Pteridophyta: surface, wall structure, and diversity based on electron microscope studies. Springer, New York.
- Vogel JC, S Jeßen, M Gibby, AC Jerymy, L Ellis 1993 Gametophytes of *Trichomanes speciosum* (Hymenophyllaceae: Pteridophyta) in central Europe. *Fern Gaz* 14:227–232.
- Wagner WH, AJ Sharp 1963 A remarkably reduced vascular plant in the United States. *Science* 142:1483–1484.
- Walp RL, GR Proctor 1946 Long-lived fern prothallia. *Am Fern J* 36:109–112.
- Watkins JE, CL Cardelús 2012 Ferns in an angiosperm world: Cretaceous radiation into the epiphytic niche and diversification on the forest floor. *Int J Plant Sci* 173:695–710.
- Watkins JE, DR Farrar 2002 A new name for an old fern from north Alabama. *Am Fern J* 92:171–178.
- 2005 Origin and taxonomic affinities of *Thelypteris* (subgen. *Stegnogramma*) *burksiorum* (Thelypteridaceae). *Brittonia* 57:183–201.
- Watkins JE, MK Mack, SS Mulkey 2007a Gametophyte ecology and demography of epiphytic and terrestrial tropical ferns. *Am J Bot* 94:701–708.
- Watkins JE, MC Mack, TR Sinclair, SS Mulkey 2007b Ecological and evolutionary consequences of desiccation tolerance in tropical fern gametophytes. *New Phytol* 176:708–717.
- Weakley AS, LD Estes, KG Mathews, CT Witsell, K Gandhi, A Ebihara 2011 New combinations, rank changes, and nomenclatural and taxonomic comments in the vascular flora of the southeastern United States. *J Bot Res Inst Tex* 5:437–455.
- Wherry E 1964 The southern fern guide, southeastern and south-midland United States. Doubleday, Garden City, NY.
- Whittingham S 2009 The Victorian fern craze. Shire, Oxford.
- Wolle F 1887 Fresh water algae of North America. Vol 1. Comenius, Bethlehem, PA.
- Zhang LB, L Zhang 2015 Didymochlaenaceae: a new fern family of eupolypods I (Polypodiales). *Taxon* 64:27–38.