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A drought-driven model for the evolution of obligate apomixis in ferns: evidence from pellaeids (Pteridaceae)

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PREMISE: Xeric environments impose major constraints on the fern life cycle, yet many lineages overcome these limitations by evolving apomixis. Here, we synthesize studies of apomixis in ferns and present an evidence-based model for the evolution and establishment of this reproductive strategy, focusing on genetic and environmental factors associated with its two defining traits: the production of "unreduced" spores ($n = 2n$) and the initiation of sporophytes from gametophyte tissue (i.e., diplospory and apogamy, respectively).

METHODS: We evaluated existing literature in light of the hypothesis that abiotic characteristics of desert environments (e.g., extreme diurnal temperature fluctuations, high light intensity, and water limitation) drive the evolution of obligate apomixis. Pellaeid ferns (Cheilanthoideae: Pteridaceae) were examined in detail, as an illustrative example. We reconstructed a plastid (*rbcL*, *trnG–trnR*, *atpA*) phylogeny for the clade and mapped reproductive mode (sexual versus apomictic) and ploidy across the resulting tree.

RESULTS: Our six-stage model for the evolution of obligate apomixis in ferns emphasizes the role played by drought and associated abiotic conditions in the establishment of this reproductive approach. Furthermore, our updated phylogeny of pellaeid ferns reveals repeated origins of obligate apomixis and shows an increase in the frequency of apomixis, and rarity of sexual reproduction, among taxa inhabiting increasingly dry North American deserts.

CONCLUSIONS: Our findings reinforce aspects of other evolutionary, physiological, developmental, and omics-based studies, indicating a strong association between abiotic factors and the establishment of obligate apomixis in ferns. Water limitation, in particular, appears critical to establishment of this reproductive mode.

KEY WORDS agamospory; apogamy; asexual; diplospory; Döpp-Manton sporogenesis; meiotic obligate apogamy; premeiotic endomitosis.

Stressful conditions associated with xeric environments have a predictable impact on the organisms inhabiting them, yet a variety of plant lineages have evolved suites of adaptations that enable them to thrive in deserts across the world (e.g., Stebbins, 1952; Kemp, 1983; Lewis and Lewis, 2005; Arakaki et al., 2011). Among these are ferns that typically rely on water for the successful transport of free-swimming sperm to complete sexual reproduction (Haufler et al., 2016). Generally associated with mesic habitats, ferns are an unexpected "poster child" for desert adaptation. Nonetheless, many fern lineages have evolved effective strategies, including an apomictic life cycle, to mitigate the challenges tied to reproduction in drought-prone regions (Woronin, 1908; Hevly, 1963; Tryon, 1968; Klekowski, 1969; Harten and Eickmeier, 1987).

The apomictic life cycle (apomixis) in ferns requires that a series of correlated changes occur in the independent sporophytic and gametophytic phases (Haufler et al., 2016; Grusz, 2016 and citations therein). These modifications—whose coordination might span ca.100–200 years, from one generation to the next—appear to be driven by environmental conditions. Available evidence suggests that there are tangible associations between obligate apomixis and a plurality of abiotic factors, including temperature, substrate composition, and water availability (e.g., Bell, 1959; Whittier, 1964a, b; Hickok, 1977b; Padhya and Mehra, 1981).

Here, we explore these connections and evaluate support for the hypothesis that obligate apomixis in ferns is not only advantageous in xeric environments, but that water limitation, in particular, is imperative for uniting the suite of traits required for the establishment of this reproductive mode in natural populations. Evidence to support this argument is gathered from the literature, especially from studies focused on the pellaeid ferns (Cheilanthoideae, Pteridaceae). Although apomixis has been observed in many large families, encompassing species from a variety of extreme habitats (such as the Dryopteridaceae; Liu et al., 2012), the pellaeids have been the focus of numerous morphological, cytogenetic, life history, and phylogenetic studies (e.g., Steil, 1911; Pickett and Manuel, 1926; Tryon and Britton, 1958; Tryon, 1968; Whittier, 1968; Rigby, 1973; Gastony, 1988; Kirkpatrick, 2007; Beck et al., 2010, 2011, 2012; Sigel et al., 2011), thus providing a robust system to evaluate evidence for critical stages in the evolution of obligate apomixis in ferns.

A MODEL FOR THE ESTABLISHMENT OF OBLIGATE APOMIXIS IN FERNS

Apomixis can be defined as the alternation of the sporophyte ($2n$) and gametophyte (n) life-history phases in the absence of sex (e.g., Haufler et al., 2016). In ferns, this adaptation is remarkably common, with an estimated 10% of species considered to be obligately apomictic (Walker, 1979; Liu et al., 2012). In other vascular plant lineages, apomixis is considerably less prevalent (e.g., <1% in angiosperms; Bicknell and Koltunow, 2004; Becks and Alavi, 2015). Despite the frequency (and stability) of obligate apomixis in ferns, a comprehensive model for its evolution is lacking. Leveraging a synthesis of previous studies, we elaborate a multi-pronged model for the evolution and establishment of this reproductive approach. We focus on apomixis by premeiotic endomitosis (PE, Grusz, 2016; APE, Mogie, 2013) because existing data indicate that it is, by far, the most widespread pathway to sporogenesis undertaken in apomictic ferns. During PE, an incomplete mitotic cell division produces spore mother cells with twice the sporophyte chromosome number, ultimately giving rise to unreduced spores ($n = 2n$). Such unreduced spores ($n = 2n$) reflect two critical prerequisites for the establishment of obligate apomixis: polyploidy and chromosome pairing control. Here, we review these cytogenetic traits and enumerate six key stages for the establishment of this reproductive adaptation in ferns, emphasizing key evidence from the literature.

Prerequisites: polyploidy and chromosome pairing control

We propose that the ability to generate and maintain polyploids is critical for the extensive proliferation of apomixis in ferns. Polyploids are usually categorized as either "paleopolyploids" or "neopolyploids" based on the time elapsed since the genome duplication event from which they arose. The vast majority of apomictic lineages are neopolyploids, having arisen relatively recently, and are easily recognized because their chromosome numbers are typically multiples of those observed in related diploids that exhibit the lowest (base) chromosome numbers in a particular lineage. Paleopolyploids, by contrast, represent lineages long since descended from whole-genome duplications (WGDs) that occurred deep within the evolutionary tree—and leptosporangiate ferns have experienced more ancient WGD events than any other green plant lineage, outside of angiosperms (Leebens-Mack et al., 2019).

Chromosome pairing control, which we hypothesize to be a second prerequisite for the origin of obligate apomixis, can be a corollary to polyploid success. The presence of three or more homoeologous (often mostly homologous) sets of chromosomes in a sporophyte ($2n$) nucleus can greatly disrupt meiosis, largely because the occurrence of multivalents and univalents during the synaptic phases of the first meiotic division leads to an unequal distribution of chromosomes to the two daughter cells. The loss of whole chromosomes in these daughter cells, in turn, leads to near complete failure of sporogenesis.

In the event of extensive multivalent and univalent formation, there are two primary options for restoring fertility, both of which involve chromosome pairing control. The first option depends on the expression of an asynaptic mutation that disrupts all forms of chromosomal association, thereby converting sporogenesis from a meiotic process to a mitotic process. This approach is taken by a relatively small number of ferns (those following the Braithwaite pathway; Braithwaite, 1964; Evans, 1964) and the vast majority of apomictic flowering plants (Hojsgaard et al., 2014). The second option, enforced bivalent formation when more than two homologues are present, is the pathway observed in most ferns, especially among polyploid apomicts generating spores via premeiotic endomitosis (PE).

In ferns, these predispositions—an evolutionary history shaped by WGD and a widespread adherence to bivalent chromosome pairing during meiosis—provide a foundation for the origin and establishment of obligate apomixis. Indeed, WGD events are rooted in the nonreductive cell divisions that are also the basis for unreduced spore production (USP) during the first and third stages of obligate apomixis establishment (see below). Meanwhile, bivalent pairing control is critical for stabilizing meiosis in polyploid sporophytes, including the vast majority of obligately apomictic ferns. Together, they allow for the stabilization of obligate apomixis, beginning with the production of chromosomally "unreduced" ($n = 2n$) spores by a sexual diploid sporophyte.

Stage 1: Facultative unreduced spore production (USP) by sexual diploid sporophytes

Leptosporangiate ferns (PPG I, 2016) are united in sharing a common approach to sporogenesis. This process is typically initiated within developing sporangia from a single sporophytic ($2n$) archesporial cell; in most cases, the archesporial cell undergoes four mitotic divisions to yield 16 spore mother cells (SMCs; Fig. 1). Meiosis then advances from each of the 16 SMCs, yielding a total of 64 spores per sporangium, each with half of the sporophytic number of chromosomes (n; Fig. 1). Notably, some clades undergo fewer than four mitotic cell divisions from each archesporial cell, leading to a correlated reduction in the final spore number per sporangium for these groups (e.g., in Cyatheaceae, Gastony and Tryon, 1976;

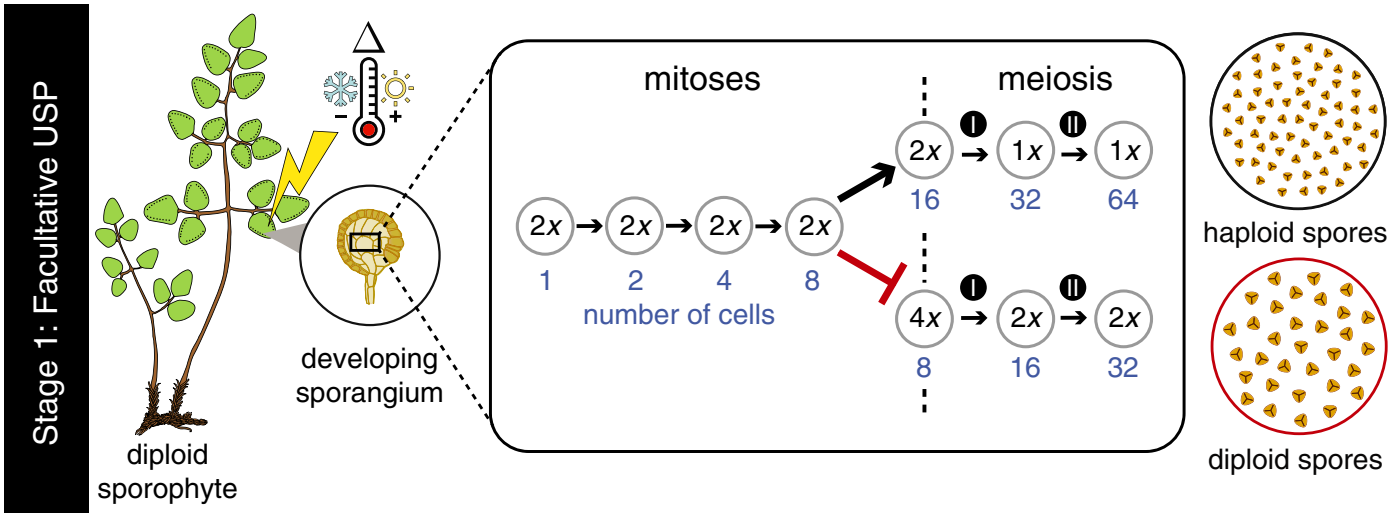

FIGURE 1. Facultative unreduced spore production (USP). Typically, leptosporangiate ferns produce 64 haploid spores per sporangium through canonical meiosis (→). However, environmental extremes during sporangial development, such as drastic fluctuations in temperature (indicated by lightning bolt), can lead to incomplete mitotic divisions in some spore mother cell precursors, thereby producing a restitution nucleus (red ⊣) with double the sporophytic chromosome number (4*x*). Cells in affected sporangia proceed through meiosis, generating 32 chromosomally unreduced spores with the same ploidy as the parent plant ($n = 2n$).

Notholaena, Gastony and Windham, 1989; and *Cheilanthes* s.s., Li et al., 2012; Grusz and Windham, 2013).

Deviations from standard sporogenesis that result in unreduced ($n = 2n$) spores are well documented in ferns, forming obligately in some species and facultatively in others (Steil, 1919; Döpp, 1932; Manton, 1950; Braithwaite, 1964; Evans, 1964; Morzenti, 1967; Haufler et al., 1985; Walker, 1985; Gastony, 1986; Gastony and Windham, 1989). In some cases, unreduced spores ($n = 2n$) can be found intermixed with canonically derived haploid (n) spores from separate sporangia on the same plant (Fig. 1), or even within the same sporangium (Mehra and Bir, 1960; Knobloch, 1966, 1969; Tryon, 1968; Debenedictis, 1969; Brouharmont, 1972a, b; Lloyd, 1973; Lin et al., 1992; Rabe and Haufler, 1992; Sigel et al., 2011; Ekrt and Koutecky, 2016; Wickell et al., 2017).

Most observations of unreduced spores in ferns are attributable to premeiotic endomitosis (PE; Grusz, 2016), also known as Döpp-Manton sporogenesis (Döpp, 1932; Manton, 1950; Walker, 1985). This process typically incorporates three (fewer in select cases) complete mitotic divisions, beginning with the archesporial cell; the fourth (last) mitotic division is incomplete, and a restitution nucleus forms (i.e., DNA replicates, but there is no subsequent cell division; Steil, 1919; Döpp, 1932; Manton, 1950; Fig. 1). As a result, only eight SMCs, each with double the sporophytic chromosome number, typically proceed to meiosis (Fig. 1). Meiosis itself then progresses normally, ultimately yielding 32 (or fewer) unreduced ($n = 2n$) spores organized in tetrads within the sporangium (Fig. 1). Döpp-Manton sporogenesis circumvents the usual 50% reduction in chromosome number that is associated with standard sporogenesis (Steil, 1919; Döpp, 1932; Manton, 1950; Gastony and Windham, 1989; Grusz, 2016; Fig. 1).

Despite widespread documentation of PE in ferns, the pressures and mechanisms guiding this form of unreduced spore production (USP) are poorly understood. Existing evidence, however, does provide some insight. First, hybridization is not required to trigger PE (Gastony, 1986). Although the presence of divergent genomes within a single cell nucleus can be problematic during *meiotic* segregation (e.g., Class II polyploidy, cf. Harlan and deWet, 1975; Morzenti, 1967), these problems are avoided in mitotic division, and it is, thus, unsurprising that PE in ferns is observed in both autopolyploid and allopolyploid lineages (Gastony and Gottlieb, 1985; Gastony and Windham, 1989). Second, environmental cues may be an important catalyst for the formation of restitution nuclei (Hickok, 1977b; Haufler et al., 1985). For example, an association has been detected between low temperature (18°C) and an increased incidence of meiotic restitution resulting from aberrant spindle formation in *Ceratopteris* (Hickok, 1977b). The association between low temperature and increased incidence of whole-genome duplication via mitotic/meiotic restitution is consistent with observations of triploid populations at the northern edge of the otherwise diploid species' ranges (e.g., in *Cystopteris protrusa*; Haufler et al., 1985).

Stage 2: Sexual reproduction between haploid and diploid gametophytes

Upon germination, unreduced ($n = 2n$) spores develop into unreduced multicellular gametophytes via mitosis. Each mature gametophyte is capable of producing functional archegonia and/or antheridia, either of which can theoretically contribute unreduced ($n = 2n$) gametes to form a polyploid sporophyte via syngamy (Klekowski, 1970). In this way, new, sexually derived polyploid sporophytes ($2n \geq 3x$) can arise via gametophytic selfing, sporophytic selfing, or sporophytic outcrossing (Gastony and Windham, 1989; Rabe and Haufler, 1992; Haufler et al., 2016), but the most common pathway to polyploid sporophyte formation is thought to be through the fusion of the relatively rare unreduced ($n = 2n = 2x$) gametes with more common reduced ($n = x$) gametes, via sporophytic selfing or sporophytic outcrossing (Haufler et al., 2016; but see Sessa et al., 2016), to yield a triploid sporophyte ($2n = 3x$; Fig. 2). Some of the strongest evidence to support this idea comes from cytological investigations of meiosis in triploid sporophytes derived

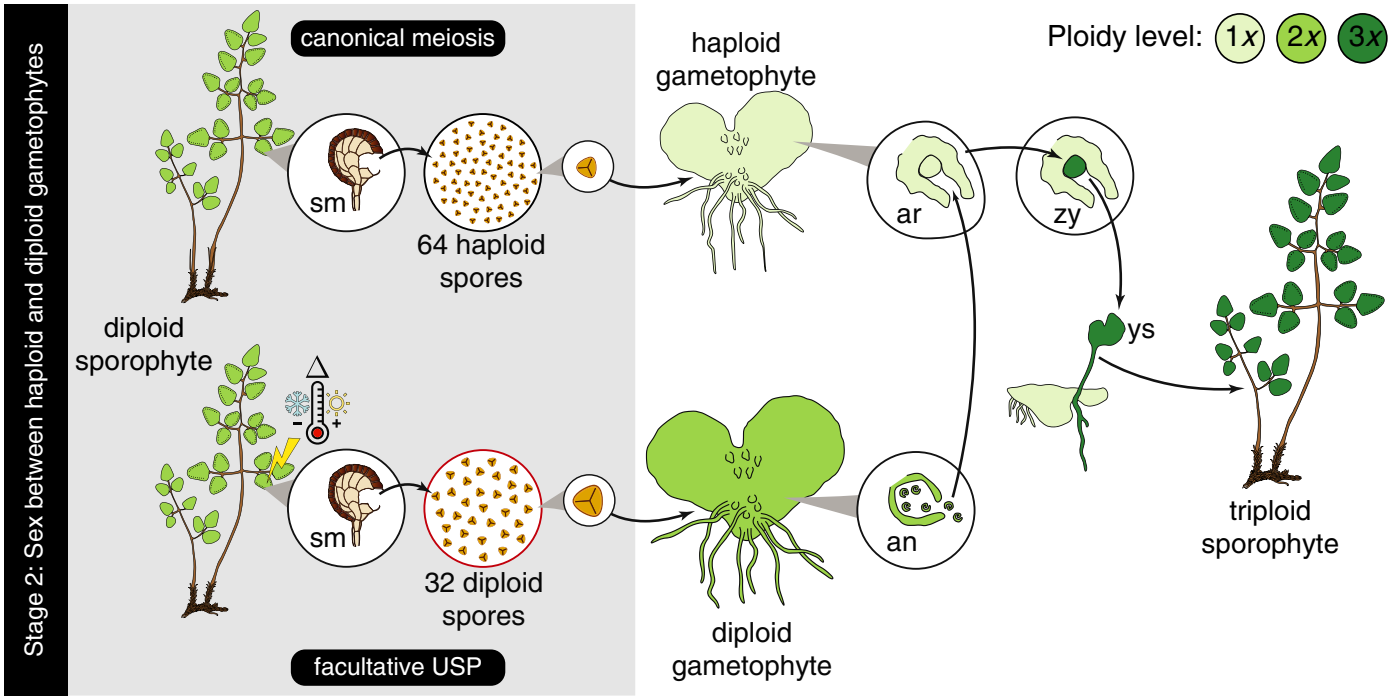

FIGURE 2. Sexual reproduction between haploid and diploid gametophytes. A typical diploid sporophyte produces 64 haploid spores within a sporangium. Spores germinate and become haploid gametophytes that, when mature, produce haploid archegonia and antheridia through mitosis. By contrast, unreduced spores become gametophytes with the same ploidy level as the parent plant ($n = 2n$). These unreduced gametophytes, in turn, produce functional unreduced gametes. As presented here, a diploid sperm derived from an unreduced gametophyte fertilizes a haploid egg on a gametophyte derived from canonical meiosis. The resulting triploid zygote develops into a triploid sporophyte. *Abbreviations:* an, antheridium; ar, archegonium; sm, sporangium; ys, young sporophyte; zy, zygote.

through this process (Morzenti, 1967; Rigby, 1973; Gastony and Gottlieb, 1985; Haufler et al., 1985; Windham and Haufler, 1985; Gastony, 1986).

In addition to engaging in sexual reproduction as described above, an unreduced diploid gametophyte ($n = 2n$) may also be capable of producing a new sporophyte via apogamy, triggered by conditions similar to those influencing apogamy in triploid gametophytes ($n = 2n = 3x$; see Stages 4, 5 below). Apomictic diploids ($n = 2n = 2x$) are common in some groups (e.g., in *Pteris* and *Dryopteris*), but the majority of documented apomictic ferns are triploid ($n = 2n = 3x$; Döpp, 1932; Manton, 1950; Walker, 1979; Haufler et al., 1985; Chao et al., 2012; Liu et al., 2012; Grusz et al., 2014). Notably, apomictic diploids are not well represented in desert-adapted lineages, but they are widely reported and diverse in monsoonal regions (Walker, 1962; Liu et al., 2012; Tanaka et al., 2014a).

Stage 3: Obligate unreduced spore production (USP) by triploid sporophytes

Regardless of their derivation, triploid sporophytes ($2n = 3x$) are characterized by having three homologous (or, in hybrids, homoeologous) copies of each chromosome per cell nucleus. Although not problematic for growth via mitosis, having an unbalanced (odd) genome copy number will severely disrupt meiotic chromosome pairing, leading to irregular cell division and, ultimately, to abortive spores (Manton, 1950; Hickok and Klekowski, 1973). Sporogenesis in such unbalanced polyploids (e.g., $2n = 3x$, $5x$) can, however, be stabilized by unreduced spore production (USP), especially involving premeiotic endomitosis (PE; Fig. 3). Following PE, meiosis is strikingly regular, and bivalent associations form between homologous chromosomes with impressive fidelity (Walker, 1985). In fact, it is not uncommon for the eight SMCs within a given sporangium to collectively produce 32 well-formed, unreduced spores via PE. Other routes to USP (e.g., Braithwaite sporogenesis; Braithwaite, 1964; Evans, 1964) are typically less stable, with multiple sporogenesis pathways sometimes proceeding from any given archesporial cell, generating inviable spores of different sizes (Mehra and Singh, 1957; Hickok, 1977a; Walker, 1985).

Environmental factors that are implicated in the initial shift to USP (see Stage 1 above) are often experienced by the triploid sporophytes derived through this process, especially if they are growing close to their progenitor(s). Additionally, any aspects of USP that are heritable (e.g., via epigenetic inheritance) will be reinforced in the triploid lineage. As discussed above, most triploid sporophytes are reported to inherit two thirds of their chromosomes from an unreduced spore (see Stages 1, 2), thereby acquiring genetic (or epigenetic) predispositions for the trait.

Heritability of USP has been widely documented in ferns. Braithwaite (1964) recognized genetic control of asynapsis during meiotic first division restitution (i.e., Braithwaite sporogenesis), consistent with observations by others (e.g., Mehra and Singh, 1957; Morzenti, 1967). Asexuality via PE (i.e., meiotic obligate apogamy or APE; Mogie, 2013) is also demonstrably heritable as a dominant trait in ferns (e.g., Morzenti, 1966; Walker, 1985; Gabancho et al., 2010). Given its dominance and the remarkable stability PE

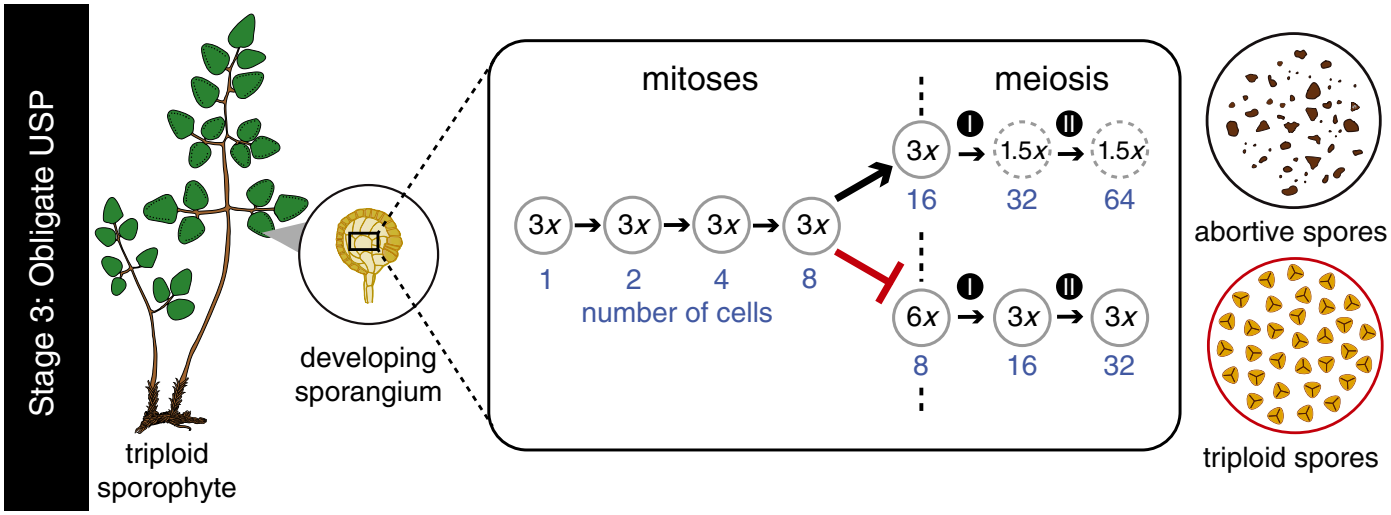

FIGURE 3. Obligate unreduced spore production (USP) in triploid sporophytes. Sporangia developing on triploid sporophytes can follow one of two paths to sporogenesis: either proceeding with normal cell division (→) or experiencing premeiotic endomitosis (red ⊣). Canonical meiosis of the 16 triploid spore mother cells (SMCs) leads to a failure in chromosome pairing and the three homologous sets of chromosomes are distributed unequally among the daughter cells, leading to the production of ca. 64 abortive spores per sporangium. Premeiotic endomitosis, by contrast, allows odd-numbered polyploids to enter meiosis with a duplicated (6*x*) chromosome complement and thus an even chromosome number; meiosis proceeds normally, resulting in 32 functional triploid spores ($n = 3x = 2n$).

affords to sporogenesis, it is not surprising that this is the most frequently followed route to USP.

Stage 4: Drought prevents sexual reproduction in triploid gametophytes

The next, and arguably most critical, stage in the establishment of obligate apomixis is the failure of sexual reproduction in first-generation triploid gametophytes ($n = 2n = 3x$; Fig. 4A). Because sexual reproduction inhibits apogamy (Duncan, 1941; Whittier, 1976), any barrier to sex is a boon to apogamous sporophyte development ($n = 2n = 3x$; Manton, 1950; Gastony and Windham, 1989). Reproductive barriers may be physical or physiological (Nayar and Kaur, 1971) and associated with either abnormal gametophyte development or abiotic factors (Duncan, 1941; Gastony and Haufler, 1976; Gastony and Windham, 1989), but the most common explanation for the prevention of sex in ferns is a lack of environmental water (Lang, 1898; Steil, 1939, 1951; Duncan, 1941; Manton, 1950; Debenedictis, 1969). Water is necessary for motile sperm to reach the archegonium (Gastony and Haufler, 1976; Haufler et al., 2016) and may also be required for effectively opening the archegonial neck (Sheffield and Bell, 1987). Therefore, in the absence of water, even reproductively competent bisexual gametophytes face a rigid barrier to sporophyte production (Debenedictis, 1969).

Associations between drought and apogamy have been documented in several spore-bearing lineages, including lycophytes (e.g., *Selaginella tenerrima*; Kornas and Jankun, 1983) and homosporous ferns (e.g., *Thelypteris* and *Dryopteris*; Bell, 1959). Within ferns, broad phylogenetic and biogeographic studies have further revealed increased occurrences of apomixis in desert and monsoonal climates, both of which are characterized by strong seasonality of precipitation and, thus, periodic or persistent water scarcity (Liu et al., 2012; Tanaka et al., 2014a; Grusz et al., 2014; K. T. Picard et al., unpublished manuscript).

Along with functioning as a physical barrier to sex, dry conditions could also, theoretically, impede pheromone and hormone signalling, e.g, via antheridiogen(s) or cytokinins, respectively, during gametophyte development (Sun, 2014; Tanaka et al., 2014b; Romanenko, 2020); however, this possibility remains unexplored. Tanaka et al. (2014b) found that in sexually reproducing *Lygodium*, the antheridiogen gibberellin A_9 methyl ester (GA_9-Me) is excreted by early-germinating gametophytes and then transferred through hydrophobic interactions to nearby, late-developing prothalli (there, it is converted into the bioactive gibberellin G_4 that is required for the downstream inhibition and promotion of archegonial and antheridial growth). Thus, the interruption of the antheridiogen signaling pathway by drought could reasonably impede normal gametangial development.

Stage 5: Apogamous initiation of triploid sporophytes

In the absence of sexual reproduction, a fern gametophyte is destined to one of three fates: (1) senescence and death; (2) persistence, i.e., vegetative growth, possibly with the development of more complex metabolic and abiotic interactions (which sometimes leads to facultative apogamy; Walp, 1951; Debenedictis, 1969; Cordle et al., 2007); or (3) production of an apogamous sporophyte (i.e., without syngamy; Farlow, 1894; Debenedictis, 1969; Fig. 4B). Although water limitation is usually key to preventing sexual reproduction in ferns, a lack of water, alone, is not sufficient for triggering apogamous growth (Steil, 1919; Klekowski, 1969; Gastony and Haufler, 1976; but see Bell, 1959, for possible implications of desiccation on apogamous development). Instead, studies have shown that apogamy relies upon a suite of interactions involving exposure to light, sugar(s), and hormones.

Since the first informed observation of apogamy in ferns (Farlow, 1874), extensive insight has been gained regarding the conditions that facilitate this form of asexual reproduction. Most studies have focused on facultative (i.e., induced) apogamy, which is distinguishable from obligate apogamy by the timing of apogamous development,

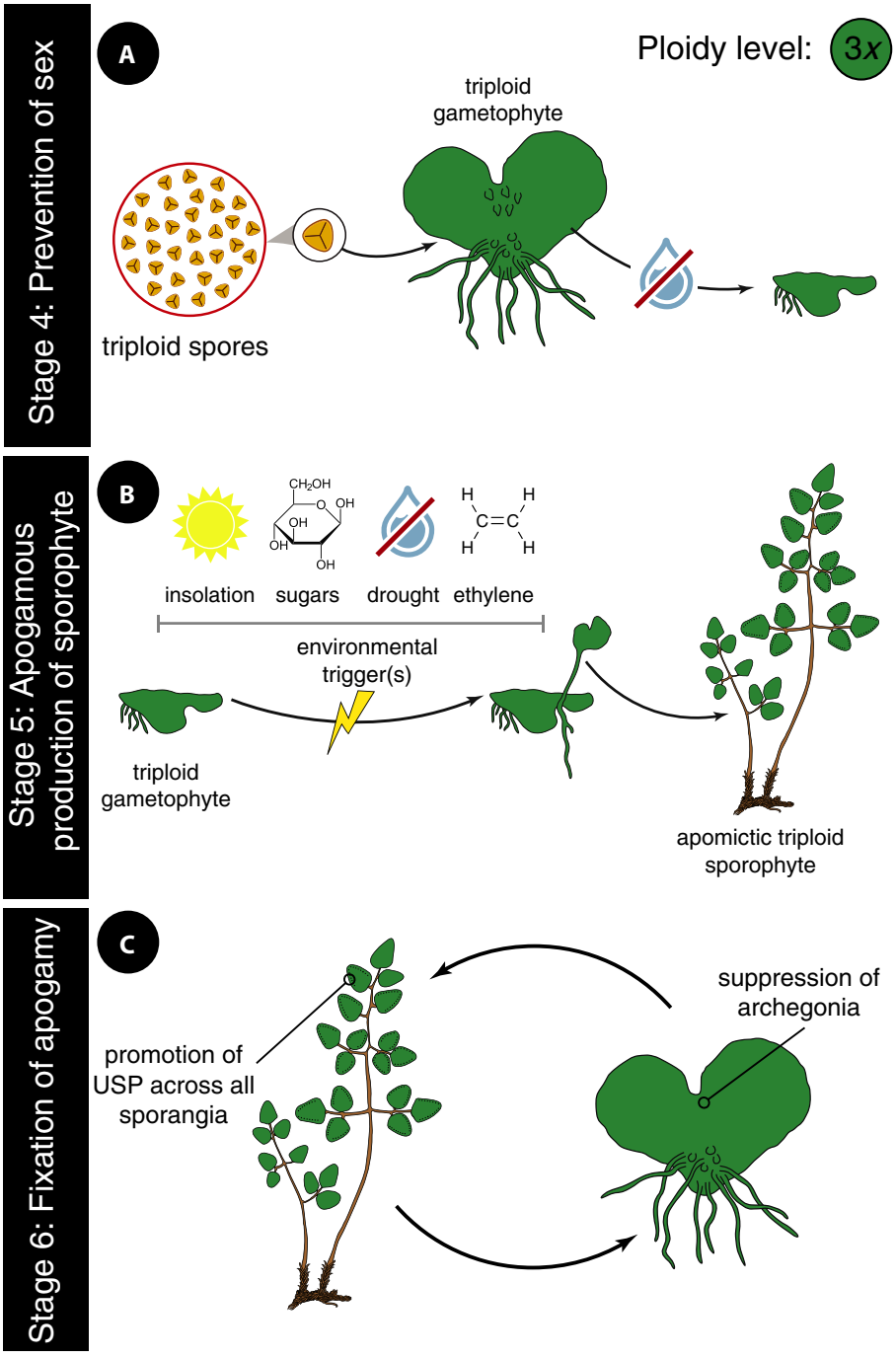

FIGURE 4. Final stages of obligate apomict formation. In (A), triploid spores germinate and develop into triploid gametophytes. The low availability of free water in xeric habitats prevents sexual reproduction. In (B), environmental triggers such as light, excess sugars, and phytohormones induce the apogamous formation of a sporophyte from gametophytic tissues. In (C), over time, selection acts jointly on the sporophyte and gametophyte, promoting unreduced spore production (USP) across most sporangia, and suppressing archegonia, respectively.

the point of origin of sporophyte tissue on the subtending prothallium, and the order of appearance of the first vascular tissue, leaf, root, and stem. Facultative apogamy usually involves the production of (sometimes numerous) sporophytic ($2n$) buds that arise late in gametophyte development. Such budding gametophytes are generally large and irregular (Duncan, 1943; Whittier, 1964a, b), with apogamous sporophytes distributed haphazardly across the prothallium (Farlow, 1894; Debenedictis, 1969; Whittier, 1974; Elmore and Whittier, 1975; Gabancho et al., 2010). In cases of obligate apogamy, however, the sporophyte arises earlier in development from a well-defined area of the gametophyte cushion (usually immediately behind the apical notch), and at roughly the same timepoint that archegonia would appear in sexual prothalli (Duncan, 1943; Debenedictis, 1969).

Although facultative apogamy does not appear to involve specific genetic control (Whittier and Steeves, 1960), the failure to induce apogamy in normally sexual gametophytes—despite prevention of sex and exposure to light and sugar—may indicate a genetic predisposition, possibly related to prothallial thickening and/or meristematic growth before the initiation of an apogamous sporophyte (Lang, 1898; Duncan, 1941; Whittier and Steeves, 1962; Steil, 1951). In stark contrast, obligate apogamy appears to be a highly regulated, heritable, dominant trait (Döpp, 1932; Walker, 1962; Morzenti, 1966; Gabancho et al., 2010).

Many studies have examined the biotic and abiotic forces shaping apogamy in ferns (see Sheffield and Bell, 1987). Together, this body of work illustrates that exposure to light is a key trigger for inducing apogamy and that exogenous sugars (especially sucrose) are a critical metabolic requirement that also shape osmotic potential for the developing apogamous sporophytes (Bell, 1959; Whittier and Steeves, 1960, 1962; Bristow, 1962; Whittier, 1964a, 1974, 1976; Whittier and Pratt, 1971; Elmore and Whittier, 1975; Padhya and Mehta, 1981; Cordle et al., 2007). Experimental studies have demonstrated that exposure to light or to sugar, alone, is insufficient to induce apogamy (Whittier and Steeves, 1960, 1962; Whittier, 1974, 1975; Padhya and Mehta, 1981). Rather, light and a threshold concentration of sugar (dependent upon osmotic potential) are needed (Bell, 1959; Whittier and Steeves, 1960, 1962; Bristow, 1962; Whittier, 1974, 1975; Padhya and Mehta, 1981).

Once the critical roles of light and sugar on apogamous growth were established experimentally, further studies revealed that plant hormones and pheromones also play key roles in the apogamous response (Whittier, 1966; Elmore and Whittier, 1973, 1975; Menéndez et al., 2006; Tanaka et al., 2014b; Romanenko, 2020). Contrary to expectations, Elmore and Whittier (1973, 1975) demonstrated that, in the

presence of supplemental sugar, ethylene serves as a likely regulator of apogamous sporophyte development in ferns. These authors examined several distinct strains of *Pteridium aquilinum* and showed that gametophytes of all strains responded with apogamous growth in the presence of exogenous ethylene (plus light and sucrose). However, when the gametophytes were exposed only to endogenous ethylene in otherwise similar conditions, not all strains proceeded with apogamy. Elmore and Whittier (1975) hypothesized that the asymmetry of this response among lineages could be the result of genetic variation for the production of endogenous ethylene.

In addition to ethylene, subsequent studies have highlighted the critical roles of auxins and, especially, gibberellins, in the commitment to apogamy. Menéndez et al. (2006) showed that in *Dryopteris affinis* subsp. *affinis* the addition of supplemental auxin (NAA) or gibberellin (GA_3) to gametophyte culture media promoted apogamous sporophyte development. They also observed a significant increase in endogenous auxin (IAA) and gibberellin (GA_9) before differentiation of the embryo from gametophyte tissue. A significant uptick in gibberellin (GA_4) was subsequently detected during early development of the apogamous embryo, coinciding with a visible browning of the apical notch, followed by an observable increase in gibberellins GA_1 and, especially, GA_3, during elongation of the corresponding sporophyte. A more detailed understanding of the physiological mechanisms driving apogamous sporophyte development remains elusive, but comparative studies such as Menéndez et al. (2006) are scratching the surface of the apogamous response.

Alongside physiological comparisons, transcriptomic and proteomic data have provided additional comparative evidence for the roles of hormones in the commitment to apogamy. Using RNA-sequencing, Cordle et al. (2012) observed a significant upregulation of genes during the commitment to induced apogamy in *Ceratopteris* with homologs related to apogamy in angiosperm and bryophyte model systems. Similarly, the transition from one- to two-dimensional growth in apogamous gametophytes of *Dryopteris affinis* is significantly correlated with increases in the expression of genes related to reproduction, regulation of meristem growth, sucrose metabolism, and auxin signalling—as well as epigenetic regulators (Wyder et al., 2020). Complementing RNA-sequencing approaches, proteomic surveys further detected an uptick in the presence of proteins related to both reproduction and phytohormone functioning (e.g., auxin, cytokinin, ethylene, abscisic acid, brassinosteroids, and jasmonic acid) during apogamous sporophyte development (Grossman et al., 2017).

Stage 6: Fixation of meiotic obligate apomixis

We propose that the evolution of obligate apomixis in ferns is rooted in facultative processes, driven primarily by exposure to abiotic conditions that are strongly associated with arid environments, including extreme diurnal fluctuations in temperature, high light intensity, and limited water availability (Fig. 4C). We hypothesize that facultative and obligate apomixis, therefore, represent two ends of a spectrum, the latter expressing a dominant suite of adaptive traits (USP and apogamy) shaped by strong natural selection.

In ferns, distinctions between facultative and obligate apomixis are apparent in all stages of the life cycle, including unreduced spore production (USP) and apogamous sporophyte development. Facultative apomicts undergo USP irregularly, following multiple routes to sporogenesis, sometimes from the same archesporial cell (Braithwaite, 1964; Brouharmont, 1972a, b). By contrast, obligate apomicts produce unreduced spores with remarkable precision; triploids tend to be vigorously fertile, reliably yielding 32 robust, well-formed spores per sporangium, usually via premeiotic endomitosis (PE; Fig. 3).

Differences are also apparent from the start of spore germination through to gametophyte maturation and the initiation of apogamy. Facultative apomicts tend to arise from long-lived, irregular gametophytes that, despite producing functional gametangia, fail to undergo sexual reproduction (e.g., Debenedictis, 1969; Whittier, 1974, 1975; Gabancho et al., 2010). In cases of obligate apogamy, prothalli develop more rapidly than facultative or sexual equivalents (Duncan, 1941; Whittier, 1965, 1970; Gabriel y Galan, 2011; Haufler et al., 2016). One or both kinds of gametangia (archegonia and antheridia) can be absent (e.g., Whittier, 1965; Morzenti, 1966), but in many cases antheridia are functional, readily producing viable sperm (Duncan, 1943; Walker, 1962, 1985; Whittier and Steeves, 1960; Whittier, 1965, 1970; Gabriel y Galan, 2011; Liu et al., 2012), while archegonia are abortive or absent altogether (Steil, 1919; Gastony and Haufler, 1976; Laird and Sheffield, 1986).

Given these distinctions, how does obligate apomixis result from facultative incidents, especially considering that the required mutations span two independent phases of the fern life cycle—USP in the sporophyte and apogamy in the gametophyte? Convincing evidence to support the hypothesis that both obligate USP and obligate apogamy have facultative origins comes from the cheilanthoid fern *Bommeria pedata* ($2n = 3x$). Gastony and Haufler (1976) conducted cytogenetic and morphological studies of *B. pedata* and found well-formed, unreduced spores being produced through PE alongside irregular, abortive spores resulting from failed attempts at canonical sporogenesis in an odd-ploidy sporophyte ($2n = 3x$; Fig. 3). Their cytological observations proved especially valuable because most evidence for PE is derived from presumably older apomictic lineages, in which PE has long since reached fixation.

Gametophytes of *B. pedata* ($n = 2n = 3x$) also appeared developmentally intermediate; they produced functional antheridia and structurally complete archegonia with necrotic eggs, but in the absence of water resorted to producing an apogamous sporophyte ($2n = n = 3x$; Gastony and Haufler, 1976). An active antheridiogen response (Atallah and Banks, 2015) was further observed in *B. pedata* by Haufler and Gastony (1978), who proposed that young apomictic lineages may retain the (presumably redundant) antheridiogen response simply by chance, having yet to experience a loss-of-function mutation. The loss of an antheridiogen response would necessarily limit sperm production and, thus, the length of time for a reticulate apogamous complex to readily form—especially if antheridiogens can stimulate the development of functional antheridia even if the apomictic lineage does not regularly produce functional gametangia.

Work by Morzenti (1967) highlights another possible early transitional phase in the establishment of obligate apomixis. She found that spores produced by the triploid hybrid *Asplenium plenum* were mostly abortive, with the exception of a few sporangia containing up to 16 large spores, each with the sporophytic chromosome number ($n = 2n$). These spores resulted from an irregular meiosis, with only univalents observed during metaphase. Morzenti (1967) followed germination and development of the large spores, which formed gametophytes with functional gametangia and, in some cases, apogamous sporophytes, thus providing critical evidence for the dual heritability of USP and apogamy.

These examples of transitional phases leading to obligate apomixis are further bolstered by studies showing that environmentally

induced phenotypes can be epigenetically inherited (Holeski et al., 2012; Lämke and Bäurle, 2017). Epigenetic modifications, such as DNA methylation and histone modifications, can be influenced by exposure to abiotic stressors, including extreme temperature (hot or cold), desiccation, and hyperosmotic conditions (Kinoshita and Seki, 2014; Lämke and Bäurle, 2017). In angiosperms, epigenetic modifications are associated with phytohormone (e.g., ethylene) signaling (Campos-Rivero et al., 2017; Zuo et al., 2018), as well as unreduced spore production (USP) and ovule development (Grimanelli, 2012 and citations therein). However, the degree to which these epigenetic modifications are heritable, i.e., transgenerational, is mostly unexplored (Doyle and Coate, 2020). Insight from a detailed examination of DNA methylation in multiple generations of apomictic dandelions (*Taraxacum*) suggests that such epigenetic modifications are mostly heritable and are widely dispersed throughout the genomes sampled (Voerhoeven et al., 2010). Notably, Wyder et al. (2020) recently found a significant increase in the expression of genes related to epigenetic regulation during the transition to apogamy in *Dryopteris affinis* subsp. *affinis*.

Natural selection is expected to be strong in desert habitats, driving rapid adaptive evolution (Stebbins, 1952). When adaptive traits that characterize obligate apomixis in ferns (i.e., USP and apogamy) arise in a desert lineage, they should move quickly to fixation because, in their absence, sporophytes are destined to suffer the inherent, fatal challenges of a water-starved environment (Figs. 4C, 5). Once it is fixed within a lineage, obligate apomixis is often succeeded by significant range expansion, such that apomicts greatly exceed the geographical and ecological ranges of their sexual relatives (e.g., Evans, 1964; Tryon, 1968; Gastony and Haufler, 1976; Wickell et al., 2017). Even though geographical parthenogenesis is widespread among apomictic ferns, with many asexual lineages growing in wet habitats that are amenable to sexual reproduction, we argue that obligate apogamy must originate in conditions of water limitation. However, such origins can be obscured by subsequent hybridization and/or range expansion into mesic or ever-wet habitats.

MATERIALS AND METHODS

Taxon sampling

Our multilocus phylogenetic study included 46 pellaeid ingroup taxa (genera *Argyrochosma*, *Astrolepis*, *Paraceterach*, *Paragymnopteris*, and *Pellaea*) and three outgroup taxa from the genus *Myriopteris* (Appendix 1).

DNA sequencing and phylogenetic analyses

Genomic DNA was extracted from silica-dried material as described by Schuettpelz and Pryer (2007). The plastid loci *rbcL*, *atpA*, and *trnG-trnR* were amplified and sequenced using the protocols and primers outlined by Schuettpelz et al. (2006) and Sigel et al. (2011). Chromatograms were assembled and edited in Sequencher 5.4 (Gene Codes Corp., Ann Arbor, MI, USA). Seventy-three newly generated sequences have been deposited into GenBank (Appendix 1).

Sequences for each plastid region were aligned with MUSCLE (Edgar, 2004) as implemented in AliView v1.26 (Larsson, 2014), then manually refined. Ambiguously aligned regions, restricted to *trnG-trnR*, were excluded from downstream analyses. Each locus was analyzed separately under maximum likelihood (ML) in RAxML-HPC v8.2.12 (Stamatakis, 2014) on the CIPRES computing cluster (Miller et al., 2010) using the GTRGAMMA model of nucleotide substitution, the rapid hill-climbing algorithm, and 1000 bootstrap replicates. Majority-rule consensus trees from each analysis were compared to identify any highly supported topological conflicts (i.e., ML bootstrap support ≥ 70%) before concatenation.

For the three-gene data set, PartitionFinder2 v2.1.1 (Lanfear et al., 2017) was used to determine the best model of nucleotide substitution for each locus, with *rbcL* partitioned by codon position. A second ML analysis was performed on the concatenated data set in RAxML using the same parameters as described above. Finally, a Bayesian inference (BI) was performed using MrBayes v3.2.6 (Ronquist et al., 2012) on CIPRES. Four independent runs, each with four chains (one cold, three heated), were conducted for 10,000,000 generations, with trees sampled every 1000 generations. Log files were inspected in Tracer v1.7.1 (Rambaut et al., 2018) to assess both convergence and proper mixing and the first 25% of trees were discarded as burn-in. The remaining trees were used to calculate a majority-rule consensus tree and posterior probabilities (PP).

EMPIRICAL EVIDENCE FOR THE ESTABLISHMENT OF OBLIGATE APOMIXIS IN PELLAEID FERNS

The model outlined above (Figs. 1–5) is based on evidence from across ferns, but many of the critical insights stem from research on one particular clade (pellaeids). The pellaeid lineage belongs to the Pteridaceae, one of the most diverse fern families in terms of both number of species (ca. 1200; PPG I, 2016) and breadth of ecological adaptations (Schuettpelz et al., 2009). In addition to a wide variety of taxa inhabiting "typical" fern habitats (i.e., shaded forest floors), the Pteridaceae includes lineages that are emergent-aquatic (Parkerioideae), epiphytic (Vittarioideae), or have evident adaptations to xeric rupestral habitats (Cheilanthoideae; sensu PPG I, 2016). The last group, commonly referred to as cheilanthoids, includes more than 400 fern species traditionally divided among four large genera (*Cheilanthes*, *Notholaena*, *Pellaea*, and *Doryopteris*) and about 20 smaller genera (many of these nested within the larger genera, rendering them nonmonophyletic).

Among cheilanthoids, the so-called pellaeid ferns account for about 60 species that receive strong support as a monophyletic group (Fig. 6). Morphologically diverse, this clade does not correspond to any classification proposed before the advent of molecular phylogenetic studies. However, a well-supported lineage congruent with the circumscription of pellaeids adopted here was identified by Kirkpatrick (2007) and subsequently referred to as the "pellaeid clade" by Windham et al. (2009). Existing analyses provide strong support for a sister relationship between the pellaeids and the myriopterid clade (Windham et al., 2009; Grusz and Windham, 2013), justifying the choice of three myriopterid species as the outgroup for our phylogenetic reconstruction (Fig. 6).

The pellaeid ferns initially segregate into two well-supported clades (Fig. 6) consisting of the genus *Argyrochosma* (J.Sm.) Windham (with 19 accepted species; Tropicos, 2020) and a heterogeneous group referred to as "core pellaeids" by Sigel et al. (2011). The latter group includes two of the four sections of *Pellaea* [*Pellaea* and *Platyloma* (J.Sm.) Hook. & Baker] recognized by Tryon et al. (1990). Well-sampled molecular phylogenies from Kirkpatrick (2007) and Eiserhardt et al. (2011) revealed that

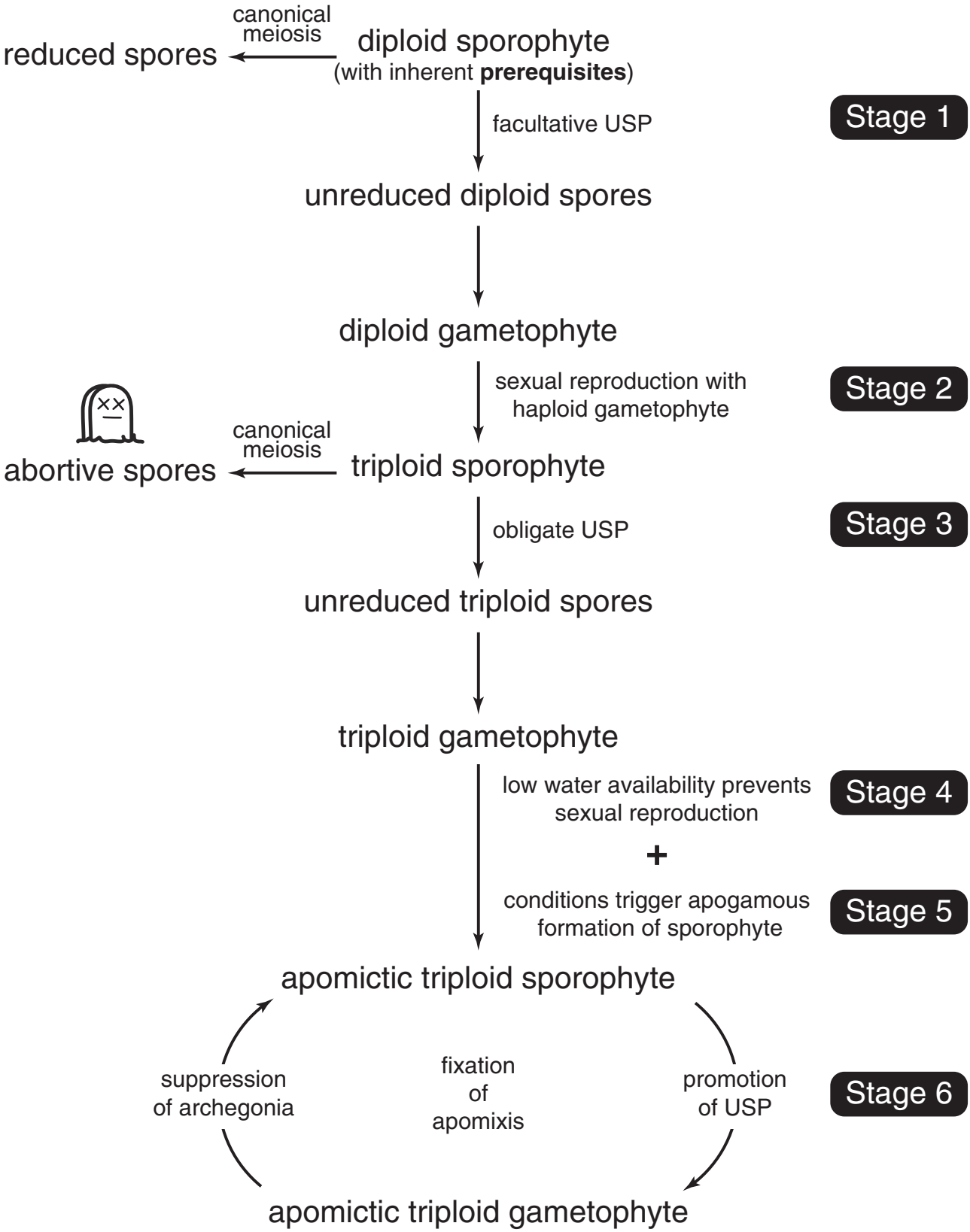

FIGURE 5. Schematic summary of the stages required for the establishment of obligate apomixis in ferns, including stepwise products and their potential alternative pathways.

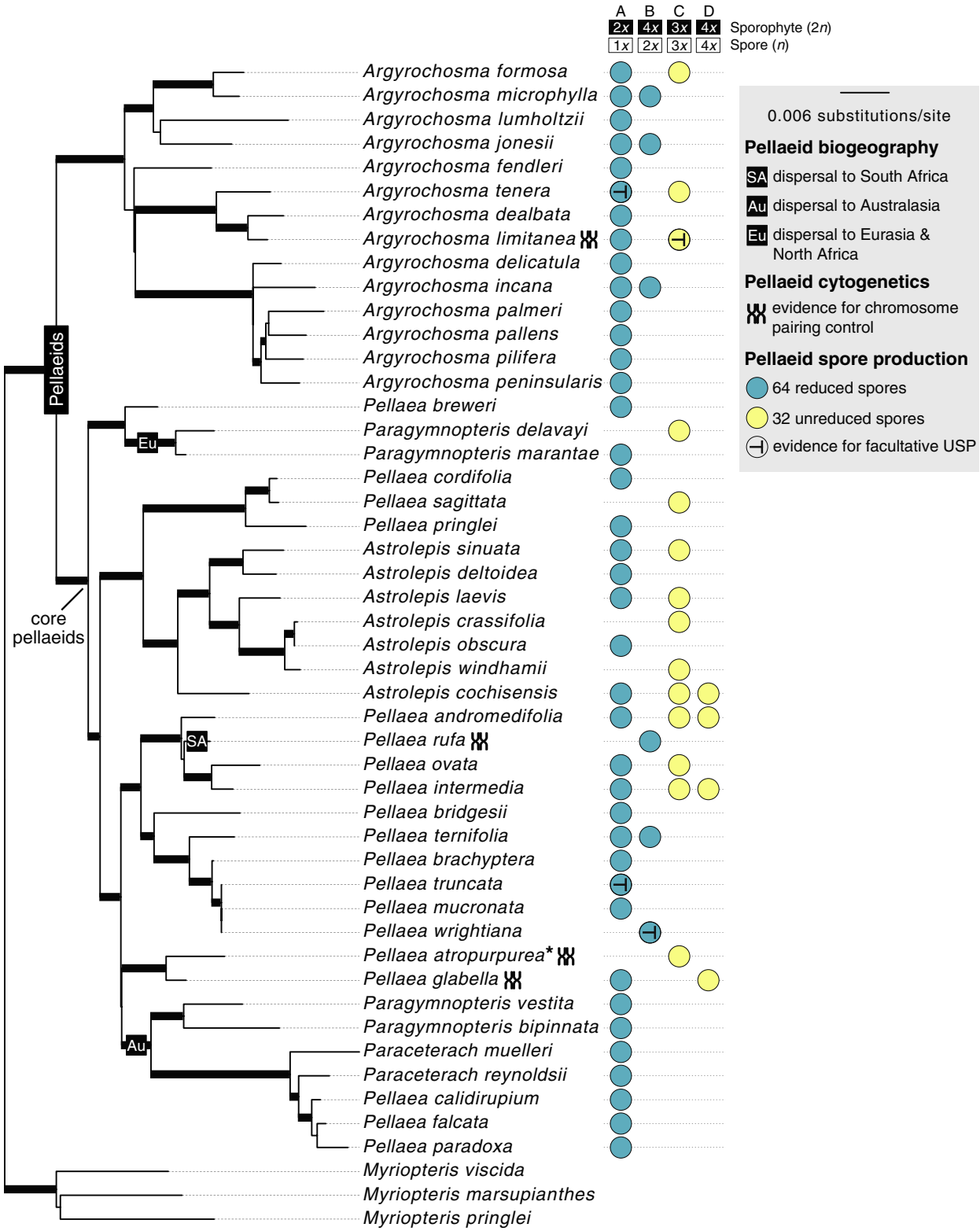

FIGURE 6. Phylogenetic relationships of 46 pellaeid taxa, inferred from maximum likelihood (ML) analysis of three plastid loci (*rbcL*, *trnG-trnR*, *atpA*). Thickened branches correspond to ML bootstrap support ≥75% and Bayesian inference posterior probability values ≥0.95. The type species of *Pellaea* (*P. atropurpurea*) is identified by an asterisk. Black boxes on branches signify known dispersal events out of the Americas. Taxa for which chromosome pairing control has been observed are noted. Spore production patterns observed in various cytotypes are indicated by colored circles, with evidence for facultative unreduced spore production (USP) marked as ⊣.

the other two species groups that Tryon et al. (1990) assigned to *Pellaea* (as sections *Holcochlaena* Hook. & Baker and *Ormopteris* (J.Sm. ex J.Sm.) R.M.Tryon & A.F.Tryon) are only distantly related and cannot be included in *Pellaea* or the broader pellaeid clade.

Among the core pellaeids, the only previously recognized groups that prove to be monophyletic are *Pellaea* section *Platyloma* (represented by *Pellaea calidirupium* Brownsey & Lovis, *P. falcata* (R.Br.) Fée, and *P. paradoxa* Hook.) and the genus *Astrolepis* D.Benham & Windham (Fig. 6). However, these two clades are nested within *Paraceterach* Copel. (recently transferred to *Pellaea* by Field, 2020) and *Pellaea* section *Pellaea,* respectively. Also nested within section *Pellaea* are four species commonly assigned to the genus *Paragymnopteris* K.H.Shing, which is, itself, nonmonophyletic. Two of these [including the type species *Paragymnopteris marantae* (L.) K.H.Shing] diverged early in the evolution of core pellaeids, whereas the other two [*Paragymnopteris bipinnata* (Christ) K.H.Shing and *P. vestita* (Hook.) K.H.Shing] are well supported as sister to the *Paraceterach*/section *Platyloma* clade. The nomenclature of the pellaeid clade remains problematic due to the continued insistence by some researchers (e.g., Fraser-Jenkins and Dulawat, 2009) that *Paragymnopteris marantae* is the type species of *Notholaena* (see Yatskievych and Smith, 2003 for counterarguments). If this position were accepted, *Notholaena* would supplant *Pellaea* as the oldest generic name applicable to the pellaeid clade.

Evidence for polyploidy and chromosome pairing control as prerequisites for apomixis in pellaeids

Pellaeid ferns exhibit extensive neopolyploidy and the distribution of ploidy levels within the clade is mapped on our phylogenetic tree (Fig. 6), that includes 46 of the ca. 60 recognized pellaeid species. Chromosome counts confirming these ploidies are available for 35 taxa (Rice et al., 2015), and the strong correlation between spore size and ploidy among pellaeids (Tryon, 1968; Beck et al., 2010; Sigel et al., 2011) allows us to infer the ploidies of the 11 remaining species. In Fig. 6, a blue circle in Column A ($2n = 2x$ sporophyte, $n = 1x$ spore) indicates that a taxon includes at least some sexual diploid individuals. Circles in Columns B–D identify species that include neopolyploid populations. Twenty-one (46%) of the taxa in this tree are either exclusively polyploid or include both polyploid and sexual diploid cytotypes.

Among the sampled species showing evidence of neopolyploidy, seven currently are known only as polyploids; these include two sexual tetraploids and five obligately apomictic triploids (Fig. 6). Fourteen additional taxa encompass both sexual diploid and sexual polyploid populations; the latter include sexual tetraploids (Column B), apomictic triploids (Column C), and apomictic tetraploids (Column D). Neopolyploidy is scattered throughout the pellaeid tree; among the major clades, it is absent only from the lineage that dispersed from the New World to Australasia (extending from *Paragymnopteris bipinnata* to *Pellaea paradoxa* in Fig. 6). It is especially prevalent among members of the nested genus *Astrolepis*, where it has been shown to be a relatively recent evolutionary phenomenon (Beck et al., 2011).

The relative youth of polyploid lineages is apparent throughout the tree, with polyploids confined to branch tips (i.e., there are no exclusively polyploid clades). The data presented in Fig. 6 indicate that there have been at least 24 origins of polyploid cytotypes among the sampled species, and each of these polyploids that have been adequately studied show evidence of multiple, independent origins (Windham, 1988, 1993b; Benham, 1989; Beck et al., 2012). Considering the two documented ancestral paleopolyploidy events (Huang et al., 2020) that resulted in the high base chromosome numbers in the clade, the occurrence of neopolyploid cytotypes in 46% of the sampled species, and the evidence for multiple origins of several of these polyploid cytotypes, it is clear that polyploidy, a key element of our drought-driven hypothesis (see Stage 1, Fig. 1), has played a central role in the evolution of pellaeid ferns.

To date, evidence of chromosome pairing control via enforced bivalent formation has been noted in four pellaeid species: *Argyrochosma limitanea* (Maxon) Windham; *Pellaea atropurpurea* (L.) Link; *P. glabella* Mett. ex Kuhn; and *P. rufa* A.F.Tryon. The last is known only as a sexual tetraploid, whereas the others encompass at least some apomictic polyploid populations (Fig. 6). Based on a combination of cytogenetic and isozyme data, Gastony (1991) reported extensive tetrasomic inheritance in tetraploid plants of *P. rufa*. Although this observation indicated that all four chromosome sets were homologous, the plants formed only bivalents during meiosis, providing strong support for the hypothesis that some form of chromosome pairing control was preventing the formation of multivalents.

Further evidence of pairing control via enforced bivalent formation in pellaeids emerges from comparative cytogenetic analyses of sporangia occurring on the same plant but following different sporogenetic pathways. This approach to understanding chromosome homology was first pursued by Manton (1950), who found an ideal subject in apomictic triploid *Pellaea atropurpurea*. She discovered that her live plant of this species was producing both types of sporangia, as illustrated here in Fig. 3. The majority had undergone premeiotic endomitosis (PE) and, as a result, the spore mother cells (SMCs) entering meiosis were hexaploid and consistently formed 87 bivalents, allowing meiosis to proceed normally and mature sporangia to produce 32 well-formed spores. However, a small number of sporangia on the same plant failed to double the chromosome number of the SMCs via PE. These SMCs entered meiosis as triploids, and their chromosome pairing was highly irregular (including multivalents and univalents), leading to meiotic failure and the production of ca. 64 malformed, nonviable spores (Fig. 3).

Rigby (1973) replicated Manton's results on additional populations of *P. atropurpurea* and conducted similar studies on *P. glabella*. The latter species includes two slightly divergent but geographically isolated sexual diploid taxa [subsp. *missouriensis* (Gastony) Windham and subsp. *occidentalis* (E.Nels.) Windham] and two similarly cryptic apomictic tetraploids [subsp. *glabella* and subsp. *simplex* (Butters) Á.Löve & D.Löve]. Again, two types of sporangia were present on individual plants. Those that began meiosis without undergoing PE contained tetraploid SMCs that formed multivalents, which prevented the successful completion of the process. By contrast, sporangia that did experience PE produced octoploid SMCs that consistently formed 116 bivalents. Thus, duplication of all chromosomes in the SMCs produced via the PE pathway had the effect of eliminating multivalents and restoring fertility.

Argyrochosma limitanea provides one final example of pairing control through enforced bivalent formation. Aside from a single inferred sexual diploid population (see Stage 1 discussion below), this species is an obligate triploid apomict that undergoes PE to produce hexaploid SMCs that consistently form 81 bivalents

during meiosis (Windham and Yatskievych, 2003). Interestingly, one plant from Coahuila, Mexico also produced sporangia that experienced two sequential PE events, resulting in dodecaploid (12*x*) SMCs. Despite a quadrupling of chromosome number, these SMCs exhibited normal pairing, forming 162 bivalents (Sigel et al., 2011). Such strict chromosomal associations in the face of rampant homology provides convincing evidence for genetic pairing control in the PE sporogenetic pathway.

Evidence for facultative unreduced spore production (USP) in pellaeids (Stage 1)

Leptosporangiate ferns (the major group to which pellaeids belong) provide a unique opportunity to investigate USP because spore number per sporangium is easily assessed in these plants and the cytogenetic processes involved in USP dramatically reduce the already finite number of spores produced in each sporangium (most commonly from 64 to 32). To date, evidence of facultative USP has been noted in four pellaeid fern species: *Argyrochosma tenera* (Gillies ex Hook.) M.Kessler & A.R.Sm., *A. limitanea*, *Pellaea truncata*, and *P. wrightiana* Hook. (Fig. 6).

***Evidence from* Argyrochosma**—*Argyrochosma tenera* is an Andean species that occupies middle to high elevation, xeric to semixeric habitats characterized by extreme diurnal temperature fluctuations that often exceed 20°C during the winter/early spring dry season. Sigel et al. (2011) sampled individuals from six populations of this taxon under the name *A. nivea* (Poir.) Windham var. *tenera* (Gillies ex Hook.) Ponce. Four of these produced an abundance of sporangia containing 32 well-formed spores in the triploid size range (averaging 64.6 μm; Sigel et al., 2011) intermixed with a few sporangia containing ca. 64 malformed, nonviable spores. These observations suggest that most plants of *A. tenera* have reached the later stages of apomictic evolution (Fig. 3), wherein all attempts at canonical meiosis (represented by the sporangia with abortive contents) fail and the only option for reproduction via spores is USP.

Sigel et al. (2011) inferred sexual reproduction in two individual plants of *A. tenera* (one each from Argentina and Bolivia), both of which showed a mix of 64- and 32-spored sporangia. In these cases, spores from 64-spored sporangia were well-formed, trilete (i.e., clearly formed in tetrads via meiosis) and similar in size to those of *Argyrochosma dealbata*, the most closely related sexual diploid species; these were interpreted as normal haploid (reduced) spores. The spores contained within 32-spored sporangia were also well-formed and trilete, but their average diameter was about 1.34–1.38× that of the putative haploid spores. This range exceeds the mean expected spore diameter increase associated with a doubling of chromosome number in the related genus *Adiantum* L. (Barrington et al., 1986) and thus strongly supports the inference that these larger spores are the unreduced products of meiosis.

Argyrochosma limitanea occurs in and around the Sonoran and Chihuahuan Deserts along the border between Mexico and the United States. Published chromosome counts and spore measurements spanning the geographic range of the species reveal that the majority of populations are obligate apomicts (Sigel et al., 2011). A single specimen from Nuevo Leon, Mexico, with 64 well-formed spores per sporangium is inferred to be a sexual diploid (Fig. 6). All other plants examined showed a predominance of sporangia containing 32 large, well-formed spores averaging 61.7 μm in diameter (Sigel et al., 2011). Cytogenetic analyses revealed that these plants are triploid ($n = 81$) and that the 32-spored sporangia had experienced a PE event, halving the number of SMCs (from 16 to eight) and doubling the chromosome number of these cells (Windham and Yatskievych, 2003). Meiosis from these momentarily hexaploid cells proceeds normally, resulting in tetrads of unreduced, trilete spores that germinate to form apomictic triploid gametophytes.

As is true of many apomictic ferns (Rigby, 1973; Walker, 1979), the triploid sporophytes of *A. limitanea* produce a small number of sporangia that fail to undergo PE and enter meiosis with three rather than six sets of chromosomes. Sporogenesis in these sporangia is unsuccessful, resulting in the production of ca. 64 malformed, mostly nonviable spores (see Fig. 3). More intriguing from the standpoint of facultative USP was the discovery of an apomictic triploid plant of *A. limitanea* from Coahuila, Mexico in which 25% of the sporangia contain 16 well-formed spores instead of the usual 32. These spores average 82.7 μm in diameter (Sigel et al., 2011), 1.23× larger than those produced by 32-spored sporangia on the same individual. Sixteen-spored sporangia are sufficiently common in this plant to be readily observed during cytogenetic analyses. These studies reveal that the 16-spored sporangia experience two sequential PE events, further reducing the number of SMCs (from eight to four) and doubling the chromosome number once again. The resultant SMCs begin meiosis as dodecaploids (Sigel et al., 2011), show strict bivalent pairing during the first meiotic division, and complete the second division by forming tetrads of unreduced, trilete spores that are hexaploid. The ultimate fate of these hexaploid spores (whether sexual or apomictic) is unknown, but they provide further evidence of a genetic predisposition for facultative USP via PE.

***Evidence from* Pellaea**—*Pellaea truncata* and *P. wrightiana* are two closely related taxa largely confined to xeric or semixeric habitats in the southwestern United States (Windham, 1993a). *Pellaea truncata* is a diploid species that is strongly xeric-adapted and one of the few ferns to occur in the low desert mountains near the mouth of the Colorado River (at elevations as low as 600 m a.s.l.). *Pellaea wrightiana*, a tetraploid that arose through hybridization between *P. truncata* and the largely Mexican species *P. ternifolia* (Windham, 1988), is somewhat less tolerant of drought and is rarely encountered below 1200 m a.s.l. Neither *P. truncata* nor *P. wrightiana* is especially cold tolerant, and they reach their upper elevational limits around 2500 m and 2900 m a.s.l., respectively (Windham, 1993a).

Both *P. truncata* and *P. wrightiana* reproduce sexually, and the vast majority of sporangia analyzed contained 64 spores (Windham, 1988). Nevertheless, plants producing a few 32-spored sporangia are occasionally encountered, and one case is especially informative with regard to possible environmental triggers for USP. *Pellaea truncata* and *P. wrightiana* often grow together, and one of these mixed populations on Elden Mountain (Arizona) has been studied in some detail (Windham, 1983, 1988). It occurs at an elevation of 2200 m a.s.l. (approaching the upper elevation limits of *P. truncata*) on a south-facing slope occupied by an entire community of plant species much more common at lower elevations. The primary environmental stressor at this site appears to be extreme diurnal temperature variation, with the exposed, rocky, south-facing slope contributing to unusually warm daytime temperatures, and high elevation, wind exposure, and low humidity promoting rapid temperature drops of 20°C or more at night.

Interestingly, the Elden Mountain population is the only one studied to date in which both *P. truncata* and *P. wrightiana* show evidence of contemporaneous USP (Windham, 1983). The critical specimens, growing a few decimeters apart, were collected on the same date after multiyear exposure to essentially identical environmental conditions in what amounts to a natural common garden experiment. Despite their divergent genetic backgrounds, the two species growing intermixed at this site produced roughly equal proportions (ca. 10%) of 32-spored sporangia. Cytogenetic analyses of the sampled sporophytes yielded the anticipated diploid and tetraploid chromosome complements expected for *P. truncata* and *P. wrightiana*, respectively. And, although chromosome counts are not available for the putative unreduced spores, their ploidy can be inferred with some degree of confidence.

In diploid *P. truncata*, the 16 SMCs from sporangia following the dominant sporogenetic pathway (reductive meiosis) exhibited 29 bivalents and produced 64 well-formed, trilete, haploid spores averaging 38 μm in diameter. Spores contained within 32-spored sporangia on these plants were morphologically similar but substantially larger (1.21×), averaging 46 μm. Although slightly less than the average spore size increase associated with a doubled chromosome number (Barrington et al., 1986), there are additional reasons to assert that these larger spores represent unreduced diplospores. Foremost among these is the fact that the putative unreduced spores produced by *P. truncata* are nearly identical in size to those contained within the 64-spored sporangia of tetraploid *P. wrightiana*. Cytogenetic studies of the dominant sporogenetic pathway (reductive meiosis) in the Elden Mountain plants of *P. wrightiana* documented that the SMCs formed 58 bivalents, and the process yielded 64 diploid spores averaging 46 μm in diameter. Spores contained within the 32-spored sporangia of these plants were substantially larger (1.17×), averaging 54 μm but their inferred status as products of USP needs to be confirmed by cytogenetic analyses.

Evidence for sexual reproduction between haploid and diploid gametophytes in pellaeids (Stage 2)

Fourteen (30%) of the pellaeid species included in our study (indicated by yellow circles in Column C of Fig. 6) include populations undergoing obligate triploid apomixis (i.e., producing well-formed spores only in 32-spored sporangia). Nine of these 14 also encompass diploid sporophyte populations that reproduce sexually, and three exhibit apomictic tetraploid populations presumably produced by crosses between the diploid and triploid cytotypes. Under the current taxonomy, all are considered autopolyploids that arose through intraspecific gametophytic crossing.

Currently, five species of pellaeids are known only as apomictic triploids. Two of these, *Pellaea atropurpurea* and *Paragymnopteris delavayi* (Baker) K.H.Shing, are inferred to be autotriploids derived from undiscovered (possibly extinct) sexual diploid cytotypes. This scenario is supported by reports of multivalent formation in the non-PE sporangia of *P. atropurpurea* (Manton, 1950; Rigby, 1973; see earlier discussion on chromosome pairing control) and the morphological isolation of both taxa, which argues against potential origins through interspecific hybridization.

Three of the five pellaeids known only as apomictic triploids are confirmed or suspected allotriploids (produced by interspecific outcrossing). Two of these [*Astrolepis crassifolia* (Houlston & T.Moore) D.M.Benham & Windham and *Pellaea sagitatta* (Cav.) Link] are digenomic allotriploids, containing two sets of chromosomes from one parental species and one set from the other. In the case of *A. crassifolia*, our plastid gene tree (Fig. 6) indicates that a recently described taxon, *A. obscura* Beck & Windham, is the maternal parent, and isozyme data (Benham, 1989) identify *A. laevis* (M.Martens & Galeotti) Mickel [formerly *A. beitelii* (Mickel) D.M.Benham & Windham] as the other parent. In the case of *P. sagitatta*, our plastid tree suggests that *P. cordifolia* (Sessé & Moc.) A.R.Sm. is the maternal parent; the other parent remains unknown. The third allotriploid in our data set has been confirmed as trigenomic, a hybrid with genetic contributions from three different parental species. This taxon (*Astrolepis windhamii* D.M.Benham) derived its plastid genome (and one third of its nuclear content) from a recent ancestor of *A. obscura* (Fig. 6). Isozyme analyses (Benham, 1989) reveal that this triploid also contains nuclear genomes derived from *A. cochisensis* (Goodd.) D.M.Benham & Windham and *A. sinuata* (Lag. ex Sw.) D.M.Benham & Windham. These observations drive home the point that apomictic triploid lineages, so common in ferns, arise from crosses between haploid and diploid gametophytes and that this process has occurred countless times during the evolution of extant pellaeid biodiversity.

Evidence for obligate unreduced spore production (USP) by triploid sporophytes in pellaeids (Stage 3)

Unreduced spores germinate to form unreduced (diploid) gametophytes that have three options to complete the life cycle: (1) undergo gametophytic selfing (Haufler et al., 2016) to produce a sexual autotetraploid; (2) regenerate a diploid sporophyte through apogamy; or (3) exchange gametes with surrounding, predominantly haploid gametophytes to produce a triploid sporophyte (Fig. 2). The first option may have produced the rare sexual autotetraploid cytotypes reported for *Argyrochosma jonesii* (Maxon) Windham and *A. microphylla* (Mett. ex Kuhn) Windham (Fig. 6), but there is no evidence at present that these species or this pathway have played a major role in the origin of apomixis among pellaeids. A diploid sporophyte resulting from the second option could go on to be sexual (and thus indistinguishable from the original sexual diploid) or apomictic; apomictic diploids are unknown among pellaeids and thus tangential to this discussion. The third option, production of triploid sporophytes through gamete exchange between rare, USP-derived (diploid) gametophytes and normal haploid gametophytes (Fig. 2) is hypothesized to be the primary pathway to apomixis among the pellaeid ferns, the majority of which show an outcrossing breeding system (Gastony and Gottlieb, 1985; Windham, 1988; Soltis and Soltis, 1992).

The resulting triploid sporophyte relies, then, on a genetic predisposition for USP to successfully complete sporogenesis. When the triploid sporophyte attempts canonical meiosis, rather than USP, the uneven pairing of homologous chromosomes (forming univalents and multivalents) will lead to the production of abortive spores. Because vegetative reproduction is limited in most pellaeid species, repetitive USP giving rise to sporangia with 32 viable triploid spores is the only real option for the long-term survival and reproduction of the lineage. Pellaeids clearly have made extensive use of this process. As indicated previously, 30% of the pellaeid species sampled in our analyses include populations exhibiting obligate triploid apomixis (Fig. 6). All show an abundance of 32-spored sporangia, as would be expected from lineages that have survived the selective bottleneck represented by Stage 3 (Fig. 3). Four taxa include apomictic tetraploids; three of these are inferred autopolyploids originating through crosses between known

sexual diploid and apomictic triploid cytotypes (with the latter contributing sperm). Only *Pellaea glabella* lacks an apomictic triploid progenitor to explain the origin of its apomictic tetraploid cytotype, but genetic studies suggest that such a "bridge" triploid does (or at least did) exist (Gastony, 1988).

Evidence that drought prevents sexual reproduction in triploid gametophytes in pellaeids (Stage 4)

Like many other members of the Cheilanthoideae, pellaeid ferns typically grow in exposed, rocky habitats that experience periodic (and often long-term) drought. Their sporophytes show a variety

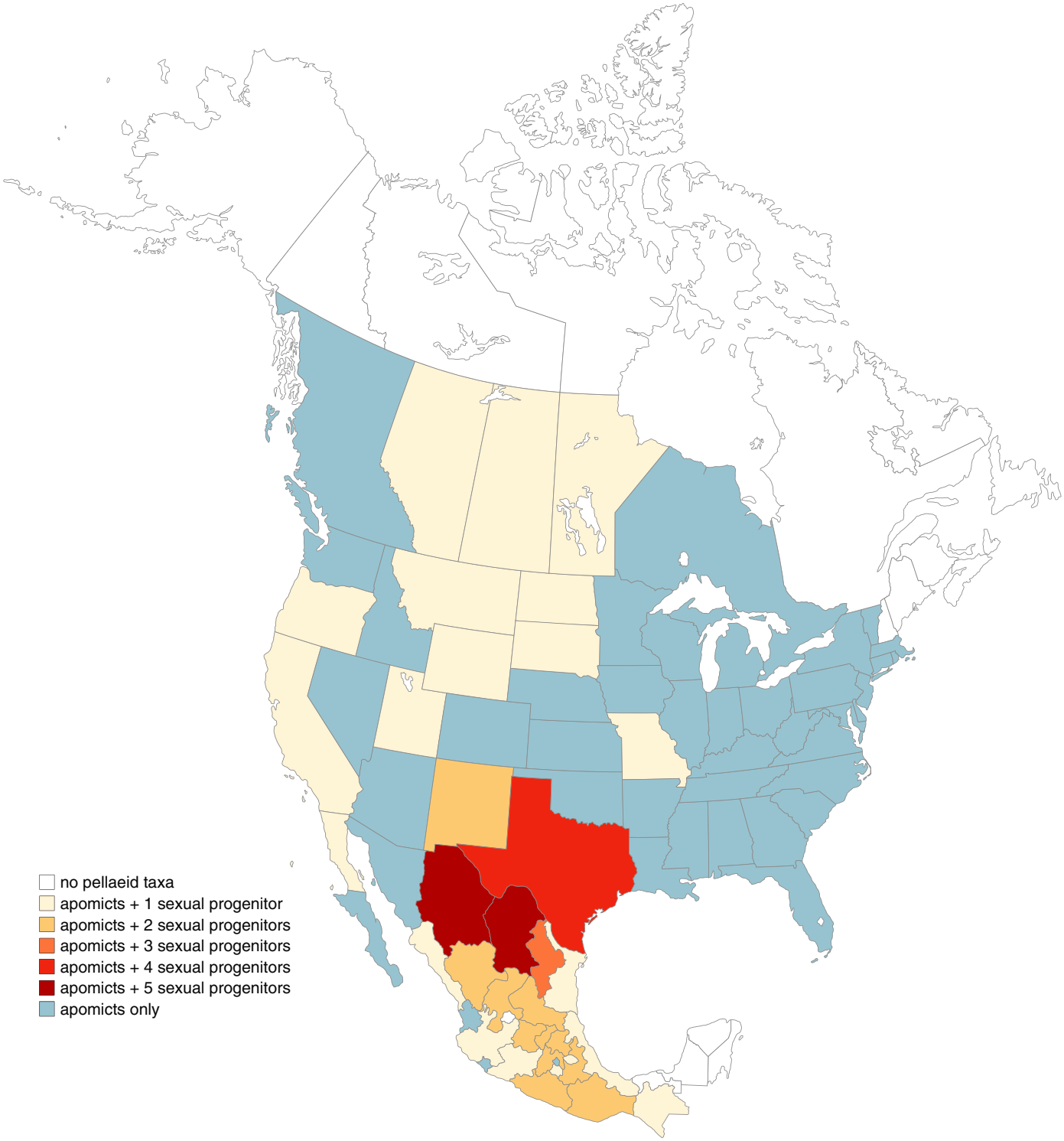

FIGURE 7. Distribution of apomictic pellaeid taxa and their sexual progenitors in Canada, the United States, and Mexico. The elevated occurrence of obligately apomictic taxa and the decreased presence of sexual progenitor taxa within increasingly drier deserts (Adams and Comrie, 1997) is notable from east to west.

of adaptations to xeric environments, among them microphylly, an unusually thick cuticle, sclerified leaf axes, and the ability to restore photosynthesis after long periods of dormancy (Hevly, 1963). The gamete-producing phase of the life cycle can survive drought conditions that kill most other plants, setting a record of 5% recovery of individual gametophytes after 5 years of desiccation (Pickett, 1931). The global distribution of pellaeids reflects the success of these adaptations, with species occurring in xeric or semi-xeric habitats on every continent except Antarctica.

Although members of the pellaeid lineage are globally distributed, species diversity is concentrated in the Americas, and it is likely that the clade originated there. The successive sister groups [the myriopterid and *Cheilanthes skinneri* (Hook.) T.Moore clades; Windham et al., 2009] are, with the sole exception of the deeply nested taxon *Myriopteris rawsonii* (Mett. ex Kuhn) Grusz & Windham, exclusively American. With regard to the distribution of pellaeids, all species occurring in other regions of the world are nested within American clades. There is evidence of four independent dispersals out of the Americas, three of which are indicated in our phylogenetic tree (Fig. 6). Two of these involve small clades of taxa that diversified in, and are endemic to, the regions to which they dispersed. The species *Paragymnopteris marantae* and *P. delavayi* represent one such lineage, which is currently confined to Eurasia/north Africa but strongly supported as sister to *Pellaea breweri* D.C.Eaton from western North America. The largest non-American clade is endemic to the Australasian region, represented in our tree (Fig. 6) by seven sampled species, including *Paragymnopteris vestita* and *Pellaea paradoxa*. There were also two dispersals giving rise to isolated endemic species: *Pellaea rufa* (known only from southern Africa; Fig. 6) and *Argyrochosma connectens* (C. Chr.) G.M.Zhang (endemic to Sichuan, China but not sampled here; see Wang et al., 2015).

Within the Americas, pellaeid distributions are heavily skewed toward North America. Only 10 species occur in South America, six of which are confined to the continent or outlying islands. By contrast, North America including Mexico has 42 recognized species, 38 of which are endemic. This region thus includes 70% of pellaeid species diversity and accounts for ca. 75% of all apomictic pellaeids, providing the best opportunity to explore the origins of apomixis. In Fig. 7, we show the distribution of pellaeid apomicts and the sexual diploids directly participating in their origins spanning Mexico, the United States, and Canada. Apomicts and their immediate progenitors (indeed, all pellaeids) are absent from the Arctic (too cold), the Maritime Provinces and parts of New England (too cold and wet), and the Yucatan (too wet). Large areas of the United States, as well as several states in Mexico, support one or more apomictic taxa but none of their sexual progenitors. The progenitors are instead heavily concentrated along the Mexico/U.S. border in the states of Chihuahua, Coahuila, and Texas (Fig. 7). This region is dominated by the Chihuahuan Desert, the largest of the three "hot deserts" of North America in which the relatively sparse precipitation (averaging 150–400 mm/yr) is largely confined to the summer months.

Based on the distribution of apomicts and their progenitors (Fig. 7), it is evident that the drier areas of the continent (see Adams and Comrie, 1997) figure prominently in both the origin and success of apomixis. One unexpected outcome of this geographic analysis is the discovery that the two hottest deserts on the continent, the Sonoran and Mohave Deserts, are devoid of diploid taxa that have given rise to apomicts. Apomicts are diverse in the Sonoran Desert, covering large portions of Sonora (six species) and Arizona (seven species), but their progenitors are absent, indicating that this region may function as an apomictic sink, providing appropriate habitat for already established apomictic lineages, but not for their origin.

The Mojave Desert is a relatively low elevation, hot desert centered in southern Nevada and eastern California (including Death Valley). Precipitation here is generally limited to the winter months and is often less dependable than that of the Chihuahuan or Sonoran Deserts. The number of pellaeids in the Mojave Desert is relatively low, and the habitat does not appear to encourage either the origin or accumulation of apomictic lineages. As seen in Fig. 7, apomictic pellaeids also occur in more temperate climates throughout much of eastern North America. However, none of the sexual diploid progenitors occur east of the Mississippi River, reinforcing the idea that extended dry periods may play a crucial role in the origin of fern apomixis. The selective establishment of apomictic pellaeids in eastern North America likely reflects the scattered distribution of suitable habitats (i.e., exposed rock outcrops), coupled with the demonstrably superior ability of apomicts to colonize distant, downwind locations based on long-distance dispersal by single spores (Peck, 1985).

Evidence for factors driving apogamous production of triploid sporophytes in pellaeids (Stage 5)

The sexual diploid progenitors of apomictic pellaeids tend to occur in habitats that are drier than those occupied by most fern species (Fig. 7). The concentration of such taxa in the Chihuahuan Desert suggests that this area represents the "cradle" of apomixis for the North American lineages and the xeric conditions that predominate there for much of the year could stall sexual reproduction for long periods, as envisioned in Stage 4 of our model. Whereas many ferns might succumb to extended drought, pellaeids have a reputation for long-term survival of gametophytes under harsh conditions (Pickett, 1931). Their resilience in the face of drought provides an extended timeline for the action of the various environmental triggers that have been shown to induce gametophytes to produce sporophytes in the absence of fertilization.

The known triggers of apogamous sporophyte production have not been extensively studied in pellaeids specifically, but most would certainly be operative in the xeric habitats occupied by the sexual diploid progenitors and their nascent triploid offspring (Fig. 4B). Abiotic triggers arise from exposure to a variety of stressful conditions, including water deficit, extreme temperature fluctuations, and intense sunlight (all common in the Chihuahuan Desert). Known biotic triggers include elevated sucrose availability (common under prolonged insolation) and ethylene accumulation (common in enclosed spaces such as rock cracks where gametophytes grow in the desert). Thus, opportunities abound for sex-starved pellaeid gametophytes to produce sporophytes without fertilization.

Evidence for fixation of meiotic obligate apomixis in pellaeids (Stage 6)

To date, there have been no targeted studies among pellaeids to ascertain whether the relative proportion of PE sporangia increases as apomictic lineages mature. Indeed, it is difficult to establish the age of (or even define) individual apomictic lineages (Beck et al., 2011). Another complicating factor is the fact that the proportion of PE

sporangia can vary considerably among leaves on the same sporophyte, influenced by variation in their exposure to environmental triggers (see Fig. 1) that occur with some frequency in the habitats occupied by both apomicts and their sexual diploid progenitors.

Nevertheless, there is some anecdotal evidence for the hypothesis that older apomictic lineages are, in general, more successful at PE than recently formed triploids. In *Pellaea*, hybrids between the diploid species *P. truncata* and tetraploid *P. wrightiana* are relatively common but restricted to the region of parental sympatry. Nearly all appear to be F1 individuals that combine the alleles of in situ parental populations (Windham, 1988). More than 99% of the sporangia produced by these triploid hybrids contain ca. 64 malformed, nonviable spores. However, a single individual from southern New Mexico exhibited a few 32-spored sporangia, and the unreduced (triploid) nature of one of these spores was confirmed by a gametophytic chromosome count of $n = 87$ (Windham, 1988), thus representing a recently formed triploid initiating Stage 3, showing early signs of facultative USP but not yet established as a self-regenerating apomict.

The above example stands in sharp contrast to the situation observed in *P. atropurpurea*. Judging from its wide distribution (Guatemala to British Columbia and Quebec), this apomictic triploid has existed for quite some time. So long, in fact, that its sexual diploid progenitor(s) are unknown (either extremely rare or extinct). As would be expected under Stage 6 of our model, this mature apomict is very successful at USP, with the proportion of sporangia containing 32 well-formed spores often exceeding 90%. Comparable levels of viable spore production have been observed in three other broadly distributed apomictic pellaeids [e.g., *Astrolepis sinuata*, *Pellaea ovata* (Desv.) Weath., and *P. sagittata* (Cav.) Link], whose ranges extend from the southwestern United States or northern Mexico to the central Andes of South America.

Among pellaeids, the apomictic cytotypes of *Pellaea andromedifolia* are thought to have originated recently based on their relative rarity (Tryon, 1968), and this group provides evidence suggesting how lineages curtail the production of functional archegonia. Although these young, geographically restricted lineages continue to produce some cordate gametophytes with some archegonia, the latter appear to be nonfunctional (Pray, 1968a). These archegoniate gametophytes mature slowly, making them noncompetitive with members of their cohort that produce sporophytes apogamously. Among older apomictic pellaeids (those whose range far exceed the distributions of their sexual diploid progenitor[s]), gametophytes almost never produce archegonia. Taxa showing this pattern include *Pellaea atropurpurea* and *P. glabella* (Pickett and Manuel, 1926; Pray, 1968b), *P. ovata* and *P. intermedia* (Tryon, 1968; Pray, 1970), and *Argyrochosma nivea* (Gabriel y Galán, 2011). In each of these examples, the failure of apomictic gametophytes to produce archegonia is most likely due to heterochrony (Pryer and Hearn, 2009). Gametophytes of sexual pellaeids require several months to mature, proceeding through a series of developmental landmarks including phase a, an initial filamentous phase; phase b, formation of a two-dimensional prothallium; phase c, initiation of a meristem leading to a classic cordate gametophyte; phase d, a unisexual phase defined by the protandrous development of antheridia; and phase e, the development of archegonia to attain bisexuality (Pray, 1968a, b, 1970).

By contrast, well-established apomictic lineages greatly abbreviate the process outlined above, rarely completing the formation of a cordate gametophyte (phase c) before initiating a new sporophyte. Antheridia often are produced, but on a shorter timeline, appearing early in phase c or even toward the end of phase b. The time from spore germination to sporophyte formation is much shorter in the apomicts, often spanning just 6 to 8 weeks, and occurring in just over 3 weeks in sterile cultures of *Pellaea glabella* (Whittier, 1968), where 50% of apomictic gametophytes had initiated sporophytes before the appearance of any archegonia on the sexual diploid progenitor. These observations are congruent with progenesis, a form of heterochrony in which development to the mature state (in this case, initiation of a sporophyte) is accelerated by decreasing the age of maturation (Pryer and Hearn, 2009). Progenesis is common among organisms that occupy habitats wherein the availability of critical resources (such as water) is unpredictable (Gould, 1977; Stearns, 1992). Under such circumstances, the benefits of early maturation are obvious, and thus, it is not surprising that this process may play a significant role in the establishment of apomixis among pellaeid ferns.

CONCLUSIONS

Historically, the relationship between drought and obligate apomixis in ferns has been considered circumstantial, centered on the idea that, although apomicts do exceedingly well in dry environments, their origins are not necessarily linked directly to these habitats. Based on the evidence presented here, we instead propose that this relationship is most likely a causal one, in which the abiotic conditions that characterize water-starved environments actually drive the adaptive evolution of unreduced spore production (USP) and apogamy, and, consequently, the fixation of obligate apomixis in ferns.

Biogeographical studies are consistent with this hypothesis, revealing that obligately apomictic fern lineages are significantly more common in environments that are drought-prone or at least seasonally water-limited (e.g., monsoonal; Liu et al., 2012; Tanaka et al., 2014a; K. T. Picard et al., in revision). Additional studies focused on the geographical distribution of reproductive mode and ploidy level in ferns are, however, needed. Combining these with phylogenetic and ecological data (especially related to water availability) will almost certainly reinforce this association. Future studies should also focus on the uneven phylogenetic distribution of obligate apomixis across ferns, examining whether lineages with a higher incidence of apomixis face more extreme hydrological conditions (e.g., as epiphytes). Moreover, studies should determine if an antheridiogen response system plays a critical role in the proliferation of obligate apomixis by inducing sperm production in reticulate species complexes; or, if a genetic propensity for prothallial thickening shapes the irregular phylogenetic distribution of this syndrome. It is our hope that the model provided here, complemented with extensive evidence from the pellaeids, will serve as a long-overdue basis for examining the evolutionary origins, and implications, of obligate apomixis in ferns.

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AUTHOR CONTRIBUTIONS

This project was conceived of and organized by A.L.G., with project development contributed by M.D.W. and K.M.P. Data and analyses were undertaken by K.P., A.L.G., and E.S. Manuscript preparation, including writing, editing, and revision was done by A.L.G., M.D.W., K.P., K.M.P., E.S., and C.H.H.

DATA AVAILABILITY

DNA sequences used in this study are deposited in GenBank (Appendix 1).

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APPENDIX 1. Sample information. ***Taxon*** Authority: Fern DNA database number, *Voucher* (Herbarium), Locality, GenBank accessions for *rbcL*, *atpA*, *trnG-trnR*, in that order.

Argyrochosma dealbata (Pursh) Windham: 4562, *Brooks 16997* (DUKE), USA, HQ846421, HQ846372, HQ846468; ***Argyrochosma palmeri*** (Baker) Windham: 6384, *Correll 28817* (MICH), Mexico, HQ846458, HQ846410, HQ846463; ***Argyrochosma peninsularis*** (Maxon & Weath.) Windham: 6225, *J.L.L de la Luz 9784* (MO), Mexico, HQ846440, HQ846391, HQ846490; ***Argyrochosma delicatula*** (Maxon & Weath.) Windham: 4561, *Windham 482* (DUKE), Mexico, HQ846420, HQ846371, HQ846469; ***Argyrochosma fendleri*** (Kunze) Windham: 3776, *Metzger et al. 120* (DUKE), USA, HQ846413, HQ846363, FN565504; ***Argyrochosma formosa*** (Liebm.) Windham: 4560, *Windham 539* (DUKE), Mexico, HQ846419, HQ846370, HQ846471; ***Argyrochosma incana*** (C. Presl) Windham: 3198, *Schuettpelz 491* (DUKE), USA, EU268771, HQ846362, HQ846472; ***Argyrochosma jonesii*** (Maxon) Windham: 3844, *Windham 3437* (DUKE), USA, EU268772, HQ846365, HQ846473; ***Argyrochosma limitanea*** (Maxon) Windham: 3179, *Schuettpelz 472* (DUKE), USA, HQ846412, HQ846360, EU268668; ***Argyrochosma lumholtzii*** (Maxon & Weath.) Windham: 4974, *Fishbein 4458* (MO), Mexico, HQ846424, HQ846375, HQ846475; ***Argyrochosma microphylla*** (Mett. ex Kuhn) Windham: 4583, *Worthington 34623* (DUKE), USA, HQ846423, HQ846374, HQ846476; ***Argyrochosma tenera*** (Gillies ex Hook.) M.Kessler & A.R.Sm.: 5855, *Beck St.G. 23950* (UC), Bolivia, HQ846432, HQ846383, HQ846480; ***Argyrochosma pallens*** (Weath.) Windham: 5054, *Yatskievych 89-280* (IND), Mexico, HQ846426, HQ846377, HQ846481; ***Argyrochosma pilifera*** (R.Tryon) Windham: 5055, *Yatskievych 89-287* (MO), Mexico, HQ846427, HQ846378, HQ846482; ***Astrolepis cochisensis*** Benham: 4716, *Windham 3490* (UT), USA, MW057268, MW057292, MW057316; ***Astrolepis crassifolia*** (Houlston & T.Moore) D.M.Benham & Windham: 4553, *Windham 494* (DUKE), Mexico, MW057269, MW057293, MW057317; ***Astrolepis deltoidea*** (Baker) J.Beck & Windham: 5828, *Hatch 279* (US), Guatemala, MW057270, MW057294, MW057318; ***Astrolepis laevis*** (M.Martens & Galeotti) Mickel: 5060, *Yatskievych & Gastony 89-265* (IND), Mexico, MW057271, MW057295, MW057319; ***Astrolepis obscura*** J.Beck & Windham: 6143, *Pérez 3559* (NY), mexico, MW057272, MW057296, MW057320; ***Astrolepis sinuata*** (Lagasca ex Swartz) D.M.Benham & Windham: 5831, *Correll P795* (US), Peru, JQ855927, JQ855915, JQ855898; ***Astrolepis windhamii*** D.M.Benham: 3138, *Schuettpelz 431* (DUKE), USA, KF961768, KF961705, JF929936; ***Myriopteris marsupianthes*** (Fée) T.Reeves ex Mickel & A.R.Smith: 6158, *Jankiewicz 13* (UC), Mexico, KF961803, KF961739, KF961864; ***Myriopteris pringlei*** (Davenp.) Grusz & Windham: 3209, *Schuettpelz 502* (DUKE), USA, HM003031, HM003027, HM003035; ***Myriopteris viscida*** (Davenp.) Grusz & Windham: 3822, *Metzger et al. 169* (DUKE), USA, KF961821, KF961757, KF961880; ***Paraceterach muelleri*** (Hook.) Copel.: 4979, *van der Werff 11472* (MO), Australia, MW057273, MW057297, MW057321; ***Paraceterach reynoldsii*** (F.Muell.) Tindale: 6193, *Albrecht 5405* (MEL), Australia, MW057274, MW057298, MW057322; ***Paragymnopteris bipinnata*** (Christ) K.H.Shing: 6076, *Rothfels 2713* (DUKE), Cultivated, MW057275, MW057299, MW057323; ***Paragymnopteris delavayi*** (Baker) K.H.Shing: 4565, *Yatskievych 32964* (MO), China, HQ846422, HQ846373, HQ846467; ***Paragymnopteris marantae*** (L.) R.M.Tryon: 3736, *Yatskievych et al. 12816* (MO), China, EF452161, EU268763, EU268711; ***Paragymnopteris vestita*** (Wall. ex C.Presl) K.H.Shing: 2976, *Schuettpelz 331* (DUKE), Cultivated, MW057276, MW057300, MW057324; ***Pellaea andromedifolia*** (Kaulf.) Fée: 5084, *Gastony 86-8* (IND), USA, MW057277, MW057301, MW057325; ***Pellaea atropurpurea*** (L.) Link: 2957, *Schuettpelz 312* (DUKE), Cultivated, EF452162, JQ855925, JQ855913; ***Pellaea brachyptera*** (T.Moore) Baker: 5623, *Oswald 8751* (JEPS), USA, MW057278, MW057302, MW057326; ***Pellaea breweri*** D.C. Eaton: 3930, *Windham 3447* (DUKE), USA, EU268808, EU268764, EU268712; ***Pellaea calidirupium*** Brownsey & Lovis: 4980, *Lovis s.n.* (IND), New Zealand, MW057279, MW057303, MW057327; ***Pellaea cordifolia*** (Sessé & Moc.) A.R.Sm.: 666, *Gastony 87-3* (IND), USA, MW057280, MW057304, MW057328; ***Pellaea falcata*** (R. Br.) Fée: 3892, *Nagalingum 21* (DUKE), Australia, MW057281, MW057305, MW057329; ***Pellaea glabella*** Mett. ex Kuhn: 5085, *Gastony 83-41* (IND), USA, MW057282, MW057306, MW057330; ***Pellaea intermedia*** Mett. ex Kuhn: 3188, *Schuettpelz 481* (DUKE), USA, EF452163, EU268765, EU268713; ***Pellaea mucronata*** (D.C.Eaton) D.C.Eaton: 3834, *Metzger et al. 181* (DUKE), USA, MW057283, MW057307, MW057331; ***Pellaea ovata*** (Desv.) Weath.: 5058, *Gastony & Yatskievych 86-45* (IND), USA, MW057284, MW057308, MW057332; ***Pellaea paradoxa*** Hook.: 3909, *A. Ford 3421* (NSW), Australia, MW057285, MW057309, MW057333; ***Pellaea pringlei*** Davenp.: 5028, *Steinmann 4728* (MO), Mexico, MW057286, MW057310, MW057334; ***Pellaea rufa*** A.F.Tryon: 5047, *Bean B-1 s.n. 5* (IND), South Africa, MW057287, MW057311, MW057335; ***Pellaea sagittata*** (Cav.) Link: 5029, *Steinmann 1939* (MO), Mexico, MW057288, MW057312, MW057336; ***Pellaea ternifolia*** (Cav.) Link: 4478, *Schuettpelz 992* (DUKE), Ecuador, MW057289, MW057313, MW057337; ***Pellaea truncata*** Goodd.: 3137, *Schuettpelz 430* (DUKE), USA, EF452164, EU268766, EU268714; ***Pellaea wrightiana*** Hook.: 4912, *Windham & Yatskievych 780* (DUKE), USA, MW057290, MW057314, MW057338; ***Pellaea bridgesii*** Hook. ex Baker: 7581, *P. Alexander 1025* (DUKE), USA, MW057291, MW057315, MW057339.