

Review

A current perspective on apomixis in ferns

Amanda L. Grusz*

Department of Biology, University of Minnesota Duluth, 1035 Kirby Drive, Swenson Science Building 207, Duluth, MN 55803

*Author for correspondence. E-mail: algrusz@d.umn.edu

Received 29 September 2016; Accepted 1 November 2016; Article first published online xx Month 2016

Abstract This review provides a synopsis of apogamous reproduction in ferns and highlights important progress made in recent studies of fern apomixis. First, a summary of the apomictic fern life cycle is provided, distinguishing between two pathways to diploid spore production that have been documented in apomictic ferns (premeiotic endomitosis and meiotic first division restitution) and briefly discussing the evolutionary implications of each. Next, recent trends in fern apomixis research are discussed, exposing a shift in focus from the observation and characterization of apomixis in ferns to more integrated studies of the evolutionary and ecological implications of this reproductive mode. Peer-reviewed contributions from the past decade are then summarized, spanning the identification of new apomictic lineages through to the developmental, phylogenetic, and population genetic insights that have been made in studies of fern apomixis during that time. Gaps in our understanding are also discussed, including the extent and implications of recombinant apomixis in ferns, the possible reversibility of reproductive mode (from apomictic to sexual) in ferns, and the genomic causes and consequences of apomixis in seed free vascular plants. To conclude, future directions for fern apomixis research are proposed in the context of modern technological advances and recent insights from studies of apomixis in other groups.

Key words: apogamy, evolution of sex, meiosis, pteridophytes.

“...apomixis is an escape from sterility, but it is an escape into a blind alley of evolution...”

—Darlington, 1939

A renaissance is underway in fern biology, propelled, in part, by technological advances being embraced by a growing research community. In particular, modern, cost-effective tools are enabling new inquiries into the unique fern life cycle, which alternates between independent, free-living sporophyte and gametophyte generations (Haufler et al., 2016). Among the many aspects of fern reproduction that are being examined in light of new approaches—and an improved understanding of fern evolution as a whole—is their propensity for apogamous reproduction (i.e., apomixis). This form of asexual reproduction (by spore or seed) is more common in ferns than in any other group of vascular plants. Nearly 10% of the ferns for which reproductive mode has been determined exhibit apomixis (Walker, 1985; Liu et al., 2012), compared to less than 1% in flowering plants (Bicknell & Koltunow, 2004; Becks & Alavi, 2015). Given that data on reproductive mode are lacking for the vast majority of ferns, this relative contribution of apomicts to fern diversity may well be an underestimate (Liu et al., 2012). The elevated frequency and distinctive meiotic characteristics of apomixis in ferns, which are elaborated upon below, suggest that this form of asexuality may play a more dynamic role in fern evolution than previously considered.

Since its discovery in ferns by Farlow (1874), major advances have been made in the study of apomixis in ferns, providing a strong foundation for contemporary investigations (e.g., Döpp, 1932; Manton, 1950; Mehra & Singh, 1957; Braithwaite, 1964; Klekowski, 1973; Walker, 1985). And while the long-term evolutionary implications of this reproductive strategy in ferns are just beginning to be considered from an experimental and/or theoretical perspective, increasing scientific contributions offer cause for optimism. This review, in recognition of the Next Generation Pteridology Symposium (Washington, DC, 2015), reflects on recent progress made in the study of fern apomixis. It highlights broad trends in the field, revealing an underlying shift in research focus from studies identifying and characterizing apomixis in ferns to those increasingly centered on the ecological and evolutionary implications of apogamous reproduction in the group. This progression is, however, ongoing and many significant gaps remain in our understanding of apomixis in ferns, especially compared to recent advances in related groups.

Here, I review essential aspects of apomixis in ferns. I begin by discussing key features of the apomictic fern life cycle. I then summarize important contributions since the last meeting of the international pteridological community, more than a decade ago (International Pteridophyte Symposium, Edinburgh, UK, 2004); these span pteridological topics from growth and development, to reticulate evolution, and the long-term implications of apomixis in ferns. Finally, I touch on notable gaps in our understanding of this reproductive

approach in ferns, pointing to recent progress in apomixis research within angiosperms. With these gaps in mind, I briefly address new tools and techniques that may be useful for further exploration of fern apomixis and suggest directions for future study.

What Is Apomixis in Ferns?

Most broadly defined as, simply, asexual reproduction (i.e., “apo” = without, “misis” = mixing/sex), apomixis encompasses both vegetative reproduction and asexual reproduction through the alternation of generations (including apospory, apogamy, and parthenogenesis). Here, I focus exclusively on apogamous alternation between sporophyte and gametophyte generations (Fig. 1A). In ferns, this involves two distinct modifications to the typical, sexual life cycle: the production of unreduced diplospores via meiosis (diplospory), and the apogamous development of a new sporophyte from somatic gametophyte tissue without the fusion of sperm and egg (apogamy; Fig. 1A). First, sporogenesis takes place within the fern sporangium, where spores are generated through meiosis. In sexual lineages, meiosis produces haploid cells (spores), each of which contains half the parental number of chromosomes. Apomictic ferns, by contrast, follow one of two alternative spore-generating pathways to yield chromosomally unreduced diplospores: either premeiotic endomitosis (PE; also referred to as Döpp-Manton sporogenesis; Steil, 1919; Döpp, 1932; Manton, 1950; Fig. 1B) or meiotic first division restitution (MFDR; also known as Braithwaite sporogenesis; Braithwaite, 1964; Walker, 1985; Fig. 1B). These diplospores, each having (or exceeding) one full chromosome complement, then germinate. The resulting prothallia are capable of generating new sporophytes from somatic cells, which are

usually located near the apical notch. Occasionally archegonia are observed, but they are typically abortive. Diploid (or polyploid) gametophytes have been known to make functional antheridia, but the viability of sperm contained therein is largely dependent upon the pathway to diplospory undertaken in the parent plant (i.e., PE or MFDR; the latter generally producing lethargic and/or inviable sperm).

Premeiotic endomitosis (PE) and meiotic first division restitution (MFDR) involve different modifications to standard sporogenesis, each with its own evolutionary implications. Both PE and MFDR usually begin with three successive mitotic divisions originating from the archesporial cell (Fig. 1B). At this stage, apomicts undergoing PE attempt a fourth and final mitotic division, but it is incomplete. From this, a single spore mother cell results, with twice the parental chromosome complement (Fig. 1B). This spore mother cell proceeds through an otherwise normal, reductive meiosis and ultimately yields half the number of spores per sporangium compared to its sexual counterparts (e.g., 32 vs. 64 spores per sporangium, respectively)—each with the same number of chromosomes per nucleus as the parent sporophyte ($n = 2n$; Döpp, 1932; Manton, 1950; Fig. 1A). Unlike PE, the MFDR pathway undergoes a normal (i.e., complete) cell division in the final mitosis preceding meiosis (Fig. 1B). Instead, the first cellular division of meiosis is incomplete; chromosomes fail to pair (only univalents are observed) and a restitution nucleus forms prior to entering meiosis II (Braithwaite, 1964; Walker, 1985).

While both PE and MFDR occur in ferns, few studies go so far as to distinguish between these two approaches to sporogenesis. Based on our current understanding, the former is far more common across ferns (Walker, 1985). The higher incidence of PE is probably attributable to genome stability imparted by high fidelity in bivalent chromosome

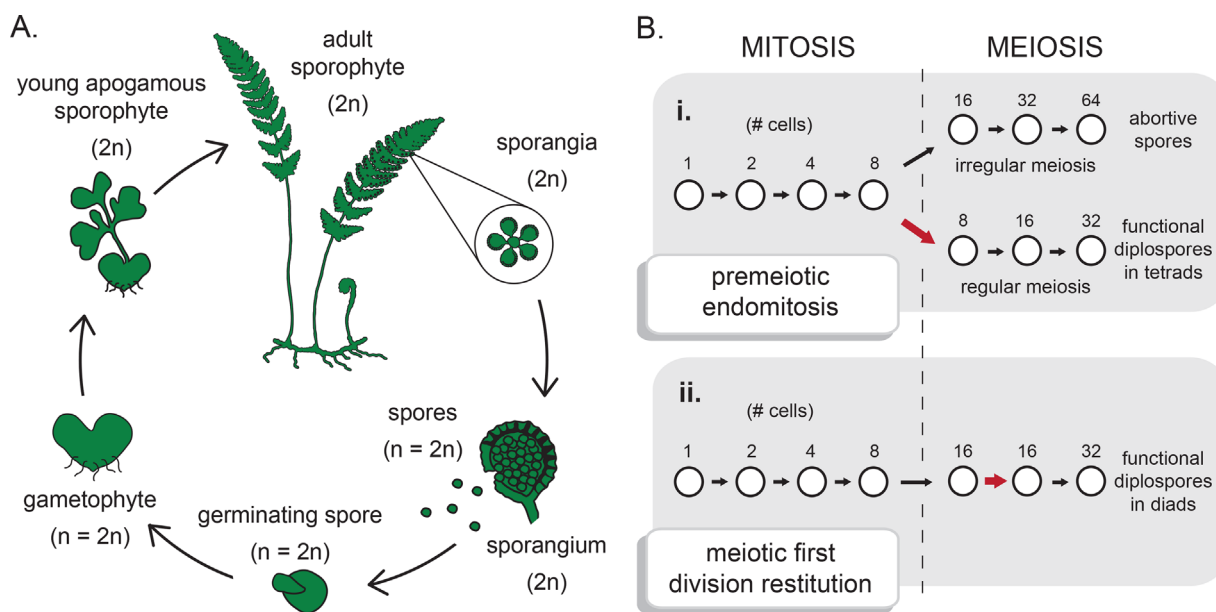


Fig. 1. Apogamous fern life cycle. **A**, Apogamous alternation of generations in ferns (n = haploid, i.e., gametophytic chromosome number; $2n$ = diploid, i.e., sporophytic chromosome number). **B**, Sporogenesis in apogamous ferns (red arrows indicate incomplete cell division): **i**, premeiotic endomitosis (Döpp-Manton sporogenesis); **ii**, meiotic first division restitution (Braithwaite sporogenesis).



pairing (Gastony & Windham, 1989) following premeiotic genome duplication. Meanwhile, MFDR is less frequently observed in ferns, most likely owing to difficulties associated with chromosome migration during meiosis, particularly in odd-numbered polyploids.

As with ferns, the distribution of the PE versus MFDR pathways is similarly inconsistent across the broader eukaryotic tree of life. Both are important avenues for producing diploid meicytes in eukaryotes (Fig. 1B), but outside of ferns PE is most commonly associated with parthenogenetic animals (Stenberg & Saura, 2009). By contrast, MFDR is more typical among flowering plants (e.g., in *Antennaria*, *Hieracium*, and *Taraxacum*; Gustafsson, 1946, 1947; Ozias-Akins & van Dijk, 2007). Parallels between the non-reductive meiotic pathways in apomictic ferns as compared to animals and angiosperms offer exciting opportunities for investigating the evolutionary implications of either apomictic pathway across eukaryotes, through a pteridological lens.

Apomixis in Ferns, Since 2004

Since the 2004 International Pteridophyte Symposium (Edinburgh, UK), more than 75 peer-reviewed articles have been published on apomixis in ferns. A *Web of Science* search for associated papers issued since that meeting (restricted to search terms: '(APOG* OR APOM*) AND (FERN* OR PTERIDOPHYT*)') returned an increase in annual contributions spanning a variety of topics in fern reproductive biology (Figs. 2A, 2B). It further revealed a transition over time from studies focused heavily on the identification (spore counts, sporophyte development) and characterization (spore measurements and morphology, cytogenetics, gametophyte biology, physiology, taxonomy) of apomixis in ferns, to integrated explorations of the evolution (population dynamics, biogeography, reticulate origins, phylogenetics) and ecology of apomictic lineages (Fig. 2A). Together, these studies have improved our grasp of apomixis in ferns; they also highlight that the designation of reproductive mode in ferns is ongoing, and little is known about the broader implications of apomixis in this major lineage of vascular plants. In the following section, I review many of these recent contributions and discuss noteworthy findings in fern apomixis research over the last decade.

Newly discovered apomictic lineages

New accounts of apomixis in ferns have, with the exception of Hypodematiaceae (Wang et al., 2014), been mostly confined to the largest leptosporangiate families: Dryopteridaceae (Lu et al., 2006; Xiang et al., 2006; Guo & Liu, 2013), Polypodiaceae (Wang et al., 2011), Pteridaceae (Huang et al., 2006, 2011; Chao et al., 2010; Martinez, 2010), and Aspleniaceae (Dyer et al., 2012). These new records have frequently combined spore studies with chromosome counts to distinguish reproductive mode, in some cases also documenting the apogamous development of offspring sporophytes. Other studies have focused exclusively on observations of gametangial development (or lack thereof) and the apogamous growth of sporophytes from somatic gametophyte tissue (e.g., in *Asplenium*; Regalado Gabancho & Gabriel y Galán, 2010). Together, these contributions underline the continued value

Publications

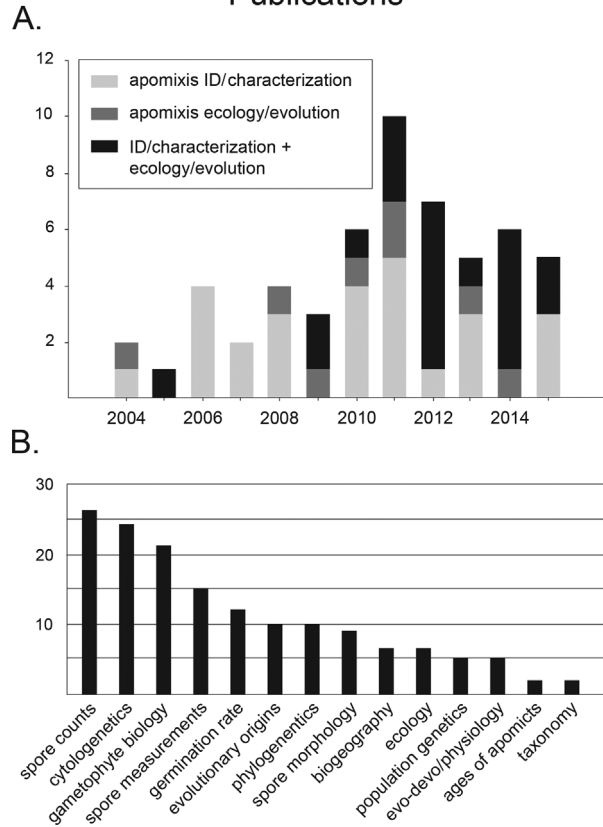


Fig. 2. Publications on fern apomixis, 2004–2015. **A**, The number of studies on fern apomixis published annually since 2004: studies focused exclusively on identification and characterization of apomixis (light grey), studies focused more broadly on ecology and evolution of apomixis (dark grey), and those focused on identification and characterization as well as ecology and evolution of apomixis (black). **B**, The number of studies on fern apomixis published since 2004 related to particular topics in fern biology based on a *Web of Science* search restricted to the years 2004–2015 and terms '(APOG* OR APOM*) AND (FERN* OR PTERIDOPHYT*)'.

of basic microscopy for illuminating macro- and micro-morphological characteristics of apomictic taxa, and illustrate that cytological techniques remain critical for determining chromosome number and chromosome pairing regimes in cryptic apomictic groups.

Along with the discovery of previously unrecognized apomictic lineages have come new observations and expanded sampling within already recognized apomictic fern taxa. For example, one trend that has emerged is the tendency for apomictic individuals to attempt multiple meiotic pathways (Sigel et al., 2011; Hernández et al., 2015; Ekt & Koutecky, 2016). In their elegant follow-up to Manton's work in *Dryopteris* (1950), Ekt & Koutecky (2016) observed variously abortive, haploid, and diploid spores, among and even within individual sporangia on a single plant. Using flow cytometry, they also documented viable polyhaploid (2.5x) gametophytes derived through apomixis from a 5x parent.

	# species	% with apomixis	# genera	% with apomixis
Saccolomataceae	18	—	1	—
Lindsaeaceae	237	4%	9	33%
Dennstaedtiaceae	122	—	10	—
Pteridaceae	1234	6%	53	28%
Dryopteridaceae	2151	3%	26	15%
Hypodematiaceae	22	13%	2	50%
Didymochlaenaceae	1	—	1	—
Nephrolepidaceae	19	—	1	—
Lomariopsidaceae	69	—	4	—
Tectariaceae	250	<1%	7	14%
Oleandraceae	15	—	1	—
Davalliaceae	65	3%	1	100%
Polypodiaceae	1620	<1%	65	8%
Cysopteridaceae	37	—	3	—
Rhachidosoraceae	8	—	1	—
Diplaziopsidaceae	4	—	2	—
Desmophlebiaceae	2	—	1	—
Hemidictyaceae	1	—	1	—
Aspleniaceae	730	3%	2	100%
Thelypteridaceae	1004	<1%	30	13%
Woodsiaceae	39	—	1	—
Athyriaceae	650	2%	3	100%
Blechnaceae	265	<1%	24	4%
Onocleaceae	5	20%	4	25%

Fig. 3. Distribution of apomixis across the leptosporangiate fern phylogeny. Data on reproductive mode, including number of species per family, % of apomictic species per family, number of genera per family, and % of genera containing one or more apomictic species per family. Summarized from Liu et al. (2012); <http://darwintree.cn/special/topic/fern/home.jsp>; family-level classification and estimated number of species/genera per family based on PPG I (2016).

Many other studies of apomictic ferns have likewise adopted flow cytometry (standardized with known chromosome counts) for genome size estimation and ploidy-level determination (Schneller & Krattinger, 2010; Ootsuki et al., 2011; Chao et al., 2012; Dyer et al., 2013; Chen et al., 2014; Hori et al., 2014, 2015). And though flow cytometry has greatly improved our understanding of these polyploid apomictic groups, recent work by Dyer et al. (2013) cautions that inferring ploidy-level using correlations between nuclear DNA content and spore length should be restricted to intraspecific comparisons. **These results further emphasize that empirical observations of chromosome numbers remain critical for establishing species-level correlations with genome size estimates based on flow cytometry and/or measurements of spore diameter.**

Advancing developmental perspectives

Investigations into the gametophytic and sporophytic development of apomictic ferns are basic to our understanding of this reproductive mode. Over the past decade, multiple

studies have characterized reproductive development in apomictic lineages (e.g., *Cyrtomium*, Soare, 2008; *Dryopteris*, Soare et al., 2010; *Argyrochosma*, Gabriel y Galán, 2011; *Asplenium*, Dyer et al., 2012; *Polystichum*, Migliaro & Gabriel y Galán, 2012; and *Cyrtogonellum*, Guo & Liu, 2013). Others have taken experimental approaches, targeting chemical cues and differential gene expression that accompany apogamous reproduction. For example, Menéndez et al. (2006) explored the stimulatory role played by auxins and gibberellins in apogamous embryo development and found that naphthalene-acetic acid (NNA) and gibberellic acid (GA_3) promote apogamy in *Dryopteris affinis* sp. *affinis*. More recently, Cordle et al. (2012) built upon extensive previous studies of induced apogamy in ferns by examining gene expression in connection with induced apogamy. Their analyses showed that stress- and metabolism-related genes experience up-regulation during commitment to apogamy in *Ceratopteris richardii* when compared to *Pteridium aquilinum*. Complementing recent advances in fern physiology as a whole (regardless of reproductive mode; e.g., Pittermann et al., 2013, 2015),

observational and experimental studies like these continue to provide critical insight into the biology of apomictic, seed-free vascular plants.

Recent phylogenetic insights

Following precipitous decreases in costs of DNA sequencing, molecular sequence data are being used more frequently to examine aspects of apomixis in ferns from a phylogenetic context. Phylogenetic inferences of species' relationships combined with current surveys of reproductive mode in ferns indicate that apomixis is widespread in some clades, but is otherwise asymmetrically distributed across the fern phylogeny (Liu et al., 2012). Within leptosporangiate ferns, nearly half of the families contain one or more apomictic taxa, apomixis appearing more frequently in the largest among them (Fig. 3; based on data summarized from Liu et al., 2012). Recent analyses by Liu et al. (2012) reveal that in ferns as a whole the frequency of apomixis is significantly correlated with species diversity; nevertheless, a further comparative probe into the polystichoid ferns did not expose any significant relationship between apomixis and diversification rates (Liu et al., 2012). These results are in agreement with recent studies showing that apomictic lineages themselves are apparently young, with initial estimates placing the ages of extant apomictic ferns in relatively recent evolutionary time (e.g., ≤ 0.4 my in *Astrolepis*, Beck et al., 2011; < 8 my for the polystichoid ferns, Liu et al., 2012; < 15 my for the crown groups of apogamous ferns in Japan, Tanaka et al., 2014).

Because few studies distinguish between MFDR and PE, it remains to be explored whether either of these pathways individually correlates with species-richness or diversification across the group. Current evidence indicates that PE is more frequently found in the most species-rich families, such as Dryopteridaceae and especially, Pteridaceae (Fig. 3). Conversely, the few examples of MFDR in ferns have been documented either in relatively small leptosporangiate families (e.g., Athyriaceae and Aspleniaceae) or less-frequently within large ones (e.g., Polypodiaceae; Fig. 3). Future in-depth surveys of reproductive mode in ferns will help to shed light on the impact of either meiotic pathway on fern evolution.

DNA sequence data have been especially useful for deconstructing reticulate relationships within apomictic complexes. Plastid-derived nucleotide sequences, along with illuminating the distribution of apomixis in ferns, have also been indispensable for placing apomictic lineages in a (presumably) maternal (Gastony & Yatskiyevych, 1992; Vogel et al., 1998; Guillon & Raquin, 2000) phylogenetic context (e.g., Liu et al., 2010; Sigel et al., 2011; Grusz et al., 2014; Li et al., 2012). Meanwhile, the development of many low-copy, nuclear DNA markers (Ishikawa et al., 2002; Schuettpelz et al., 2008; Chen et al., 2012; Rothfels et al., 2013) has dramatically streamlined the inference of bi-parental precursors to extant polyploid lineages, helping to elucidate the independent origins that often comprise apomictic polyploid taxa (e.g., in *Cheilanthes* [= *Myriopteris* Fée emend. Grusz & Windham], Grusz et al., 2009; *Crepidomanes*, Nitta et al., 2011; *Astrolepis*, Beck et al., 2012; *Pteris*, Chao et al., 2012; *Asplenium*, Dyer et al., 2012; *Dryopteris*, Ebihara et al., 2012; Hori et al., 2014; and *Davallia*, Chen et al., 2014). More recently, the adoption of genotyping-by-sequencing has also proven

powerful for exploring the evolution of widespread apomictic taxa and deciphering cryptic lineages in ferns (Wickell, 2015).

Few investigations have gone beyond untangling reticulate relationships to examine the broader evolutionary significance of apomixis in ferns. Recent studies, however, have made strides in this direction by uniting fossil, environmental, and molecular evidence (Liu et al., 2012; Tanaka et al., 2014). Tanaka et al. (2014) integrated such data to explore correlations between apomixis and abiotic factors in the ferns of Japan. Surprisingly, these authors found that the frequency of apomixis among Japanese ferns declined with increasing latitude and elevation (temperature being correlated along both gradients). Their analyses also uncovered a strong correlation between increased seasonality of precipitation and the incidence of apomixis in the ferns of Japan, thought to be driven by the Asian monsoon (Tanaka et al., 2014). Apomixis is regularly observed within lineages that experience extreme fluctuations in water availability (e.g., within Pteridaceae; Fig. 3). Given that plants from more seasonal and/or dry environments are restricted in their time for reproduction and their ability to reproduce sexually (because drought limits the motility of sperm), it is not surprising that apomixis frequently arises in geographic and/or phylogenetic groups that experience these conditions (Haufler et al., 2016).

New views on genotypic diversity

Since 2004, multiple studies have focused on genotypic diversity and its dynamic nature in apomictic ferns. Recent population surveys have used a variety of molecular tools to examine genotypic diversity in apomictic groups, including allozymes, simple sequence repeats (SSRs), inter-simple sequence repeats (ISSRs), and amplified fragment length polymorphisms (AFLPs). Their results support the claim that apomictic lineages have low levels of genotypic diversity across and, especially, within populations (e.g., in *Myriopteris*, Grusz & Pryer, 2015; and *Dryopteris*, Schneller & Krattinger, 2010; Peredo et al., 2013), particularly when compared to limited sampling of a single obligate outcrosser (i.e., *Blechnum spicant*; Peredo et al., 2013). However, extensive sampling of apomictic taxa within *Cyrtomium* (Ootsuki et al., 2011) revealed comparatively high genetic diversity in these lineages across Japan. This is speculated to have resulted from niche differentiation among existing apomictic lineages and/or the frequent formation of new clones from sexual progenitors.

Other studies have examined the heritability of fixed genotypes in apomictic ferns. Generally speaking, apomicts are assumed to produce genotypically clonal (i.e., identical) offspring (Hand & Koltunow, 2014; Verhoeven & Preite, 2014), but emerging research in ferns illustrates that these generalizations are not attributable to all apomictic groups. Following the suggestion of Klekowski (1973) and preliminary evidence from Bierhorst (1975) and Ishikawa et al. (2003), recent studies have examined the extent to which apomictic polyploid ferns are able to produce genotypically variable offspring (Ootsuki et al., 2012; Grusz, 2014). These studies find evidence that, unlike with MFDR (Fig. 4B), the genome duplication that precedes meiosis in PE apomicts can facilitate the pairing of, and recombination between, non-identical hom(oe)ologous chromosomes during meiosis (Fig. 4C).

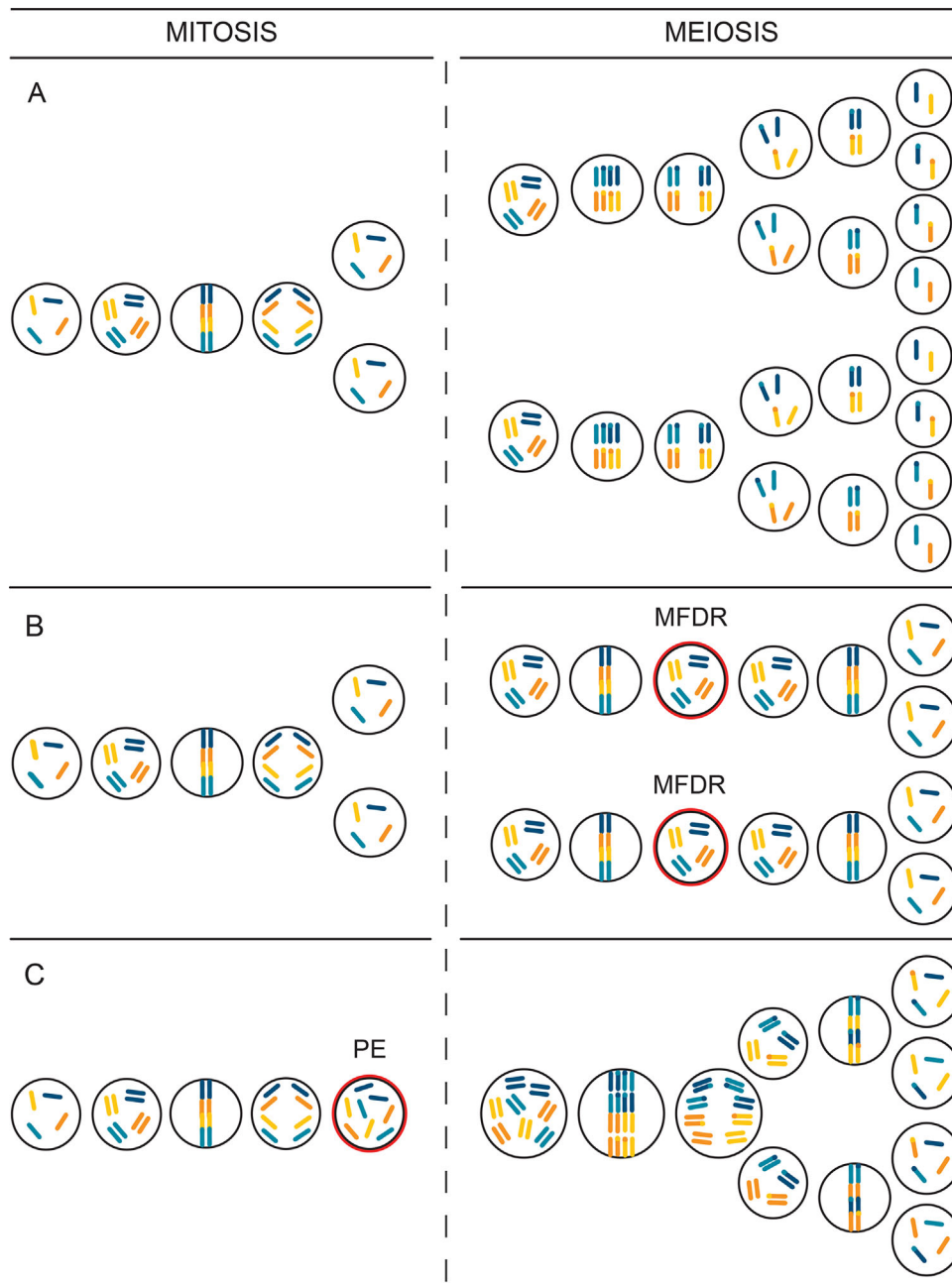


Fig. 4. Mitosis and meiosis for a diploid organism with two chromosomes. **A**, Sexual reproduction. **B**, Apomixis by meiotic first division restitution (MFDR). **C**, Apomixis by premeiotic endomitosis (PE). Homologous chromosomes indicated by similar color shades.

The retention of functional meiosis in PE apomicts has been previously hypothesized to confer an evolutionary advantage, possibly as a means of generating genotypic diversity (DeBenedictis, 1969; Klekowski, 1973). Results from Ootsuki et al. (2012) and Grusz (2014) support this postulation, that PE could bestow long-term benefits in apomictic ferns, whose evolutionary potential may more closely resemble a selfing mating system than the purely clonal apomixis with which they are often associated.

The discovery of recombinant apomixis in ferns has major consequences for interpreting mating system evolution in the

group, particularly when compared to reproductive mode in angiosperms. Asexual reproduction through seed is considered to be strictly clonal in most flowering plants. In ferns, given the evidence for genetic recombination resulting from PE (Figs. 1B, 4C), the most appropriate corollaries of clonal apomixis in angiosperms may be either apomixis by MFDR (Figs. 1B, 4B) or vegetative clonality. In some cases, gametophytic selfing in ferns (Hafler et al., 2016) could resemble clonal apomixis in angiosperms. For example, although gametophytic selfing does not yield progeny that are genetically identical to the parent sporophyte per se, it

does, in theory, result in completely homozygous offspring. In generations thereafter, continued gametophytic selfing would yield genetically identical progeny. Meanwhile, recombinant apomixis, as facilitated by PE, involves genetic mixing among parent alleles (Fig. 4C). Hence, with the added benefit of diplospory, this genetic mixing in some apomictic ferns is likely to have evolutionary consequences that resemble long-term selfing in angiosperms. Future studies examining the extent of recombinant apomixis more broadly across ferns will illuminate the degree to which this evolutionary interpretation is applicable.

Apomixis in Ferns, Beyond 2016

Bolstered by new, affordable approaches and a reinvigorated research community, pteridologists are poised to expand the study of apomixis and its evolutionary implications in ferns. Unique reproductive characteristics of ferns, including independent gametophyte and sporophyte life phases and the potential for recombinant apomixis in some lineages, offer unprecedented opportunities to explore the dynamic nature of asexuality. Below, I elaborate on possible future directions for studies of fern apomixis and touch on emerging technologies that may facilitate these endeavors.

Exploring the implications of recombinant apomixis

The discovery that apomixis via PE in ferns can generate genotypically distinct offspring is among the most exciting revelations in recent pteridological research (Ishikawa et al., 2003; Ootsuki et al., 2012; Grusz, 2014). But whether this process is widespread among apomictic ferns remains unexplored. Future studies expanding observations of this phenomenon will undoubtedly reveal additional recombinant apomictic lineages. With this in mind, it will be useful to examine whether the extent of recombination varies among lineages, and, if so, what aspects of meiosis influence such variation. For example, do recombinant processes behave similarly in apomictic autopolyploid vs. allopolyploid lineages? Is reciprocal recombination the most common result of crossing over, or does gene conversion predominate? Could this have implications for the evolution of genome size, synteny, and structure? If present, does variation in recombination rate among PE apomicts have long-term evolutionary consequences for diversification? Furthermore, what are the evolutionary implications of a mixed reproductive mode in ferns (including recombinant apomixis) as it relates to population dynamics in apomictic groups; e.g., in the context of frozen niche variation models (Vrijenhoek, 1984)? Development of molecular markers for genotyping apomictic lineages (and their offspring) will be necessary to answer many of these questions. The impending release of multiple annotated fern genomes (Li & Pryer, 2014; Sessa et al., 2014) will facilitate marker development for these surveys and provide previously unparalleled opportunities to examine recombination and its effects in ferns from a genome-wide perspective.

Evaluating irreversible evolution of apomixis in ferns

Several recent studies have proposed that apomixis in the angiosperms may be a reversible trait (Koltunow et al., 2011; Cosendai et al., 2013; Hojsgaard et al., 2014). These findings

represent a paradigm shift in the study of apomixis as it relates to organismal evolution. This reinterpretation has been implicated to explain the increased frequency of apomixis in large angiosperm clades (Hojsgaard et al., 2014), as well as the role of apomixis in rapid range expansion following reversals to sexual reproduction (Cosendai et al., 2013). While preliminary evidence from the polystichoid ferns does not reveal a significant correlation between apomixis and shifts in diversification rates (Liu et al., 2012), further examination, either more broadly among ferns or across other large clades that exhibit apomixis, may yield interesting results. It is important to keep in mind, however, that if apomixis is more frequently obligate in ferns, there may be fewer opportunities for reversals to sexuality compared with flowering plants, which are often facultatively apomictic. But the preliminary evidence from Ekrt & Koutecky (2016) indicates that there is much to learn about the extent and implications of facultative apomixis in the group. Extended surveys of apomixis across ferns, specifically distinguishing between PE and MFDR types, will be critical for large-scale testing for reversibility of reproductive mode in the group.

Investigating genome dynamics of apomixis in ferns

Contemporary studies have brought to light many exciting discoveries regarding the epigenetic regulation of apomixis in angiosperms (Verhoeven & Preite, 2014; Zappacosta et al., 2014). Recent research has revealed that apomixis genes are highly methylated in some lineages (e.g., in *Paspalum*; Podio et al., 2014) and that epigenetic modifications are involved in the regulation of embryo and endosperm development in asexual flowering plants (Hojsgaard et al., 2014). Because DNA methylation can be influenced by abiotic stressors (Rapp & Wendel, 2005; Verhoeven et al., 2010) and has been shown to be heritable in some contexts (Feng et al., 2010; Ferreira de Carvahlo et al., 2016), it is reasonable to infer that epigenetic modification could lead to the heritable expression of apomixis in response to environmental cues.

DNA methylation has also been shown to be important for the regulation and silencing of transposable elements (TEs) in some groups (Verhoeven & Preite, 2014). Proliferating TEs can cause extreme genome restructuring, but can also function as transcriptional regulators (particularly as enhancers; Xie et al., 2013), and serve as potential sources of novel genetic variation (Lisch, 2013; Belyayev, 2014). Interestingly, TE proliferation has been hypothesized to result from several large-scale, genome altering events, including polyploidy, hybridization, changes in mating system, and changes in environment (Belyayev, 2014), all of which are often intimately related to apomixis in ferns.

In ferns, virtually nothing is known about the role of epigenetic modifications or TEs in the expression of apomixis. Some authors have speculated on the potential for TE activation to influence genome size variation among apomictic polyploid hybrids (Dyer et al., 2013; but see Clark et al., 2016), and others have presented data revealing a high relative proportion of TEs in some fern lineages (Wolf et al., 2015), but little has been done to explore the relationship of these traits to asexual reproduction. Unique aspects of meiosis in apomictic ferns could provide insight into the role, if any, of TEs in apomictic reproduction across eukaryotes. Likewise, further investigations into the epigenetic regulation of apomixis in ferns will surely provide evolutionary insight into its broader

implications for fern genome dynamics, such as gene silencing that occurs in diploidized groups with high chromosome numbers (Haufler, 2002; Clark et al., 2016). By leveraging next-generation sequencing for characterizing TEs, including their distribution and abundance, we can gain a better understanding of their role in fern apomixis. Next-generation approaches will also be critical for examining methylation and its evolutionary implications in apomictic ferns.

Conclusions

Substantial progress has been made in our understanding of apomixis in ferns during the last decade. From the discovery of new apomictic lineages to broad comparative studies that explore the implications of apomixis for diversification in ferns, each contribution has helped to clarify the role of apomixis in fern evolution and explain its high incidence within these seed free vascular plants. Even so, reproductive mode is still undetermined for most ferns, and much remains to be explored regarding the origin of apomictic taxa (e.g., frequencies of autopolyploid vs. allopolyploid lineages; Gastony & Windham, 1989; Barker et al., 2016) and the significance of apomixis for fern population dynamics, colonization and biogeography, and even speciation. Traditional morphological and cytological techniques combined with new technologies—e.g., long-read next-generation sequencing for assessing parentage in apomictic polyploids (Rothfels et al., 2016), or whole genome/next-generation sequencing for exploring TE proliferation and DNA methylation in apomictic lineages—offer exciting prospects for future studies of apomixis and its evolution in ferns.

Acknowledgements

The author would like to thank the editors and two anonymous reviewers for providing valuable comments. Thanks also to my colleagues at the Smithsonian Institution and the University of Minnesota Duluth for their feedback, enthusiasm, and support, and to MD Windham and KM Pryer for cultivating my interest in the evolutionary dynamics of asexual reproduction in ferns.

References

Barker MS, Arrigo N, Baniaga AE, Li Z, Levin DA. 2016. On the relative abundance of autopolyploids and allopolyploids. *New Phytologist* 210: 391–398.

Beck BB, Allison JR, Pryer KM, Windham MD. 2012. Identifying multiple origins of polyploid taxa: A multilocus study of the hybrid cloak fern (*Astrolepis integerrima*; Pteridaceae). *American Journal of Botany* 99: 1857–1865.

Beck BB, Windham MD, Pryer KM. 2011. Do asexual polyploid lineages lead short evolutionary lives? A case study from the fern genus *Astrolepis*. *Evolution* 65: 3217–3229.

Becks L, Alavi Y. 2015. Using microevolution to explain the macroevolutionary observations for the evolution of sex. In: Serrelli E, Gontier N eds. *Macroevolution*. Switzerland: Springer. 279.

Belyayev A. 2014. Bursts of transposable elements as an evolutionary driving force. *Journal of Evolutionary Biology* 27: 2473–2584.

Bicknell RA, Koltunow AM. 2004. Understanding apomixis: Recent advances and remaining conundrums. *The Plant Cell* 16: S228–S245.

Bierhorst DW. 1975. The apogamous life cycle of *Trichomanes pinnatum*—A confirmation of Klekowski's prediction on homoeologous pairing. *American Journal of Botany* 62: 448–456.

Braithwaite AF. 1964. A new type of apogamy in ferns. *New Phytologist* 63: 293–305.

Chao YS, Liu HY, Huang YM, Chiou WL. 2010. Reproductive traits of *Pteris cadieri* and *P. grevilleana* in Taiwan: Implications for their hybrid origin. *Botanical Studies* 51: 209–216.

Chao YS, Dong SY, Chiang YC, Liu HY, Chiou WL. 2012. Extreme multiple reticulate origins of the *Pteris cadieri* complex (Pteridaceae). *International Journal of Molecular Sciences* 13: 4523–4544.

Chen CW, Ngan LT, Hidayat A, Evangelista L, Nooteboom HP, Chiou WL. 2014. First insights into the evolutionary history of the *Davallia repens* complex. *Blumea* 59: 49–58.

Chen WC, Kuo LY, Wang CN, Chiou WL. 2012. Development of PCR primer sets for intron 1 of the low-copy gene *LEAFY* in Davalliaceae. *American Journal of Botany* 99: e223–e225.

Clark J, Hidalgo O, Pellicer J, Liu HM, Marquardt J, Robert Y, Christenhusz M, Zhang S, Gibby M, Leitch IJ, Schneider H. 2016. Genome evolution of ferns: evidence for relative stasis of genome size across the fern phylogeny. *New Phytologist* 210: 1072–1082.

Cordle AR, Irish EE, Cheng CL. 2012. Gene expression associated with apogamy commitment in *Ceratopteris richardii*. *Sexual Plant Reproduction* 25: 293–304.

Cosendai AC, Wagner J, Ladinig U, Rosche C, Hörandl E. 2013. Geographical parthenogenesis and population genetic structure in the alpine species *Ranunculus kuepferi* (Ranunculaceae). *Heredity* 110: 560–569.

Darlington CD. 1939. *The evolution of genetic systems*. Cambridge: Cambridge University Press.

DeBenedictis VMM. 1969. *Apomixis in ferns with special reference to sterile hybrids*. Ph.D. Dissertation. Ann Arbor: The University of Michigan.

Dopp W. 1932. Die apogamie bei *Aspidium remotum* Al. Br. *Planta* 17: 87–152.

Dyer RJ, Pellicer J, Savolainen V, Leitch IJ, Schneider H. 2013. Genome size expansion and the relationship between nuclear DNA content and spore size in the *Asplenium monanthes* fern complex (Aspleniaceae). *BMC Plant Biology* 13: 219.

Dyer RJ, Savolainen V, Schneider H. 2012. Apomixis and reticulation in the *Asplenium monanthes* fern complex. *Annals of Botany* 110: 1515–1529.

Ebihara A, Matsumoto S, Kato M. 2012. Origin of *Dryopteris shibipedis* (Dryopteridaceae), a fern species extinct in the wild. *Journal of Plant Research* 125: 499–505.

Ekrt L, Koutecky P. 2016. Between sexual and apomictic: Unexpectedly variable sporogenesis and production of viable polyploids in the pentaploid fern of the *Dryopteris affinis* agg. (Dryopteridaceae). *Annals of Botany* 117: 97–106.

Farlow WG. 1874. An asexual growth from the prothallus of *Pteris cretica*. *Journal of Cell Science* 14: 266–272.

Feng S, Cokus SJ, Zhang X, Chen PY, Bostick M, Goll MG, Hetzel J, Jain J, Strauss SH, Halpern ME, Ukomadu C, Sadler KC, Pradhan S, Pellegrini M, Jacobsen SE. 2010. Conservation and divergence of methylation patterning in plants and animals. *Proceedings of the National Academy of Sciences USA* 107: 8689–8694.

- Ferreira de Carvahlo J, Oplaat C, Pappas N, Derks M, de Ridder D, Verhoeven KJF. 2016. Heritable gene expression differences between apomictic clone members in *Taraxacum officinale*: Insights into early stages of evolutionary divergence in asexual plants. *BMC Genomics* 17: 203.
- Gabriel y Galán JM. 2011. Gametophyte development and reproduction of *Argyrochosma nivea* (Pteridaceae). *Biología* 66: 50–54.
- Gastony GJ, Windham MD. 1989. Species concepts in pteridophytes: The treatment and definition of agamosporous species. *American Fern Journal* 79: 65–77.
- Gastony GJ, Yatskievych G. 1992. Maternal inheritance of the chloroplast and mitochondrial genomes in cheilanthoid ferns. *American Journal of Botany* 79: 716–722.
- Grusz AL. 2014. *Evolutionary patterns and processes in the desert-adapted fern genus Myriopteris (Pteridaceae)*. Ph.D. Dissertation. Durham: Duke University.
- Grusz AL, Pryer KM. 2015. Development of microsatellite markers for the apomictic triploid fern *Myriopteris lindheimeri* (Pteridaceae). *Applications in Plant Sciences*. doi: 10.3732/apps.1500061.
- Grusz AL, Windham MD, Pryer KM. 2009. Deciphering origins of apomictic polyploids in the *Cheilanthes yavapensis* complex (Pteridaceae). *American Journal of Botany* 96: 1636–1645.
- Grusz AL, Windham MD, Yatskievych G, Huiet L, Gastony GJ, Pryer KM. 2014. Patterns of diversification in the xeric-adapted fern genus *Myriopteris* (Pteridaceae). *Systematic Botany* 39: 698–714.
- Guillon J, Raquin C. 2000. Maternal inheritance of chloroplasts in the horsetail *Equisetum variegatum* (Schleich.). *Current Genetics* 37: 53–56.
- Guo ZY, Liu HM. 2013. Gametophyte morphology and development of three species of *Cyrtogonellum* Ching (Dryopteridaceae). *American Fern Journal* 103: 153–165.
- Gustafsson Å. 1946. Apomixis in higher plants. I. The mechanism of apomixis. *Lunds Universitets Årsskrift. NF. Adv.* 42: 1–66.
- Gustafsson Å. 1947. Apomixis in higher plants. III. Biotype and species formation. *Lunds Universitets Årsskrift. NF. Adv.* 43: 181–370.
- Hand ML, Koltunow AMG. 2014. The genetic control of apomixis: Asexual seed formation. *Genetics* 197: 441–450.
- Haufler CH. 2002. Homospory 2002: An odyssey of progress in pteridophyte genetics and evolutionary biology. *BioScience* 52: 1081–1093.
- Haufler CH, Pryer KM, Schuettpelz E, Sessa EB, Farrar DR, Moran R, Schneller JJ, Watkins JE, Windham MD. 2016. Sex and the single gametophyte: Revising the homosporous vascular plant life cycle in light of contemporary research. *BioScience*. doi:10.1093/biosci/biw108.
- Hernández MA, Andrada AR, Páez VA, Martínez OG. 2015. Ploidy level and obligate apogamy in two populations of *Argyrochosma nivea* var. *tenera* (Pteridaceae). *Hoehnea* 42: 233–237.
- Hojsgaard D, Klatt S, Baier R, Carman JG, Hörandl E. 2014. Taxonomy and biogeography of apomixis in angiosperms and associated biodiversity characteristics. *Critical Reviews in Plant Sciences* 33: 414–427.
- Hori K, Akitaka T, Fujimoto K, Kato J, Ebihara A, Watano Y, Murakami N. 2014. Reticulate evolution in the apogamous *Dryopteris varia* complex (Dryopteridaceae, subg. *Erythrovariae*, sect. *Variae*) and its related sexual species in Japan. *Journal of Plant Research* 127: 661–684.
- Hori K, Matsumoto M, Ebihara A, Nakato N, Murakami N. 2015. Geographical distribution of sexual and apogamous types of *Dryopteris chinensis* (Dryopteridaceae) in Japan. *Acta Phytotaxonomica Geobotanica* 66: 35–45.
- Huang YM, Chou HM, Hsieh TH, Wang JC, Chiou WL. 2006. Cryptic characteristics distinguish diploid and triploid varieties of *Pteris fauriei* (Pteridaceae). *Canadian Journal of Botany* 84: 261–268.
- Huang YM, Hsu SY, Hsieh TH, Chou HM, Chiou WL. 2011. Three *Pteris* species (Pteridaceae: Pteridophyta) reproduce by apogamy. *Botanical Studies* 52: 79–87.
- Ishikawa H, Ito M, Watano Y, Kurita S. 2003. Electrophoretic evidence for homoeologous chromosome pairing in the apogamous fern species *Dryopteris nipponensis* (Dryopteridaceae). *Journal of Plant Research* 116: 165–167.
- Ishikawa H, Watano Y, Kano K, Ito M, Kurita S. 2002. Development of primer sets for PCR amplification of the *PgiC* gene in ferns. *Journal of Plant Research* 115: 65–70.
- Klekowski EJ. 1973. Sexual and subsexual systems in homosporous pteridophytes: A new hypothesis. *American Journal of Botany* 60: 535–544.
- Koltunow AMG, Johnson SD, Rodrigues JCM, Okada T, Hu Y, Tsuchiya T, Wilson S, Fletcher P, Ito K, Suzuki G, Mukai Y, Fehrer J, Bicknell RA. 2011. Sexual reproduction is the default mode in apomictic *Hieracium* subgenus *Pilosella*, in which two dominant loci function to enable apomixis. *The Plant Journal* 66: 890–902.
- Li FW, Pryer KM. 2014. Crowdfunding the *Azolla* fern genome project: A grassroots approach. *GigaScience* 3: 1.
- Li FW, Pryer KM, Windham MD. 2012. *Gaga*, a new fern genus segregated from *Cheilanthes* (Pteridaceae). *Systematic Botany* 37: 845–860.
- Lisch D. 2013. How important are transposons for plant evolution? *Nature Reviews Genetics* 14: 49–61.
- Liu HM, Dyer RJ, Guo ZY, Meng Z, Li JH, Schneider H. 2012. The evolutionary dynamics of apomixis in ferns: A case study from polystichoid ferns. *Journal of Botany*. doi:10.1155/2012/510478.
- Liu HM, Zhang XC, Wang W, Zeng H. 2010. Molecular phylogeny of the endemic fern genera *Cyrtomidictyum* and *Cyrtogonellum* (Dryopteridaceae) from East Asia. *Organisms, Diversity, and Evolution* 10: 57–68.
- Lu JM, Li DZ, Ding WU. 2006. Chromosome numbers of four genera in the Dryopteridaceae. *Acta Phytotaxonomica Sinica* 44: 516–522.
- Manton I. 1950. *Problems of cytology and evolution in the Pteridophyta*. Cambridge: Cambridge University Press.
- Martinez O. 2010. Gametophytes and young sporophytes of four species of the fern genus *Pteris* (Pteridaceae) naturalized in the American continent. *Revista de Biología Tropical* 58: 89–102.
- Mehra PN, Singh G. 1957. Cytology of the Hymenophyllaceae. *Journal of Genetics* 55: 379–393.
- Menéndez V, Villacorta NF, Revilla MA, Gotor V, Bernard P, Fernández H. 2006. Exogenous and endogenous growth regulators on apogamy in *Dryopteris affinis* (Lowe) Fraser-Jenkins sp. *affinis*. *Plant Cell Reports* 25: 85–91.
- Migliaro G, Gabriel y Galán JM. 2012. Gametophyte development and reproduction of the Asian fern *Polystichum polyblepharum* (Roem. ex Kunze) C. Presl (Dryopteridaceae, Polypodiopsida). *Plant Biosystems* 146: 368–373.
- Nitta JH, Ebihara A, Motomi I. 2011. Reticulate evolution in the *Crepidomanes minutum* species complex (Hymenophyllaceae). *American Journal of Botany* 98: 1782–1800.
- Ootsuki R, Sato H, Nakato N, Murakami N. 2012. Evidence of genetic segregation in the apogamous fern species *Cyrtomium fortunei* (Dryopteridaceae). *Journal of Plant Research* 125: 605–612.

- Ootsuki R, Shinohara W, Suzuki T, Murakami N. 2011. Genetic variation in the apogamous fern *Cyrtomium fortunei* (Dryopteridaceae). *Acta Phytotaxonomica Geobotanica* 62: 1–14.
- Ozias-Akins P, van Dijk PJ. 2007. Mendelian genetics of apomixis in plants. *Annual Reviews of Genetics* 41: 509–537.
- Peredo EL, Mendez-Couz M, Revilla M, Fernandez H. 2013. Mating system in *Blechnum spicant* and *Dryopteris affinis* ssp. *affinis* correlates with genetic variability. *American Fern Journal* 103: 27–39.
- Pittermann J, Brodersen C, Watkins JE. 2013. The physiological resilience of fern sporophytes and gametophytes: Advances in water relations offer new insights into an old lineage. *Frontiers in Plant Science*. doi: 10.3389/fpls.2013.00285.
- Pittermann J, Watkins JE, Cary KL, Schuettelpelz S, Brodersen C, Smith AR, Baer A. 2015. The structure and function of xylem in seed free vascular plants: An evolutionary perspective. In: Hacke U ed. *Ecological and functional xylem anatomy*. Springer International.
- Podio M, Cáceres ME, Samoluk SS, Seijo JG, Pessino SC, Ortiz JPA, Pupilli F. 2014. A methylation status analysis of the apomixis-specific region in *Paspalum* spp. suggests an epigenetic control of parthenogenesis. *Journal of Experimental Botany* 65: 6411–6424.
- PPG I. 2016. A community-derived classification for extant lycopods and ferns. *Journal of Systematics and Evolution*. doi: 10.1111/jse.12229.
- Rapp RA, Wendel JF. 2005. Epigenetics and plant evolution. *New Phytologist* 168: 81–91.
- Regalado Gabancho L, Gabriel y Galán JM. 2010. Sexuality and apogamy in the Cuban *Asplenium auritum-monodon* complex (Aspleniaceae). *Plant Systematics and Evolution* 289: 137–146.
- Rothfels CJ, Larsson A, Li FW, Sigel E, Huiet L, Burge DO, Ruhsam M, Graham S, Stevenson DW, Wong GKS, Korall P. 2013. Transcriptome-mining for single-copy nuclear markers in ferns. *PLoS ONE* 8: e76957.
- Rothfels CJ, Pryer KM, Li FW. 2016. Next generation polyploid phylogenetics: rapid resolution of hybrid polyploid complexes using PacBio single-molecule sequencing. *New Phytologist*. doi: 10.1111/nph.14111
- Schneller J, Krattinger K. 2010. Genetic composition of Swiss and Austrian members of the apogamous *Dryopteris affinis* complex (Dryopteridaceae, Polypodiopsida) based on ISSR markers. *Plant Systematics and Evolution* 286: 1–6.
- Schuettelpelz E, Grusz AL, Windham MD, Pryer KM. 2008. The utility of nuclear *gapCp* in resolving polyploid fern origins. *Systematic Botany* 33: 621–629.
- Sessa EB, Banks JA, Barker MS, Der JP, Duffy AM, Graham SW, Hasebe M, Langdale J, Li FW, Marchant DB, Pryer KM. 2014. Between two fern genomes. *GigaScience* 3: 1.
- Sigel EM, Windham MD, Huiet L, Yatskievych G, Pryer KM. 2011. Species relationships and farina evolution in the cheilanthoid fern genus *Argyroschisma* (Pteridaceae). *Systematic Botany* 36: 554–564.
- Soare LC. 2008. In vitro development of gametophyte and sporophyte in several fern species. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 36: 13–19.
- Soare LC, Vşoiu E, Dobrescu CM. 2010. In vitro culture and regeneration of the fern *Dryopteris affinis*, species growing in a protected area. *Romanian Biotechnological Letters* 15: 45–54.
- Steil WN. 1919. A study of apogamy in *Nephrodium hirtipes*. *Annals of Botany* 33: 109–132.
- Stenberg P, Saura A. 2009. Cytology of asexual animals. In: Schön I, Martens K, van Dijk P eds. *Lost sex*. Dordrecht: Springer.
- Tanaka T, Yuichi I, Mitsuru H. 2014. Ecological and phylogenetic approaches for diversification of apogamous ferns in Japan. *Plant Systematics and Evolution* 300: 2041–2050.
- Verhoeven KJF, Preite V. 2014. Epigenetic variation in asexually reproducing organisms. *Evolution* 68: 644–655.
- Verhoeven KJF, van Dijk PJ, Biere A. 2010. Changes in genomic methylation patterns during the formation of triploid asexual dandelion lineages. *Molecular Ecology* 19: 315–324.
- Vogel JC, Russel SJ, Rumsey FJ, Barrett A, Gibby M. 1998. Evidence for maternal transmission of chloroplast DNA in the genus *Asplenium* (Aspleniaceae, Pteridophyta). *Acta Botanica* 111: 247–249.
- Vrijenhoek RC. 1984. Ecological differentiation among clones: The frozen niche variation model. In: Wöhrmann K, Loeschcke V eds. *Population biology and evolution*. Berlin: Springer-Verlag. 217–231.
- Walker TG. 1985. Some aspects of agamospory in ferns—The Braithwaite system. *Proceedings of the Royal Society B: Biological Sciences* 86: 59–68.
- Wang RX, Shao W, Liu L. 2014. Cytotaxonomic study of *Hypodematium* (Hypodematiaceae) from China. *Phytotaxa* 161: 101–110.
- Wang RX, Shao W, Lu SG, Zhou SY, Liang SC. 2011. Species in the Polypodiaceae from Southern China. *American Fern Journal* 101: 307–316.
- Wickell D. 2015. Does asexuality confer a short term advantage? A case study in the fern *Myriopteris gracilis* (Pteridaceae). MSc. Thesis. Wichita: Wichita State University.
- Wolf PG, Sessa EB, Marchant DB, Li FW, Rothfels CJ, Sigel EM, Gitzendanner MA, Visger CJ, Banks JA, Soltis DE, Soltis PS. 2015. An exploration into fern genome space. *Genome Biology and Evolution* 7: 2533–2544.
- Xiang JY, Cheng X, Wu SG. 2006. Chromosome numbers of 13 species in the genus *Dryopteris* (Dryopteridaceae) from Yunnan, China. *Acta Phytotaxonomica Sinica* 44: 304–319.
- Xie M, Hong C, Zhang B, Lowdon RF, Xing X, Li D, Zhou X, Lee HJ, Maire CL, Ligon K, Gascard P. 2013. DNA hypomethylation within specific transposable element families associates with tissue-specific enhancer landscape. *Nature Genetics* 45: 836–841.
- Zappacosta DC, Ochogavía AC, Rodrigo JM, Romero JR, Meier MS, Garbus I, Pessino SC, Echenique VC. 2014. Increased apomixis expression concurrent with genetic and epigenetic variation in a newly synthesized *Eragrostis curvula* polyploid. *Scientific Reports* 4: 4423. doi: 10.1038/srep04423.