Sex and the Single Gametophyte: Revising the Homosporous Vascular Plant Life Cycle in Light of Contemporary Research

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Homosporous vascular plants are typically depicted as extreme inbreeders, with bisexual gametophytes that produce strictly homozygous sporophytes. This view is promulgated in textbook life cycles despite ample evidence that natural populations of most species regularly outcross. We review research on a variety of mechanisms, including genetic load, asynchronous production of eggs and sperm, and pheromonal control of gamete production, that actively promote heterozygosity in ferns and lycophytes. Evolution of the land plants cannot be reconstructed without accurate depictions of the unique life cycle that has helped make ferns the second most diverse lineage of vascular plants on Earth. With revised illustrations and definitions, we provide scientists, educators, and students with a contemporary understanding of fern and lycophyte reproduction, revealing them as evolutionarily dynamic and exploiting a wide range of mating systems.

Keywords: ferns, breeding systems, life cycles, genetics, botany

L ife cycles provide one of the most effective conceptual frameworks for exploring the evolution of land plants (Qiu et al. 2012). More than a century and a half ago, Hofmeister (1851) described the life cycles of various land plants, identifying common elements as well as unique features that characterize each major lineage. This led to a revolution in plant classification wherein most major groups could be defined by distinctive aspects of their gametophytes and sporophytes (see box 1 for definitions of key words). In this system, ferns and lycophytes are distinguished from all other plants by a life cycle featuring fully independent gametophyte and sporophyte phases (also referred to as generations), with the sporophyte being dominant (i.e., the most obvious and generally the longest lived). Recent classifications based on fossil evidence and molecular phylogenetic studies have only reinforced the conclusion that changes in life cycles have been a major correlate and driver of evolution in plants (Gerrienne and Gonez 2011, Qiu et al. 2012).

Despite the scientific and pedagogical value of this evolutionary construct, many students struggle to comprehend the "alternation of generations" (Sheffield 2008) and other essential features of land plant life cycles. In part, the challenge for students involves the fundamental differences between the meiotic products of animals and plants. In animals, meiosis directly yields unicellular gametes (eggs and sperm); in plants, meiosis yields spores. Before plants can produce gametes, the spores must germinate and divide mitotically to produce multicellular gametophytes. These gametophytes, in turn, mature and develop gametangia: in ferns and lycophytes, the sperm-releasing antheridia and egg-containing archegonia. As mammals with a built-in capacity for self-awareness, students identify more readily with the animal life cycle than that of plants and are baffled in particular by the gametophyte phase.

Compounding student confusion is the traditional textbook depiction that inbreeding predominates in homosporous vascular plants (i.e., most ferns and some lycophytes). From early childhood, students learn that inbreeding is detrimental and a social taboo. But pick your favorite textbook (e.g., Graham et al. 2006, Mauseth 2012, Evert and Eichorn 2013, Reece et al. 2014), turn to the figure of the fern life cycle, and there, at the center of it all, is likely a single bisexual gametophyte simultaneously producing both eggs and sperm (see Morris et al. 2013 for an improved, albeit too simplified, alternative). When these mitotically formed and genetically identical gametes fuse during fertilization, the product is a zygote that is homozygous at all loci

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Box 1. Glossary.

Alternation of generations: A reproductive cycle characteristic of plants that alternates between two distinct, multicellular phases. The sporophyte phase produces spores (usually through meiosis). The spore-derived gametophyte phase produces gametes, usually has a chromosome number half that of the sporophyte, and initiates new sporophytes through gametic fusion (but see *apomixis* below).

Apomixis: Reproduction that does not involve gametic fusion (i.e., fertilization or syngamy) or a change in ploidy. In apomictic ferns, new sporophytes arise directly from gametophytic tissue.

Agamospermy: Production of a new sporophyte directly from a gametophyte without syngamy.

Archegonium (plural = archegonia): A gametangium that produces an egg.

Antheridiogen: A pheromone usually generated by a mature (meristematic) gametophyte that induces nearby immature (ameristic) gametophytes to become precociously and exclusively male.

Antheridium (plural = antheridia): A gametangium that produces sperm.

Gametangium (plural = gametangia): Structures formed by gametophytes that produce and enclose gametes (eggs and sperm).

Gametophyte: In ferns (and other homosporous vascular plants), a multicellular, independent organism that usually produces sperm- and egg-forming gametangia (antheridia and archegonia respectively): can be unisexual or bisexual (hermaphroditic).

Gametophytic selfing: Formation of a zygote through the fusion of genetically identical egg and sperm produced by a single bisexual (hermaphroditic) gametophyte.

Genetic load: A calculation of the fitness reduction of an average individual in a population owing to the presence of deleterious genes.

Heterosporous: Describes species whose sporophytes produce two different kinds of spores: megaspores that yield only egg-producing female gametophytes and microspores that yield only sperm-producing male gametophytes.

Homosporous: Describes species whose sporophytes produce only one kind of spore, each of which germinates to yield a unisexual or bisexual (hermaphroditic) gametophyte.

Mixed mating: Describes reproduction in hermaphroditic plant species in which both selfing and outcrossing occur.

Spore: A propagule produced by a sporophyte, usually through meiosis in ferns and other land plants. Spores germinate to yield multicellular gametophytes.

Sporophyte: A multicellular plant that produces spores.

Sporophytic outcrossing: Formation of a zygote through fusion of genetically distinct egg and sperm that are produced by separate gametophytes arising from spores of two different sporophytes.

Sporophytic selfing: Formation of a zygote through fusion of genetically distinct egg and sperm that are produced by sibling gametophytes arising from spores of the same sporophyte.

(i.e., lacking any allelic variation). The natural assumption, often reinforced by text associated with the figure, is that homosporous vascular plants regularly initiate sporophytes in this manner, which is the most extreme form of inbreeding possible (see figure 1, gametophytic selfing). Given these textbook diagrams, why shouldn't students view ferns as an evolutionary dead end? Without genetic variation, how have homosporous vascular plants survived, much less diversified?

Only recently have we fully appreciated the disparity between the fern life cycle traditionally presented in textbooks and what is actually happening in nature. Indeed, through the 1970s, most studies of fern breeding systems seemed to support the apparent paradox: Controlled laboratory experiments involving isolated gametophytes showed that most became bisexual and produced sporophytes (reviewed in Lloyd 1974b, Klekowski 1979). However, the relevance of these laboratory observations to natural populations was unclear. In the 1980s, with the advent of isozyme genetics, it became possible to actually assess genetic variation in fern sporophyte populations. These studies revealed that, despite the *potential* for "gametophytic selfing," nearly all sporophytes in natural populations of most diploid fern species arose through outcrossing between eggs and sperm from two genetically different gametophytes (Soltis DE and Soltis PS 1992, Ranker and Geiger 2008). In hindsight, this result should not have been too surprising: The very same laboratory studies of fern gametophytes that indicated the occurrence of extreme inbreeding through gametophytic selfing also revealed mechanisms that could promote outcrossing. For example, the sequence of gametangial maturation appeared to favor temporally unisexual gametophytes, and some species possessed a pheromonal (antheridiogen) system that established populations of largely unisexual gametophytes (reviewed by Lloyd 1974b). Nonetheless, just the *possibility* of bisexual gametophytes

and the associated proximity of antheridia and archegonia continue to dominate textbook depictions of ferns as consummate inbreeders.

With this article, we seek to set the record straight and illustrate a revised life cycle based on the results of contemporary research on the breeding systems of ferns and lycophytes. The facts are clear: Studies of ferns reveal that most are characterized by the capacity for both inbreeding and outcrossing and quite effectively maintain the genetic diversity necessary for long-term evolutionary success (reviewed by Sessa et al. 2016).

Revealing the complex possibilities of the homosporous vascular plant life cycle

The life cycles of all sexually reproducing land plants alternate between two distinct *generations*, or phases: a gametophytic (*n*) phase and a sporophytic (*2n*) phase that are produced by meiosis (reduction division) and syngamy (fertilization), respectively (Qiu et al. 2012). Each of these phases is multicellular, but they vary (among major lineages) in duration and size (see figure 2 in Qiu et al. 2012, figure 16 in Gerrienne and Gonez 2011). The exact relationships of the major bryophyte lineages (mosses, liverworts, and hornworts) to one another and to vascular plants are still unresolved (Wickett et al. 2014), but all three bryophyte lineages feature a long-lived gametophyte phase and an ephemeral sporophyte phase that is nutritionally dependent on the more prominent gametophyte. The origin of vascular plants was apparently accompanied by a shift in life cycle whereby the sporophyte became the *dominant* (larger, longer-lived) phase. In seed plants, gametophytes are always unisexual, with the female gametophytes attached to (and nutritionally dependent on) sporophytes. In *seed-free* homosporous vascular plants (lycophytes and ferns), gametophytes are diminutive but nonetheless fully independent of the sporophytes and potentially bisexual. Within vascular plants, ferns are sister to seed plants, together forming a large euphyllophyte clade (Pryer et al. 2001), and lycophytes are, in turn, sister to euphyllophytes. Therefore, gametophytic independence is inferred to be the plesiomorphic character state for the euphyllophytes, whereas gametophytic dependence in the seed plant lineage is derived.

Homosporous ferns have a wide range of breeding system options (figure 1). At one extreme, some species do indeed regularly exhibit gametophytic selfing (e.g., Lloyd 1974a). At the other end of the spectrum, some species are obligate outcrossers and have redundant mechanisms to promote such a breeding system (e.g., Haufler and Soltis 1984). Recent work has shown that most homosporous ferns (up to 70% of the species examined) are capable of initiating sporophyte progeny *in vitro* via gametophytic selfing as well as via sporophytic selfing or sporophytic outcrossing (Sessa et al. 2016). Demonstrating such mating system flexibility in laboratory cultures highlights crucial options that may be exploited in nature to maximize success in the face of varying ecological conditions. In fact, the flexible homosporous

life cycle can be seen as a significant advantage over the more limited and canalized alternatives available to seed plants. Although it is true that gametophytic selfing can have negative evolutionary consequences, its occasional occurrence in species with low genetic load may be an effective ecological strategy for single-spore migration. The potentially bisexual gametophytes of homosporous ferns open the door to gametophytic selfing (see figure 1), an option simply unavailable to seed plants with their strictly unisexual gametophytes. In combination with much smaller dispersal units (spores averaging 50 micrometers versus seeds typically much more than 200 micrometers long), gametophytic selfing can give homosporous ferns an advantage in long distance dispersal and the establishment of new populations (e.g., Ranker et al. 1994). In this sense, the evolution of the seed, with all of its demonstrated benefits, nonetheless can be viewed as a narrowing of the reproductive niche space.

To gain perspective on the origin and diversification of land plants, students need to appreciate the dynamic evolutionary history of the plant life cycle and be familiar with the variations that characterize each major lineage. Here, we focus on how the homosporous vascular plants (more than 99% of ferns and about one-third of lycophytes) have explored and exploited a set of morphological, physiological, ecological, and genetic innovations to maintain and expand their evolutionary potential over nearly half a billion years of Earth's history. The primary mating system options available to homosporous vascular plants are contrasted in table 1, and several attributes that can affect breeding systems are discussed below.

Gametophyte ontogenesis. The elusive and often ephemeral nature of the diminutive gametophyte phase is one of the reasons seed-free vascular plants were previously referred to as *cryptogams*, reflecting the mysterious nature of their reproductive biology. In the early twentieth century, F. O. Bower, a pioneer of fern biology, published a monumental three-volume summary of the state of knowledge of the ferns. In that work, Bower (1923–1928) stated that "the constituent tissues of the gametophyte in the Filicales [ferns] show a much lower scale of differentiation, while its external form, which is without any regular sequence or relation of parts, is frequently very simple" and "these facts detract heavily from the value of the gametophyte for comparative purposes" (vol. 1, p. 273). He did admit, however, "that knowledge of the prothalli [gametophytes] of ferns is far less complete than that of the sporophyte-plant, and present views may be subject to drastic revision as the facts are more fully disclosed by further investigation." Indeed, subsequent detailed work by A. G. Stokey and L. R. Atkinson (summarized in Atkinson 1973) and Nayar and Kaur (1971) demonstrated that the gametophyte phase could yield valuable perspectives and characters for reconstructing the evolutionary history of ferns.

All the same, ontogenetic and morphological studies were based almost exclusively on laboratory cultures obtained by
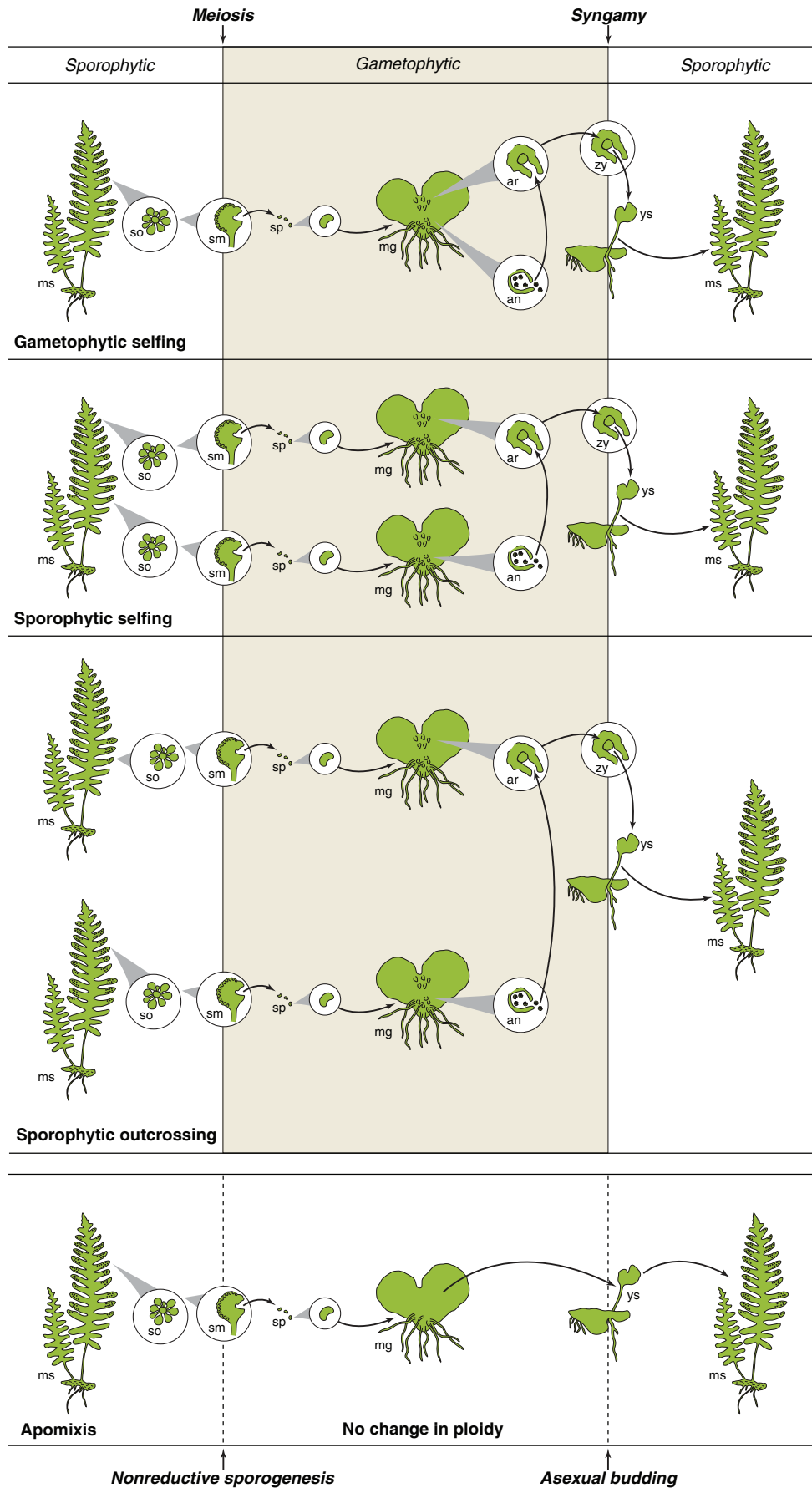
Table 1. Expectations at the ends of the spectrum of breeding-system options in sexually reproducing, diploid, homosporous vascular plants.	
Gametophytic Selfing	**Sporophytic Outcrossing**
Gametophytes bisexual: gametangia appear simultaneously	Gametophytes unisexual or with delayed bisexuality (gametangia appear sequentially)
Genetic load absent	Genetic load substantial
Antheridiogen system absent	Antheridiogen system present
Gametophyte isolate potential high	Gametophyte isolate potential low
Populations with little genetic variability	Populations with substantial genetic variability

Note: The gametophyte ontogenetic patterns, numbers of lethal genes, pheromonal systems, gametophyte isolate potential, and levels of variability anticipated under extreme inbreeding (gametophytic selfing) versus outcrossing systems are contrasted. See the text for more discussion.

germinating spores in vitro. Although informative about the structure of gametangia and, in some cases, the sequence of gametangial maturation, these studies could not shed much light on the reproductive biology of natural populations. A more complete understanding of the effects of asynchronous gametangial initiation (promoting temporally unisexual gametophytes) on breeding systems arose from later studies of gametophyte reproductive biology (summarized in Lloyd 1974b, Klekowski 1979, and Cruden and Lloyd 1995). Likewise, in developing a key to gametophytes in some natural populations and contrasting laboratory and field observations of gametophyte populations, Cousens and colleagues (1985) provided a glimpse of what information could be gained through field studies. They further noted that the rarity of *safe sites* (areas suitable for the growth of gametophytes) led to the concentration of gametophytes into distinct populations, which might explain the frequent co-occurrence of developmentally and genetically different individuals. Together, these studies demonstrated that coordinating the study of natural populations of both sporophytes and gametophytes produces a much more complete and dynamic picture of the factors contributing to the distribution and maintenance of genetic diversity in ferns.

Genetic load. In the late 1960s, Edward Klekowski launched a series of mating studies (Klekowski 1967, 1969a, 1969b) to test his hypotheses about fern genetic systems (Klekowski and Baker 1966, Klekowski 1973). His goal was to demonstrate that ferns harbored little genetic load (few deleterious

genes) and therefore were able to initiate sporophytes from a single gametophyte in which the eggs and sperm were genetically identical. In most cases, Klekowski was able to demonstrate that at least some isolated gametophytes of the many species he tested were able to initiate new sporophytes (e.g., Klekowski 1970a, 1970b). Concluding that ferns were habitual inbreeders, Klekowski hypothesized that these plants evolved high chromosome numbers (and active polyploid genomes) to buffer them against the genetic consequences of the extreme inbreeding resulting from frequent gametophytic selfing (Klekowski 1973). These observations appeared consistent with the idea that the breeding systems of homosporous ferns and lycophytes were fundamentally different from those of other vascular plants. However, just as ontogenetic studies showed asynchrony in the initiation and maturation of gametangia, there was also evidence of significant genetic load in some fern species (Lloyd 1974b). Nonetheless, the notion of ferns as extreme inbreeders was established and continued to hold sway over rising contradictory evidence for the next several decades.

Antheridiogen and the control of sex expression. In the middle of the twentieth century, Döpp (1950) reported that reusing growth media that had supported female gametophytes of bracken fern (*Pteridium aquilinum*) induced precocious antheridium formation in developing gametophytes of *Pteridium*, as well as in those of the wood fern *Dryopteris filix-mas*. Subsequent workers (e.g., Näf 1962, Voeller 1964, 1971) showed that these and several additional fern species

Figure 1. Life-cycle variants observed in homosporous vascular plants. In sexually reproducing species, spores are produced in sporangia by meiosis. These spores germinate and divide mitotically to form multicellular gametophytes that are photosynthetic and nutritionally independent. The lower surfaces of mature gametophytes bear flask-shaped archegonia and spherical antheridia that produce eggs and sperm, respectively, by mitosis. When an egg is fertilized, a zygote is formed that divides mitotically and differentiates into a mature sporophyte, which is rooted in the ground and maintains itself by photosynthesis. In asexual (apomictic) species, sporophytes yield unreduced spores that germinate and form independent gametophytes mitotically; sporophytes are initiated on the gametophytes without syngamy. The background shading indicates relative ploidy level (no shading, sporophytic ploidy level resulting from past or present syngamy; shading, gametophytic ploidy level produced by meiosis). Sessa and colleagues (2016) included an alternative (circular) representation of the sexual life cycle variants depicted here. Abbreviations: an, antheridium; ar, archegonium; mg, mature gametophyte; ms, mature sporophyte; sm, sporangium; so, sorus; sp, spore; ys, young sporophyte; zy, zygote.

produced and responded to a pheromonal substance that came to be known as *antheridiogen*. In multispore cultures of antheridiogen-sensitive species, the earliest-maturing gametophytes (those with defined meristems) typically become archegoniate (female) and secrete antheridiogen into the substrate, inducing adjacent, less developed gametophytes to become exclusively antheridial (male). As reviewed by Schneller and colleagues (1990), there are obvious breeding system implications associated with the ability of an individual sporophyte to control the sex ratio of the gametophytes formed from its spores in a given population (Schneller 2008, Tanaka et al. 2014). Unfortunately, owing to the challenge of studying gametophytes in nature, there have been few attempts to demonstrate the operation of antheridiogen in situ (but see Tryon and Vitale 1977). Nonetheless, by correlating laboratory studies of gametophyte development with population genetic analyses of natural sporophyte populations, it has been possible to show that species producing and responding to antheridiogen are highly outcrossing (e.g., *Bommeria hispida*; Haufler and Soltis 1984), whereas species whose gametophytes show variation in response to antheridiogen have sporophyte populations with variable levels of outcrossing (e.g., *Hemionitis palmata*; Schneller et al. 1990).

The spores of many ferns require light to germinate, and those that penetrate far enough into the soil horizon to avoid light become part of a "spore bank" (Dyer and Lindsay 1992). In addition to affecting sex ratios in susceptible fern populations, antheridiogen can also trigger dark germination of spores that lie dormant in spore banks. Once they germinate in the dark, these subterranean, antheridiogen-activated spores produce small, male gametophytes whose sperm can fertilize eggs formed by surficial female gametophytes (Schneller 1988, Haufler and Welling 1994). Individuals producing antheridiogen can thereby mine the genetic variability stored in spore banks, augmenting standing diversity. Clearly, the antheridiogen system is a potent physiological force in fern reproductive biology, promoting outcrossing and enhancing genetic variability in gametophyte and sporophyte populations alike.

Reproductive potential of isolated fern gametophytes. Despite having ontogenetic and antheridiogen-based mechanisms that promote outcrossing, many fern species retain some potential to generate new sporophytes through gametophytic selfing (often referred to as *isolate potential*). As Peck and colleagues (1990) emphasized, a set of requirements must be met before a single spore can establish a new population: It must be able to germinate, the resulting gametophyte must become bisexual, and it must self-fertilize and initiate a new sporophyte with adequate viability to successfully inhabit the new site. Having the capability to establish a new sporophyte from a single spore dispersal event provides a huge ecological advantage, and some species appear highly adapted to this lifestyle. For example, *Nephrolepis exaltata* appears to have little to no genetic load (Lloyd 1974a) and is a pioneer species that is one of

the first to establish on recent lava flows. The majority of fern species analyzed through *in vitro* gametophyte culture demonstrate a capacity for gametophytic selfing (Sessa et al. 2016) that would potentially allow them to colonize new habitats via this means (depending on the amount of genetic load). Through somatic mutation or with the arrival of additional spores, these species can subsequently exploit sporophytic selfing or even sporophytic outcrossing (figure 1) to enhance genetic variability. Some normally outcrossing species appear to also have a reproductive assurance strategy whereby their initially unisexual gametophytes can eventually attain bisexuality and undergo gametophytic selfing even if a second individual never shows up to allow outcrossing (Klekowski 1970b).

Contemporary research makes it clear that fern species vary considerably in their capacity to generate sporophytes from single gametophytes. This variability has been reported between species within genera (with some being highly adapted inbreeding colonists and others being highly outcrossing), as well as within particular species (e.g., Testo et al. 2015). Single spores can effect long-distance migration but then subsequently incorporate new genetic diversity and become highly outcrossing (Schneller and Holderegger 1996). Although the traditional textbook depiction of ferns undergoing gametophytic selfing does illustrate one of the ecological benefits of homospory, promoting the assumption that ferns *primarily* establish new populations via this means (and therefore routinely have "high isolate potential") is inaccurate and misleading. The only long-term comparative study of sporophytes that result from different breeding systems (Schneller 1987) shows that although it may be possible to initiate individuals through gametophytic or sporophytic selfing, such inbred individuals are neither as vigorous nor as successful as those formed via sporophytic outcrossing.

Gametophyte variability. The heart-shaped gametophytes depicted in figure 1 are convenient "placeholders" recognizable to biologists familiar with the fern life cycle. However, gametophytes of ferns have evolved significant morphological and physiological diversity, even among those expressing heart-shaped morphologies. This variability, often correlating with systematic (Nayar and Kaur 1971) and/or ecological (Farrar et al. 2008) categories, also relates to reproductive strategies (Dassler and Farrar 1997). Gametophytes of many species tolerate freezing and drought, high and low light intensities, and other environmental extremes (Farrar and Gooch 1975, Sato and Sakai 1981, Watkins et al. 2007b, Pittermann et al. 2013). They can also be perennial, expanding through vegetative propagation into large clones that are meters in diameter and decades or more in age (Watkins and Cardelus 2012, Farrar 2016).

Diversity in gametophyte form and physiology has enabled ferns to radiate into the wide range of habitats they must first colonize through gametophyte establishment—habitats including dark, moist forest floors; extremely bright, exposed forest canopies; tropical regions never experiencing

freezing temperatures; the coldest of northern latitudes and mountain tops where gametophytes formed from spores released in late summer must overwinter; and rocky desert extremes where gametophytes produce sporophytes despite the rare presence of environmentally available water (Liu et al. 2012). Some gametophytes tolerate environmental extremes exceeding that of their sporophyte counterparts and can therefore occur beyond the ecological and geographical ranges of their species' sporophytes (Farrar 1998, Ebihara et al. 2013, Duffy et al. 2015). In this sense, gametophytes continually test habitat suitability.

Gametophyte diversity and adaptability have also produced diverse reproductive strategies. A short-lived gametophyte, developing from a spore germinating in isolation (e.g., subsequent to long-distance migration), only has the option of gametophytic selfing, producing a homozygous sporophyte. However, if the resulting population endures, it can eventually incorporate genetic variation resulting from later arriving spores (Schneller and Holderegger 1996). Long-lived gametophytes have the added option of persisting (for years or decades) in the gametophytic phase, increasing the chance that a second spore migrant will introduce additional variation. Both strategies exploit the superior dispersal capacity of fern spores (Dassler and Farrar 2001, Farrar 2016).

Genetic variability correlates with mating system. Past reviews of seed plant population genetics (e.g., Hamrick and Godt 1996) have documented that breeding system type has a significant effect on genetic diversity: Populations of species with inbreeding systems typically have less genetic variation than do those with outcrossing systems. Similar studies of fern populations have demonstrated that very few have the depauperate genetic profiles expected of plants with a history of significant gametophytic selfing (Soltis PS and Soltis DE 1990, Soltis DE and Soltis PS 1992). More recent, broader-scale analyses of sexual gametophytes grown in vitro indicate that about 64% of the nearly 100 species investigated were capable of both gametophytic and sporophytic selfing (see figure 1), whereas 32% only produced sporophytes via crossing between gametophytes (either sporophytic selfing or sporophytic outcrossing) and only about 4% engaged exclusively in gametophytic selfing (Sessa et al. 2016). The potential for such *mixed-mating* systems in ferns highlights their flexibility and may allow them to establish new populations through single spore dispersal (via gametophytic selfing) while simultaneously maintaining significant genetic variation (via sporophytic selfing or sporophytic outcrossing).

Apomictic reproduction. Production of a new sporophyte directly from a gametophyte without syngamy (termed *agamospory*, a type of apomixis; see figure 1) has arisen independently in a diversity of fern families (Liu et al. 2012). Estimates of the proportion of fern species using this reproductive pathway range from 3% (Liu et al. 2012) to about 10% (Walker 1984). Although apomictic ferns occur in a variety of habitats, they are especially common in seasonally dry environments (Beck et al. 2011, Grusz et al. 2014). In these species, sporophytes give rise to unreduced spores through one of two cytogenetic pathways (see Walker 1966 for review). The resulting gametophytes have the same chromosome number as the progenitor sporophyte (most often triploid) and initiate new sporophytes by mitosis (asexual budding) instead of syngamy (figure 1). This budding occurs early in the development of the gametophyte, usually well before the point at which the gametophytes of sexual species produce gametangia. Therefore, apomixis both accelerates the life cycle and bypasses the ecological restrictions imposed by syngamy, which depends on having sufficient water in the environment to allow free-swimming sperm to reach an egg. This breeding system has clear advantages in xeric habitats where the moisture otherwise needed to complete the fern life cycle is temporally unpredictable, in short supply, or available for a very limited time.

Polyploidy. Wood and colleagues (2009) calculated that about 31% of fern speciation events are accompanied by an increase in ploidy, and about 44% of extant fern species can be called *neopolyploids*—that is, they have chromosome numbers that are multiples of basic generic chromosome numbers (Manton and Vida 1968). Polyploidy has a strong influence on fern breeding systems because it conceals some of the accumulated genetic load. The gametophytes of polyploids have at least two full sets of chromosomes (i.e., are diploid or polyploid themselves) and therefore avoid the intense selection imposed on free-living haploid organisms. Polyploid ferns tend to show less inbreeding depression than their diploid relatives (Masuyama and Watano 1990) and higher rates of gametophytic selfing (Soltis PS and Soltis DE 2000). Plants with these characteristics should be more efficient colonizers, which might help to explain why polyploid ferns frequently have more extensive distributions than their primarily outcrossing diploid congeners. Testo and colleagues (2015) demonstrated that ploidy level and hybrid status can have major effects on gametophyte biology and sporophyte formation. Their data suggest that polyploids themselves show considerable variability in antheridiogen potency and influence, sperm swimming time, size, and gametangial morphology. Clearly, the effect of polyploidy on gametophyte biology is an area crucially in need of further investigation.

Revising the homosporous vascular plant life cycle illustration, with updated terminology

The disconnect between reality (reflected in the contemporary research summarized above) and what is diagrammatically presented in most current textbooks undermines the teaching of both plant biology and the basic tenets of evolutionary theory. Also contributing to the continuing confusion about fern breeding or mating systems is the terminology currently applied to the variable pathways

by which fern gametophytes initiate sporophytes. This terminology was coined by Klekowski (1973, 1979), who proposed using *intragametophytic selfing*, *intergametophytic selfing*, and *intergametophytic crossing* to distinguish the three mating options available to homosporous vascular plants. Founded on his hypotheses and experimental results, these terms emphasized the gametophyte phase of the life cycle and the unique potential that he hypothesized ferns possessed for extreme inbreeding. However, these terms are easily confused (especially in verbal communication), they are not in sync with those used in other plant groups, and they are associated with hypotheses that have been falsified by recent studies (reviewed by Haufler 2014, Sessa et al. 2016). We agree with Cruden and Lloyd (1995) that, wherever possible, the specialized terminology relating to life cycle variants should be simplified to help students understand the unique features of the different phases, and, in particular, the complexities of independent gametophytes. Here, we propose a revised set of terms that coordinate more directly with those applied to seed plant life cycles. These descriptive terms are coupled with a novel diagram (figure 1) that more accurately portrays the life cycle options available to homosporous vascular plants.

***Gametophytic selfing* (replaces *intragametophytic selfing*).** Syngamy unites genetically identical gametes (egg and sperm) produced by the same gametophyte. In diploid species, gametophytic selfing results in a zygote that is homozygous at every gene locus. Among vascular plants, this breeding system is unique to homosporous ferns and lycophytes; such extreme inbreeding (resulting in 0% heterozygosity and therefore no allelic variability) can only be approximated in heterosporous plants after many generations of selfing. Among diploid homosporous vascular plants, the probability of gametophytic selfing leading to a viable sporophyte is low (Schneller 1987) and appears to represent a balancing act between short-term ecological benefits and a long-term evolutionary detriment.

***Sporophytic selfing* (replaces *intergametophytic selfing*).** This life-cycle variant is equivalent to *selfing* as applied to seed plants. Spores produced by a single sporophyte form a population of sibling gametophytes, and the sperm from one sib fertilizes the egg of a neighboring sib. Thus, the egg and sperm involved in syngamy come from different meiotic products of a single sporophyte. The use of the term *sporophytic selfing* facilitates comparisons with the life cycles and breeding systems of heterosporous vascular plants, which are rarely presented from the perspective of the gametophyte phase. It also differentiates this mating system from the *gametophytic selfing* discussed above.

***Sporophytic outcrossing* (replaces *intergametophytic crossing*).** When the sperm and egg that fuse to initiate a new zygote come from gametophytes formed by spores from different parental sporophytes, outcrossing occurs. Using the term *sporophytic outcrossing* is in line with our goal of seeking a common and consistent set of breeding system terms applicable across vascular plants. Outcrossing predominates in the diploid fern sporophyte populations surveyed to date, and *sporophytic outcrossing* ensures that this terminology consistently emphasizes the life-cycle stage that is most influenced by the outcome of the mating system.

Conclusions

Rather than portraying ferns and other homosporous seed-free vascular plants as persistent and extreme inbreeders constrained by their bisexual gametophytes, textbooks need to illustrate the unique diversity of breeding systems available to these plants. Texts should also note the predominance of mixed mating systems, which are characteristic of a majority of ferns studied to date (Sessa et al. 2016). Mixed mating in seed-free vascular plants has advantages that seed plants lack. Ferns and lycophytes that can use gametophytic selfing to establish new populations through wind dispersal of single, minute spores, have an advantage over seed plants in long distance dispersal. Sporophytes produced by gametophytic selfing can be viewed as a mechanism by which the first migrant of a species to a distant location may persist until the arrival of a second migrant. Outcrossing can then elevate genetic variability to a level comparable to what could have resulted from the far less likely scenario of two genetically different spores landing simultaneously in reproductive proximity. Even in the absence of later migrants, genetic variability can be added through mutation and infused into the population by subsequent outcrossing.

Therefore, the fern life cycle should not be depicted as a "weak link" or vestige of early land plants but rather as an intricate adaptation that has supported the long and continued success of ferns as important floristic components throughout the world. The simplistic "one-size-fits-all" illustration in current textbooks of a single heart-shaped, bisexual gametophyte undergoing extreme inbreeding does a great disservice not only to ferns and lycophytes but also to the disciplines of ecology, genetics, and evolution. The thoughtful student is left to conclude, incorrectly, that ferns and lycophytes do not follow basic principles of variation and selection and therefore should be considered enigmatic curiosities in the broader context of plant evolution. A more accurate picture of the life cycle of seed-free vascular plants is made available here, and we encourage the authors of biology textbooks to adopt these improvements in their new editions.

field of fern research. More than 40 years later, in January 2008, OTS offered its second course in tropical fern biology. Twenty-three students and six faculty members attended from the United States, Brazil, Colombia, Cuba, Spain, and Switzerland. This publication was sparked by lively discussions among that critical mass of fern biologists attending the 2008 course. We are grateful to OTS for re-igniting this course and for sponsoring subsequent courses in 2013 and 2015. We are indebted to Chris Martine and Jae Cantley for providing valuable comments on an early draft of the article and to the anonymous reviewers for helpful critiques of our manuscript. KMP is grateful to David Haig for constructive conversations about the fern life cycle and for helping to develop the new terminology presented here.

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